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ON MOBIUS MEANS

by

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ON MOBIUS MEANS

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The well-known Mobius Function is defined by the relations:

$\mu(1) = 1$,

$\mu(n) = 0$ if n has a square factor > 1;

and $\mu(n) = (-1)^r$ when n is a product of r different primes ≥ 2. We define

$$\mu_1(n) = \sum_{t=1}^{n} \mu(t), \quad \mu_2(n) = \sum_{t=1}^{n} \mu_1(t).$$

In 1946, Professor Viggo Brun conjectured that on the average

$$\frac{1}{n}\mu_2(n-1) \approx -2 + \frac{12}{n} - \frac{\beta}{n^2},$$

where $\beta = 18$. C. L. Siegel opined that

$$\beta = \frac{2\pi^2}{\zeta(3)} = 16{\cdot}42\ldots$$

The study made here was undertaken at the suggestion of Brun whose attention was attracted by H. Gupta's paper * 'On a Conjecture of Miller'. We have studied in fact, the function

$$F(n) \equiv \{\mu_2(n-1) + 2n - 12\}n.$$

In the table that follows are recorded values of $F(n)$ and $T_k(n)$ for values of $n \leqslant 750$, $k \leqslant 5$; where

$$T_k(n) = \frac{\sum_{t=1}^{n} T_{k-1}(t)}{n},$$

and

$$T_0(t) = F(t).$$

The first minimum for $T_k(n)$ occurs for

$k = 1$ at $n = 6$; for $k = 2$ at $n = 18$; for $k = 3$ at $n = 50$; for $k = 4$ at $n = 200$ and for $k = 5$ at $n = 539$.

In fact,

$T_1(6) = -14{\cdot}667$; $T_4(200) = -14{\cdot}846$;

$T_2(18) = -14{\cdot}542$; $T_5(539) = -14{\cdot}712$.

$T_3(50) = -14{\cdot}441$;

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* *Jour. Indian Math. Soc.*, 13, 1949, 85–90.

n	$F(n)$	T_1	T_2	$-T_3$	$-T_4$	$-T_5$
1	−10	−10·000	−10·000	10·000	10·000	10·000
2	−14	−12·000	−11·000	10·500	10·250	10·125
3	−15	−13·000	−11·667	10·889	10·463	10·238
4	−16	−13·750	−12·188	11·214	10·651	10·341
5	−15	−14·000	−12·550	11·481	10·817	10·436
6	−18	−14·667	−12·903	11·718	10·967	10·525
7	−14	−14·571	−13·141	11·921	11·103	10·607
8	−16	−14·750	−13·342	12·099	11·228	10·685
9	−18	−15·111	−13·539	12·259	11·342	10·758
10	−20	−15·600	−13·745	12·408	11·449	10·827
11	−11	−15·182	−13·876	12·541	11·548	10·893
12	−12	−14·917	−13·982	12·659	11·641	10·955
13	−13	−14·769	−14·024	12·764	11·727	11·014
14	−28	−15·714	−14·145	12·863	11·808	11·071
15	−30	−16·667	−14·313	12·960	11·885	11·125
16	−16	−16·625	−14·458	13·053	11·958	11·177
17	0	−15·647	−14·528	13·140	12·028	11·227
18	0	−14·778	−14·542	13·218	12·094	11·276
19	0	−14·000	−14·513	13·286	12·156	11·322
20	−20	−14·300	−14·502	13·347	12·216	11·367
21	−42	−15·619	−14·556	13·404	12·273	11·410
22	−44	−16·909	−14·663	13·462	12·327	11·451
23	−23	−17·174	−14·772	13·519	12·378	11·492
24	−24	−17·458	−14·884	13·576	12·428	11·531
25	−25	−17·760	−14·999	13·632	12·477	11·569
26	−26	−18·077	−15·117	13·690	12·523	11·605
27	0	−17·407	−15·202	13·746	12·568	11·641
28	28	−15·786	−15·223	13·798	12·612	11·676
29	58	−13·241	−15·154	13·845	12·655	11·709
30	60	−10·800	−15·009	13·884	12·696	11·742
31	31	− 9·452	−14·830	13·914	12·735	11·774
32	− 32	−10·156	−14·684	13·938	12·773	11·806
33	− 99	−12·848	−14·628	13·959	12·809	11·836
34	−136	−16·471	−14·683	13·981	12·843	11·866
35	−140	−20·000	−14·834	14·005	12·876	11·894
36	−108	−22·444	−15·046	14·034	12·909	11·923
37	− 74	−23·838	−15·283	14·068	12·940	11·950
38	− 76	−25·211	−15·545	14·107	12·971	11·977
39	− 39	−25·564	−15·802	14·150	13·001	12·003
40	40	−23·925	−16·005	14·196	13·031	12·029
41	123	−20·341	−16·110	14·243	13·060	12·054
42	168	−15·857	−16·104	14·287	13·089	12·079
43	172	−11·488	−15·997	14·327	13·118	12·103
44	132	− 8·227	−15·820	14·361	13·147	12·127
45	90	− 6·044	−15·603	14·389	13·174	12·150
46	46	−4·913	−15·371	14·410	13·201	12·173
47	47	−3·809	−15·125	14·425	13·227	12·195
48	0	−3·729	−14·887	14·435	13·252	12·217
49	− 49	−4·653	−14·679	14·440	13·276	12·239
50	−100	−6·560	−14·516	14·441	13·300	12·260

n	$F(n)$	T_1	T_2	$-T_3$	$-T_4$	$-T_5$
51	−153	− 9·431	−14·416	14·441	13·322	12·281
52	−156	−12·250	−14·375	14·440	13·344	12·301
53	−159	−15·019	−14·387	14·439	13·364	12·321
54	−216	−18·741	−14·468	14·439	13·384	12·341
55	−275	−23·400	−14·630	14·443	13·403	12·360
56	−280	−27·982	−14·868	14·450	13·422	12·379
57	−285	−32·491	−15·178	14·463	13·440	12·398
58	−232	−35·931	−15·535	14·481	13·458	12·416
59	−118	−37·322	−15·905	14·506	13·476	12·434
60	− 60	−37·700	−16·268	14·535	13·494	12·452
61	0	−37·082	−16·609	14·569	13·511	12·469
62	0	−36·484	−16·930	14·607	13·529	12·486
63	63	−34·905	−17·215	14·648	13·547	12·503
64	128	−32·359	−17·452	14·692	13·565	12·520
65	195	−28·862	−17·627	14·737	13·583	12·536
66	330	−23·424	−17·715	14·783	13·601	12·552
67	402	−17·075	−17·705	14·826	13·619	12·568
68	408	−10·824	−17·604	14·867	13·638	12·584
69	414	− 4·667	−17·417	14·904	13·656	12·599
70	490	2·400	−17·134	14·936	13·674	12·615
71	497	9·366	−16·760	14·962	13·692	12·630
72	432	15·236	−16·316	14·980	13·710	12·645
73	365	20·027	−15·818	14·992	13·728	12·660
74	222	22·757	−15·297	14·996	13·745	12·674
75	150	24·453	−14·767	14·993	13·762	12·689
76	76	25·132	−14·242	14·983	13·778	12·703
77	0	24·805	−13·735	14·967	13·793	12·717
78	0	24·487	−13·246	14·945	13·808	12·731
79	− 79	23·177	−12·784	14·917	13·822	12·745
80	−240	19·888	−12·375	14·886	13·835	12·759
81	− 405	14·642	−12·042	14·850	13·848	12·772
82	− 574	7·463	−11·804	14·813	13·859	12·785
83	− 664	− 0·627	−11·669	14·775	13·870	12·798
84	− 840	−10·619	−11·657	14·738	13·881	12·811
85	−1020	−22·494	−11·784	14·704	13·890	12·824
86	−1118	−35·232	−12·057	14·673	13·900	12·837
87	−1131	−47·828	−12·468	14·647	13·908	12·849
88	−1056	−59·284	−13·000	14·629	13·915	12·861
89	− 979	−69·618	−13·636	14·618	13·924	12·873
90	− 990	−79·844	−14·372	14·615	13·932	12·885
91	−1001	− 89·967	−15·203	14·621	13·940	12·896
92	− 920	− 98·989	−16·113	14·638	13·947	12·908
93	− 837	−106·925	−17·090	14·664	13·955	12·919
94	− 658	−112·787	−18·108	14·701	13·963	12·930
95	− 380	−115·600	−19·134	14·747	13·971	12·941
96	0	−114·396	−20·126	14·803	13·980	12·952
97	388	−109·216	−21·045	14·868	13·989	12·963
98	686	−101·102	−21·862	14·939	13·999	12·973
99	990	− 90·081	−22·551	15·016	14·009	12·984
100	1300	− 76·180	−23·087	15·097	14·020	12·994

n	$F(n)$	T_1	T_2	$-T_3$	$-T_4$	$-T_5$
101	1616	−59·426	−23·447	15·179	14·031	13·004
102	1836	−40·843	−23·617	15·262	14·043	13·014
103	1957	−21·447	−23·596	15·343	14·056	13·025
104	1976	− 2·240	−23·391	15·420	14·069	13·035
105	1995	16·781	−23·008	15·492	14·083	13·045
106	1808	34·623	−22·465	15·558	14·096	13·054
107	1926	52·299	−21·766	15·616	14·111	13·064
108	1836	68·815	−20·927	15·665	14·125	13·074
109	1744	84·183	−19·963	15·705	14·140	13·084
110	1540	97·418	−18·896	15·734	14·154	13·094
111	1221	107·540	−17·757	15·752	14·168	13·103
112	1008	115·580	−16·566	15·759	14·183	13·113
113	791	121·558	−15·344	15·756	14·197	13·123
114	456	124·491	−14·117	15·741	14·210	13·132
115	0	123·409	−12·921	15·717	14·223	13·142
116	− 348	119·345	−11·781	15·683	14·236	13·151
117	− 702	112·325	−10·720	15·640	14·248	13·160
118	−1062	102·373	− 9·762	15·591	14·259	13·170
119	−1309	90·513	− 8·919	15·535	14·270	13·179
120	−1440	77·758	− 8·197	15·473	14·280	13·188
121	−1573	64·116	−7·600	15·408	14·289	13·197
122	−1708	49·590	−7·131	15·341	14·298	13·206
123	−1722	35·187	−6·787	15·271	14·306	13·215
124	−1612	21·903	−6·555	15·201	14·313	13·224
125	−1500	9·728	−6·425	15·130	14·320	13·233
126	−1386	− 1·349	−6·385	15·061	14·325	13·242
127	−1270	−11·339	−6·424	14·993	14·331	13·250
128	−1280	−21·250	−6·540	14·927	14·335	13·259
129	−1290	−31·085	−6·730	14·863	14·339	13·267
130	−1170	−39·846	−6·985	14·803	14·343	13·275
131	−1179	−48·542	−7·302	14·746	14·346	13·283
132	−1320	−58·174	−7·687	14·692	14·349	13·292
133	−1463	−68·737	−8·146	14·643	14·351	13·299
134	−1474	−79·224	−8·677	14·598	14·353	13·307
135	−1350	−88·637	−9·269	14·559	14·354	13·315
136	−1224	− 96·985	− 9·914	14·525	14·356	13·323
137	−1096	−104·277	−10·603	14·496	14·357	13·330
138	−1104	−111·522	−11·334	14·473	14·357	13·338
139	−1251	−119·719	−12·114	14·456	14·358	13·345
140	−1540	−129·864	−12·955	14·446	14·359	13·352
141	−1833	−141·943	−13·870	14·441	14·359	13·359
142	−1988	−154·944	−14·863	14·444	14·360	13·367
143	−2002	−167·860	−15·933	14·455	14·361	13·373
144	−1872	−179·694	−17·070	14·473	14·361	13·380
145	−1740	−190·455	−18·266	14·499	14·362	13·387
146	−1460	−199·151	−19·505	14·533	14·363	13·394
147	−1029	−204·796	−20·765	14·576	14·365	13·400
148	− 592	−207·412	−22·027	14·626	14·367	13·407
149	− 149	−207·020	−23·268	14·684	14·369	13·413
150	150	−204·640	−24·477	14·749	14·371	13·420

n	$F(n)$	T_1	T_2	$-T_3$	$-T_4$	$-T_5$
151	453	−200·285	−25·642	14·822	14·374	13·426
152	608	−194·967	−26·756	14·900	14·378	13·432
153	765	−188·693	−27·814	14·985	14·382	13·438
154	924	−181·468	−28·812	15·074	14·386	13·445
155	930	−174·297	−29·750	15·169	14·391	13·451
156	1092	−166·179	−30·625	15·268	14·397	13·457
157	1256	−157·121	−31·431	15·371	14·403	13·463
158	1264	−148·127	−32·169	15·477	14·410	13·469
159	1431	−138·195	−32·836	15·586	14·417	13·475
160	1760	−126·331	−33·420	15·698	14·426	13·481
161	2093	−112·547	−33·912	15·811	14·434	13·487
162	2592	− 95·852	−34·294	15·926	14·443	13·493
163	3097	− 76·264	−34·552	16·039	14·453	13·499
164	3444	− 54·799	−34·675	16·153	14·463	13·504
165	3795	− 31·467	−34·656	16·265	14·474	13·510
166	3984	− 7·277	−34·491	16·375	14·486	13·516
167	4342	18·766	−34·172	16·482	14·498	13·522
168	4536	45·655	−33·697	16·584	14·510	13·528
169	4732	73·385	−33·063	16·682	14·523	13·534
170	4930	101·953	−32·269	16·773	14·536	13·540
171	4959	130·357	−31·318	16·858	14·550	13·546
172	4988	158·599	−30·214	16·936	14·564	13·552
173	5017	186·682	−28·960	17·005	14·578	13·557
174	4372	213·609	−27·566	17·066	14·592	13·563
175	4550	238·389	−26·046	17·117	14·606	13·569
176	4224	261·034	−24·415	17·159	14·621	13·575
177	3894	281·559	−22·686	17·190	14·635	13·581
178	3738	300·978	−20·868	17·211	14·650	13·587
179	3759	320·296	−18·962	17·221	14·664	13·593
180	3600	338·517	−16·976	17·219	14·678	13·599
181	3439	355·646	−14·917	17·207	14·692	13·605
182	3094	370·692	−12·799	17·182	14·706	13·611
183	2562	382·667	−10·638	17·147	14·719	13·617
184	2208	392·587	− 8·446	17·099	14·732	13·624
185	1850	400·465	− 6·236	17·041	14·745	13·630
186	1674	407·312	− 4·012	16·970	14·757	13·636
187	1309	412·134	− 1·787	16·889	14·768	13·642
188	1128	415·941	0·435	16·797	14·779	13·648
189	945	418·741	2·648	16·694	14·789	13·654
190	760	420·537	4·848	16·581	14·799	13·660
191	382	420·335	7·023	16·457	14·807	13·666
192	− 192	417·146	9·159	16·324	14·815	13·672
193	− 772	410·984	11·241	16·181	14·822	13·678
194	−1552	400·866	13·249	16·029	14·828	13·684
195	−2145	387·810	15·170	15·869	14·834	13·690
196	−2940	370·832	16·985	15·702	14·838	13·695
197	−3743	349·949	18·675	15·527	14·842	13·701
198	−4752	324·182	20·218	15·347	14·844	13·707
199	−5771	293·553	21·591	15·161	14·846	13·713
200	−7000	257·085	22·769	14·971	14·846	13·718

n	$F(n)$	T_1	T_2	$-T_3$	$-T_4$	$-T_5$
201	− 8241	214·806	23·724	14·779	14·846	13·724
202	− 9292	167·743	24·437	14·585	14·845	13·730
203	−10150	116·916	24·893	14·390	14·843	13·735
204	−10812	63·343	25·081	14·197	14·839	13·741
205	−11480	7·034	24·993	14·006	14·835	13·746
206	−11948	− 51·000	24·624	13·818	14·830	13·751
207	−12213	−109·754	23·975	13·636	14·825	13·756
208	−12480	−169·226	23·046	13·459	14·818	13·761
209	−12749	−229·416	21·838	13·290	14·811	13·766
210	−12810	−289·324	20·357	13·130	14·803	13·771
211	−12660	−347·953	18·611	12·980	14·794	13·776
212	−12720	−406·811	16·607	12·840	14·785	13·781
213	−12780	−464·404	14·348	12·712	14·775	13·786
214	−12626	−521·234	11·846	12·598	14·765	13·790
215	−12265	−575·809	9·112	12·497	14·754	13·795
216	−11664	−627·144	6·167	12·410	14·744	13·799
217	−11067	−675·253	3·027	12·339	14·732	13·803
218	−10246	−719·156	−0·286	12·284	14·721	13·808
219	− 9198	−757·872	−3·745	12·245	14·710	13·812
220	− 7920	−790·427	−7·321	12·222	14·699	13·816
221	−6630	−816·851	−10·984	12·217	14·687	13·820
222	−5106	−836·171	−14·701	12·228	14·676	13·824
223	−3791	−849·422	−18·444	12·256	14·666	13·827
224	−2688	−857·629	−22·191	12·300	14·655	13·831
225	−1575	−860·818	−25·918	12·361	14·645	13·835
226	−452	−859·009	−29·604	12·437	14·635	13·838
227	908	−851·225	−33·224	12·529	14·626	13·842
228	2052	−838·491	−36·756	12·635	14·617	13·845
229	3206	−820·830	−40·180	12·755	14·609	13·848
230	4140	−799·261	−43·480	12·889	14·601	13·852
231	4851	−774·801	−46·646	13·035	14·595	13·855
232	5336	−748·461	−49·671	13·193	14·588	13·858
233	5825	−720·249	−52·549	13·362	14·583	13·861
234	6084	−691·171	−55·278	13·541	14·579	13·864
235	6345	−661·230	−57·856	13·730	14·575	13·867
236	6844	−629·428	−60·278	13·927	14·572	13·870
237	7347	−595·772	−62·538	14·132	14·571	13·873
238	8092	−559·269	−64·625	14·344	14·570	13·876
239	8604	−520·929	−66·534	14·562	14·570	13·879
240	8880	−481·758	−68·264	14·786	14·570	13·882
241	9158	−441·759	−69·814	15·014	14·572	13·885
242	9196	−401·934	−71·186	15·247	14·575	13·888
243	9234	−362·280	−72·384	15·482	14·579	13·890
244	9272	−322·795	−73·411	15·719	14·584	13·893
245	9310	−283·478	−74·268	15·958	14·589	13·896
246	9348	−244·325	−74·959	16·198	14·596	13·899
247	9139	−206·338	−75·491	16·438	14·603	13·902
248	9176	−168·504	−75·866	16·678	14·612	13·905
249	9213	−130·827	−76·087	16·916	14·621	13·908
250	9500	− 92·304	−76·152	17·153	14·631	13·911

n	$F(n)$	T_1	T_2	$-T_3$	$-T_4$	$-T_5$
251	9789	−52·936	−76·059	17·388	14·642	13·913
252	9828	−13·726	−75·812	17·620	14·654	13·916
253	9867	25·328	−75·412	17·848	14·666	13·919
254	10160	65·228	−74·859	18·072	14·680	13·922
255	10710	106·972	−74·146	18·292	14·694	13·925
256	11008	149·555	−73·272	18·507	14·709	13·928
257	11308	192·973	−72·236	18·716	14·724	13·931
258	11352	236·225	−71·040	18·919	14·741	13·935
259	11137	278·313	−69·691	19·115	14·758	13·938
260	11180	320·242	−68·192	19·304	14·775	13·941
261	11223	362·015	−66·543	19·485	14·793	13·944
262	11266	403·634	−64·749	19·658	14·812	13·948
263	11572	446·099	−62·806	19·822	14·831	13·951
264	11616	488·409	−60·718	19·977	14·850	13·954
265	11660	530·566	−58·487	20·122	14·870	13·958
266	11970	573·571	−56·111	20·257	14·890	13·961
267	12015	616·423	−53·592	20·382	14·911	13·965
268	12328	660·123	−50·929	20·496	14·932	13·968
269	12643	704·669	−48·120	20·599	14·953	13·972
270	12690	749·059	−45·168	20·690	14·974	13·976
271	12737	793·295	−42·074	20·769	14·995	13·980
272	12512	836·379	−38·844	20·835	15·017	13·983
273	12285	878·315	−35·484	20·889	15·038	13·987
274	11782	918·109	−32·004	20·929	15·060	13·991
275	11550	956·771	−28·409	20·956	15·081	13·995
276	11316	994·304	−24·703	20·970	15·103	13·999
277	11080	1030·715	−20·893	20·970	15·124	14·003
278	10564	1065·007	−16·987	20·956	15·145	14·007
279	10323	1098·190	−12·990	20·927	15·166	14·011
280	10080	1130·268	− 8·907	20·884	15·186	14·016
281	9835	1161·246	−4·742	20·827	15·206	14·020
282	9306	1190·128	−0·505	20·754	15·226	14·024
283	8490	1215·922	3·793	20·668	15·245	14·028
284	7384	1237·641	8·137	20·566	15·264	14·033
285	6270	1255·298	12·513	20·450	15·282	14·037
286	4862	1267·909	16·903	20·320	15·300	14·042
287	3157	1274·491	21·285	20·175	15·316	14·046
288	1728	1276·066	25·642	20·016	15·333	14·050
289	289	1272·650	29·957	19·843	15·348	14·055
290	−1160	1264·262	34·213	19·656	15·363	14·060
291	−2910	1249·918	38·390	19·457	15·377	14·064
292	−4380	1230·637	42·474	19·245	15·391	14·069
293	−5860	1206·437	46·446	19·020	15·403	14·073
294	−7644	1176·333	50·289	18·785	15·414	14·078
295	−9440	1140·346	53·984	18·538	15·425	14·082
296	−10952	1099·493	57·516	18·281	15·435	14·087
297	−12474	1053·791	60·871	18·015	15·443	14·091
298	−14006	1003·255	64·033	17·739	15·451	14·096
299	−15249	948·900	66·993	17·456	15·458	14·100
300	−16200	891·737	69·742	17·165	15·464	14·105

n	$F(n)$	T_1	T_2	$-T_3$	$-T_4$	$-T_5$
301	−17157	831·774	72·274	16·868	15·468	14·110
302	−17818	770·020	74·584	16·565	15·472	14·114
303	−18180	707·478	76·673	16·258	15·474	14·119
304	−18240	645·151	78·543	15·946	15·476	14·123
305	−18300	583·036	80·197	15·630	15·476	14·128
306	−18054	522·131	81·641	15·313	15·476	14·132
307	−17806	462·430	82·881	14·993	15·474	14·136
308	−17864	402·928	83·920	14·672	15·472	14·141
309	−17922	343·624	84·761	14·350	15·468	14·145
310	−17670	285·516	85·408	14·028	15·464	14·149
311	−17727	227·598	85·866	13·707	15·458	14·153
312	−18096	168·868	86·132	13·387	15·451	14·158
313	−18467	109·329	86·206	13·069	15·444	14·162
314	−19154	47·981	86·084	12·753	15·435	14·166
315	−19530	−14·171	85·766	12·440	15·426	14·170
316	−19908	− 77·126	85·250	12·131	15·415	14·174
317	−20288	−140·883	84·537	11·826	15·404	14·178
318	−20988	−206·440	83·622	11·526	15·392	14·181
319	−22011	−274·793	82·498	11·231	15·378	14·185
320	−22720	−344·934	81·163	10·942	15·365	14·189
321	−23433	−416·860	79·611	10·660	15·350	14·192
322	−23828	−489·565	77·844	10·386	15·335	14·196
323	−24548	−564·050	75·856	10·118	15·318	14·199
324	−24948	−639·306	73·649	9·860	15·302	14·203
325	−25350	−715·342	71·221	9·610	15·284	14·206
326	−25754	− 792·147	68·573	9·371	15·266	14·209
327	−25833	− 868·725	65·707	9·141	15·247	14·213
328	−25584	− 944·076	62·628	8·922	15·228	14·216
329	−25333	−1018·207	59·343	8·715	15·208	14·219
330	−24750	−1090·121	55·860	8·519	15·188	14·222
331	−23832	−1158·828	52·190	8·336	15·167	14·224
332	−23240	−1225·337	48·342	8·165	15·146	14·227
333	−22644	−1289·658	44·324	8·007	15·125	14·230
334	−22044	−1351·796	40·144	7·863	15·103	14·233
335	−21105	−1410·761	35·813	7·733	15·081	14·235
336	−19824	−1465·562	31·344	7·616	15·059	14·238
337	−18535	−1516·214	26·752	7·514	15·036	14·240
338	−17576	−1563·728	22·047	7·427	15·014	14·242
339	−16611	−1608·115	17·238	7·354	14·991	14·244
340	−15300	−1648·385	12·339	7·296	14·968	14·246
341	−13981	−1684·551	7·363	7·253	14·946	14·249
342	−12312	−1715·626	2·325	7·225	14·923	14·251
343	−10633	−1741·624	− 2·759	7·212	14·901	14·252
344	− 8944	−1762·561	− 7·875	7·214	14·878	14·254
345	− 7245	−1778·452	−13·007	7·231	14·856	14·256
346	− 5882	−1790·312	−18·144	7·263	14·834	14·258
347	− 4154	−1797·163	−23·271	7·309	14·813	14·259
348	− 2784	−1799·988	−28·376	7·369	14·791	14·261
349	− 1396	−1798·831	−33·449	7·444	14·770	14·262
350	− 350	−1794·691	−38·481	7·533	14·750	14·264

n	$F(n)$	T_1	T_2	$-T_3$	$-T_4$	$-T_5$
351	702	−1787·578	−43·464	7·635	14·729	14·265
352	1760	−1777·500	−48·391	7·751	14·710	14·266
353	2824	−1764·464	−53·252	7·880	14·690	14·268
354	3540	−1749·480	−58·044	8·021	14·671	14·269
355	3905	−1733·552	−62·764	8·176	14·653	14·270
356	4628	−1715·682	−67·406	8·342	14·635	14·271
357	5355	−1695·877	−71·968	8·520	14·618	14·272
358	5728	−1675·140	−76·446	8·710	14·602	14·273
359	6462	−1652·474	−80·836	8·911	14·586	14·274
360	6840	−1628·883	−85·136	9·123	14·571	14·274
361	7220	−1604·371	− 89·345	9·345	14·556	14·275
362	7602	−1578·939	− 93·460	9·577	14·542	14·276
363	8349	−1551·590	− 97·477	9·819	14·529	14·276
364	9100	−1522·327	−101·391	10·071	14·517	14·277
365	9855	−1491·156	−105·199	10·332	14·506	14·278
366	10930	−1457·082	−108·892	10·601	14·495	14·278
367	11744	−1421·112	−112·468	10·878	14·485	14·279
368	12144	−1384·250	−115·924	11·164	14·476	14·280
369	12546	−1346·499	−119·259	11·457	14·468	14·280
370	12950	−1307·859	−122·471	11·757	14·461	14·280
371	12985	−1269·334	−125·562	12·064	14·454	14·281
372	13392	−1229·922	−128·531	12·377	14·448	14·281
373	13801	−1189·625	−131·376	12·696	14·444	14·282
374	13838	−1149·444	−134·098	13·020	14·440	14·282
375	13500	−1110·379	−136·701	13·350	14·437	14·283
376	13160	−1072·426	−139·190	13·685	14·435	14·283
377	12818	−1035·581	−141·568	14·024	14·434	14·284
378	12852	− 998·841	−143·836	14·367	14·434	14·284
379	12886	− 962·206	−145·995	14·715	14·435	14·284
380	12540	− 926·674	−148·049	15·066	14·436	14·285
381	12192	− 892·241	−150·002	15·420	14·439	14·285
382	12224	− 857·906	−151·856	15·777	14·442	14·286
383	12639	− 822·666	−153·607	16·137	14·447	14·286
384	12672	− 787·523	−155·258	16·499	14·452	14·286
385	12705	− 752·478	−156·809	16·864	14·458	14·287
386	12352	− 718·528	−158·264	17·230	14·466	14·287
387	12384	− 684·672	−159·625	17·598	14·474	14·288
388	12416	− 650·907	−160·891	17·967	14·483	14·288
389	12448	− 617·234	−162·064	18·338	14·493	14·289
390	12090	− 584·651	−163·148	18·709	14·503	14·289
391	12121	− 552·156	−164·142	19·081	14·515	14·290
392	12544	− 518·747	−165·047	19·453	14·528	14·290
393	12969	− 484·427	−165·860	19·826	14·541	14·291
394	13790	− 448·198	−166·576	20·198	14·556	14·292
395	15010	− 409·063	−167·190	20·570	14·571	14·292
396	16632	− 366·030	−167·692	20·942	14·587	14·293
397	18262	− 319·108	−168·074	21·312	14·604	14·294
398	19502	− 269·306	−168·328	21·682	14·622	14·295
399	21147	− 215·632	−168·447	22·050	14·640	14·296
400	22400	− 159·092	−168·423	22·416	14·660	14·297

n	$F(n)$	T_1	T_2	$-T_3$	$-T_4$	$-T_5$
401	23659	−99·696	−168·252	22·779	14·680	14·298
402	24522	−33·448	−167·929	23·140	14·701	14·299
403	24986	23·648	−167·454	23·498	14·723	14·300
404	25856	87·589	−166·822	23·853	14·745	14·301
405	26730	153·373	−166·032	24·204	14·768	14·302
406	27608	220·995	−165·078	24·551	14·793	14·303
407	28083	289·452	−163·962	24·894	14·818	14·304
408	28968	359·743	−162·678	25·232	14·843	14·306
409	29857	431·863	−161·224	25·564	14·869	14·307
410	30340	504·810	−159·600	25·891	14·896	14·308
411	30414	577·582	−157·806	26·212	14·924	14·310
412	30900	651·180	−155·843	26·526	14·952	14·312
413	31388	725·603	−153·708	26·834	14·981	14·313
414	32292	801·850	−151·400	27·135	15·010	14·315
415	33200	879·918	−148·915	27·429	15·040	14·317
416	34528	960·803	−146·248	27·714	15·070	14·318
417	35862	1044·499	−143·392	27·992	15·101	14·320
418	37020	1132·000	−140·341	28·260	15·133	14·322
419	38967	1222·298	−137·089	28·520	15·165	14·324
420	39900	1314·388	−133·633	28·770	15·197	14·326
421	40837	1408·266	−129·970	29·011	15·230	14·328
422	41356	1502·929	−126·101	29·241	15·263	14·331
423	42300	1599·376	−122·022	29·460	15·297	14·333
424	43248	1697·604	−117·730	29·668	15·331	14·335
425	44200	1797·609	−113·224	29·865	15·365	14·338
426	45156	1899·390	−108·499	30·050	15·399	14·340
427	45689	2001·941	−103·557	30·222	15·434	14·343
428	46652	2106·264	− 98·394	30·381	15·469	14·346
429	47619	2212·354	− 93·007	30·527	15·504	14·348
430	48160	2319·209	− 87·397	30·659	15·539	14·351
431	48272	2425·828	−81·566	30·778	15·575	14·354
432	47952	2531·213	−75·518	30·881	15·610	14·357
433	47630	2635·367	−69·257	30·970	15·646	14·360
434	46872	2737·295	−62·791	31·043	15·681	14·363
435	45675	2836·002	−56·127	31·101	15·717	14·366
436	44036	2930·498	−49·277	31·142	15·752	14·369
437	42389	3020·792	−42·252	31·168	15·787	14·372
438	41172	3107·895	−35·059	31·177	15·822	14·376
439	39610	3190·815	−27·711	31·169	15·857	14·379
440	37400	3268·564	−20·220	31·144	15·892	14·382
441	35280	3341·152	−12·597	31·102	15·926	14·386
442	33150	3408·593	− 4·857	31·042	15·961	14·389
443	30567	3469·898	2·986	30·966	15·995	14·393
444	27528	3524·083	10·917	30·871	16·028	14·397
445	24475	3571·164	18·917	30·759	16·061	14·400
446	21854	3612·157	26·974	30·630	16·094	14·404
447	19668	3648·076	35·075	30·483	16·126	14·408
448	17920	3678·933	43·211	30·318	16·158	14·412
449	16164	3707·737	51·372	30·136	16·189	14·416
450	13950	3730·498	59·548	29·937	16·219	14·420

n	$F(n)$	T_1	T_2	$-T_3$	$-T_4$	$-T_5$
451	11726	3748·226	67·727	29·721	16·249	14·424
452	9944	3761·934	75·900	29·487	16·279	14·428
453	8154	3771·629	84·058	29·236	16·307	14·432
454	6810	3778·322	92·196	28·969	16·335	14·436
455	5915	3783·018	100·307	28·685	16·362	14·441
456	4560	3784·721	108·387	28·384	16·389	14·445
457	3199	3783·440	116·429	28·067	16·414	14·449
458	1374	3778·179	124·424	27·734	16·439	14·454
459	0	3769·948	132·366	27·386	16·463	14·458
460	−1380	3758·752	140·250	27·021	16·486	14·462
461	−2766	3744·599	148·068	26·641	16·508	14·467
462	−4620	3726·494	155·814	26·246	16·529	14·471
463	−6019	3705·445	163·480	25·837	16·549	14·476
464	−7888	3680·459	171·060	25·412	16·568	14·480
465	−9765	3651·544	178·545	24·974	16·586	14·485
466	−12116	3617·708	185·925	24·521	16·603	14·489
467	−14010	3579·961	193·193	24·055	16·619	14·494
468	−16380	3537·312	200·338	23·575	16·634	14·498
469	−18760	3489·770	207·352	23·083	16·648	14·503
470	−20680	3438·345	214·226	22·578	16·660	14·508
471	−23079	3382·044	220·952	22·061	16·672	14·512
472	−25016	3321·879	227·522	21·532	16·682	14·517
473	−26961	3257·856	233·929	20·992	16·691	14·522
474	−28440	3190·983	240·167	20·441	16·699	14·526
475	−30400	3120·266	246·230	19·880	16·706	14·531
476	−32368	3045·710	252·112	19·308	16·711	14·535
477	−34344	2967·325	257·804	18·728	16·716	14·540
478	−36328	2885·117	263·300	18·137	16·718	14·544
479	−37841	2800·094	268·596	17·539	16·720	14·549
480	−39840	2711·260	273·685	16·932	16·721	14·554
481	−41847	2618·624	278·560	16·318	16·720	14·558
482	−43380	2523·191	283·217	15·696	16·718	14·562
483	−44436	2425·967	287·654	15·068	16·714	14·567
484	−45980	2325·954	291·865	14·434	16·710	14·571
485	−47530	2223·159	295·847	13·794	16·704	14·576
486	−48600	2118·584	299·598	13·150	16·696	14·580
487	−49674	2012·234	303·114	12·500	16·688	14·584
488	−51240	1903·111	306·393	11·847	16·678	14·589
489	−52812	1791·219	309·430	11·190	16·666	14·593
490	−53900	1677·563	312·222	10·530	16·654	14·597
491	−54992	1562·147	314·767	9·867	16·640	14·601
492	−56580	1443·972	317·062	9·203	16·625	14·605
493	−58174	1323·042	319·103	8·537	16·609	14·610
494	−59280	1200·364	320·887	7·870	16·591	14·614
495	−60885	1074·939	322·410	7·203	16·572	14·618
496	−62496	946·772	323·669	6·536	16·552	14·621
497	−64113	815·867	324·659	5·869	16·530	14·625
498	−65238	683·229	325·379	5·204	16·508	14·629
499	−66866	547·860	325·825	4·541	16·484	14·633
500	−69000	408·764	325·991	3·880	16·458	14·636

n	$F(n)$	T_1	T_2	$+T_3$	$-T_4$	$-T_5$
501	−71142	265·948	325·871	−3·221	16·432	14·640
502	−72790	120·418	325·462	−2·567	16·404	14·643
503	−73941	− 26·821	324·762	−1·916	16·375	14·647
504	−75600	−176·768	323·766	−1·270	16·346	14·650
505	−77265	−329·418	322·473	−0·629	16·314	14·654
506	−78430	− 483·767	320·880	0·007	16·282	14·657
507	−80106	− 640·813	318·983	0·636	16·249	14·660
508	−81788	− 800·551	316·779	1·258	16·214	14·663
509	−83476	− 962·978	314·265	1·873	16·179	14·666
510	−85680	−1129·090	311·435	2·480	16·142	14·669
511	−87381	−1297·881	308·285	3·079	16·104	14·672
512	−88576	−1468·346	304·815	3·668	16·066	14·674
513	−89775	−1640·483	301·023	4·248	16·026	14·677
514	−90978	−1814·292	296·908	4·817	15·986	14·680
515	−91670	−1988·769	292·470	5·376	15·944	14·682
516	−91848	−2162·915	287·711	5·923	15·902	14·684
517	−92026	−2336·731	282·635	6·458	15·859	14·687
518	−91686	−2509·220	277·245	6·981	15·815	14·689
519	−91863	−2681·385	271·545	7·490	15·770	14·691
520	−91520	−2852·229	265·537	7·987	15·724	14·693
521	−91175	−3021·754	259·228	8·469	15·678	14·695
522	−91350	−3190·966	252·618	8·937	15·630	14·697
523	−91525	−3359·864	245·711	9·389	15·582	14·698
524	−92224	−3529·452	238·506	9·826	15·534	14·700
525	−92925	−3699·730	231·005	10·248	15·485	14·701
526	−93628	−3870·696	223·207	10·653	15·435	14·703
527	−93806	−4041·351	215·115	11·041	15·385	14·704
528	−93456	−4210·697	206·733	11·411	15·334	14·705
529	−93104	−4378·737	198·065	11·764	15·283	14·706
530	−92750	−4545·475	189·115	12·099	15·231	14·707
531	−92925	−4711·915	179·885	12·415	15·179	14·708
532	−93100	−4878·058	170·377	12·712	15·127	14·709
533	−93275	−5043·906	160·594	12·989	15·074	14·710
534	−92916	−5208·461	150·540	13·247	15·021	14·710
535	−93090	−5372·725	140·216	13·484	14·968	14·711
536	−92728	−5535·701	129·627	13·701	14·914	14·711
537	−92364	−5697·393	118·776	13·896	14·861	14·711
538	−91460	−5856·803	107·669	14·071	14·807	14·712
539	−90013	−6012·937	96·313	14·223	14·753	14·712
540	−88560	−6165·802	84·717	14·354	14·699	14·712
541	−87101	−6315·405	72·886	14·462	14·645	14·712
542	−86178	−6462·753	60·828	14·548	14·591	14·711
543	−84708	−6606·851	48·549	14·610	14·538	14·711
544	−82688	−6746·706	36·058	14·650	14·484	14·711
545	−80660	−6882·327	23·363	14·666	14·430	14·710
546	−78078	−7012·722	10·477	14·658	14·377	14·709
547	−74939	−7136·901	− 2·590	14·626	14·324	14·709
548	−72336	−7255·878	−15·826	14·571	14·271	14·708
549	−69723	−7369·661	−29·221	14·491	14·219	14·707
550	−67100	−7478·262	−42·764	14·387	14·167	14·706

n	$F(n)$	T_1	T_2	T_3	$-T_4$	$-T_5$
551	−64467	−7581·690	− 56·447	14·258	14·115	14·705
552	−61272	−7678·956	− 70·256	14·105	14·064	14·704
553	−58065	−7770·069	− 84·179	13·928	14·014	14·703
554	−54292	−7854·043	− 98·204	13·727	13·964	14·701
555	−49950	−7929·892	−112·315	13·498	13·914	14·700
556	−46148	−7998·629	−126·499	13·246	13·865	14·698
557	−42332	−8060·269	−140·743	12·970	13·817	14·697
558	−39060	−8115·824	−155·035	12·669	13·770	14·695
559	−35776	−8165·306	−169·365	12·343	13·723	14·693
560	−31920	−8207·725	−183·719	11·993	13·677	14·692
561	−28050	−8243·094	−198·085	11·618	13·632	14·690
562	−24728	−8272·427	−212·452	11·220	13·588	14·688
563	−20831	−8294·734	−226·808	10·797	13·544	14·686
564	−17484	−8311·026	−241·142	10·350	13·502	14·684
565	−14125	−8321·317	−255·443	9·880	13·461	14·681
566	−10188	−8324·615	−269·700	9·386	13·420	14·679
567	− 5670	−8319·933	−283·898	8·869	13·381	14·677
568	− 1135	−8307·285	−298·023	8·328	13·343	14·674
569	+ 3414	−8286·685	−312·063	7·765	13·306	14·672
570	7410	−8259·147	−326·005	7·180	13·270	14·670
571	11991	−8223·683	−339·837	6·572	13·235	14·667
572	16015	−8181·306	−353·546	5·942	13·201	14·665
573	20055	−8132·028	−367·121	5·291	13·169	14·662
574	24682	−8074·861	−380·549	4·619	13·138	14·659
575	28760	−8010·817	−393·819	3·926	13·108	14·657
576	32832	−7939·910	−406·920	3·213	13·080	14·654
577	36928	−7862·149	−419·840	2·480	13·053	14·651
578	40460	−7778·547	−432·572	1·727	13·028	14·648
579	44004	−7689·112	−445·104	0·955	13·004	14·645
580	48140	−7592·855	−457·428	0·165	12·981	14·643
581	52290	−7489·786	−469·532	−0·643	12·960	14·640
582	57036	−7378·918	−481·404	−1·469	12·940	14·637
583	61215	−7261·261	−493·033	−2·313	12·922	14·634
584	65992	−7135·827	−504·408	−3·172	12·905	14·631
585	70785	−7002·629	−515·516	−4·048	12·890	14·628
586	75594	−6861·679	−526·345	−4·939	12·876	14·625
587	81006	−6711·990	−536·883	−5·846	12·864	14·622
588	85848	−6554·575	−547·117	−6·766	12·854	14·619
589	90706	−6389·446	−557·036	−7·700	12·845	14·616
590	96170	−6215·617	−566·627	−8·648	12·838	14·613
591	101061	−6034·100	−575·878	− 9·608	12·832	14·610
592	106560	−5843·907	−584·777	−10·579	12·829	14·607
593	112077	−5645·052	−593·310	−11·562	12·826	14·604
594	117018	−5438·549	−601·467	−12·555	12·826	14·601
595	121975	−5224·408	−609·237	−13·558	12·827	14·598
596	126352	−5003·643	−616·610	−14·570	12·830	14·595
597	130743	−4776·261	−623·578	−15·590	12·835	14·592
598	135746	−4541·274	−630·129	−16·617	12·841	14·589
599	140166	−4299·693	−636·255	−17·652	12·849	14·586
600	144000	−4052·527	−641·949	−18·692	12·859	14·583

n	$F(n)$	T_1	T_2	$-T_3$	$-T_4$	$-T_5$
601	147846	−3799·784	−647·203	19·738	12·870	14·580
602	151102	−3542·472	−652·013	20·788	12·884	14·578
603	153765	−3281·597	−656·374	21·842	12·898	14·575
604	156436	−3017·164	−660·282	22·899	12·915	14·572
605	159115	−2749·177	−663·735	23·959	12·933	14·569
606	161802	−2477·640	−666·728	25·019	12·953	14·567
607	163890	−2203·558	−669·260	26·081	12·975	14·564
608	165376	−1927·934	−671·330	27·142	12·998	14·562
609	166866	−1650·768	−672·938	28·202	13·023	14·559
610	167750	−1373·062	−674·086	29·261	13·050	14·556
611	168025	−1095·815	−674·776	30·318	13·078	14·554
612	168912	− 818·024	−675·011	31·371	13·108	14·552
613	169801	− 539·690	−674·790	32·421	13·139	14·549
614	170078	− 261·811	−674·117	33·466	13·172	14·547
615	170970	16·615	−672·994	34·506	13·207	14·545
616	171248	294·588	−671·423	35·540	13·243	14·543
617	171526	572·110	−669·408	36·567	13·281	14·541
618	171186	848·184	−666·962	37·587	13·320	14·539
619	170225	1121·814	−664·062	38·599	13·361	14·537
620	168640	1392·005	−660·746	39·602	13·404	14·535
621	167049	1658·763	−657·011	40·597	13·448	14·533
622	165452	1922·096	−652·865	41·581	13·493	14·532
623	164472	2183·011	−648·313	42·555	13·539	14·530
624	164112	2442·513	−643·359	43·518	13·587	14·529
625	163750	2700·606	−638·009	44·469	13·637	14·527
626	163386	2957·291	−632·266	45·408	13·688	14·526
627	163647	3213·574	−626·132	46·334	13·740	14·524
628	163280	3468·457	−619·612	47·247	13·793	14·523
629	162911	3721·943	−612·710	48·146	13·848	14·522
630	163170	3975·035	−605·428	49·031	13·903	14·521
631	163429	4227·735	−597·768	49·900	13·960	14·520
632	163056	4479·046	−589·735	50·754	14·019	14·520
633	162681	4728·970	−581·333	51·593	14·078	14·519
634	162938	4978·511	−572·563	52·414	14·138	14·518
635	163830	5228·671	−563·427	53·219	14·200	14·518
636	165360	5480·450	−553·924	54·006	14·263	14·517
637	166894	5733·846	−544·054	54·776	14·326	14·517
638	168432	5988·859	−533·814	55·526	14·391	14·517
639	169335	6244·487	−523·206	56·258	14·456	14·517
640	170240	6500·730	−512·231	56·971	14·523	14·517
641	171147	6757·588	−500·890	57·663	14·590	14·517
642	171414	7014·062	−489·184	58·336	14·658	14·517
643	171038	7269·154	−477·119	58·987	14·727	14·518
644	170016	7521·866	−464·698	59·617	14·797	14·518
645	168990	7772·205	−451·927	60·225	14·867	14·518
646	167314	8019·173	−438·814	60·811	14·938	14·519
647	164985	8261·779	−425·367	61·374	15·010	14·520
648	162000	8499·029	−411·594	61·915	15·083	14·521
649	159005	8730·934	−397·507	62·432	15·156	14·522
650	156650	8958·502	−383·114	62·925	15·229	14·523

n	$F(n)$	T_1	T_2	$-T_3$	$-T_4$	$-T_5$
651	154287	9181·740	−368·421	63·395	15·303	14·524
652	151264	9399·658	−353·439	63·840	15·378	14·525
653	148231	9612·263	−338·178	64·260	15·452	14·527
654	144534	9818·566	−322·648	64·655	15·528	14·528
655	140170	10017·576	−306·861	65·025	15·603	14·530
656	136448	10210·305	−290·829	65·369	15·679	14·532
657	132714	10396·764	−274·562	65·687	15·755	14·534
658	128968	10576·964	−258·070	65·980	15·831	14·536
659	124551	10749·914	−241·366	66·246	15·908	14·538
660	119460	10914·626	−224·463	66·485	15·985	14·540
661	114353	11071·113	−207·374	66·699	16·061	14·542
662	108568	11218·390	−190·115	66·885	16·138	14·544
663	103428	11357·469	−172·698	67·045	16·215	14·547
664	97608	11487·364	−155·137	67·177	16·292	14·550
665	91770	11608·090	−137·448	67·283	16·368	14·552
666	85248	11718·661	−119·646	67·362	16·445	14·555
667	78706	11819·091	−101·747	67·413	16·521	14·558
668	72812	11910·398	− 83·765	67·438	16·598	14·561
669	66900	11992·595	− 65·713	67·435	16·674	14·564
670	61640	12066·696	− 47·605	67·405	16·749	14·568
671	55693	12131·712	−29·454	67·349	16·825	14·571
672	50400	12188·659	−11·272	67·265	16·900	14·574
673	45091	12237·548	6·928	67·155	16·974	14·578
674	39092	12277·392	25·133	67·018	17·049	14·582
675	33750	12309·203	43·332	66·855	17·122	14·586
676	28392	12332·994	61·512	66·665	17·196	14·589
677	23018	12348·777	79·661	66·449	17·268	14·593
678	16950	12355·563	97·767	66·206	17·341	14·597
679	10185	12352·367	115·815	65·938	17·412	14·602
680	4080	12340·201	133·792	65·645	17·483	14·606
681	− 2043	12319·081	151·686	65·326	17·553	14·610
682	− 7502	12290·018	169·484	64·981	17·623	14·614
683	−13660	12252·023	187·174	64·612	17·692	14·619
684	−20520	12204·111	204·743	64·218	17·760	14·624
685	−27400	12146·295	222·176	63·800	17·827	14·628
686	−33614	12079·589	239·461	63·358	17·893	14·633
687	−39848	12004·006	256·585	62·892	17·959	14·638
688	−45408	11920·558	273·536	62·403	18·023	14·643
689	−50986	11829·257	290·310	61·892	18·087	14·648
690	−55890	11731·113	306·891	61·357	18·150	14·653
691	−60117	11627·136	323·274	60·800	18·212	14·658
692	−65048	11516·334	339·448	60·222	18·272	14·663
693	−69993	11398·716	355·407	59·622	18·332	14·668
694	−74952	11274·291	371·140	59·002	18·390	14·674
695	−79230	11144·069	386·641	58·360	18·448	14·679
696	−82824	11009·057	401·903	57·699	18·504	14·685
697	−86428	10869·262	416·921	57·018	18·560	14·690
698	−89344	10725·690	431·690	56·318	18·614	14·696
699	−91569	10579·346	446·207	55·599	18·667	14·702
700	−93100	10431·233	460·472	54·862	18·718	14·707

n	$F(n)$	T_1	T_2	$-T_3$	$-T_4$	$-T_5$
701	— 94635	10281·352	474·481	54·107	18·769	14·713
702	— 96876	10128·706	488·234	53·334	18·818	14·719
703	— 99123	9973·299	501·726	52·544	18·866	14·725
704	—100672	9816·132	514·957	51·738	18·913	14·731
705	—102225	9657·208	527·924	50·916	18·958	14·737
706	—104488	9495·530	540·626	50·078	19·002	14·743
707	—106050	9332·099	553·061	49·225	19·045	14·749
708	—106908	9167·918	565·229	48·357	19·086	14·755
709	—107768	9002·987	577·130	47·475	19·126	14·761
710	—109340	8836·307	588·763	46·579	19·165	14·768
711	—111627	8666·879	600·124	45·670	19·202	14·774
712	—113020	8494·706	611·212	44·747	19·238	14·780
713	—116219	8319·792	622·024	43·812	19·273	14·786
714	—117810	8143·140	632·558	42·864	19·306	14·793
715	—118690	7965·751	642·814	41·905	19·337	14·799
716	—120288	7786·626	652·791	40·935	19·368	14·805
717	—121890	7605·766	662·488	39·954	19·396	14·812
718	—122778	7424·173	671·906	38·963	19·423	14·818
719	—122949	7242·847	681·045	37·961	19·449	14·825
720	—123840	7060·788	689·906	36·950	19·474	14·831
721	—124732	6877·994	698·488	35·930	19·496	14·838
722	—124906	6695·468	706·794	34·902	19·518	14·844
723	—125079	6513·207	714·825	33·865	19·538	14·850
724	—124528	6332·211	722·584	32·820	19·556	14·857
725	—123975	6152·477	730·074	31·768	19·573	14·864
726	—123420	5974·003	737·297	30·708	19·588	14·870
727	—122863	5796·785	744·256	29·642	19·602	14·877
728	—123032	5619·823	750·953	28·570	19·614	14·883
729	—123201	5443·114	757·390	27·492	19·625	14·890
730	—123370	5266·658	763·567	26·408	19·634	14·896
731	—124270	5089·453	769·485	25·320	19·642	14·903
732	—124440	4912·500	775·144	24·226	19·648	14·909
733	—124610	4736·798	780·548	23·128	19·653	14·916
734	—125514	4558·346	785·695	22·026	19·656	14·922
735	—125685	4381·144	790·586	20·920	19·658	14·928
736	—125856	4204·192	795·224	19·812	19·658	14·935
737	—126027	4027·487	799·610	18·700	19·657	14·941
738	—125460	3852·030	803·746	17·585	19·654	14·948
739	—124891	3677·817	807·635	16·469	19·650	14·954
740	—125060	3503·847	811·279	15·350	19·644	14·960
741	—125229	3330·119	814·678	14·230	19·637	14·967
742	—126140	3155·631	817·833	13·109	19·628	14·973
743	—127796	2979·384	820·742	11·986	19·618	14·979
744	—130200	2800·379	823·403	10·864	19·606	14·985
745	—132610	2618·620	825·813	9·740	19·593	14·992
746	—134280	2435·110	827·970	8·618	19·578	14·998
747	—135207	2250·850	829·875	7·495	19·562	15·004
748	—136136	2065·841	831·527	6·373	19·544	15·010
749	—137067	1880·083	832·927	5·253	19·525	15·016
750	—137250	1694·576	834·076	4·134	19·504	15·022

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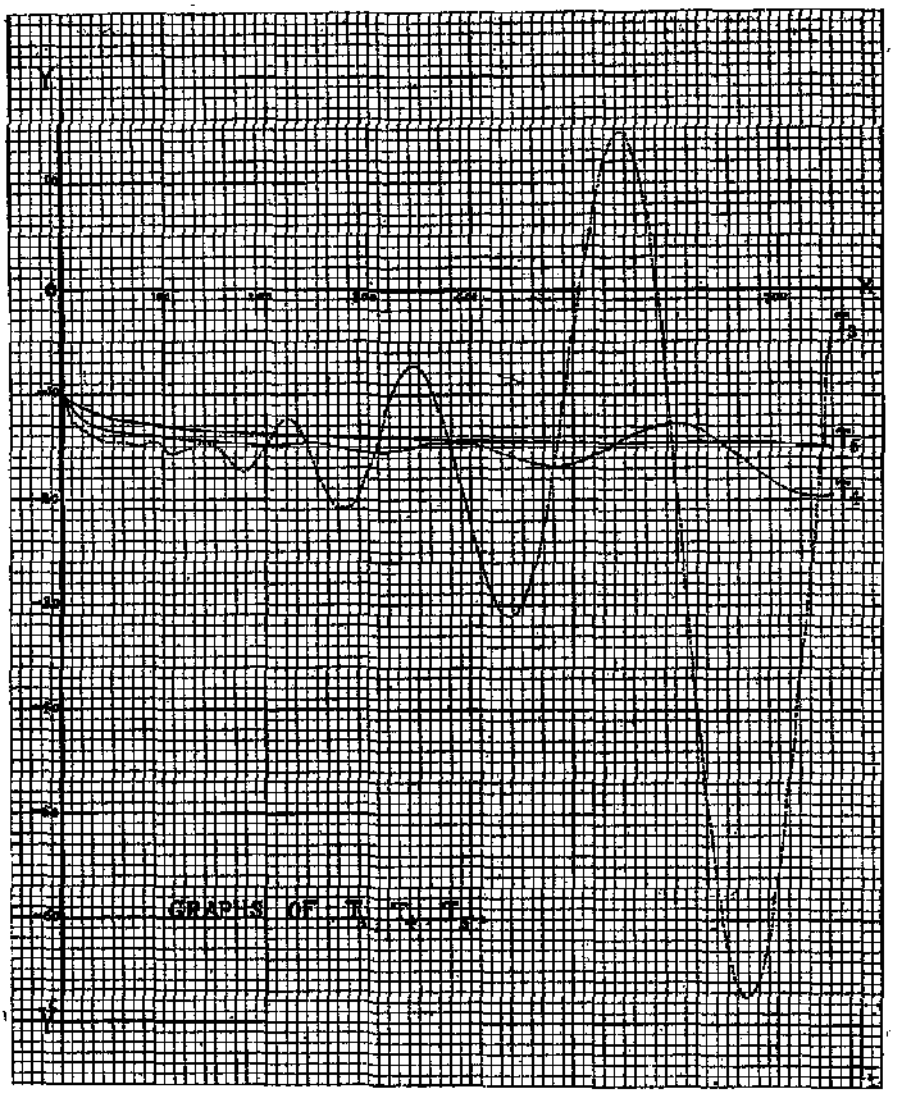

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CHLORAMINE-B AS VOLUMETRIC REAGENT. PART I

by

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CHLORAMINE-B AS VOLUMETRIC REAGENT. PART I

By Apar Singh, *Panjab University College, Hoshiarpur*

Chloramine-B is the sodium salt of N-chloro benzene sulphonamide and has the formula $C_6H_5SO_2N(Na)Cl.3H_2O$ (molecular weight, 267·5). Its high equivalent weight, ready solubility in water and moderate stability are worthy of note and recommend it as a volumetric oxidising agent. When kept in bottle of blue glass, the freshly prepared solution was found to lose about 2-3% of its oxidising power in the course of a fortnight at ordinary temperature. The aqueous solution reacts as if it were a hypochlorite and has the great advantage that it is far more stable than sodium hypochlorite.

$$C_6H_5SO_2N\begin{matrix}Na\\Cl\end{matrix} + H_2O \longrightarrow C_6H_5SO_2NH_2 + NaOCl.$$

B. N. Affana's eve (Sverdlovsk. Agr. Inst. Zavodskaya Lab., 1950, 16, 1011-1012) has reported that Chloramine-B is a good substitute for Chloramine-T in analytical chemistry.

In the present investigation arsenious oxide, tartaremetic, potassium sulphocyanide, hydrazine sulphate, stannous chloride, ferrous sulphate mercurous chloride, thallous chloride, potassium sulphite, sodium bisulphite, sulphurous acid were pre-oxidised with iodine monochloride and the liberated iodine was oxidised with standard Chloramine-B to iodine monochloride, iodine cyanide and complex IBr_2 in the presence of excess of concentrated hydrochloric acid, hydrocyanic acid and a saturated solution of potassium bromide respectively. Potassium iodide was titrated against standard Chloramine-B directly without pre-oxidation with iodine monochloride. Hydrogen peroxide was pre-oxidised with hypo-iodous acid (5 c.c. of 0·5 *M* iodine monochloride+20 c.c. of 2·5 *N* sodium hydroxide).

Experimental

Iodine Cyanide Method.—A known weight of each substance in solution was taken in a stoppered conical flask. To it was added 5 c.c. of 0·5 *M* iodine monochloride and the mixture was allowed to stand for about 5 minutes to complete the oxidation. About 15 c.c. of 0·5 *N* KCN, at least 50 c.c. of 2·5 *N* HCl, about 1 to 2 gms. of sodium chloride and some starch solution were added and the titration with 0·1 *N* Chloramine-B was carried out slowly, with constant shaking, to the discharge of the iodine starch blue

colour, which appeared at the beginning of the titration. In these titrations normality of the solution with respect to hydrochloric acid was maintained at about 1 *N*.

Iodine Bromide Method.—A known weight of the substance was pre-oxidised as before with 5 c.c. of iodine monochloride in a conical flask. To it was added 5 c.c. of concentrated hydrochloric acid, 22·0 gms. of potassium bromide and 5 c.c. of carbon tetrachloride and the mixture was diluted to about 100 c.c. with water and titrated against standard Chloramine-B solution. The conical flask was stoppered and vigorously shaken during the titration, the carbon tetrachloride layer acquired purple colour due to iodine. Addition of small volumes of Chloramine-B solution was continued, shaking vigorously after each addition until the carbon tetrachloride layer was faintly violet. The Chloramine-B was then added dropwise, with shaking after the addition of each drop until the carbon tetrachloride layer became colourless. The end point was very sharp.

Iodine Monochloride Method.—A known weight of each substance in solution was treated with 5 c.c. of iodine monochloride, 60 c.c. of hydrochloric acid and 5 c.c. of chloroform. The mixture was cooled to room temperature and titrated against standard Chloramine-B solution until the carbon-tetrachloride layer became colourless. In these titrations, the normality of the solution with respect to hydrochloric acid was kept between 4 *N* to 7 *N* even at the end point.

In the case of potassium sulphite, sodium bisulphite, and sulphurous acid the solutions were added to 10–15 c.c. of iodine monochloride contained in a titration flask. Iodine monochloride reacted with them quantitatively liberating iodine without any loss of sulphur dioxide.

In the following tables, the iodine monochloride procedure will be referred to as method A, the iodine cyanide procedure as B and iodine bromide as C.

Method B is not applicable in the case of mercurous chloride and thallous chloride because of the failure of the iodine starch reaction.

TABLE I

Arsenious oxide.				Tartaremetic.			
Taken (g.)	Found (g.)			Taken (g.)	Found (g.)		
	A	B	C		A	B	C
·04946	·04938	·04932	·04980	·16690	·16879	·16879	·16881
·06429	·06430	·06431	·06430	·28373	·28696	·28527	·28527
·08408	·08514	·08462	·08463	·35040	·35279	·35279	·35279
·10386	·10384	·10433	·10436	·38387	·38486	·38486	·38655

TABLE II

Potassium sulphocyanide.				Hydrazine sulphate.			
Taken (g.)	Found (g.)			Taken (g.)	Found (g.)		
	A	B	C		A	B	C
·01617	·01609	·01618	·01628	·03200	·03242	·03208	·03208
·02103	·02109	·02109	·02104	·04160	·04164	·04164	·04197
·02748	·02763	·02755	·02773	·05440	·05461	·05494	·05494
·03396	·03402	·03399	·03408	·06720	·06723	·06757	·06757

TABLE III

Stannous chloride.				Ferrous sulphate.			
Taken (g.)	Found (g.)			Taken (g.)	Found (g.)		
	A	B	C		A	B	C
·1098	·1078	·1090	·1090	·2780	·2780	·2780	·2780
·1279	·1275	·1280	·1280	·3614	·3600	·3614	·3614
·1866	·1849	·1860	·1860	·4176	·4176	·4180	·4180
·2018	·2015	·2014	·2015	·4879	·4879	·4893	·4907

TABLE IV

Mercurous chloride.				Thallous chloride.			
Taken (g.)	Found (g.)			Taken (g.)	Found (g.)		
	A	B	C		A	B	C
·3034	·3024	..	·3045	·1260	·1259	..	·1265
·3232	·3227	..	·3227	·2617	·2617	..	·2627
·3720	·3720	..	·3723	·3030	·3048	..	·3053
·4199	·4196	..	·4201	·3290	·3286	..	·3298

TABLE V

Potassium sulphite.				Sodium bisulphite.			
Taken (g.)	Found (g.)			Found (g.)	Taken (g.)		
	A	B	C		A	B	C
·07562	·07505	·07584	·07584	·06970	·06968	·06968	·06968
·1020	·1019	·1023	·1020	·09048	·09048	·09048	·09043
·1323	·1312	·1319	·1327	·10754	·10750	·10756	·10756
·1390	·1387	·1387	·1383	·14180	·14170	·14140	·14190

TABLE VI

Sulphurous acid.				Hydrogen peroxide.			
Taken (g.)	Found (g.)			Taken (g.)	Found (g.)		
	A	B	C		A	B	C
·04100	·04114	·04102	·04114	·001853	·001836	·001845	·001845
·05347	·0533	·0533	·05412	·00290	·00289	·00295	·00295
·0656	·0656	·0656	·06601	·00629	·00625	·00627	·00627

TABLE VII

Potassium iodide.

Taken (g.)	Found (g.)		
	A	B	C
·0830	·0826	·0830	·0830
·1079	·1079	·1079	·1079
·1411	·1409	·1411	·1411
·1743	·1743	·1743	·1743

From the above results it is concluded that arsenious oxide, tartaremetic, potassium sulphocyanide, hydrazine sulphate, stanous chloride, ferrous sulphate, mercurous chloride, thallous chloride, potassium sulphite, sodium bisulphite, sulphurous acid, hydrogen peroxide and potassium iodide can be estimated volumetrically with standard Chloramine-B after pre-oxidation with iodine monochloride or hypo-iodous acid in the presence of concentrated hydrochloric acid, hydrocyanic acid or saturated solution of potassium bromide respectively.

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GAMETOGENESIS AND FERTILIZATION IN *ISOPARORCHIS EURYTREMUM*

by

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GAMETOGENESIS AND FERTILIZATION IN *ISOPARORCHIS EURYTREMUM*

By Om Parkash Dhingra,
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Introduction

The Digenea is a group of parasitic trematodes, which has been attracting a good deal of attention from the cytologists since the beginning of this century, but all the previous workers without exception have confined themselves to the study of chromosomes. To the best of my knowledge the present investigation is the first attempt to work out the gametogenesis and fertilization processes of a trematode species, *Isoparorchis eurytremum*, with modern cytological fixatives. I have not only followed and studied the chromosomes in all stages of gametogenesis and fertilization, but I have also studied the cytoplasmic inclusions such as the mitochondria and the Golgi elements in spermatogenesis. These cytoplasmic inclusions have not been studied by me in oogenesis and fertilization on account of the insuperable difficulty of fixing the shelled eggs with fixatives lacking strong fat-solvents. Nevertheless the study of spermatogenesis with modern fixatives has been extremely profitable.

Certain remarkable features with regard to the maturation of the egg and the process of fertilization in *Isoparorchis eurytremum* have been brought out in this investigation. The egg leaves the ovary when it is still in the oocyte stage, and takes its abode in the oviduct, where the sperm enters the oocyte. It is after the sperm has entered the oocyte that a shell is formed round the egg and the egg undergoes maturation divisions. But this is not all. Both the sperm pronucleus and the female pronucleus, instead of conjugating directly, pass into a resting stage and it is after some time that chromosomes appear in the pronuclei and the process of the union of the pronuclei is accomplished.

Previous Work

Cable (1931) has given an historical account of the chromosomes in trematodes. It appears that there has been a great deal of confusion about the chromosomes in this group of animals. The haploid number of chromosomes in the cases recorded so far has not exceeded fourteen, but there have been some conflicting counts. Von Janicki (1903) and Kathariner (1904) have counted eight diploid chromosomes in *Gyrodactylus*, but Gille (1914) recorded twelve diploid chromosomes instead of eight. Goldschmidt (1905) counted ten diploid chromosomes in *Zoogonus mirus*, while Schreiners (1908), Gregoire (1909) and Wassermann (1913) stated that the diploid number in *Zoogonus mirus* was twelve instead of ten.

In the monogenetic forms it has not been known for a long time which of the two meiotic divisions is the reductional division. Goldschmidt (1902) in *Polystomum integerrimum* was unable to say whether the first or second maturation division was reductional. Likewise Von Janicki (1903) and Gille (1914), working on *Gyrodactylus elegans*, failed to give any information on the subject.

Goldschmidt (1905) in *Zoogonus* described a unique phenomenon of 'Primärtypus'. According to him, the first maturation division is equational when the chromosomes split longitudinally; in the second maturation division two groups of haploid numbers are separated and drawn to daughter cells. In such a process synapsis as such is absent. A number of workers studying the same material have, however, discarded this view. Anderson (1935), Chen (1937), Rees (1939), Pennypacker (1940), Jones (1945), Britt (1947) and Willmott (1950) seem to agree that the maturation of germ cells in essential aspects is similar in the various trematodes studied. Willey and Koulish (1950) and Willey and Godman (1951) have, however, stated that in the absence of an heteromorphic pair of chromosomes, it is not possible to say as to which of the two maturation divisions is reductional.

Some of the early stages in gametogenesis have been interpreted differently from the accepted scheme. Chen (1937) while working on the oogenesis of *Paragonimus kellicotti*, has described a leptotene stage after synapsis. Pennypacker (1940) following Wilson's scheme has proposed the names 'diakinesis', 'prophase' or 'Post spireme' for the leptotene stage of Chen. This author has stated that Chen's leptotene corresponds with his own figure 3 of *Pneumonoeces mediopiexus*. Rees (1939) in *Parorchis acanthus* has drawn figures 16 and 17 as 'zygotene' and 'pachytene' respectively, but Pennypacker (1940) considers these as metaphases or as late prophase stages. Willmott (1950) has stated that in *Gigantocotyle bathycotyle*, the primary spermatocyte undergoes the characteristic nuclear changes as outlined by White (1946).

The importance of chromosomes in taxonomy has been stressed by White (1940) and Britt (1947). Giving evidence based on chromosome study, Britt (1947), has supported the view of McMullen (1937) that *Lecithodendriidae* shows relationship with the families *Plagiorchiidae* and *Reniferidae* and that *Allocreadidae* should not be put along with *Plagiorchiidae* as held by Faust (1939), and Dollfus (1930). Analysing the work on about fifty species of various genera and families, Britt has brought out a new idea of 'Aneuploidy' as the basis of speciation in this group of hermaphroditic animals, and has rejected polyploidy as suggested by White (1940) for other groups of hermaphroditic animals. In this vast group of parasitic trematodes there is still a large number of families and genera in which chromosomes have not been studied, and until this is done it will be premature to pass final judgment on the claims of Britt.

As has already been stated none of the previous workers had employed fixatives without fat-solvents, and for that reason the study of cytoplasmic inclusions in this group of animals has been completely neglected. Dingler (1910) suggested that since the whole of the cytoplasmic material is left behind in the form of a residual mass, the sperm is only nuclear.

Unfortunately no serious attempt seems to have been made to determine the fate of these cytoplasmic structures, which play an important rôle in spermatogenesis in general.

The chromatic granules are the only cytoplasmic inclusions to which special attention has been paid by some workers. Gelei (1913) in *Dendrocoelum lacteum* and Pennypacker (1940) in *Pneumonoeces similiplexus* have observed certain dark granules in the developing oocytes. Gelei (1913) has claimed their origin from the oocyte nucleus, while Pennypacker (1940) is not definite about it. However, none of the workers has described any such granules in the spermatogenesis of trematodes.

The existence of chromatic granules in the egg cells of trematodes has given rise to certain speculations regarding the relationship of these granules to the differentiation of the germ cells.

Halkin (1902), Cable (1931), Chen (1937), Rees (1939), Markell (1943), Britt (1944), Jones (1945), and Willmott (1950) have all studied fertilization. All these workers except Jones have shown that the haploid pronuclei prior to fertilization go into a resting stage. Jones (1945) in *Macravestibulum kepneri* has, however, stated that there is no such resting stage.

Markell (1943) and Britt (1944) have observed a little of cytoplasm around the polar bodies after they are liberated into the nutritive material but Jones (1945) has failed to observe any cytoplasm associated with the polar bodies after their migration. Jones has further maintained that the first polar body, when discharged from the oocyte, enlarges and becomes lobed but does not divide.

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Material and Methods

The specimens of *Isoparorchis eurytremum* were obtained from the air-bladder of a freshwater fish, *Wallagonia attu*. The collection was made at Rupar and Ludhiana and from a local fish shop in Hoshiarpur, in the months of October, November and December, 1951. These trematodes survive for at least two days during winter in the air-bladder after death. The infection occurs in about 50% of these fishes, and the range of infection is from a few to twenty trematodes in a single infected fish. The size of the trematodes is fairly large. There are two rounded testes which lie, one on either side of the ventral sucker. The single ovary is tubular and lies towards the posterior side. The uterus lies close to it on its anterior side.

The specimens were washed in normal saline, and the testes were taken out with the help of a fine forceps by removing the skin. On removal they were directly put into the various fixatives like Bouin, Flemming-without-acetic acid, Champy, Kolatchev, Altmann and Da-Fano. Fixation

in Bouin's fluid for 24 hours gave good results for chromosome study. Flemming-without-acetic acid and Champy for 48 hours and 24 hours respectively proved successful for the mitochondria and the Golgi body. Kolatchev's method for the Golgi body was quite successful, and Altmann's fixative gave better results in smears than in sections. Smears were made in F.W.A., Bouin's, Kolatchev's and Altmann's fixatives. After Bouin's, F.W.A. and Champy's fixatives 0·5% iron haematoxylin was used as stain.

For section cutting the material was passed through various grades of alcohol, cleared in cedar-wood oil and embedded in paraffin. Sections were cut 10–14 microns thick for chromosome study, and 6–8 microns thick for cytoplasmic inclusions.

For the maturation stages of the egg and fertilization the proximal portion of the uterus was taken out and transferred to Carnoy's fluid for four hours. The entire trematodes were also put in this fixative and the uteri were taken out afterwards. After removing the material from the fixative, it was thoroughly washed in 80% alcohol for a few hours and then transferred to 70% alcohol for storage. For staining a mixture of Iron-acetocarmine and Ehrlich's haematoxylin in equal parts was used. The material was directly put into this mixture of stains for twenty-four hours. As it does not overstain, no differentiation is needed after this mixture. For permanent preparations the material after staining was passed through different mixtures of absolute alcohol and acetic acid in the ratios of 1 : 3, 2 : 2 and 3 : 1 and then it was put in absolute alcohol. It was cleared in xylol and mounted in canada balsam. Before mounting, the proximal portion of the uterus was teased very gently with the help of fine needles to liberate the eggs and spread them uniformly over the slide.

Chromosome counts were made from spermatogonial mitosis and the meiotic divisions in both spermatogenesis and oogenesis. Segmentation stages of the fertilized eggs were also helpful in counting the diploid number of chromosomes.

Observations

(a) *Spermatogonia*

The male germ cells are loosely packed in the testis. The spermatogonia also occur loosely in great abundance close to the wall of the testis, but sometimes groups of spermatogonia are met with. The spermatocytes lie inner to the peripheral zone of spermatogonia, while the spermatids and spermatozoa are more centrally located.

The spermatogonia are of three types:—primary, secondary and tertiary. The primary spermatogonia occur singly and are the smallest; and they have very little cytoplasm (Plate I, figs. 1, 2). They contain one or two karyosomes in their nuclei with finely granular chromatin. No cytoplasmic granule can be made out in the primary spermatogonia. As the first spermatogonial division approaches the nuclear wall disappears and the chromosomes are arranged on the spindle. Their individual identity, however, cannot be defined on account of their compact arrangement on the spindle (Plate I, fig. 3).

The secondary spermatogonia occur in groups of twos. They remain connected by a protoplasmic strand, which probably represents the spindle-remains. The pairs are not easily differentiated as they are closely packed up. *In size the secondary spermatogonia are bigger than the primary.* They also have one or two karyosomes in their nuclei, which stain brilliantly as in the case of primary spermatogonia (Pl. I, fig. 4).

In the course of the second spermatogonial mitosis 20 diploid chromosomes can be easily counted in the metaphase as seen from above (Pl. I, fig. 5). In the side view of the equatorial plate, however, the diploid number cannot be determined with accuracy as the chromosomes overlap and aggregate (Pl. I, fig. 6). In division the chromosomes split, but the movement of the chromosomes on the spindle is irregular (Pl. I, fig. 7). Centrosomes can sometimes be observed at the poles of the spindle of the second spermatogonial mitosis.

The division of the secondary spermatogonia results in the formation of a group of four cells, which are connected by spindle remains. They are called the tertiary spermatogonia. It is not always easy to distinguish the tertiary groups, as they are sometimes encroached upon by the primary and secondary spermatogonia. The individual cells of the tertiary groups are, however, very much like the preceding ones, but their nuclei are slightly bigger than the nuclei of the secondary spermatogonia. They may have one or two karyosomes as observed in the primary and secondary spermatogonia (Pl. I, fig. 8).

The mitochondria appear for the first time in the tertiary spermatogonia as a mass of granules forming a cap close to the nucleus. The exact location of this mass varies, but it is generally seen towards the point of attachment of the cell (Pl. I, fig. 9). The mitochondria have not been traced through the spermatogonial division, as the cells are very small and the chromosomes when placed at the equator make these granules obscure.

The Golgi body exists in the tertiary spermatogonia invariably as a single minute granule (Pl. I, fig. 10). Twenty chromosomes (diploid) can be easily counted in the metaphases of the third spermatogonial mitosis (Pl. I, fig. 11). *Twenty chromosomes go to each pole, but the splitting* of chromosomes and their migration to the poles is not simultaneous. As a result of this, the chromosomes are scattered all over the spindle during the anaphases (Pl. I, figs. 12, 13). It is difficult to count 20 chromosomes in later anaphases on account of the close aggregation of some of them (Pl. I, fig. 14). During the telophases the chromosomes fuse to form irregular chromatic masses (Pl. I, fig. 15). Cytoplasmic cleavage in all the four tertiary spermatogonia now takes place, with the result that eight cells are formed. These grow quickly and are thus converted into primary spermatocytes. During spermatogonial division a minute centrosomal granule at each pole of the spindle can be seen.

(*b*) *Primary Spermatocytes*

Primary Spermatocytes occur in clusters of 8 cells, resulting from the division of tertiary spermatogonia. They are connected with each other by protoplasmic strands.

The nucleus is generally excentric in position (Pl. I, fig. 16). In the resting stage the spermatocyte nucleus shows no structure, save for the presence of a faint reticulum and deeply staining nucleolus (Pl. I, fig. 16). In the subsequent leptotene stage the nuclear threads become prominent, but there is no indication of polarization of these threads (Pl. I, fig. 17). The number of loops in the leptotene stage could not be determined, but when the process of pairing begins, some of the threads appear double (Pl. I, fig. 18). The threads become more conspicuous and double, but they are still scattered (Pl. I, fig. 19). With the further advance of the pairing process, the polarization of the threads is indicated (Pl. I, fig. 20). As the pairing process is completed, polarization becomes more pronounced, and the syndesis or the synapsis stage is reached (Pl. I, fig. 21). The number of loops at the completion of polarization corresponds to the haploid number of chromosomes (Pl. I, fig. 21). The loops contract and give rise to tetrads (Pl. I, fig. 22). They condense further and reach the end of prophase (Pl. I, fig. 23), when the nuclear wall breaks down and they come to lie on the meiotic spindle. The tetrad nature is apparently lost to view because of an extreme condensation of chromatin (Pl. I, figs. 24–26). These bivalent chromosomes are at first scattered all over the spindle (Pl. II, fig. 27), but soon they arrange themselves in the form of a plate at the equator (Pl. II, fig. 28). The synaptic mates then separate (Pl. II, fig. 29). With the beginning of the anaphase two groups of homologous chromosomes are formed (Pl. II, fig. 30). The identity of the chromosomes is retained even at the late anaphase stage (Pl. II, fig. 31). At the telophase stage, however, the daughter nuclei form compact masses (Pl. II, figs. 32, 33).

In the primary spermatocyte the mitochondria exist in the form of juxta-nuclear mass (Pl. II, figs. 34–36). The mitochondrial mass gradually spreads out with the advance of meiotic activity, so much so that during the first meiotic division the mitochondrial granules are dispersed throughout the cytoplasm except for the peripheral region (Pl. II, figs. 37, 38). The mitochondria appear to be sorted out more or less equally during the division stages of the primary spermatocyte (Pl. II, fig. 39).

A fragment of the Golgi body is passed on to the primary spermatocyte in the last spermatogonial division (Pl. II, figs. 35, 36). This rounded granule starts growing until it is differentiated into two regions—a deeply impregnated thick cortex and a light central core (Pl. II, figs. 40, 41; Pl. III, fig. 42). During meiosis I, the Golgi body divides into two and each granule moves towards one of the two poles (Pl. II, figs. 38, 39; Pl. III, figs. 43, 44).

In the resting spermatocyte the centrosome is seen situated close to the nucleus (Pl. I, fig. 16). During first meiosis the centrosome seems to divide into two centrosomes, which take up their position at the respective poles of the spindle (Pl. II, figs. 27, 28).

When the primary spermatocyte divides, a furrow starts from the outer free border of the dividing cell and extends gradually towards the centre, where the cells of a cluster meet (Pl. II, fig. 33). In the dividing cluster the furrows meet at the centre, the cells are drawn out, and thus a cluster of secondary spermatocytes with double the number of cells, i.e.,

16 is formed. The daughter chromosomal masses do not form definite nuclei till after cytoplasmic cleavage is completed.

(c) *Secondary Spermatocytes*

The clusters of secondary spermatocytes are composed of 16 cells each. The secondary spermatocytes are smaller than the primary spermatocytes (Pl. III, fig. 45).

In the cytoplasm the mitochondria again assume the form of a cloudy rounded mass close to the nucleus (Pl. III, fig. 46). The single Golgi body again grows and is differentiated into two regions, a chromophilic cortex and a chromophobic medulla (Pl. III, figs. 47, 48). At this stage the Golgi body has reached its largest size, almost ready for division. As the chromosomes divide during meiosis II, the Golgi body is also fragmented into two parts each moving towards opposite poles (Pl. III, figs. 49–53).

The centrosome is sometimes seen in close relation with the nucleus of the resting secondary spermatocyte (Pl. III, fig. 45).

During meiosis II, the changes observed in the mitochondrial mass are similar to those described earlier for meiosis I.

After a resting period there follows the second meiotic division. The haploid number of 10 chromosomes can be counted in the metaphase (Pl. III, fig. 54). Each chromosome now splits into two, and the daughter chromosomes move towards the respective poles (Pl. III, fig. 55).

In late anaphase II, the chromosomes are aggregated closely, and a reliable count of their number cannot be easily made (Pl. III, fig. 55). As the chromosomes are further drawn to the poles, they form compact chromatic masses, one at each pole (Pl. III, figs. 56, 57). These masses do not form definite nuclei till cytoplasmic cleavage is completed.

(d) *Spermateleosis*

The spermatids are seen in clusters of 32 cells. Like the primary and secondary spermatocytes, these are also attached to each other (Pl. III, fig. 58). A variable number of chromatin granules is seen in the spermatid nucleus during the early stages. The spermatid in early stages has a mass of mitochondria lying on one side of the nucleus (Pl. III, fig. 59). A Golgi granule is invariably seen (Pl. III, fig. 60), and a centrosome is sometimes seen close to the nucleus (Pl. III, fig. 58).

In the early spermatid the Golgi granule is rather small. It again shows a growth period before the nucleus elongates. At the height of its growth, it is differentiated into an outer thick rim and an inner light core (Pl. III, fig. 61). This is followed by a decrease in the size of the Golgi body, when the inner lighter core disappears, and we see a deeply impregnated undifferentiated rounded structure (Pl. III, fig. 62). With the advance of spermateleosis, the Golgi body gradually decreases in size and ultimately it disappears from view altogether (Pl. III, figs. 63–65). Even the residual mass of the spermatid cluster does not show any structure, which may be considered as the remnant of the Golgi body.

In the early spermatid the nucleus is rounded and lies towards the outer end of the cell. To begin with the centrosome is situated at the

anterior narrow end of the cell (Pl. III, fig. 66), but it soon starts moving backwards (Pl. III, fig. 67), till ultimately it takes up its position at the base of the nucleus (Pl. III, fig. 68). The centrosome at this stage lies between the nucleus and the cell membrane, and two flagella are given off posteriorly from it. The mitochondria are seen scattered throughout the cytoplasm of the cell (Pl. IV, fig. 69). The nucleus now becomes oxyphilic, and there can be seen one or two granules in it. It becomes more and more elongated in the antero-posterior axis and looks spindle-shaped (Pl. IV, figs. 70–73). Subsequently the nucleus becomes wavy (Pl. IV, figs. 75, 76), The Golgi granule has by this time degenerated, and disappears from view. With further development the nucleus becomes basiphilic and its elongation continues (Pl. IV, figs. 77, 78). The cytoplasm with its contents shrivels up towards the centre of the spermatid cluster and the elongated sperm nucleus completely withdraws itself from the cytoplasm, which is cast off (Pl. IV, fig. 81). The sperm, when set free in the testis, is very long and filamentous with two flagella (Pl. IV, fig. 79). The centrosome from which the flagella arise is also sometimes visible (Pl. IV, fig. 80). The sperm nucleus is of uniform width, though slightly tapering towards the posterior end. It is difficult to measure the length of the sperms, as they form bundles and as they are too long. Sperms of different sizes have been recorded by me; and it is very probable that the elongation of the nucleus continues even after the sperm is set free in the testis.

The residual masses of the spermatid clusters are seen in the form of big ball-like structures and are loaded with mitochondrial granules (Pl. IV, fig. 81). They also show the holes from which the sperms come out, after the retraction of the cytoplasm.

(*e*) *Maturation and Fertilization*

Observations have also been made on various stages in egg development, which occur after the shell has been secreted. The shell encloses an oocyte with a sperm towards the opercular end and four to seven yolk globules towards the opposite side. The sperm enters the egg before the shell is formed; and after its penetration into the oocyte, the sperm lies coiled close to the nucleus (Pl. IV, fig. 82). On the maturation of the oocyte ten bivalent chromosomes are spread on the spindle (Pl. IV, fig. 83). The sperm lies coiled, a little away from the spindle. Soon the chromosomes clump together and form a deeply stained mass at the equator, (Pl. IV. fig. 84). The fused chromosomal mass divides into two halves; and now the sperm nucleus begins to condense (Pl. V, fig. 85). One chromosomal mass, which is the first polar body, moves into a cytoplasmic tube, and the other travels towards the opercular end. The condensed sperm nucleus shows a reticular appearance (Pl. V, fig. 86). The formation of the second polar body starts immediately after the separation of the first. The chromosomal mass of the oocyte unravels into 10 haploid chromosomes (Pl. V, fig. 87). Soon the spindle is formed, and the chromosomes get themselves arranged on it (Pl. V, fig. 88). The cytoplasm of the oocyte shows a protuberance to receive the second polar body. Metaphase II appears to be of long duration, and its chromosomes are often agglutinated.

The first polar body at this stage elongates, and divides into two unequal halves, which remain enclosed together into a little piece of cytoplasm for some time (Pl. V, figs. 89, 90). The agglutinated chromosomal mass of metaphase II divides into two halves, forming the second polar body and the female pronucleus (Pl. V, fig. 91). The second polar body moves into the cytoplasmic tube (Pl. V, fig. 92). This now constricts and thus completely encloses the second polar body (Pl. V, fig. 93), which is detached from the mature ovum and lies amongst the yolk globules. The male and the female pronuclei, which were hitherto very compact, now lose their compactness and become irregular and reticular in appearance (Pl. V, fig. 94). The two pro-nuclei now become vesicular with faint granules of chromatin inside each and remain in a state of rest for some time (Pl. V, fig. 95).

After a period of rest, the process of cell cleavage sets in. The twenty diploid chromosomes are set free in the cytoplasm of the mature oocyte (Pl. V, fig. 96). Soon the chromosomes are arranged on a spindle (Pl. V, fig. 97), and then their division starts. In the anaphase it is not easy to count 20 chromosomes at each pole on account of their close aggregation (Pl. V, fig. 98). The telophase chromosomes now fuse to form a chromatic mass at each pole. Nuclear division, having been completed, is followed by cytoplasmic division, which results in the formation of two blastomeres of unequal size (Pl. V, fig. 99). Their nuclei are soon reorganized (Pl. V, fig. 100). It is, however, to be noted that the daughter cells when formed are separated. The diploid chromosome number (20) has been definitely confirmed by a study of early segmentation (Pl. V, figs. 101–103). The second cleavage affects the bigger cell, which too divides unequally. The third cleavage also affects the bigger cell. The smaller cell formed in the first cleavage division, which remains undivided in the subsequent few stages has often been called the 'Propagatory cell'.

(f) Chromosomes

It has been shown above that the diploid number of chromosomes in *Isoparorchis eurytremum* is 20 and the haploid 10.

The chromosomes are rather small. There is a pair of chromosomes about 3μ long; another pair 2μ; still another is $1{\cdot}5\mu$. But the rest of them are quite small not exceeding 1μ. The largest pair of chromosomes is J-shaped and the second in length is rod-shaped. The third one is also rod-like. All the other chromosomes, which are quite small, cannot be said to be rounded or elongated.

Discussion

The present work on spermatogenesis agrees in some essential respects with the findings of a number of previous workers in other species of trematodes. Dingler (1910) in *Dendrocoelum lanceolatum*, Cable (1931) in *Cryptocotyle lingua*, Anderson (1935) in *Proterometra macrostoma*. Chen (1937) in *Paragonimus kellicotti*, and Willmott (1950) in *Gigantocotyle bathycotyle* have all shown that during the process of spermatogenesis the successive

stages remain crowded together and form clusters. The clusters of secondary spermatogonia, tertiary spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids consist of 2, 4, 8, 16 and 32 cells respectively. The clusters described by some of the previous workers are in the form of syncytia, and when the successive stages follow, only the nuclei are doubled. But in *Isoparorchis eurytremum* the clusters are of cells attached to one another at a point. There is doubling of cells and not the nuclei alone. A similar condition has also been reported by Anderson (1935) in *Proterometra macrostoma*.

Dingler (1910) described the differentiation of the spermatid into spermatozoon. Cable (1931), agreeing with Dingler, described the transformation as follows:—

'There begins a gradual elongation of the nuclei accompanied by a condensation of chromatin. The spermatids are at first ovate with the pointed end drawn out and directed towards the centre of the cluster. As they become longer and thinner, they stain more intensely and lose their granular appearance. They then become twisted into a confused mass, which later seems to become oriented in respect to the vas deferens— The spermatozoon does not appear to have a differentiated head or other parts such as are found in the male gametes of many animals.'

This appears to be an incomplete description of spermateleosis. No body seems to have stated definitely, whether the trematode sperm is to be classed with flagellate or non-flagellate sperms.

I have found that the process of spermateleosis in *Isoparorchis eurytremum* is quite simple. Once the centrosome has taken up its position at the base of the nucleus and the flagella have appeared, the nucleus starts elongating towards the centre of the cluster of spermatids without losing its orientation. There occurs only a slight twisting of the nucleus during late stages of its elongation without any rotation of the anterior or posterior ends of the nucleus. The whole elongated filamentous part of the sperm arising from the nucleus may be said to be the head and the posterior region having two flagella and a centrosome can be called the tail.

The head and tail described by Chen (1937) in *Paragonimus kellicotti*, cannot be compared with the head and tail of the sperm of *Isoparorchis eurytremum*. In *Paragonimus*, as stated by Chen (1937), the head and the tail are two differentiated regions of chromatin. The head is thick and short, but the tail is thin and long. In *Isoparorchis*, as observed by me, the region of the sperm formed from the chromatin is the head alone and the flagella, which arise from the centrosome, constitute the tail. The sperm of *Isoparorchis* is to be classed with the flagellate sperms. The sperm is, however, unique in having two flagella and only one centrosome, which remains undivided.

Cable (1931) ascribed the elongation of the spermatids to the presence of cytoplasmic strands radiating from the centre of a spermatid syncytium to the individual nuclei. I have not noticed such strands in the case of *Isoparorchis eurytremum*, though the elongation of the spermatid nucleus is almost of the same type. It is possible that the cytoplasmic strands described by Cable in *Cryptocotyle* are the differentiated cytoplasmic zones

having mitochondrial granules. In *Isoparorchis*, on the other hand, the mitochondria are scattered in the whole of cytoplasm, and thus the differentiated zones are absent.

Dingler (1910) has stated that the spermatid nuclei only go to form the spermatozoa, while the cytoplasmic material is left behind in the form of abandoned residual mass. Cable (1931) saw only the residual cytoplasmic masses and agreed with what Dingler (1910) had said. But the cytoplasmic components have not been described by these workers.

Indeed, information regarding the two categories of cytoplasmic structures, viz. the Golgi elements and mitochondria in the Trematoda is meagre. In *Isoparorchis* the mitochondria in the spermatogonia are in the form of a cloudy mass lying on one side of the nucleus. In the primary and secondary spermatocytes the mitochondria are found scattered throughout the cytoplasm almost evenly. In the earliest spermatid also the mitochondria are seen in the form of a cloudy mass, but during spermformation these are once again scattered uniformly. Ultimately they are left behind with the residual cytoplasm. The 32 ripe sperms of a cluster wriggle out from the free border and leave behind a massive cytoplasmic mass containing all the mitochondria. The residual mass assumes a globular form, as has also been observed by Rees (1939) in *Parorchis acanthus*.

The casting out of the mitochondria without forming any sperm structure has also been described recently in some other animals. Sharma (1950) has stated that mitochondria seem to have no function in the spermformation of the spider *Plexippus paykulli*. Nath and Bhatia (1953) have very recently described a typical mitochondrial nebenkern in the early spermatids of *Lepisma domestica*, which is typical of insect spermatogenesis, but the nebenkern completely pales off without forming the sheath of the axial filament. The climax is reached in the spermatogenesis of a Hoshiarpur dragon-fly, *Sympetrum hypomelas* (Selys), in which the mitochondria are conspicuous by their absence—what to talk of their forming a nebenkern (Nath and Rishi 1953). All these instances and that of *Isoparorchis* clearly point towards the conclusion that the mitochondrial material as such is not an essential component of the ripe sperm.

Another cytoplasmic component, which has been investigated by the author in trematode spermatogenesis for the first time is the Golgi body. This is first seen as a granule in the spermatogonia. In the spermatocyte it is seen as a small body, which grows to a big vesicle, showing a differentiated cortex and a core.

During meiosis I, the Golgi vesicle is seen dividing. In the secondary spermatocytes it grows from a smaller to a bigger body. Here also it is differentiated into a deeply impregnated outer rim and a lighter inner region. It divides again during meiosis II, and thus a single Golgi granule is transmitted to each spermatid. In the spermatid it starts growing at first but later on degenerates gradually in the cytoplasm and is ultimately lost to view.

My observations on the structure of the Golgi body in *Isoparorchis* would allow me to say that the single vesicular and deeply impregnated Golgi granule corresponds to the lipoid-containing substance of Baker

(1945). The lighter region appears very late when the Golgi body has attained a fairly big size. It is only at this stage that the chromophilic and chromophobic regions are marked.

The complete degeneration of the Golgi material at different stages of spermateleosis has been traced both in the non-flagellate and flagellate sperms. Nath (1932, 1937 and 1942), working on the non-flagellate sperms of the Decapod Crustacea, has shown that the Golgi material completely degenerates at varying stages of spermateleosis without forming the acrosome. Nath and Sharma (1953) have also made similar observations in the millipede, *Thyroglutus malayus*.

Amongst the flagellate sperms, teleostean fishes seem to have no product of the Golgi body in the ripe sperm. Vaupel (1929) in *Lebistes reticulatus* has shown that the Golgi bodies are sloughed off, without forming any sperm component.

I agree with Cable (1931), Pennypacker (1940) and Jones (1945) that the maturation of the oocyte occurs after the shell is formed in the ootype. The condensation of the sperm-nucleus is continued till anaphase of meiosis I in the oocyte. This is more or less the case in *Pneumonoeces* as observed by Pennypacker (1940) and in *Macravestibulum* as noted by Jones (1945). The polar bodies when liberated into the nutritive material of the oocyte, gather a little cytoplasm around them. In this respect I agree with Markell (1943) and Britt (1944), who have observed a little of cytoplasm around the polar bodies, and differ from Jones (1945), who failed to see any such cytoplasm.

Jones (1945) found that the first polar body when formed enlarges and becomes lobed, but does not divide. In *Isoparorchis eurytremum*, however, the first polar body is seen dividing into two unequal halves.

The absence or presence of a resting stage in the pronuclei prior to fertilization has been discussed by many workers. Cable (1931), Halkin (1902), Chen (1937), Rees (1939), Markell (1943), Britt (1944) and Willmott (1950) have all observed male and female pronuclei gradually going into resting stage before the diploid chromosomes are set free or a fusion nucleus is formed. Jones (1945) has stated that the male and female pronuclei go into prophase directly, there being no resting stage. In *Isoparorchis eurytremum*, however, the pronuclei go into a resting stage prior to karyogamy.

It may be stated that the separation of the germ cell at the first cleavage stage as stressed by Ishii (1934) and Chen (1937), required further observations in *Isoparorchis*. Anyhow, it has been observed that the smaller cell formed by the first cleavage remains unaffected in the subsequent two cleavage divisions.

Summary

1. The processes of spermatogenesis, maturation of the ovum and karyogamy have been worked out in detail in *Isoparorchis eurytremum*.

2. The haploid number of chromosomes is 10 and the diploid 20. The chromosomes have been counted in male meiosis I and II and in segmentation of the egg.

3. The mitochondria are in the form of a juxta-nuclear cap-like mass in spermatogenesis. In spermatocytes they are more or less uniformly distributed in the cytoplasm. The mitochondria are left behind in the residual cytoplasmic mass and do not contribute towards the formation of any part of the ripe sperm.

4. The Golgi body occurs as a single granule in spermatogenesis. It grows and divides during spermatogenesis. At certain stages it reveals a duplex structure. In the spermatid it completely disappears from view.

5. The single centrosome of the spermatid has never been seen dividing. Two flagella arise from the centrosome.

6. Polar bodies are extruded with a little of cytoplasm.

7. The first polar body divides into two unequal halves.

8. The sperm and ovum nuclei reorganize themselves into a resting stage before they unite in karyogamy.

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Explanation of Figures

Abbreviations:

C,—centrosome; *G*,—Golgi body; *M*,—mitochondria; *N*,—nucleus; *P*,—polar body; P_1,—first polar body; P_2,—second polar body; *PR*,—propagatory cell; *S*,—sperm; *Y*,—yolk cell.

Plate I

Figs. 1, 2. Primary spermatogonia, showing one and two karyosomes in their nuclei. Bouin's and Iron Haematoxylin.

Fig. 3. Metaphase of primary spermatogonium. Bouin's and Iron Haematoxylin.

Fig. 4. A group of secondary spermatogonia, showing one or two karyosomes. Bouin's and Iron Haematoxylin.

Fig. 5. Metaphase of secondary spermatogonium, showing diploid number of 20 chromosomes. Bouin's and Iron Haematoxylin.

Fig. 6. Metaphase of secondary spermatogonium (side view). Centrosome and spindle are seen. Bouin's and Iron Haematoxylin.

FIG. 7. Anaphase of secondary spermatogonium. Bouin's and Iron Haematoxylin.

FIG. 8. A group of four tertiary spermatogonia. Bouin's and Iron Haematoxylin.

FIG. 9. Tertiary spermatogonium, showing mitochondrial mass. Altmann's and Acid Fuchsin.

FIG. 10. Tertiary spermatogonium, showing Golgi body. Kolatchev.

FIG. 11. Metaphase of tertiary spermatogonium, showing diploid number of 20 chromosomes. Bouin's and Iron Haematoxylin.

FIG. 12. Metaphase of tertiary spermatogonium (side view). Centrosome and spindles are also seen. *Bouin's and Iron Haematoxylin.*

FIGS. 13, 14. Early and late anaphases of tertiary spermatogonia. Bouin's and Iron Haematoxylin.

FIG. 15. Telophase of tertiary spermatogonium. Bouin's and Iron Haematoxylin.

FIG. 16. Section through a cluster of primary spermatocytes, showing 6 of the 8 cells in a resting stage. Bouin's and Iron Haematoxylin.

FIG. 17. Nucleus of primary spermatocyte, showing leptotene stage. Bouin's and Iron Haematoxylin.

FIGS. 18–20. Nuclei of primary spermatocytes, showing zygotene stages. Bouin's and Iron Haematoxylin.

FIG. 21. Nucleus of primary spermatocyte, showing a bouquet stage. Bouin's and Iron Haematoxylin.

FIG. 22. Nucleus of primary spermatocyte, showing diplotene stage. Bouin's and Iron Haematoxylin.

FIG. 23. Nucleus of primary spermatocyte, showing diakinesis stage. Bouin's and Iron Haematoxylin.

FIGS. 24–26. Primary spermatocytes in metaphase, showing haploid number of 10 chromosomes. Bouin's and Iron Haematoxylin.

Plate II

FIGS. 27, 28. Primary spermatocytes in metaphase showing chromosomes, spindles, centrosomes and Golgi remains. Bouin's and Iron Haematoxylin.

FIG. 29. Primary spermatocyte in early anaphase. Bouin's and Iron Haematoxylin.

FIGS. 30, 31. Primary spermatocytes, showing two successive stages of anaphase. Haploid number of chromosomes can be counted in each set. Bouin's and Iron Haematoxylin.

FIGS. 32, 33. Primary spermatocytes in telophase, showing centrosomes and Golgi remains. Bouin's and Iron Haematoxylin.

FIG. 34. Primary spermatocyte from a smear, showing a mitochondrial cap. Altmann's and Acid Fuchsin.

FIG. 35. Primary spermatocyte from a smear, showing mitochondrial mass and a Golgi body. F.W.A. and Iron Haematoxylin.

FIG. 36. Primary spermatocyte, showing mitochondrial cap, a Golgi body and a centrosome. F.W.A. and Iron Haematoxylin.

FIG. 37. Primary spermatocyte in metaphase. F.W.A. and Iron Haematoxylin.

FIGS. 38, 39. Primary spermatocytes in telophase, showing division and separation of the Golgi body. F.W.A. and Iron Haematoxylin.

FIGS. 40, 41. Primary spermatocytes, showing the growth of the Golgi body. Kolatchev's method on smears.

Plate III

FIG. 42. Primary spermatocyte, showing Golgi body grown to its maximum size and indicating duplex structure. Kolatchev (smear).

FIG. 43. Primary spermatocyte at metaphase. Note the Golgi body has decreased in size and its inner lighter core has disappeared. Kolatchev (smear).

FIG. 44. Primary spermatocyte at metaphase, showing the Golgi body divided into two. Kolatchev (smear).

FIG. 45. Section through a cluster of secondary spermatocytes showing 9 of 16 cells in a resting stage. Bouin's and Iron Haematoxylin.

FIG. 46. Secondary spermatocyte in resting stage, showing a mitochondrial mass. Altmann's and Acid Fuchsin (smear).

FIGS. 47, 48. Secondary spermatocytes, showing the growth of the Golgi body. Kolatchev (smear).

FIGS. 49, 50. Secondary spermatocytes at metaphase, showing division of the Golgi body. Kolatchev (smear).

FIG. 51. Secondary spermatocyte, showing the Golgi bodies reaching the poles when the cell is about to divide. Kolatchev (smear).

FIGS. 52, 53. Secondary spermatocyte at metaphase and telophase. Note the mitochondria scattered and the Golgi body divided. F.W.A. and Iron Haematoxylin.

FIG. 54. Secondary spermatocyte at metaphase showing 10 chromosomes. Bouin's and Iron Haematoxylin.

FIGS. 55, 56. Secondary spermatocyte at late anaphase and telophase. Bouin's and Iron Haematoxylin.

FIG. 57. Secondary spermatocyte at telophase. Note the cleavage begins from the outer border. Bouin's and Iron Haematoxylin.

FIG. 58. Section through a cluster of spermatids, showing 17 of the 32 cells. Centrosome is seen in some of the cells. Bouin's and Iron Haematoxylin.

FIG. 59. A spermatid, showing mitochondrial mass. Altmann's and Acid Fuchsin (smear).

FIGS. 60, 61. Spermatids, showing the growth of the Golgi body. Kolatchev (smear).

FIGS. 62–65. Successive stages in the degeneration of the Golgi body during spermateleosis. Kolatchev (smear).

FIGS. 66–68. Spermatids, showing the movement of the centrosome. Bouin's and Iron Haematoxylin.

Plate IV

FIG. 69. Spermatid, showing lightly stained oval nucleus, mitochondrial granules a Golgi body, a centrosome and two filaments. F.W.A. and Iron Haematoxylin.

FIGS. 70–73. Successive stages in the elongation of the spermatid nucleus. F.W.A. and Iron Haematoxylin.

FIGS. 74–76. Spermatids, showing further elongation of the spermatid nucleus and the degeneration of the Golgi body. F.W.A. and Iron Haematoxylin.

FIGS. 77, 78. Spermatids, showing a change in the intensity of the nucleus. Note the mitochondria are shrivelled up. F.W.A. and Iron Haematoxylin.

FIG. 79. A mature sperm. F.W.A. and Iron Haematoxylin.

FIG. 80. Basal part of the sperm, showing the centrosome from which two flagella arise. F.W.A. and Iron Haematoxylin.

FIG. 81. A residual mass of the spermatid cluster loaded with residual mitochondrial mass. F.W.A. and Iron Haematoxylin.

FIG. 82. Egg, showing a primary oocyte with a sperm in the cytoplasm and some yolk cells whose outlines alone are drawn.

FIG. 83. Egg, showing primary oocyte in metaphase and the sperm lying away from the spindle in a coiled condition. Carnoy's and Iron-Acetocarmine with Ehrlich's Haematoxylin.

FIG. 84. Egg, showing chromosomal mass at metaphase and a coiled sperm outside the spindle. Carnoy's and Iron-Acetocarmine with Ehrlich's Haematoxylin.

Plate V

FIG. 85. Egg, showing the division of the chromosomal mass into two and a condensed globular sperm. Carnoy's and Iron-Acetocarmine with Ehrlich's Haematoxylin.

FIG. 86. Egg, showing the separation of the first polar body into a cytoplasmic tube and a condensed sperm. Carnoy's and Iron-Acetocarmine with Ehrlich's Haematoxylin.

FIG. 87. Egg, showing a detached first polar body, a condensed sperm and the secondary Oocyte at metaphase. Carnoy's and Iron-Acetocarmine with Ehrlich's Haematoxylin.

FIG. 88. Egg, showing the chromosomes of the meiosis II arranged on the spindle and the sperm in a condensed form. Carnoy's and Iron-Acetocarmine with Ehrlich's Haematoxylin.

FIG. 89. Egg, showing the chromosomes of meiosis II on the spindle and the first polar body dividing. Carnoy's and Iron-Acetocarmine with Ehrlich's Haematoxylin.

FIG. 90. Egg, showing the chromosomal mass of meiosis II on the spindle, the sperm condensed and the polar body divided. Carnoy's and Iron-Acetocarmine with Ehrlich's Haematoxylin.

FIGS. 91–93. Eggs, showing successive stages in the formation and liberation of the second polar body. Note the sperm is still condensed. Carnoy's and Iron-Acetocarmine with Ehrlich's Haematoxylin.

FIG. 94. Egg, showing male and female pronuclei changing their texture. The female pronucleus is bigger than the male. Carnoy's and Iron-Acetocarmine with Ehrlich's Haematoxylin.

FIG. 95. Egg, showing male and female pronuclei of equal size, having reached the resting stage. Carnoy's and Iron-Acetocarmine with Ehrlich's Haematoxylin.

FIG. 96. Egg, showing male and female pronuclei at metaphase. Diploid number (20) of chromosomes is clear. Carnoy's and Iron-Acetocarmine with Ehrlich's Haematoxylin.

FIGS. 97–99. Eggs, showing successive stages in the 1st cleavage division. Carnoy's and Iron-Acetocarmine with Ehrlich's Haematoxylin.

FIG. 100. Egg, showing two-celled stage. One cell is larger than the other. Carnoy's and Iron-Acetocarmine with Ehrlich's Haematoxylin.

FIG. 101. Egg, showing the larger cell of a two-cell embryo at metaphase. Carnoy's and Iron-Acetocarmine with Ehrlich's Haematoxylin.

FIG. 102. Egg, showing the larger cell of a two-cell embryo at telophase. Carnoy's and Iron-Acetocarmine with Ehrlich's Haematoxylin.

FIG. 103. Egg, showing a three-cell stage of an embryo. One cell at metaphase. Carnoy's and Iron-Acetocarmine with Ehrlich's Haematoxylin.

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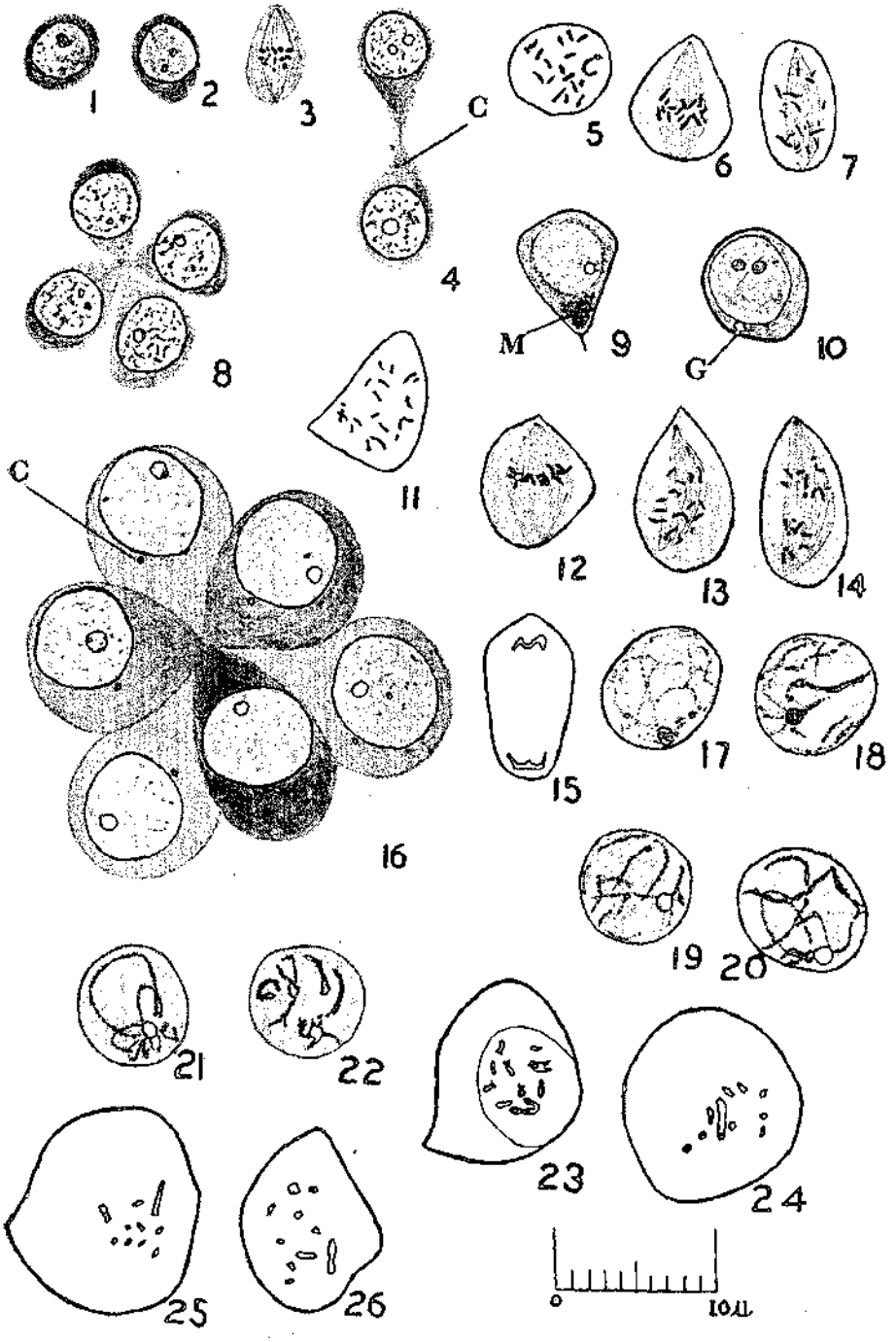
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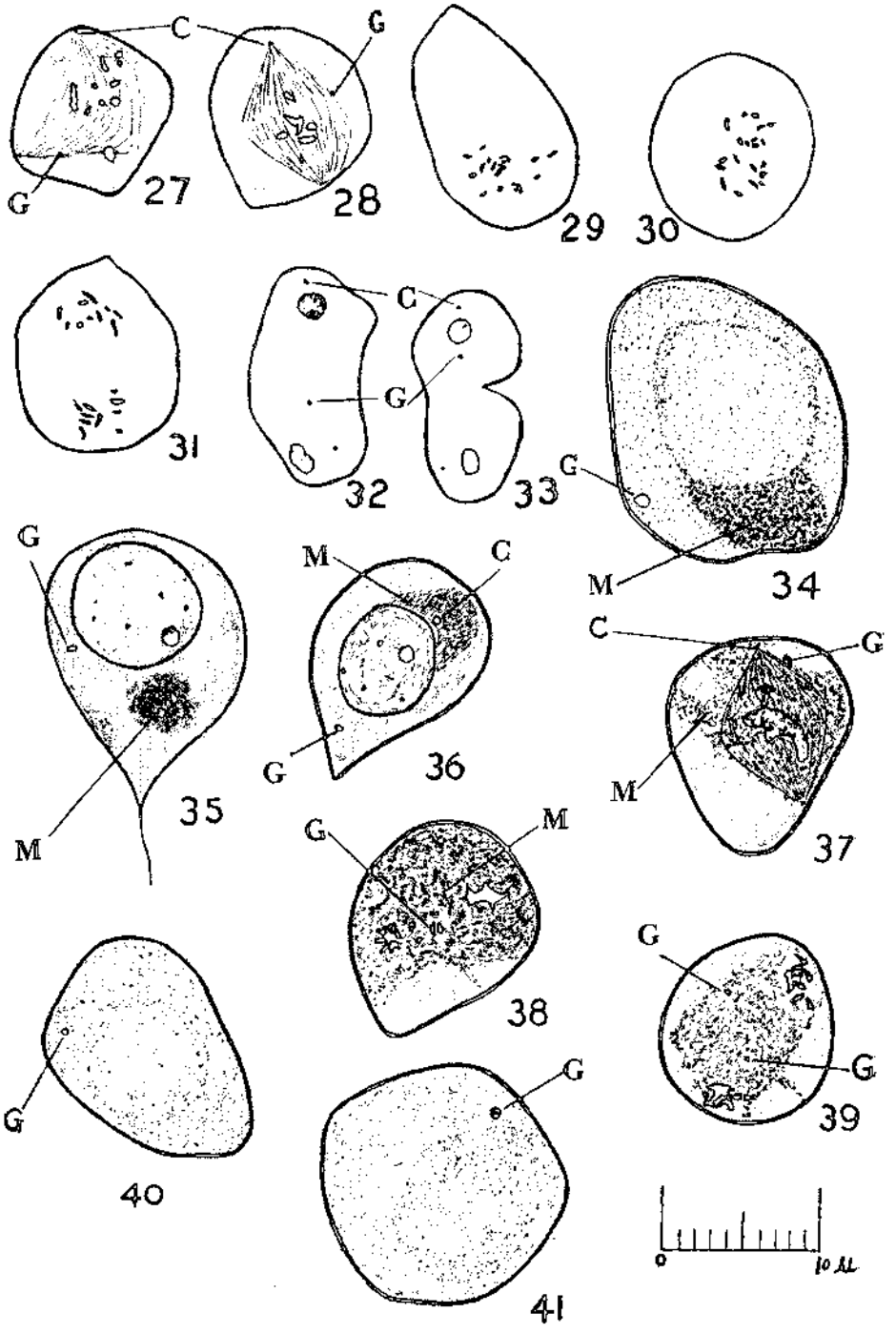
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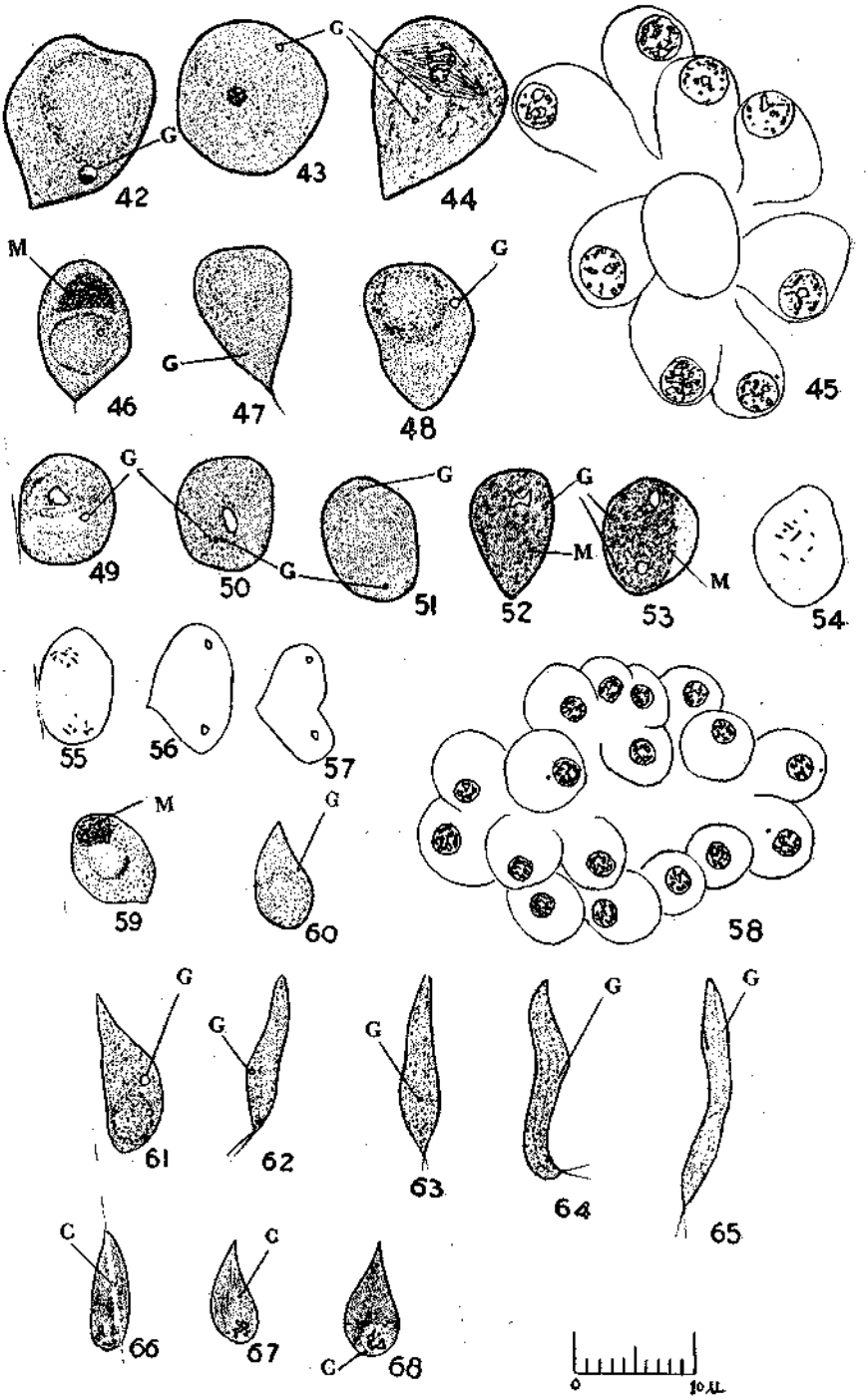
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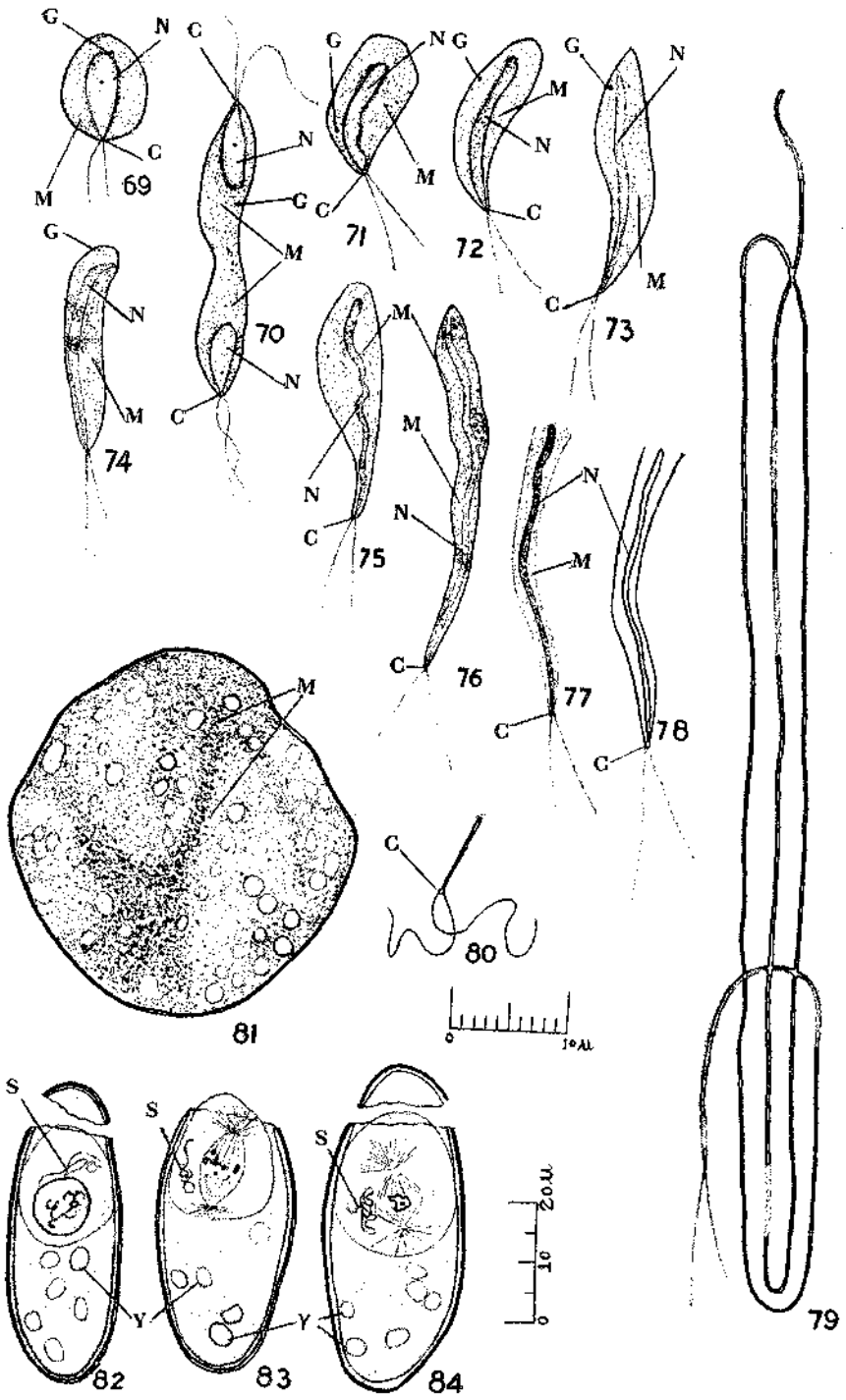
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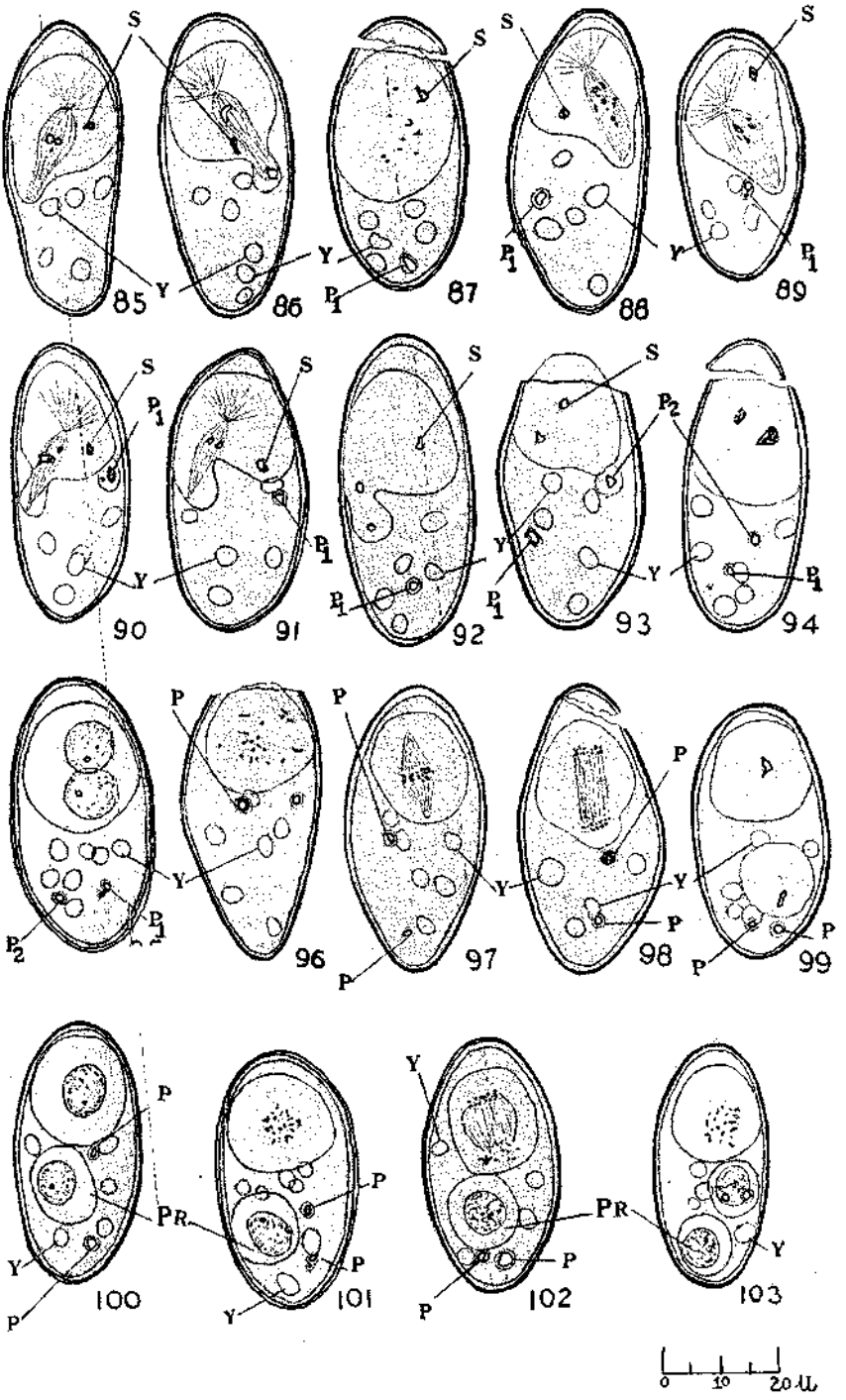
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March, 1954

STUDIES ON INSECT SPERMATOGENESIS

No. II. Lepidoptera

On the Formation of the Acrosome and Mitochondrial Nebenkern in *Prodenia litura* Fabr. and *Anaphaeis* sp.

by

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STUDIES ON INSECT SPERMATOGENESIS

No. II. Lepidoptera

On the Formation of the Acrosome and Mitochondrial Nebenkern in *Prodenia litura* Fabr. and *Anaphaeis* sp.

By Sukhdev Raj Bawa, *University Research Scholar, Zoology Department, Panjab University, Hoshiarpur.*

Introduction

Gatenby (1917) in *Smerinthus* gives a clear account of the direct origin of the acrosome from the Golgi elements—his acroblasts. The mitochondrial nebenkern, in his opinion, is of the nature of a 'spireme'. But his account is incomplete inasmuch as he has not studied the later development of the acrosome and the formation of the sheath of the tail region of the sperm.

Bowen (1922) in his paper on Lepidoptera conceives the mitochondrial nebenkern to be of the nature of 'plate-work' rather than a 'spireme'. Moreover, in his opinion, the tail sheath is formed not from the fragmented 'spireme' as suggested by Gatenby but by the 'central substance' granules appearing in the chromophobic part of the mitochondrial nebenkern. Regarding acrosome formation, Bowen states that 'the early steps in the formation of the acrosome cannot be analyzed with any satisfaction in *Callosamia* on account of very small size of the acrosome in this species'. In *Pygaera* his 'material begins at a point where the acrosome is already nearing completion'.

Nath (1925), who on an invitation from Professor Gatenby examined the latter's slides on *Operabia*, *Smerinthus*, and *Pieris*, came to the conclusion that 'the mitochondrial nebenkern does not directly form the tail-sheath, but undergoes a progressive dwindling and ultimately disappears'. The sheath-forming substance, according to Nath, 'arises as a new substance in the chromophobic cavity round the nebenkern'.

In view of the above-mentioned controversy with regard to the exact formation of the acrosome and the tail-sheath of the sperm in the Lepidoptera, I undertook work on *Prodenia litura* and *Anaphaeis* sp. Some of the observations made on fixed preparations of *Prodenia litura* have been confirmed by the study of the fresh material under the Phase Contrast Microscope.

The investigations recorded in this paper—the second in the series 'Studies on Insect Spermatogenesis'—were conducted in the Department of Zoology, Panjab University, Hoshiarpur, Panjab (I.). I am greatly indebted to Professor Vishwa Nath for providing me with the necessary research facilities and also for correcting the manuscript of this paper.

Material and Methods

The material was obtained from larvae and from a small number of pupae of *Prodenia litura* and *Anaphaeis* sp. The eggs of *Prodenia litura* were collected in large numbers deposited on the leaves of the Cauliflower during September and October. These eggs were reared in the laboratory at room temperature. The larvae of *Anaphaeis* sp. can be obtained feeding on *Amaryllis grandiflora* in December and January. Both the species were available in various fields and gardens in the vicinity of the University Zoological Laboratory, Hoshiarpur.

The testes of fully grown larvae and young pupae were transferred to the fixing fluids directly after dissection. A number of fixatives, viz., Champy, Flemming, Flemming-without-acetic and Bouin were tried. Best results were obtained in Flemming-without-acetic, while Bouin's fluid acted as control. After the usual process of dehydration the material was embedded in paraffin wax and sections were cut at 4–6 μ and stained in 0·5% iron-haematoxylin.

Observations

As there is not much variation in the behaviour of the cytoplasmic inclusions, viz., Golgi bodies and the mitochondria, in the process of sperm-formation in the two species under discussion (*Prodenia litura* and *Anaphaeis* sp.), it has been considered best to record the findings together in one communication.

(*a*) *Spermatogonia.*—In the spermatogonium of *Prodenia litura* the mitochondria exist in the form of a juxta-nuclear mass of faintly-staining granules. The Golgi bodies can be seen as darkly-staining crescents embedded in the mitochondrial mass (Pl. I, Fig. 1).

But under the Phase Contrast the Golgi bodies appear as vesicles in the fresh material and never as crescents (Pl. II, Fig. 27).

In *Anaphaeis* sp., on the contrary, the mitochondria and the Golgi bodies of the spermatogonium exist in the form of faintly-staining vesicles and deeply-staining granules respectively (Pl. III, Fig. 39). Before the spermatogonium enters the growth period, the vesicular mitochondria spread themselves out and enclose the nucleus all round (Pl. III, Fig. 40).

(*b*) *Spermatocytes.*—In the resting primary spermatocyte of *Prodenia litura* the mitochondria, which were granular in the spermatogonium now assume a vesicular appearance but remain juxta-nuclear in position. The Golgi crescents can be located in the neighbourhood of the nucleus. A darkly-staining nucleolus can also be observed at this stage in the interior of the nucleus (Pl. I, Fig. 2). Later on, when the mitochondria have reached a considerable size, the Golgi bodies tend to move away from the nucleus. As the chromosomes arrange themselves along the nuclear wall prior to the nuclear division, the Golgi bodies have moved considerably away from the nucleus but still lie amongst the mitochondrial vesicles (Pl. I, Figs. 3, 4). In the polar view of metaphase I, the mitochondria are seen to be spread out throughout the cell encircling the chromosome plate (Pl. I, Fig. 5).

Similar observations have been made in the fresh material of *Prodenia litura* under Phase Contrast, but the Golgi bodies are now clearly seen as complete spheres, each having an outer chromophilic cortex and an inner chromophobic medulla, instead of crescents (Pl. II, Figs. 28, 29).

In the resting primary spermatocytes of *Anaphaeis* sp. the vesicular mitochondria are seen distributed throughout the cytoplasm of the cell with a few Golgi granules of appreciable size lying amongst them (Pl. III, Fig. 41). In a fully grown spermatocyte the mitochondrial vesicles have enormously grown in size, giving the cytoplasm of the cell a frothy appearance (Pl. III, Fig. 42).

During the first meiotic division, in both *Prodenia litura* and *Anaphaeis* sp., the mitochondria seem to be distributed almost in equal proportions to the two daughter cells, and they get much stretched during the telophase (Pl. I, Fig. 7; Pl. III, Fig. 43). In *Prodenia litura* the mitochondria are caught up in the area of spindle fibres, while in *Anaphaeis* sp., they make a mantle round the spindle. Two rod-like centrioles towards each pole of the first meiotic spindle can be clearly made out in *Anaphaeis* sp. (Pl. III, Fig. 43), but these centrioles lie far away from the poles of the spindle. This, however, is certainly a later development in this species, as during metaphase I of the *P. litura* a small rod-like centriole lies at the very apex of the spindle at each end (Pl. I, Fig. 6).

In both the species, the second meiotic division follows immediately the first, there being no resting stage intervening between the two meiotic divisions. The behaviour of the Golgi bodies and the mitochondria during the second meiotic division is more or less similar to that in the first meiotic division (Pl. I, Figs. 8–10; Pl. III, Figs. 44, 45).

In the fresh material of *Prodenia litura* studied under the Phase Contrast, the spindle fibres have been observed in a number of dividing cells. The vesicular mitochondria are aggregated towards the two apices of the spindle and a few Golgi bodies, revealing a duplex structure, can also be observed being distributed almost evenly to the two daughter cells. The mid-body appears as a highly refractive plate (Pl. II, Figs. 30, 31).

(c) Spermatelecosis.—In the early spermatids of *Prodenia litura* and *Anaphaeis* sp., the mitochondria collect together to form a compact mass towards one side of the cell (Pl. I, Figs. 11, 12; Pl. III, Fig. 46). It will be recalled that the mitochondria have been described as vesicles, each vesicle showing a distinct chromophilic cortex and a central chromophobic medulla. Soon they begin to coalesce to form the mitochondrial nebenkern. It need hardly be mentioned that in optical sections the mitochondrial vesicles appear as rings.

There are important differences in the structure of the mitochondrial nebenkern in the two species. In both the species a nebenkern characteristic of insect spermatogenesis is formed, but in *Prodenia litura* a remarkable and unique event takes place in the later stages of the differentiation of the mitochondrial nebenkern. Here in this species the central chromophilic part of the nebenkern, instead of dwindling in size and disappearing ultimately, is actually ejected out of the nebenkern in the tail region of the sperm, where it is sloughed off along with some Golgi

material. This unique process has been fully studied in the fresh material also under the Phase Contrast (Pl. II, Figs. 32–38). A typical insect nebenkern, consisting of a central chromophilic core and a peripheral envelope of mitochondrial vesicles can be seen (Pl. II, Figs. 34, 35). Gradually the chromophilic walls of the peripheral mitochondrial vesicles break down and a spacious chromophobic peripheral space is produced surrounding the chromophilic core (Pl. II, Fig. 36). Subsequently the chromophilic core is thrown out of the nebenkern posteriorly in the tail region, where it will be sloughed off along with the Golgi remnants (Pl. II, Figs. 37, 38). It may also be noted that the 'central substance', which ultimately forms the sheath of the axial filament, appears in the peripheral chromophobic cavity sometime before the ejectment of the chromophilic core.

Reverting to the fixed material of *Prodenia litura*, the running together of the mitochondrial vesicles, the formation of the nebenkern with a chromophilic core and a peripheral chromophobic space, the appearance of the 'central substance' in this space, and finally the ejectment of the chromophilic core can be studied very clearly (Pl. I, Figs. 11–21; Pl. II, Figs. 22–24).

The process of the differentiation of the mitochondrial nebenkern in *Anaphaeis* sp., are more conventional as compared with *Prodenia litura*, except that the size of the nebenkern is much larger than that of the nucleus. The details of the process can be studied clearly by a reference to (Pl. III, Figs. 46–66).

In an early spermatid of *Prodenia litura* the Golgi bodies appear as crescents distributed in the outer region of the cell immediately surrounding the mitochondrial mass, while in *Anaphaeis* sp., the Golgi bodies are in the form of granules or crescents (Pl. I, Figs. 11, 12; Pl. III, Figs. 46, 48, 49).

A study of the living material of *Prodenia litura* under Phase Contrast reveals that the Golgi bodies are really complete spheres and show a duplex structure. Each Golgi body has an outer chromophilic cortex enclosing an inner chromophobic medulla (Pl. II, Figs. 32, 33 and 36–38). The crescents so often seen in the sectioned material seem to be the optical sections of the Golgi spheres.

When the mitochondrial nebenkern has been formed, the Golgi bodies tend to collect round the spermatid nucleus (Pl. I, Figs. 13, 14; Pl. III, Fig. 49), and soon a deeply-staining granule, the acrosomal granule, appears amongst the Golgi bodies (Pl. I, Fig. 15; Pl. III, Fig. 50). The acrosomal granule seems to grow in size probably at the expense of the Golgi bodies. During further growth of the acrosomal granule the outer multiple structure formed by the fusion of the Golgi bodies progressively gets simplified (Pl. I, Figs. 16, 17; Pl. III, Figs. 51, 52). Finally the acrosomal granule is seen enclosed in a single envelope—the acrosomal vesicle (Pl. III, Figs. 54–56). The acrosomal vesicle subsequently disappears and the acrosomal granule, which has much increased in size by this time, prepares to form the acrosome of the mature sperm.

It is to be noted that not a single Golgi body can be seen in the tail of the sperm during late spermateleosis in *Anaphaeis* sp. It would appear,

therefore, that all the Golgi bodies in the spermatid of this species participate in the formation of the acrosomal granule. But in *Prodenia litura*, judging from the fact that a few Golgi bodies are seen being sloughed off, it would appear that not all the Golgi bodies participate in the formation of the acrosomal granule.

In Figs. 18–21, Pl. I and Figs. 22–24, Pl. II and Figs. 57–63, Pl. III are shown the stages of acrosome development. The acrosome to start with is a massive granule lying in close contact with the nucleus. Subsequently it assumes a spindle shape, and then it elongates. Ultimately it is seen projecting behind the spermatid nucleus (Pl. II, Figs. 25, 26). But as the spermatids during late spermateleosis in both the species are very much attenuated, I have been unable to trace the later history of acrosome elongation.

Lastly I might make reference to the spindle fibres of meiotic divisions. So far, in conformity with the colloidal conception of protoplasm, it is generally believed that the spindle fibres of the dividing cells are simply the coagulation effects caused by the fixing fluids. But I have seen the spindle fibres in the living testicular material of *Prodenia litura* under Phase Contrast (Pl. II, Figs. 30, 31).

Discussion

It will be recalled that in both the Lepidopteran species under discussion the mitochondria are vesicular in nature in the spermatocytes, the vesicles being larger in *Anaphaeis* sp. than in *Prodenia litura*. These observations are in accord with those of Gatenby (1917).

In the early spermatid the mitochondrial vesicles fuse to form a typical insect nebenkern. The study of the fresh testicular material of *Prodenia litura* under Phase Contrast and of the sectioned material of *P. litura* and *Anaphaeis* sp., reveals that the mitochondrial nebenkern is of the nature of a 'plate-work' as conceived by Bowen (1922) and Nath (1925) rather than a 'spireme' as suggested by Gatenby (1917).

The various stages of nebenkern differentiation have been very carefully studied in certain species of Lepidoptera by Bowen (1922) and Nath (1925), with whom I am in close agreement as far as *Anaphaeis* sp., is concerned. But in *Prodenia litura* it is to be carefully noted that the central chromophilic core of the mitochondrial nebenkern instead of disappearing completely *in situ* is actually ejected out of the nebenkern in the tail region of the sperm. The rejected chromophilic part of the nebenkern has been seen and figured by me in the tail region along with the sloughed off Golgi bodies in the fresh testicular material of *Prodenia litura* under Phase Contrast. This unique event in the process of differentiation of the nebenkern has not been recorded by any previous worker on insect spermatogenesis.

Diverse opinions have been expressed regarding the sperm-tail sheath formation in Lepidoptera. Gatenby (1917) considers that the 'spireme' (chromophilic substance) of the nebenkern breaks up into thick threads which spread out in the chromophobic cavity of the drawn out mitochondrial

nebenkern. These threads finally form the tail sheath, but Gatenby has not figured the necessary stages to justify his contention. Bowen (1922), on the other hand, is of the opinion that the tail sheath is formed by 'central substance' granules—an altogether new product appearing in the chromophobic cavity of the nebenkern—and not by the fragmentation of the 'spireme' as conjectured by Gatenby (1917). Nath (1925), working on a cross between *Oporabia dilutata* and *O. autumnata*, suggests that as the chromophilic substance is undergoing condensation, its substance may somehow be transformed into the sheath substance (central substance of Bowen), which is simultaneously developing in the chromophobic cavity of the mitochondrial nebenkern. My observations in *Prodenia litura* and *Anaphaeis* sp. in this respect are fully in accord with Bowen's and Nath's.

The Golgi bodies in the fixed preparations of *Prodenia litura* and *Anaphaeis* sp. appear in the form of crescents or granules. But a thorough study of the fresh material of *P. litura* under Phase Contrast reveals Golgi bodies as complete spheres. I am in close agreement with Baker (1949), Nath (1944), Nath and Bhatia (1953), etc., etc., that the essential structure of the Golgi elements is granular or vesicular. It has been suggested by Nath that the crescents very often observed in the sectioned material are really the optical sections of the Golgi spheres.

Gatenby (1917) for the first time gives a clear account of the direct origin of the acrosome of the Golgi bodies, his acroblasts, in the sperm of the Lepidopteran, *Smerinthus*. To quote Gatenby's own words: 'When formed the acroblasts are quite spherical, and their wall is of equal thickness, not more bulging or thicker on one side than the other', Within each acrosomal vesicle there differentiates, according to Gatenby an acrosomal granule. Gatenby thinks that the acrosome is finally formed by the running together of several acroblasts.

Later Bowen (1922), working on *Pygaera bucephala*, interprets the acrosome as a differentiation product of the Golgi bodies. He confirms Gatenby inasmuch as there is one acrosomal granule to each acrosomal vesicle (Gatenby's acroblast) and the fusion of the acrosomal granules into one large granule, which becomes the acrosome. But he differs from Gatenby inasmuch as the acrosomal vesicles are sloughed off after secreting the acrosomal granules.

I am unable to confirm the findings of both Gatenby and Bowen, who have described one acrosomal granule to each acrosomal vesicle. I have never observed in either *Prodenia litura* or in *Anaphaeis* sp., at any stage of spermateleosis, more than one acrosomal granule to all the participating Golgi bodies (acroblasts of Gatenby or acrosomal vesicles of Bowen).

It will be recalled that in *Anaphaeis* sp. all the Golgi elements come together in the vicinity of the nucleus to form the acrosomal granule; and at no stage in spermateleosis there can be found any Golgi body in the tail region of the sperm ready to be sloughed off. As the acrosomal granule grows in size, the acrosomal vesicles fuse together to form a single vesicle enclosing the acrosomal granule. Gradually the acrosomal vesicle disappears from view as the acrosomal granule within it is growing in volume. The conclusion, therefore, is irresistible that the acroblasts, far from being

sloughed off, are completely used up in the formation of the acrosomal granule.

In *Prodenia litura*, in which a few Golgi elements can be seen being sloughed off in the tail region, there is no evidence that these are the same Golgi elements which participated in the formation of the acrosomal granule. The size of the acrosomal vesicles is much smaller in this species, and it would appear that they are completely used up in the formation of the acrosomal granule before they have any chance to fuse together to form one single vesicle as in *Anaphaeis* sp.

Summary

1. In this communication, the behaviour of the Golgi bodies and the mitochondria in the male germ cells during spermatogenesis has been described in two species of Lepidoptera, viz., *Prodenia litura* and *Anaphaeis* sp.

2. The mitochondria are granular in the spermatogonium of *Prodenia litura*, but they are vesicular in *Anaphaeis* sp.

3. The mitochondria increase in size considerably during growth of the spermatocyte in both the species.

4. A typical insect mitochondrial nebenkern is formed in both the species; the chromophilic core dwindles gradually and finally disappears completely in *Anaphaeis* sp., but in *Prodenia litura* the chromophilic core gets extruded into the spermatid tail, where it is sloughed off.

5. The mitochondrial nebenkern is of the nature of 'plate-work' (Bowen, 1922) rather than a 'spireme' (Gatenby, 1917).

6. The 'central substance' (sheath substance of Nath) forms the tail sheath.

7. The Golgi bodies are granular in *Anaphaeis* sp., but complete Golgi spheres revealing a duplex structure can be observed in fresh living material of *Prodenia litura* under Phase Contrast.

8. According to my investigations the acrosome is formed directly from the Golgi bodies, there being some important differences between my findings on the one hand and those of Gatenby and Bowen on the other.

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Explanation of Lettering in the Plates

A.—Acrosome; *A.g.*—Acrosomal granule; *A.f.*—Axial filament; *C.*—Centriole; *C.B.*—Chromophobic area; *C.L.*—Chromophilic area; *Ch.*—Chromosomes; *C.s.*—Central substance; *G.*—Golgi bodies; *M.*—Mitochondria; *M.n.*—Mitochondrial nebenkern; *M.B.*—Mid-body; *N.*—Nucleus; N_1.—Nucleolus; *S.f.*—Spindle fibres; *S.r.*—Spindle remnants.

Explanation of the Plates

Pl. I, Figs. 1–21; Pl. II, Figs. 22–26; and Pl. III, Figs. 39–66 have been drawn with a Camera Lucida at the table level with Spencer 10× eyepiece with oil immersion objective giving a magnification of approximately 1,700 times. All the figures have been selected from sectioned material fixed in Flemming-without-acetic and stained with 0·5% iron-haematoxylin.

Pl. II, Figs. 27–38, are drawn from the fresh material studied under Phase Contrast Microscope.

Plate I

Figs. 1–21. *Prodenia litura*

Fig. 1. Resting spermatogonium.
,, 2. Early primary spermatocyte showing nucleus, vesicular mitochondria and crescent-shaped Golgi bodies.
,, 3. Resting primary spermatocyte.
,, 4. Primary spermatocyte showing late prophase.
,, 5. Polar view metaphase I.
,, 6. Side view metaphase I.
,, 7. Telophase I.
,, 8. Polar view metaphase II.
,, 9. Side view metaphase II.
,, 10. Telophase II.
Figs. 11, 12. Early spermatids showing mitochondrial vesicles aggregating to form a compact mass and a few scattered Golgi bodies.
,, 13, 14. Spermatids showing mitochondrial nebenkern and a few Golgi bodies collecting near the nucleus.
Fig. 15. Spermatid showing the formation of the acrosomal granule.
,, 16. Spermatid showing the axial filament traversing the mitochondrial nebenkern and the acrosomal granule with a single Golgi body.
,, 17. Spermatid showing differentiation of the nebenkern into outer clear chromophobic and inner darkly staining chromophilic portion.
,, 18. Spermatid showing the disappearance of the acrosomal vesicle and the elongation of the mitochondrial nebenkern.
,, 19. Spermatid showing the appearance of the 'central substance' granules in the chromophobic area of the nebenkern.
Figs. 20, 21. Spermatids showing massive acrosome in close contact with the nucleus, extruded chromophilic core and a few Golgi bodies in the tail region.

Plate II

Figs. 22–26. *Prodenia litura*

Figs. 22–26. Spermatids showing the progressive development and elongation of the acrosome.

Figs. 27–38. *Prodenia litura* (Phase Contrast).

FIG. 27. Resting spermatogonium.

„ 28. Resting primary spermatocyte showing vesicular mitochondria and Golgi bodies.

„ 29. Primary spermatocyte. Late prophase.

FIGS. 30, 31. Telophase II showing the spindle fibres and almost equal distribution of the mitochondria and the Golgi bodies.

FIG. 32. Early spermatid showing the compact mitochondrial mass, spherical Golgi bodies and the spindle fibre remains.

„ 33. Spermatid showing a few Golgi bodies collecting in the vicinity of the nucleus.

FIGS. 34, 35. Spermatids showing the condensation of the mitochondrial nebenkern and the formation of the acrosomal granule.

FIG. 36. Elongating spermatid showing the acrosome and the appearance of the 'central substance' granules in the chromophobic area of the nebenkern.

FIGS. 37, 38. Spermatids showing the extrusion of the chromophilic substance of the nebenkern and a few Golgi bodies in the tail region.

PLATE III

Figs. 39–66. *Anaphaeis* sp.

FIG. 39. Spermatogonium showing vesicular mitochondria and a few Golgi granules.

„ 40. Spermatogonium before the growth period.

„ 41. Primary spermatocyte.

„ 42. Resting primary spermatocyte.

„ 43. Telophase I showing mitochondria, faintly staining spindle fibres and two rod-like centrioles on either side of the spindle.

„ 44. Newly formed secondary spermatocyte.

„ 45. Telophase II.

„ 46. Earliest spermatid.

„ 47. Early spermatid showing the formation of the mitochondrial nebenkern.

FIGS. 48, 49. Spermatids showing differentiation of the mitochondrial nebenkern and a few Golgi bodies in the form of granules and crescents.

FIG. 50. Spermatid showing the appearance of the 'central substance' granules in the chromophobic cavity of the nebenkern and the aggregation of the Golgi bodies in the formation of the acrosomal granule.

FIGS. 51–56. Spermatids showing the elongation of the nebenkern and the gradual dwindling of the chromophilic substance. The growth in size of the acrosomal granule can also be seen.

„ 57–63. Spermatids showing the later development stages of the acrosome.

„ 64–66. T.S. spermatid tail region showing the 'central substance' granules in the partitioned chromophobic cavity of the nebenkern.

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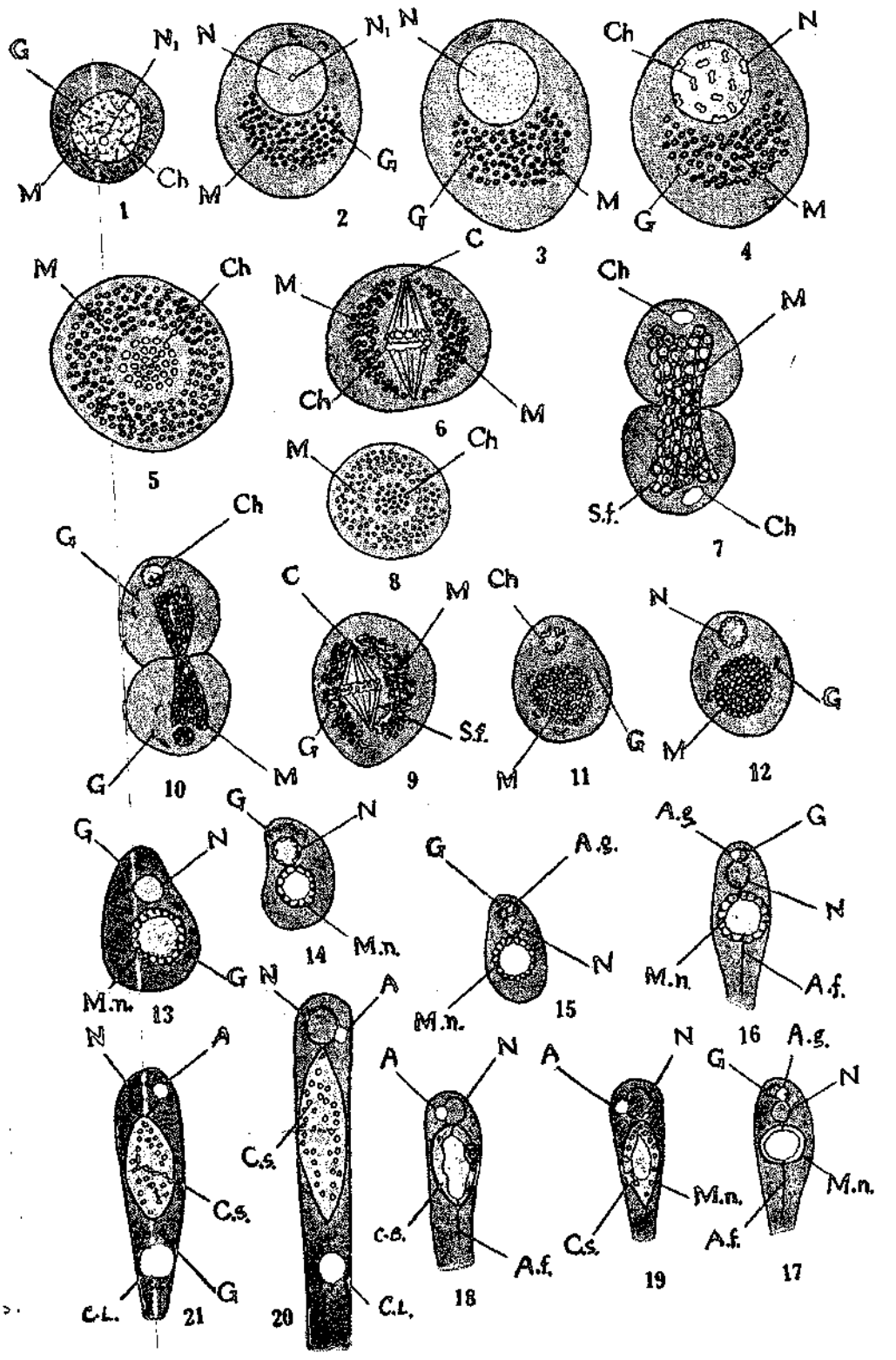
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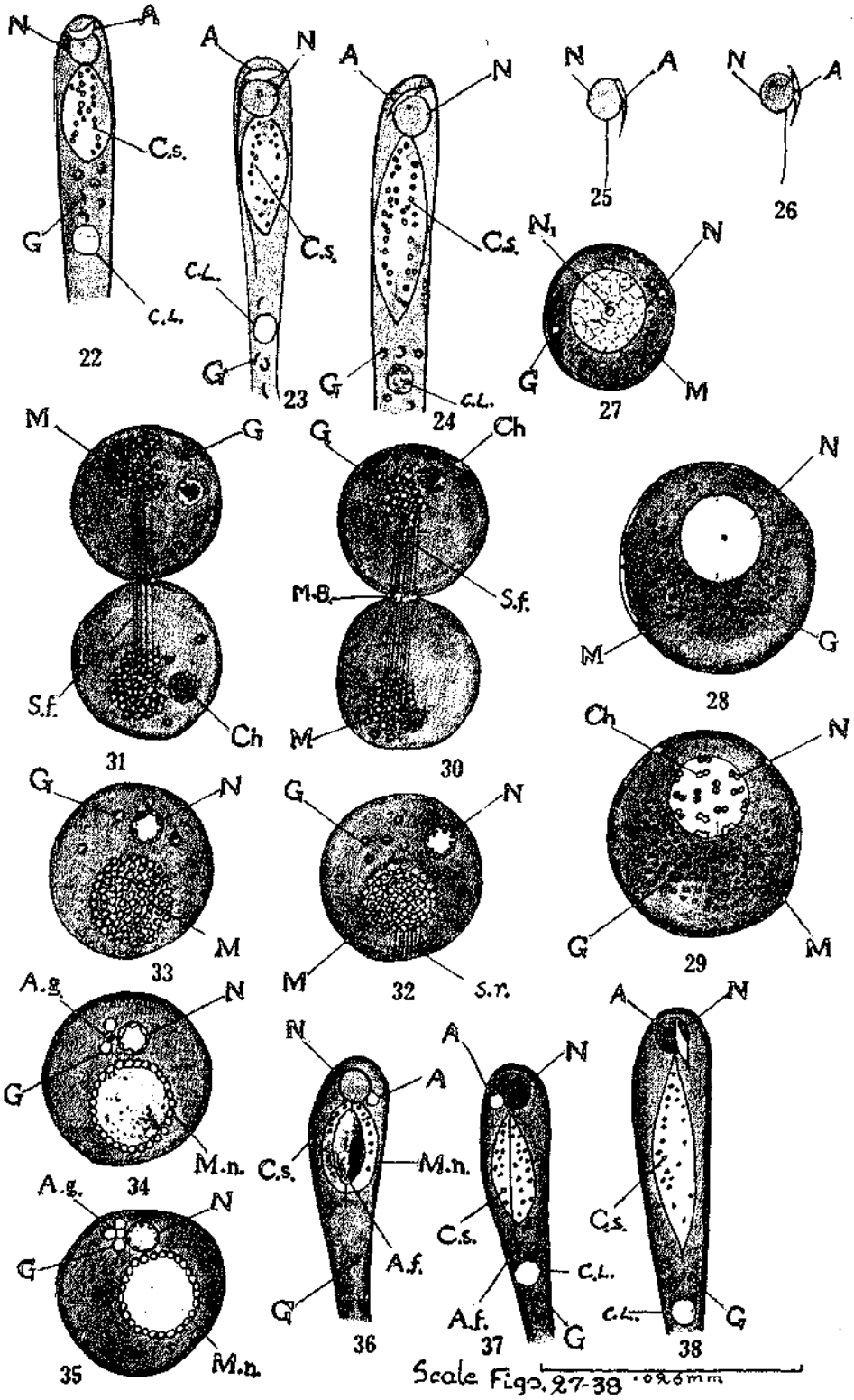
PLATE II.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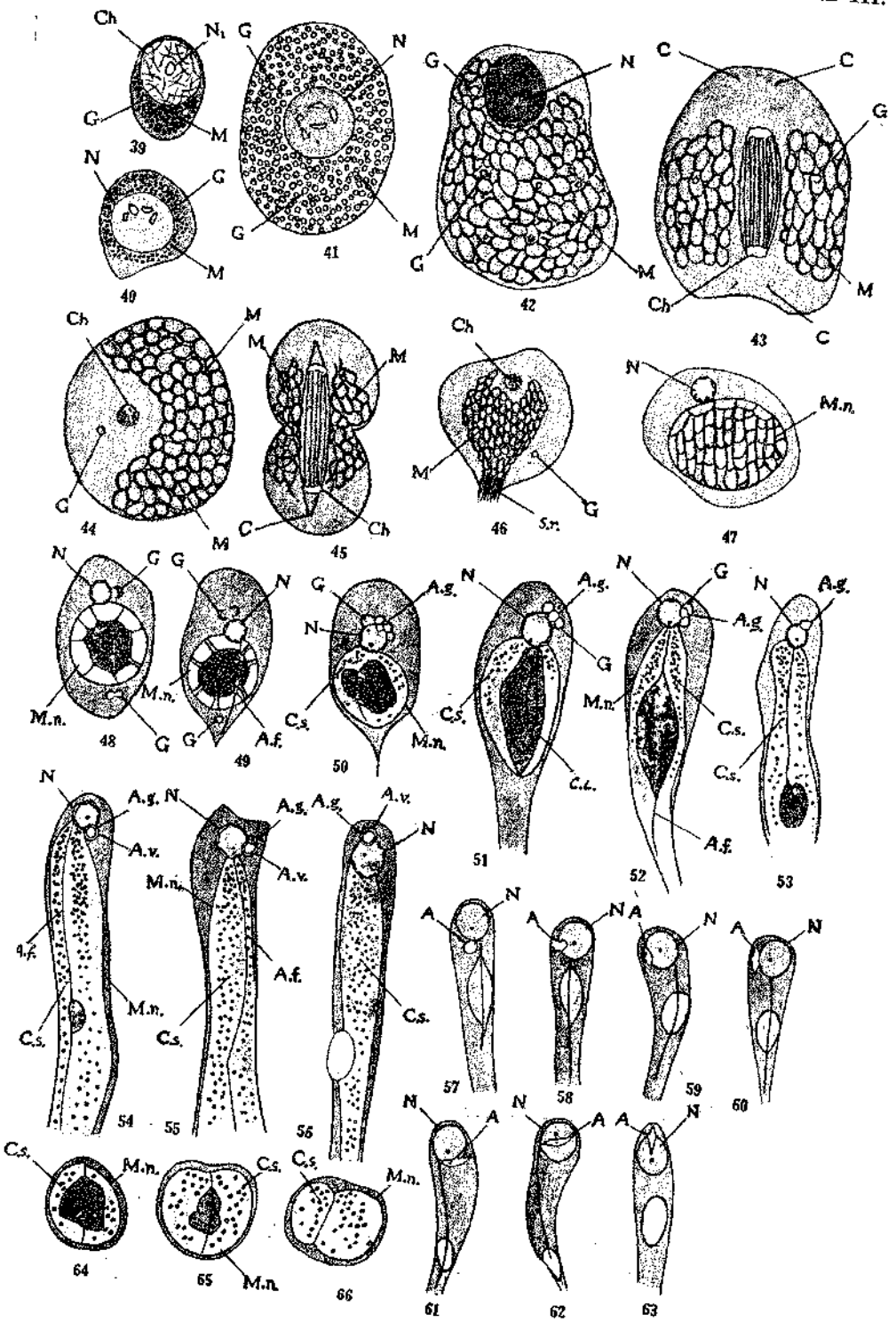
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FISH SPERMATOGENESIS WITH PARTICULAR REFERENCE TO THE FATE OF THE CYTOPLASMIC INCLUSIONS

II. Spermatogenesis of Elasmobranchs (Pleurotremata)

by

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FISH SPERMATOGENESIS WITH PARTICULAR REFERENCE TO THE FATE OF THE CYTOPLASMIC INCLUSIONS

II. Spermatogenesis of Elasmobranchs (Pleurotremata)

By Hem Sagar Vasisht, *Junior Research Scholar, Government of India, Department of Zoology, Panjab University, Hoshiarpur*

Introduction

In the first paper of this series, the spermatogenesis of nine species of Rays and Skates (Vasisht, 1953) was worked out with particular reference to the fate of the Golgi bodies and the mitochondria in spermateleosis. It was shown that in all the species of Rays and Skates the acrosome is formed directly from the Golgi bodies, and the mitochondria form the sheath of the middle-piece.

In this, the second paper of the series, the spermatogenesis of three species of sharks (*Chiloscyllium griseum, Carcharinus limbatus* and *Sphyrna blochii*) has been worked. The study of shark spermatogenesis has fully confirmed my conclusions arrived in the spermatogenesis of Rays and Skates.

The names of the previous workers on fish spermatogenesis has been listed in the first paper of this series. Here reference may be made again to a recent paper on the spermatogenesis of *Chiloscyllium griseum* by Ratnavathy (1941). This paper has been fully discussed by me in the present communication.

My thanks are due to the staff of the Taraporewala Aquarium, Bombay, Central Marine Fisheries, Mandapam Camp, and the Research Assistants at Krusadai and Pamban Islands of Madras Fisheries for the help, which they all rendered to me for collecting the fish.

I am also indebted to Dr. Vishwa Nath for suggesting this problem and correcting the manuscript of this paper.

Material and Technique

The spermatogenesis of the three sharks has been worked out in this paper. They all belong to the order Lamniformes, sub-class Selachii and class Chondrichthyes. The species are: (1) *Chiloscyllium griseum*, (Muller and Henle), (2) *Carcharinus limbatus*, (Muller and Henle), and (3) *Sphyrna blochii* (Day).

This work was started in the month of April, 1951. I visited different fishing grounds in the suburbs of Bombay and around Pamban Island. Generally I used to dissect the fish on the spot, but sometimes it was considered necessary to take the catch to the laboratory.

The male genital organs of these sharks are built on the same plan as described by Thillayampalam (1928) in *Scoliodon*. The testes are a pair of buff or flesh-coloured elongated structures, each with a vas deferens opening to the outer world through the grooves in the claspers. These claspers are present only in the male Elasmobranchs. The testes are situated below the kidneys, extending from the base of the liver to the rectal glands. They are attached to the body wall by a fold of peritoneum. Immediately after the testes were dissected out, they were put in 0·75% solution of sodium chloride, cut into small pieces of not more than 1 millimeter in diameter, and transferred to separate capsules containing different fixatives.

Smears were also prepared for the study of sperms. For smears Flemming-without-acetic acid was diluted with an equal quantity of distilled water.

The testicular material was mainly fixed in Flemming-without-acetic acid, Champy, Altmann, Kolatchew and Bouin's fluid. Amongst all the fixatives used Flemming-without-acetic acid proved to be the best. Very satisfactory results were obtained after fixation in F.W.A. for 24 or 48 hours. This was followed by 0·5% iron haematoxylin.

Observations

As the pre-spermateleosis stages of the three sharks, viz., *Chiloscyllium griseum*, *Carcharinus limbatus* and *Sphyrna blochii*, dealt with in this paper are more or less similar, it has been considered useful to give a comparative account, with occasional references to the existing variations.

Spermatogonia

The earliest spermatogonia, as is usual in the spermatogenesis, are more or less rounded cells with comparatively large nuclei. The nucleus hardly reveals any structure save for the presence of a deeply-staining, prominent nucleolus and a few chromatin granules. The nucleus is central in position with a layer of attenuated cytoplasm surrounding it. The cytoplasm is clear and does not contain any granules (Pl. I, Fig. 1; Pl. III, Fig. 39; and Pl. IV, Fig. 68).

As the spermatogonium grows in size, a few feebly-staining granules make their appearance in the cytoplasm. These are the mitochondria (Pl. I, Figs. 2 and 3; Pl. III, Fig. 40; and Pl. IV, Figs. 69 and 70). Embedded amongst the mitochondrial granules are a few bigger and more chromophilic bodies. These are the Golgi bodies (Pl. I, Fig. 3; Pl. III, Fig. 40; and Pl. IV, Fig. 70).

Primary spermatocyte

The primary spermatocyte is the largest cell in the testis. It generally has a large, excentric, vesicular nucleus, often containing a nucleolus. The mitochondria which were originally small dust-like granules grow in size appreciably into bigger granules during the growth period (Pl. I, Figs. 4 to 8; Pl. III, Figs. 41 to 43; and Pl. IV, Figs. 71 to 73).

The deeply-staining Golgi bodies are either in the form of granules or crescents or vesicles. They are very few in number as compared with the mitochondria and are more often than not in the form of crescents. The Golgi bodies do not lie in a particular position but are scattered amongst the mitochondria (Pl. I, Figs. 4 to 8; Pl. III, Figs. 41 to 43; and Pl. IV, Figs. 71 to 73).

Only a few division stages (Meiosis I) in *Chiloscyllium griseum* have been noticed and figured by me. During the division of the primary spermatocyte a well-defined spindle can be observed. Two prominent centrosomes are also seen, one at each pole. During metaphase I the chromosomes appear as deeply-stained compact mass (Pl. I, Fig. 9). During telophase I the two daughter masses formed by the splitting of a single chromosomal mass migrate towards the poles, one moving towards one pole and the other to the opposite pole (Pl. I, Fig. 10). In late telophase I (Pl. I, Fig. 11) the two daughter nuclei are formed and the centrosomes disappear from the scene.

During the first meiotic divisions the mitochondria are seen as pale vesicles, and they are sorted out roughly into two equal parts to the two secondary spermatocytes. But in the few dividing cells which appear in my preparations fixed with F.W.A. I have failed to observe any Golgi bodies.

Secondary spermatocyte

The secondary spermatocytes are similar to the primary spermatocytes but are definitely smaller than the latter (Pl. I, Figs. 12 and 13; Pl. III, Figs. 44 to 47; and Pl. IV, Fig. 74). The secondary spermatocytes have the usual complement of mitochondria and Golgi bodies. There is a definite inter-kinetic resting stage, when the nucleus of the secondary spermatocyte is reformed.

In *Chiloscyllium griseum* metaphase II shows the chromosomes jumbled up in a single mass (Pl. I, Fig. 14) as in metaphase I. This single chromatic mass lies at the equator of the spindle, while at each pole there is a distinct centrosome. In Pl. I, Fig. 15, is shown a telophase II. During the late telophase II the two daughter nuclei, i.e., the spermatid nuclei are formed (Pl. I, Fig. 16). Here also the mitochondria appear as vesicles and they are distributed, more or less equally, to the two daughter spermatids and the Golgi bodies fail to come up in this technique, i.e., F.W.A.

Spermateleosis

For the sake of convenience and to avoid possible confusion, individual account of each fish is dealt with hereafter.

Chiloscyllium griseum

In *Chiloscyllium griseum*, the earliest spermatid, in F.W.A. technique, shows a deeply-stained excentric nucleus, a juxta-nuclear mass of mitochondrial granules and a few Golgi bodies (Pl. I, Fig. 17). Pl. I, Fig. 18, is drawn from Kolatchew and shows a single vesicular Golgi body. A few

Golgi bodies seem to come together and form a slightly bigger structure called the pro-acrosome (Pl. I, Figs. 19 and 20). Pl. I, Fig. 21, is drawn from a smear and shows very satisfactorily the arrangement of different structures in the cell. The pro-acrosome, sooner or later, settles down on the nucleus (Pl. I, Figs. 19 and 20) and is transformed into an acrosome (Pl. I, Figs. 22 to 26). The acrosome is at first pear-shaped, but ultimately it becomes wavy (Pl. II, Figs. 35 to 37). The point at which the acrosome attaches itself to the nucleus is the anterior end of the future sperm head. The cytoplasm now gradually gets attenuated and at the same time the sperm nucleus elongates (Pl. II, Figs. 27 to 32). A few of the Golgi bodies which do not take part in the formation of the pro-acrosome are sloughed off along with the residual cytoplasm (Pl. I, Figs. 20 and 26; and Pl. II, Figs. 29 to 32 and 35).

As the pro-acrosome is approaching the nucleus the granular mitochondria, are becoming vesicular. These mitochondria form a compact mass towards the posterior end of the spermatid (Pl. I, Figs. 23 to 26). As the spermatid grows the mitochondria become more localized around the axial filament (Pl. II, Figs. 27 to 31). Most of these mitochondria form the sheath of the axial filament in the region of the middle-piece, while a few are sloughed off along with the residual cytoplasm (Pl. II, Figs. 33 to 35).

At about the first appearance of the pro-acrosome and its contact with the spermatid nucleus, there appears in the spermatid two closely situated centrosomes, just within the cell membrane (Pl. I, Figs. 19 and 20). One of these centrosomes migrates towards the nucleus and becomes the proximal centrosome, the other remains as the distal one. An axial filament springs up from the proximal centrosome connecting it with the distal. At the same time the distal centrosome gives off the extra-cellular part of the axial filament, which is, indeed, the posterior extension of the intracellular part (Pl. I, Figs. 23 to 25).

As the nucleus begins to elongate the cytoplasm starts attenuating, so much so that it cannot be made out round the head region, and exists in the form of a very thin sheath in the region of the middle-piece, whose anterior and posterior ends are determined by the proximal and the distal centrosomes respectively (Pl. II, Figs. 33 to 35). By this time the mitochondria of the middle-piece region have completely disappeared. Immediately behind the middle-piece the residual cytoplasm, containing the unused mitochondria and Golgi bodies, forms a prominent bleb as shown in Figs. 33 and 34 of Pl. II. Soon after, this bleb shifts down the axial filament (Pl. II, Fig. 35), and is ultimately sloughed off (Pl. II, Fig. 36), the axial filament behind the middle-piece remaining absolutely naked.

The fully mature sperm has a cylindrical deeply-staining nucleus with a wavy acrosome in front, middle-piece behind and a naked axial filament forming the tail (Pl. II, Fig. 36).

Sertoli cells, which presumably perform the two-fold function of nourishment and anchorage for the ripening sperms, are met with just within the walls of the sperm follicles or cysts (Pl. II, Fig. 38). These cells are large with large nuclei like the typical nurse-cells, to which bundles of ripening sperms are attached. Subsequently the sertoli cells get loosened

and come to lie amongst the sperm bundles, where the sloughed off residual cytoplasm of the sperm can also be seen.

Carcharinus limbatus

The earliest spermatid has a vesicular excentric nucleus, granular mitochondria and a few granular and crescent-shaped Golgi bodies (Pl. III, Fig. 49). As the transformation of the spermatid into the sperm proceeds, the nucleus starts taking up a deep homogeneous stain with iron-haematoxylin and becomes compact, showing no structure whatsoever in its interior (Pl. III, Figs. 50 to 55).

Most of the Golgi bodies come close together (Pl. III, Fig. 53), and ultimately fuse to form a bigger structure called the pro-acrosome (Pl. III, Fig. 54). This hovers round the nucleus for some time and ultimately fuses with it (Pl. III, Figs. 55 and 59). Later on the pro-acrosome is transformed into the acrosome, which is generally pear-shaped (Pl. IV, Figs. 65 to 67) but sometimes it assumes the form of a small filament (Pl. IV, Figs. 62 and 63).

During spermateleosis, the mitochondria lose their staining capacity (Pl. III, Figs. 50 to 55), and ultimately fuse to form a homogeneous, faintly-staining mass (Pl. III, Figs. 56 and 57). This is the mitochondrial nebenkern, which elongates when the cytoplasm of the spermatid is drawn out. It seems to be traversed by the two axial filaments (Pl. III, Figs. 59 to 61). In other words, the mitochondrial nebenkern forms the sheath of the axial filament in the region of the middle-piece, and finally disappears from view as such (Pl. IV, Fig. 67).

In *Carcharinus limbatus* the history of the centrioles is quite complicated inasmuch as there are three granular centrosomes instead of the usual two, hitherto described in Elasmobranchs. To begin with one finds two granular centrosomes near the cell membrane (Pl. III, Fig. 51), which soon get apart (Pl. III, Fig. 52); and an axial filament now appears between the two (Pl. III, Figs. 53 and 54). Thus we have a proximal and a distal centrosome. At the same time the extra-cellular part of the axial filament, which is a continuation of the intra-cellular part, puts in its appearance. Soon after the stage represented in Pl. III, Fig. 54, with a single proximal centrosome and a single intra-cellular axial filament, there appear in the spermatid two proximal centrosomes each connected with the single distal centrosome by an intra-cellular axial filament (Pl. III, Fig. 55). It is difficult to say whether the second proximal centrosome is formed by the division of the originally single proximal centrosome or by the division of the distal centrosome. With the lengthening out of the cytoplasm of the spermatid, the two intra-cellular filaments also lengthen out and coil round each other in the region of the middle-piece (Pl. III, Figs. 59 and 61; and Pl. IV, Figs. 63 to 66). In the ripe or nearly ripe sperm, however, there is again only one intra-cellular filament, probably formed by the fusion of the two filaments (Pl. IV, Fig. 67).

The ripe sperm has a deeply-stained bean-shaped nucleus with a conical acrosome in front, a middle-piece behind and a naked axial filament forming its tail. It may be noted that the nucleus of the ripe sperm of

Carcharinus limbatus is much smaller as compared with *Chiloscyllium griseum.*

Sphyrna blochii

The earliest spermatid of *Sphyrna blochii* shows a vesicular nucleus with a few chromatin threads, juxta-nuclear mitochondria and a few Golgi granules or crescents or vesicles (Pl. IV, Figs. 75 and 76). The Golgi bodies have a distinct tendency to come close together and lie in groups (Pl. V, Figs. 77 and 78). The Golgi bodies of one group fuse to form a bigger structure, called the pro-acrosome (Pl. V, Fig. 80). This is soon attached to the nucleus (Pl. V, Figs. 81 and 82), and is ultimately transformed into an acrosome (Pl. V, Figs. 83 and 84), which soon becomes conical in form (Pl. V, Figs. 85 to 89). The Golgi elements which do not take part in the formation of the pro-acrosome, are sloughed off along with the residual cytoplasm (Pl. V, Figs. 86, 88 and 89).

The mitochondria of the spermatid come closer and ultimately fuse to form a compact structure taking a feeble homogeneous stain (Pl. V, Figs. 82 to 86). This is the mitochondrial nebenkern. With the drawing out of the cytoplasm the mitochondrial nebenkern also lengthens out and ultimately it forms the sheath of the middle-piece, the axial filament running through it.

There are two centrosomes and their relationship with the axial filament is exactly the same as in *Chiloscyllium griseum* (Pl. V, Figs. 78 to 80 and 85 to 90).

As in *Chiloscyllium griseum* and *Carcharinus limbatus*, the rounded spermatid nucleus of *Sphyrna blochii*, which stains deeply and homogeneously, gets elongated to form the sperm nucleus, but it does not grow to the same length as in *Chiloscyllium griseum.*

The ripe sperm of *Sphyrna blochii* has a short nucleus with a conical acrosome in front, the middle-piece behind, and a tail formed by the axial filament only (Pl. V, Fig. 90).

Discussion

Golgi bodies and the acrosome

In the first paper of this series dealing with the nine species of Elasmobranchs (Vasisht, 1953), I have fully discussed the work of older workers of the last century, such as Hermann (1882), Jensen (1883), Swaen and Masquelin (1883), and Moore (1895) on the Elasmobranch sperm. It was concluded by me that all these workers, in spite of the faulty technique used by them, gave faithful accounts of what they saw in their preparations. A reference to my earlier paper referred to above on the spermatogenesis of Rays and Skates will clearly show that all these workers saw the Golgi bodies fused to form an acrosome, but they could not possibly have been expected to use modern terminology at a time when the Golgi apparatus had not been even discovered.

The only recent paper on the Elasmobranch sperm is by Ratnavathy (1941), who worked on the sperm of the shark, *Chiloscyllium griseum*,

which forms also the subject matter of the present communication. According to her, '... the Golgi bodies of the spermatid fuse together in such a manner that an almost homogeneous mass of Golgi substance is obtained which may now be designated as the acroblast. This structure always occupies that pole of the spermatid which is destined to become the anterior end of the sperm and is diametrically opposed to that region of the cell where the centrosome has settled down. The acroblast very soon elaborates within its substance a single small vesicle, which, however, soon enlarges and becomes prominent. When it reaches its maximum dimensions it is gradually pushed out of the acroblast and deposited close against the nuclear wall, the acroblast is still maintaining its connection with the vesicle for a longer or shorter time. Soon it is filled with a dense substance so that the transparent appearance of the vesicle is lost, and when this change of appearance is effected the acroblast, which up to now has been observed clinging to the wall of the vesicle like a lump, gradually separates itself and proceeds in a posterior direction to be later expelled from the substance of the spermatid. The vesicle together with the dense substance that it encloses comes to be known as the acrosome. This structure presently assumes a conical appearance and persists through the later stages of sperm formation as a thin short apical filament, until in the metamorphosed spermatozoon it presents the appearance of a spirally twisted filament.' I agree with Ratnavathy (1941) that the acrosome in *Chiloscyllium griseum* sperm is screw-like, and arises from the Golgi bodies. But I have never come across, in my material, either the 'acroblast' or the 'acrosomal vesicle' described by her. In all the twelve species of Elasmobranchs studied by me the earliest spermatid has a few Golgi bodies, which soon come together to form a pro-acrosome, the fore-runner of the acrosome. But there is no indication whatsoever of the Golgi bodies secreting the acrosome.

Mitochondria and the middle-piece

With regard to mitochondria it is of interest to note that, like the Golgi bodies, their behaviour in spermateleosis in general is uniform in all the twelve species of Elasmobranchs inasmuch as the mitochondria invariably form the sheath of the axial filament in the region of the middle-piece.

Centrosomes and the axial filament

Hermann (1882) described the distal centrosome as 'renflement' (swelling). Jensen (1883) has called it 'button'. Moore (1895) has mentioned the distal centrosome as 'bead' in his paper on Elasmobranchs. From the account of these workers it would appear that the distal centrosome of the sperm in these Elasmobranchs is granular. My studies of the sperm of twelve species of Elasmobranch fishes has convinced me that the accounts of the above-named workers, describing the distal centrosome of the sperm as a granule, is correct.

But a few workers like Suzuki (1899) and Ratnavathy (1941) have described the distal centrosome of the sperm as ring-like and funnel-like

respectively. Ratnavathy (1941), working on *Chiloscyllium griseum*, gives a very elaborate account of 'the phenomenon of the cataclysmic disruption of the centrosome, entailing the abnormal formation of the axial filament in between the two products of centrosomal division'. The result of this 'cataclysmic disruption' is that, according to Ratnavathy, the distal centrosome becomes 'funnel-like'. I am unable to support this account of Ratnavathy. In my preparations of the sperm of the *Chiloscyllium* and other eleven species of Elasmobranchs the distal centrosome is invariably granular in form.

Summary

1. The spermatogenesis of the three Elasmobranch sharks, viz., *Chiloscyllium griseum*, *Carcharinus limbatus* and *Sphyrna blochii*, has been worked out in this paper.

2. A thorough study of the cytoplasmic inclusions in the spermatogonia, spermatocytes and in spermateleosis has been made.

3. The earliest spermatid has an excentrically placed nucleus, a few Golgi elements and granular mitochondria.

4. During spermateleosis a pro-acrosome is formed directly by the fusion of a few Golgi bodies. This fuses with the nucleus and is directly transformed into an acrosome.

5. The mitochondria in, *Chiloscyllium griseum* become vesicular in the late spermatids, whereas in *Carcharinus limbatus* and *Sphyrna blochii* they remain granular. They form the sheath of the middle-piece.

6. In *Chiloscyllium griseum* and *Sphyrna blochii* the two centrosomes, proximal and distal, are situated at the anterior and posterior ends of the middle-piece respectively. In *Carcharinus limbatus* there are two proximal centrosomes and one distal.

7. The unused Golgi bodies and the mitochondria are sloughed off along with the residual cytoplasm.

8. The ripe sperm in *Chiloscyllium griseum* has an elongated nucleus with a short wavy acrosome in front, a prominent middle-piece and a long flagellum. In the sperm of *Carcharinus limbatus* and *Sphyrna blochii*, however, the nucleus is much smaller.

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Explanation of Lettering in Plates

A.—Acrosome; A_1.—Proacrosome; *A.f.*—Axial filament; *C.*—Centrosome; *Cs.*—Centrosomes; C_1.—Proximal centrosome; C_2.—Distal centrosome; *C.G.*—Chromatin granules; *Ch.*—Chromosomes; *G.*—Golgi body; *M.*—Mitochondria; *M.p.*—Middle-piece; *M.s.*—Mitochondrial nebenkern; *N.*—Nucleus; N_1—Nucleolus; *N.f.*—Nuclear filament; *R.C.*—Residual cytoplasm; *S.f.*—Spindle fibres.

Explanation of Figures and Plates

All figures have been drawn with a camera lucida at the table level with Beck 10× eyepiece and oil immersion objective giving a magnification of approximately 1,700. All figures (except Pl. I, Fig. 21; Pl. II, Figs. 27 and 31 to 37; Pl. III, Figs. 59 to 61; Pl. IV, Figs. 62 to 67; and Pl. V, Figs. 89 and 90 which are drawn from smears) have been drawn from sectioned material fixed either in Flemming-without-acetic acid or Champy followed by 0·5% iron haematoxylin; and Kolatchew.

Plate I (*Chiloscyllium griseum*)

Fig. 1. Earliest spermatogonium (F.W.A.).
„ 2. Spermatogonium showing the mitochondria (F.W.A.).
„ 3. Late spermatogonium (F.W.A.).
Figs. 4 and 5. Primary spermatocytes (F.W.A.).
„ 6 to 8. Primary spermatocytes showing the grown-up mitochondria in addition to the Golgi bodies (F.W.A.).
Fig. 9. Metaphase I (F.W.A.).
„ 10. Telophase I (F.W.A.).
„ 11. Late telophase I (F.W.A.).
Figs. 12 and 13. Secondary spermatocytes (F.W.A.).
Fig. 14. Metaphase II (F.W.A.).
„ 15. Telophase II (F.W.A.).
„ 16. Late telophase II (F.W.A.).
„ 17. Spermatid (F.W.A.).
„ 18. Spermatid (Kolatchew).
Figs. 19 and 20. Spermatids showing the pro-acrosome, centrosomes and the vesicular mitochondria (F.W.A.).
Fig. 21. Spermatid drawn from a smear (F.W.A.).
„ 22. Spermatid with an acrosome (F.W.A.).
Figs. 23 to 26. Late spermatids showing the elongating nuclei, acrosomes and the axial filaments (F.W.A.).

Plate II (*Chiloscyllium griseum*)

Figs. 27 to 32. Late spermatids (F.W.A.).
„ 33 to 35. Early sperms showing the blebs of residual cytoplasm (F.W.A.).
Fig. 36. Fully ripe sperm (F.W.A.).
„ 37. Diagrammatic representation to show the intra-nuclear filament and the twisted acrosome (F.W.A.).
„ 38. *Sertoli cells and the arrangement of sperm bundle in the testicular cyst or* foliole (F.W.A.).

Plate III (*Carcharinus limbatus*)

Fig. 39. Earliest spermatogonium (F.W.A.).
„ 40. Spermatogonium showing mitochondria and Golgi bodies (F.W.A.).
Figs. 41 to 43. Primary spermatocytes (F.W.A.).
„ 44 to 47. Secondary spermatocytes (F.W.A.).
Fig. 48. Spermatid (Kolatchew).
„ 49. Early spermatid (F.W.A.).
„ 50. Spermatid showing condensed nucleus (F.W.A.)
„ 51. Spermatid showing two centrosomes (F.W.A.).
„ 52. Spermatid (F.W.A.).
„ 53. Spermatid showing the Golgi bodies coming near one another and the appearance of an axial filament (F.W.A.).
„ 54. Proacrosome is formed (F.W.A.).
„ 55. Spermatid showing three centrosomes (F.W.A.).
Figs. 56 to 58. Mitochondrial nebenkern is formed (F.W.A.).
Fig. 59. Elongating spermatid (F.W.A.).
Figs. 60 and 61. Acrosome is formed (F.W.A.).

Plate IV (*Carcharinus limbatus*)

Figs. 62 and 63. Filamentous acrosome (F.W.A.).
„ 64 to 66. Note the early sperm stages with a bean-shaped nucleus and the conical acrosome (F.W.A.).
Fig. 67. Mature sperm (F.W.A.).

(*Sphyrna blochii*)

Fig. 68. Earliest spermatogonium (Champy).
„ 69. Spermatogonium showing the mitochondrial granules (Champy).
„ 70. Late spermatogonium showing the Golgi bodies in addition to the mitochondria (F.W.A.).
Figs. 71 to 73. Primary spermatocytes (F.W.A.).
Fig. 74. Secondary spermatocyte (F.W.A.).
„ 75. Earliest spermatid (F.W.A.).
„ 76. Spermatid with a condensed nucleus and showing different structures of Golgi bodies (Champy).

Plate V (*Sphyrna blochii*)

Figs. 77 and 78. The Golgi bodies are showing a distinct tendency to come closer (F.W.A.).
Fig. 79. A small intra-cellular filament has appeared between the two centrosomes (F.W.A.).
„ 80. Pro-acrosome is formed (F.W.A.).
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„ 82. Elongating spermatid (F.W.A.).
„ 83. Spermatid showing the directly transformed acrosome (F.W.A.).
Figs. 84 and 85. Late spermatids showing elongated nuclei and the axial filaments (F.W.A.).
„ 86 and 87. Late spermatids showing the receding cytoplasm (F.W.A.).
„ 88 and 89. Early sperms with the residual cytoplasm (Champy).
Fig. 90. Mature sperm constituted by a small nucleus with an acrosome in front, a long middle-piece behind and an axial filament (F.W.A.).
„ 91. Sertoli cells and the arrangement of sperm bundle in the follicle of the testis (F.W.A.).

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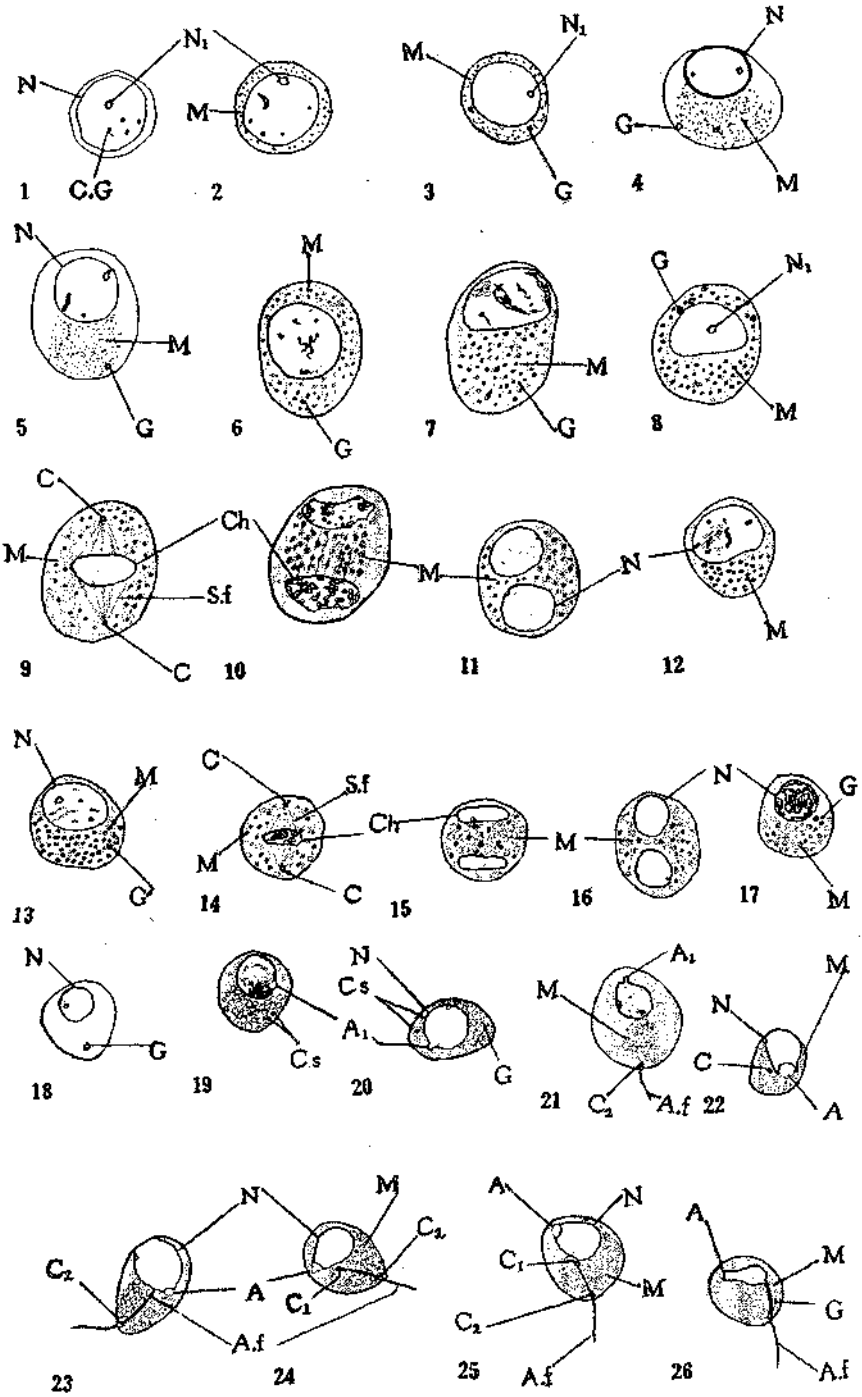
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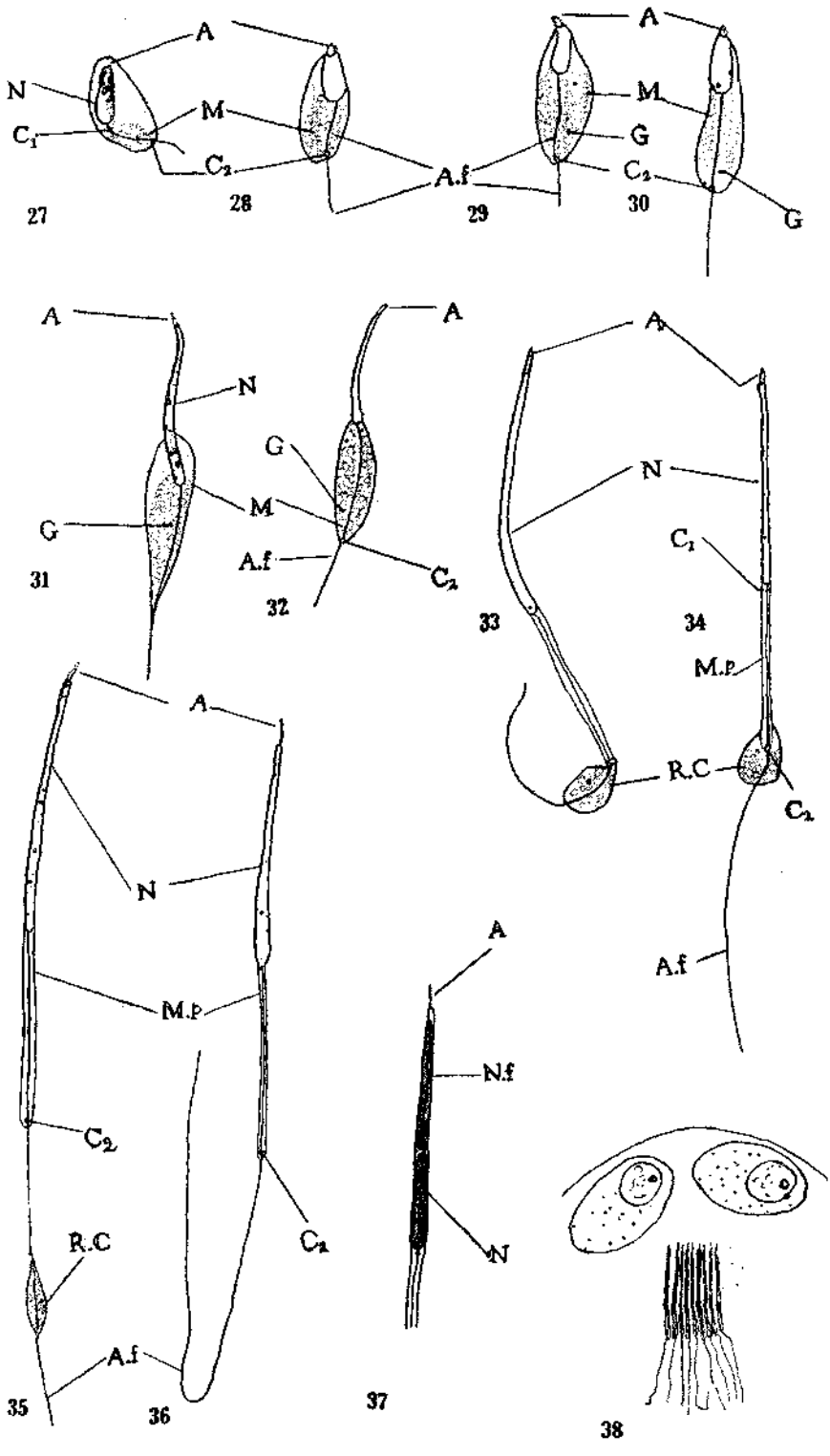
PLATE II.



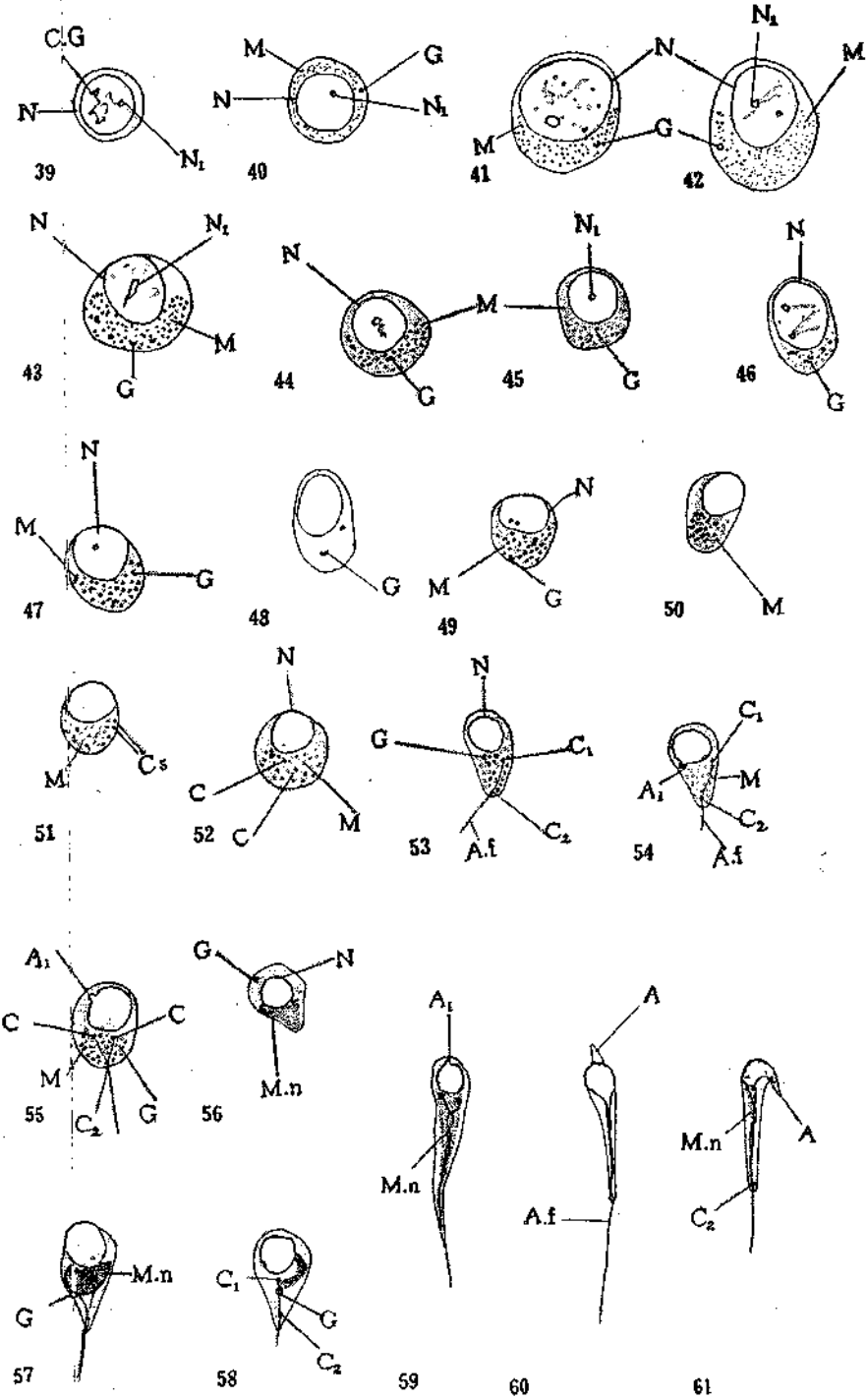

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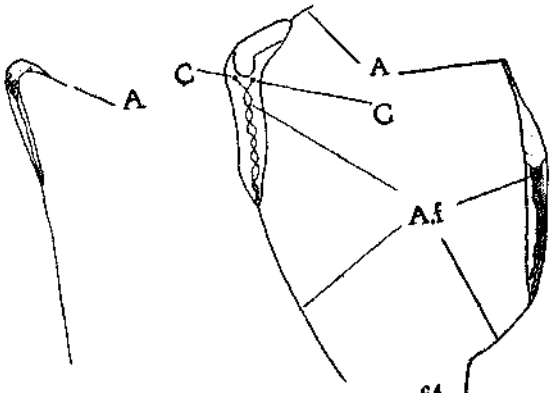
PLATE IV.



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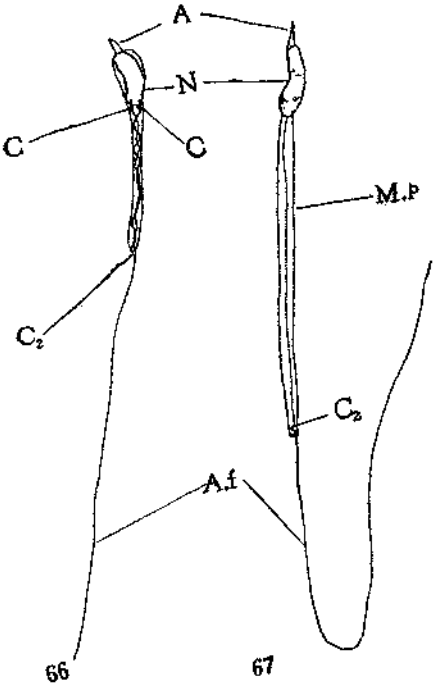

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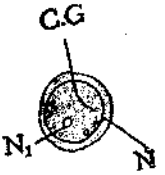

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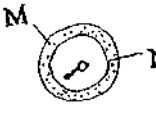

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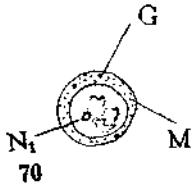

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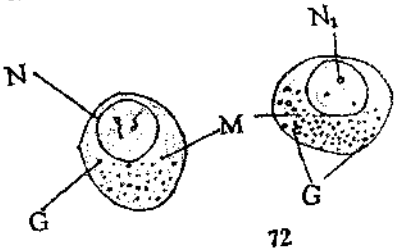

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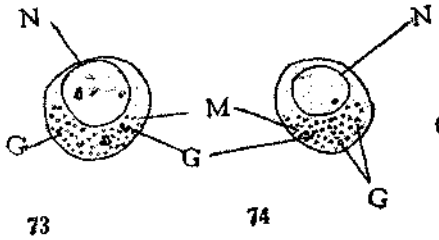

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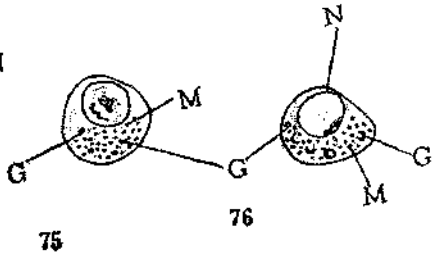

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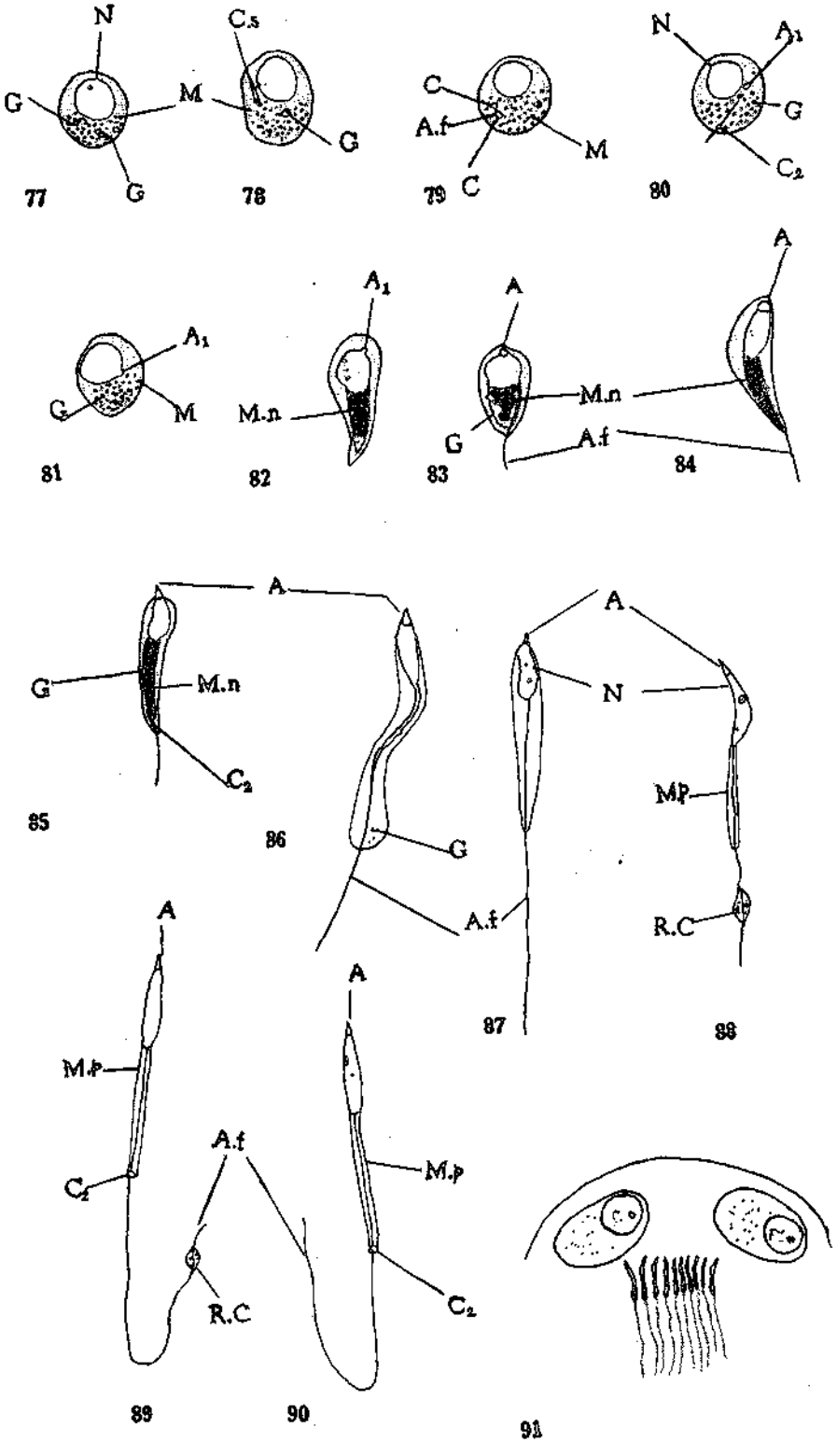
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CHLORAMINE-B AS A VOLUMETRIC REAGENT

Part III—Determination of Metals

by

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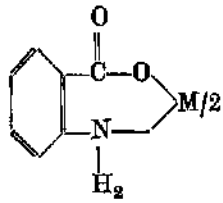
CHLORAMINE-B AS A VOLUMETRIC REAGENT

Part III. Determination of Metals

By Apar Singh, *Panjab University College, Hoshiarpur, Panjab (India)*

Anthranilic acid reacts with zinc, cobalt, nickel, cadmium, lead, etc., to form insoluble Chellate compounds which are represented by the following formula:—



The precipitation of these metals quantitatively have been studied very thoroughly by Goto (*J. Chem. Soc. Japan*, 1934, **55**, 1156–63; C.A., 1935, **29**, 1029), and Funk and his co-workers (*Z. Anal. Chem.*, 1933, **93**, 241–7; *ibid.*, 1934, **96**, 385–8; *ibid.*, 1935, **101**, 85–88). The organic component of the precipitate is determined by quantitative bromination with Chloramine-B in the presence of excess potassium bromide and is used to calculate the amount of the metallic substance.

The brominating reagent consists of a standard solution of Chloramine-B, used in conjunction with potassium bromide in an acid medium. The organic metallic complex is dissolved in hydrochloric acid, treated with a measured excess amount of Chloramine-B.

The reactions involved are:

$$Me^{+++} + 2C_7H_7O_2N = Me(C_7H_6O_2N)_2 + 2H^+$$

$$Me(C_7H_6O_2N)_2 + 2HCl = Me^{++} + 2C_7H_7O_2N + 2Cl^-$$

$$C_6H_5SO_2N(Na)Cl + 2KBr + 2HCl = C_6H_5SO_2NH_2 + 2KCl + NaCl + Br_2$$

$$C_7H_7O_2N + 3Br_2 = C_6H_2Br_3NH_2 + CO_2 + 3HBr.$$

General Procedure of Bromination.—The precipitate of the complex formed was digested, filtered on a sintered crucible and washed free from the reagent. The precipitate was dissolved in concentrated hydrochloric acid and the solution transferred to an iodine flask, diluted with water so that it was 2-3 *N* with respect to hydrochloric acid. The solution was cooled thoroughly and mixed with 10 c.c. of 20% potassium bromide and a known excess of standard Chloramine-B solution to brominate completely the whole of the anthranilic acid and to leave an excess equivalent to 20–30 c.c. of *N*/10 sodium thiosulphate. During the bromination period, the iodine flask was stoppered and the cup was kept filled with 5 c.c. of 40%

potassium iodide solution to prevent the escape of bromine. The potassium iodide was finally added to the flask and the iodine liberated was titrated against sodium thiosulphate using starch as the indicator. A starch solution should be added just near the end point.

Experimental

Preparation of the Reagent.—Three per cent sodium anthranilate reagent was prepared by dissolving 3·0 gm. of A.R. anthranilic acid (twice recrystallised) in 22 c.c. of 1·0 *N* sodium hydroxide solution. The solution was filtered and the filtrate was diluted to 100 c.c. and treated very carefully with small quantities of anthranilic acid until slightly acidic to litmus.

Determination of Zinc.—A known weight of zinc sulphate was dissolved in water. To it was added a very dilute solution of sodium-carbonate drop by drop with stirring till slightly turbid and was then acidified slightly with dilute acetic acid. The solution was diluted to 150 c.c., heated to boiling, mixed with 20 c.c. of 3 per cent sodium anthranilate solution and allowed to stand for 2-3 hours. The precipitate was filtered and washed with a cold solution of the reagent which was prepared by diluting the reagent solution with 15–20 times as much water. The precipitate was sucked dry, washed several times with alcohol and dried to a constant weight at 105–110°C. The precipitate was dissolved in 4 *N* hydrochloric acid in a glass stoppered bottle and titrated with a measured excess of Chloramine-B solution in conjunction with potassium bromide solution. The results of the various determinations are recorded in Table I.

Table I

$ZnSO_4 . 7H_2O$ taken. (g.)	$Zn(C_7H_6O_2N)_2$ obtained.	*N*/10 Chloramine-B used. (c.c.)	Wt. of the $Zn(C_7H_6O_2N)_2$ w.r. to C.B. used.	$ZnSO_4 . 7H_2O$ found.
0·03825	0·0456	16·30	0·04583	0·039038
0·04335	0·05160	18·40	0·05174	0·044068
0·05100	0·0606	21·50	0·06045	0·051493
0·06375	0·0758	26·70	0·07508	0·063947

1 c.c. of *N*/10 Chloramine-B = 0·002812 gm. $Zn(C_7H_6O_2N)_2$ = 0·002395 gm. $ZnSO_4 . 7H_2O$.

Determination of Cobalt.—A known weight of cobalt salt (say 0·1 gm.) was dissolved in 250 c.c. of distilled water. It was heated to boiling and mixed with 15–20 c.c. of 3 per cent sodium anthranilate solution. The solution was allowed to stand over-night and filtered through sintered crucible. The precipitate was washed thoroughly with a cold solution prepared by diluting 5 c.c. of the reagent solution with 95 c.c. of water and finally several times with alcohol. A red precipitate corresponding to the formula $CO(C_7H_6O_2N)_2$ obtained was dried to a constant weight at 105–107°C. It was dissolved in 4 *N* HCl and titrated against standard Chloramine-B in the presence of potassium bromide as usual.

Results of the titrations are summarized in Table II.

TABLE II

$CoCl_2 . 6H_2O$ taken. (g.)	$Co(C_7H_6O_2N)_2$ obtained. (g.)	$N/10$ Chloramine-B used. (c.c.)	$Co(C_7H_6O_2N)_2$ corresponding to C.B. used. (g.)	$CoCl_2 . 6H_2O$ found. (g.)
0·03315	0·0452	16·40	0·04523	0·03251
0·0390	0·0540	19·60	0·05405	0·03887
0·04680	0·0654	23·80	0·06564	0·04719
0·0585	0·0812	29·60	0·08163	0·05869
0·0975	0·1360	49·30	0·13597	0·09776

1 c.c. of $N/10$ C.B. = 0·002758 gm. of $Co(C_7H_6O_2N)_2 = 0·001983$ gms. of $CoCl_2 . 6H_2O$.

Determination of Nickel.—The determination of nickel is similar to cobalt. Anthranilic acid gives a light green precipitate of nickel anthranilate when added to a hot neutral solution of nickel salt.

The results of various titrations are recorded in Table III.

TABLE III

$NiCl_26H_2O$ taken. (g.)	$Ni(C_7H_6O_2N)_2$ obtained. (g.)	$N/10$ Chloramine-B used. (c.c.)	$Ni(C_7H_6O_2N)_2$ corresponding to C.B. used. (g.)	$NiCl_2 . 6H_2O$ found. (g.)
0·0200	0·02740	9·90	0·02728	0·01960
0·0260	0·03615	13·20	0·03638	0·03366
0·0400	0·0552	20·30	0·05594	0·04019
0·0600	0·0830	30·50	0·08406	0·06039

1 c.c. of $N/10$ Chloramine-B = 0·002758 gm. $Ni(C_7H_6O_2N)_2 = 0·0019808$ gm. $NiCl_2 . 6H_2O$.

Determination of Cadmium.—A white crystalline precipitate corresponding to the formula $Cd(C_7H_6O_2N)_2$ is obtained when sodium anthranilate is added to a slightly acid or neutral solution of cadmium salt. The details for quantitative precipitation of cadmium are similar to zinc determination as discussed before. The precipitate is dissolved in 4 *N* HCl and titrated against standard excess Chloramine-B in the presence of potassium bromide as before.

The results are given in Table IV.

TABLE IV

$3CdSO_4 . 8H_2O$ taken. (g.)	$Cd(C_7H_6O_2N)_2$ obtained. (g.)	$N/10$ Chloramine-B used. (c.c.)	$Cd(C_7H_6O_2N)_2$ corresponding to C.B. used. (g.)	$3CdSO_4 . 8H_2O$ found. (g.)
0·03910	0·0592	18·50	0·05925	0·03953
0·05290	0·0806	25·00	0·08007	0·05342
0·0690	0·1067	33·20	0·10634	0·07094

1 c.c. of $N/10$ Chloramine-B = 0·003203 gms. $Cd(C_7H_6O_2N)_2 = 0·002137$ gm. $3CdSO_4 . 8H_2O$.

Determination of Lead.—A known weight of lead nitrate was dissolved in 100 c.c. of water. To it was added 30 c.c. of 3 per cent sodium anthranilate solution with constant stirring. The solution was allowed to stand for 2-3 hours and filtered through sintered crucible. The precipitate was washed repeatedly with a wash solution prepared by diluting the precipitating reagent 5-6 times with water. It was finally washed with a little alcohol and dried at 105–107°C. to a constant weight. The precipitate was dissolved in hot 10 per cent ammonium acetate solution, cooled, acidified with hydrochloric acid and titrated with standard Chloramine-B solution in conjunction with potassium bromide, as described in the general procedure.

The results of various determinations are recorded in Table V.

TABLE V

$Pb(NO_3)_2$ taken.	$Pb(C_7H_6O_2N)_2$	*N*/10 Chloramine-B used.	$Pb(C_7H_6O_2N)_2$ corresponding to C.B. used.	$Pb(NO_3)_2$ found. (g.)
0·03588	0·0512	13·00	0·05180	0·03588
0·0552	0·0790	20·05	0·07986	0·05520
0·06348	0·0910	23·00	0·09184	0·06348
0·08004	0·1154	29·10	0·11619	0·08032

1 c.c. of *N*/10 Chloramine-B = 0·003993 gms. $Pb(C_7H_6O_2N)_2$ = 0·00276 gm. $Pb(NO_3)_2$.

From the above results it is evident that zinc, cobalt, nickel, cadmium and lead can be precipitated as their metallic anthranilates and the organic component of the precipitate is determined by quantitative bromination with Chloramine-B in conjunction with excess of potassium bromide.

P.S.—In the case of zinc sulphate, cobalt chloride and nickel chloride, the solutions were made in water and their strength was determined by the usual standard methods.

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April, 1954

CHEMICAL EXAMINATION OF THE OIL FROM THE SEEDS OF *CASSIA ABSUS* LINN.

by

I. SEN GUPTA, KULWANT SINGH and RAM PARKASH SOOD

(Department of Chemistry, Panjab University College, Hoshiarpur)

Edited for the Panjab University by Vishwa Nath,
Department of Zoology, Panjab University,
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CHEMICAL EXAMINATION OF THE OIL FROM THE SEEDS OF *CASSIA ABSUS* LINN.

By I. Sen Gupta, Kulwant Singh *and* Ram Parkash Sood

(*Dept. of Chemistry, Panjab University College, Hoshiarpur*)

'The Physical properties and chemical composition of the oil from *Cassia Absus* Linn. has been studied and the empirical formula of unsaponifiable matter along with its benzoate and acetate reported.'

Introduction

Cassia Absus Linn.[8] (ver. Chaksu), an indigenous medicinal plant, belongs to the natural order of Leguminosae. It is distributed throughout India, Ceylon, Tropical Asia, Australia and Africa. The seeds are bitter, cooling, astringent to the bowels, diuretic, cure diseases of the eye, used for bronchitis pains, leucoderma, etc.

The earlier work on the composition of the seed was first reported by Dymock,[4] who reported the presence of an alkaloidal principle, the moisture content, the ash percentage and the amount of soluble extract by various solvents. Later on, Siddiqui and Ahmed,[11] found two water-soluble isomeric quaternary bases in a total yield of about 1·5%. These bases were named Chaksine and Isochaksine. A good deal of work has been carried out by various workers [7, 9, 12] on the constitution of these bases. Petroleum ether extract gave a dark yellow oil. The present investigation was initiated with a view to study the composition of the oil. Ahmed, Z.,[1] has also reported the composition of the oil. There seems to be some discrepancy regarding the percentage of glycerol and our results are at variance with those reported by him. Further the hydroxy acid has been identified as ricinoleic acid. The unsaponifiable matter on purification was found to have empirical formula $C_{22}H_{30}O$. This was further converted into the corresponding acetate and benzoate.

Experimental

Extraction of the Oil.—Four kilograms of chaksu seeds were dried in the sun for a day and then crushed and powdered. The black husk was separated and the yellow powder soaked in petroleum ether (B.P. below 100°C.) at room temperature (20–25°C.), shaking occasionally. Three extractions were made in two weeks' time. The solvent was distilled off on water bath and the last traces of it were removed in an air oven at 120°C. The total amount of the oil obtained was 86 gms.

Characteristics of the Oil.—The oil was dark green with yellowish tinge. A single drop was pale yellow in colour. The oil does not contain any nitrogen and sulphur and burns with a sooty flame. A drop of it was spread on each of the two glass slides and kept at room temperature. The films became white, sticky and dried at the end of 40 days, showing thereby that the oil belongs to the class of the semi-drying oils.

Table I records the physical and chemical constants:

TABLE I

Specific gravity at 25°C.	0·9203
Refractive index at 24·25°C. ..	1·4610
Specific rotation in chloroform $^{27°}_{D}[\alpha]$ ..	+0·03542
Saponification value	189·00
Acid value	22·125
Iodine value	117·05
R.M. value	0·847
Polenske value	0·70
Unsaponifiable matter	3·81
Acetyl value	39·96
Hehner value	87·11

Saponification.—200 gms. of the oil were saponified with alcoholic potash; the alcohol was distilled off and the residual soap dissolved in water. The unsaponifiable matter was removed with ether and the soap solution was then decomposed with dilute sulphuric acid. When heated, the fatty acids formed a clear oily layer at the top. These were removed with ether in a separating funnel, washed free of acid with water, solvent recovered, and then dried at 100°C. The acids were yellowish green with semi-solid consistency. Yield of the acids = 84%.

The constants of the mixed fatty acids are as in Table II.

TABLE II

Refractive index at 22°C. ..	1·46
Iodine value	118·20
Neutralization value ..	197·25
Mean molecular weight ..	284·40

Unsaponifiable Matter.—The yellowish brown sticky mass obtained by extracting the saponified oil with ether, washing it free of soap and alkali with water, drying over anhydrous calcium chloride and recovering the solvent. After treatment with animal charcoal and repeated crystallization from absolute alcohol, beautiful silky white flakes were obtained on cooling the alcoholic solution in ice. The crystals were collected on suction and dried in a vacuum desiccator. m.pt. 130-131°C.; $[\alpha]_{D}^{27} = 1·469$.

4·130 mgms. of the white substance gave on combustion 12·615 mgms. of CO_2 and 4·410 mgms. of H_2O. Found: C, 83·30%, H, 11·86%, O (by difference), 4·84%, the empirical formula comes out to be $C_{28}H_{39}O$.

Acetate derivative.—It was prepared by refluxing 0·1 gm. of the substance with excess of acetic anhydride in a 100 c.c. R.B. flask on a sand bath for two hours. The oily substance obtained on pouring the cooled solution in ice cold water, was chilled in ice for four hours and allowed to remain so for a day. The white-solid mass crystallized, was rubbed into small flakes with a glass rod to remove the embedded acetic anhydride, washed free of acetic acid and crystallized twice from absolute alcohol. Yield = 0·09 gm. m.pt. = 115-6°C.

Benzoate derivative.—(i) 0·05 gm. of the substance was dissolved in 5 c.c. of dry pyridine and an excess of benzoyl chloride was added to it in a 100 c.c. R.B. flask. The solution changed from yellow to scarlet red. It was allowed to stand, chilled in ice and crystallized from boiling alcohol. The product was found to be the original substance. m.pt. = 130°C. Mixed m.pt. with the original substance = 130-1°C.

(ii) The experiment was repeated by dissolving the substance in 10 c.c. of dry benzene and then adding pyridine and benzoyl chloride but the product on crystallization was again found to be the original substance.

(iii) 0·1 gm. of the substance was dissolved in 10 c.c. of dry benzene and refluxed on a water bath for six hours in a 100 c.c. R.B. flask with 0·5 gm. of pure benzoic anhydride. The yellowish residue obtained after distilling off benzene was heated at 120–140°C. for three hours in an oil bath. The yellow mass after treating with sodium hydroxide to make it free of benzoic anhydride, was extracted with ether, washed free of sodium hydroxide and sodium benzoate and kept overnight on anhydrous sodium sulphate. The yellow mass obtained on recovering the ether melted at 110–120°C. This on extracting with boiling absolute alcohol and chilling gave a white substance which was found to be the original substance. m.pt. 130°C.

The residue insoluble in alcohol was dissolved in ether and crystallized from a mixture of dry ether and absolute alcohol by chilling in ice. The m.pt. of the beautiful white flakes was found to be 142-3°C. Yield of the benzoate = 0·02 gm.

Separation of Solid and Liquid Fatty Acids.—The acids (100 gms.) were dissolved in 95% alcohol (500 c.c.) and the solution, after boiling mixed with a boiling solution of lead acetate (75 gms.) in 95% alcohol (500 c.c.), the alcohol in both cases containing 1·5% glacial acetic acid. The insoluble lead salts obtained on cooling overnight at 18–20°C., were crystallized from alcohol containing 1·5% glacial acetic acid. The solid acids were regenerated from the lead salts by treating with conc. HCl and then extracting with ether. The liquid acids were recovered from the lead salts left over on evaporation of the mixed alcohol mother liquors.

Some characteristics of saturated and unsaturated acids were studied as shown in Table III.

TABLE III

	Unsaturated acids.	Saturated acids.
Physical state	Greenish liquid.	Cream coloured solid.
Refractive index	1·4522 at 23·25°C.	..
Iodine value	136·00	2·56
Neutralization value	160·55	206·30
Percentage in total fatty acids ..	81·22	18·78
Percentage in the oil	68·23	15·77

Unsaturated Acids.—In order to determine the percentage of the hydroxy acid 17·8194 gms. of the unsaturated acids were converted into methyl acetyl esters[10] (19·0613 gms.) and fractionated[5] using modified Willstatter distillation flask under 9-10 mm. pressure.

TABLE IV

Fractions.	Temperature °C.	Wt. of the fraction obtained in gms.
S_1	201–208	14·1266
S_2	210–220	1·8934
S_3	260	1·6465
Residue	....	1·3948

The boiling point of fraction S_3 corresponds to that of methyl acetyl ricinoleic acid. The acid was liberated from fraction S_3 and residue by saponification with KOH and hydrolysis with 50% HCl. The acid obtained after purification was found to be identical with ricinoleic acid.

Iodine value	86·00
Saponification value	188·90
Wt. of S_3 fraction+residue ..	3·0413 gms.

The composition of the unsaturated acids was determined according to the method of Jamieson and Baughmann.[6] The ethereal solution of unsaturated acids was treated with bromine and then chilled at −15°C. No crystals of hexabromide derivative were obtained, showing the absence of linolenic acid in the oil. On cooling the petroleum ether solution at 0°C., tetra-bromo linoleic acid crystals separated, m.pt. 112-113°C. The filtrate was freed of the solvent on a water bath and from the bromine contents of the residue, estimated by sodium ethoxide method,[8] the percentages of dibromo ricinoleic acid, dibromo oleic acid and tetra-bromo linoleic acid were calculated. The results are given in Table V.

TABLE V

Unsaturated acids taken	..	5·1430 gms.
Tetra-bromo linoleic acid insoluble in petroleum ether	..	3·0869 gms.
Residue (di- and tetra-bromides)	..	6·5388 gms.
Bromine contents of the residue	..	45·7650%
Bromine present in the residue	..	2·9920 gms.
Ricinoleic acid in 5·1430 gms.	..	0·7392 gms.
Ricinoleic acid dibromide due to 0·7392 gm. ricinoleic acid	..	1·1360 gms.
Dibromo oleic and tetra-bromo linoleic acid	..	5·4028 gms.
Bromine in the residue due to dibromo ricinoleic acid	..	0·3965 gms.
Bromine in the residue due to dibromo oleic and tetra-bromo linoleic acid	..	2·5955 gms.
Dibromo oleic acid in the residue	..	1·6580 gms.
Tetra-bromo linoleic acid in the residue	..	3·7448 gms.
Total tetra-bromo linoleic acid	..	6·8317 gms.
Oleic acid equivalent to dibromide	..	1·0580 gms.
Linoleic acid equivalent to tetra-bromide	..	3·1900 gms.

TABLE VI

	Oleic acid.	Ricinoleic acid.	Linoleic acid.	Saturated acids.
% in total unsaturated acids..	20·57	14·37	62·01	3·05
% in total fatty acids ..	16·71	11·67	50·36	2·48
% in oil	14·03	9·80	42·31	2·08

Saturated Acids.—The acids (20·3223 gms.) obtained from the insoluble lead salts after conversion into methyl esters[2] (21·4195 gms.) were fractionally distilled as in the case of unsaturated acids. The losses on distillation were added proportionately to each fraction. The various fractions of the esters were separately hydrolyzed with alcoholic potassium hydroxide. Hydrolyzed products from fractions L_1 and L_2 on repeated crystallization from alcohol were identified to be myristic and palmitic acids in both the cases. Hydrolyzed product from fraction L_3 on repeated crystallization from alcohol and acetone gave a product melting at 69°C. showing it to be stearic acid, confirmed by taking mixed melting point with an authentic sample. The results are recorded in Table VII.

Saturated acids present in the unsaturated acids up to the extent of 2·08% of the oil were also distributed proportionately into the individual saturated acids. Table VIII shows the calculated composition of total fatty acids from chaksu oil.

TABLE VII

Fraction.	Boiling point °C.	Pressure mm.	M.pt. of ester °C.	Wt. of the methyl esters obtained in gms.	Corrected weight in gms.	Saponification value of esters.	Mean Mol.wt.		Weight of the acid ≡ corrected ester weight.	Wt. of individual acids in the fraction wt.					
							Esters.	Acids.		Myristic C_{14}.	Palmitic C_{16}.	Stearic C_{18}.	Arachidic C_{20}.	Behenic C_{22}.	Lignoceric C_{24}.
L_1	178–90	10	25	6·0679	6·1953	217·45	258·0	244·0	5·8600	2·5790	3·2810				
L_2	190–99	10	26	6·5037	6·6401	213·20	263·1	249·1	6·2870	1·0310	4·6560				
L_3	200–18	10	31	6·8331	6·9763	198·05	283·2	269·2	6·6310	..	3·6100	3·0210			
L_4	225–35	9	49	0·5797	0·5919	161·25	347·9	333·9	0·5682	..	..	..	0·1350	0·4332	
Residue ..	..	..	..	0·9950	1·0159	157·90	355·3	341·3	0·9761	..	..	..	..	0·9506	0·02550
Total weight ..	..	..	..	20·9794	21·4195	..	..	..	20·3223	4·2100	11·5470	3·0210	0·1350	1·3838	0·02550
% of acids in mixed saturated acids..										20·7200	56·8200	14·8700	0·6643	6·8110	0·12550
% in total fatty acids										3·8900	10·6700	2·7920	0·1247	1·2780	0·02356
% in oil										3·2680	8·9620	2·3460	0·1048	1·0740	0·01979

TABLE VIII

	Solid acids 15·77%	Liquid acids 68·23%	Total 84%
Saturated:			
Myristic	3·26800	0·4315	3·6995
Palmitic	8·96200	0·1836	10·1456
Stearic	2·34500	0·3097	2·6547
Arachidic	0·10480	0·0138	0·1186
Behenic	1·07400	0·1418	1·2158
Lignoceric	0·01980	0·0026	0·0224
Unsaturated:			
Oleic	....	14·0300	14·0300
Ricinoleic	....	9·8000	9·8000
Linoleic	....	42·3100	42·3100
Non-saponifiables ..	....	....	3·8100

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ON THREE NEW SPECIES OF THE GENUS *CEPHALOGONIMUS* POIRIER, 1886, FROM THE INTESTINE OF *LISSEMYS PUNCTATA PUNCTATA*

ON FIVE NEW TREMATODES OF THE GENUS *ASTIOTREMA* LOOSS, 1900, FROM THE INTESTINE OF *LISSEMYS PUNCTATA PUNCTATA* AND DISCUSSION ON THE SYNONYMITY OF TWO ALREADY KNOWN FORMS

by

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ON THREE NEW SPECIES OF THE GENUS *CEPHALOGONIMUS* POIRIER, 1886, FROM THE INTESTINE OF *LISSEMYS PUNCTATA PUNCTATA*

By N. K. Gupta, M.Sc., Ph.D., *Lecturer in Zoology, Punjab University College, Hoshiarpur*

Introduction

In this paper, three new species, namely, *Cephalogonimus indicus*, *C. kumarus* and *C. asiaticus* have been described. These new trematodes were found in the small intestine of *Lissemys punctata punctata* during the course of my investigations of helminths in vertebrates. The work was carried out in the Zoological Laboratory of the Panjab University College, Hoshiarpur.

I am thankful to Dr. Vishwa Nath for providing me facilities in the department and to Dr. H. D. Srivastava for his guidance and helpful criticism in this work. My thanks are also due to Prof. Dewan Anand Kumar, Vice-Chancellor of the Panjab University, for much kind encouragement in these studies.

Genus **Cephalogonimus** Poirier, 1886

Poirier (1886) established the genus *Cephalogonimus* for a digenetic trematode *Cephalogonimus lenoiri*, n.sp., obtained from the small intestine of an African turtle, *Tetrathyra vaillantii* in Senegal. Looss (1899) recorded the type species *C. lenoiri* from *Trionyx nilotica* in Egypt. From Canada, Stafford in 1902 described *C. americanus* which he got from the intestine of *Rana virescens* and *R. clamata*—common frogs of North America. From France, Blaizot in 1910 described the third species *C. europaeus* from the intestine of *Rana esculenta*. Luhe (1911) recorded *Distomum retusus* of Dujardin (1845) and included it in the genus. He, however, did not give any description. Nickerson (1912) added another species *C. vesicaudus* parasitic in the intestine of soft-shelled turtles (Amyda and Aspidonectes) collected from Minnesota. From the United States of America, two more species were added to the genus, one *C. amphiumae* described by Chandler in 1923 from the intestine of *Amphiuma means* and the other *C. compactus* by Stunkard in 1924 from the intestine of *Pseudomys floridana*.

From India, Moghe (1930) was the first to describe a representative of the genus—*C. emydalis*, n.sp.—from the alimentary canal of *Emyda granosa*. From California, Ingles (1932) described *C. brevicirrus* from the intestine of *Rana aurora draytoni*. In the same year from India, Sinha reported *C. magnus* from the intestine of *Trionyx gangeticus* and Pande described *C. mehri* from *Lissemys punctata punctata* and *C. gangeticus*

from *Trionyx gangeticus*. In 1934, Sinha corrected the measurements of the species *C. magnus* already described by him in 1932. Ogata (1934) from Japan described a new species *C. japonicus* from the intestine of a turtle *L' Amyda japonica*. From Burma Chatterji (1936) described *C. burmanica* from the intestine of *Emyda scutata*. Caballero and Sokoloff (1936) got a new species *C. robustus* from *Rana montezumae* in Mexico. They held *C. europaeus* as a synonym of *C. retusus* (Dujardin, 1845) on page 119 of their paper published in 1936 but later on on page 126 in the same paper they described it as a distinct species. Even in the key to the species given by the joint authors, *C. europaeus* is held valid.

Mehra (1937) from Allahabad (India) gave the description of *C. minutum* from the intestine of *Lissemys punctata punctata*. Lent and Freitas (1940) held the three species, namely, *C. retusus*, *C. americanus* and *C. europaeus* as distinct species. They also pointed out the probable mistake made by Odhner (1910) who had recorded *C. retusus* from *Rana esculenta* whereas Dujardin (1845) had mentioned *R. temporaria* as its host. They further stated that if the views of Odhner (1910) were accepted with regard to the size of eggs and incorrect measurements recorded by Dujardin, then *C. europaeus* should be treated as synonymous with *C. retusus* and *C. americanus* as a distinct species. If Dujardin's measurements were taken as correct, then *C. americanus* should be equal to *C. retusus* as had been shown by Whalton (1938). The joint authors, however, preferred not to accept these views and, therefore, they held the three species valid.

From France, Dollfus (1950) described *C. thomei* from *Petusios nigricans*. Gupta (1951) from Lucknow described *C. heteropneustus* from a fresh-water fish, *Heteropneustes fossilis* (Bloch.).

During the period from 1845–1921, three more species were assigned to this genus, but were removed from it because of their anatomical differences. These are *C. ovatus* (Rudolphi, 1803), *C. pellucidus* (V. Linstow, 1873) and *C. trachysauri* (MacCallum, 1921). The species *C. ovatus* and *C. pellucidus* have now been placed in the genus *Prosthogonimus Luhe*, 1899. Chandler (1923) rightly excluded *C. trachysauri* from the genus *Cephalogonimus*. He was, however, not aware of the work of Dollfus who had already assigned MacCallum's species *C. trachysauri* to the genus *Paradistomum* Kossack (1910). Johnston (1932) proposed that the specific name be replaced by *Paradistomum MacCallumi*.

Synonymity of the Species

Chandler (1923) pointed out that *C. retusus* (Dujardin, 1845) Odhner (1910) and *C. europaeus* Blaizot (1910) were identical and this identity was later confirmed by Stunkard (1924). Travassos (1932) and Bhalerao (1936) also expressed a similar opinion in regard to the synonymy of *C. europaeus* with *C. retusus*. Bhalerao (1936) further pointed out that Pande's (1932) species—*C. gangeticus* and Sinha's (1932) species—*C. magnus* were also identical. They were from the intestine of the same host, i.e., *Trionyx gangeticus*. But according to the law of priority the

specific name *C. magnus* has precedence over the name of *C. gangeticus*. Therefore, *C. gangeticus* should fall into the synonymy of *C. magnus*. While commenting on the characters used in separating the species, Bhalerao pointed out that *C. magnus* should be treated as a synonym of *C. amphiumae* Chandler (1923). Therefore, both the species, i.e., *C. gangeticus* and *C. magnus* be merged into the synonymy of *C. amphiumae*.

Cabellero and Sokoloff (1936) and Lent and Freitas (1940) considered *C. europaeus* as a valid species. Bhalerao (1942) pointed out that *C. mehri* and *C. minutum* were quite distinct species. With regard to the synonymy of *C. magnus* with *C. amphiumae* he revised his earlier opinion and kept the former species as distinct from the latter. He, however, maintained *C. gangeticus* as synonym of *C. magnus*. In *C. amphiumae* the intestinal caeca end very close to the posterior extremity of the body, whereas in *C. magnus* the two caeca terminate midway between the posterior end of the body and the hinder margin of the posterior testis. He further proved that *C. magnus* was merely a large variety of *C. emydalis* Moghe (1930).

Genus **Cephalogonimus** Poirier, 1886

Diagnosis

Cephalogonimidae Nicoll, 1914; Cephalogoniminae Looss, 1899. Body: elongated, elliptical, oval or rounded dorso-ventrally. Cuticle: spinous. Suckers: oral larger or smaller than the ventral sucker or both of the same size. Digestive system: oesophagus, if present, small, or absent; intestinal caeca not extending to the posterior extremity of the body. Reproductive systems: genital opening at the anterior end, median or lateral, to the right or left or slightly behind or dorsal to the oral sucker or at the anterior tip of the body; testes placed obliquely behind each other or in tandem one behind the other; cirrus sac long and curved, with posterior end broad and the anterior tubular; ovary spherical in front of the testes, to the right side or close behind the ventral sucker; receptaculum seminis and Laurer's canal present; uterus coiled particularly in the post-testicular region; vitellaria well developed, on the lateral sides of the body. Excretory system: excretory bladder Y-shaped with lateral branches.

Host ..	..	Fish, Frogs, Snakes and Chelonia.
Locality	..	Egypt, America, France, Congo Belge, Japan, Burma and India.
Type Species	..	*C. Lenoiri* Poirier, 1886.

Cephalogonimus indicus, n.sp. (Fig. 1.)

Host ..	..	*Lissemys punctata punctata.*
Location	..	Small intestine.
Locality	..	Hoshiarpur (India).

A dozen specimens of this parasite were obtained from the small intestine of *Lissemys punctata punctata* examined at Hoshiarpur. The trematode is elliptical in shape with broad anterior and narrow posterior ends. The live worm is yellowish in colour. The cuticle is beset with

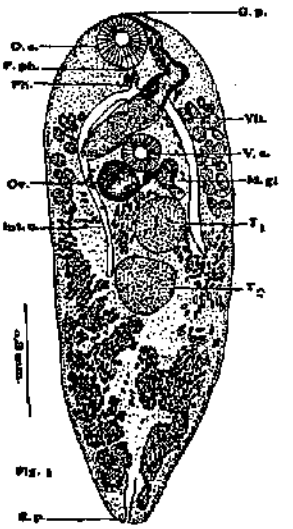

FIG. 1. Ventral view of *Cephalogonimus indicus*, n.sp.

E.P.—excretory pore; *G.p.*—genital pore; *Int.c.*—Intestinal caecum; *O.s.*—oral sucker; *Ov.*—ovary; *M.gl.*—Mehlis' gland complex; *P.ph.*—prepharynx; *Ph.*—pharynx; T_1.—anterior testis; T_2.—Posterior testis; *V.s.*—Ventral sucker; *Vit.*—vitelline glands.

very minute backwardly directed spines. They are more closely arranged in the preovarian region than behind it. In the latter region they gradually decrease in number till they disappear altogether in the posterior region of the body. The oral sucker is spherical or globular, situated subterminally at the anterior extremity with its opening facing ventrally. It measures 0·0952–0·135 × 0·135–0·1425 mm. in size. It is slightly larger than the ventral sucker. The ventral sucker, measuring 0·085–0·135 × 0·09–0·1275 mm. in size, is situated at a distance of 0·240–0·285 mm. from the anterior extremity of the body.

The oral sucker is followed by a small prepharynx which leads into a muscular pharynx, oval in outline and measuring 0·03 × 0·0578 mm. The oesophagus is inconspicuous. In specimens where it is visible, it is never more than 0·008 mm. in length. The intestinal caeca run backward terminating at the level of either the anterior margin or middle of the posterior testis. The intestinal bifurcation lies far in front of the ventral sucker.

The excretory pore is terminal at the posterior extremity of the body. The excretory bladder is Y-shaped, the main stem which is median in position extends up to the posterior border of the posterior testis where it bifurcates into lateral cornua. In some specimens the distal end of the main excretory bladder has been seen forming a small caudal vesicle. The right and left cornua extend up to the level of the base of the ovary.

The two testes may be rounded or oval and are placed somewhat obliquely one behind the other in the middle region of the body partly in between the two intestinal caeca. The anterior testis lies slightly to the left of the median line close to the left intestinal caecum and 0·048–0·06 mm. behind the ventral sucker. The posterior testis is median in position

close to the blind end of the right intestinal caecum. The former testis measures 0·095–0·15 × 0·09–0·165 mm. and the latter measures 0·105–0·150 × 0·135–0·165 mm. in size. From the anterior border of each testis arises the vas efferens, the two vasa efferentia unite near the base of the cirrus sac to form a small inconspicuous vas deferens. The cirrus sac is obliquely placed in the space between the oral sucker and the ovary with its posterior end touching or over-lapping the right intestinal caecum. It is broader posteriorly and towards the anterior end it becomes narrower. The vesicula seminalis lies in the basal part of the cirrus sac and consists of two distinct portions which are demarcated by a constriction. The proximal part is longer than the distal. The pars prostatica and ejaculatory duct are long tubular structures, the first being surrounded by the prostate gland cells. The cirrus is protrusible and without any spine. The genital pore is median in position lying at the anterior end of the body in front of the oral sucker.

The ovary is situated close to the postero-lateral border of the ventral sucker, to the right of the median line. It is almost spherical in appearance measuring 0·075–0·120 × 0·075–0·1275 mm. From its left lateral side, there arises the oviduct which soon dilates to form the ootype surrounded by the Mehlis' glands. The whole complex lies to the left of the median line, close to the postero-lateral side of the ventral sucker and in front of the anterior testis. In some specimens it has been observed that the ovary and Mehlis' gland complex are partly overlapped by the ventral sucker. The receptaculum seminis lies just behind the Mehlis' gland complex. The Laurer's canal is present. The vitelline reservior lies between the ovary and the Mehlis' gland complex. The uterus is much coiled, its descending limb passes through a space between the ovary and the anterior testis and is confined to the right side of the body, while the ascending limb to the left side, proceeds forward along the left intestinal caecum, terminating into the metraterm which opens close to the male genital opening into the genital atrium. In the post-testicular region of some specimens the coils of the uterus are arranged in such a way and are so full of eggs that their descending and ascending limbs cannot be made out.

The vitelline glands are extra-caecal and confined to the lateral sides. They commence midway between the oral and ventral suckers and extend up to the level of the middle of the anterior testis. At some places they may overlap the intestinal caeca. The number of follicles varies from 13–21 on the left and 10–21 on the right sides. The eggs are dark-brown or yellowish in colour, oval in shape, measure 0·0306–0·035 mm. in length and 0·0102–0·0105 mm. in breadth.

Relationships

Sixteen valid species have been described under the genus *Cephalogonimus*. Of these, *C. indicus*, n.sp., resembles *C. retusus* (Dujardin, 1845), *C. americanus* Stafford (1902), *C. europaeus* Blaizot (1910), *C. amphiumae* Chandler (1923), *C. emydalis* Moghe (1930), *C. brevicirrus* Ingles

(1932), *C. mehri* Pande (1932), *C. burmanica* Chatterji (1936), *C. minutum* Mehra (1937), *C. thomsi* Dollfus (1950) and *C. heteropneustus* Gupta (1951) in having the oral sucker larger than the ventral sucker. It further resembles *C. amphiumae*, *C. emydalis*, *C. mehri*, *C. burmanica*, *C. minutum* and *C. thomsi* in the position of the genital pore which is placed in front of the oral sucker, i.e., at the anterior extremity and differs from *C. retusus*, *C. americanus*, *C. europaeus*, *C. brevicirrus* and *C. heteropneutus* in which the genital pore is subterminal and lies on the dorsal side of the oral sucker.

In its oblique position of testes, the new species resembles *C. minutum* and *C. mehri* and differs from the other species in which the genital pore is situated in front of the oral sucker. It stands apart from *C. minutum* in which the body is rounded, the vitellaria are pre-acetabular in position and the intestinal caeca reaching behind the posterior testis and from *C. mehri* in the length of intestinal caeca and distribution of vitellaria. In the remaining species, i.e., *C. lenoiri* Poirier (1886), *C. vesicaudus* Nickerson (1912), *C. compactus* Stunkard (1924), *C. japonicus* Ogata (1934) and *C. robustus* Caballero and Sokoloff (1936) the oral sucker is either smaller than or equal to the ventral sucker.

Cephalogonimus kumarus, n.sp. (Fig. 2.)

Host ..	..	*Lissemys punctata punctata.*
Location	..	Intestine.
Locality	..	Hoshiarpur (India).

Only three mature specimens of *Cephalogonimus kumarus*, n.sp., were found in the intestine of a tortoise dissected in the Zoological Laboratory of the Panjab University College, Hoshiarpur. The live worms were light yellow in colour. The body is elongated, broad anteriorly and narrower posteriorly. The cuticle is spinous. The spines are sparsely distributed and are directed either posteriorly or sidewardly or even anteriorly. A few spines are also seen in the post-testicular region in which region they are mostly absent as in other species.

The worm is 1·305–1·815 mm. in length and 0·420–0·495 mm. in maximum breadth, which is across the level of the cirrus sac. The oral sucker is subterminal, measuring 0·135–0·153 mm. in length and 0·135–0·153 mm. in breadth. It is larger than the ventral sucker which measures 0·1088–0·1156 mm. in length and 0·102–0·1088 mm. in breadth. The ventral sucker is situated at a distance of 0·375–0·540 mm. from the anterior extremity. A small prepharynx is present. The pharynx is a small muscular pear-shaped structure lying behind the prepharynx and measures 0·0374–0·051 mm. in length and 0·0544 mm. in breadth. The oesophagus is an exceedingly small tubular structure arising from the base of the pharynx and soon bifurcates into two intestinal caeca which run posteriorly along the lateral sides of the body terminating at a little distance beyond the posterior testis at a distance of 0·405–0·45 mm. in front of the posterior end of the body.

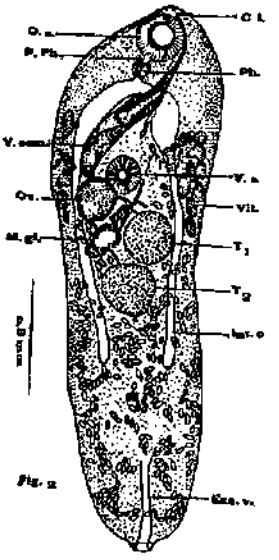

FIG. 2. Ventral view of *Cephalogonimus kumarus*, n.sp.

Ci.—Cirrus; *Exc.v.*—excretory vesicle; *Int.c.*—intestinal caecum; *O.s.*—oral sucker; *Ov.*—ovary; *M.gl.*—Mehlis' gland complex; *P.ph.*—prepharynx; *Ph.*—pharynx; T_1.—anterior testis; T_2.—posterior testis; *V.s.*—ventral sucker; *V.sem.*—vesicula seminalis; *Vit.*—vitelline glands.

The excretory system is Y-shaped, the main bladder bifurcates into two cornua at half-way between the posterior testis and the hinder end of the body. The excretory pore opens to the outside at the posterior end of the body.

The testes are spherical, placed obliquely either in the middle region or slightly anterior to it. The anterior testis is to the left of the median line close to the left intestinal caecum, measuring 0·135–0·165 × 0·135–0·165 mm. The posterior testis is almost in the median line measuring 0·135–0·165 × 0·135–0·150 mm. Both the testes are situated close together. From the anterior aspect of each testis is given off a vas efferens and the two vasa efferentia unite at the base of the cirrus sac to form a short vas deferens. The cirrus sac is a long curved structure lying obliquely in the space between the oral and the ventral suckers. The vesicula seminalis lies at the basal part of the cirrus sac. It shows a constriction at its middle region, thus dividing it into two equal portions, the proximal and the distal. The latter portion is followed by the prostatic duct which is surrounded by the prostate gland cells.

The ovary is situated to the right of the median line in the pre-testicular region, posterior to the ventral sucker. It measures 0·12–0·15 × 0·12 mm. in size. It is slightly overlapped by the ventral sucker. The oviduct arises from the left margin of the ovary and proceeds backwards and soon dilates to form the ootype which is surrounded by the Mehlis' glands. The Mehlis' gland complex lies behind the ovary and to the right of the anterior testis. The receptaculum seminis lies partly dorsal to the Mehlis' gland complex. In some specimens Mehlis' gland

complex lies opposite to the ovary and is just behind the ventral sucker. The receptaculum seminis, however, remains to the right of the median line behind the ovary. The vitelline glands are mostly extra-caecal and extend from the level of the middle of the cirrus sac to the anterior 1/4th of the anterior testis. The uterus is densely coiled in the post-testicular region. The genital pore lies subterminally a little behind the anterior extremity just in front of the oral sucker. The eggs measure 0·0216–0·034×0·012–0·0136 mm. in size. They are dark-brown or yellow in colour.

Relationships

In possessing oral sucker larger in size than the ventral sucker *Cephalogonimus kumarus*, n.sp., differs from *C. lenoiri* and *C. vesicaudus* in which oral sucker is smaller than the ventral sucker and from *C. compactus*, *C. japonicus* and *C. robustus* in which both the oral and ventral suckers are of the same size. In having the oral sucker larger than the ventral sucker the new species resembles the remaining species, viz., *C. retusus*, *C. americanus*, *C. europaeus*, *C. amphiumae*, *C. emydalis*, *C. brevicirrus*, *C. mehri*, *C. burmanica*, *C. minutum*, *C. thomsi*, *C. heteropneustus* and *C. indicus* n.sp. It, however, differs from *C. retusus*, *C. americanus*, *C. europaeus*, *C. brevicirrus* and *C. heteropneustus* in the position of the genital pore which in the latter species is placed subterminally dorsal to the oral sucker and from *C. amphiumae*, *C. emydalis* and *C. thomsi* in which the testes are placed in tandem. In the oblique position of testes *C. kumarus* resembles *C. mehri*, *C. burmanica*, *C. minutum* and *C. indicus*. But it stands apart from *C. indicus* in which the intestinal caeca terminate in the testicular region and from *C. minutum* in the shape of the body and distribution of the vitellaria. The new species *C. kumarus* differs from *C. burmanica* in the presence of oesophagus and the extent of vitelline glands. It closely resembles *C. mehri*, but it differs from it in the absence in latter of the prepharynx and oesophagus and also in the extension of vitellaria. Therefore, the new species is distinct from all the existing species. This new species has been named after my old teacher Prof. Dewan Anand Kumar at present Vice-Chancellor of the Panjab (India) University.

Cephalogonimus asiaticus, n.sp. (Fig. 3.)

Host	..	*Lissemys punctata punctata.*
Location	..	Intestine.
Locality	..	Hoshiarpur (India).

Only one specimen of *Cephalogonimus asiaticus*, n.sp., was found in the small intestine of a tortoise collected from a local pond. The worm is elliptical in shape. It measures 0·762 mm. in length and 0·315 mm. in breadth across the testicular region. It is rounded at the anterior end, while the posterior end is slightly attenuated. The body is beset with backwardly directed spines, more densely in the first half of the body as compared to the second half where they are scanty.

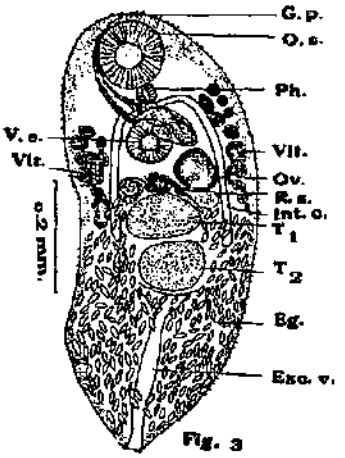

FIG. 3. Dorsal view of *Cephalogonimus asiaticus*, n.sp.

Exc.v.—excretory vesicle; *Eg.*—eggs; *G.p.*—genital pore; *Int.c.*—intestinal caecum; *O.s.*—oral sucker; *Ov.*—ovary; *Ph.*—pharynx; *R.s.*—receptaculum seminis; T_1.—anterior testis; T_2.—posterior testis; *Vit.*—vitellaria; *V.s.*—ventral sucker.

There is a large oral sucker placed subterminally at the anterior end of the body and measuring 0·1156 mm. in length and 0·119 mm. in breadth. The ventral sucker measuring 0·085 × 0·085 mm. is situated at a distance of 0·195 mm. from the anterior end of the body. The pharynx is very small and is placed behind the oral sucker. The prepharynx and the oesophagus seem to be absent. The two caeca are almost straight running posteriorly along the lateral sides of the body and the inner side of the vitellaria. They terminate at the level of the anterior border of first 1/4th of the posterior testis.

The excretory pore is terminal, placed at the posterior end of the body. It opens into the main bladder of the Y-shaped excretory system. At the base of the posterior testis, the main excretory bladder forks into two lateral vessels—the cornua which run along the lateral sides of the testes.

The two testes are transversely elongated, placed almost one behind the other in the inter-caecal region. The anterior testis measures 0·075 mm. in length and 0·12 mm. in breadth, while the posterior testis 0·09 mm. in length and 0·12 mm. in breadth. The cirrus sac is an elongated structure measuring 0·240 mm. in length and 0·06 mm. in breadth at its basal portion. It is placed obliquely in between the ovary and the oral sucker. It encloses the vesicula seminalis which is bilobed and full of sperms. The pars prostatica and the ejaculatory duct are present. The former is surrounded by the prostate gland cells. The cirrus is protrusible and without any spine. The genital pore is situated in the space between the anterior margin of the body and the oral sucker.

The ovary is almost spherical 0·075 × 0·075 in dimensions and is placed to the right of the median line close to the right intestinal caecum and the postero-lateral side of the ventral sucker in front of the anterior testis. From the postero-lateral border arises the oviduct. The Mehlis' gland

complex is situated just in front of the anterior testis, while the receptaculum seminis is placed behind the ovary. The uterus is full of eggs and is densely coiled in the post-testicular region. The coils are confined to the space between the body-wall and Y-shaped excretory system.

The vitellaria are compactly arranged along the lateral sides of the body. The vitelline glands on the right side extend for about 0·195 mm. while those of the left side for about 0·210 mm. They are at the level of forked intestinal caeca in front and at the level of the middle of the anterior testis behind. The eggs measure 0·032–0·036 × 0·012 mm.

Relationships

In having the oral sucker larger than the ventral sucker *C. asiaticus* n.sp., differs from *C. lenoiri*, *C. vesicaudus*, *C. compactus*, *C. japonicus* and *C. robustus* in which oral sucker is either smaller than or equal to the ventral sucker. In having the oral sucker larger than the ventral sucker, the new species resembles the remaining species, viz., *C. retusus*, *C. americanus*, *C. europaeus*, *C. amphiume*, *C. emydalis*, *C. brevicirrus*, *C. mehri*, *C. burmanica* *C. minutum*, *C. thomsi*, *C. heteropneustus*, *C. indicus* n.sp. and *C. kunarus* n.sp. In the extent of the intestinal caeca it differs from all the species of the group in which oral sucker is larger than the ventral sucker except *C. heteropneustus* and *C. indicus* with which it resembles closely. But the new species stands apart from *C. heteropneustus* in the position of the genital pore and the extent of vitellaria and from *C. indicus* in the absence of the oesophagus, the extent of vitellaria, tandem and transversely elongated position of the testes.

A key to the identification of all the valid species of the genus *Cephalogonimus* Poirier (1886) based on the undermentioned characters has been prepared.

(i) Size of suckers.
(ii) Length of intestinal caeca.
(iii) Position of genital pore.
(iv) Extent of vitellaria.
(v) Shape and arrangement of testes.
(vi) Cirrus sac.
(vii) Oesophagus.
(viii) Caudal vesicle.
(ix) Vesicle in vas efferens.

Key to the species of the genus *Cephalogonimus* Poirier (1886)

Oral sucker larger than the ventral sucker	A.
Oral sucker smaller than the ventral sucker	B.
Oral sucker equal to the ventral sucker	C.

A.	Intestinal caeca terminate in the post-testicular field	1.
	Intestinal caeca not terminating in the post-testicular field but in the testicular field	2.
1.	Genital pore subterminal on the dorsal side of the oral sucker ..	a.
	Genital pore not on the dorsal side of the oral sucker but in front of it	b.
a.	Genital pore median	ai.
	Genital pore not median, i.e., to the right side of median line ..	aii.
ai.	Caudal vesicle and a small vesicle in each vas efferens present ..	*C. brevicirrus* Ingles, 1932.
	Caudal vesicle and the vesicle in the vas efferens absent	*C. americanus* Stafford, 1902.
aii.	Testes irregular, tandem in position..	*C. retusus* Dujardin, 1845.
	Testes entire, placed obliquely ..	*C. europaeus* Blaizot, 1910.
b.	Testes tandem in position ..	b i.
	Testes obliquely placed ..	b ii.
bi.	Oesophagus present	b iii.
	Oesophagus absent	b iv.
biii.	Vitellaria extend from just behind the anterior end of the ventral sucker to the level of the posterior testis, on the left side, the follicles are more extensive; intestinal caeca extend nearly to the posterior extremity of the body	*C. amphiumae* Chandler, 1923.
	Vitellaria extend from the intestinal bifurcation to the level of the ends of the caeca; intestinal caeca terminating in the anterior portion of the post-testicular space ..	*C. thomsi* Dollfus, 1950.
biv.	Testes transversely elongated; vitelline follicles extend from the level of ventral sucker to the mid-way between testes and the ends of the intestinal caeca	*C. emydalis* Moghe, 1930.
	Testes rounded; vitelline follicles extend from immediate anterior of	

ventral sucker to a little behind the anterior margin of posterior testis .. .. .. *C. burmanica* Chatterji, 1936.

bii. Body rounded; vitellaria confined to the pre-acetabular region .. *C. minutum* Mehra, 1937.

Body not rounded but elongated; vitellaria both pre- and post-acetabular .. .. b ii a.

biia. Oesophagus absent; vitellaria extend from near the intestinal bifurcation to the hinder margin of the ovary.. .. .. *C. mehri* Pande, 1932.

Oesophagus present; vitellaria from half-way between the oral sucker and ventral sucker to about the middle of anterior testis .. *C. kumarus*, n.sp.

2. Testes obliquely placed; oesophagus present .. .. .. 2a.

Testes tandem in position, transversely elongated; oesphagus absent; vitellaria from the intestinal bifurcation to the middle of anterior testis .. .. *C. asiaticus*, n.sp.

2a. Genital pore in front of the oral sucker; vitellaria extend from half-way between the oral and the ventral suckers up to the middle of anterior testis .. .. *C. indicus*, n.sp.

Genital pore dorsal to the oral sucker; vitellaria few, from the middle of the ventral sucker to the hind region of the posterior testis .. *C. heteropneustus* Gupta, 1951.

B. Oesophagus present; testes ovoid, placed one behind the other; vitellaria in the anterior half of the body, posterior to the ventral sucker .. .. .. *C. lenoiri* Poirier, 1886.

Oesophagus absent; testes transversely elongated, obliquely placed; vitellaria from half-way between the oral sucker and ventral sucker to a little behind the

	posterior border of the posterior testis	*C. vesicaudus* Nickerson, 1912.
C.	Testes tandem in position ..	C_1.
	Testes obliquely placed, rounded; vitellaria from a little in front of ventral sucker to the ends of the intestinal caeca	*C. compactus* Stunkard, 1924.
C_1.	Anterior testis elliptical transversely or reniform, posterior testis ovoid laterally; cirrus sac extends a little behind the ventral sucker; vitellaria on both sides in the middle region	*C. japonicus* Ogata, 1934.
	Testes transversely elongated. Cirrus sac does not extend behind the ventral sucker; vitellaria extend from the level of the ventral sucker to the posterior edge of the posterior testis	*C. robustus* Caballero and Sokoloff, 1936.

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ON FIVE NEW TREMATODES OF THE GENUS *ASTIOTREMA* LOOSS, 1900, FROM THE INTESTINE OF *LISSEMYS PUNCTATA PUNCTATA* AND DISCUSSION ON THE SYNONYMITY OF TWO ALREADY KNOWN FORMS

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Introduction

In this paper, five new trematodes, viz., *Astiotrema nathi*, *A. hoshiarpurium*, *A. srivastavai*, *A. thapari* and *A. matthaii* have been described and their validity as new species to the science has been discussed. Two already known forms, namely, *A. amydae* Ogata (1938) and *A. foochowensis* Tang (1941) have been considered synonymous with *A. orientale* Yamaguti (1937).

The work was carried out under the supervision of Dr. Vishwa Nath, in the department of Zoology, Panjab University College, Hoshiarpur. I am much indebted to Dr. H. D. Srivastava for his criticism and to Dewan Anand Kumar for his kind encouragement in this work.

Genus **Astiotrema** Looss, 1900

Looss in 1899 created the genus *Astia* for the species *A. reniferum* (*Distomum reniferum* Looss, 1898) and *A. impletum* Looss, 1899. The former species was obtained by him in 1898 from the intestine of a turtle—*Trionyx nilotica*—and the latter in 1899 from the intestine of *Tetredon fahaka*. As the name *Astia* was found to be preoccupied, the same author, a year later, changed the name of the genus to *Astiotrema*. Stossich in 1904 added another species to the genus—*A. monticellii*—from the intestine of *Tropidonotus viperinus*. The specimens on which the species was based by Stossich had actually been collected by Monticelli (1891) who had wrongly referred them to *Distomum signatum* Dujardin (1845). Stossich (1904) also placed the parasite *Distomum crinaceum* Poirier (1886) under the genus *Astiotrema*. Odhner (1911) elaborated the description of the two species, namely, *A. reniferum* and *A. impletum* and removed *Distomum crinaceum* from the genus *Astiotrema*.

Ejsmont (1930) from Poland described *A. emydis* from the stomach and duodenum of *Emys orbicularis*. In 1931, Mehra gave the description of two species, viz., *A. elongatum* from the intestine of *Trionyx gangeticus* and *A. loossii* from the intestine of *Kachuga dhongoka*. A year later, Harshey described *A. gangeticus* from the intestine of *Lissemys punctata punctata* (Syn. *Emyda granosa*) at Allahabad. Thapar in 1933 gave the account of a new species *A. indica* from a tortoise *Chitra indica*, and

Chatterji in the same year described *A. spinosa* from *Clarias batrachus* (Linnaeus, 1785). Bhalerao in 1936 found another new species to which he gave the name *A. rami*. He also pointed out that the specimens which Odhner (1911) dealt with as *A. reniferum* were actually not of *A. reniferum*, but represented a new species to which he gave the name *A. odhneri*.

Yamaguti in 1937 from Japan obtained *A. orientale* from the intestine of *Amyda japonica*. From India again, Dayal in 1938 described *A. dassia* from the intestine of *Clarias batrachus*. In the same year from Poland, Modrzejewska redescribed *A. emydis* from *Emys orbicularis*. Ogata in 1938 from Japan gave the preliminary description of *A. amydae* and *A. fukuii* from the intestine of *Amyda maackii*. His description was without any illustration. Tang in 1941 from China added *A. foochowensis* from the intestine of a turtle *Amyda tuberculata* to the list of species of the genus.

I have added five new species, namely, *A. nathi*, *A. hoshiarpurium*, *A. srivastavai*, *A. thapari* and *A. matthaii*.

Synonymity of species

The species *Astiotrema gangeticus* Harshey (1932) has already been synonymized with *A. loossii* Mehra (1931) by Bhalerao (1936). The differences on the basis of which Harshey distinguishes *A. gangeticus* from *A. loossii* are the presence of spines on the ventral surface, continuous vitelline follicles, rounded ovary and larger size of receptaculum seminis. Bhalerao (1936) points out that in his specimens of *A. loossii* the ovary and the receptaculum seminis are similar to that of *A. gangeticus* but the vitellaria are arranged in groups on one side, while on the other side they are contiguous except for a short distance towards the anterior side. He further points out that the presence of vitelline follicles in groups or otherwise is not a point of specific importance and the presence of spines on the ventral surface should not be taken as a character for separating the two species. Therefore, *A. gangeticus* is regarded as a synonym of *A. loossii*.

In discussing the validity of his new species *A. amydae*, Ogata (1938) points out that it resembles closely *A. orientale* but differs from it in the size of its eggs. I have compared both the species very carefully and I find that *A. amydae* is only a synonym of *A. orientale*. It resembles in the smaller size of the oral sucker as compared to the ventral sucker, in the shape of testes, the extension of intestinal caeca and the vitellaria. The minor difference in size of the eggs of the species should not be taken as of sufficient importance to warrant the separation of the two species. I, therefore, regard *A. amydae* as a synonym of *A. orientale*.

Tang in 1941 describes a new species *A. foochowensis* from the intestine of *Amyda tuberculata* in Foochow. From his discussion on the validity of *A. foochowensis*, Tang does not seem to be aware of *A. orientale* Yamaguti, 1937, with which the former species resembles closely. *A. foochowensis* resembles *A. orientale* in the extent of intestinal caeca, the vitellaria, in having a smaller oral sucker and in the shape of the testes. Thus, in all essential features *A. foochowensis* and *A. orientale* are alike and should be regarded as synonymous.

Genus **Astiotrema** Looss, 1900

Diagnosis

Plagiorchiidae Luhe, 1901, emend. Ward, 1917; Plagiorchiinae Luhe, 1901 emend. Pratt, 1902. Shape and size: elliptical or elongated, flattened dorso-ventrally with somewhat rounded anterior and posterior ends, size variable. Bodywall: spinous, spines more closely arranged in the anterior region. Suckers: oral sucker may be larger or smaller than, or equal to ventral sucker; ventral sucker in the first half of the body. Digestive system: prepharynx present or absent; pharynx always present; intestinal caeca terminate either at level with posterior testis or behind it or just in front of the posterior end of the body. Reproductive system: testes rounded, ovoid, entire, notched anteriorly or lobed, placed obliquely or tandem in position; cirrus sac conical, elongated with broad basal part extending up to the front or middle or posterior margin of ventral sucker or far behind it, anterior part is tubular curving round or overlapping partly the ventral sucker; vesicula seminalis straight, thin-walled, confined basally to cirrus sac; pars prostatica short; cirrus protrusible; ovary spherical or subspherical or kidney-shaped, or lobed or entire, pre-testicular, posterior to the ventral sucker, to the right if examined ventrally, left, if seen from dorsal side; receptaculum seminis always present; uterine coils form both descending and ascending limbs passing between the testes; vitellaria arranged in groups or scattered along the lateral sides, the extent variable; genital pore median or slightly lateral to median line. Excretory system: Y-shaped.

Host and Location .. Stomach and intestine of tortoises, snakes and fishes.
Locality .. India, Egypt, Italy, Poland, Korea and Japan.
Type Species .. *Astiotrema reniferum* (Looss, 1898) Looss, 1900.

Astiotrema nathi, n.sp. (Fig. 1.)

Host .. .. *Lissemys punctata punctata*.
Location .. Intestine.
Locality .. Hoshiarpur (India).

Only two specimens of this species were obtained from the intestine of a tortoise, *Lissemys punctata punctata*, collected from one of the local ponds.

The live worm is somewhat yellowish in appearance. The contracted worm is dorso-ventrally flexed. It is elliptical in shape with rounded anterior and posterior ends, the latter is broader than the former. The worm measures 4·08–5·304 mm. in length and 0·85 mm. in maximum breadth across the middle region of the anterior testis. The body from the level of the oral sucker up to the anterior testis is studded with small spines. Some of these spines are directed sidewardly and others backwardly. In the whole mounts these can be seen prominently on the lateral

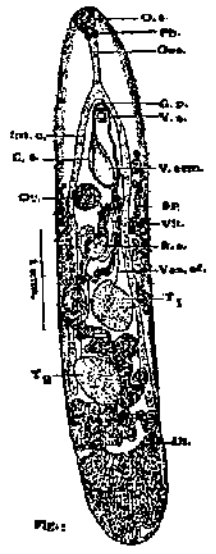

FIG. 1. Ventral view of *Astiotrema nathi*, n.sp.

O.s.—cirrus sac; *G.p.*—genital pore; *O.s.*—oral sucker; *Oes.*—oesophagus; *Ov.*—ovary; *Ph.*—pharynx; *R.s.*—receptaculum seminis; *Sp.*—spine; T_1.—anterior testis; T_2.—posterior testis; *V.s.*—ventral sucker; *V.sem.*—vesicula seminis; *Vas.eff.*—vas efferens; *Vit.*—vitellaria.

sides. They are more closely arranged in the region of the oral sucker, but they become gradually sparse posteriorly.

The oral sucker is at the anterior end and measures 0·225 mm. in breadth and 0·210 mm. in length. It is larger than the ventral sucker which measures 0·18 × 0·18 mm. The ventral sucker is situated at a distance of 0·65 mm. from the anterior end of the body. Just behind the oral sucker there is a short globular pharynx measuring 0·12 × 0·15 mm. There is no prepharynx. The oesophagus measuring 0·51–0·525 mm. in length and 0·045 mm. in width, is a long tubular structure arising from the base of the pharynx. At a distance of 0·195 mm. in front of the ventral sucker it forks into two intestinal caeca which run along the lateral sides of the body and terminate blindly in level with the hinder margin of the posterior testis. The right intestinal caecum is slightly shorter than the left.

The male genital organs consist of two testes with irregular outlines and are placed obliquely in between the two intestinal caeca. The anterior testis measures 0·465–0·495 mm. in length and 0·345–0·375 mm. in maximum breadth. It is slightly notched on its anterior side. The posterior testis measures 0·51–0·525 mm. in length and 0·39–0·465 mm. in breadth and is placed at a distance of 0·045–0·345 mm. from the anterior testis and 0·969–1·394 mm. from the posterior end of the body. The two vasa efferentia, one from the notch of the anterior testis, the other from the anterior aspect of the posterior testis, arise and unite to form a small vas deferens at the base of the cirrus sac. The cirrus sac is an elongated structure measuring 0·51–0·54 mm. in length and 0·225 mm. in maximum

breadth. It is median in position and extends posteriorly up to the level of the middle of the ovary. It encloses the vesicula seminalis, pars prostatica, ejaculatory duct and the cirrus. The vesicula seminalis is large and fills the major portion of the cirrus sac. The pars prostatica is also an elongated structure surrounded by the prostate gland cells. It occupies the tubular region of the cirrus sac. The ejaculatory duct continues into the small cirrus. The male opening lies close to the female opening in the genital atrium in the space between the ventral sucker and the intestinal fork.

The female genital organs consist of an ovary situated on the right side close to the right intestinal caecum. It is at a distance of 0·405–0·6 mm. from the ventral sucker and measures 0·25–0·3 mm. in length and 0·21–0·255 mm. in breadth. From its postero-lateral aspect arises the oviduct which, after a short course, receives the duct of the receptaculum seminis. The receptaculum seminis itself is like a thick U-shaped sac and is situated between the ovary and the anterior testis. The vitellaria are present in follicles along the lateral sides of the body. They are mostly extra-caecal in position. The right vitellaria extends from the level of the anterior margin of the ovary or base of the cirrus sac to the posterior margin of the anterior testis, while the left vitellaria extends from the level of the middle of the cirrus sac to about the middle or posterior margin of the anterior testis. The vitelline follicles on both sides overlap one another and also cover a portion of the intestinal caeca. The two vitelline ducts unite mesially in front of the receptaculum seminis and form a yolk reservoir, the duct from which opens into the ootype, which is a dilated part of the oviduct and is surrounded by the Mehlis' gland cells. The Mehlis' gland complex is median in position. The uterus is full of eggs. It first proceeds towards the posterior side of the body, where it is so much coiled that it becomes difficult to make out the descending and ascending limbs. The metraterm runs along the male genital duct and opens into a shallow atrium situated in between the intestinal fork and the ventral sucker. The eggs measure 0·0238–0·034 × 0·0102–0·0136 mm.

Relationships

In *Astiotrema nathi*, n.sp., the oral sucker is larger than the ventral sucker and in this respect it differs from all those species possessing larger ventral sucker, viz., *A. loossii* Mehra (1931), *A. spinosa* Chatterji (1933) and *A. orientale* Yamaguti (1937) and also from species with suckers of the same size, i.e., *A. indica* Thapar (1933) and *A. dassia* Dayal (1938). The new species can be distinguished from the undermentioned species in which the oral sucker is larger than the ventral sucker by the position of the latter in *A. monticellii* Stossich (1904), in equal sized testes and ovary in *A. emydis* Ejsmont (1930), in different extent of intestinal caeca and vitellaria in *A. elongatum* Mehra (1931) and *A. rami* Bhalerao (1936).

A. nathi can be separated from *A. reniferum* (Looss, 1899) by the shape of testes and extension of vitellaria in the latter. In the position of genital pore which is in front of intestinal fork and in the extension of vitellaria from the middle of oesophagus to the middle of anterior testis,

A. impletum (Looss, 1899) differs from the new species. *A. nathi* differs from *A. odhneri* (Odhner, 1911) Bhalerao (1936) in which the testes are lobed and from *A. fukuii* Ogata (1938) in the position of the testes.

Astiotrema hoshiarpurium, n.sp. (Fig. 2.)

Host	..	*Lissemys punctata punctata.*
Location	..	Intestine.
Locality	..	Hoshiarpur (India).

Only two specimens of *Astiotrema hoshiarpurium*, n.sp. were got from the intestine of a tortoise caught from one of the streams at Hoshiarpur. The parasite is elongated in shape with the anterior end more broadly rounded than the posterior end. In one of the specimens there is a slight protuberance at the anterior extremity. The body is 3·24–4·88 mm. in

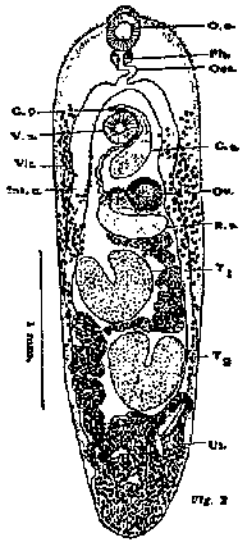

FIG. 2. Dorsal view of *Astiotrema hoshiarpurium*, n. sp.

G.p.—genital pore; *Int.c.*—intestinal caecum; *O.s.*—oral sucker; *Oes.*—oesophagus; *Ov.*—ovary; *Ph.*—pharynx; *R.s.*—receptaculum seminis; T_1.—anterior testis; T_2.—posterior testis; *Ut.*—uterus; *V.s.*—ventral sucker; *Vit.*—vitellaria.

length and 1·32–1·40 mm. in its maximum breadth round the region just in front of the anterior testis. The cuticle is covered with small spines arranged in transverse rows. The rows of spines are more closely set towards the anterior end as compared to the posterior end. They can be best seen in the living specimens.

The subterminal oral sucker measures 0·238–0·255 mm. in length and 0·204–0·289 mm. in breadth and is larger than the ventral sucker which measures 0·204–0·238 mm. long and 0·187–0·255 mm. broad. The ventral sucker is situated at a distance of 0·119–0·321 mm. from the intestinal fork. The prepharynx is absent. The globular pharynx measures 0·068–0·085×0·136–0·170 mm. The small oesophagus forks at a distance of 0·119–0·321 mm. from the ventral sucker into two intestinal caeca which terminate a little behind the posterior testis.

The two testes may be slightly or deeply notched on their anterior margins, and are placed obliquely in the inter-caecal region at 0·051–0·238 mm. wide apart. The anterior testis measures 0·374–0·527 × 0·493–0·748 mm. and the posterior testis 0·408–0·595 × 0·476–0·965 mm. The cirrus sac is large and more or less semilunar, curving round the ventral sucker. It measures 0·561–0·935 mm. in length and 0·153 mm. in its maximum breadth. It is situated to the right of the ventral sucker. The genital pore is just in front of the ventral sucker slightly to the left of the median line.

The ovary is almost spherical measuring 0·204–0·289 mm. long and 0·238–0·323 mm. broad, placed in front of the testes to the right of the median line. The oviduct arises from its left lateral aspect. The Mehlis' gland complex is situated to the left of the ovary. The uterine coils pass between the two testes filling the post-testicular region. The metraterm overlaps the ventral sucker and opens into the genital atrium. The uterine coils are filled with ova which are 0·030–0·032 mm. in length and 0·008–0·012 mm. in maximum breadth.

The vitelline glands are confined to the lateral sides of the body commencing either at the level of the intestinal fork or half-way between it and the ventral sucker to the middle or posterior margin of the posterior testis overlapping the intestinal caeca at some places.

Relationships

In having the oral sucker larger than the ventral sucker, *Astiotrema hoshiarpurium*, n.sp., resembles *A. impletum*, *A. reniferum*, *A. monticelli*, *A. emydis*, *A. elongatum*, *A. odhneri*, *A. fukuii* and *A. nathi*, and differs from *A. loossii*, *A. spinosa* and *A. orientale* in which oral sucker is smaller than ventral sucker and from *A. indica* and *A. dassia* in which both the suckers are of the same size.

The new species differs from *A. impletum* in the position of the genital pore which lies in front of intestinal bifurcation in the latter and from *A. monticellii* in which the ventral sucker is cephalad to the intestinal bifurcation. *A. hoshiarpurium* can be separated from *A. emydis* in which the testes and ovary are of equal size and the vitelline follicles confluent medially anterior to the ventral sucker and from *A. odhneri*, *A. fukuii* and *A. nathi* in the extent of intestinal caeca and of the vitellaria and in the shape of its testes. In the extent of intestinal caeca, the new species resembles *A. reniferum*, *A. elongatum* and *A. rami*, but it stands apart from them in the shape of testes and the extent of vitellaria.

Astiotrema srivastavai, n.sp. (Fig. 3.)

Host	..	*Lissemys punctata punctata*.
Location	..	Intestine.
Locality	..	Hoshiarpur (India).

A single slightly mature specimen of *Astiotrema srivastavai* was obtained from the intestine of a tortoise examined at Hoshiarpur. It is a

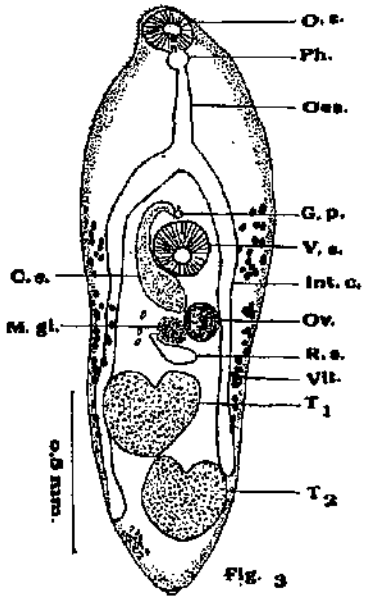

FIG. 3. Dorsal view of *Astiotrema srivastavai*, n.sp.

C.s.—cirrus sac; *G.p.*—genital pore; *Int.c.*—intestinal caecum; *M.gl.*—Mehlis' gland complex; *O.s.*—oral sucker; *Oes.*—oesophagus; *Ov.*—ovary; *Ph.*—pharynx; *R.s.*—receptaculum seminis; T_1.—anterior testis; T_2.—posterior testis; *V.s.*—ventral sucker; *Vit.*—Vitellaria.

small elliptical worm measuring 1·64 mm. in length and 0·64 mm. in maximum breadth across the region of intestinal fork. The cuticle is covered with small backwardly directed spines. The subterminal oral sucker measuring 0·102 mm. in length and 0·153 mm. in breadth is smaller than the ventral sucker which measures 0·17×0·17 mm. and is situated at a distance of 0·153 mm. from the intestinal fork. The prepharynx is absent. The pharynx is globular, 0·06×0·112 mm. in dimensions. The oesophagus is 0·221 mm. long, bifurcating 0·153 mm. in front of the ventral sucker. The right intestinal caecum terminates in the first half and the left in the the second half of the posterior testis but not extending beyond it.

The two testes are present in the second half of the body. Their anterior margins are notched. The anterior testis is 0·272 mm. long, 0·357 mm. broad, while the posterior testis measures 0·255 mm. in length and 0·272 mm. in breadth. The cirrus sac is large, curved and extends along the left margin of the ventral sucker. Posteriorly it extends up to the antero-lateral margin of the ovary. The genital pore is median just in front of the ventral sucker.

The spherical ovary measuring 0·119×0·119 mm. is situated close to the right intestinal caecum in the anterior region of the second half of the body. The Mehlis' gland complex is placed to the left of the ovary. The receptaculum seminis is small situated transversely behind the Mehlis' gland complex and inner half of the ovary.

The vitelline glands consist of small follicles confined to the lateral sides, extending from the genital pore to the posterior margin of the anterior testis. Eggs are few and measure 0·024×0·008 mm.

Relationships

The specimen described above differs from all the species of the genus *Astiotrema* in which the oral sucker is either equal to or larger than the ventral sucker. Therefore, in respect of the size of the oral sucker as compared with that of the ventral sucker, it resembles *A. loossii*, *A. spinosa* and *A. orientale*.

The extent of intestinal caeca, lobed condition of the testes, kidney-shaped ovary, the extent of vitellaria, different size of its eggs separate *A. loossii* from the new species—*A. srivastavai*. In *A. spinosa* the intestinal caeca extend behind the posterior testis, the slightly lobed testes are tandem in position, the vitellaria extend from the posterior margin of the ventral sucker to the anterior or hinder margin of the posterior testis and the size of the eggs, which distinguish it from the new species. The extent of vitellaria, the shape of testes and ovary in *A. orientale* separate it from the new species.

Pending a study of fully mature forms, this parasite is assigned tentatively to a new species on account of the length of its intestinal caeca, the equatorial position of the ovary and the location of the testes in the posterior half of the body.

Astiotrema thapari, n.sp. (Fig. 4.)

Host	..	*Lissemys punctata punctata*.
Location	..	Intestine.
Locality	..	Hoshiarpur (India).

Only one specimen of this parasite was found in the intestine of a tortoise caught in a stream in Hoshiarpur. It is an elongated flat worm, with rounded anterior and posterior ends. It is 9 mm. long and 2·28 mm. broad just in front of the anterior testis. The cuticle is spinous, the spines being small. The mouth is subterminal or terminal and lies in the centre of the oral sucker, which measures 0·408 mm. in length and 0·425 mm. in breadth. Prepharynx is absent. The globular pharynx 0·238×0·289 mm. in size, is next to the oral sucker. The oesophagus is small, bent in the whole mount probably due to the contraction. It is 0·473 mm. in length. It forks at a distance of 0·765 mm. in front of the ventral sucker into two intestinal caeca which terminate behind the posterior testis. The ventral sucker, measuring 0·408×0·425 mm., lies at a distance of 0·765 mm. from the intestinal fork, and is equal to the oral sucker.

The two testes are irregular in outline and are placed obliquely in the inter-caecal zone. The anterior testis is 0·952 mm. long and 1·037 mm. broad, lying close to the left intestinal caecum. The posterior testis is slightly larger than the anterior testis and is separated by a distance of 1·037 mm. from the latter. It is 1·071 mm. long and 1·105 mm. broad

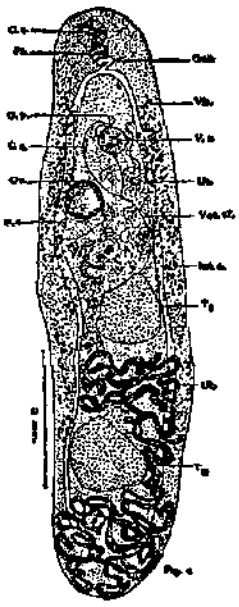

FIG. 4. Ventral view of *Astiotrema thapari*, n.sp.

G.p.—genital pore; *Int.c.*—intestinal caecum; *O.s.*—oral sucker; *Oes.*—oesophagus; *Ov.*—ovary; *Ph.*—pharynx; *R.s.*—receptaculum seminis; T_1.—anterior testis; T_2.—posterior testis; *Ut.*—uterus; *V.s.*—ventral sucker; *Vas.ef.*—vas efferens; *Vit.*—vitellaria.

and lies near the right intestinal caecum. The vas efferens arises from the anterior pointed aspect of each testis. The cirrus sac is about 1·28 mm. long and 0·40 mm. in its maximum breadth. It is placed obliquely in between the ovary and the ventral sucker, extending from the level of the middle of the ovary up to slightly in front of the anterior margin of the ventral sucker. During its course it partly overlaps the ventral sucker. The genital pore lies just in front of the ventral sucker.

The ovary is spherical measuring 0·544×0·595 mm. It is placed to the right of the median line close to the right intestinal caecum. The Mehlis' gland complex is to the left of the ovary. The receptaculum seminis, 0·765×0·357 mm. in size, lies behind the ovary. The vitellaria consist of small follicles and are confined to the lateral sides extending from the level of half-way between the intestinal fork and ventral sucker to the middle of the posterior testis. Eggs measure 0·028–0·032×0·008–0·012 mm.

RELATIONSHIPS

In *Astiotrema thapari*, n.sp., the oral sucker is equal to the ventral sucker. Therefore, it stands apart from all the species in which oral sucker is either larger or smaller than the ventral sucker. In the relation of oral sucker with the ventral sucker, the new species *A. thapari* resembles *A. indica* and *A. dassia*, but it differs from them in the shape of testes, the ovary and in the extent of the vitellaria. In the new species the cirrus sac and the ovary are on the same side.

Astiotrema matthaii n.sp. (Fig. 5.)

Host . .	. .	*Lissemys punctata punctata.*
Location	. .	Intestine.
Locality	. .	Hoshiarpur (India).

The worm is elliptical with broader anterior end and narrower posterior end. It measures 1·64 mm. in length and 0·84 mm. in maximum breadth, which is across the intestinal fork. The body is covered with minute backwardly directed spines. In the anterior region of the body these spines are prominent and are arranged closely, whereas in the posterior region they are absent.

The mouth is subterminal in position, at the anterior end of the body. It is surrounded by the oral sucker which measures 0·172 mm. in length

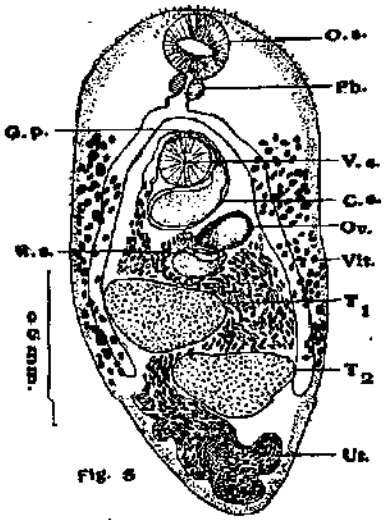

FIG. 5. Dorsal view of *Astiotrema matthaii*, n.sp.

O.s.—cirrus sac; *G.p.*—genital pore; *O.s.*—oral sucker; *Ov.*—ovary; *Ph.*—pharynx; *R.s.*—receptaculum seminis; T_1.—anterior testis; T_2.—posterior testis; *V.s.*—ventral sucker; *Vit.*—Vitellaria.

and 0·228 mm. in breadth. The mouth opens into the pharynx which is globular and measures 0·052×0·108 mm., there being no prepharynx. The pharynx is followed by a small oesophagus which is 0·034 mm. long. The intestinal bifurcation lies in front of the ventral sucker. The two intestinal caeca run along the lateral sides of the body and terminate at the level of the posterior testis. The right intestinal caecum ends in level with the second half and the left in the first half of the posterior testis.

The male genitalia consist of two testes, which are entire in their outlines and are placed obliquely in the inter-caecal region. Each testis is almost flat anteriorly and convex posteriorly. The anterior testis measures 0·224 mm. in length and 0·38 mm. in breadth and lies close to the left intestinal caecum. The posterior testis measures 0·192×0·383 mm. and lies close to the right intestinal caecum. The cirrus sac is an elongated structure measuring 0·469 mm. in length and 0·112 mm. in maximum

breadth and is placed transversely behind the ventral sucker. It curves round its right side to open at the genital pore which lies in between the intestinal bifurcation and the ventral sucker. The vesicula seminalis fills the three-fourths of the cirrus sac. The pars prostatica is small.

The female genital organs consist of tho ovary, which is transversely elongated and is placed right to the median line in front of the testes. It measures 0·119 mm. in length and 0·204 mm. in breadth. The Mehlis' gland complex lies to the left of the ovary. The receptaculum seminis is like a small bag placed mesially at the equatorial line in front of the anterior testis. It is 0·128 mm. in length and 0·240 mm. in breadth. The terminal portion of the uterus lies on the opposite side of the ventral sucker round which the cirrus sac passes. The vitelline follicles are scattered along the lateral sides of the body. They extend from the level of the ventral sucker to the middle of the posterior testis. The eggs are 0·020–0·030×0·008 mm. in size. The species has been named after the late Dr. George Matthai, my old Professor at Government College, Lahore.

Relationships

In having a larger oral sucker as compared to the ventral sucker, the new species described above is different from those species in which the oral sucker is either equal to or smaller than the ventral sucker. Amongst the species with the oral sucker larger than the ventral sucker, the new species resembles *A. odhneri*, *A. fukuii* and *A. nathi* in the extent of the intestinal caeca, the position of the genital pore and of the ventral sucker. But it differs from them in having testes of different shape, the transversely elongated ovary and the cirrus sac placed transversely behind the ventral sucker. It differs from *A. fukuii* in the position of the testes and from *A. nathi* in having a smaller oesophagus and in the extent of the vitellaria.

A key to the identification of all the valid species of the genus *Astiotrema* Looss (1900) based on the undermentioned characters has been prepared.

1. Size of suckers.
2. Position of ventral sucker.
3. Length of oesophagus.
4. Length of intestinal caeca.
5. Position of genital pore.
6. Shape and arrangement of testes.
7. Cirrus sac.
8. Shape of ovary.
9. Extent of vitellaria.

Key to the species of the genus *Astiotrema* Looss, 1900

Oral sucker larger than ventral sucker	A.
Oral sucker smaller than ventral sucker	B.
Oral sucker equal to ventral sucker	C.

A.	Ventral sucker in front of intestinal bifurcation	*A. monticellii* Stossich, 1904.
	Ventral sucker behind the intestinal bifurcation ..	1.
1.	Genital pore in front of the intestinal bifurcation ..	*A. impletum* (Looss, 1899) Looss, 1900.
	Genital pore behind the intestinal bifurcation	2.
2.	Intestinal caeca short, terminate at level of the caudal margin of posterior testis	3.
	Intestinal caeca longer, terminate behind the posterior testis ..	4.
3.	Testes with entire margins ..	3a.
	Testes lobed; vitellaria extending from the level of ventral sucker or cephalad to it up to the posterior margin of posterior testis	*A. odhneri* (Odhner, 1911) Bhalerao, 1936.
3a.	Testes tandem in position, spherical or elliptical	*A. fukuii* Ogata, 1938.
	Testes obliquely placed ..	$3a_1$.
$3a_1$.	Testes oval, anterior testis slightly notched on its anterior aspect; oesophagus long; ovary spherical; vitellaria extend from level of ovary or a little in front of it, but not reaching the ventral sucker, up to the posterior margin of anterior testis ..	*A. nathi*, n.sp.
	Testes transversely elongated, oesophagus short; ovary also transversely elongated; vitellaria from the level of ventral sucker to the middle of posterior testis	*A. matthaii*, n.sp.
4.	Vitelline follicles confluent medially to ventral sucker; testes and ovary of the same size ..	*A. emydis* Ejsmont, 1930.
	Vitelline follicles not confluent medially; testes distinctly larger than ovary	4a.

4a.	Vitellaria extend cephalad from ventral sucker but not anterior to intestinal bifurcation ..	$4a_1$.
	Vitellaria do not extend cephalad to ventral sucker	4a.
$4a_1$.	Testes with margins irregular, not deeply notched	*A. elongatum* Mehra, 1931.
	Testes deeply notched from anterior aspects only ..	*A. hoshiarpurium*, n.sp.
$4a_2$.	Testes spherical or irregular; cirrus sac and uterus on the opposite sides of the ventral sucker ..	*A. rami* Bhalerao, 1936.
	Testes broader than long; cirrus sac and terminal portion of the uterus on one side of ventral sucker; ovary near the middle of body	*A. reniferum* (Looss, 1898) Looss, 1900.
B.	Intestinal caeca terminate behind the posterior testis ..	B_1.
	Intestinal caeca terminate near the level of caudal margin of posterior testis	B_2.
B_1.	Testes deeply lobed, ovary kidney-shaped; vitellaria extend to or beyond the posterior testis	*A. loossii* Mehra, 1931. Syn. *A. gangeticus* Harshey, 1932.
	Testes rounded, margins smooth, ovary rounded; vitellaria extend from posterior margin of ventral sucker to area between testes ..	*A. spinosa* Chatterji, 1933.
B_2.	Testes with entire margins, vitellaria extend from the level of ventral sucker or slightly behind it to near the ends of intestinal caeca	*A. orientale* Yamaguti, 1937. Syn. *A. amydae* Ogata, 1938. *A. foochowensis* Tang, 1941.
	Testes notched on their anterior aspects, caudad in position; ovary near the equatorial line of the body; vitellaria extend from the level of genital pore to the hinder margin of anterior testis	*A. srivastavai*, n.sp.

C.	Testes tandem in position, slightly lobed on all sides; ovary oval; vitellaria extend from ventral sucker to the hinder margin of posterior testis	*A. dassia* Dayal, 1938.
	Testes obliquely placed ..	C_1.
C_1.	Testes lobed, wider than long; ovary with posterior margin deeply indented; vitellaria extending from the level of ventral sucker to the anterior end of posterior testis	*A. indica* Thapar, 1933.
	Testes not lobed, irregular in shape, ovary spherical; vitellaria extending from the pre-acetabular region, i.e., between the intestinal bifurcation and ventral sucker to the middle of posterior testis	*A. thapari*, n.sp.

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TAXONOMIC VALUES OF CHROMOSOMES AND CYTOPLASMIC INCLUSIONS IN A DIGENETIC TREMATODE—*PHYLLODISTOMUM SPATULA*

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TAXONOMIC VALUES OF CHROMOSOMES AND CYTOPLASMIC INCLUSIONS IN A DIGENETIC TREMATODE— *PHYLLODISTOMUM SPATULA*

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Introduction

Though the complicated life cycle of the digenetic trematodes has been engaging the attention of many workers, a comprehensive study of germ cells in the adults has for long been neglected. The first satisfactory account of the germ cells was given by Dingler (1910), but since then for about twenty years the workers have been interested in the maturation phenomena of the germ cells alone. The accounts of chromosomes have been meagre and accidental. During the last decade, however, development of the germ cells has been reported in a dozen species, and the chromosome number has been recorded in nearly 50 species. These observations form a very small percentage of the total number of recorded species in this parasitic group of trematodes. Further cytological observations are also required for establishing valid basis of relationship within the groups of Platyhelminthes, as has been stressed by White (1940), Jones (1945) and Britt (1947).

The rôle of cytomorphological characters other than the chromosomes has not been stressed in establishing close relationship of the allied members. It may be stated that the cytological investigations in *Probolitrema californiense* (Markell, 1943) and *Gorgoderina attenuata* (Willey and Koulish, 1950), which belong to the same family *Gorgoderidae*, show some common characteristics in so far as the behaviour of cytoplasmic inclusions in the oocytes is concerned. In view of these existing cytological resemblances in two different genera of the same family, it was felt that similar observations on some other genera of this family might well establish the cytological characteristics of this family. The hope was well realized. It was discovered that the spermatogonia in *Phyllodistomum spatula* also lie irregularly in patches along the peripheral border, and they are not easily distinguished from one another or the primordial germ cells, as reported in the other two genera. Similarly a large amount of food material appears and develops in the oocytes, as it does in the species of *Probolitrema* (Markell, 1943), and *Gorgoderina* (Willey and Koulish, 1950). These indicated trends of similar behaviour show how the characters described by Markell and Willey and Koulish can be considered as family characters.

In the present account an attempt has also been made to describe the process of spermatogenesis with special reference to the behaviour of the

chromosomes and the cytoplasmic inclusions of the oocytes, which have been interpreted as reserve food material.

Five specimens of the adult *Phyllodistomum spatula* were obtained from the urinary bladder of a freshwater fish, *Ophiocephalus punctatus* and fixed in Bouin's fluid for 24 hours. Sections were cut 8 microns thick and were stained with 0·5% iron haematoxylin.

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Observations

The trematodes are characterized by the possession of a leaf-like body with a discoidal posterior region, sharply set off from the anterior stalk-like part by a groove. They range from 2·7 mm. to 4·0 mm. in length. There is a pair of testes lying almost symmetrically, and a single ovary, which is entire. The testes lie just behind the ventral sucker, and the single ovary, which is postero-lateral to the ventral sucker, lies just in front of the left testis. The testes measure about 0·35 mm. in diameter, and the ovary nearly 0·25 mm.

Chromosomes

The chromosomes are highly pycnotic in the spermatogonial divisions. Full diploid number was seen only in a few gonial metaphases. Out of the 16 diploid chromosomes, there are four pairs of large chromosomes varying from 2·5 μ to 4·5 μ in length, and the other four pairs are small ones, not exceeding 1·5 μ each. The largest pair, which is almost 4·5 μ, has unequal arms with a submedian attachment constriction. The second in length is a straight one, about 3·5 μ. The third pair is J-shaped, nearly 2·5 μ, and the fourth one, which also measures 2·5 μ, is V-shaped with a median attachment constriction. In meiosis I of the male germ cells, 8 haploid chromosomes were very often seen. The same number was also seen in the meiosis II of the spermatocytes. In the oocyte divisions also the chromosome number has been determined. The cleavage divisions were, however, not observed.

Spermatogenesis

The gross morphology of the testes indicated that the primordial germ cells together with the spermatogonia lie peripherally, and the spermatocytes together with the spermatids lie centrally. The primordial germ cells and the spermatogonia are seen to lie in irregularly scattered patches. Sometimes they are seen to extend towards the interior of the testes (Pl. I, Fig. 1). These cells are very often closely packed together, and their

individual boundaries cannot be easily observed. Each cell has a characteristic nucleolus. It is rather difficult to distinguish the spermatogonia from the primordial germ cells. The primary, secondary and tertiary spermatogonia occur haphazardly within the groups of such cells, and are not easily distinguishable from each other except when in metaphase. The size of these cells varies from 6 μ to 8 μ in diameter. Judged from the metaphases, the primary spermatogonia are the largest. In the metaphase plate the chromosomes are often clumped together, but in one instance full diploid number (16) has been counted (Pl. I, Fig. 2). The spindle and the centrosomes are, however, seen in the side view (Pl. I, Fig. 3). The centrosomes may not be very often seen, as they are very minute. During anaphase the chromosomes split up into identical sets and are drawn towards the respective poles (Pl. I, Fig. 4). The secondary and tertiary spermatogonia during metaphases hardly show any recognizable difference in size. Their chromosomes are also pycnotic, but appear slightly thicker. In a few instances of these cells the diploid number of chromosomes (16) has, however, been observed (Pl. I, Figs. 5 and 6).

The tertiary spermatogonia on division result in the formation of a group of 8 cells, which enlarge to become the primary spermatocytes. The primary spermatocytes are met with in any part of the lumen of the testis. They show distinct outlines of cell boundaries, and measure from 10 μ to 12 μ in diameter. The nucleus of the primary spermatocyte does not show any distinct nucleolus. An indistinct nucleolus may, however, be seen in a few cells (Pl. I, Fig. 7). The resting stage is organized into the leptotene threads, when long threads become visible, but their course is not easily determined (Pl. I, Fig. 8). In the following zygotene stage the process of pairing starts and the chromosomes appear as double threads. During this stage the threads are seen to lie in different directions (Pl. I, Fig. 9). In the subsequent pachytene stage the bivalent chromosomes are seen to form loops with their ends directed towards one side (Pl. I, Fig. 10). This stage has, however, not been observed frequently. The bivalents then open out slightly, but are still held together at the chiasmata. They lose their orientation and thus form the diplotene stage (Pl. I, Fig. 11). The chromosomes still retain their woolly appearance. The condensation of the chromosomes continues and they spread out in the nucleus, thus reaching the diakinesis stage (Pl. I, Fig. 12). A little later the chromosomes assume a smooth contour, and typical tetrads are seen (Pl. I, Fig. 13). The nuclear wall then breaks down, and the end of prophase is reached, when the bivalent chromosomes are set free in the cytoplasm. By this time the chromosomes have condensed further and their tetrad nature is apparently lost to view. In the polar view of the metaphase plates the haploid number of chromosomes (8) is distinctly seen (Pl. I, Figs. 14 and 15). When the chromosomes are arranged on the spindle, they start segregating and moving towards the poles simultaneously. In the late anaphase stage, however, two chromatic bridges are often seen, which indicate the late segregation of two tetrads (Pl. I, Fig. 16). Centrioles and spindles are occasionally met with at this stage. As the chromosomes reach the telophase stage, cytoplasmic cleavages appear and

gradually extend inward to form a cluster of 16 cells—the secondary spermatocytes (Pl. I, Fig. 17). The chromatin mass of each cell is re-organized into a resting nucleus, which does not show any nucleolus.

The secondary spermatocytes of a cluster undergo simultaneous division. On reaching the end of the prophase stage, the haploid chromosomes (8) form the metaphase plate (Pl. I, Fig. 18). These chromosomes are shorter than those seen in meiosis I. The chromosomes then divide simultaneously, and are drawn towards the poles (Pl. I, Fig. 19). At the telophase stage the division of the secondary spermatocyte cluster is effected in the manner of primary spermatocyte cluster (Pl. I, Fig. 20). As the division is completed a cluster of 32 cells, the spermatids, is formed, and this brings us to the end of the meiotic behaviour of the chromosomes (Pl. I, Fig. 21).

Sufficient stages have not been observed to make a critical analysis of the process of spermateleosis. The rounded nuclei of the spermatids become elongated and pointed towards the outer free border. The nuclei at this stage are seen to have a weak staining capacity (Pl. II, Fig. 22). The ripe sperms are thread-like, and stain uniformly throughout their length (Pl. II, Fig. 23).

Oogenesis

The single ovary lies anterior to the left testis and measures about 0·22 mm. in diameter. The oogonia occur close to the peripheral region and measure about 8 μ to 9 μ (Pl. II, Fig. 24). The division stages of the oogonia have not been found in any of the sections.

The oocytes lie inner to the peripheral zone of the oogonia and occupy almost the entire ovary. They measure about 15 μ to begin with, but attain a maximum size of 25 μ. The young oocytes soon begin to undergo nuclear changes. The resting stage is organized to form a leptotene stage, when their threads are visible (Pl. II, Fig. 25). During synapsis, some of the threads appear thick (Pl. II, Fig. 26), and as the pairing is completed the chromosomal threads are orientated towards one side of the nucleus (Pl. II, Fig. 27). The nucleus then enters a diffuse stage, when it is apparently comparable with the presynaptic resting stage (Pl. II, Fig. 28). It can, however, be distinguished by the presence of a well-developed darkly staining nucleolus and the larger size of the oocyte itself. As the growth of the oocyte proceeds, one or two darkly staining masses appear in the cytoplasm (Pl. II, Fig. 29). With the further growth of the oocyte, the cytoplasmic bodies also increase in size, and they unite to form one mass, which continues growing (Pl. II, Fig. 30). When the oocyte attains its maximum size, it leaves the ovary and enters the fertilization or the ovarian space. Here the vitelline cells are poured, and the sperms move. A single sperm enters the oocyte and lies in a coiled condition in the cytoplasm. The cytoplasmic mass of the oocyte has by now attained its maximum size (Pl. II, Figs. 31 and 32). The vitelline cells, which are characterized by the presence of a large number of chromatic granules in their nuclei, are applied to the sides of the oocyte and secrete the shell membrane, which surrounds the oocyte. Some of the vitelline cells are

stored inside the shell in the form of small granules, which nourish the egg at later stages of development. The activities of the oocyte to form the polar bodies are set in at this stage.. Thick threads appear from the diffuse stage of the oocyte nucleus, which are converted into a haploid number of chromosomes (Pl. II, Fig. 33). The chromosomes very often appear as an agglutinated mass or masses and thus their number is not countable (Pl. II, Fig. 34). The sperm at this stage is seen to lie on one side of the configuration division. The cytoplasmic mass is also seen as a well-developed structure. On division, the first polar body is formed as a chromatic mass, which is subsequently set off from the mother oocyte (Pl. II, Figs. 35 and 36). The stages of the 2nd meiotic divisions have, however, not been traced. In the two-celled stage—the cytoplasmic mass is still present as a rounded globular mass, lying freely in the nutritive material. The polar bodies seem to have been absorbed by now, as they are not seen (Pl. II, Fig. 37). In the three-celled stage the cytoplasmic mass is found to have degenerated (Pl. II, Fig. 38), and in the four-celled stage it completely disappears from view (Pl. II, Fig. 39).

Some of the oocytes in the ovarian space have been observed to degenerate. The degeneration starts with the extrusion of chromatin granules from the nucleus, and gradually the size of the cell decreases. The nuclear membrane becomes indistinct and only the chromatic granules are visible in the cell, which ultimately are absorbed probably for nourishment (Pl. II, Figs. 40–43).

Discussion

The observations on spermatogenesis suggest a great similarity of the general trends, as have been observed by a number of previous workers in the digenetic trematodes, regarding the organization of the germ cells. Three spermatogonial divisions and two subsequent meiotic divisions result in the formation of a cluster of 32 spermatids. Variations have, however, been reported in the present study, which can be described as family characters. The spermatogonia occur irregularly along the periphery in patches. They are not distinguishable either from each other or from the primordial germ cells. The primordial germ cells and the spermatogonia are characterized by the presence of one nucleolus each, and except for a slight difference of size show no cytomorphological character to differentiate them. The spermatogonia among themselves show no appreciable size differences and thus in the resting stage cannot be easily distinguished from each other. Markell (1943) in *Probolitrema californiense* and Willey and Koulish (1950) in *Gorgoderina attenuata* also observed that the primordial germ cells are irregularly distributed in closely clumped groups and lie peripherally, and the spermatogonia, which are somewhat larger, appear among the primordial germ cells, but are not easily distinguishable from them. They further noticed that the primary, secondary and tertiary spermatogonia are irregularly arranged and they cannot be easily distinguished from each other. The similar behaviour of the primordial germ cells and the spermatogonia in three different genera of the family *Gorgoderidae* can be described as a characteristic of this family.

Another cytological feature common to *Probolitrema californiense* (Markell, 1943), *Gorgoderina attenuata* (Willey and Koulish, 1950) and *Phyllodistomum spatula* is the appearance of large globular masses and their subsequent growth in the primary oocytes. It appears very probable that this character is at the family rank. Although such bodies have also been observed in some other trematodes, they are not so large as found in this family. Schubmann (1905) and Schellenberg (1911) in *Fasciola hepatica* and Anderson (1935) in *Proterometra* observed granules in the oocytes and interpreted them as the degenerated oogonial cells, ingested by the normal oocytes. Pennypacker (1940) also observed small granules in the developing oocytes of a frog lung fluke, whose nature was not clear to him. I have observed similar bodies in another digenetic trematode, but they were not so large as found in *Phyllodistomum spatula*.

The importance of cytomorphological characters, other than the chromosomes, in taxonomy have also been recorded in other groups of animals. Nath (1942) in his work on the sperm of Decapod Crustacea built up an evolutionary scheme of the sperm in that group based on the study of centrosomes, which coincides with the scheme of classification within that group evolved by the taxonomist, who relies on the study of seemingly unimportant external characters. Similarly Nath and Gill (1949) reported the formation of a prominent mitochondrial ring in the spermatocytes of the scorpion, *Buthus acutecarinatus*, which behaves in exactly the same manner as in the scorpion *Centrurus* (Wilson, 1915), whereas in other species of scorpions, such as *Palamnaeus* (Nath, 1925), the mitochondrial spheres do not fuse to form a ring. Nath and Gill pointed out the parallelism between this cytological affinity of *Buthus* and *Centrurus* and their close relationship as emphasized by the taxonomist, who had placed both these genera in the family *Buthidae*.

The behaviour of the reserve food material in the developing oocytes has thrown some light on their doubtful nature. Markell (1943) and Willey and Koulish (1950) noticed that in the ovary these bodies appear in the developing oocytes and develop for some time, but they start degenerating before the oocytes leave the ovary. They could not, therefore, positively consider them as reserve food material. In *Phyllodistomum spatula* the reserve food material has been found to develop within the ovary and to retain its full identity even when it has left the ovary or when the shell has been formed. It is only at the three-celled stage of the embryo that this mass starts degenerating. The present study points towards the conclusion that the large globular mass is of the nature of reserve food material. It may not be easy to answer as to why these bodies start degenerating within the ovary of *Probolitrema* and *Gorgoderina*, as reported by Markell (1943) and Willey and Koulish (1950) respectively, but it becomes quite evident from the present study that identical bodies, which appear in the oocytes of the three different genera of the family *Gorgoderidae*, are of the nature of reserve food material. The difference in their stage of degeneration may be a generic behaviour.

The degeneration of some of the oocytes in the region of the ovarian space is not clear to me. Why should they degenerate in the ovarian

space, where the yolk globules are present, for nourishment, and not in the ovary, where they can serve a better purpose for nourishment? Kathariner (1904) and Gille (1914) have reported the association of some nuclei with the fully developed oocytes, when it leaves the ovary. Gille regarded them as abortive oocytes, which disintegrate to nourish the chief oocytes. Such degenerating oocytes have not been observed in *Phyllodistomum spatula.*

In the testes of *Phyllodistomum spatula* nothing has been observed, which could be compared with the degenerating oocytes of Anderson (1935) and Willey and Koulish (1950), or the globules of Dingler (1910) and John (1953), or again the pycnotic cells of Chen (1937). The testes appear to be nourished by the body fluids.

The nuclear changes in preparation of the meiosis of the male and female germ cells are very much like those described by Willey and Koulish (1950), Willmott (1950) and Willey and Godman (1951). The characteristic condensation stage or synezesis of Pennypacker (1940) and Willey and Godman (1951) has, however, not been observed, either in the spermatocytes or oocytes of *Phyllodistomum.* The oocyte nucleus passes through a diffuse stage before the emergence of the tetrads.

The chromosome study has already been made in three species of the family *Gorgoderidae.* Markell (1943) in *Probolitrema californiense* reported 6 haploid chromosomes. Britt (1947) in *Gorgoderina attenuata* and *Gorgodera amplicava* recorded 7 and 8 as the haploid numbers respectively. In *Phyllodistomum spatula,* which belongs to the same family, the haploid number of chromosomes is 8. This number falls within the recorded limits of this family. Though this number corresponds with the recorded number of the genus *Gorgodera,* the morphology of the chromosomes show differences in the two genera. In *Gorgodera amplicava* there is only one pair of long chromosomes, but in *Phyllodistomum spatula* there are four pairs of long chromosomes. On the basis of chromosome morphology the genera are distinct.

Summary

1. The process of spermatogenesis in *Phyllodistomum spatula* with special reference to the behaviour of the chromosomes has been described.

2. Some stages of oogenesis have been reported and the history of the reserve food material in the oocytes is traced. Some well-developed oocytes have been seen to degenerate in the ovarian space.

3. The haploid number (8) and the diploid number (16) has been counted, and the morphology of chromosomes has been described.

4. The cytomorphological characters—the behaviour of the primordial germ cells and the spermatogonia, and the presence of large reserve food masses have been brought out as family characters of the family *Gorgoderidae.*

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Abbreviations

Ch—Chromosomes; Pb_1—First Polar-body; *rf*—Reserve food material; *sp*—sperm.

Explanation of Plates

Plate I

Fig. 1. Primordial germ cells and spermatogonia along the wall of the testis.
„ 2. Spermatogonium, showing a diploid number (16) of chromosomes in metaphase.
„ 3. Spermatogonium, showing metaphase chromosomes in side view. Spindles and centrosomes are also seen.
„ 4. Spermatogonium, showing chromosomes in anaphase.
„ 5. Secondary spermatogonia in metaphase.
„ 6. Tertiary spermatogonia in metaphase.
„ 7. Primary spermatocytes in the resting stage.
„ 8. Primary spermatocyte, showing the leptotene stage.
„ 9. Primary spermatocyte, showing a zygotene stage.

FIG. 10. Primary spermatocyte, showing a bouquet stage.
FIGS. 11 and 12. Primary spermatocytes, showing the diplotene stage.
FIG. 13. Primary spermatocyte, showing the diakinesis stage.
FIGS. 14 and 15. Primary spermatocytes in metaphase.
FIG. 16. Dividing primary spermatocytes.
„ 17. Secondary spermatocytes in the resting stage.
„ 18. Secondary spermatocytes in metaphase.
„ 19. Secondary spermatocytes in anaphase.
„ 20. Dividing secondary spermatocytes.
„ 21. Spermatids in the resting stage.

PLATE II

FIG. 22. Developing spermatids.
„ 23. Mature sperms in the residual mass.
„ 24. Oogonium.
„ 25. Primary oocyte, showing the leptotene threads.
„ 26. Primary oocyte, showing the zygotene stage.
„ 27. Primary oocyte, showing the pachytene stage.
„ 28. Primary oocyte, showing the diffuse stage. The reserve food material has also appeared.
FIGS. 29 and 30. Primary oocytes, showing the reserve food material fused to form a single body.
FIG. 31. Primary oocyte, showing a coiled sperm and the reserve food material in the cytoplasm.
„ 32. Primary oocyte, showing a condensed sperm and the reserve food material.
„ 33. Primary oocyte in metaphase. The sperm and the reserve food material are lying away from the meiotic chromosomes of the oocyte.
„ 34. Primary oocyte in division. The chromosomes are agglutinated. The sperm is lying quiescent and the reserve food material is prominent.
FIGS. 35 and 36. Primary oocytes, showing the formation of the first polar body. The sperm is still a condensed mass and the reserve food material is unaffected.
FIG. 37. Egg, showing a two-celled stage. Reserve food material is seen as a rounded mass.
„ 38. Egg, showing a three-celled stage. Reserve food material is seen degenerating.
„ 39. Egg, showing a four-celled stage. Reserve food material has been consumed completely.
FIGS. 40 to 43. Primary oocytes, showing successive stages of degeneration in the ovarian spaces.

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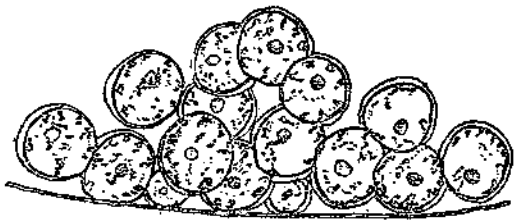

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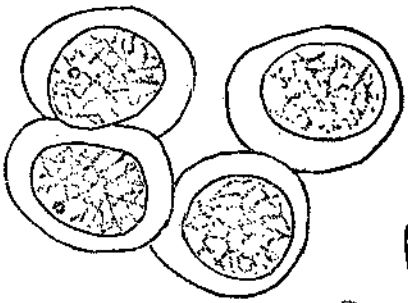

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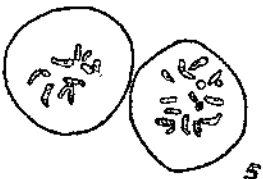

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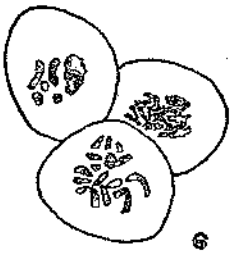

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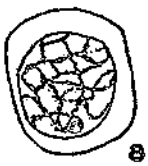

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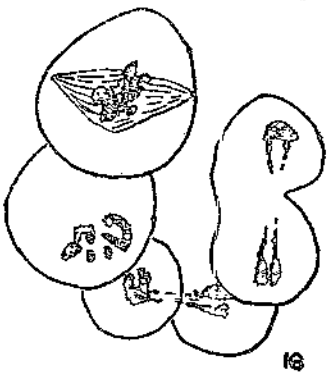

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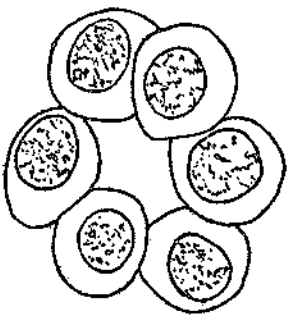

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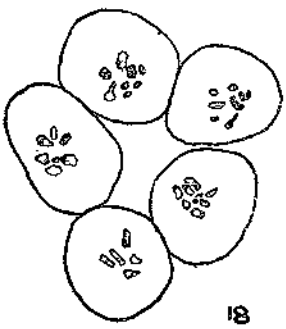

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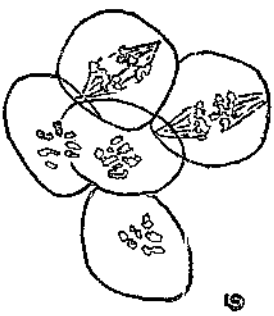

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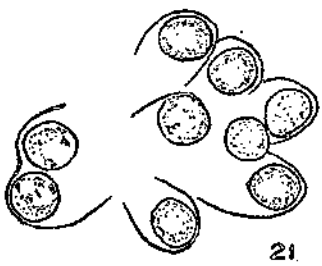

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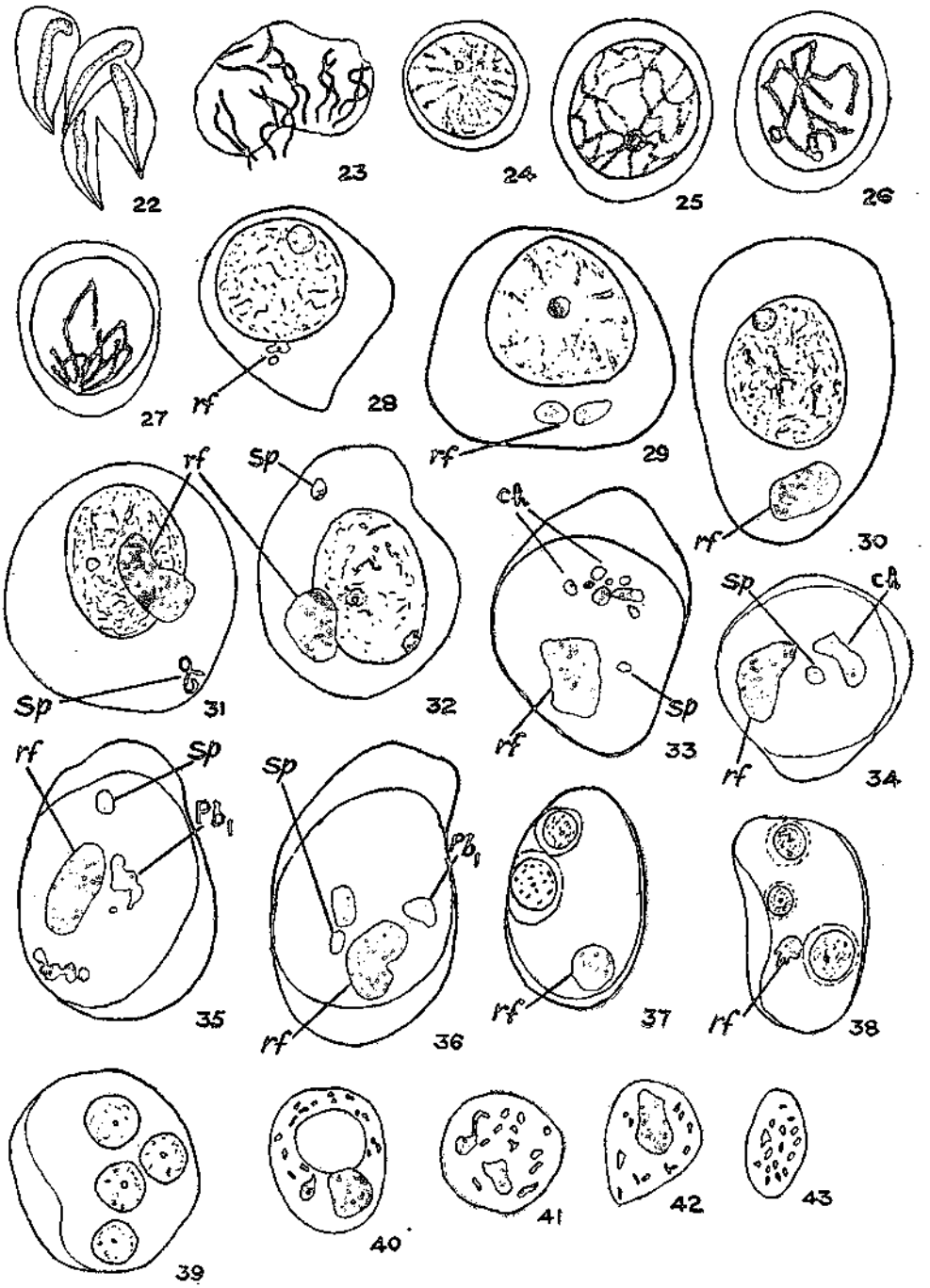
PLATE II.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RESEARCH BULLETIN
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No. 52 | CHEMISTRY | Pp. 111-116

July, 1954

THE PHENOMENON OF CONTINUOUS FREEZING OF CAPILLARY-HELD WATER AT DECREASING TEMPERATURES

by

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THE PHENOMENON OF CONTINUOUS FREEZING OF CAPILLARY-HELD WATER AT DECREASING TEMPERATURES

By Balwant Rai Puri, Lekh Raj Sharma *and* M. L. Lakhanpal
(*Department of Chemistry, Panjab University College, Hoshiarpur*)

The authors reported recently (4) a dilatometric technique for determining freezing points of water held in porous bodies and showed that the sorbed water does not freeze even at temperatures well below its normal freezing point and that the temperature at which freezing commences is a function of the vapour pressure of the adsorbent. It was also found during those investigations that the entire amount of the sorbed water does not freeze at one particular temperature. On the contrary, the freezing continues as the temperature is lowered. Foote and Saxton (1, 2) and Jones and Gortner (3) also deduced from similar measurements that water adsorbed on silica gel continues to freeze as the temperature is lowered and that even at −48° a certain portion remains unfrozen. This water was considered by them as 'bound' water. Robinson (5, 6, 7) carried out similar investigations and described the water which does not freeze at −20° as 'bound' water.

It appears that the real reason for the continuous freezing is that water held up in a porous body is actually contained in capillary pores of different radii and since according to the well-known laws of capillarity, as embodied in the Kelvin equation, the negative pressure to which a liquid is subject is related with the radius of curvature of the liquid meniscus, it follows that water condensed in capillary pores of decreasing radii will have decreasing vapour pressures and will consequently freeze at decreasing temperatures. It may not be correct to describe the adsorbed water as 'bound' if it does not freeze at −20° or even at −48° as suggested by the previous workers, since it can be easily shown by calculations that water held up in capillary pores of radius <10 Å will require much lower temperatures to freeze.

Since each freezing temperature corresponds to a definite value of relative vapour pressure (p/p_0) of the liquid, according to the well-known thermodynamic relationship, it follows that the amount of water that freezes between any temperature interval should be equal to the amount of water that is adsorbed by the porous body between the corresponding relative vapour pressure interval. The explanation offered in the above paragraph, for the anomalous behaviour of adsorbed water below its normal freezing point, therefore, permits of experimental verification in a simple manner. The work described in this paper was undertaken with this objective in view. The apparatus and the technique employed was the same as described in the earlier work (4).

Experimental

A sample of silica gel, prepared fresh in the laboratory by precipitation from sodium silicate solution of density 1·185 by the addition of an equivalent amount of HCl, and a sample of bentonite were taken for these investigations. Their known weights were allowed to come to equilibrium with 97 per cent relative humidity and then placed in the dilatometer (4) and cooled to a temperature of about −50° in a bath of carbon dioxide snow mixed with alcohol. The temperature of the bath was allowed to rise gradually, thereafter, by radiation and the position of the ligroin meniscus was noted after about every 1° rise of temperature. The rate of rise of temperature was quite slow, the temperature rising by 1° in about half an hour.

On plotting the positions of the ligroin meniscus against the corresponding temperatures, the points were seen to fall on two curves with different slopes (Figs. 1 and 2) and the temperature, at which the abrupt change in the slope was noticed, was taken as the temperature at which the phase transformation from the solid to liquid state was just completed. Obviously, this would also give the temperature at which the capillary-held water would just commence to freeze, if gradually cooled. This temperature is seen to be about 0·3° in each case as required at 97 per cent humidity (4).

Table 1

Comparison of the amount of water that freezes within a certain temperature range and that which is adsorbed within the corresponding vapour pressure range in the case of Silica gel.

Temperature range of fusion. (°C.)	Corresponding vapour pressure range.	Displacement between the two curves within the temperature range of fusion. (cm.)	Change of volume calculated from the displacement (1 cm. = 0·198 c.c.) (c.c.)	Amount of water that freezes per 100 gm. of the material within the temp. range. Column $4 \times 100 / 0{\cdot}09 \times W^*$ (gm.)	Amount of water adsorbed per 100 gm. of the material within the corresponding vapour pressure range. (gm.)
0·25– 6·83	0·975–0·860	1·08	0·214	6·67	5·72
0·25–12·62	0·975–0·756	1·64	0·333	10·39	9·56
0·25–19·10	0·975–0·653	1·95	0·386	12·05	12·14
0·25–26·42	0·975–0·557	2·30	0·455	14·20	13·77
0·25–33·67	0·975–0·472	2·77	0·548	17·12	15·75
0·25–40·85	0·975–0·404	3·41	0·675	21·07	18·82

* W is the actual weight of the substance used in the experiment.

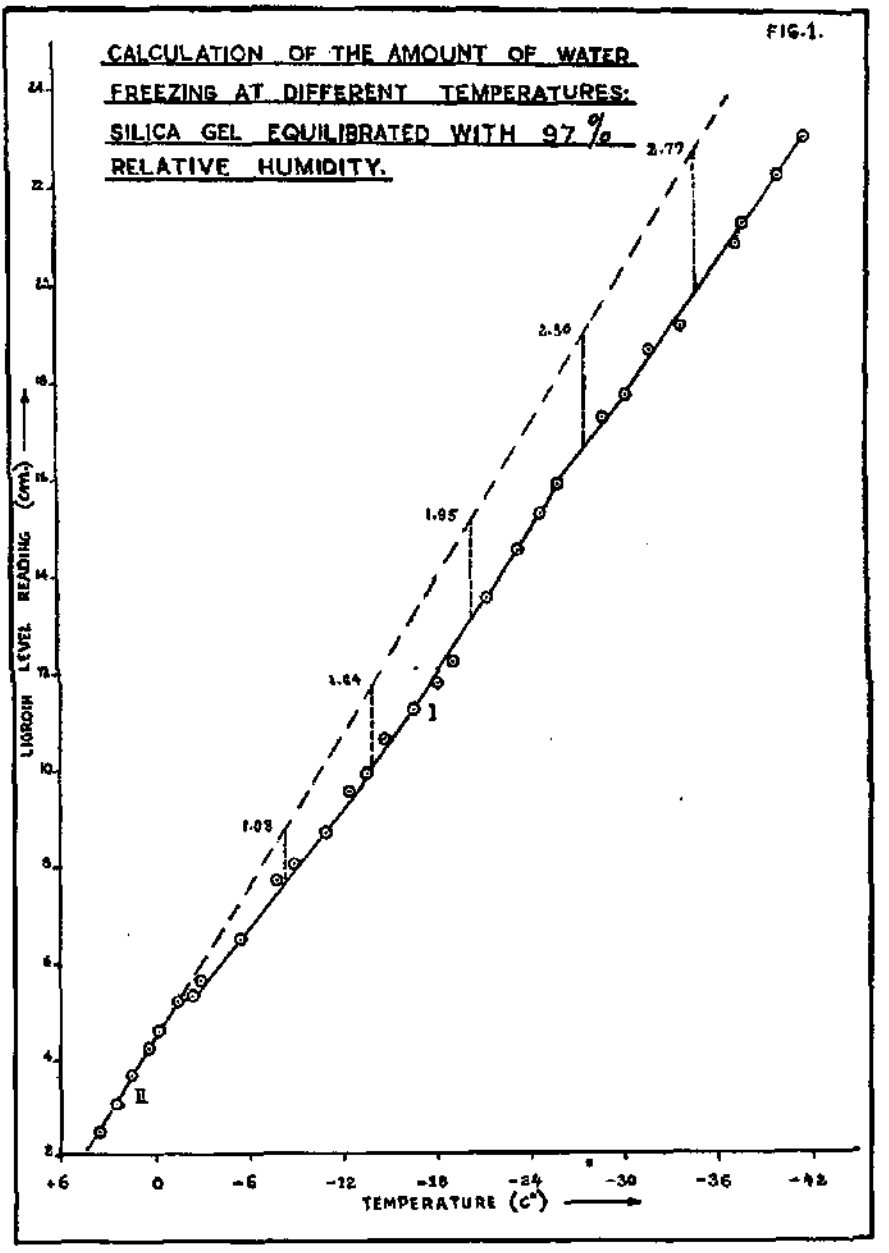
FIG.1.
CALCULATION OF THE AMOUNT OF WATER FREEZING AT DIFFERENT TEMPERATURES: SILICA GEL EQUILIBRATED WITH 97 % RELATIVE HUMIDITY.
LIGROIN LEVEL READING (cm.)
TEMPERATURE (C°)
+6
0
-6
-12
-18
-24
-30
-36
-42
1.03
1.64
1.95
2.56
2.77
I
II

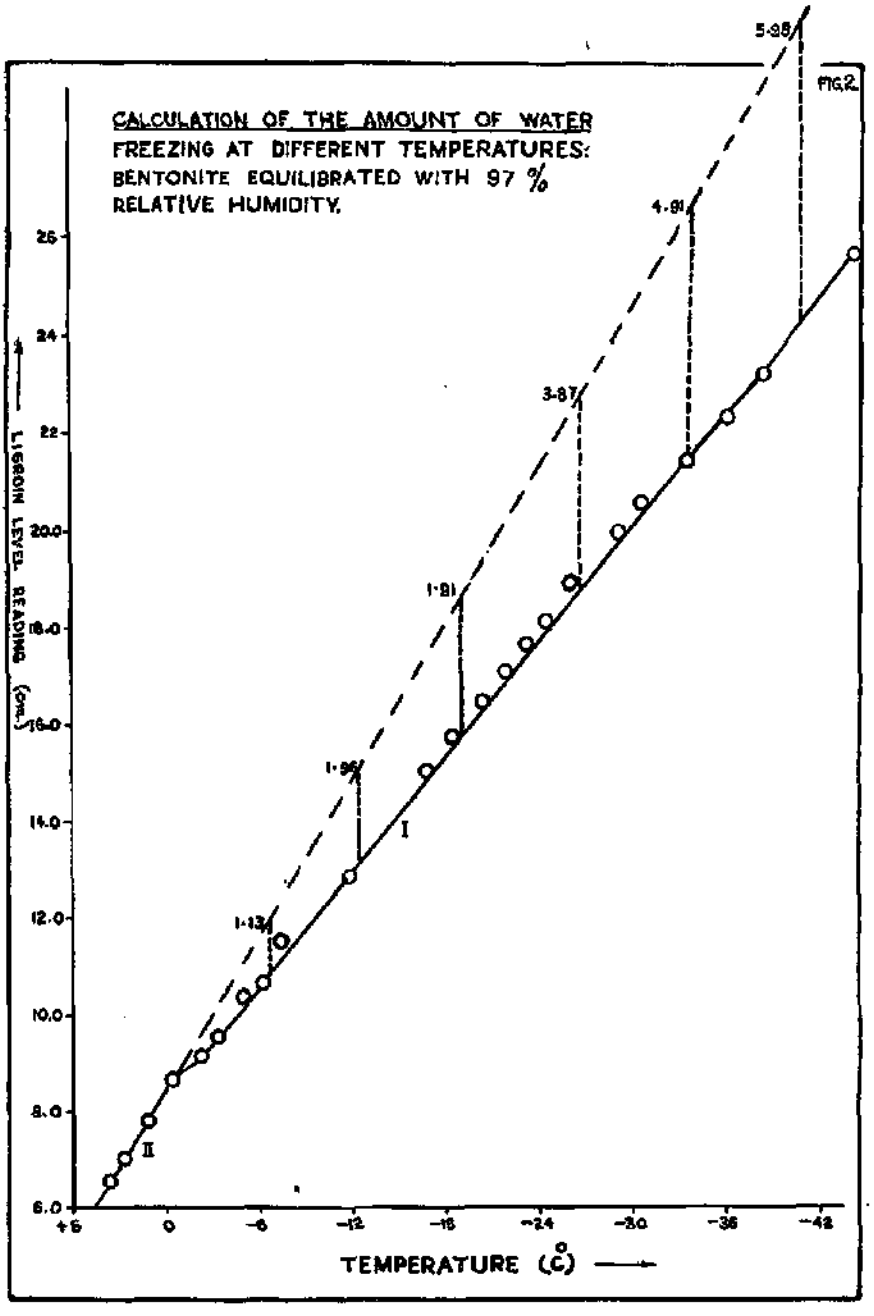
FIG.2.
CALCULATION OF THE AMOUNT OF WATER FREEZING AT DIFFERENT TEMPERATURES: BENTONITE EQUILIBRATED WITH 97 % RELATIVE HUMIDITY.
5·98
4·91
3·87
1·91
1·96
1·13
I
II
LISBON LEVEL READING (cms.)
TEMPERATURE (C°)
+5
0
-6
-12
-18
-24
-30
-36
-42
6.0
8.0
10.0
12.0
14.0
16.0
18.0
20.0
22
24
26

TABLE 2

Comparison of the amount of water that freezes within a certain temperature range and that which is adsorbed within the corresponding vapour pressure range in the case of Bentonite.

Temperature range of fusion. (°C.)	Corresponding vapour pressure range.	Displacement between the two curves within the temperature range of fusion. (cm.)	Change of volume calculated from the displacement (1 cm. = 0·198 c.c.) (c.c.)	Amount of water that freezes per 100 gm. of the material within the temp. range. $\frac{\text{Column 4} \times 100}{0{\cdot}09 \times W^*}$ (gm.)	Amount of water adsorbed per 100 gm. of the material within the corresponding vapour pressure range. (gm.)
0·25– 6·83	0·975–0·860	1·13	0·224	2·49	2·41
0·25–12·62	0·975–0·756	1·96	0·388	4·33	4·13
0·25–19·10	0·975–0·653	2·91	0·576	6·43	6·52
0·25–26·42	0·975–0·557	3·87	0·766	8·55	8·23
0·25–33·07	0·975–0·472	4·91	0·972	10·85	11·92
0·25–40·85	0·975–0·404	5·98	1·184	13·21	14·33

* W is the actual weight of the substance used in the experiment.

If the curve obtained after the attainment of this temperature (curve II) is produced towards decreasing temperatures as shown in Figs. 1 and 2, it is seen that the relative displacement between the two curves increases as the temperature is lowered. This is obviously due to the progressive freezing of the capillary condensed moisture. The amount of water that freezes on cooling up to a particular temperature can be calculated from the magnitude of the displacement between the two curves at that temperature. These values at different temperatures are given in Tables 1 and 2 for the silica gel and bentonite respectively. The various steps in the calculations will be clear from these tables. The values of relative vapour pressure corresponding to these freezing temperatures (4, Table 2) are also given in the tables. The amount of water adsorbed at the various vapour pressures, as obtained from the adsorption isotherms, are given in the last column of each table.

It is seen that the amount of water that freezes at a particular temperature is almost the same as is held up in the micropores of the adsorbent at the corresponding relative vapour pressures. These results are fairly important in showing that the entire amount of moisture taken up by porous bodies at comparatively higher humidity intervals is held up by capillary forces and that the theory of poly-molecular layer cannot fit in under such conditions.

Summary

The continuous freezing of capillary condensed moisture in the case of porous bodies has been shown to be due to the existence of water in the micro-capillary pores of different radii. The amount of water that freezes within a particular temperature interval is almost equal to the amount that is adsorbed between the corresponding humidity interval as required from capillary considerations.

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RESEARCH BULLETIN
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No. 53 ZOOLOGY Pp. 117–120

August, 1954

ON *MEHRAORCHIS TIGRINARUM*, A NEW SPECIES FROM THE STOMACH OF *RANA TIGRINA*

by

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ON *MEHRAORCHIS TIGRINARUM*, A NEW SPECIES FROM THE STOMACH OF *RANA TIGRINA*

By N. K. Gupta, M.Sc., Ph.D., *Lecturer in Zoology, Panjab University College, Hoshiarpur*

Introduction

In this paper, a new trematode, namely, *Mehraorchis tigrinarum* belonging to the genus *Mehraorchis* Srivastava, 1933, has been described.

Genus **Mehraorchis** Srivastava, 1933.

The genus *Mehraorchis* was established by Srivastava in 1933 for a new digenetic trematode *Mehraorchis ranarum* found in cysted condition in the body cavity of *Rana cyanophlyctis* at Sitapur.

Bhalerao in 1936 recorded *M. ranarum* from the bile ducts and gall bladder of *R. tigrina* in Nagpur. In his paper he elucidated certain points in which his specimens differed from the original description given by Srivastava, 1933.

I have obtained another species of the genus *Mehraorchis* from the stomach of *Rana tigrina* at Hoshiarpur. With the establishment of a second species, the necessity arose for the revision of the characters of the genus which has been done.

Mehraorchis tigrinarum, n.sp.

Host : *Rana tigrina.*
Location : Stomach.
Locality : Hoshiarpur.

Many specimens of *Rana tigrina* were dissected and examined from time to time for the collection of trematode parasites. Only once I got three specimens of *Mehraorchis tigrinarum*, n.sp., from the stomach of *Rana tigrina*. Two of them were cut in transverse and longitudinal serial sections and of the third a stained permanent preparation was made.

The live worm is somewhat ovoid (Fig. 1). It is 5·08 mm. long and 2·84 mm. broad at the region just behind the ventral sucker. The cuticle is spinous, the spines being very small and a few in number. The oral sucker, 0·33 mm. long and 0·42 mm. broad, is either subterminal or terminal in position. It is smaller than the ventral sucker which measures 0·629–0·476 mm. and is placed at a distance of 1·56 mm. from the anterior end of the body. The prepharynx is present (Fig. 2). Its presence has been verified by cutting longitudinal sections. In whole mounts it is not visible due to the contraction of the pharynx which overlaps the oral sucker. It is 0·034 mm. long and 0·0408 mm. broad. It is followed by the pharynx

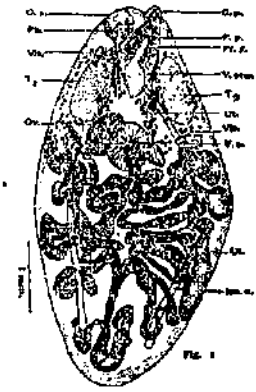

FIG. 1. Ventral view of *Mehraorchis tigrinarum*, n.sp.

G.p., genital pore; *Int.c.*, intestinal caecum; *O.s.* oral sucker; *Ov.*, ovary; *P.p.*, pars prostatica; *Ph.*, pharynx; *Pr.g.*, prostate gland; T_1., right testis; T_2., left testis; *Ut.*, uterus; *V.s.*, ventral sucker; *V.sem.*, vesicula seminalis; *Vit.*, vitellaria.

which measures 0·240 × 0·255 mm., in whole mount and 0·1836 × 0·1462 mm in longitudinal sections. In whole mounts it is covered over by the vitellaria and a portion of the cirrus sac. The oesophagus bifurcates into two intestinal caeca which run parallel to the lateral sides, ending blindly at a little distance in front of the posterior end of the body.

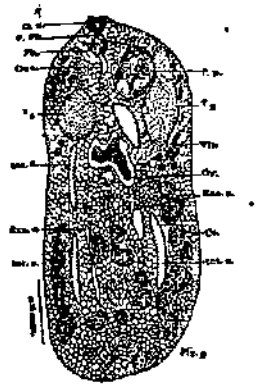

FIG. 2. Longitudinal section showing the prepharynx, the pharynx, the testes, the ovary and the intestinal caeca.

Exc.c., excretory canal; *Int.c.*, intestinal caecum; *O.s.*, oral sucker; *Oes.*, oesophagus; *Ov.*, ovary; *P.p.*, pars prostatica; *P.ph.*, prepharynx; *Ph.*, pharynx; T_1., right testis; T_2., left testis; *Ut.*, uterus; *Vit.*, vitellaria.

The testes are confined to the anterior region, one on either side of the body. They are oval or irregular in shape. The right testis measures 0·510–0·850 × 0·357–0·595 mm., while the left 0·765–0·969 × 0·272–0·425 mm. Vas efferens arises from the inner lateral side of each testis and both the vasa efferentia unite to form the vas deferens before penetrating into the cirrus sac. The vesicula seminalis is a U-shaped structure lying in the posterior region of the cirrus sac. The vesicula seminalis is continued further into pars prostatica which is an elongated structure surrounded by

the prostate gland cells. Each cell shows a distinct round nucleus. The cirrus sac 1·020 mm. long and 0·510 mm. broad, is well developed. It is broad in the middle, while the ends are bluntly pointed. The knob-shaped cirrus enclosing the ductus ejaculatorius opens into the genital atrium.

The ovary, measuring 0·391–0·663 mm. in length and 0·272–0·408 mm. in breadth, is irregular or kidney-shaped and is situated just behind the right testis partly overlapping the ventral sucker. The receptaculum seminis and Laurer's canal are present. The vitelline glands are restricted to the ventral surface under the intestinal caeca and the testes. They extend from the level of the base of the pharynx up to the posterior margin of the ventral sucker. The vitelline ducts run posteriorly to open into the median yolk reservoir. The uterus is very much coiled, extending beyond the blind ends of the intestinal caeca. The ascending limb runs along the left lateral side of the body and then it runs to the right of the left testis and opens through the metraterm into the genital atrium. The eggs are numerous, small and operculated and measure 0·0204–0·034 × 0·0102–0·0136 mm. The excretory system is Y-shaped, the stem being small as compared to the cornua.

In the oral sucker (Fig. 3), next to the cuticle the circular muscles are present in small bands and over them are a few interior longitudinal muscle fibres. The exterior longitudinal muscles are very poorly developed and lie beneath the limiting membrane. The radial muscle fibres are in groups extending from the circular muscles to the limiting membrane. The sub-cuticular cells are only a few and scattered in the oral sucker.

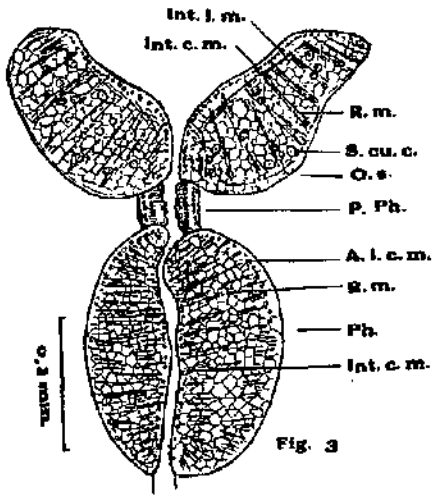

Fig. 3. Oral sucker, prepharynx and pharynx in a longitudinal section.

A.l.c.m., anterior lateral circular muscles; *Int.c.m.*, interior circular muscles; *Int.l.m.*, interior longitudinal muscles; *O.s.*, oral sucker; *P.ph.*, prepharynx; *Ph.*, pharynx; *R.m.*, radial muscles; *S.cut.c.*, sub-cuticular cells.

In the pharynx (Fig. 3) the interior circular muscle fibres are present in bands. There are also anterior lateral circular muscles present in bands. The radial muscles of the pharynx are many and thinner than those of the oral sucker. The sub-cuticular cells are lacking.

Relationships

Mehraorchis tigrinarum, n.sp., differs from *M. ranarum* Srivastava (1933) in the smaller size of the oral sucker as compared to that of the ventral sucker and the uterine coils extending beyond the blind ends of the intestinal caeca.

Emended Diagnosis of the Genus *Mehraorchis* Srivastava, 1933

Pleurogenetinae. Shape and size: ovoid and small. Cuticle: spinous. Suckers: small, oral smaller or larger than the ventral. Digestive system: prepharynx present; pharynx present; oesophagus slender, moderately long; intestinal caeca not coiled, reaching the posterior end of the body. Reproductive system: testes irregular in outline and situated in the anterior part of the body, one on each side in between the body-wall and oesophagus and intestinal caeca; cirrus sac muscular, partly in front of the left testis; ovary kidney-shaped or irregular in outline, to the right side, partly overlapping the ventral sucker; vitelline glands restricted to the anterior region beneath the oesophagus, intestinal caeca and testes, extending from near the middle of the cirrus sac to the level of the posterior margin of the ventral sucker, meeting anteriorly in the median line; Laurer's canal and receptaculum seminis present; genital atrium deep, eversible, situated on the left body margin in level with the pharynx; uterus mainly post-ovarian extending beyond the blind ends of the intestinal caeca; metraterm present. Eggs numerous, small operculate.

In an encysted condition in the body cavity or free in the stomach or bile ducts and gall-bladder of Amphibians.

Key to the Species of the Genus *Mehraorchis* Srivastava, 1933

1. Oral sucker larger than the ventral sucker; uterus not extending beyond the blind ends of the caeca. *M. ranarum* Srivastava, 1933.
2. Oral sucker smaller than the ventral sucker; uterus extends beyond the blind ends of the caeca. .. *M. tigrinarum*, n.sp.

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ON A NEW SPECIES OF THE GENUS *OMMATOBREPHUS* NICOLL, 1914, FROM THE INTESTINE OF *NATRIX PISCATOR* IN LUDHIANA

by

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By N. K. Gupta, M.Sc., Ph.D., *Lecturer in Zoology*,
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Introduction

This paper presents the description of *Ommatobrephus nicolli*, a new species found in the small intestine of *Natrix piscator* caught from Budha Nala, a rivulet near Ludhiana.

The worm is leaf-like, broad and rounded posteriorly and tapering anteriorly (Fig. 1). It measures 3·60 mm. in length and 1·76 mm. in breadth in the region behind the ventral sucker. The integument is without spines. The oral sucker lies at the anterior extremity of the worm and measures 0·238 × 0·255 mm. It is smaller than the ventral sucker which is 0·578 mm. in length and 0·561 mm. in breadth and is placed at a distance of 0·204 mm. posterior to the intestinal fork and at about one-third distance from the anterior end of the body.

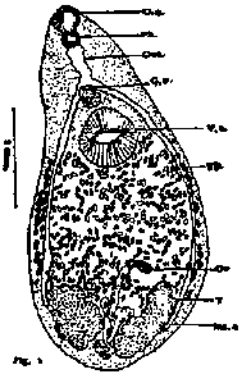

Fig. 1. Ventral view of *Ommatobrephus nicolli*, n.sp.

G.p., genital pore; *Int.c.*, intestinal caecum; *O.s.*, oral sucker; *Oes.*, oesophagus; *Ov.*, ovary; *Ph.*, pharynx; *T.*, testis; *V.s.*, ventral sucker; *Vit.*, vitellaria.

The prepharynx is very small and is visible only in the living condition of the worm. The pharynx is a muscular elongated structure, measuring 0·153 mm. in length and 0·170 mm. in breadth. The oesophagus is moderately long measuring 0·340 mm. in length and 0·136 mm. in maximum breadth, which is just behind the pharynx. The oesophagus bifurcates at a little

distance in front of the ventral sucker into two intestinal caeca, which run along the lateral walls of the body, terminating near the posterior end. The right intestinal caecum is slightly shorter than the left and terminates at the level of the first quarter of the right testis, while the latter terminates at the middle of the left testis.

The testes are lobulated, placed laterally partly in the inter-caecal region and partly behind the blind ends of the intestinal caeca near the posterior extremity of the body. They are longer than broad. The right testis is shorter in length and broader in width than the left testis. Posteriorly both the testes converge towards the median plane of the body. The right testis measures 0·680 mm. in length and 0·459 mm. in breadth. The left testis is 0·883 mm. in length and 0·272 mm. in width. The two testes are separated from each other by a fold of uterus and the receptaculum seminis. The vasa efferentia are long slender ducts, each arises from almost the anterior aspect of each testis. The cirrus sac is oval and lies in the space between the intestinal bifurcation and the ventral sucker, and measures 0·221 × 0·272 mm. in size. The vesicula seminalis fills the entire space of the cirrus sac except the little anterior portion which lodges the pars prostatica and the ductus ejaculatorius.

The ovary is transversely elongated and measures 0·102 × 0·136 mm. in dimensions. It lies to the left of the body in front of the left testis. It is very much smaller than the testes. The oviduct arises from its right border. It is then surrounded by the Mehlis' glands which lie to the right of the ovary. The oviduct receives the duct of the receptaculum seminis which is a looped structure, one loop overlapping the other. The vitellaria are *lateral and mostly extra-caecal. A few of them overlap the caeca.* They extend from the level of the posterior end of the ventral sucker to the level of anterior border or one-fourth of the testes or a little in front of the blind ends of the caeca. The uterus harbours a large number of eggs. The uterine coils are confined to the region behind the ventral sucker and in between the intestinal caeca. The genital pore lies ventral to the intestinal bifurcation. The ova are operculated and measure 0·068–0·080 × 0·028–0·048 mm.

Relationships

The genus *Ommatobrephus* Nicoll (1914) comprises two species and one variety, viz., *Ommatobrephus singularis* Nicoll (1914), *Ommatobrephus lobatum* Mehra (1928) and *Ommatobrephus lobatum* var. *najii* Mehra (1928). The new species, *Ommatobrephus nicolli*, resembles *Ommatobrephus singularis* in the position of genital pore, but differs from it in the greater length of the intestinal caeca, lobed testes and in the extension of the vitellaria. With regard to the lobed condition of the testes, the new species resembles *Ommatobrephus lobatum* and *Ommatobrephus lobatum* var. *najii*, but it stands apart from them in the more cephalad position of the genital pore, i.e., ventral to intestinal bifurcation and the greater length of the intestinal caeca and the vitellaria. The new species has been named after Dr. William Nicoll who erected the genus.

Key to the species of the Genus *Ommatobrephus* Nicoll, 1914.

I. Intestinal caeca extending to the anterior region of the second half of the body, i.e., much in front of the testes; testes oval and entire; genital pore ventral to the intestinal bifurcation; vitellaria from the level of the middle of the body to in front of the testes or much behind the intestinal caeca .. .. *Ommatobrephus singularis* Nicoll, 1914.

II. Intestinal caeca reaching the posterior region of the second half of the body, but stop slightly in front or in the level of the anterior border of the testes or at the middle of the testes; testes lobed; genital pore in between the intestinal bifurcation and ventral sucker; vitellaria extending up to the blind ends of the intestinal caeca or little beyond them .. .. *Ommatobrephus lobatum* Mehra, 1928.

III. Intestinal caeca terminate in the testicular zone; testes lobed; genital pore ventral to intestinal bifurcation; vitellaria stop a little in front of the blind ends of the intestinal caeca .. *Ommatobrephus nicolli*, n.sp.

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ON A NEW SPECIES OF THE GENUS *GANEO* KLEIN, 1905, FROM THE INTESTINE OF *RANA CYANOPHLYCTIS*

by

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ON A NEW SPECIES OF THE GENUS *GANEO* KLEIN, 1905, FROM THE INTESTINE OF *RANA CYANOPHLYCTIS*

By N. K. Gupta, M.Sc., Ph.D., *Lecturer in Zoology, Panjab University College, Hoshiarpur*

Introduction

The new species *Ganeo panjabensis* found along with other digenetic trematodes from the intestine of *Rana cyanophlyctis* has been described here. A key to all the species known is also given. The work was carried out in the Department of Zoology, Panjab University College, Hoshiarpur.

Genus **Ganeo** Klein, 1905.

The Genus *Ganeo* Klein (1905) comprises nine species up to now. Klein in 1905 obtained a new distome from the intestine of *Rana hexadactyla* from Madras, to which he assigned the name *Ganeo glottoides* under a new genus *Ganeo* and placed it provisionally in the sub-family pleurogenetinae Looss (1899). Skrjabin in 1922 got a new variety *G. glottoides* var. *africana* in the collection made by Prof. Dogiel and Sokolov in British East Africa. Four years later, Bhalerao (1926) reported the type species *G. glottoides* Klein (1905) from *Rana tigrina*, a common frog of India. Mehra and Negi in 1928 recorded another new species, namely, *G. tigrinum*, and a new variety, *G. glottoides* var. *madrasensis*. Bhalerao (1936) reported *G. korkei* which he had collected from *Rana tigrina* at Nagpur in 1928. Srivastava (1933) added two more species to the genus, namely, *G. gastricus* and *G. attenuatum*, the former species from the stomach and the latter from the intestine of *Rana cyanophlyctis* at Sitapur (India).

Pande in 1937 reported the eighth species, *G. kumaonensis* from the intestine of *Rana cyanophlyctis* in the Kumaon hills. In 1950, Kaw described the ninth species, *G. srinagarensis* from *Rana cyanophlyctis* in Kashmir. The author has added another species, i.e., *G. panjabensis*, n.sp., from *Rana cyanophlyctis* at Hoshiarpur.

Genus **Ganeo** Klein, 1905.

Diagnosis

Lecithodendriidae Odhner, 1910; Pleurogenetinae Looss, 1899. Shape: moderately elongated, elliptical, oval or oblong. Cuticle: partly or wholly spinous. Suckers: ventral is placed at the margin of anterior or middle third of the body length. Digestive system: oesophagus short or moderately long; intestinal caeca extending about or beyond three-fifths of body length but never touching the posterior extremity. Reproductive system: testes in the posterior part of the anterior third or half of the body, obliquely placed behind each other or opposite, one on each side of the median line;

ovary post-testicular; vitellaria confined to lateral sides beneath the intestinal caeca, the extent variable; pseudocirrus sac present or absent; uterus transversely coiled in the posterior region of the body; genital pore on left lateral side of the anterior region of the body at level of the oesophagus or the intestinal fork. Excretory system: bladder U- or V-shaped, with or without a short median stem, excretory pore terminal or subterminal.

Parasitic in the intestine of Amphibians.

Locality: India, British East Africa.

Type species: *G. glottoides* Klein, 1905.

Ganeo panjabensis, n.sp.

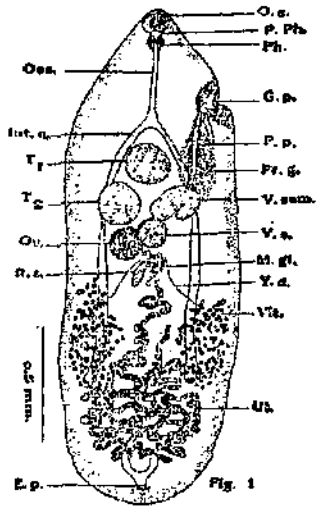

Fig. 1

FIG. 1. Ventral view of *Ganeo panjabensis*, n.sp.

E.p., excretory pore; *G.p.*, genital pore; *Int.c.*, intestinal caecum; *M.gl.*, Mehlis' gland complex; *O.s.*, oral sucker; *Oes.*, oesophagus; *Ov.*, ovary; *P.p.*, pars prostatica; *P.ph.*, prepharynx; *Ph.*, pharynx; *Pr.g.*, prostate glands; *R.s.*, receptaculum seminis; T_1, testis (first); T_2, testis (second); *Ut.*, uterus; *V.s.*, ventral sucker; *V.sem.*, vesicula seminalis; *Y.d.*, yolk duct.

Host: *Rana cyanophlyctis.*
Location: Intestine.
Locality: Hoshiarpur (India).

Only one specimen of *Ganeo panjabensis*, n.sp., was found along with other digenetic trematodes from the intestine of *Rana cyanophlyctis*. The body is dorso-ventrally flattened with broad round posterior end and tapering anterior end. The living specimen shows marked power of contraction and expansion. The body measures 1·89 mm. in length and 0·78 mm. in maximum breadth which is at the level of the testicular region. The body around the pharynx and partly around the oesophagus is surrounded by closely arranged spines, the pointed ends of which are directed towards the posterior end. Behind this region of the body, the number of spines goes on decreasing up to post-testicular region beyond which the spines disappear altogether.

The oral sucker is subterminal, placed at the anterior end. It measures 0·075 mm. in length and 0·12 mm. in breadth. The ventral sucker measuring 0·135×0·135 mm., is situated at a distance of 0·87 mm. from the anterior end. It is larger than the oral sucker. The mouth is placed at the bottom of the oral sucker and leads posteriorly into a small prepharynx, which in turn is followed by a pear-shaped pharynx measuring 0·045 mm. in length and 0·09 mm. in breadth. The oesophagus is moderately long, 0·3 mm. in length and 0·03 mm. in width. At a distance of 0·465 mm. from the anterior end it forks into two intestinal caeca which gradually diverge to run parallel along the lateral sides of the body, terminating at a distance of 0·465 mm. from the posterior end.

The two testes are almost spherical, the anterior one 0·18×0·195 mm. in size, is placed just posterior to the intestinal fork in the median line. The posterior testis measuring 0·195×0·195 mm. is placed obliquely at a distance of 0·03 mm. from the anterior testis, in front of the ovary and partly covering the right intestinal caecum. The vesicula seminalis is swollen and coiled like a hook, lying to the left of the posterior testis in front of the ventral sucker. Its distal end partly covers the left intestinal caecum. The pars prostatica is a long narrow duct measuring 0·285 mm. in length and is surrounded by a large number of prostate gland cells. The pars prostatica is followed by a short ejaculatory duct which opens into the genital atrium.

The ovary is almost a rounded structure measuring 0·135×0·135 mm. in size and is placed just behind the posterior testis in between the right intestinal caecum and the ventral sucker. From its postero-lateral side is given off an oviduct which soon receives the duct of the receptaculum seminis. The latter is like a cucumber in shape, lying behind the ovary and the ventral sucker. The two vitelline ducts, one from each side of vitellaria, open into a small median yolk reservoir lying just behind the ventral sucker. The ootype is surrounded by the Mehlis' glands. The vitellaria lie ventral to the intestinal caeca extending from the level of the posterior border of the receptaculum seminis to the terminal ends of the intestinal caeca. The uterus is much coiled, containing eggs at different stages of development. The uterine coils are confined to the space between the two intestinal caeca, behind the blind ends of which they expand transversely reaching the forked excretory bladder. The terminal part of the uterus is not clearly visible in the specimen. The deep genital atrium lies on the left body margin at a distance of 0·375 mm. from the anterior end. The eggs measure 0·0238–0·034 mm. in length and 0·0068–0·0102 mm. in breadth. The excretory bladder is U-shaped and opens through a very small wide duct into the excretory pore situated subterminally on the ventral surface a little in front of the posterior end of the body.

Relationships

Ganeo panjabensis, n.sp., differs from all the species of the genus in the more caudad position of the vitellaria which beginning from a point markedly behind the Mehlis' gland-complex extend up to the blind ends of the intestinal caeca. In the other species, the vitellaria begin more

anteriorly at the level of the posterior testis or ovary and terminate far in front of the ends of the caeca. It differs further from *G. glottoides* Klein (1905), *G. glottoides* var. *madrasensis* Mehra and Negi (1928) and *G. glottoides* var. *africana* Skrjabin (1922) in the size of ventral sucker as compared to the oral sucker, in the absence of pseudocirrus sac. It also differs from *G. attenuatum* Srivastava (1933) in which the ventral sucker is equal to the oral sucker and the excretory bladder is V-shaped.

KEY TO THE SPECIES OF THE GENUS *GANEO* KLEIN, 1905.

Ventral sucker smaller than oral sucker ..... A
Ventral sucker equal to oral sucker ..... B
Ventral sucker larger than oral sucker C

A.	Pseudocirrus sac straight or slightly curved; oesophagus long; intestinal caeca to 3/5ths body length	*G. glottoides* Klein, 1905.
	Pseudocirrus sac strongly curved; oesophagus small; left intestinal caecum reach ⅔ths body length, the right reaches the posterior end of the body	*G. glottoides* var. *madrasensis* Mehra and Negi, 1928.
B.	Pseudocirrus sac present; metraterm absent; excretory bladder U-shaped	*G. glottoides* var. *africana* Skrjabin, 1922.
	Pseudocirrus sac absent; metraterm present; excretory bladder V-shaped	*G. attenuatum* Srivastva, 1933.
C.	Intestinal caeca extending beyond vitellaria ..	C 1.
	Intestinal caeca not extending beyond vitellaria	*G. panjabensis*, n.sp.
C 1.	Excretory bladder V-shaped ..	*G. korkei* Bhalerao, 1936.
	Excretory bladder U-shaped ..	C 2.
C 2.	Excretory pore ventral	*G. kumaonensis* Pande, 1937.
	Excretory pore subterminal ..	C 3.
C 3.	Excretory bladder without a median stem	*G. gastricus* Srivastava, 1933.
	Excretory pore with a median stem ..	C 4.
C 4.	Testes placed obliquely	*G. tigrinum* Mehra and Negi, 1928.
	Testes side by side (one on each side) ..	*G. srinagarensis* Kaw, 1950.

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September, 1954

ON TWO NEW SPECIES OF TREMATODE PARASITES FROM BIRDS IN HOSHIARPUR

by

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ON TWO NEW SPECIES OF TREMATODE PARASITES FROM BIRDS IN HOSHIARPUR

By N. K. Gupta, M.Sc., Ph.D. *and* B. K. Dhillon, M.Sc.,
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Introduction

Trematode parasites are quite common in birds. In 1951-52, a large collection of trematodes was made from different birds in Hoshiarpur. Out of this collection we have found two new forms, viz., *Cardiocephalus halcyonis* and *Parastrigea duboisi* belonging to the family Strigeidae Railliet (1919). The former species was obtained from the intestine of *Halcyon smyrnensis smyrnensis* (Kingfisher) and the latter from the gut of *Pseudogyps bengalensis* (Vulture) and *Aquila rapax vinthiana* (Eagle). The other trematodes collected have also been arranged here hostwise.

This work was carried out in the zoological laboratory of the Panjab University College, Hoshiarpur. We are very thankful to Dr. Vishwa Nath who very kindly provided the laboratory facilities.

Family Strigeidæ Railliet, 1919.

(I) Subfamily Cotylurini Dubois, 1936.

Genus **Cardiocephalus** Szidat, 1928.

Cardiocephalus halcyonis, n.sp. (Fig. 1).

The specimens of *Cardiocephalus halcyonis*, n.sp. were collected from the intestine of *Halcyon smyrnensis smyrnensis* (Kingfisher) shot at Hoshiarpur.

The live worm is transparent and somewhat whitish in appearance. The body is elongated and divided into two anterior and posterior regions by a very conspicuous constriction. It is 1·206–1·9497 mm. long and 0·2412–0·4824 mm. broad. The anterior segment is pyriform with the apex directed anteriorly. It is much smaller than the posterior segment and measures 0·2412–0·402 × 0·2412–0·4824 mm. The posterior segment is subcylindrical and measures 1·005–1·5678 × 0·3216–0·5628 mm.

The suckers are feebly developed. The oral sucker is subterminal, transversely elongated and measures 0·0451–0·0533 × 0·0861–0·0902 mm. It is larger than the ventral sucker which is also transversely elongated and measures 0·0328–0·041 × 0·0369–0·0615 mm. The holdfast organ is not very conspicuous and lies behind the ventral sucker.

The muscular pharynx is elongated and measures 0·0287–0·0451 × 0·0287–0·0492 mm. It is followed by a short oesophagus which is 0·0287–0·065 mm. in length. The oesophagus bifurcates into two intestinal caeca

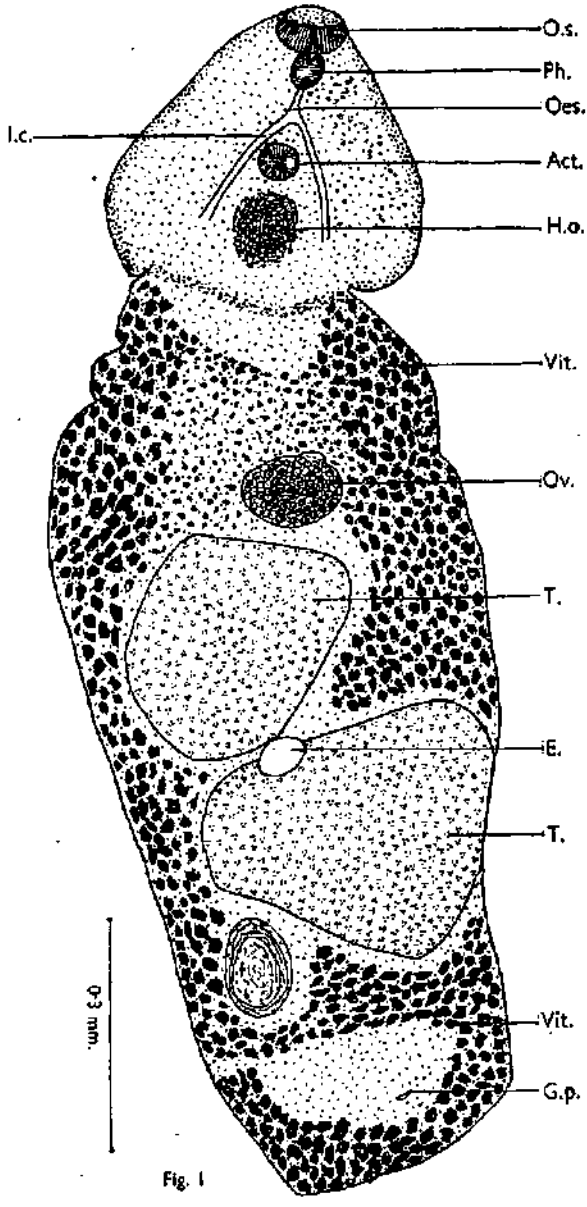

Fig. 1

FIG. 1. *Cardiocephalus halcyonis*, n.sp.

Act.—Acetabulum; *E.*—Egg; *G.p.*—Genital pore; *H.o.*—Holdfast organ; *I.c.*—Intestinal caecum; *O.s.*—Oral sucker; *Oes.*—Oesophagus; *Ov.*—Ovary; *Ph.*—Pharynx; *T.*—Testis; *Vit.*—Vitellaria.

which are smooth and their blind ends terminate near the posterior end of the body.

The testes are two in number and lie one behind the other in the posterior segment of the body. The anterior testis is subpentagonal and is slightly towards the right of the median line. It measures 0·252–0·294 × 0·252–0·364 mm. The posterior testis is subtriangular with its left lateral

border coinciding with the left lateral wall of the body. It measures 0·28–0·35×0·266–0·392 mm. The vesicula seminalis is present behind the posterior testis towards the right side of the median line.

The ovary is oval or rounded in shape and lies in the pre-testicular region. It measures 0·084–0·14×0·098–0·154 mm. The genital pore lies a little in front of the posterior extremity of the body. The vitellaria are confined only to the posterior region. They cover all the spaces between the organs and the bodywall but towards the sides they are densely distributed. The vitelline follicles are of medium size. The eggs are small, oval and thin shelled, 0·0964×0·0533 mm. in size.

Relationships

The genus *Cardiocephalus* Szidat (1928) comprises the species *C. brandesii* Szidat (1928), *C. hilli* (Johnston, 1904) Szidat, 1928, *C. longicollis* (Rudolphi, 1819) Szidat, 1928, *C. musculosus* (Johnston, 1904) Szidat, 1928, and *C. physalis* (Lutz, 1926) Dubois, 1937.

The new species *C. halcyonis* differs from *C. brandesii* in having smaller ratio between the lengths of anterior and posterior segments, different shape and position of the testes and the ovary, and from *C. hilli* in which there is a marked extra constriction in the post-testicular region, in the extent of vitellaria and in possessing the region of the copulatory purse larger than the breadth of the anterior segment. In the shape and size of the segments and in the position and shape of genital organs, the new species stands apart from *C. longicollis*.

The new species *C. halcyonis* also deviates from *C. physalis* and *C. musculosus* in which the hinder region of the second segment bulges laterally.

Specific diagnosis

Shape and size: Body elongated, divided into anterior and posterior segments by a prominent constriction; anterior segment pyriform, smaller than posterior, 0·2412–0·402×0·2412–0·4824 mm.; posterior segment much longer than the anterior, subcylindrical, 1·005–1·5678×0·3216–0·5628 mm. *Colour*: White and transparent. *Suckers*: Oral larger than ventral, transversely elongated, subterminal, 0·0451–0·0533×0·0861–0·0902 mm.; ventral also transversely elongated, 0·0328–0·041×0·0369–0·0615 mm.; holdfast organ inconspicuous. *Gut*: Pharynx smaller than oral sucker, slightly elongated, 0·0287–0·0451×0·0287–0·0492 mm.; oesophagus short, 0·0287–0·065 mm. long; intestinal caeca smooth, reach almost the posterior end of the body. *Genital systems*: Male: testes two, post-ovarian, anterior testis subpentagonal, slightly towards the right of median line, 0·252–0·294×0·252–0·364 mm.; posterior testis subtriangular, 0·28–0·35×0·266–0·392 mm.; vesicula seminalis present. Female: ovary oval or rounded, pretesticular, 0·084–0·14×0·098–0·154 mm.; genital pore a little in front of posterior end of the body; vitellaria confined to the posterior segment. *Eggs*: oval small and thin shelled, 0·0964×0·0533 mm. in size.

(2) Subfamily Strigeinæ Railliet, 1919.

Genus **Parastrigea** Szidat, 1928.

Parastrigea duboisi, n.sp. (Fig. 2).

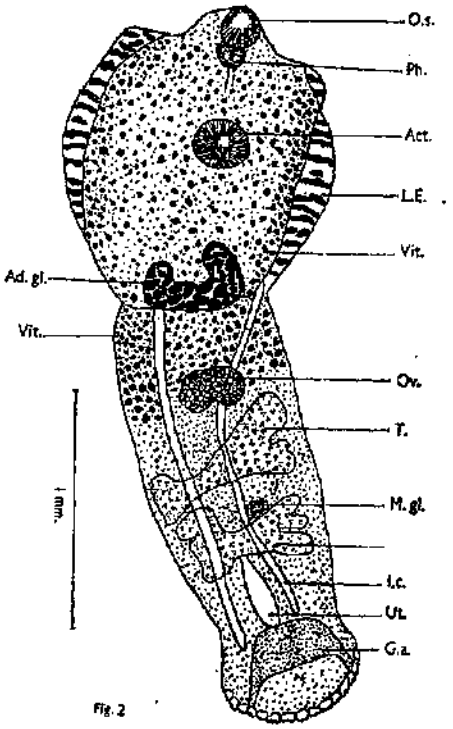

FIG. 2. *Parastrigea duboisi*, n.sp.

Act.—Acetabulum; *Ad.gl.*—Adhesive gland; *G.a.*—Genital atrium; *I.c.*—Intestinal caecum; *L.e.*—Lateral expansion; *M.gl.*—Mehlis' gland; *O.s.*—Oral sucker; *Ov.*—Ovary; *Ph.*—Pharynx; *T.*—Testis; *Ut.*—Uterus; *Vit.*—Vitellaria.

About a half dozen worms of this new species were collected from the intestines of *Pseudogyps bengalensis* (Vulture) and *Aquila rapax vindhiana* (Eagle) shot at Hoshiarpur.

The worm is elongated, having anterior and posterior segments. It measures 1·5276–3·2964 mm. in length and 0·644–1·3668 mm. in breadth. The anterior segment is somewhat globular, the posterior barrel-shaped and is much longer than the anterior one. Its breadth varies from 0·4824–1·0452 mm. In the anterior segment there are present two medium sized lateral expansions in which are also confined the vitelline follicles.

The oral sucker is rounded, subterminal and measures 0·1025–0·182 × 0·0861–0·21 mm. It is smaller than the ventral sucker which is transversely elongated and measures 0·1476–0·28 × 0·154–0·308 mm. The adhesive-gland is trough-shaped. In some specimens it is elliptical in shape. It measures 0·154–0·257 × 0·266–0·616 mm. It lies at the base of the anterior segment close to the union of the two segments.

The pharynx is partially overlapped by the oral sucker. It is spherical and varies 0·0615–0·14×0·0433–0·14 mm. The oesophagus is short and measures 0·0328 mm. in length. The intestinal caeca are smooth and lie dorsally to the testes. Their blind ends terminate a little anterior to the genital atrium.

The two testes are lobed and elongated transversely. They are post-ovarian and lie one behind the other. The lobes are very conspicuous on the lateral margins of each testis. The anterior testis measures 0·42× 0·686 mm., while the posterior 0·42×0·588 mm.

The ovary lies in the anterior part of the second segment of the body, i.e. a little behind the body constriction. Its shape varies from oval to oblong with a conspicuous notch at its posterior border. It measures 0·154–0·196×0·28–0·462 mm. The Mehlis' gland complex is a small structure and lies in between the two testes. The uterus is convoluted and opens into the genital atrium. The vitellaria are present in both the anterior and the posterior segments of the body. They extend anteriorly up to the level of the posterior border of the pharynx. Along the lateral sides of the body, they are densely distributed as compared to the middle region. They are also present in the lateral expansions of the anterior segment. In the posterior segment they extend up to the level of the middle of the ovary. The vitelline glands are conspicuously absent in the entire post-ovarian region.

Relationships

There are three species, namely, *Parastrigea cincta* (Brandes, 1888) Szidat, 1928, P. *intermedia* Tubangui, 1932, and P. *robusta* Szidat, 1928, known to the genus *Parastrigea*. The new species *P. duboisi* differs from all the three already known forms in the shape of the anterior segment, testes and adhesive organ, the size of the lateral expansions and the distribution of the vitellaria.

The species has been named after the name of Dr. George Dubois whose work on strigeid worms is well known.

Specific diagnosis

Shape and size: Body elongated, divided into anterior and posterior segments, 1·5276–3·2964 mm. long and 0·644–1·3668 mm. broad; the anterior segment somewhat globular, the posterior barrel-shaped. The former is longer than the posterior and 0·4824–1·0452 mm. in breadth with two medium sized lateral expansions. *Suckers*: oral rounded, subterminal, smaller than ventral, 0·1025–0·182×0·0861–0·21 mm.; ventral sucker transversely elongated, well developed, 0·1476–0·28×0·154–0·308 mm.; adhesive gland trough-shaped, oval or elliptical, 0·154–0·257× 0·266–0·616 mm., lies at the posterior part of the anterior half and close to the union of two segments. *Gut*: prepharynx, slightly overlapped by the oral sucker, spherical, 0·0615–0·14×0·0433–0·14 mm.; oesophagus short, 0·0328 mm. long; intestinal caeca smooth reaching a little in front of the genital atrium. *Genital systems*: Male: testes two, lobed, transversely

elongated, post-ovarian, lie one behind the other; anterior testis 0·42× 0·686 mm.; posterior testis 0·42×0·588 mm. Female: ovary lies a little behind the body constriction, oval or oblong sometimes with a conspicuous notch at its posterior border, 0·154–0·196×0·28–0·462 mm.; Mehlis' gland complex inter-testicular, small; uterus convoluted, opens into the genital atrium; vitellaria present in both anterior and posterior segments, in the anterior they occupy the whole post-pharyngeal region, in the posterior they are confined up to the middle of ovary.

TREMATODE PARASITES RECORDED FROM BIRDS IN HOSHIARPUR

Name of Host.	Name of Parasite.
Tringa hypoleucos (Sand piper) ..	*Cyclocoelum mehrii* Khan, 1935.
Lobivanellus indicus indicus (Lapwing). ..	*Haemetotrephus nebularium* Khan, 1935. *Paramonostomum elongatum* Yamaguti, 1934. *Neodiplostomum tytense* Patwardhan, 1935. *Neodiplostomum pseudoattenuatum* (Dubois, 1928) Dubois, 1932. *Neodiplostomum* sp.
Ardeola grayii (Paddy bird) ..	*Echinochasmus megavitellus* Lal, 1939. *Strigea falconis* Verma, 1936.
Astur badius dussumieri (Hawk) ..	*Echinochasmus bagulai* Verma, 1935. *Strigea falconis eaglesa* Verma, 1936. *Neodiplostomum pseudoattenuatum* (Dubois, 1928) Dubois, 1932. *Neodiplostomum lucidum* La Rue and Bosma, 1927. *Neodiplostomum* sp.
Falco jugger (Luggar falcon) ..	*Echinochasmus bagulai* Verma, 1935.
Pseudogyps bengalensis (Vulture) ..	*Parastrigea duboisi*, n.sp. *Strigea globocephala* Verma, 1936. *Neodiplostomum lucidum* La Rue and Bosma, 1927. *Neodiplostomum* sp.
Aquila rapax vindhiana (Eagle) ..	*Parastrigea duboisi*, n.sp. *Strigea globocephala* Verma, 1936.
Egretta garzetta garzetta (Egret) ..	*Strigea falconis eaglesa* Verma, 1936.
Halcyon smyrnensis smyrnensis (Kingfisher). ..	*Cardiocephalus halcyonis*, n.sp.

Name of Host.	Name of Parasite.
Sterna aurantia (Tern)	*Bolbophorus orientalis* Vidyarthi, 1938. *Alaria robusta* Verma, 1936.
Sarcogyps calvus (King Vulture) ..	*Neodiplostomum* sp.

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RESEARCH BULLETIN
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ON *ENCYCLOMETRA VITELLATA*, A NEW SPECIES FROM WATER-SNAKE, *NATRIX PISCATOR*

by

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ON *ENCYCLOMETRA VITELLATA*, A NEW SPECIES FROM WATER-SNAKE, *NATRIX PISCATOR*

By N. K. Gupta, M.Sc., Ph.D., *Lecturer in Zoology, Panjab University College, Hoshiarpur*

Two specimens of *Natrix piscator* collected from Budha Nala, an arm of Sutlej river at Ludhiana, were examined for helminths. They were found to harbour many specimens of *Encyclometra vitellata*, n.sp. The worm (Fig. 1) is flat, elongated with round anterior end and gradually tapering posterior end. The body is smooth without any spines. The oral sucker is smaller than the ventral sucker. It is placed subterminally at the anterior end and measures 0·646–0·714 mm. in length and 0·680–0·765 mm. in breadth. Histologically, the oral sucker consists of interior circular, exterior circular and radial muscles. There are also 2-3 bands of circular muscles at the posterior end as an indication of posterior sphincter muscles. The ventral sucker measures 0·918–1·118 mm. in length and 0·935–1·156 mm. in breadth. It is composed of anterior external circular, anterior internal circular, posterior external circular and posterior internal circular muscles. There are also present in it exterior and interior longitudinal and radial muscles.

The prepharynx is quite distinct. The pharynx is also quite prominent and measures 0·323–0·425 mm. long and 0·340–0·493 mm. broad. Instead of interior circular muscles, it possesses interior longitudinal muscles. The oesophagus is very small and is surrounded by oesophageal gland cells. Intestinal caeca are lateral in position, terminating a little in front of the posterior end of the body. The right caecum is always smaller than the left. The intestinal bifurcation lies at a distance of 0·527–1·020 mm. in front of the ventral sucker.

The testes are oval or subspherical, placed one behind the other in the median line behind the ventral sucker and much in front of the posterior end of the body. The anterior testis lies at about the middle of the body and measures 0·289–0·498 mm. in length and 0·255–0·476 mm. in breadth. The posterior testis is just behind the anterior testis but lies in the second half of the body. It is 0·340–0·544 mm. long and 0·340–0·476 mm. broad. The cirrus sac lies transversely in front and slightly to the left of the ventral sucker and measures 0·629–0·731 mm. in length and 0·221–0·238 mm. in breadth. The vesicula seminalis is a coiled structure and lies at the base of the cirrus sac. The pars prostatica is an enlongated tubular structure covered by the prostate gland cells. The ejaculatory duct is long and coiled before it opens at the genital pore which is situated to the left of the ventral sucker and just close to the left intestinal caecum.

The ovary is transversely or obliquely elongated and lies just behind the ventral sucker either in the median line or to the right of it. It

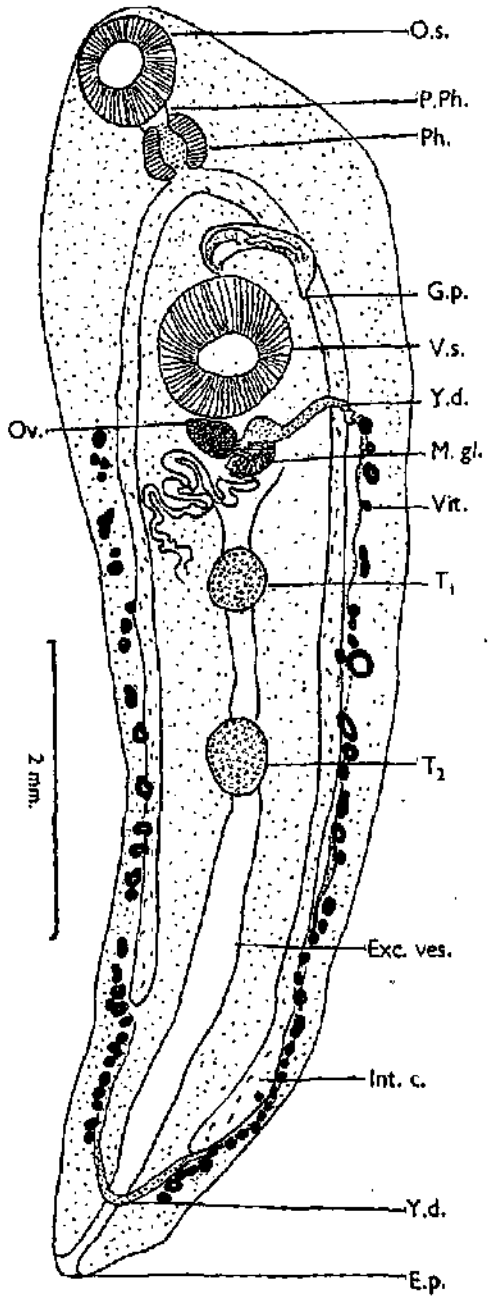

Fig. 1

FIG. 1. Ventral view of *Encyclometra vitellatu*, n.sp.

E.p.—Excretory pore; *Exc.ves.*—Excretory vesicle; *G.p.*—Genital pore; *Int.c.*—Intestinal caecum; *M.gl.*—Mehlis' gland complex; *O.s.*—Oral sucker; *Ov.*—Ovary; *P.ph.*—Prepharynx; *Ph.*—Pharynx; T_1.—Anterior testis; T_2.—Posterior testis; *V.s.*—Ventral sucker; *Vit.*—Vitellaria; *Y.d.*—Yolk duct.

measures 0·238–0·357 mm. in length and 0·255–0·391 mm. in breadth. The distance between the ovary and the anterior testis is almost equal to the distance between the latter and the posterior testis. The Mehlis' gland-complex lies close behind the ovary and to the left of it. The oviduct emerges from the left margin of the ovary and it is joined by the tubular receptaculum seminis and the duct of the yolk reservoir. The Laurer's canal is present.

The uterine coils are inter-caecal running along the inner sides of the intestinal caeca, leaving space in the middle where the excretory vesicle runs up to the posterior border of the ovary. The vitelline glands are extra-caecal, extending either from the level of the ovary or slightly behind it to a little in front of the posterior end of the body. The main vitelline ducts proceed anteriorly where they give off two transverse vitelline ducts which open into the yolk reservoir. An interesting feature noticed in this species is the union of two vitellaria by another transverse vitelline duct at the posterior end of the body.

The eggs measure 0·060–0·088 mm. in length and 0·028–0·040 mm. in maximum breadth.

The excretory vesicle is Y-shaped, the two lateral cornua are short.

Relationships

The new species *Encyclometra vitellata* differs from *E. caudata* (Polonio, 1859), *E. japonica* Yoshida and Ozaki (1929), *E. microrchis* Yamaguti (1933) and *E. asymmetrica* Wallace (1936) in having an additional transverse vitelline duct lying in the posterior region of the body.

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ON *PLEUROGENES (TELOGONELLA) SAWANENSIS*, N.SP., PARASITIC IN THE INTESTINE OF *RANA CYANOPHLYCTIS* WITH A DISCUSSION ON THE SYSTEMATIC POSITION OF THE GENUS *PLEUROGENES* LOOSS, 1896

by

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By N. K. Gupta, M.Sc., Ph.D., *Lecturer in Zoology, Panjab University College, Hoshiarpur*

Genus **Pleurogenes** Looss, 1896

Rudolphi in 1819 described *Distoma clavigerum* from the intestine of Anurans, which was subsequently described by V. Linstow in 1888 as *Dist. negletum*. Olsson (1876) described *Dist. medians* from the intestine of Anurans. Sonsino (1894) described *Dist. tacapense* parasitic in the intestine of reptile—*Chamaelea basiliscus*, which was changed to *Dist. tacapensis* by Looss two years later. The genus *Pleurogenes* was created by Looss in 1896 with *Dist. clavigerum* as its type species. In 1898, Looss named *Dist. tacapense* as *Pleurogenes tener* and a year later transferred it to the genus *Prosotocus* Looss (1899). He also pointed out the synonymity of *Dist. negletum* with *Dist. clavigerum* and *Dist. tacapense* with *Dist. medians*.

A distome parasitic in frogs in America was described in 1900 as *Dist. arcanum* and *Dist. medians* by Nickerson and Stafford respectively. Luhe (1901) described *Pleurogenes gastroporus* from the intestine of *Rana cyanophlyctis* in India. Stafford in 1904 created the genus *Loxogenes* for *Dist. arcanum* Nickerson (1900) on account of the position of its genital pore midway between the left intestinal caecum and the body margin. Klein (1905) assigned *Prosotocus tener* Looss (1899) to the genus *pleurogenes*. He also described a new species, *Pleurogenes sphericus* from the intestine of *Rana hexadactyla* and *Dist. arcanum* Nickerson (1900) found encysted in the liver, pyloric region and around the neck of the urinary bladder of some representatives of the family Ranidae. Johnston in 1912 described *Pleurogenes freycineti* and *P.solus*, n.spp., parasitic in the intestine of *Hyla freycineti* and *H. aurea* respectively.

Travassos in 1921 split up the genus *Pleurogenes* into two genera *Pleurogenes* and *Pleurogenoides*. The former genus comprised the species in which intestinal caeca extend beyond the ventral sucker and the latter genus those having short caeca, never extending beyond the centre of the ventral sucker. Ozaki in 1926 added another new species *Pleurogenes lobatus*, from the bile duct of Japanese frogs—*Polypedates buergeri*. In the same year Isaitschikow described *Pleurogenes intermedius*. Mehra and Negi in 1928 described *Pleurogenes gastroporus* var. *equalis*, n.var. The joint authors referred the species *Loxogenes arcanum* to the genus *Pleurogenes*. They divided the genus *Pleurogenes* into two subgenera, *Pleurogenes* (*Pleurogenes*), *Pleurogenes* (*Telogonella*) on the basis of the length of the intestinal caeca and the position of the genital pore. They separated under the

subgenus *P.* (*Pleurogenes*) those species in which the intestinal caeca reach near middle of body about the level of ventral sucker, and the genital pore lies in front of the intestinal bifurcation near the oral sucker. The subgenus *P.* (*Telogonella*) comprised the species having longer intestinal caeca extending much behind the ventral sucker and reaching about the last quarter of the body and the genital opening at level with or behind intestinal bifurcation about midway between the pharynx and the ventral sucker.

Tubangui in 1928 recorded a new species, *P. taylori*, from the intestine of *R. vittegera* in philippine and maintained the genus *Loxogenes* for *Pleurogenes arcanum*. Fuhrmann (1928) also maintained the genus *Loxogenes*. Travassos in 1930 described *P. stromi*, n.sp., from the intestine of *R. esculenta* and maintained his genera *Pleurogenes* and *Pleurogenoides* as valid. He reaffirmed this view in a subsequent paper the following year. Africa (1930) recorded *P. loossi* from *Rana esculenta*.

Piguleswsky in 1931 described *P. minus* parasitic in the intestine of pike—*Esox lucios*. Krull in 1933 retained the genus *Loxogenes* and added to it another new species, *Loxogenes bicolor*. Srivastava in 1934 suppressed the genera *Loxogenes* and *Pleurogenoides* and retained the *Pleurogenes* and its two subgenera (*Pleurogenes*) and (*Telogonella*). He described two more new species, *Pleurogenes orientalis* and *Pleurogenes sitapuri* from *R. cyanophlyctis*. Yamaguti (1936) got another new species, *P. japonicus* from *Rana nigromaculata*. Pande (1937) gave the account of *Pleurogenes pabdai*, n.sp., from a fish—*Callichrous pabda*. Bhalerao (1936) dropped the variety *Pleurogenes gastroporus* var. *equalis* Mehra and Negi (1928).

Kaw in 1943 maintained the genera *Pleurogenes* Looss (1896) emend. *Travassos (1921)*, *Pleurogenoides Travassos (1921)*, *Loxogenes Krull (1933)* emend. Kaw (1943) and added a new genus, *Pleurolobatus* for *P. lobatus* Ozaki (1926). He described one new species, *Pleurogenoides bufonis*, from the intestine of a toad—*Bufo viridis*. He removed the species *Loxogenes arcanum* (Nickerson, 1900) Stafford (1904) from the genus *Loxogenes* Krull (1933) to the genus *Pleurogenoides*. In the genus *Loxogenes* emend. Kaw (1943) he included *Loxogenes bicolor* Krull (1933).

Kaw in 1945 formed another new genus *Loxogenoides* for *Loxogenes bicolor* as the type species. *Loxogenes arcanum* (Nickerson, 1900) Stafford (1904) was removed from the genus *Loxogenes* and was put under the genus *Pleurogenoides*. Under the international rules of zoological nomenclature he could not do so, therefore, he created a new genus *Loxogenoides* for the reception of *Loxogenes bicolor* Krull (1933).

Pleurogenes (Telogonella) sawanensis, n.sp. (Fig. 1.)

Host: *Rana cyanophlyctis*.
Location: Intestine.
Locality: Hoshiarpur (India).

Only two specimens of *Pleurogenes* (*Telogonella*) *sawanensis*, n.sp., were found in the intestine of *Rana cyanophlyctis* dissected in Zoological Laboratory of the Panjab University College, Hoshiarpur. The body is ovoid, 0·54–0·64 mm. long and 0·39–0·42 mm. broad across the region between

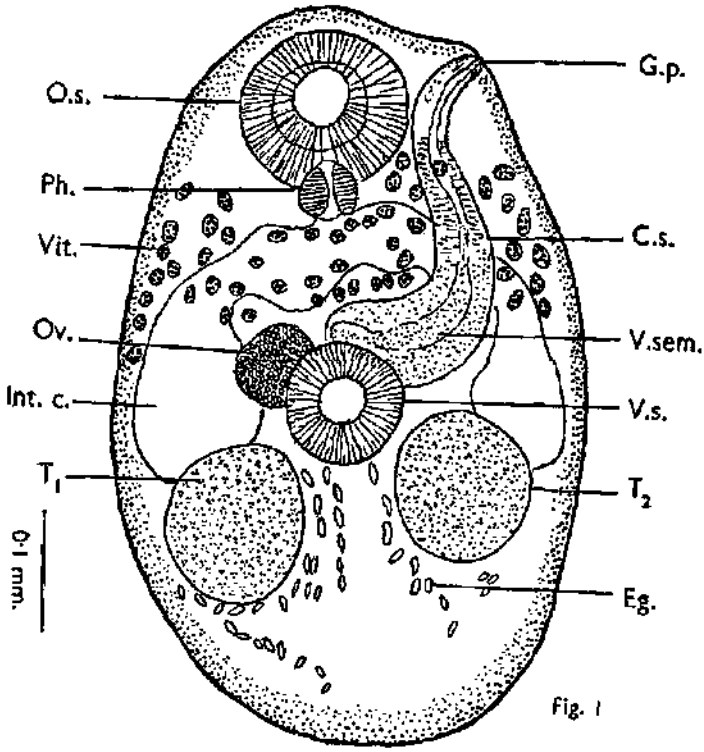

FIG. 1. Ventral view of *Pleurogenes* (*Telogonella*) *sawanensis*, n.sp.

Eg.—Egg; *C.s.*—Cirrus sac; *G.p.*—Genital pore; *Int.c.*—Intestinal caecum; *O.s.*—Oral sucker; *Ov.*—Ovary; *Ph.*—Pharynx; T_1.—Right testis; T_2.—Left testis; *V.s.*—Ventral sucker; *V.Sem.*—Vesicula seminalis; *Vit.*—Vitellaria.

the ovary and the testes. The cuticle of the body is beset with small backwardly directed spines. The suckers are broader than long. The oral sucker, measuring 0·1292–0·136 mm. in length, 0·153–0·156 mm. in breadth, is situated ventrally close to the anterior end of the body. The ventral sucker, measuring 0·1054–0·119×0·1292–0·1428 mm., is situated at a distance of 0·27–0·3 mm. from the anterior extremity of the body. A little portion of the ventral sucker is in the first half and the major portion in the second half of the body.

The oral sucker is followed by a short pharynx which measures 0·0476–0·0612×0·0646–0·068 mm. Oesophagus is absent. From the base of the pharynx arise the two intestinal caeca which first diverge towards the lateral sides, then proceed posteriorly terminating slightly behind the ventral sucker. During their course, each intestinal caecum shows two dilatations, one near the bifurcation of the caeca, the other at the blind end.

The testes are placed one on each side of the median line, at the junction of middle and posterior thirds of the body and postero-lateral to the ventral sucker. The right testis is globular, measuring 0·1088–0·1292 mm. in length and 0·0952–0·1292 mm. in breadth. The left testis 0·119–0·1326×0·0952–0·119 mm. in dimensions. The cirrus pouch extends from the genital pore to the front of the ventral sucker, with its terminal part partially overlapping

it. It is a curved structure, measuring 0·15 mm. in length and 0·06 mm. in maximum breadth. Its basal portion contains coiled vesicula seminalis, while its anterior part contains the pars prostatica surrounded by the prostate gland cells and the ejaculatory duct. The cirrus is quite conspicuous and it opens into the genital atrium which in turn opens on the left body margin at the level of the oral sucker.

The ovary is almost spherical, 0·051–0·0646 × 0·0578–0·0646 mm. in size, lies at the junction of first and second halves of the body, to the right of the median line, close to the right intestinal caecum and the antero-lateral border of the ventral sucker. The coils of the uterus are a few, lying behind and in between the testes. The vitellaria lie scattered extending from behind the oral sucker to the level of the middle of the ovary. The *eggs measure 0·017–0·024 × 0·0068–0·0102 mm. in size.*

The excretory pore is on the ventral side, the excretory bladder is V-shaped, the cornua extending up to the antero-lateral sides of the testes.

Relationships.

On account of its intestinal caeca extending behind the ventral sucker, the new species belongs to the subgenus, *Pleurogenes (Telogonella)*. This subgenus comprises *P. claviger* (Rudolphi, 1819), *P. intermedius* Isaitschikow (1926), *P. lobatus* Ozaki (1926), *P. loossi* Africa (1930), *P. bicolor* (Krull, 1933) and *P. orientalis* Srivastava (1934).

In *P. (Telogonella) sawanensis*, n.sp., the main stem of the excretory bladder is much smaller than the cornua. In this character it resembles all the other species of the subgenus except *P. bicolor* in which the main stem of the excretory bladder is much longer than the cornua. The new species stands apart from *P. lobatus*, in which the gonads are lobulated, from *P. loossi* in which the testes are obliquely situated one behind the other and *P. claviger* and *P. orientalis* in which the testes are symmetrically situated one on each side behind the termination of the intestinal caeca. It further differs from *P. orientalis* in the position of the ovary and the extension of the cirrus sac. The new species also differs from *P. intermedius* in the position of the testes and the genital pore which in the latter lies inwards to the body margin.

Discussion on the Systematic Position of the Genus *Pleurogenes* Looss, 1896.

Looss in 1896 established the genus *Pleurogenes*. Stafford in 1904 created the genus *Loxogenes* for *Distomum arcanum* Nickerson (1900) on account of the position of the genital opening which lies on the ventral surface midway between the left intestinal caecum and the body margin. As suggested by Stafford (1904), Travassos in 1921 created a new genus *Pleurogenoides* for those species of the genus *Pleurogenes* in which intestinal caeca are short and never extend behind the ventral sucker. The genus *Pleurogenes* was retained by him for *Pleurogenes claviger*. Mehra and Negi (1928) dropped the genera *Loxogenes* and *Pleurogenoides* and split up the

genus *Pleurogenes* into two subgenera, *P.* (*Pleurogenes*) and *P.* (*Telogonella*), on the basis of the length of the intestinal caeca and position of the genital pore. In the former subgenus the intestinal caeca reach near the middle of body about the level of ventral sucker and the genital opening lies in front of the intestinal bifurcation near the oral sucker. In the latter the intestinal caeca extend much behind the ventral sucker, reaching about the last quarter of body length and the genital opening lies at level with or behind the intestinal bifurcation, about midway between the pharynx and the ventral sucker. Srivastava (1934) followed Mehra and Negi (1928) in maintaining the two subgenera. Travassos in 1930 and 1931 maintained the two genera, *Pleurogenes* and *Pleurogenoides*. Kaw (1943) created a new genus *Pleurolobatus* for *Pleurogenes lobatus* Ozaki (1926). He also emended the genus *Loxogenes* and removed its type species *L. arcanum* (Nickerson, 1900) Stafford (1904) to the genus *Pleurogenoides* and instead placed *L. bicolor* Krull (1933) as its only representative. Two years after, the same author suppressed the genus *Loxogenes* and proposed a new genus *Loxogenoides* for *L. bicolor*.

The genus *Loxogenoides* has been suppressed by Mehra and Negi (1928) and Srivastava (1934). Tubangui (1928), Fuhrmann (1928), Krull (1933) and Macy (1936) are in favour of retaining this genus. Kaw (1945) changed the genus *Loxogenes* to *Loxogenoides*. This genus was created by Stafford on account of the different position of the genital pore. But there are species such as *P. sphericus* and *P. intermedius* in which the genital pore lies far inwards to the left body margin. In *Dist. arcanum* Nickerson (1900) for which this genus was created, the position of the genital pore is disputable. Stafford (1904) points out its presence on the dorsal surface behind the pharynx. Osborn (1912) describes its presence near the intestinal fork. Since the genital pore also lies far inwards in some other species, it is necessary to drop the genera *Loxogenes* and *Loxogenoides* Kaw (1945).

The genus *Pleurogenoides*, as we have already stated, was created by Travassos (1921) for those species in which the intestinal caeca never extend behind the ventral sucker. Srivastava (1934) points out that the two genera, *Pleurogenes* and *Pleurogenoides* could be accepted only so long as the generic differences between them are of absolute value and the intermediate forms connecting them do not exist. The two genera—*Pleurogenes* and *Pleurogenoides*—are now connected by intermediate forms, such as *P. intermedius*, *P. lobatus*, *P. orientalis* and *P. sawanensis*, n.sp. It, therefore, becomes necessary to drop the genus *Pleurogenoides*. The genus *Pleurolobatus*, based only on the lobed character of its gonads, is also untenable, for it resembles in all its other characters the genus *Pleurogenes*. The lobed nature of the gonads can at best be regarded as of specific value in classification.

The classification adopted by Mehra and Negi (1928) is a convenient arrangement and has been adopted.

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RESEARCH BULLETIN

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MICROPHOTOGRAPHS DEMONSTRATING THE VACUOME, GOLGI BODIES, MITOCHONDRIA AND NUCLEOLAR EXTRUSIONS IN THE FRESH EGGS OF FROG AS STUDIED UNDER THE PHASE CONTRAST MICROSCOPE

by

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Department of Zoology, Panjab University College, Hoshiarpur

AN EXAMINATION OF THE YOLK-NUCLEUS OF THE SPIDER PLEXIPPUS PAYKULLI UNDER THE PHASE CONTRAST MICROSCOPE

by

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MICROPHOTOGRAPHS DEMONSTRATING THE VACUOME, GOLGI BODIES, MITOCHONDRIA AND NUCLEOLAR EXTRUSIONS IN THE FRESH EGGS OF FROG AS STUDIED UNDER THE PHASE CONTRAST MICROSCOPE

By Vishwa Nath *and* Sudarshan Kumar Malhotra, *Department of Zoology, Panjab University College, Hoshiarpur*

Introduction

In 1931 Nath published a paper demonstrating the Vacuome and the Golgi apparatus as independent cytoplasmic components in the fresh eggs of the frog. His conclusions may be summarized as follows:—

In *Rana tigrina* small vacuoles with watery contents appear throughout the cytoplasm when the oocyte measures about ·3 mm. The vacuoles are stainable with neutral red and cannot be blackened with Da Fano or Kolatchev, however heavy the impregnations. In the course of oogenesis the vacuoles grow enormously in size, the biggest vacuole measuring as much as ·02 mm. At the same time their contents grow denser and they look slate-coloured. They can now be seen in fresh oocytes even without the aid of neutral red. These vacuoles, however, do not condense the albuminous yolk within their interior, contrary to what happens in the fish *Ophiocephalus*. The albuminous yolk arises directly from the mitochondrial granules, which form a characteristic ring at the periphery of the cytoplasm. In the earliest oocytes the mitochondrial granules are arranged in a circum-nuclear fashion, but ultimately they are uniformly dispersed throughout the cytoplasm. The Golgi elements can be seen in the fresh egg-cells from the earliest to the most advanced stages. In the undifferentiated germ cells and in the earliest oocytes they are in the form of dark-greyish refractile granules. In later stages many of them grow into tiny vesicles, each vesicle showing a dark-greyish cortex and a less refractile medulla. In the earliest oocytes they are juxta-nuclear. The Golgi elements are intensely blackened with Da Fano and Kolatchev, and they cannot be stained in neutral red.

When the oocyte measures about 1·2 mm. many of the Golgi elements form the fatty yolk, stainable with Sudan III and Scharlach R (Nath, 1932).

In the present paper we have recorded our observations on the living oocytes of *Rana tigrina*, as examined and microphotographed under the Phase Contrast Microscope. We are publishing here five such microphotographs, giving an excellent demonstration of the Golgi elements, mitochondria and watery vacuoles as independent cytoplasmic cell-components.

We have observed nucleolar extrusions in *Rana tigrina* and *R. Cyanophlyctis*, which have not been recorded by any previous worker.

One of us (S. K. M.) has gone over the whole oogenesis of the frog, employing all the methods previously employed by Nath (1931, 1932). In addition Sudan IV (B.D.H.) and Scharlach R (Rubrum Scarlatinum, B.P.C., B.D.H.) were employed for staining the *fatty* yolk. We have confirmed in every detail the conclusions of Nath (1931 and 1932).

It is our very pleasant duty to thank Mr. H. S. Vasisht, Demonstrator in Zoology, for preparing the microphotographs.

Observations

Plate I, figure 1 represents a part of the peripheral region of a living oocyte of *Rana tigrina*, measuring 0·45 mm. in diameter. The mitochondrial granules (*M*) have formed the characteristic ring at the periphery, and give rise directly to the albuminous yolk. The Golgi granules (*G*) can be clearly seen in the general cytoplasm, but here and there they have collected to form irregular masses, rods or crescents, which are all artefacts. The Golgi elements shine as highly refractile and jet-black granules in sharp contrast with the much smaller and greyish mitochondria, forming the background. The watery vacuoles, which are small at this stage, have mostly coalesced, forming whitish irregular patches.

Plate I, figure 2 is a microphotograph of a part of the general cytoplasm of an oocyte, measuring 0·456 mm. in diameter. Here the watery vacuoles, the small greyish mitochondria forming the background, and the refractile Golgi granules, many of which are aggregated in small groups, can be very clearly seen side by side. Plate I, figure 3 is a microphotograph of a part of the cytoplasm of a bigger oocyte (0·6 mm. in diameter). It will be noticed that the watery vacuoles have grown in size, and appear as the most prominent cell-components.

Nath (1931) examined the oocytes of *Rana tigrina* at Lahore (now in Pakistan) during the winter months of hibernation. He did not examine oocytes after the month of March. One of us (S. K. M.) prepared Bouin's preparations of the oocytes of *R. tigrina* at Hoshiarpur, collected during the months of April and May, and did not observe any nucleolar activity in the form of nucleolar extrusions. But oocytes of frogs collected in the month of June showed nucleolar extrusions (Pl. I, fig. 4). This is a microphotograph of a living oocyte, measuring 0·12 mm. in diameter. The nuclear membrane is out of focus and a large number of nucleoli can be seen in the nucleus. Some of these nucleoli seem to be extruded into the cytoplasm, where they quickly disappear. They rapidly lose their rounded form, and, becoming granular, completely break down and disappear. Small Golgi granules can also be seen in the background. We have not noticed nucleolar extrusions in oocytes measuring less than about 0·12 mm., but they are met with in slightly older oocytes (Pl. I, fig. 5). They completely disappear long before the yolk-forming mitochondrial ring is established at the periphery of the oocyte.

In figure 6, Plate II, is represented a section of an oocyte of *R. tigrina*, fixed in Bouin's fluid and stained with iron haematoxylin, showing a few nucleolar extrusions.

In figure 7, Plate II, is represented a part of an oocyte of *Rana cyanophlyctis*, as seen under the Phase Contrast. There is a fairly large number of nucleolar extrusions, which look exactly like the nucleoli, a large number of Golgi vesicles, and a background of small greyish mitochondrial granules.

Discussion

The oocytes of *Rana tigrina* seem to be an ideal material for the demonstration of the watery vacuoles (vacuome), Golgi bodies and mitochondria side by side in the living cell. It also seems to be a very favourable material for demonstrating the origin of albuminous yolk bodies directly from the mitochondrial granules.

Nath (1931), who examined oocytes of *R. tigrina* at Lahore during the winter months of hibernation, did not observe any nucleolar activity. One of us (S. K. M.) also did not observe any nucleolar activity in the oocytes of *R. tigrina* collected in the months of April and May. But prominent nucleolar extrusions have been observed and described by us in the oocytes of *R. tigrina* collected in the month of June. It would thus appear that there is a seasonal nucleolar activity in the oocytes of *Rana tigrina*.

In *R. cyanophlyctis*, which is a much smaller species with a warty skin, however, nucleolar activity has been observed even in the month of April. This earlier nucleolar activity on the part of this species of frog seems to be closely related to the fact stated by Nath (1931) that in *R. cyanophlyctis* the eggs 'ripen' earlier than in *R. tigrina*.

It must, however, be repeated that in both the species of frog described here the nucleolar extrusions disappear completely and quickly, without making any visible contribution to yolk-formation.

Summary

1. The living oocytes of *Rana tigrina* have been studied under the Phase Contrast Microscope and microphotographed.

2. Watery, neutral red-staining, vacuoles (vacuome), argentophilic and osmiophilic Golgi elements, not stainable with neutral red, and mitochondrial granules have been demonstrated side by side in the living oocytes of *R. tigrina* as independent cell-components.

3. There is a seasonal nucleolar activity in the oocytes of *R. tigrina*. Throughout the period of winter hibernation and during the following months of April and May, no nucleolar extrusions have been observed, but oocytes of frogs collected in the month of June showed prominent nucleoli, which had been extruded into the cytoplasm. The extrusions however, quickly disappear and do not make any visible contribution to yolk-formation.

4. The albuminous yolk bodies arise directly from the mitochondria, which form a characteristic ring at the periphery of the oocyte.

5. In the oocytes of *R. cyanophlyctis* nucleolar extrusions have been detected even in the month of April.

Explanation of Plates

All the figures of Plate I are microphotographs of the oocytes of *Rana tigrina*, as studied under the Phase Contrast. Leitz Dialux Phase Contrast Microscope with ×10 Periplanatic Eye-piece and 40 : 1 objective (Apochromatic dry system), giving a magnification of 500 times, and Leica Camera were used.

Plate II, figure 6 is a part of a section of an oocyte of *R. tigrina*, measuring 0·224 mm. × 0·196 mm., fixed in Bouin's fluid and stained with iron haematoxylin.

Plate II, figure 7 is a part of a living oocyte of *R. cyanophlyctis*, measuring 0·24 mm. in diameter, studied under the Phase Contrast, and drawn free hand.

Lettering

G—Golgi body; *M*—Mitochondria; *N*—Nucleus; *N.E.*—Nucleolar extrusions; *NU*—Nucleolus.

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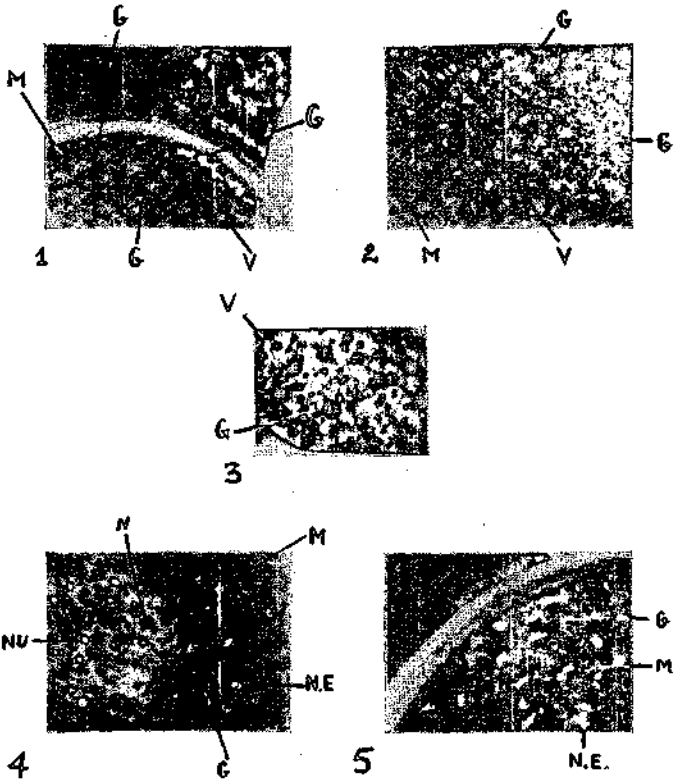
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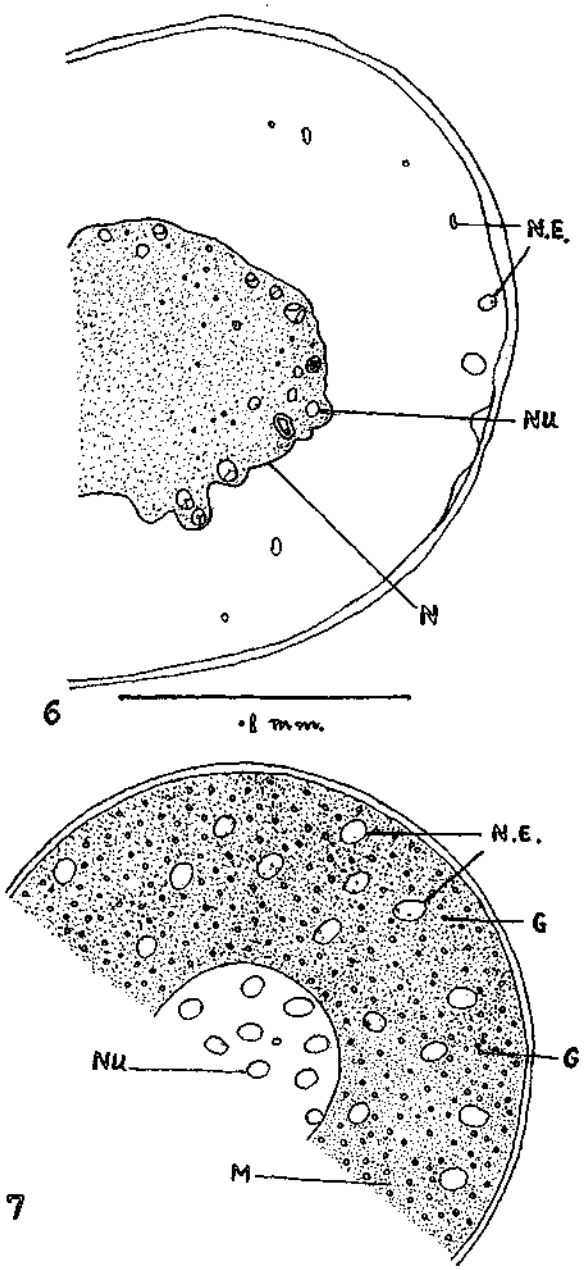
PLATE II.



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AN EXAMINATION OF THE YOLK-NUCLEUS OF THE SPIDER PLEXIPPUS PAYKULLI UNDER THE PHASE CONTRAST MICROSCOPE

By VISHWA NATH *and* RAJINDAR M. DHAWAN, *Department of Zoology, Panjab University College, Hoshiarpur*

INTRODUCTION

Sukh Dyal and Nath (1933) published a paper on the nature of the 'Yolk-nucleus' of spiders, with particular reference to the yolk-nucleus of *Plexippus paykulli*. In addition to the routine laboratory methods of fixation and staining employed by them, they examined in detail living oocytes of this spider. Their conclusions may be summarized as follows.

In the oogonium the mitochondria and the Golgi elements exist in the form of a juxta-nuclear mass. With the growth of the oogonium this juxta-nuclear mass begins to spread out in the cytoplasm. Both these cell elements can be easily observed *intra vitam* under an ordinary microscope. The mitochondria appear as greyish granules and the Golgi elements as more refractile and perfectly spherical bodies. Throughout oogenesis the mitochondria remain as small granules, but some of the Golgi spheres grow slightly in size.

Immediately after the mitochondria and the Golgi elements have uniformly dispersed in the cytoplasm and the nucleus has become slightly ex-centric, the characteristic yolk-nucleus puts in its appearance. The yolk-nucleus was never observed by the authors in oocytes measuring 0·08 mm. or less. In living oocytes studied in a drop of normal saline the yolk-nucleus appears as a spherical capsule which, of all the cell-components, stands out most prominently in the cytoplasm. The cortex of the capsule is made of concentrically arranged threads, and the medullary region contains dark-greyish, refractile, spherical bodies. The former are the mitochondrial threads which, according to the authors, are formed by a concentric alignment of mitochondrial granules, and the latter are the Golgi spheres. Some Golgi bodies also exist along the mitochondrial fibres. According to the authors the yolk-nucleus is a structure of remarkable solidity, so much so that it retains its spherical form even after the egg is ruptured, and it may even drop out in the process of section-cutting.

The authors also demonstrated Golgi and mitochondrial elements in the form of granules distributed uniformly throughout the cytoplasm. Albuminous yolk granules appear for the first time at the periphery of the oocyte. They arise *de novo* in the cytoplasm without any visible association either with the mitochondria or the Golgi elements. Gradually they grow in size and invade the interior of the cell. Sukh Dyal and Nath did

not examine advanced oocytes, so that they did not describe any fatty yolk.

In this paper we have recorded our observations on this material as examined under the Phase Contrast, and we are also publishing a microphotograph of the yolk-nucleus. One of us (R. M. D.) has also used all the fixatives previously employed by Sukh Dyal and Vishwa Nath (1933); and we are glad to confirm all the conclusions arrived at by these authors. Sudan IV has also been employed by us on this material.

It is our very pleasant duty to thank Mr. H. S. Vasisht, Demonstrator in Zoology, for preparing the microphotographs.

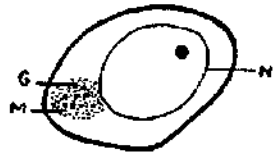
Observations

Text-figures 1 and 2 and figures 3 to 7, Plate I, have been drawn free hand, and they are of living oocytes as examined under the Phase Contrast microscope. The size of each oocyte has been recorded.

Text-fig. 1.

Young oogonium measuring 0·012 mm. in diameter.

Text-fig. 1 represents what we consider as the youngest oogonium. There is a juxta-nuclear mass, consisting of greyish mitochondrial granules in which are embedded a few slightly refractile Golgi granules. These latter shine as jet-black granules, bigger than the mitochondrial granules. The rest of the cytoplasm is absolutely free from granulation of any type, and appears as a hyaline jacket for the nucleus. There is no nucleolus. Text-fig. 2 represents a slightly grown up oogonium in which a rounded nucleolus has appeared in the nucleus, but the juxta-nuclear mass of mitochondrial and Golgi elements is still intact.



Text-fig. 2.

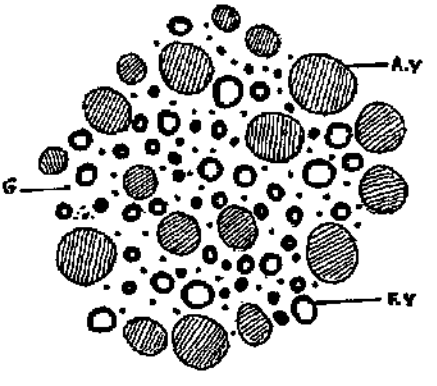
A bigger oogonium, measuring 0·032 mm. in diameter.

Plate I, fig. 1 represents a young oocyte in which nurse-cells are seen attached to one pole and the juxta-nuclear mass has divided into two parts, lying opposite each other. The nucleolus has grown and has become irregular in outline. In the nurse-cells a few Golgi elements can be seen as highly refractile granules, but there are no traces of mitochondria.

The characteristic yolk-nucleus appears for the first time when the oocyte measures about 0·082 mm. in diameter (Pl. I, fig. 2). By this time all the mitochondrial and Golgi granules of the juxta-nuclear mass of the oogonium have spread out uniformly throughout the cytoplasm. The Golgi granules seem to have grown in size by this time. The yolk-nucleus appears as a tightly-packed capsule, consisting of concentrically arranged cortical mitochondrial fibres and medullary Golgi granules. The latter shine as highly refractile, jet-black granules in sharp contrast with the greyish mitochondrial fibres. Gradually the yolk-nucleus grows in size, and the medullary Golgi bodies also grow appreciably (Pl. I, figs. 3 to 6).

In Plate I, fig. 7 is represented an oocyte, measuring 0·31 mm. The yolk-nucleus has grown in size, but remains intact showing no indication of fragmentation. Its medullary Golgi bodies have, however, grown appreciably in size. The mitochondrial granules and the Golgi bodies of the general cytoplasm remain uniformly dispersed, and have not appreciably grown in size. But the albuminous yolk bodies, which appear *de novo* for the first time at the periphery of the oocyte, have grown in size and are invading the interior of the oocyte.

Plate II, fig. 8 is a microphotograph of the yolk-nucleus, as seen under the Phase Contrast. Although the cortical and concentrically arranged mitochondrial fibres do not appear in the microphotograph, their characteristic concentric arrangement is betrayed by the concentrically arranged Golgi bodies along their course, especially on the right side of the photograph. The medullary Golgi bodies are out of focus and are appearing in the form of white spheres. The background of the general cytoplasm is represented by the very small and greyish mitochondrial granules. Against this background the bigger and more refractile Golgi bodies stand out prominently, also dispersed throughout the cytoplasm. Nevertheless, at several places the Golgi granules aggregate and form rods and small irregular masses, which must be interpreted as artefacts. Such a rod can be seen very clearly at *X* in the microphotograph as having been formed by the alignment of the Golgi granules.



TEXT-FIG. 3

Crushed contents of an advanced oocyte stained in Scharlach R or Sudan IV.

Scharlach R (Rubrum Scarlatinum B.P.C., B.D.H.) and Sudan IV (B.D.H.) have been employed to ascertain if the Golgi bodies contain any fat. It may be stated very definitely that the Golgi bodies of *Plexippus paykulli* are not stainable with these fat dyes till a very late stage in oogenesis. In Text-fig. 3 are represented diagrammatically the contents of a crushed oocyte of an advanced age stained with Scarlach R or Sudan IV. At *A.Y* are the albuminous yolk bodies, and at *F.Y* are the fatty yolk spheres, which have arisen directly from the Golgi bodies. The fatty yolk spheres appear red, and the albuminous yolk spheres do not stain at all with these fat dyes.

Summary

1. The oocytes of *Plexippus paykulli* have been studied under the Phase Contrast microscope, and the characteristic 'Yolk-nucleus' has been photographed.

2. A juxta-nuclear mass of greyish mitochondrial granules and bigger, highly refractile Golgi bodies can be very clearly seen in the earliest oogonium.

3. This juxta-nuclear mass fragments and spreads out throughout the cytoplasm till the mitochondrial granules and the Golgi bodies are dispersed uniformly.

4. The mitochondrial granules remain unchanged throughout oogenesis, the albuminous yolk appearing *de novo* at the periphery of advanced oocytes. But in still more advanced oocytes the Golgi bodies become fatty inasmuch as they begin to stain brilliantly with Scharlach R and Sudan IV, and thus give rise directly to the fatty yolk.

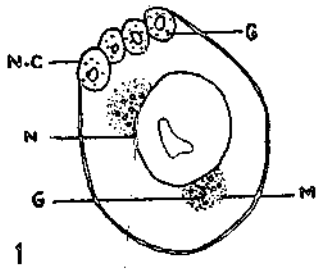
5. The essential form of the Golgi bodies is granular or spherical, but due to artificial alignment or aggregation Golgi rods and irregular Golgi bodies may be formed.

6. Golgi bodies can be very clearly seen and photographed in the nurse-cells also.

7. The characteristic yolk-nucleus of *Plexippus paykulli* appears for the first time when the oocyte measures about 0·082 mm. in diameter. It appears as a tightly-packed capsule, consisting of concentrically arranged mitochondrial fibres and medullary Golgi granules. The latter shine as highly refractile, jet-black granules in sharp contrast with the greyish mitochondrial fibres. The Golgi bodies also exist along the course of mitochondrial fibres. Gradually the yolk-nucleus grows in size, and so do its Golgi bodies. The yolk-nucleus seems to remain intact for a very long time in oogenesis, but its ultimate fate is still unknown.

Explanation of figures

All figures have been drawn free hand, but the measurement of the living cells have been given in every case. Leitz Dialux Phase Contrast microscope with ×10 Periplanatic Eye-piece and 40 : 1 objective (Apochromatic dry system) giving a magnification of 500 times was used. The microphotograph in Plate II, was taken with Leica Camera, and the photograph was enlarged about six times.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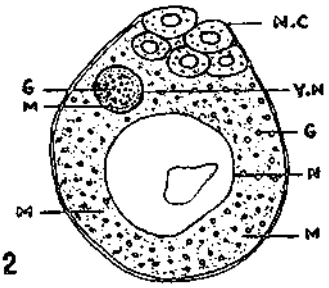
1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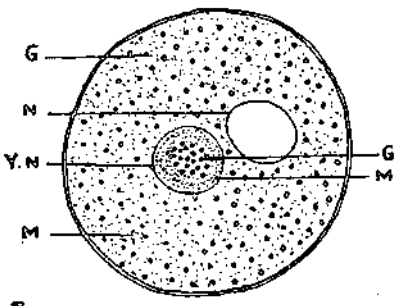
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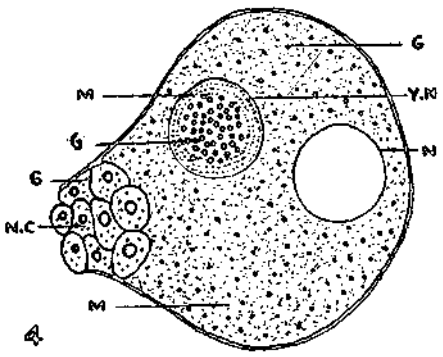
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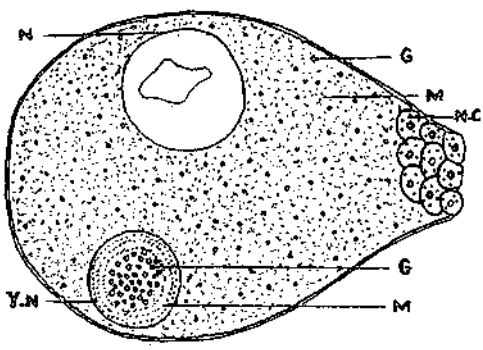
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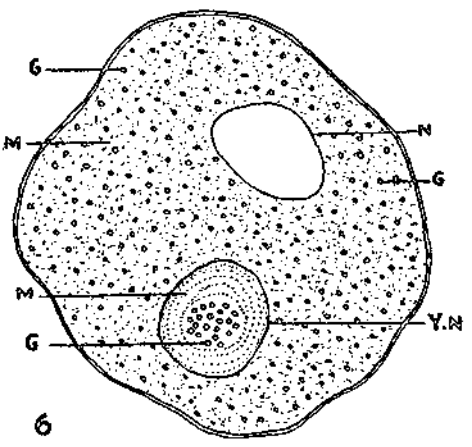
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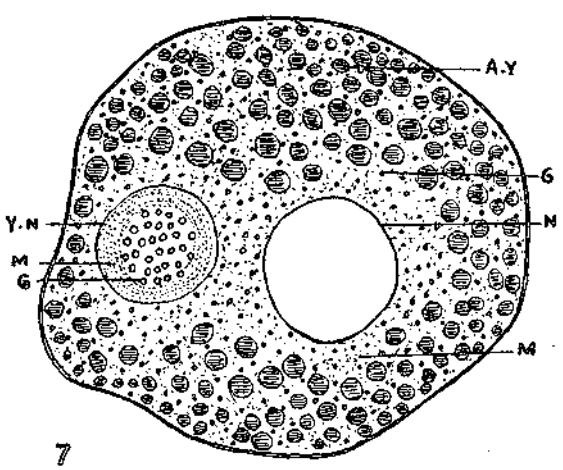
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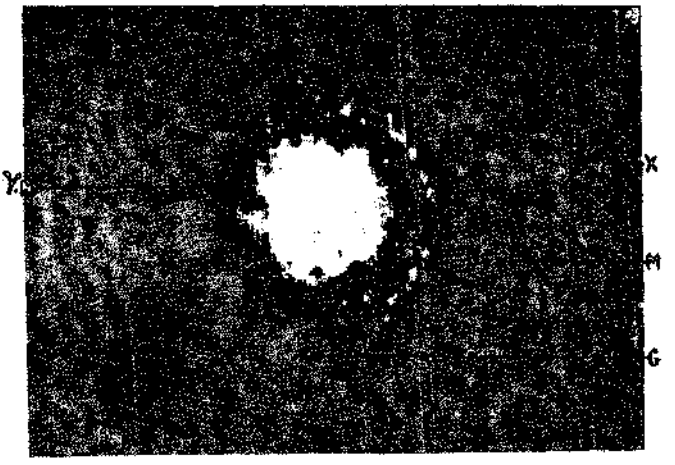
7

PLATE II.



Lettering

A.Y—Albuminous yolk; *F.Y*—Fatty yolk; *G*—Golgi body; *M*—Mitochondria; *N*—Nucleus; *N.C*—Nurse-cells; *Y.N*—Yolk-nucleus; *X*—Golgi rod.

Plate I

Fig. 1. An oocyte measuring 0·054 mm. in diameter.
„ 2. An oocyte measuring 0·082 mm. in diameter.
„ 3. An oocyte measuring 0·095 mm. in diameter.
„ 4. An oocyte measuring 0·12 mm. in diameter.
„ 5. An oocyte measuring 0·20 mm. in diameter.
„ 6. An oocyte measuring 0·25 mm. in diameter.
„ 7. An oocyte measuring 0·31 mm. in diameter.

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SPERMATOGENESIS OF A DIGENETIC TREMATODE, *CYCLOCOELUM BIVESICULATUM*

by

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SPERMATOGENESIS OF A DIGENETIC TREMATODE, *CYCLOCOELUM BIVESICULATUM*

By Om Parkash Dhingra, *Late Panjab University Research Scholar, Department of Zoology, Hoshiarpur.*

Introduction

There are some fundamental, controversial issues in the literature of the few cytological investigations of the adult digenetic trematodes. Many have regarded the sperm only a nuclear structure. Woodhead (1931) reported it partly cytoplasmic, and Dhingra (1954) observed a biflagellate sperm in *Isoparorchis eurytremum*.

The cytology of the adult flukes is also drawing the attention of many workers because of certain peculiar structures reported occasionally in the testes and the ovaries. Dingler (1910) and John (1953) have reported some globules, present at the periphery of the testis in the form of clusters of variable size and form, or scattered singly amongst the primordial germ cells. Another peculiarity has been reported by Markell (1943) and John (1953) in the form of ball-like structures met with in the peripheral region of the testis, but sometimes in the lumen. Markell (1943) has considered them as vacuolated cells, while John (1953) regarded them as pycnotic primordial spermatogonia. Chen (1937) observed some masses of small cells with scanty but granular cytoplasm and clear nuclei. These he regarded as degenerating spermatogonia. Anderson (1935) described nuclear degeneration or pycnosis of the spermatocytes and oocytes. Willey and Koulish (1950) reported similar pycnosis of the germ cells, which they compared with the degenerating oocytes of Anderson (1935). Still another abnormality is the occurrence of the oocytes in the testis, as reported by Anderson (1935).

The rôle and significance of these structures is highly problematical. Dingler (1910) attributed a nutritive function to the globules he found in his material. Anderson (1935), Chen (1937) and others, who reported a mass of pycnotic or degenerating cells in the testis, also assigned a nutritive rôle to these cells.

The author in this paper has described the spermatogenesis of a digenetic trematode, *Cyclocoelum bivesiculatum*. The specimens were obtained from the air sacs of a Red-billed Blue Magpie—*Urocissa erythrorhyncha* Boddaert. Several specimens were obtained on a single occasion from a male bird, in July, 1951, at Kulu (Punjab, I). The testes were fixed in Bouin's fluid for 24 hours and even longer. Sections were cut 8–14 μ thick.

ACKNOWLEDGMENTS

I wish to express my deep gratitude to Dr. Vishwa Nath, Professor and Head of the Zoology Department, Panjab University, for his help in preparing this paper and his constant encouragement and advice.

OBSERVATIONS

There are two rounded testes measuring from 0·65 mm. to 1·0 mm. in diameter and lying one behind the other between the intestinal caecae in the posterior part of the body. The anterior testis is not in line with the posterior, but is deflected to the right or left of the median line. The posterior testis lies in the median plane in the intestinal loop. There is a single ovary, which lies between the two testes. It is also rounded and measures from 0·2 mm. to 0·3 mm. in diameter.

Each testis is comparable to a hollow ball, filled with the maturing germ cells. The germ cells lie free in the testis cavity. There is hardly any zoning of the different stages of the germ cells, except that the primordial germ cells and the spermatogonia are generally to be found in the peripheral region. The spermatocytes, spermatids and the mature sperms may be present in any region.

Spermatogonia

The primordial germ cells and the spermatogonia, which are often found along the testicular wall, occur in the form of irregular patches of varying forms and sizes. At places there are no such cells. The primordial germ cells occur close to the testicular wall, but may also be present anywhere in the patch. Very often the outlines of these cells are difficult to make out, and there are no definite criteria for distinguishing them from the primary spermatogonia. The nuclei of the primordial germ cells are generally in the resting stage, and hardly show any chromatin except for one or two prominent nucleoli (Pl. I, fig. 1).

The primary spermatogonia, which measure from 12–14 μ in diameter, occur singly amongst the primordial and other spermatogonial cells. They are the largest of all the cells to be found in the testis, except the primary spermatocytes. The nuclei of these cells are often in the resting stage with a very prominent nucleolus. Sometimes, however, two nucleoli are seen. The cytoplasm shows a centrosomal granule lying close to the nucleus (Pl. I, fig. 2). The stages between the resting and metaphase stages have not been traced, as they seem to be rare. In the metaphase the diploid number of chromosomes (20) has been counted (Pl. I, fig. 3). The centrosomes and the spindle are sometimes seen in the side view (Pl. I, fig. 4). The chromosomes split and the daughter halves move towards the opposite poles (Pl. I, fig. 5). The cytoplasmic cleavage then follows, which leads to the formation of two cells remaining in close association.

These twin cells, which are nearly of the same size and form, are the secondary spermatogonia. Each measures about 10-11 μ in diameter. Like the primary spermatogonia the secondary spermatogonia are also

often seen in the resting stage and their nuclei have one or two nucleoli each. In the cytoplasm a centrosome is often seen (Pl. I, fig. 6). As the twin secondary spermatogonia divide simultaneously, they are easily recognised from the primary spermatogonium in mitosis. During metaphase the diploid number is countable only in some of the cells, as the chromosomes are often overlapping (Pl. I, fig. 7). The chromosomes then split simultaneously in both the cells, and the cytoplasmic cleavages are also simultaneous. This results in the formation of a group of four cells—the tertiary spermatogonia.

The tertiary spermatogonia, which may be recognised as a group of four cells, are found mixed up with the primary and secondary spermatogonia. In size they are smaller than the primary and secondary spermatogonia, and measure each 8-9 μ in diameter. As these cells are also very often in the resting stage, their nuclei do not reveal any structure save for the presence of some lightly stained chromatic granules and one or two prominent nucleoli. The cytoplasm very often contains a centrosome, lying close to each nucleus (Pl. I, fig. 8). The four cells undergo simultaneous nuclear division, and then reach the metaphase quickly (Pl. I, fig. 9). The small size of the cells and the chromosomes, coupled with the overcrowding of the latter, makes the chromosome count very difficult. The chromosomes are, however, seen in the same state in all the four cells. When the telophase stage is reached, the cytoplasmic cleavages set in (Pl. I, fig. 10). The division results in the formation of a cluster of eight cells, which grow to form the primary spermatocytes.

Spermatocytes

The clusters of the primary spermatocytes, which consist of eight cells each, are found in any region of the lumen of the testis. Individually these cells are the largest to be found in the sequence of sperm formation. Each spermatocyte measures from 12 μ to 17 μ. In the resting stage the nucleus is seen to have some chromatic granules and a nucleolus (Pl. I, fig. 11). The nuclear contents are activated for the meiotic behaviour of the chromosomes, and they are first organised to form the leptotene threads (Pl. I, fig. 12). In the subsequent zygotene stage the pairing of the homologous chromosomal threads sets in, but, as this process is very quick, it is rather difficult to trace the associates of the paired threads (Pl. I, fig. 13). The threads then enter the pachytene stage, when their open ends are oriented towards the nucleolus, and the looped condition now becomes evident. The number of the loops is rather difficult to determine, but they are never more than the haploid number of the chromosomes (Pl. I, fig. 14). In the forthcoming diplotene stage, the loops contract and become shorter. At first there appears a fused mass due to the shortening and overlapping of the loops, but soon the loops are spread out in the whole of the nucleus as distinct homologous pairs. Chiasmata are rather pronounced (Pl. I, fig. 15). At this stage, which may be called the diakinesis, ten bivalents can be counted. The bivalents then become thicker and shorter, and now stain uniformly. A centrosome is seen lying close to the nuclear wall. The centrosomal granule soon divides and the two resultant granules move

apart and ultimately come to lie just opposite each other (Pl. I, figs. 16 and 17). When the nuclear wall breaks down, the bivalent chromosomes are seen to lie on the spindle. In the metaphase plate, they are easily counted as ten thick and heavily stained rods or granules (Pl. I, fig. 18). The centrioles and the spindle can be observed in the side view (Pl. I, fig. 19). The homologous chromosomes then separate at the metaphase stage, and a diploid number of chromosomes can be counted (Pl. I, fig. 20). The resulting chromosomes are, however, small and short. The homologous sets then move apart towards the opposite poles. At the anaphase also the sets sometimes display a haploid number (Pl. I, fig. 21). When the telophase stage is reached the chromosomes are fused, and thus one thick mass near each pole is seen. The centrosomes are, however, not visible at this stage (Pl. II, fig. 22). When the segregation of homologous chromosomes in the cluster is completed, the cytoplasmic cleavages put in their appearance. A depression starts from the outer side of each dividing cell and gradually extends inward towards the centre of the cluster. This results in the formation of a cluster of 16 cells—the secondary spermatocytes.

The cells of the secondary spermatocyte cluster measure each from 9 μ to 10 μ in diameter. Soon after formation, their nuclei are reorganized into a resting stage. A centrosomal granule at this stage becomes visible (Pl. II, fig. 23). After a brief resting stage thick chromatic granules appear and the chromosomes are formed from these granules. The nuclear wall then disappears and the haploid number of chromosomes is spread on the metaphase plate (Pl. II, fig. 24). The chromosomes are short and thin as compared to the corresponding stage of the primary spermatocytes. In the side view the centrosomes and spindle become visible (Pl. II, fig. 25). The chromosomes split at the metaphase stage, and often two haploid sets of chromosomes are seen (Pl. II, fig. 26). The daughter chromosomes then move apart towards the poles. When the telophase stage is reached, there is a single compact chromatic mass near each pole (Pl. II, fig. 27). The centrosomes at this stage are not seen. Probably they are masked by the chromatic masses. Following the chromosomal divisions, there is a gradual ingrowing from the outer free borders of the cells in the cluster. This results in cytoplasmic division, and a resultant cluster of 32 cells, the spermatids, is formed.

Spermateleosis

In general appearance the spermatid clusters look like the secondary spermatocyte clusters, except for the larger number of cells and their smaller size. Each spermatid measures about 7–9 μ in diameter. When the spermatid nuclei start reorganizing the centrosome in each spermatid becomes visible (Pl. II, fig. 28). In a little more advanced stage a space appears around each spermatid nucleus, and a centrosome is seen lying close to this space (Pl. II, fig. 29). Gradually the centrosome in each cell comes to lie at the base of the nucleus (Pl. II, fig. 30). The spermatid nuclei then become elongated and appear ovate with the pointed end towards the outer free border, where a centrosomal granule is distinctly seen. The nuclei, however, show a weak staining reaction (Pl. II, figs. 31

and 32). Subsequently the spermatid nuclei become basiphilic, and as the nuclei become longer and thinner, they stain more and more intensely. A flagellum appears to arise from the centrosome (Pl. II, fig. 33), but as development proceeds the nucleus seems to pass insensibly into the axial filament. In the development process of the spermatid nuclei, the latter show coiling within the cytoplasm (Pl. II, fig. 34). When the sperms become mature, they leave the spermatid rosette and come to lie outside as spindle-shaped structures with the anterior half slightly thicker than the posterior. The sperms are generally seen in bundles. The evacuated spermatid rosettes look globular, and they are seen nearly in all the sections (Pl. II, fig. 35). The number of such globules is much larger in the mature worms.

A number of unusual structures have been noted in the present investigation. Very often at the periphery of the testis are observed certain small granules of different sizes, occurring either singly or in groups (Pl. II, fig. 36). They stain intensely with iron haematoxylin. They are also seen to extend towards the centre of the testis, but these latter are smaller than those seen at the periphery. Sometimes these granules are seen to lie amongst the spermatogonia.

Clear, faintly stained big globules of different sizes have been observed in the peripheral region. Occasionally such globules are seen in the interior of the testis. They are also found in the nourishing mass of the oocytes, met with in the testis (Pl. II, fig. 37).

Sometimes the spermatogonia fail to undergo proper mitotic divisions and are seen to exhibit a polyaster condition, or the chromosomes may be found scattered in the whole of the cytoplasm (Pl. III, figs. 38–40). Occasionally the spermatogonia either in the peripheral region or in the testis cavity are seen degenerating. Such cells are characterized by the presence of a big chromatic mass with a clear space around them (Pl. III, figs. 41–43).

Very often the testis shows fully developed oocytes and a nourishing mass associated with them. The formation of these structures can be traced from the patches of spermatogonial and primordial germ cells lying in the peripheral region. There first appear a few nuclei, which look like the yolk-cell nuclei amongst the primordial and the spermatogonial cells, lying close to the testicular wall (Pl. III, fig. 44). Later the germ cells seem to be concerned with the formation of deeply staining globules, which start appearing in such a mass of cells (Pl. III, figs. 45-46). The whole mass at this stage or even earlier breaks its contact with the testicular wall and becomes free to move in the lumen of the testis. Formation of the deeply staining globules continues and the normal mode of multiplication of germ cells is also exhibited. Some of the spermatogonia reach the spermatocyte-typed stage but not beyond that (Pl. III, fig. 47). The growth of the spermatocyte-typed cells continues and they reach the size of a fully grown oocyte, measuring 28 μ in diameter (Pl. III, fig. 48). These oocytes are generally seen in a post synaptic diffuse stage. The formation of the globules from the germ cells continues and very often these globules encircle the yolk cell nuclei, and the whole mass appears to be formed of

big cells (Pl. IV, fig. 49). Degeneration of the oocytes and the nourishing mass follows, and ultimately the globules, the yolk cell nuclei and the spermatogonia disappear. The degeneration of the oocytes starts with the appearance of chromatic globules inside their nuclei and small granules in the cytoplasm (Pl. IV, fig. 50). The degeneration of the nourishing mass and oocytes goes on simultaneously (Pl. IV, fig. 51). The oocytes very often leave the nourishing mass, and lie free in the testis cavity. The degenerated nourishing mass is also seen occasionally in the testis cavity (Pl. IV, fig. 52). Further degeneration of the oocytes in the testis cavity leads to its gradual decrease in size with the chromatic mass lying scattered in the cytoplasm (Pl. IV, figs. 53–57).

Chromosomes

The chromosome number as determined from *meiosis I and meiosis II* is 10 haploid, and the diploid number as determined from the mitotic figures is 20. The mitotic chromosomes are very often crowded and their critical study is rather difficult.

The largest pair of chromosomes is J-shaped and measures about 3·0 μ in length. In the meiotic stages it appears rod like. The second pair which measures 2·5 μ in length is straight. The third and the fourth pairs are again straight and they measure nearly 2 μ each. The rest of the chromosomes are quite short, hardly exceeding 1 μ.

Discussion

It is concluded that the process of spermatogenesis is essentially similar to that described in other digenetic trematodes. The outlines of the cells in each type of cluster remain distinct, as also reported by Anderson (1935), John (1953) and Dhingra (1954). The prophase stages of the spermatocytes run in conformity with the general plan outlined by Willmott (1950), Willey and Godman (1951), John (1953) and Dhingra (1954). Syndesis or the contraction figure which, according to Pennypacker (1940) and Willey and Godman (1951) occurs before the pachytene stage, has not been observed, and it appears that such a stage is not essential for all the trematodes.

The most important point, which can be emphasized in this communication, is the presence of a single centrosomal granule in the spermatid, which takes up its position at the base of the spermatid nucleus and forms the entire tail of the sperm along with the axial filament. The centrosome in the spermatid of the digenetic trematodes has not been reported before except in *Isoparorchis eurytremum* (Dhingra, 1954) where the centrosome also comes to lie at the base of the spermatid nucleus and gives off two flagella. In a dioecious form, *Schistosoma japonicum*, however, Severinghaus (1927) has reported a centrosome dividing in the spermatid.

It seems difficult to point out the biological significance of the abnormal structures reported in the testicular material, but it looks evident from their degenerating behaviour that they contribute in the nourishment

of the testes. The small globules seen in the peripheral region resemble those described by Dingler (1910) in *Dicrocoelium lanceolatum* and John (1953) in *Fasciola hepatica*. Such globules are seen to lie close to the peripheral wall and also to extend in the interior of the testis. From their gradual decrease in size as they extend towards the interior of the testis, it is presumed that they are consumed for nourishment. They might possibly be some protein products because they are not affected even by long fixation in Bouin's fluid. Dingler (1910) also attributed a nutritive function to them, but John (1953) failed to produce any evidence in favour of Dingler's contention.

The big globules which are seen at the periphery and occasionally in the interior of the testis are comparable with those described by Markell (1943) and John (1953), but there is no evidence to show that they are the products of large vacuolated cells as Markell considers them or the pycnotic primordial spermatogonia as John puts them. They are, however, shown to decrease gradually in size, which both John and Markell have failed to record.

The degeneration of certain cells in the peripheral region due to abnormal chromosomal divisions has not been reported before. Anderson (1935), Chen (1937), and Willey and Koulish (1950) reported pycnosis, which is characterized by the extreme condensation of the chromatic mass. Such a degeneration is also reported in the present investigation.

The formation and the degeneration of the oocytes in the testis has never been described in such a detail previously. Anderson (1935) in the testis of *Proterometra macrostoma* observed oocytes, which he believed were formed from the detached spermatocytes. The nourishing mass seen associated with the developing oocytes has never been reported before.

Summary

1. The behaviour of the nucleus during the spermatogenesis of *Cyclocoelum bivesiculatum* has been described. The chromosome number is 10 haploid and 20 diploid.

2. The history of the centrioles has been traced and their morphological rôle in the ripe sperms is discussed.

3. The sperm is both nuclear and cytoplasmic.

4. The unusual structures met with in the testes include small globules which stain darkly; bigger globules which stain lightly; oocytes with nourishing mass; cells which fail to undergo proper division; and the pycnotic cells. The nature, origin, and fate of some of these abnormalities are also described.

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Abbreviations

A.G.—Small globules; *C.*—Centrosome; *CH.G.*—Chromatic granules; *N.*—Nucleus; *Ooc.*—Oocyte; *Py.Sp.*—Pycnotic primordial spermatogonium; *Sp.*—Spermatogonium; *Y.C.N.*—Yolk cell nucleus.

Explanation of Plates

Plate I

Fig. 1. Primordial spermatogonia.
„ 2. Primary spermatogonium in the resting stage, showing a centrosomal granule.
„ 3. Primary spermatogonium in metaphase, showing a full diploid number (20) of chromosomes.
„ 4. Primary spermatogonium in metaphase, showing centrioles at each end of spindle.
„ 5. Primary spermatogonium in telophase with the chromosomes clumped.
„ 6. Secondary spermatogonia in a group of two cells. A centrosomal granule in each cell is seen.
„ 7. Secondary spermatogonia in metaphase, showing nearly the diploid number (20) of chromosomes.
„ 8. A group of four tertiary spermatogonia in the resting stage. A centrosomal granule in each cell is distinct.
„ 9. Tertiary spermatogonia in metaphase, showing full diploid number (20) of chromosomes in two of these cells.
„ 10. Three of the four tertiary spermatogonia in anaphase stages.
„ 11. Three of the eight primary spermatocytes in the resting stage.
„ 12. Four of the eight primary spermatocytes, showing the leptotene threads.
„ 13. Four of the eight primary spermatocytes, showing the zygotene stage.
„ 14. Primary spermatocyte, showing the bouquet stage.

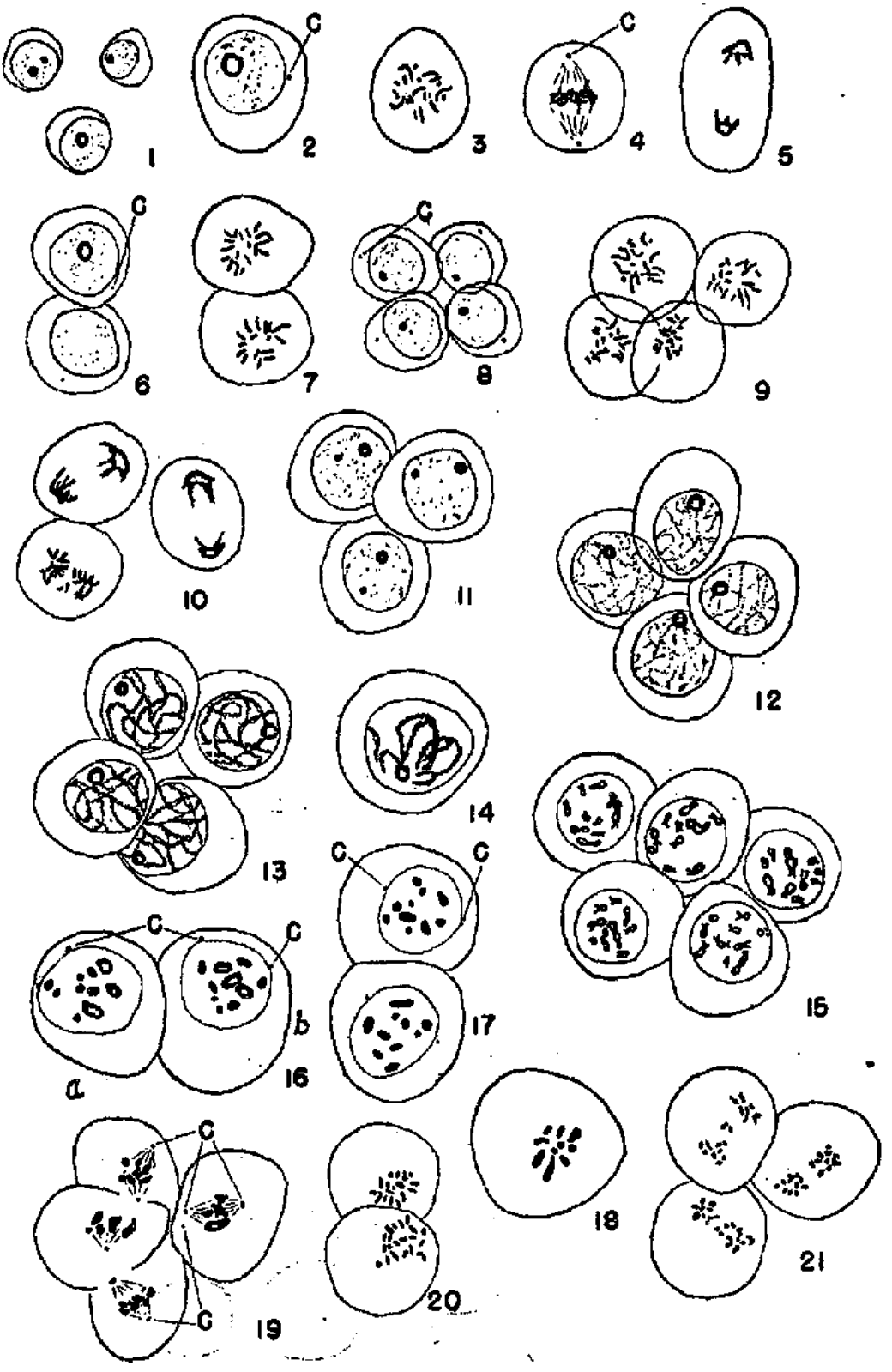
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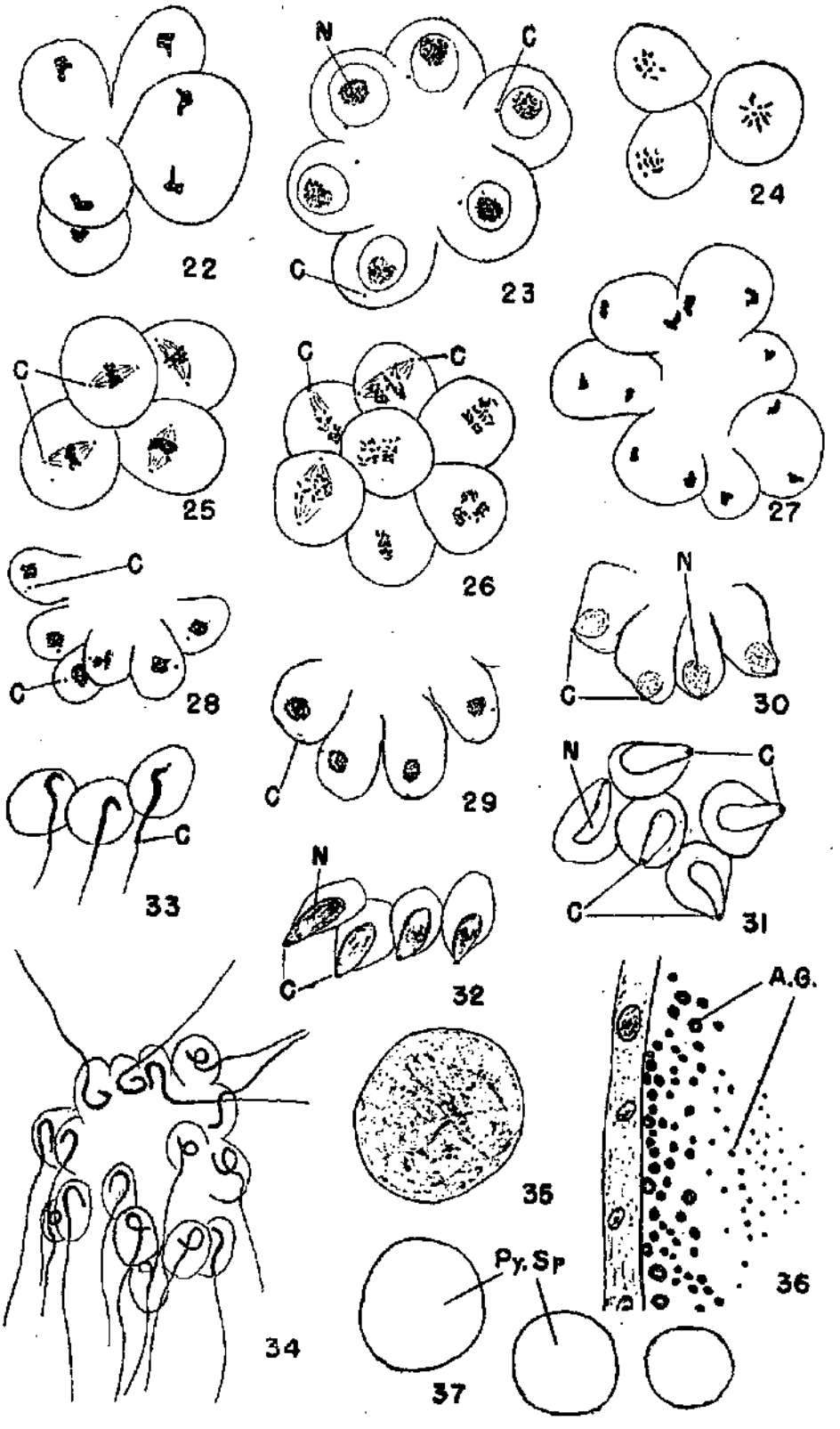
PLATE II.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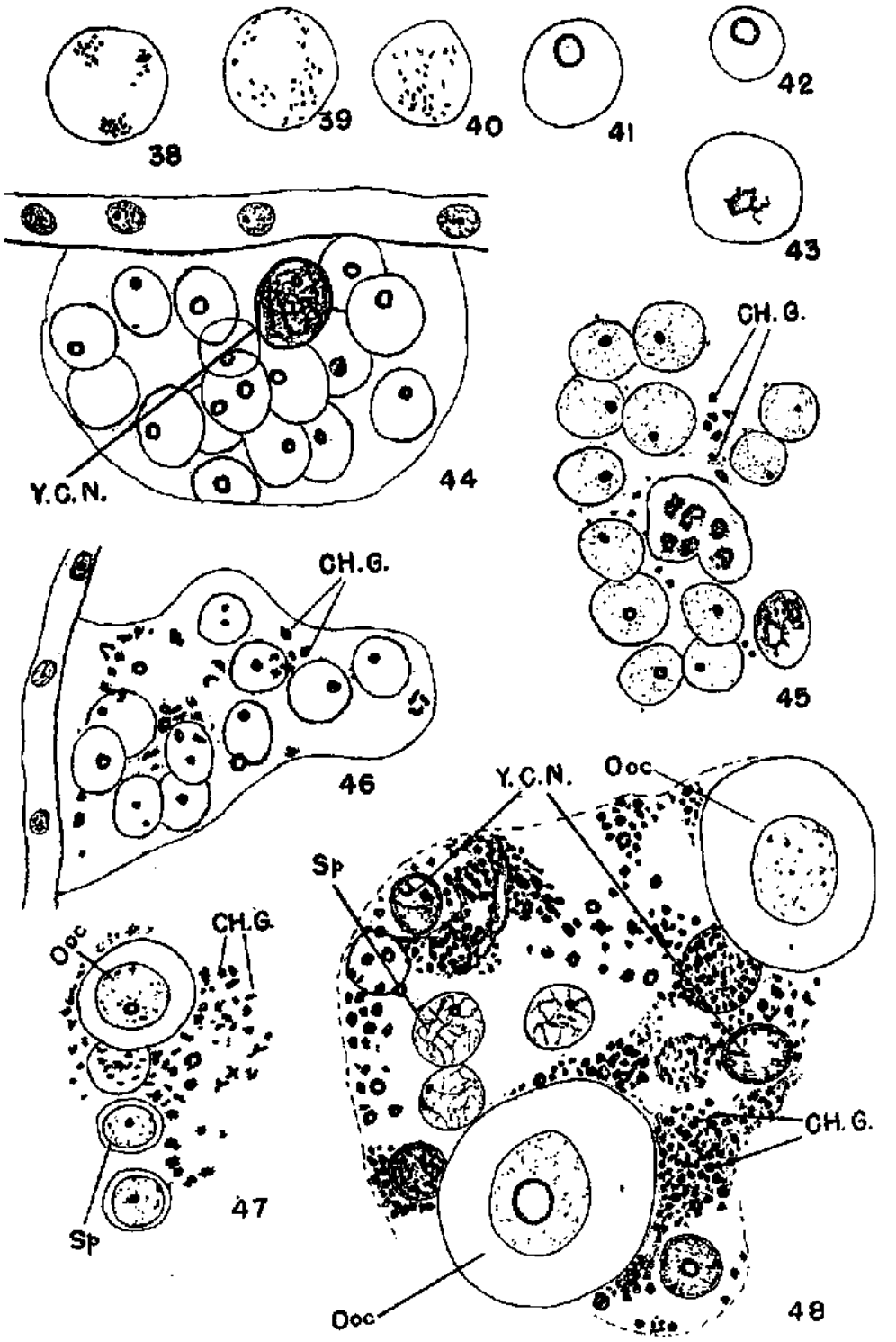
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PLATE IV.

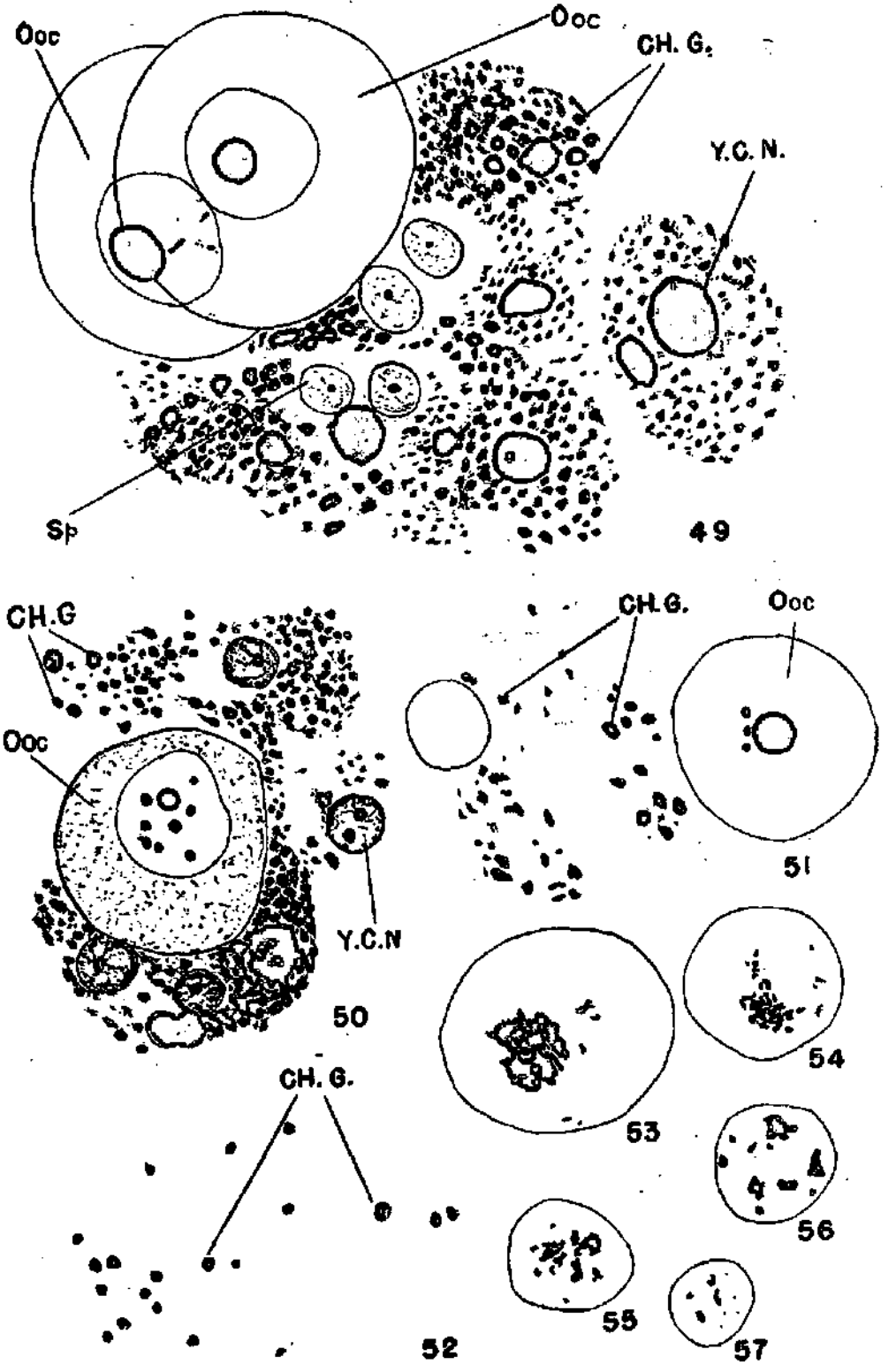
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Fig. 15. Primary spermatocytes, showing the diakinesis stage.

„ 16. Primary spermatocytes, showing the tetrads. In (*a*) there is a single centrosomal granule, but in (*b*) it has divided into two granules.

„ 17. Primary spermatocytes, showing the tetrads. The two centrosomal granules have come to lie opposite each other.

„ 18. Primary spermatocyte in metaphase, showing a haploid number (10) of chromosomes.

„ 19. Primary spermatocytes in metaphase, showing the spindles and centrosomes. In one cell the centrosome at the pole has divided into two.

„ 20. Primary spermatocytes in polar view, showing the separation of the bivalent chromosomes.

„ 21. Primary spermatocytes in anaphase. The haploid number (10) is seen in some of these sets.

Plate II

Fig. 22. Primary spermatocytes in telophase. The chromosomes are clumped and the cytoplasmic cleavages have appeared in some cells.

„ 23. Part of a rosette of secondary spermatocytes. The chromatin in each nucleus has loosened and a centrosome in each cell is seen.

„ 24. Secondary spermatocytes in metaphase, showing the haploid number of chromosomes.

„ 25. Secondary spermatocytes in metaphase, showing the spindles and the centrosomes at the poles of the spindle.

„ 26. Secondary spermatocytes in anaphase. Spindles and centrosomes are seen in some of the cells.

„ 27. Secondary spermatocytes in telophase. The chromosomes have clumped and the cytoplasmic cleavages have appeared in some cells.

„ 28. A part of the spermatid rosette, showing the condensed chromatin and the centrosomal granule in each cell.

„ 29. A part of the spermatid rosette, showing the nuclei with the chromatin in the centre. A centrosomal granule near each nucleus is seen.

„ 30. Spermatids, showing the nuclei reorganized and the centrosomal granule lying at the base of each spermatid nucleus.

„ 31. Spermatids, showing weakly stained ovate nuclei with a centrosomal granule at the tip of each spermatid nucleus.

„ 32. Spermatids, showing the nuclei pulling out of the cytoplasm.

„ 33. Spermatids, showing strongly basiphilic nuclei and the centrosomes from where the flagella are given off.

„ 34. Spermatid rosette, showing almost mature sperms.

„ 35. Residual cytoplasmic mass.

„ 36. Small globules as seen near the periphery.

„ 37. Some of the pycnotic primordial spermatogonia from the peripheral region.

Plate III

Figs. 38–40. Spermatogonia in which normal mitotic divisions have failed.

Figs. 41–43. Pycnotic spermatogonial cells.

Fig. 44. A patch of spermatogonial cells along the wall of the testis in which a yolk cell nucleus has formed.

Figs. 45-46. A patch of spermatogonial cells in which the chromatic granules have formed and are being formed.

Fig. 47. A small patch of spermatogonial cells with chromatic granules and a young oocyte.

„ 48. Well-developed oocytes associated with a mass of cells and granules.

Plate IV

Fig. 49. Two fully developed oocytes in the post-synaptic diffuse stage associated with some spermatogonia, yolk cell nuclei and the chromatic granules.

,, 50. Degenerating oocyte surrounded by the nourishing mass.

,, 51. A little more advanced stage of degeneration of the oocyte. The nourishing mass has dwindled.

,, 52. Remnants of the nourishing mass.

Figs. 53–57. Stages in the degeneration of the oocytes found free in the testis cavity.

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October, 1954

FISH SPERMATOGENESIS WITH PARTICULAR REFERENCE TO THE FATE OF THE CYTOPLASMIC INCLUSIONS

III. Spermatogenesis of Actinopterygii (Cyprinodontidæ)

by

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FISH SPERMATOGENESIS WITH PARTICULAR REFERENCE TO THE FATE OF THE CYTOPLASMIC INCLUSIONS

III. Spermatogenesis of Actinopterygii (Cyprinodontidae)

By Hem Sagar Vasisht, *Department of Zoology, Panjab University, Hoshiarpur*

Introduction

In two previous papers of this series (Vasisht, 1953 and 1954) I have published my findings on the spermatogenesis of twelve Elasmobranch species (Hypo- and Pleurotremata), with particular reference to the fate of the Golgi bodies and mitochondria. It has been found that in all the twelve Elasmobranchs the acrosome is directly formed from the Golgi body or bodies, and the mitochondria form the sheath of the middle-piece.

In this, the third paper of the series, the spermatogenesis of three Teleost fishes of sub-family Pœciliinæ of the family Cyprinodontidæ has been worked out. The study of these fishes has once again confirmed my conclusions arrived at in the two previous papers.

On reviewing the previous literature on Teleost fishes, it is noted that all workers have figured sperms without showing the acrosome. Ballowitz (1895) in *Salmo* and *Clupea*, Retzius (1905) in a pike *Esox*, Duesberg (1918) in *Fundulus*, Turner in a perch, and Essenberg (1923) in *Xiphophorus hellerii* have figured no acrosome.

Vaupel (1929) was the first worker who demonstrated the presence of Golgi bodies and the mitochondria in *Lebistes reticulatus*. During spermateleosis the mitochondria arrange themselves behind the nucleus and the Golgi bodies are sloughed off without forming the acrosome. Consequently, the sperm of this fish is also said to have no acrosome. Lall (1945), in his preliminary communication to the 'Indian Science Congress' on the sperm formation of seven Teleostean fishes once again confirmed the observations of the previous workers, i.e., he found no acrosome.

I am thankful to Dr. H. G. Kewalramni, Curator, Taraporewala Aquarium, Bombay, for the supply of live fishes free of cost. I am also obliged to Dr. Vishwa Nath for suggesting this problem and for helping me to prepare the manuscript for the press.

Material and Technique

The spermatogenesis of three Teleosts has been worked out in this paper. They all belong to the sub-family pœciliinæ of the family Cyprinodontidæ of the class Actinopterygii. The names of these fishes are: (1) *Lebistes reticulatus*, (2) *Xiphophorus hellerii*, and (3) *Gambusia wrayi*.

The testes are two small oval creamish structures, lying in the dorsal wall of the body cavity below the swim bladder, just anterior to the cloacal

opening. They are partly fused, and are covered by a delicate membrane. The two vasa deferentia from the testes open into the urinogenital sinus.

Immediately after the testes were dissected out, they were put in normal saline, cut into small pieces, and were transferred to capsules containing different fixatives. The testicular material was mainly fixed in Flemming-without-acetic acid, Champy and Bouin. The material was fixed in F.W.A. and Champy for 24 hours and was washed in running water for the same period. Sections were cut 5 μ thick. Smears were also prepared for the study of sperms. The sections and smears were stained with 0·5% iron hæmatoxylin.

The testicular material of *Gambusia wrayi* was also studied fresh under the Phase Contrast Microscope.

Observations

As the process of spermatogenesis in the three Teleost species dealt with in this paper is more or less similar, it has been considered best to give a comparative account of these three species.

Spermatogonia

The spermatogonia in all the three fishes studied are small rounded cells with vesicular nuclei. The nucleus is large and central, with a thin layer of cytoplasm surrounding it. It contains chromatin granules, chromatin threads and a distinct darkly-stained nucleolus (Pl. I, Figs. 1, 2 and 18; and Pl. II, Fig. 35). In the majority of the spermatogonia the cytoplasm does not reveal any granules (Pl. I, Fig. 1; and Pl. II, Fig. 35), but in a few of them there can be observed some dust-like granules. These are the mitochondria. In addition to these granular mitochondria there can be seen occasionally one or two comparatively bigger and more refringent granules, which are the Golgi elements (Pl. I, Figs. 2 and 18).

Primary spermatocyte

The primary spermatocyte is the largest cell in the testis. The excentric nucleus is vesicular and contains a prominent nucleolus in addition to the chromatin threads and granules. As the spermatocyte grows the juxta-nuclear mitochondria grow in size from the dust-like granules to appreciably bigger granules (Pl. I, Figs. 3, 4 and 19; and Pl. II, Figs. 36 and 37). The Golgi bodies are big darkly-staining granules scattered either amongst the mitochondrial mass or outside it. They are, however, fewer in number as compared to the mitochondria.

Secondary spermatocyte

The secondary spermatocytes are smaller than the primary, but are bigger than the spermatogonia (Pl. I, Figs. 5 and 20; and Pl. II, Fig. 38). They can be easily distinguished from the spermatogonia by their excentric nucleus, larger mitochondria and Golgi bodies, and an increase in the volume of the cytoplasm. In *Gambusia wrayi* one also notices Golgi crescents and vesicles in addition to the Golgi granules (Pl. II, Fig. 38).

In spite of my best efforts I have not been able to find any useful division stage.

Spermateleosis

The earliest spermatid has a vesicular excentric nucleus with a few chromatin granules and threads. The mitochondria are small granular structures, lying on one side of the nucleus. Amongst the granular mitochondria are also met with a few Golgi bodies, which are comparatively bigger and more refringent (Pl. I, Figs. 6 and 21; and Pl. II, Figs. 39 and 40). At a little later stage the nucleus starts showing condensation till ultimately it takes up a homogeneously dark stain.

In the spermatid of all the three Teleosts one of the Golgi granules moves towards the nucleus. This granule may now be termed as the pro-acrosome, as it is the fore-runner of the future acrosome. The pro-acrosome ultimately takes its position at the anterior end of the nucleus (Pl. I, Figs. 9 to 12; and Pl. II, Figs. 26, 27, 43 and 44). The first step in the transformation of the pro-acrosome into the acrosome is the appearance of a very lightly staining triangular area, the pro-acrosomal granule, now reduced in size, lying at the apex of the triangle (Pl. I, Figs. 13 to 15; and Pl. II, Figs. 28 to 31, 45 and 46). Gradually the pro-acrosomal granule completely vanishes from the apex (Pl. I, Figs. 16 and 17; Pl. II, Figs. 32 to 34, 47 and 48). The acrosome which stains very feebly lies at the anterior end of the sperm head.

Simultaneously when the pro-acrosome is being transformed into an acrosome the mitochondria become more concentrated around the axial filament (Pl. I, Figs. 8 to 11, 24 and 25; and Pl. II, Figs. 26, 42 and 43). These now fuse to form a homogeneous mass, the mitochondrial nebenkern in the region of the middle-piece (Pl. I, Figs. 12 to 14; and Pl. II, Figs. 27, 28 and 44 to 47). The mitochondrial nebenkern soon loses its staining capacity, and forms a sheath of the axial filament in the region of the middle-piece (Pl. I, Figs. 15 to 17; and Pl. II, Figs. 32 to 34 and 48).

The behaviour of the centrioles is not very clear, as the spermatids are of a very small size. But from the study of these three species it seems that there appears a small filament between two granular centrosomes (Pl. I, Fig. 23; and Pl. II, Fig. 40). Due to the growth of this intra-cellular filament one of the centrosomes is carried near the base of the nucleus and the other towards the cell wall. These are the proximal and distal centrosomes respectively. From the distal centrosome is given out an extra-cellular axial filament, which forms the end-piece of the sperm (Pl. I, Figs. 8 to 17, 24 and 25; and Pl. II, Figs. 26 to 34 and 42 to 48). Sometimes in all the three species the proximal centriole becomes rod-shaped and can be seen lying on the nucleus (Pl. I, Fig. 17; and Pl. II, Figs. 29 and 33).

As the nucleus begins to elongate the cytoplasm starts attenuating, so much so that it cannot be made out round the nucleus and exists in the form of a thin sheath in the region of the middle-piece. In all these three species of fish the spermatid nucleus remains in a spherical condition for a long time (Pl. I, Figs. 10 to 14, 23 and 24; and Pl. II, Figs. 26 to 28 and 43). It condenses into a bean-shaped structure at a very late stage in

spermateleosis (Pl. I, Figs. 15 to 17 and 25; and Pl. II, Figs. 29 to 34 and 44 to 48).

As is clear from the figures and from the account of the acrosome formation only one Golgi body becomes the pro-acrosome. The rest are sloughed off along with the blebs of residual cytoplasm (Pl. II, Fig. 32).

The ripe sperm of these fishes has a bean-shaped nucleus, having a small conical acrosome in front, middle-piece behind and a naked axial filament forming the tail (Pl. I, Figs. 16 and 17; and Pl. II, Figs. 33, 34 and 48).

Discussion

The main conclusions of my investigations on the spermatogenesis of the three species of Teleost fishes, which form the subject matter of this paper, are (1) that the teleostean sperm has a small acrosome which is formed by the direct transformation of a Golgi body, the pro-acrosome, and (2) that there is well-defined middle-piece in the sperm, which is limited by the proximal centrosome in front and by the distal centrosome behind, the mitochondria forming a sheath of the axial filament in this region. The axial filament of the middle-piece is prolonged posteriorly into the end-piece of the sperm which is devoid of any cytoplasmic or mitochondrial sheath.

As will be clear from the accounts of the earlier workers on Teleosts they did not describe any acrosome in the sperm of these fishes. Nor did any one, except Jean Vaupel (1929) and Lall (1945), make even a mention of the Golgi bodies. Since Lall has not published a detailed account of his paper, it remains for me to discuss the paper of Vaupel (1929) only, who worked out in detail the spermatogenesis of *Lebistes reticulatus*, one of the three species investigated by me. But unfortunately Jean Vaupel's technique was defective so far as the study of Golgi bodies and mitochondria was concerned. All the fixatives employed by her contained acetic acid except osmic vapour fixation. Apart from this there are only a few Golgi granules present in the spermatid of *Lebistes reticulatus*, which completely escaped her notice. She described the Golgi apparatus in the spermatocyte as 'a cap-shaped reddish mass of denser consistency than the remainder of the cytoplasm', which ultimately is sloughed off during spermateleosis. Likewise the mitochondria in her figures are greatly distorted by the fixatives used by her, although she has figured them correctly in the middle-piece of the maturing sperm. In view of the faulty technique used by Vaupel it is impossible to accept her finding that there is no acrosome in the sperm of *Lebistes reticulatus*. My findings on *Lebistes reticulatus*, *Xiphophorus hellerii* and *Gambusia wrayi*, run counter to those of Vaupel's, so far as the acrosome is concerned. The Teleosts are no exception to the general plan of the acrosome formation in spermateleosis. It becomes quite clear from my observations that there is a definite acrosome in the sperm of these fishes. In all the three species under investigation I have observed a small acrosome in the living sperm even under an ordinary microscope and in the case of *Gambusia wrayi* this has been further confirmed by the study of fresh testicular material under

Phase Contrast Microscope. Under this microscope the acrosome is seen as a very conspicuous conical structure in front of the nucleus of the sperm. But in fixed preparations the tiny acrosome is liable to collapse, and keeping in view the fact that the acrosome always stains poorly, it has completely escaped the notice of all the previous workers.

Finally, it may be repeated that in all the three Teleost species studied by me the acrosome is formed directly from a Golgi body.

Summary

1. In this paper the spermatogenesis of three Teleosts, viz., *Lebistes reticulatus*, *Xiphophorus hellerii* and *Gambusia wrayi*, has been worked out with particular reference to the fate of the Golgi bodies and the mitochondria.

2. A thorough study of the cytoplasmic inclusions in the spermatogonia, spermatocytes and in the spermateleosis has been made.

3. The earliest spermatid has an excentrically placed nucleus, a few granular Golgi elements, and a large number of mitochondrial granules.

4. During spermateleosis one of the Golgi bodies advances towards the nucleus and is called the pro-acrosome. This pro-acrosome is directly transformed into an acrosome.

5. The granular mitochondria fuse to form the mitochondrial nebenkern, which in turn gives rise to the sheath of the middle-piece.

6. The ripe sperm has a bean-shaped nucleus with a short conical acrosome in front, a middle-piece and a long tail behind.

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Explanation of Lettering in the Plates

A.—Acrosome; A_1.—Pro-acrosome; *A.f.*—Axial filament; *C.*—Centrosome; C_1.—Proximal centrosome; C_2.—Distal centrosome; *C.G.*—Chromatin granules; *G.*—Golgi body; *M.*—Mitochondria; *M.p.*—Middle-piece; *M.n.*—Mitochondrial nebenkern; *N.*—Nucleus; N_1.—Nucleolus; *R.C.*—Residual cytoplasm.

Explanation of Figures and Plates

All figures have been drawn with Camera Lucida at table level with Beck 10× eyepiece and oil immersion objective. All figures (except Pl. I, Figs. 16 to 17 and 25; and Pl. II, Figs. 29 to 34 and 46 to 48 which are drawn from smears) have been drawn from sectioned material fixed in Flemming-without-acetic acid followed by 0·5% iron hæmatoxylin.

Plate I

Figs. 1–17. *Lebistes reticulatus*

Fig. 1. Earliest spermatogonium.
" 2. Late spermatogonium showing the mitochondria and the Golgi body.
Figs. 3 and 4. Primary spermatocytes.
Fig. 5. Secondary spermatocyte.
" 6. Early spermatid.
" 7. Spermatid with a condensed nucleus.
" 8. Spermatid showing the appearance of axial filament.
Figs. 9 to 11. Spermatids each showing the pro-acrosome and the mitochondrial concentration round the axial filament.
Fig. 12. The mitochondria have fused to form the mitochondrial nebenkern.
Figs. 13 to 15. The pro-acrosomes have started transforming into acrosomes.
Fig. 16. Early sperm.
" 17. Mature sperm.

Figs. 18–25. *Xiphophorus hellerii*

Fig. 18. Spermatogonium showing the granular mitochondria and the Golgi body.
" 19. Primary spermatocyte showing the excentric nucleus, juxta-nuclear granular mitochondria and a few Golgi granules.
" 20. Secondary spermatocyte.
" 21. Earliest spermatid.
" 22. Spermatid showing the condensed nucleus and other cytoplasmic inclusions.
" 23. Spermatid with two centrosomes and a small filament in between them.
" 24. The axial filament has come out of the cell.
" 25. The proximal centrosome is clearly seen on the nucleus. The mitochondria are seen round the axial filament.

Plate II

Figs. 26–34. *Xiphophorus hellerii*

Fig. 26. Spermatid showing the pro-acrosome.
" 27. Spermatid with the mitochondrial nebenkern.
Figs. 28 to 30. Pro-acrosome has started transforming into an acrosome.
" 31 and 32. Note the Golgi remnant in the region of the middle-piece and the bleb of residual cytoplasm.
" *33 and 34. Mature sperms.*

FIGS. 35–48. *Gambusia wrayi*

FIG. 35. Spermatogonium.

FIGS. 36 and 37. Primary spermatocytes showing the excentric nuclei, juxta-nuclear mitochondria and the Golgi granules. Also note the nucleoli.

FIG. 38. Secondary spermatocyte showing the crescent-shaped, vesicular and granular Golgi bodies in addition to the other cell components.

„ 39. Earliest spermatid.

„ 40. Spermatid showing two centrosomes, intra-cellular axial filament, mitochondria and the Golgi bodies.

„ 41. Note the pro-acrosome.

„ 42. Note the axial filament in the spermatid.

„ 43. The spermatid has elongated and the mitochondria are arranged round the axial filament.

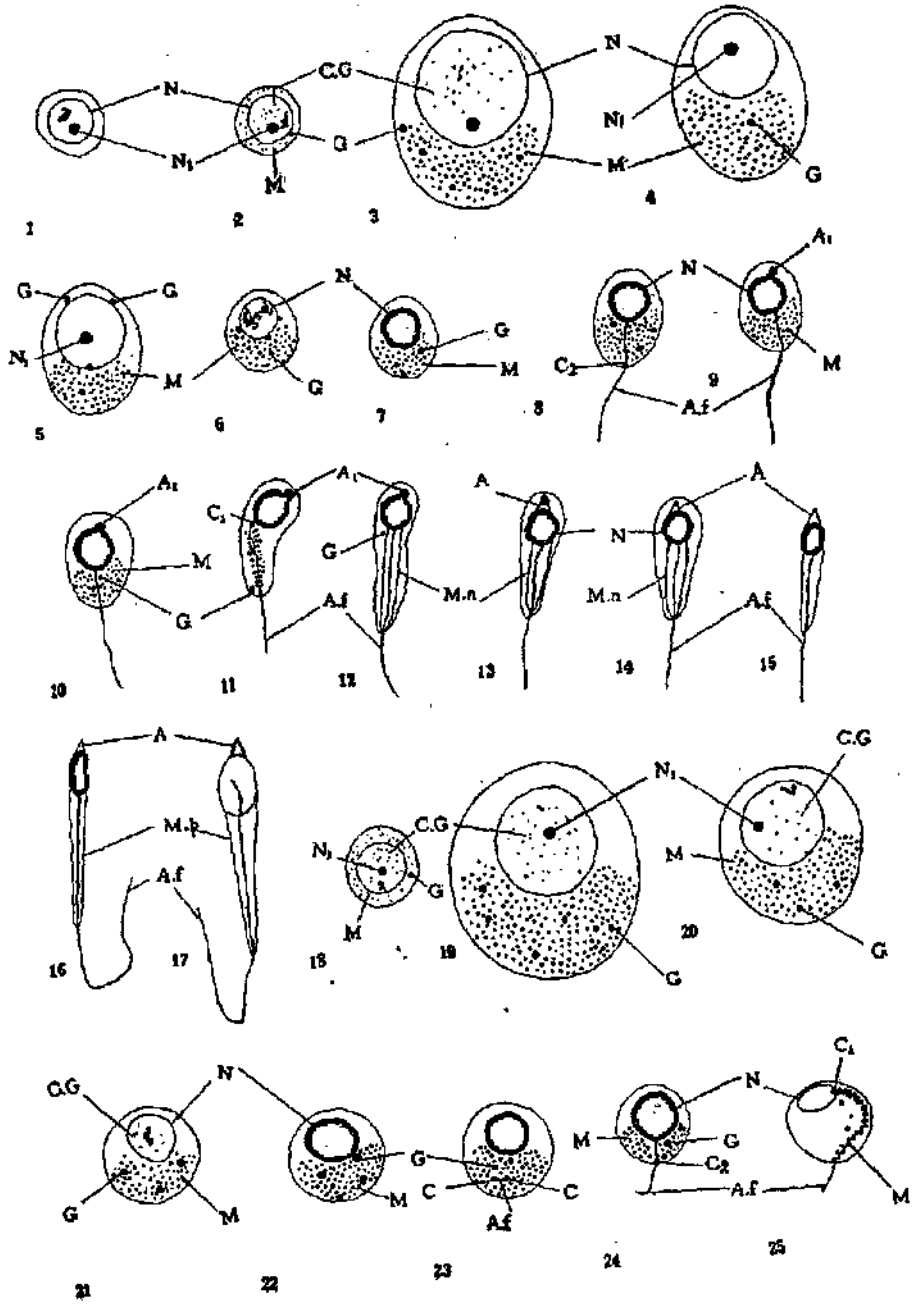
„ 44. Mitochondria have fused to form the mitochondrial nebenkern.

FIGS. 45 and 46. Pro-acrosome has started transforming into an acrosome.

FIG. 47. The elongated spermatid showing the mitochondrial nebenkern, the Golgi remnant and the distal centrosome.

„ 48. Fully ripe sperm with an oval nucleus having a conical acrosome in front, middle-piece behind and the tail.

PLATE I.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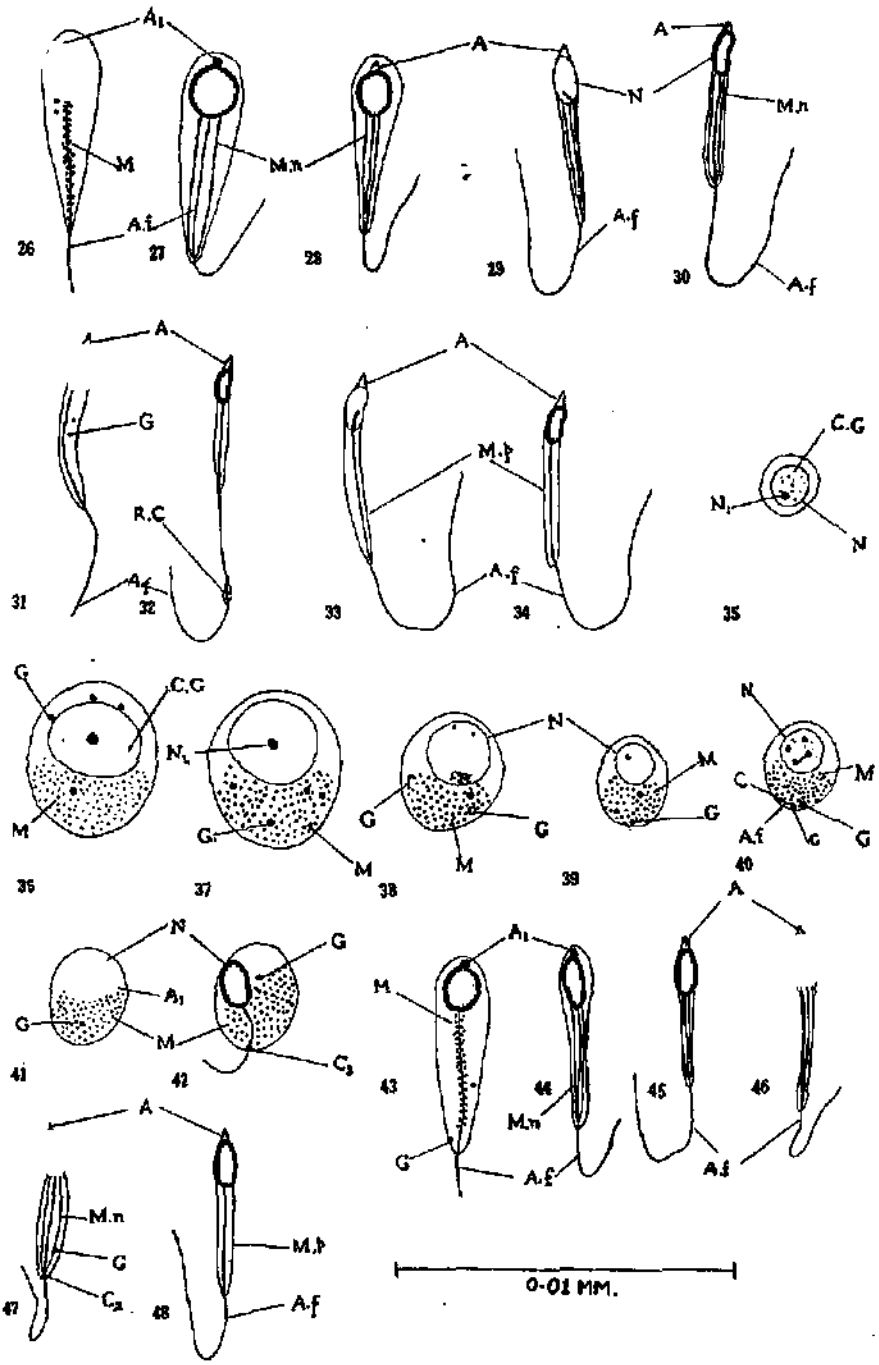

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A PRELIMINARY NOTE ON SOME BIRDS OF THE HOSHIARPUR DISTRICT, PANJAB (I)

by

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(Department of Zoology, Panjab University College, Hoshiarpur)

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The bird fauna of many districts of the Panjab (I) has been, to some extent, recorded by a number of workers. Of these Whistler occupies the most prominent position as he has described the birds of the various places which he happened to visit in connection with his duties as an officer in the Indian Police, viz. Hissar (1915), Dalhousie (1916), Ambala (1918*a* and *b*), Ludhiana (1919*a*), Fagoo (1919*b*), Simla (1920 and 1928) and Kulu (1926). Jones (1919*a*) had already given a long list of the birds found in the Simla Hills and in the same year (1919*b*) he also published further notes on the birds of the Ambala district. Similarly Basil-Edwardes (1926) added some notes on the birds uncommon in, or unrecorded from the Simla Hills. Betham (1916) described a few birds breeding at Ferozepore and Hingston (1921) published a list of the birds collected from the Dhauladhar Range in the immediate vicinity of Dharmsala. Currie (1916), while describing the birds nesting on the Kaisapur Jhil and at Malikpur in the Gurdaspur district, also mentions about one or two birds from Hoshiarpur. Excepting Currie none of the above stated workers seems to have paid any attention to the study of the avi-fauna of the district Hoshiarpur. Since 1948, when the Department of Zoology of the Panjab University was established at Hoshiarpur, the need for such a study began to be felt acutely and the present work was, therefore, started in October, 1951. Although the 'summer visitors' had left by that time, yet the 'residents' and the 'winter visitors' were quite encouraging to start with. The lack of a miniature rifle to shoot the small birds and the general scarcity of cartridges (nos. 8–10) in the market were the main difficulties encountered.

The district Hoshiarpur, being situated at the foot of the Shiwalik Hills, provides quite a large variety of habitat for the birds. Considerable areas of Una, Garhshankar and Hoshiarpur *tehsils* comprise a dry hilly country with scrub jungle, light forests and dense undergrowth, thus providing an ideal habitat for the order Gallinæ (game birds). The river Beas which flows through a part of the *tehsil* Dasuya and a few permanent and semi-permanent 'Chos' (small streamlets) of the district are inhabited by the birds belonging to the orders Charadriiformes and Anseres (water birds). The plains of the district are rich in birds belonging to the orders Passeres (crows, shrikes, bulbuls and babblers, etc.), Coraciiformes (barbets, parrots, cuckoos and wood-peckers, etc.), Accipitres (birds of prey) and Columbæ (pigeons and doves). The abundance of fruit trees and insect life,

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due to healthy climatic conditions, attract a number of 'summer and winter visitors'.

The birds were collected mainly from Garhshankar and its vicinity, Gagret, Hoshiarpur and its vicinity, Mukerian and Bankhandi. The collection includes forty-three families belonging to the above-mentioned seven orders. In all ninety-four sub-species of birds have been collected.

As the description of the birds in our collection agrees (except for very minor variations in measurements and shades) with that given by Stuart Baker (1922, 1924, 1926, 1927, 1928 and 1929) it has been considered best to adopt the same numbers and nomenclature as used by him. We have added only the local name of the bird, wherever known, and the locality from which it has been collected.

1. (1) *Corvus corax laurencei* (Ghogar Kan, Pahari Kan).—It arrives in the plains of this district in winter, the earliest date of observation being the middle of October, when a solitary pair was seen near the Bhangi Cho. Most of the flocks were seen in the months of December and January. These birds, however, became scarce from April onwards. A solitary pair was noted near the Railway station, Hoshiarpur, as late as the last week of May and one of them was shot which turned out to be a male.
2. (5) *Corvus coronoides levaillanti* (Jungli Kan).—It is a permanent resident of the hilly areas of this district. In the plains it is not very common during summer, although they are found in considerable numbers in winter. They do not, however, disappear totally in summer. A single bird was observed near the kitchen of the college hostel in company with house crows as late as the end of May. It did not observe the natural shyness of the race and came frequently in the verandah of the kitchen to collect bits of bread, etc.
3. (11) *Corvus splendens splendens* (Kan).—The house crow is a strictly resident bird and it breeds in June and July.
4. (26) *Dendrocitta rufa rufa*.—It is also a strictly resident species and is actively making nests in April, May and June.
5. (53) *Parus major kaschmiriensis*.—It is mainly an arboreal form but also visits bushes and scrub jungle. A number of them was noticed at Bharwain, Gagret and Bankhandi, but could not find any in the plains. They appear to be very methodical in hunting insects.
6. (110) *Sitta castaneiventris castaneiventris*.—This bird is purely arboreal in habit and is found in all the well-wooded parts of the district. It was first observed in the middle of December. In April it was very common and a pair of them was shot.
7. (185) *Turdoides terricolor sindianus*.—This bird is very common throughout the district. Its breeding season commences in June and July after the monsoons. The nest which is made in some tree or thick bush is a fairly deep cup of grass stems and roots which are put together rather loosely.

8. (192) *Argya caudata caudata.*—It is again a very common bird of the district.

9. (195) *Argya malcolmi.*—Very common throughout the district.

10. (404) *Molpastes hæmorrhous intermedius* (Bulbul).—This bird is found in large numbers throughout the district. The breeding season lasts from February to August. The nest which is in the form of a neat cup is made up of dry grass stems, fine twigs and shoots. It is lined with fine roots and hair intermingled with dry leaves.

11. (405) *Molpastes leucogenys leucogenys* (Bulbul).—This bird shows a local movement in the district. In the months of March, April, May, June, July and August it leaves the plains and goes uphill while in the winter months, i.e., from September to February, it is very common in the plains. Its breeding season also lasts from March to August. The nest of this bird is in the form of a well-constructed cup which is light and fragile, to look at, but is quite strong otherwise.

12. (495) *Saxicola torquata indica.*—It is a common bird of the district and breeds from April to July. The nest which is cup-shaped is made up of coarse grass and fine twigs, etc.

13. (506) *Œnanthe picata.*—This bird also is quite common throughout the district.

14. (507) *Œnanthe capistrata.*—It is a very rare bird. Only one specimen was seen in a bush near the Model Town, Hoshiarpur.

15. (531) *Phœnicurus ochrurus phœnicuroides.*—This bird is very commonly met with in the gardens and groves of the district during winter.

16. (535) *Rhyacornis fuliginosa fuliginosa.*—This bird is a winter visitor to the plains of the district. It is purely a water bird and is found near about the streams in the hills.

17. (557) *Saxicoloides fulicata cambaiensis.*—It is one of the common garden birds and is found throughout the district.

18. (558) *Copsychus saularis saularis.*—It is also a very common garden bird of the district.

19. (700) *Rhipidura aureola aureola.*—It is comparatively a rare bird.

20. (706) *Lanius excubitor lahtora.*—This bird is very common at the foot of the hills. Two specimens were shot—one from Birumpur and the other from Garhshankar.

21. (710) *Lanius vittatus.*—This bird is common throughout the district. One specimen was shot near the Model Town, Hoshiarpur.

22. (714) *Lanius schach erythronotus.*—This bird is also available throughout the district and is abundant near the foot of the hills. One specimen was shot at Khanpur.

23. (731) *Tephrodornis pondiceriana pallida.*—This bird is abundant in the semi jungle areas at the foot of the hills. Three specimens were shot from Birumpur and Chak Sadho.

24. (745) *Pericrocotus peregrinus peregrinus.*—This bird is commonly met with in the gardens, groves and thickets of the district. The

only pair shot from Khanpur was, however, too badly damaged for purposes of preservation.

25. (766) *Dicrurus macrocercus macrocercus* (Kalchiri).—It is one of the most familiar birds and is found throughout the district.

26. (767) *Dicrurus macrocercus albirictus* (Kalchiri).—Two specimens which were, to look at, just like the last bird were shot at Garhshankar and Khanpur. A closer observation, however, showed that their underwing coverts, long upper tail coverts and the feathers from the lower breast to the under tail coverts were all edged with white to a variable degree. Such characters, according to Ali (1935), are shown by the young first year birds of both the Indian races of *Dicrurus macrocercus*, namely *albirictus* (Northern India) and *peninsularis* (Southern India). It, therefore, appears to us that these two specimens are the young first year birds of *Dicrurus macrocercus albirictus*. The last bird also should be classified, according to Ali (1935), as *Dicrurus macrocercus albirictus*.

27. (813) *Orthotomus sutorius sutorius*.—This bird is again very common throughout the district. It is usually found in the gardens and also in the open cultivation. Three specimens were shot from Khanpur, Sajna and the Model Town, Hoshiarpur but, on account of their small size, they were too badly damaged for purposes of preservation. *Orthotomus sutorius sutorius* is, however, the Ceylonese race, according to Ali (1946), the Indian race being *guzerata*.

28. (953) *Oriolus oriolus kundoo*.—This bird is commonly met with during the mango season. A single male was shot from near the Railway level crossing, Hoshiarpur.

29. (974) *Sturnus vulgaris porphyronotus* (Tiliar, Kala-tiliar).—This bird is very common in winter when it is on its migratory trip to this district.

30. (996) *Acridotheres tristis tristis* (Myna, Lali, Sharakh).—Very common throughout the district.

31. (998) *Acridotheres ginginianus* (Lali, Myna, Sharakh).—Common throughout the district. One typical specimen was shot from near the Model Town, Hoshiarpur. Three more specimens in which the legs and feet were orange yellow in colour instead of pale yellow were also shot out of a small flock of about 20 to 30 birds feeding in a field on the bank of the cho near Sajna.

32. (1008) *Ploceus philippinus* (Baya, Bijra).—A common bird of the district. Two males were shot—one from Garhshankar and the other from Khanpur.

33. (1036) *Amandava amandava*.—This bird was quite abundant in the months of November and December along the cho banks and could be easily identified by its striking colour. A pair of them was shot near Khanpur. Both of these birds were, however, very much damaged for skinning purposes. No

nests were observed. According to Stuart Baker (1926) this bird is not available in the Panjab.

34. (1096) *Passer domesticus indicus* (Chiri).—It is the most common bird of the district.

35. (1157) *Hirundo smithii filifera*.—This bird is commonly met with near the permanent ponds and the river Beas. It was seldom seen sitting. No nests or colonies were observed.

36. (1166) *Motacilla alba dukhunensis* (Balkatara).—This bird is found throughout the district and is abundant near water. One pair was shot from the Model Town, Hoshiarpur.

37. (1174) *Motacilla cinerea caspica* (Balkatara).—This bird is exclusively a bird of the small streams.

38. (1215) *Alauda gulgula gulgula* (chiri).—This bird is found throughout the district and is abundant on cultivated lands. A single specimen was shot near the Bhangi cho but it could not be preserved due to the damage done to its feathers. According to Whistler (1949), however, the pale bird of the Panjab and the United Provinces is *A. g. punjaubi*.

39. (1248) *Zosterops palpebrosa elwesi*.—This bird is again found throughout the district and is abundant in small groves, gardens and thickets. One male was shot at Khanpur.

40. (1278) *Leptocoma asiatica asiatica* (Kali piddi).—This bird is common throughout the district in Spring when the flowers are in plenty. Two males were shot—one from Jehan Khelan and the other from the Model Town, Hoshiarpur. It is one of the summer visitors to this district arriving in April and departing early in September.

41. (1375) *Leiopicus mahrattensis blanfordi* (Tarkhan, Kath Khora).—This bird is found in gardens and wooded country-side, generally along the foot of the hills (Shiwaliks). A solitary male was shot from Jehan Khelan and a pair from Khanpur.

42. (1394) *Brachypternus benghalensis benghalensis* (Tarkhan, Kath-khora).—This bird is found throughout the district and is very common in groves and gardens. About five specimens were shot from the various places.

43. (1430) *Thereiceryx zeylanicus caniceps*.—It is also found throughout the district, in gardens and cultivation.

44. (1447) *Xantholæma hæmacephala lutea*.—This bird is in abundance in this district from the month of April to September. A pair of them was shot from Khanpur and a single male from near the Model Town, Hoshiarpur. According to Stuart Baker (1927), however, this bird is said to be rare in the Panjab.

45. (1457). *Hierococcyx sparveroides*.—This bird is also available in abundance in this district during the summer months (i.e. from April to September). It was observed for the first time late in March. A few birds were shot from Khanpur and Jehan Khelan. Stuart Baker (1927), however, does not mention the Panjab State under the distribution of this bird.

46. (1475) *Eudynamis scolopaceus scolopaceus* (Koel).—It is a very common bird and is found throughout the district in mango season. It is one of the summer visitors arriving in the end of March and leaving in the end of September. Two birds were shot from near the Model Town, Hoshiarpur.

47. (1487) *Taccocua leschenaulti sirkee.*—This bird inhabits jungles and comparatively calm thickets and groves. Two specimens were shot from Birumpur and Khanpur.

48. (1490) *Centropus sinensis sinensis.*—This bird is abundant throughout the district in open cultivation.

49. (1497) *Psittacula eupatria nepalensis* (Ra tota).—Found practically throughout the district wherever there are numerous large trees. Some of the specimens were collected from Sajna, Birumpur, Khanpur and the Model Town, Hoshiarpur.

50. (1500) *Psittacula krameri manillensis* (Katha tota). Most abundant and well-known bird. Several specimens were shot at Birumpur, Jehan Khelan and Hoshiarpur. According to Ali (1946), however, *manillensis* is the S. India-Ceylon race while the N. India-Assam-Burma race is *borealis*.

51. (1517) *Coracias benghalensis benghalensis* (Nil-Kanth, Garud).—This is a common bird of the district. Specimens were shot from Birumpur, Khanpur and Jehan Khelan.

52. (1523) *Merops orientalis orientalis.*—This bird is found throughout the district. Specimens were shot from Dhaggam, Garhshankar, the Model Town, Hoshiarpur and the Bhangi cho near Hoshiarpur.

53. (1531) *Ceryle rudis leucomelanura* (Machhi-khora).—It is very commonly met with throughout the district, along the water tracts and water collections. Specimens were shot from the ponds near Hoshiarpur, Garhshankar and Khanpur.

54. (1535) *Alcedo atthis pallasii* (Nikka Machhi-khora).—This bird is found in considerable numbers along the river-side areas of Mukerian and Bala chaur. It is also seen occasionally near the village ponds or permanent chos. A single male was shot in Khanpur Cho.

55. (1550) *Halcyon smyrnensis smyrnensis* (Machhi-khora).—Very common throughout the district along the permanent water tracts and near the temporary ponds. Many specimens were shot from the various places.

56. (1575) *Lophoceros birostris* (Dhan chiri).—This bird is also commonly met with throughout the district. One specimen was shot from the Cemetery Garden, Hoshiarpur. According to Stuart Baker (1927) and Ali (1946), however, this bird is not found in the Panjab.

57. (1579) *Upupa epops epops* (Hud-hud).—This bird is abundant throughout the district in winter when it is on a migratory trip to the Panjab. Two males were shot from Garhshankar and a single female from the Model Town, Hoshiarpur.

58. (1581) *Upupa epops orientalis* (Hud-hud).—It is a resident bird, abundantly found throughout the district.

59. (1596) *Micropus affinis affinis* (Ababil).—It is a very common bird of the district.

60. (1625) *Caprimulgus indicus indicus*.—This bird is found along the foot of the Shiwalik hills. Only one specimen was collected from the Nara Rest House.

61. (1684) *Athene brama indica* (ulu, chugad).—Very common throughout the district. Specimens were shot from Bassi Ghulam Hussain, Jehan Khelan, the Model Town, Hoshiarpur and Garhshankar.

62. (1704) *Pandion haliaëtus haliaëtus* (Machhli mar).—Only one specimen (male) was seen and shot from Sajna cho—a swampy area, in winter.

63. (1706) *Sarcogyps calvus* (Raj-gidh).—Very common throughout the district. One male was shot from near the Railway station, Hoshiarpur.

64. (1713) *Pseudogyps bengalensis* (Gidh).—This bird is also very commonly met with throughout the district. One male was shot from Hoshiarpur and a female from Shah-Nur-Jamal. According to Stuart Baker (1928), however, this bird is comparatively rare in the Panjab.

65. (1714) *Neophron percnopterus percnopterus* (Chitta Gidh, Sufed Gidh).—Very common throughout the district. Specimens were shot from the Bhangi cho, Hoshiarpur.

66. (1721) *Falco jugger* (Laggar).—A single male was shot from Garhshankar.

67. (1740) *Cerchneis tinnunculus tinnunculus*.—Very common throughout the district in winter. Specimens were shot from Jehan Khelan, Khanpur and Bankhandi.

68. (1749) *Aquila rapax vindhiana* (Wokab, Ookaab).—Only one specimen (male) was shot from Birumpur.

69. (1774) *Butastur teesa* (Baz.)—Very common throughout the district. Specimens were shot from Garhshankar, Padrana and Hoshiarpur.

70. (1787) *Milvus migrans govinda* (ill, cheel).—This is again a very common bird of the district. Specimens were shot from Garhshankar and the Model Town, Hoshiarpur.

71. (1803) *Astur badius dussumieri* (Shikra).—This bird is also very common throughout the district. Specimens were shot from Garhshankar, the Model Town, Hoshiarpur, Khanpur and the Bhangi cho, Hoshiarpur.

72. (1856) *Columba livia intermedia* (Kabutar, Jungli Kabutar, Gola Kabutar).—Abundantly found throughout the district. Specimens were shot from Garhshankar, Birumpur and Khanpur.

73. (1873) *Streptopelia chinensis suratensis* (Chitur-mitri ghughi, chitkabri-fakhta).—This bird is not very common in this district.

A few birds were observed near Bassi Ghulam Hussain and single bird was seen near the Model Town, Hoshiarpur.

74. (1877) *Streptopelia senegalensis cambaiensis* (Chhoti ghughi).—Very common bird of the district. Specimens were shot from Jehan Khelan, Sajna, Garhshankar and the Model Town, Hoshiarpur.

75. (1879) *Streptopelia decaocto decaocto* (Ghughi, Fakhta).—This is the commonest dove of the district. Specimens were shot from Mukerian, Hoshiarpur and Birumpur.

76. (1881) *Œnopopelia tranquebarica tranquebarica* (Gérvi Ghughi).—This bird is not so common as the two preceding birds, i.e. the brown dove and the ring-dove but is still found in considerable numbers throughout the district. One male was shot from Khanpur.

77. (1897) *Pavo cristatus* (Mor, Boder ♀).—Very abundant in the hill-side areas of Garhshankar, Hoshiarpur, Una, Mukerian and Bala chaur. Specimens were shot from Bankhandi and Garhshankar.

78. (1903) *Gallus bankiva murghi* (Jungli murgha, Ban murgha, Ban Kukkar ♂; Jungli murghi, Ban murghi, Ban Kukri ♀).—Very common in the jungles of Shiwaliks.

79. (1918) *Gennæus hamiltonii* (Kolsa).—This bird is also found in the Shiwalik forests.

80. (1950) *Coturnix coturnix coturnix* (Batera).—This bird is abundant in winter in the cereal crops and the bushes of the semi-jungle areas of the district. Specimens were shot from Bankhandi.

81. (1976) *Francolinus francolinus asiæ* (Kala tittar).—This bird is very common at the foot of the hills, in high grasses and also up to small elevations, in bush jungles and tall grasses. Specimens were shot at Bankhandi.

82. (1984) *Francolinus pondicerianus interpositus* (Tittar).—Very common throughout the district.

83. (2047) *Burhinus œdicnemus indicus* (Karbanak).—This bird is found on the sandy tracts of the dry chos throughout the district. A single male was shot from the chohal cho.

84. (2052) *Cursorius coromandelicus*.—Only once in the month of December a few birds of this species were observed running in the freshly ploughed fields. One of these birds was shot and this turned out to be a male.

85. (2080) *Sterna aurantia*.—This bird is found throughout the district along the water tracts, permanent and semi-permanent ponds. Three specimens were shot from the pond near the Railway level crossing, Hoshiarpur.

86. (2114) *Charadrius dubius curonicus*.—Only once in the month of January a few birds were noticed in the Khanpur cho and shot.

87. (2125) *Lobivanellus indicus indicus* (Tatteeri).—This bird is very common throughout the district.

88. (2145) *Tringa hypoleucos* (chaha).—It is found throughout the district along the water tracts and near the permanent and semi-permanent ponds. Specimens were shot from Garhshankar, Sajna and Mukerian.

89. (2218) *Ardea cinerea rectirostris*.—This is purely a water bird and is found in the permanent ponds and water ducts of the district. Two specimens were shot from the pond near the Railway level crossing, Hoshiarpur.

90. (2222) *Egretta alba alba* (Bara Bagla).—This bird is found near water, running water tracts or permanent ponds. One specimen was shot from Mukerian.

91. (2225) *Egretta garzetta garzetta* (Bagla, Chitta Bagla).—Found near running water or permanent ponds throughout the district.

92. (2226) *Bubulcus ibis coromandus* (Badami Bagla).—Very common throughout the district, feeding in fields near the grazing cattle.

93. (2229) *Ardeola grayii* (Bagla).—Very commonly found throughout the district. It is essentially a water bird.

94. (2271) *Nettion crecca crecca* (Murghabi, Jal-Kukarri).—Very common at Nangal, Mukerian and Bala chaur. A few stragglers often visit the permanent ponds. One specimen was shot from Khanpur pond.

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SPERMATOGENESIS OF A DIGENETIC TREMATODE *COTYLOPHORON ELONGATUM*

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SPERMATOGENESIS OF A DIGENETIC TREMATODE *COTYLOPHORON ELONGATUM*

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Introduction

The review of the literature on the spermatogenesis of the adult digenetic trematodes suggests that the cytological investigations are inconclusive in many respects. Cytoplasmic structures have been completely investigated only in one instance of *Isoparorchis eurytremum* (Dhingra, 1954*a*). The existing information on the composition of the sperm is, however, conflicting. Most of the workers have considered the sperm as only a nuclear product, but Woodhead (1931) and Dhingra (1954*a*) have reported that a part of the sperm is cytoplasmic.

In view of the conflicting opinions with regard to the nature of the ripe sperm and the urgent necessity of the chromosome study together with cytoplasmic components in this group of digenetic trematodes, I undertook the present investigation on the spermatogenesis of *Cotylophoron elongatum*.

Cotylophoron elongatum is found in the stomach of sheep. The material was collected from the slaughter house at Hoshiarpur during the months of March and April, 1953. The testes were taken out and directly put into the fixatives like Bouin's fluid, Allen's modification B-15, Carnoy (6 : 3 : 1), Flemming-without-acetic, and Champy. Serial sections were cut 6–12 microns thick and were mostly stained with 0·5% Iron Haematoxylin. Carnoy's fixed material was stained with Aceto-carmine. Smears were made in Bouin's, F.W.A. and Carnoy's fluids. Smears proved more helpful than the sections.

All drawings were made with the help of a Camera lucida. Observations were made with 1/12th oil immersion objective and ×10 ocular.

Acknowledgments

I am greatly thankful to Dr. Vishwa Nath, Professor and the Head of the Zoology Department, Panjab University, for his kind guidance, valuable suggestions and the corrections he made in the manuscript of this paper. My thanks are also due to Dr. H. R. Mehra, Professor and the Head of the Zoology Department, Allahabad University, who very kindly identified the trematodes for me.

Observations

There are two testes lying antero-posteriorly in the middle region between the two intestinal caeca. The testes are slightly lobed. The

wall of each testis is formed of fibrous tissue. In the testis-cavity the germ cells lie in a somewhat unorganized manner. The primordial germ cells and the spermatogonia are, however, often found near the periphery, but they do not form any definite lining all around. In the mature specimens, which have chiefly been used in the present investigation, such cells are very few and they may just be seen in small patches near the periphery. In the immature specimens the primordial germ cells are more in abundance.

Primordial germ cells

The primordial germ cells, which lie in the peripheral region, are seen to occur in patches. These cells are quite small and their diameter ranges from 3·0 μ to 6·0 μ. Very often the nuclear outlines are difficult to mark out from the cell membranes. These cells are often seen in the resting stage with one or two small nucleoli (Pl. I, fig. 1). In the F.W.A. fixed material, however, the cytoplasm shows some mitochondrial granules, lying in the juxta-nuclear region (Pl. I, fig. 2). These cells undergo multiplicative divisions, but the prophase reorganizing stages of the chromosomes are rather difficult to trace. In a few instances mitotic divisions have been observed, but the chromosomes are so much clumped together that the full diploid number (16) has not been determined (Pl. I, fig. 3).

Spermatogonia

An unknown number of divisions of the primordial germ cell leads to the formation of the primary spermatogonia, which measure about 8·0 μ to 11·5 μ in diameter. Invariably such cells are seen to have two nucleoli, but a morphological difference between the primordial germ cells and the primary spermatogonia is rather difficult to establish. The largest individual cells lying away from the patches of primordial germ cells have been regarded as the primary spermatogonia (Pl. I, fig. 4). A mass of mitochondrial granules has been observed in the cytoplasm (Pl. I, fig. 5). In the metaphase plate the chromosomes are seen to lie distinctly, and the full diploid number (16) is countable (Pl. I, fig. 6). The mitochondrial granules are, however, not well demonstrated during division stages.

On division the two resultant secondary spermatogonial nuclei start reorganizing in the original undivided cytoplasmic mass (Pl. I, fig. 7). When the two nuclei are reorganized a mass of mitochondrial granules, corresponding to each daughter cell, becomes visible (Pl. I, fig. 8). The secondary spermatogonia show some further growth and attain a diameter of 10 μ. The mitochondrial mass by now is partly dispersed in the cytoplasm and partly condensed to form a small stained mass (Pl. I, fig. 9). Cytoplasmic cleavage then sets in, but it is not complete inasmuch as the two secondary spermatogonia retain a cytoplasmic connection (Pl. I, fig. 10). Very often these cells are seen to have a single nucleolus. In the following mitosis early prophase stages are difficult to trace, but occasionally chromatin threads are seen (Pl. I, fig. 11). Following this the chromosomes are dispersed in the cytoplasm and, during metaphase, full diploid number (16) is easily counted. The two cells again show a closer connection (Pl. I, fig. 12), and may sometimes show a common investing cytoplasm. The

chromosomes of the two secondary spermatogonia divide together and reach the telophase stage (Pl. I, fig. 13).

The four daughter nuclei remain enclosed in a common cytoplasm (Pl. I, fig. 14). Cytoplasmic demarcations then follow. At this stage each of these four tertiary spermatogonia measures about 4 μ in diameter and shows two nucleoli. The mitochondria are seen in the F.W.A. fixed material (Pl. I, fig. 15). A further growth in the tertiary spermatogonia is noticed, and each cell attains a diameter of 8 μ (Pl. I, fig. 16). Like the secondary spermatogonia, the tertiary spermatogonia are also very close together during mitotic divisions, and may occasionally show a common investing cytoplasmic sheath. As the division of the four tertiary spermatogonia is simultaneous, the metaphase chromosomes of all the tertiary spermatogonia can be seen in the same cluster. The chromosomes are often countable, and the full diploid number (16) can be determined in all the four tertiary spermatogonia (Pl. I, fig. 17). As the divisions proceed a resultant cluster of 8 cells with distinct outlines and united to each other at a point are seen (Pl. I, fig. 18).

Spermatocytes

The primary spermatocyte clusters consist of 8 cells each and are united to each other at a point. Individually these cells are the largest to be seen in the sequence of spermatogenesis. The spermatocytes measure each from 9·0 μ to 12·5 μ in diameter, and their nuclei are 5·5 μ to 9·5 μ in diameter. With the onset of meiosis, the resting stage is organized into the leptotene threads, which are seen scattered in the whole of the nucleus (Pl. I, fig. 19). The individual ends of the threads, however, cannot be seen. In the subsequent zygotene stage the threads are thicker and deeply stained. Sometimes a few pairing threads can be followed for some distance (Pl. I, fig. 20). The process of polarization is then completed and the threads are seen directed towards the nucleolus (Pl. I, fig. 21). The number of loops is rather difficult to determine due to their overlapping, but they are not seen to exceed the haploid number (8) of chromosomes. After this pachytene stage, in which the chromosomal bands appear thicker than the leptotene threads, the loops lose their orientation and start spreading out in the nucleus (Pl. I, fig. 22). At places the members of a homologous pair are seen separated, but at certain points they are held together. The chromosomes show further condensation and their contours become smooth. This then leads to a typical diakinesis stage, when thick tetrads become visible in the nucleus (Pl. I, figs. 23, 24 and 25). The tetrads condense further and appear thicker (Pl. I, fig. 26). The nuclear wall then breaks down and the haploid number (8) of chromosomes comes to lie in the cytoplasm. In the polar view of the metaphase plate, this number is easily determined (Pl. I, figs. 27 and 28). The chromosomes are then aligned on the spindle, and a small transversely elongated centrosomal granule is seen at either end of the spindle (Pl. I, fig. 29). At each end the centrosomal granule divides (Pl. I, figs. 30 and 31). The homologous chromosomes then separate and pass to the poles (Pl. I, fig. 32).

Except for the centrosomes, which are occasionally met with, the mitochondria are the only cytoplasmic inclusions, which have been observed in the present investigation. In the newly formed cluster of the primary spermatocytes, where the spermatocytes measure each only 9 μ in diameter, the mitochondria are seen as a mass of lightly-stained granules, lying in the usual juxta-nuclear position (Pl. I, fig. 33). A part of this mass becomes deeply-stained and appears in one or two irregular masses. The remaining granules are scattered in the cytoplasm (Pl. I, figs. 34 and 35). As the cells grow, the well-stained mass also increases and tends to remain close to the nucleus (Pl. II, fig. 36). With the onset of the meiotic activities of the chromosomes, the well-stained mitochondrial mass becomes more deeply stained and lies often on or close to the nucleus. A few mitochondrial granules, however, remain dispersed in the cytoplasm (Pl. II, fig. 37). The close proximity of the stained mass to the nucleus continues even during the diplotene and the diakinesis stages (Pl. II, figs. 38 and 39). As the nuclear wall breaks down and the chromosomes are liberated in the cytoplasm, the deeply staining mitochondrial mass is seen to lie away from the metaphase chromosomes (Pl. II, fig. 40). Soon the intensity of the mitochondrial mass fades away and instead of a single mass there appear two to four lightly stained masses (Pl. II, figs. 41, 42 and 43). This probably has been effected by the division of that mass. Small mitochondrial granules, however, remain dispersed in the cytoplasm.

On the simultaneous completion of the first meiotic division in the 8 cells of the primary spermatocyte cluster, the daughter nuclei are reorganized before cytoplasmic cleavages set in. In each of the resultant secondary spermatocyte, there is seen a small, but well-stained mitochondrial mass and some dispersed mitochondrial granules (Pl. II, fig. 44). Cytoplasmic divisions then follow and a cluster of 16 cells is formed (Pl. II, fig. 45). In this newly formed secondary spermatocyte cluster the nuclei are faintly stained and show no nucleoli in them. The nuclei, however, increase in size and attain a diameter of 4·5 μ. In preparation for the 2nd meiotic division, the nuclei of the secondary spermatocytes undergo reorganization, and there appear condensed chromatic structures, corresponding to the chromosomes (Pl. II, fig. 46). The mitochondria are, however, seen scattered uniformly in the whole of the cytoplasm. In the ensuing stage, the chromosomes appear in the metaphase as rods or granules. The individual cells of the cluster may not, however, exhibit exactly identical phases (Pl. II, fig. 47). In certain metaphase II stages, the chromosomes appear very much like those of metaphase I, but are much smaller (Pl. II, fig. 48). Centrosomes and spindles are sometimes met with (Pl. II, fig. 49). As the chromosomes divide and the telophase stage is reached, a chromatic mass at each pole is formed (Pl. II, fig. 50). The daughter nuclei are then reorganized, but the cytoplasmic cleavages do not set in for some time. At this stage the mitochondria are seen scattered in the whole of the cytoplasm, and a centrosomal granule becomes evident in close relation with each nucleus (Pl. III, fig. 51). The spermatid nuclei increase in size and the cytoplasmic cleavages are effected. A spermatid cluster of 32 cells is thus formed (Pl. III, fig. 52).

Spermateleosis

The individual cells of the 32-spermatid cluster show distinct outlines towards the outer free side. The spermatid nuclei are weakly stained and show no nucleoli. Soon they come to lie close to the cell wall towards the outer free boundaries of the spermatids. A centrosomal granule in each is seen between the nucleus and the cell membrane, but the mitochondrial granules remain scattered in the cytoplasm. The spermatid nucleus then begins to elongate. At first the rounded nucleus becomes ovoid with the pointed end towards the free border and the centrosomal granule lying between the nucleus and the cell boundary. The oxyphilic nature of the nucleus becomes more pronounced (Pl. III, fig. 53). Subsequently the nucleus becomes more elongated (Pl. III, figs. 54 and 55). In a little more advanced stage the nucleus becomes basiphilic, but no other appreciable change is noticed (Pl. III, fig. 56). As the nucleus becomes more and more elongated, it protrudes slightly towards the free border. The centrosomal granule at this stage is seen to lie at the base of the nucleus and a small but broad lightly stained flagellum becomes visible (Pl. III, fig. 57). The nuclear elongation continues and with this the flagellum is also seen to elongate. The mitochondria, however, remain scattered in the cytoplasm (Pl. III, figs. 58 and 59). On further elongation the nucleus becomes curved or somewhat coiled in the cytoplasmic mass of the spermatid cluster (Pl. III, fig. 60). Towards the close of development the spermatid nucleus straightens up and starts protruding out of the cytoplasmic mass. The flagellum at the same time shows further elongation (Pl. III, fig. 61). When the sperm is about to mature the flagellum becomes deeply-stained, and a distinct centrosomal granule cannot be made out. The whole structure appears spindle-shaped with both the ends pointed (Pl. III, fig. 62). The 32 sperms, which develop together in the common cytoplasmic mass of the spermatid cluster, wriggle out and leave behind a massive residual mass. The mature sperm as set free in the testis cavity appears spindle-shaped with both ends pointed. The anterior half, which is formed from the nucleus, is, however, thicker than the posterior half, which constitutes the flagellum (Pl. III, fig. 63). The residual cytoplasmic mass on being evacuated by the sperms assumes a globular appearance. The mass is loaded with the mitochondrial granules, which have been left behind. Such globules are often seen in the testis cavity (Pl. III, fig. 64).

Chromosomes

It has been shown above that the haploid number of chromosomes is 8 and the diploid 16. The mitotic chromosomes were studied from the metaphase plates of the primary, secondary and tertiary spermatogonia, while the meiotic chromosomes were studied from the metaphase plates of primary and secondary spermatocytes. Generally speaking, the chromosomes are well-spaced, but sometimes they are too crowded for a critical study. The number of chromosomes has been found to be constant in a number of specimens of this species.

In Figure 65, Plate III, the mitotic chromosomes have been grouped in pairs according to their length; and in Figure 66, Plate III, are arranged the meiotic chromosomes according to their length as met with in the diakinesis and metaphases.

The longest pair of chromosomes is nearly 3 μ. The second pair, which is also long, measures about 2·5 μ. The third pair is J-shaped with a sub-median construction, and each measures 2 μ. The fourth and fifth pairs are respectively 1·75 and 1·5 μ in length. The rest of three pairs are quite small and measure each from 1 μ to 1·25 μ in length.

Discussion

In the presence of three spermatogonial and two meiotic divisions with the formation of clusters, the sequence of stages in the spermatogenesis of *Cotylophoron elongatum* resemble many other forms. But unlike other forms, the nuclear reorganizations of the newly formed daughter cells in this species precede cytoplasmic divisions, and thus for sometime the daughter cells remain in a common investing membrane.

The most significant point worthy of note in the present investigation is the absence of the Golgi material. The presence of the Golgi body in *Isoparorchis eurytremum* (Dhingra, 1954*a*) up to the spermateleosis stages, where it gradually degenerates, and its absence in *Cotylophoron elongatum* is an example of its degeneration in different species at varying stages of spermatogenesis. Nath (1932, 1937 and 1942), working on the non-flagellate sperms of the Decapod Crustacea, has also shown the degeneration of the Golgi material at varying stages of spermatogenesis.

Regarding the mitochondria it has been observed that they exist as a pale juxta-nuclear mass during the resting stages, but at the time of nuclear activity some of the mitochondria are condensed to form a deeply-stained mass. This behaviour is particularly well pronounced during the meiotic activities of the primary spermatocytes, when a deeply stained mitochondrial mass is seen in close relation with the nucleus. Gradually the mitochondrial mass disappears from view. During spermateleosis the mitochondria remain scattered in the cytoplasm and are left behind in the residual mass without forming any sperm component. In *Isoparorchis eurytremum* (Dhingra, 1954*a*) where the Golgi material is present, the mitochondria are not seen to have condensed in a thick mass at any stage. The casting out of the mitchondria without forming any sperm component has also been observed by Dingler (1910) in *Dicrocoelium lanceolatum* and Dhingra (1954*a*) in *Isoparorchis eurytremum*. Observations in other groups of animals as made by Sharma (1950) in *Plexippus paykulli*, Nath and Bhatia (1953) in *Lepisma domestica*, and Nath and Rishi (1953) in *Sympetrum hypomelas* also point towards the conclusion that the mitochondrial material as such is not always an essential component of the ripe sperm.

Another important conclusion of this investigation is that the sperm consists of a nucleus, a centrosome and a flagellum, the entire cytoplasmic mass having been cast off. This confirms my previous observations on *Isoparorchis eurytremum* (1954*a*) and *Cyclocoelum bivesiculatum* (1954*b*). I

am unable to agree with Anderson (1935), Rees (1939), Markell (1943) and Willmott (1950), who have all regarded the sperm as a nuclear structure alone.

My observations on the residual cytoplasmic masses are in accord with those of Dingler (1910), Anderson (1935) Rees (1939), Willey and Godman (1951) and Dhingra (1954*a*, *b*), who have all described them as globular masses formed on the evacuation of the sperms from the spermatid rosette. Markell (1943), however, stated that as the sperms of *Probolitrema* mature the cytoplasmic mass in which they are embedded becomes more and more broken down. The disintegration of the cytoplasm seems to be a constant feature of sperm formation in the group. Criticizing those who described residual cytoplasmic globules, Markell stated: 'It is difficult to understand how this cytoplasm could after liberating the sperm, round up to form the spherical, compact residual cytoplasmic masses'. According to him the residual cytoplasmic masses may just be vacuolated cells, which appear in the peripheral region as seen in *Probolitrema*. To me the interpretation of Markell (1943) appears doubtful. How are we going to account for the abundant mitochondrial granules seen in the globular masses? The vacuolated cells of Markell are some peculiar structures, which appear in some forms and are distinct from the residual cytoplasmic bodies.

The chromosome investigations have been made in four genera and five species of the family *Paramphistomidae*. In *Zygocotyle lunata* Willey and Godman (1951) counted 7 haploid and 14 diploid chromosomes. In *Gigantocotyle bathycotyle* as reported by Willmott (1950*a*), there are 6 haploid and 12 diploid, and in *Paramphistomum hiberniae* and *P. Scotiae* (Willmott, 1950*b*) there are 6–8 and 8 haploid chromosomes respectively. In *Cotylophoron elongatum* also there are 8 haploid and 16 diploid chromosomes. On the basis of chromosome number and their morphology the genus *Zygocotyle* appears distinct from the three other genera. The chromosomes of the genus *Gigantocotyle* look like those of *Paramphistomum* and *Cotylophoron*, but their number is less. Unfortunately Willmott (1950*b*) has not given a satisfactory account of the chromosomes in the two species of *Paramphistomum*, and thus it is difficult to compare the chromosomes of the genus *Cotylophoron* with those of *Paramphistomum*, which show a much closer relation both in their number and form.

Summary

1. Spermatogenesis in *Cotylophoron elongatum* has been described in detail. The haploid number of chromosomes is 8 and the diploid 16.

2. The mitochondria are present at all the stages up to late spermateleosis, when they are all sloughed off along with the entire cytoplasm. They are, however, more significant during meiotic activity when they condense to form a well-stained mass.

3. The Golgi material has not been observed at any stage during spermatogenesis.

4. The centrosomes have been seen both at meiosis I and meiosis II. In the spermatid a centrosomal granule is seen at the base of spermatid from where the flagellum arises.

5. The entire sperm consists of a nucleus, a centrosome and a flagellum.

6. The cast-off cytoplasmic mass occurs in the form of globules in the lumen of the testis, loaded with mitochondria.

7. On the basis of chromosomes number the three genera *Zygocotyle*, *Gigantocotyle* and *Paramphistomum* in which the chromosomes number have been recorded for a few species appear distinct. The chromosome number of *Cotylophoron*, however, corresponds with that of *Paramphistomum*.

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Explanation of Figures

Abbreviations :—

C—Centrosome; *CH*—Chromosomes; *F*—Flagellum; *M*—Mitochondria; *N*—Nucleus.

Plate I

Fig. 1. A group of primordial germ cells. Bouin's and Iron Haematoxylin.

Fig. 2. A group of primordial germ cells, showing the mitochondria (smear). F.W.A. and Iron Haematoxylin.

FIG. 3. Primordial germ cell in metaphase. Bouin's and Iron Haematoxylin.

FIG. 4. Young primary spermatogonium, showing two nucleoli. Bouin's and Iron Haematoxylin.

FIG. 5. Full grown primary spermatogonium, showing mitochondrial granules in the cytoplasm and two nucleoli in the nucleus (Smear). F.W.A. and Iron Haematoxylin.

FIG. 6. Primary spermatogonium in metaphase, showing the diploid number (16) of chromosomes. Bouin's and Iron Haematoxylin.

FIG. 7. Primary spermatogonium at late telophase. Bouin's and Iron Haematoxylin.

FIG. 8. Newly formed secondary spermatogonia invested in a common cytoplasmic sheath, showing mitochondrial granules (Smear). F.W.A. and Iron Haematoxylin.

FIG. 9. Young secondary spermatogonia, still enclosed in a common cytoplasmic sheath. The mitochondria are partly condensed and partly dispersed (Smear). F.W.A. and Iron Haematoxylin.

FIG. 10. Fully developed secondary spermatogonia distinctly marked off. Each nucleus contains one nucleolus (Smear). Bouin's and Iron Haematoxylin.

FIG. 11. Secondary spermatogonia, showing the chromosomes in prophase (Smear). Bouin's and Iron Haematoxylin.

FIG. 12. Secondary spermatogonia in metaphase (Smear). F.W.A. and Iron Haematoxylin.

FIG. 13. Secondary spermatogonia in telophase. F.W.A. and Iron Haematoxylin.

FIG. 14. Newly formed tertiary spermatogonia invested in a common cytoplasmic sheath. Bouin's and Iron Haematoxylin.

FIG. 15. Young tertiary spermatogonia, showing the mitochondria (Smear). F.W.A. and Iron Haematoxylin.

FIG. 16. Fully developed tertiary spermatogonia, occurring in a group of four. Each nucleus has two nucleoli (Smear). Bouin's and Iron Haematoxylin.

FIG. 17. A group of four tertiary spermatogonia in metaphase (Smear). F.W.A. and Iron Haematoxylin.

FIG. 18. A cluster of eight primary spermatocytes (Smear). F.W.A. and Iron Haematoxylin.

FIG. 19. Primary spermatocyte, showing leptotene threads (Smear). Bouin's and Iron Haematoxylin.

FIG. 20. Primary spermatocyte, showing zygotene stage (Smear). Bouin's and Iron Haematoxylin.

FIG. 21. Primary spermatocyte, showing a bouquet stage (Smear). Bouin's and Iron Haematoxylin.

FIG. 22. Primary spermatocyte, showing a diplotene stage (Smear). Bouin's and Iron Haematoxylin.

FIGS. 23–26. Primary spermatocytes, showing diakinesis stages (Smear). Carnoy's and Aceto-carmine.

FIGS. 27 and 28. Primary spermatocytes, in metaphase. Bouin's and Iron Haematoxylin.

FIGS. 29–31. Primary spermatocytes in metaphase, showing the division of the centrosomes at the poles. Bouin's and Iron Haematoxylin.

FIG. 32. *Primary spermatocyte at telophase. Bouin's and Iron Haematoxylin.*

FIG. 33. Young primary spermatocyte, showing a juxta-nuclear mass of mitochondrial granules (Smear). F.W.A. and Iron Haematoxylin.

FIGS. 34 and 35. Primary spermatocytes, showing the mitochondria, partly condensed and partly dispersed (Smear). F.W.A. and Iron Haematoxylin.

PLATE II

FIGS. 36–39. Primary spermatocytes, showing the condensed mitochondrial mass gradually increasing with the progressing prophase of the nucleus (Smear). F.W.A. and Iron Haematoxylin.

FIG. 40. Primary spermatocytes at metaphase, showing the condensed mitochondria mass lying away from the chromosomes (Smear). F.W.A. and Iron Haematoxylin.

FIGS. 41–43. Primary spermatocytes at metaphase, showing the mitochondrial mass divided. The staining intensity of this mass has decreased (Smear). F.W.A. and Iron Haematoxylin.

FIG. 44. A cluster of newly formed secondary spermatocytes, showing 10 of 16 cells. The nuclei have reorganized themselves before the cytoplasmic cleavages. Mitochondria are partly condensed and partly dispersed (Smear). F.W.A. and Iron Haematoxylin.

FIG. 45. A part of the secondary spermatocyte cluster, showing the cytoplasmic cleavages in progress (Smear). F.W.A. and Iron Haematoxylin.

FIG. 46. A part of the secondary spermatocyte cluster, showing the chromosomes condensing in the nuclei. The mitochondria are spread out in the cytoplasm (Smear). F.W.A. and Iron Haematoxylin.

FIG. 47. A cluster of secondary spermatocytes, showing the chromosomes at metaphases and anaphases (Smear). F.W.A. and Iron Haematoxylin.

FIG. 48. Secondary spermatocyte at metaphase showing a haploid number (8) of chromosomes. Bouin's and Iron Haematoxylin.

FIG. 49. Secondary spermatocyte at metaphase, showing the spindle and the centrioles. Bouin's and Iron Haematoxylin.

FIG. 50. A part of the cluster of secondary spermatocytes showing the telophase stages. Bouin's and Iron Haematoxylin.

PLATE III

FIG. 51. A part of the newly formed cluster of spermatids, showing the nuclei reorganized before the cytoplasmic cleavages set in. A centrosomal granule is seen lying close to each nucleus and the mitochondria are spread out (Smear). F.W.A. and Iron Haematoxylin.

FIG. 52. A part of the cluster of spermatids, showing the nuclei coming close to the cell boundary and the centrosomal granules lying between the cell membranes and the spermatid nuclei (Smear). F.W.A. and Iron Haematoxylin.

FIG. 53. A part of the spermatid cluster, showing the nuclei ovoid in form. A centrosomal granule is seen lying at the base of each nucleus and the mitochondria are spread out (Smear). F.W.A. and Iron Haematoxylin.

FIGS. 54, 55. Developing spermatids, showing the nuclei elongated, mitochondria spread out and a centrosomal granule at the base of each spermatid nucleus (Smear). F.W.A. and Iron Haematoxylin.

FIG. 56. Developing spermatids, showing the nuclei turned basophilic (Smear). F.W.A. and Iron Haematoxylin.

FIG. 57. Developing spermatids, showing a flagellum arising in each spermatid from the centrosome (Smear). F.W.A. and Iron Haematoxylin.

FIGS. 58–61. Developing spermatids, showing the nuclei and the flagella elongating. The mitochondria remain scattered (Smear). F.W.A. and Iron Haematoxylin.

FIG. 62. A part of the spermatid cluster, showing the maturing sperms (Smear). F.W.A. and Iron Haematoxylin.

FIG. 63. Mature sperms (Smear). F.W.A. and Iron Haematoxylin.

FIG. 64. Residual cytoplasmic mass, showing the mitochondria (Smear). F.W.A. Iron Haematoxylin.

FIG. 65. Mitotic chromosomes, grouped according to their length.

FIG. 66. (*a*, *b*, *c* and *d*) Meiotic chromosomes arranged according to their length as met with in diakinesis and in meiotic metaphase.

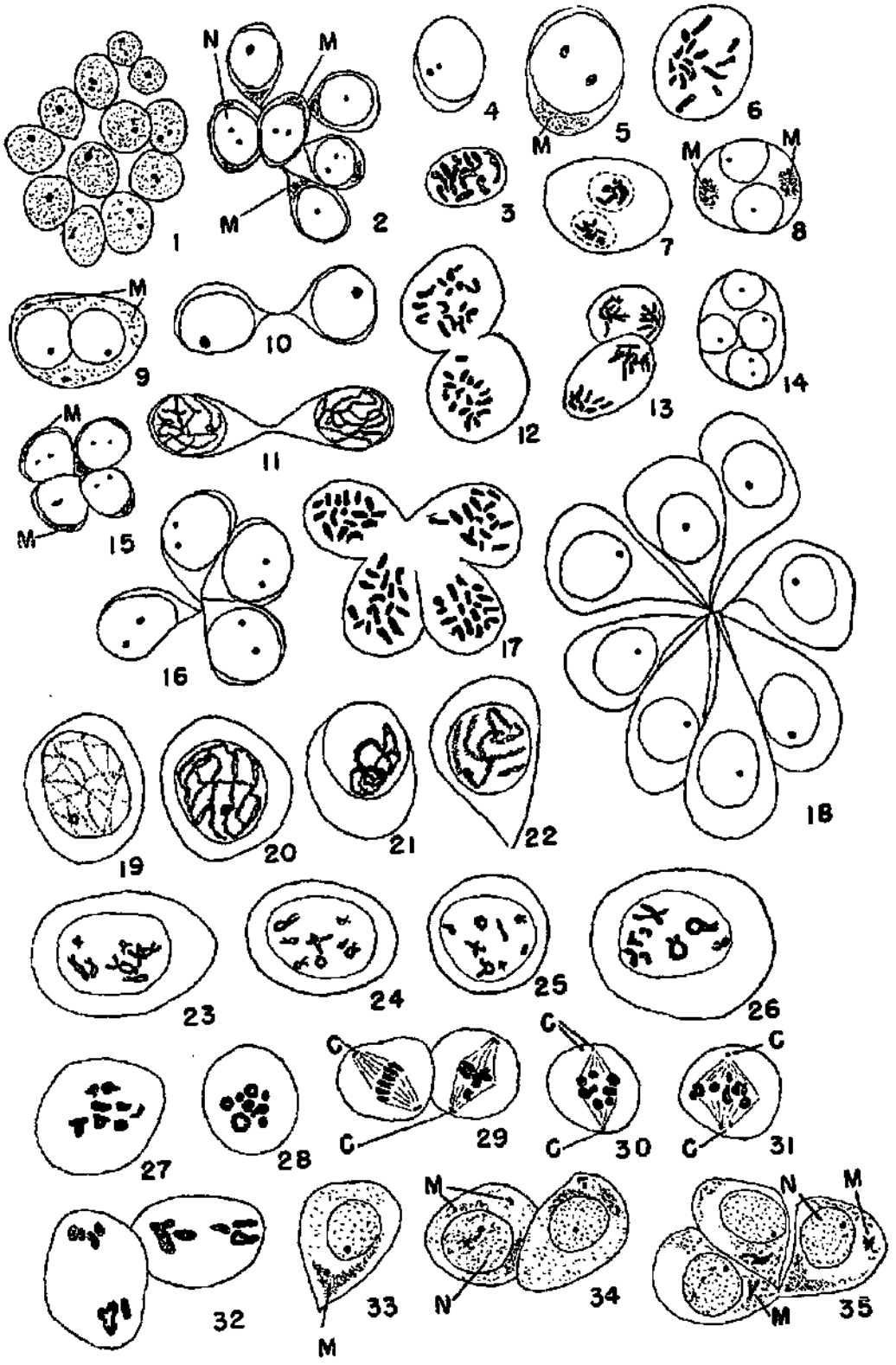
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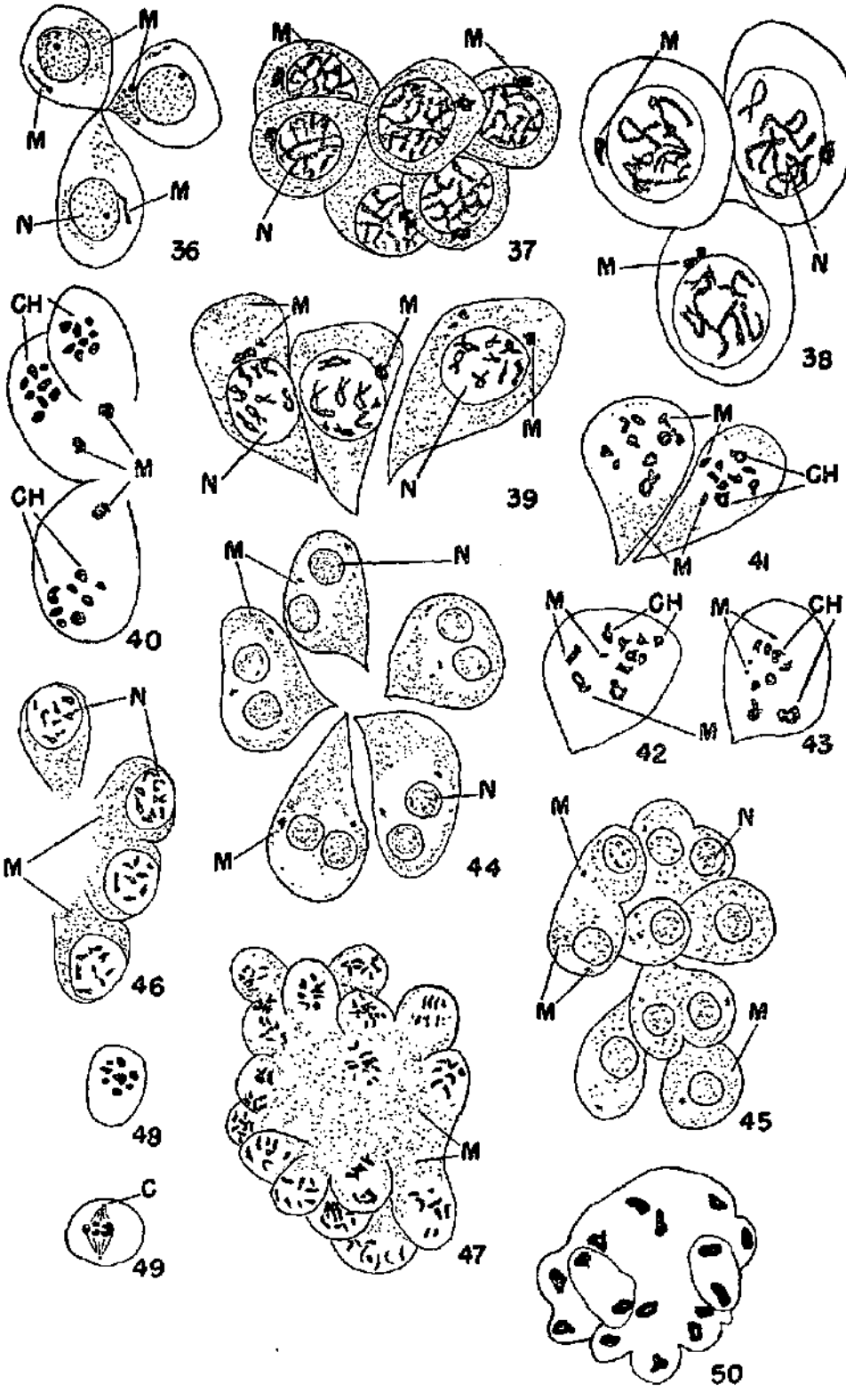
PLATE II.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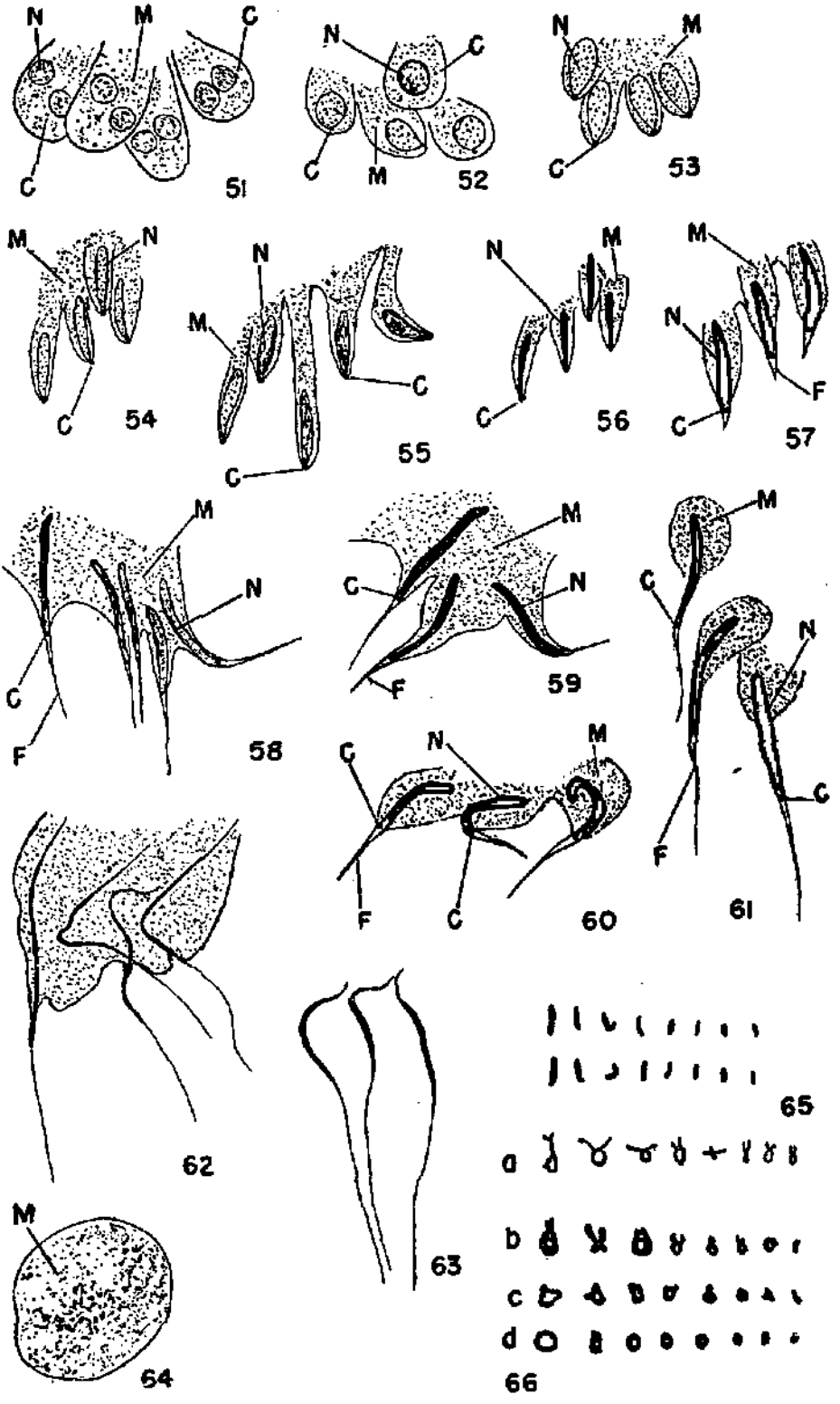

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SPERMATOGENESIS OF A DIGENETIC TREMATODE *GASTROTHYLAX CRUMENIFER*

by

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SPERMATOGENESIS OF A DIGENETIC TREMATODE *GASTROTHYLAX CRUMENIFER*

By Om Parkash Dhingra, *Late Panjab University Research Scholar, Department of Zoology, Hoshiarpur.*

Introduction

Previous to my investigations on *Isoparorchis eurytremum* (1954*a*), *Cyclocoelum bivesiculatum* (1954*b*) and *Cotylophoron elongatum* (1955), the main interest of the cytologists in the study of adult digenetic trematodes was centred round the chromosome investigations alone. My studies have served to open the field of cytoplasmic inclusions in the germ cells. The Golgi bodies and the mitochondria have been traced through many stages in *Isoparorchis eurytreinum*. They, however, form no sperm component. The Golgi bodies are lost during the process of spermateleosis, and the mitochondria are left behind in the residual cytoplasmic mass. In *Cotylophoron elongatum* the mitochondria alone are present and they are left behind in the residual mass. A centrosomal granule in the spermatids has been observed for the first time and the flagellum is seen to arise from it. The sperm, which was held by many as a nuclear product alone, has been shown both as nuclear and cytoplasmic (centrosome and flagellum).

The present paper records my observations on the cytoplasmic structures and their rôle in sperm formation in *Gastrothylax crumenifer*. The chromosome study has also been made. The degenerating cells, as reported by Anderson (1935), Chen (1937), and Willey and Koulish (1950), and the oocytes, as reported by Anderson (1935) in *Proterometra* and Dhingra (1954*b*) in *Cyclocoelum* in the testis, have also been recorded.

Gastrothylax crumenifer is found in the stomach of the sheep. The material was collected from a slaughter house at Hoshiarpur in the month of May, 1953. The testes were taken out with the help of fine forceps and were directly put into Bouin's fluid and Flemming-without-acetic. Serial sections were cut 6–10 μ in thickness and were stained with iron-haematoxylin.

All drawings were made with the help of Camera lucida. Observations were made with 1/12th oil immersion objective and ×10 Ocular.

Acknowledgments

I am highly thankful to Dr. Vishwa Nath, Professor and the Head of the Zoology Department, Panjab University, under whose guidance these investigations were made, for correcting the manuscript and his valuable critical suggestions.

Observations

There are two testes lying side by side close to the acetabulum. The wall of each testis is rather thick and is formed of fibrous tissue. The germ cells lie free in the testis cavity and occur in an unorganized manner. The primordial germ cells and the spermatogonia are, however, seen close to the testicular wall.

The primordial germ cells, as they develop from the testis wall, remain enclosed in a common cytoplasmic mass and undergo many nuclear divisions (Pl. I, fig. 1). Gradually these cells become detached from the common mass and come to lie near the testis wall. The cellular outlines of these cells are rather indistinct and they measure each from 4 to 6 μ in diameter.

Spermatogonia

After repeated divisions the primordial germ cells develop into the primary spermatogonia, which occur along the testis wall, scattered amongst secondary and tertiary spermatogonia. The primary spermatogonia measure each about 7–9 μ in diameter with their nuclei 6–8 μ each in diameter. The nuclei contain each one or two nucleoli in them (Pl. I, fig. 2). In the F.W.A. fixed material, the mitochondrial mass is seen lying on one side of the nucleus in the cytoplasm (Pl. I, fig. 3). At the beginning of nuclear activity, the chromatin undergoes necessary reorganization and forms chromosomal threads (Pl. I, fig. 4). Ultimately the chromosomes are formed and they get aligned on the equator. The spindle and the centrosomes also become visible (Pl. I, fig. 5). In the polar view of the metaphase plate full diploid number (14) is seen (Pl. I, figs. 6 and 7). In the anaphase stage, the chromosomes in some of these cells remain distinct in each set (Pl. I, fig. 8). On reaching the telophase, however, only a compact chromatic mass is seen at each pole (Pl. I, fig. 9). Occasionally the primary spermatogonial cells were seen to attain a diameter of 9 to 11 μ each (Pl. I, fig. 10), and in one case only these large cells were seen to divide (Pl. I, fig. 11). The chromosomes in each anaphase set were clearly visible and they were almost identical with those of the normal spermatogonia.

When a primary spermatogonium divides, the two resultant cells, the secondary spermatogonia, remain together. Invariably they are seen to have only one nucleolus each (Pl. I, fig. 12). In the cytoplasm the mitochondrial mass is seen lying on one side of the nucleus (Pl. I, fig. 13). As in the primary spermatogonia, the most common prophase stage is that when chromosomal threads are seen scattered in the nucleus (Pl. I, fig. 14). On the breaking down of the nuclear wall, the chromosomes come to lie on the equator, and in the polar view the full diploid number (14) becomes evident (Pl. I, fig. 15). As the chromosomes divide and reach the telophase stage, a compact chromatic mass is seen at each pole (Pl. I, fig. 16).

As the division of the secondary spermatogonia is completed, a cluster of four cells, the tertiary spermatogonia, is formed. The four cells remain together and are smaller than the primary and secondary spermatogonia. Each cell measures 4–6 μ in diameter. The nucleus in each is seen to have two nucleoli (Pl. I, fig. 17). The mitochondria in the cytoplasm occupy

the same juxta-nuclear position as seen in the primary and secondary spermatogonia (Pl. I, fig. 18). In the metaphase the chromosomes are often clumped together and their count is not easy (Pl. I, fig. 19). The groups of four tertiary cells show almost simultaneous divisions, and at the telophase stage a chromatic mass is seen near each pole of each cell (Pl. I, fig. 20).

Spermatocytes

The tertiary spermatogonia on division result in the formation of a cluster of eight cells, which grow to form the primary spermatocyte cluster. Each primary spermatocyte measures from 10 to 14 μ in diameter and its nucleus from 6 to 8 μ. The mitochondria in the young spermatocyte are seen in the form of a deeply stained juxta-nuclear mass (Pl. I, fig. 21). As the spermatocytes grow, the mitochondria become lightly stained, and appear as a cloudy mass (Pl. I, fig. 22). The mitochondrial mass then spreads out in the whole of the cytoplasm (Pl. I, fig. 23). The small mitochondrial granules then coalesce and become deeply stained (Pl. I, figs. 24 and 25). When the primary spermatocytes divide the mitochondria get arranged near the periphery (Pl. I, fig. 26).

The prophase activities of the nuclei lead to the formation of the diakinesis stages, when seven tetrads are seen held together at points (Pl. I, fig. 27). As further condensation of the tetrads is continued, the nuclear wall breaks down and the tetrads (7 in number) lie freely in the cytoplasm (Pl. I, fig. 28). In the lateral view of the metaphase plates the spindle and the centrosomes are also seen (Pl. I, fig. 29). When the division of the chromosomes is effected, chromatic masses are quickly formed, which move on the spindle and reach the telophase stage (Pl. I, figs. 30 and 31).

The simultaneous divisions in the primary spermatocyte cluster lead to the formation of a cluster of 16 cells, the secondary spermatocyte cluster. In the young secondary spermatocytes the nuclei are lightly stained and show no nucleoli in them. In the cytoplasm the mitochondrial mass is scattered on one side, and a centrosomal granule is visible close to the nucleus (Pl. II, fig. 32). With the onset of the nuclear activity, the chromosomal threads make their appearance (Pl. II, fig. 33), and at the end of the prophase, when the nuclear wall breaks down, the chromosomes come to lie in the cytoplasm. The mitochondria at this stage are scattered near the periphery (Pl. II, fig. 34). The metaphase chromosomes are quite distinct (Pl. II, fig. 35). In the side view of the metaphase plate the spindles and the centrosomes are clearly seen (Pl. II, fig. 36). As the division proceeds, the chromosomes are drawn towards the poles, and on reaching the telophase stage they form a compact mass near each pole (Pl. II, fig. 37).

Spermateleosis

The secondary spermatocyte clusters divide to form clusters of 32 cells, the spermatid clusters. In the newly formed spermatids the nuclei are lightly stained. The mitochondria are seen as a condensed mass, lying on one side of the nucleus; and a centrosomal granule in each spermatid is visible at the base of the nucleus towards the outer free side (Pl. II, fig. 38).

The mitochondrial mass then breaks up into smaller granules, and the nuclei start picking up some stain (Pl. II, figs. 39 and 40). The spermatid nuclei then elongate. At first they become ovate with the pointed ends towards the outer free border, where a centrosomal granule at the base of each nucleus can be easily detected. The mitochondria, however, remain scattered in the cytoplasm as well-stained granules (Pl. II, fig. 41). As development proceeds the nuclei become slightly more elongated (Pl. II, figs. 42–44). The spermatid nuclei elongate more and more and they become pointed even towards the anterior side. The mitochondria, however, remain scattered in the cytoplasm (Pl. II, figs. 45 and 46). The staining capacity of the spermatid nuclei then starts changing. The change is, however, gradual. First of all the anterior half of the spermatid nucleus becomes deeply stained, and gradually the basiphilic nature extends downwards (Pl. II, figs. 47, 48 and 49). The flagellum during all these stages remains oxyphilic, but subsequently this also stains deeply (Pl. II, fig. 50). The ripe sperms, when they come out of the spermatid rosette, appear spindle-shaped with the anterior half thicker than the posterior (Pl. II, fig. 51). The residual cytoplasmic mass, which is left behind on the evacuation of the sperms from the spermatid rosette, looks globular and is loaded with the mitochondrial granules. (Pl. II, fig. 52.)

Chromosomes

As already pointed out, there are 7 haploid and 14 diploid chromosomes. In figure 53 are arranged the mitotic chromosomes according to their length. The longest pair of chromosomes, which is 4·5 μ in length, is J-shaped. The second pair of chromosomes, which is V-shaped, is 4 μ in length. The third pair has a median constriction, and measures 3·5 μ. The fourth pair is 3·5 μ, and is slightly curved. The rest of chromosomes appear rod-like, measuring 1 to 2 μ in length.

In figure 54 are arranged the meiotic chromosomes as they appear in the late prophase.

Irregularities

Occasionally in the testis, near its periphery, are seen some cells with their chromatin condensed into a deeply stained mass. These are probably the degenerating spermatogonia (Pl. II, fig. 55). A few nuclei are also seen, which do not appear to be those of the male germ cells (Pl. II, fig. 56). In addition to these some fully developed oocytes have been observed (Pl. II, fig. 57).

Discussion

An important point brought out in the present investigation is the presence of a centrosomal granule in the spermatid from where the flagellum arises. Though the tail has been described as cytoplasmic by Woodhead (1931), only Dhingra in *Isoparorchis eurytremum* (1954*a*), *Cyclocoelum bivesiculatum* (1954*b*) and *Cotylophoron elongatum* (1955) has referred to the presence of a centrosomal granule in the spermatids. In the composition of the sperm enters a nucleus, a centrosome and a flagellum. Anderson

(1935), Rees (1939), Markell (1943) and Willmott (1950) have, however, regarded the sperm as a purely nuclear product.

The presence of the mitochondrial mass in the spermatogonial cells is in accordance with my observations in *Isopararchis eurytremum* (1954*a*). In the spermatocytes the mitochondrial mass is seen on one side of the nucleus and, as the cell grows, it spreads out in the cytoplasm. As they do so, the mitochondria coalesce to form deeply stained granules. During meiosis I, however, the mitochondria again become amorphous and remain so in meiosis II, which follows. In the spermatids the mitochondria again condense into a small well-stained mass, which breaks up into two or three granules. The mitochondrial granules remain as such, till ultimately they are left behind in the residual cytoplasmic mass. These changes in the staining reactions of the mitochondria have also been reported by me in *Cotylophoron elongatum* (1955). It may be noted that the mitochondria condense into deeply-staining bodies in *Cotylophoron elongatum* (Dhingra, 1955) and *Gastrothylax* alone, where the Golgi material is absent and not in *Isopararchis eurytremum* (Dhingra, 1954*a*), where the Golgi material is present.

The significance of the degenerating spermatogonia is not very clear to me. They probably contribute to the nourishment of the testis. Anderson (1935) reported that the most usual stage in development, during which degeneration or pycnosis occurs, is that of the primary spermatocytes, but he maintained that it is possible that there may be a relationship between these cells and the oocytes, which appear in the testis. Willey and Koulish (1950) in the testis of *Gorgodernia attenuata* reported groups of cells similar to the degenerating oocytes of Anderson. Chen (1937) also observed what she regarded as the degenerating spermatogonia in the testis.

Regarding the presence of oocytes in the testis of *Gastrothylax crumenifer* I cannot say more than that a few of them have been seen. Anderson (1935) in the testis of *Proterometra macrostoma* observed oocytes, which he believed were formed from the detached spermatocytes. Dhingra (1954*b*) in *Cyclocoelum bivesiculatum* traced the formation and the degeneration of the oocytes in the testis and observed nourishing masses associated with the oocytes, which had never been reported before.

Summary

1. The process of spermatogenesis in *Gastrothylax crumenifer* has been worked out. The haploid number of chromosomes is 7 and the diploid 14.

2. The mitochondria, which are present in all the earlier stages of spermatogenesis, are left behind in the residual cytoplasmic mass without forming any sperm component.

3. A centrosomal granule is present in the spermatids and from it arises the flagellum.

4. The sperm consists of an elongated nucleus, a centrosome and a flagellum (axial filament).

5. Peculiar structures in the testis include the degenerating spermatogonia, some unidentified nuclei and the oocytes.

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Explanation of Plates

Abbreviations:

C—centrosome; *F*—flagellum; *M*—mitochondria; *N*—nucleus.

Plate I

Fig. 1. Primordial germ cells, showing their relation with the testicular wall and their 'cysted' condition.
Fig. 2. Primary spermatogonium, showing two nucleoli.
Fig. 3. Primary spermatogonium, showing a single nucleolus and the mitochondria.
Fig. 4. Primary spermatogonium in prophase.
Fig. 5. Primary spermatogonium in metaphase, showing the chromosomes, spindle and the centrosomes.
Figs. 6 and 7. Primary spermatogonia in metaphase, showing the full diploid number (14) of chromosomes.
Fig. 8. Primary spermatogonium in anaphase, showing the diploid number (14) of chromosomes in each set.
Fig. 9. Primary spermatogonium in telophase.
Fig. 10. An unusually large primary spermatogonium.
Fig. 11. An unusually large primary spermatogonium in anaphase.
Fig. 12. Secondary spermatogonia, showing a single nucleolus in each.
Fig. 13. Secondary spermatogonia, showing the mitochondria in the cytoplasm.
Fig. 14. Secondary spermatogonia in prophase.
Fig. 15. Secondary spermatogonia in metaphase, showing the diploid number (14) of chromosomes.
Fig. 16. Secondary spermatogonia, showing the telophase.
Fig. 17. Tertiary spermatogonia in a cluster of four cells. Each cell has two nucleoli.
Fig. 18. Tertiary spermatogonia, showing the mitochondria in each cell.
Fig. 19. Three of the four tertiary spermatogonia in metaphase, showing the chromosomes rather overlapping.
Fig. 20. Three of the four tertiary spermatogonia in telophase.
Fig. 21. Four of the eight primary spermatocytes, showing the condensed mitochondria.

FIG. 22. A primary spermatocyte, showing the mitochondria having formed the amorphous mass.

FIG. 23. A primary spermatocyte, showing the mitochondria spread out in the whole of cytoplasm.

FIG. 24. A primary spermatocyte, showing the mitochondria coalescing into deeply stained granules.

FIG. 25. A primary spermatocyte, showing the mitochondria as deeply stained granules.

FIG. 26. Four of the eight primary spermatocytes in metaphase, showing the mitochondria occurring peripherally.

FIG. 27. A primary spermatocyte, showing diakinesis.

FIG. 28. Four of the eight primary spermatocytes in metaphase, showing 7 tetrads.

FIG. 29. A primary spermatocyte in metaphase, showing 7 tetrads, spindle and centrosomes.

FIG. 30. A primary spermatocyte in anaphase. Spindle and centrosomes are also seen.

FIG. 31. A primary spermatocyte in telophase.

PLATE II

FIG. 32. Four of the sixteen secondary spermatocytes, showing lightly stained nuclei without any nucleoli, a centrosomal granule in each and the mitochondria spread on one side of each nucleus.

FIG. 33. Five of the sixteen secondary spermatocytes, showing prophase.

FIG. 34. Seven of the sixteen secondary spermatocytes in metaphase, showing the chromosomes and the mitochondria.

FIG. 35. Five of the sixteen secondary spermatocytes in metaphase, showing the haploid number (7) of chromosomes of meiosis II.

FIG. 36. Secondary spermatocytes in metaphase, showing the chromosomes, spindle and the centrosomes.

FIG. 37. Six of the sixteen secondary spermatocytes in telophase.

FIG. 38. Six of the 32 spermatids, showing a condensed mitochondrial mass in each and a centrosomal granule at the base of each nucleus.

FIGS. 39 and 40. Spermatids, showing the mitochondria broken up into granules and the nuclei slightly basiphilic.

FIG. 41. Spermatids, showing the nuclei slightly elongated with a centrosomal granule at the tip of each nucleus. The mitochondrial granules are condensed.

FIG. 42. Spermatids, showing the nuclei slightly more elongated.

FIG. 43. Spermatids, showing a flagellum arising from each centrosome.

FIGS. 44–46. Spermatids, showing the successive stages of development with the flagellum gradually lengthening out.

FIGS. 47–49. Spermatids, showing a further change in the intensity of the nuclei.

FIG. 50. Spermatids, showing the nuclei tapering into flagella.

FIG. 51. Ripe sperms.

FIG. 52. Residual cytoplasmic mass loaded with the mitochondrial bodies.

FIG. 53. Mitotic chromosomes arranged according to their length.

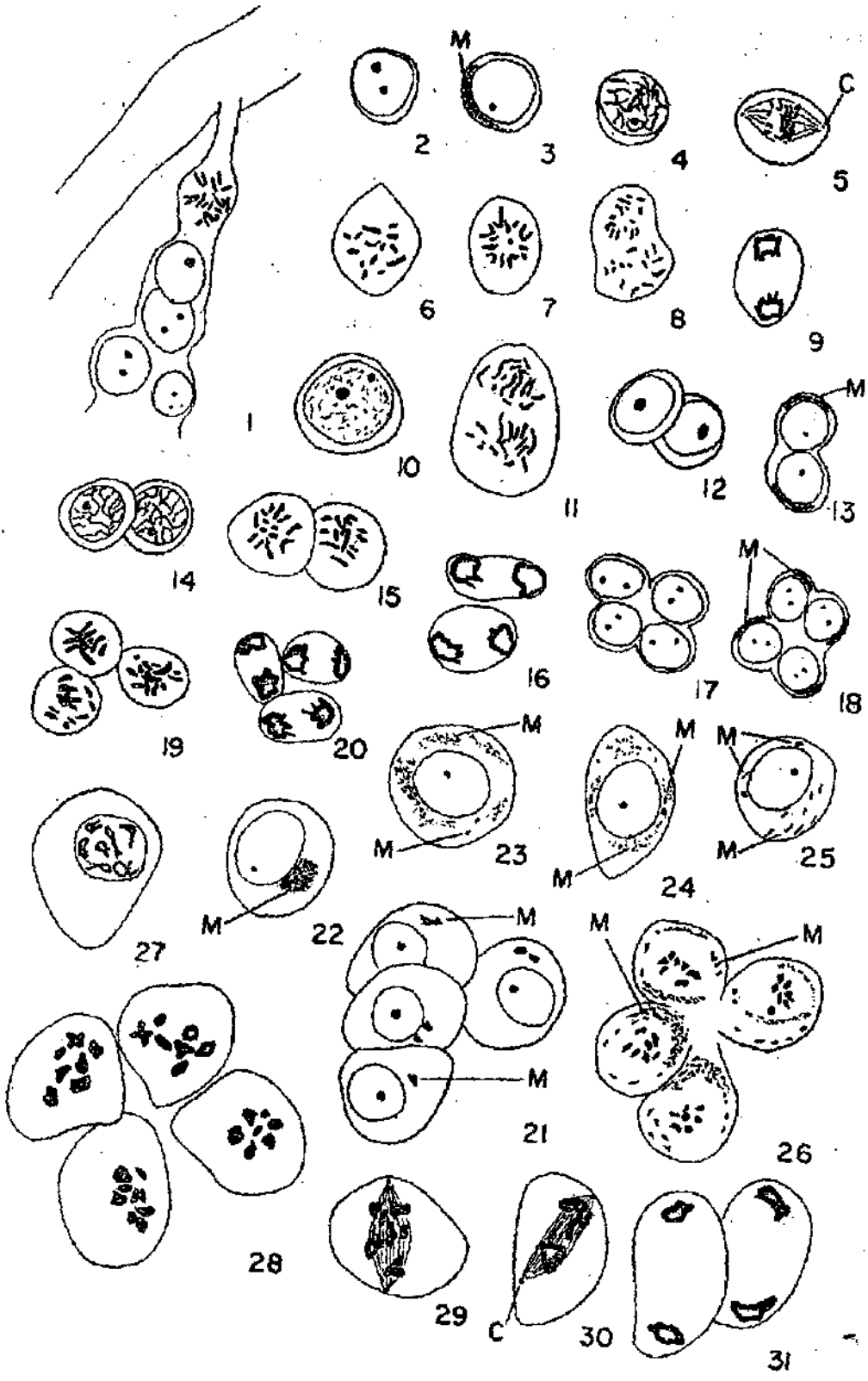
FIG. 54. Meiotic chromosomes as they appear in diakinesis.

FIG. 55. Degenerating spermatogonia, showing the nuclei, condensed to form chromatic masses.

FIG. 56. An unidentified nucleus in the testis cavity.

FIG. 57. A well-developed oocyte from the testis cavity.

PLATE I.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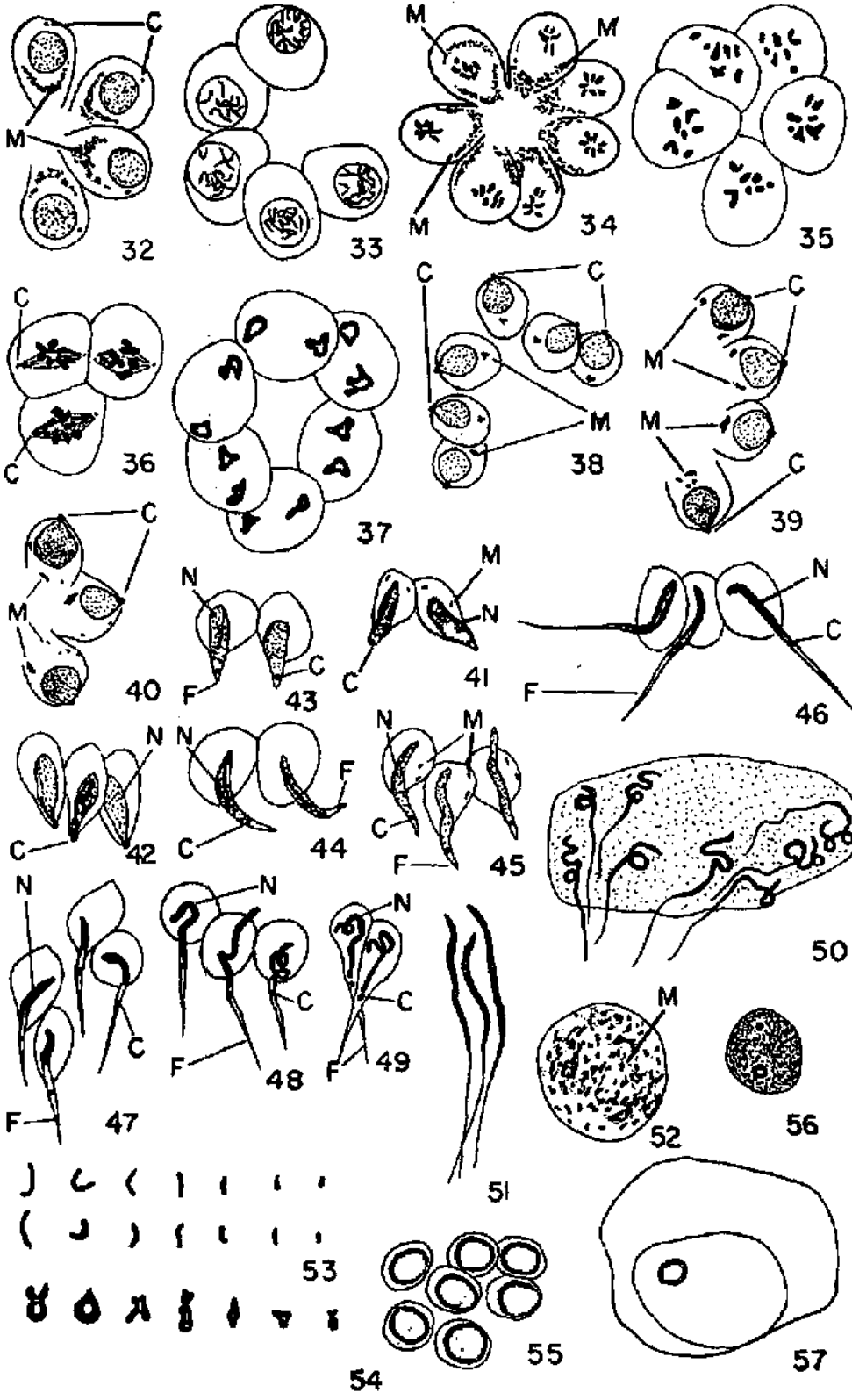

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GAMETOGENESIS, FERTILIZATION AND CLEAVAGE IN *ASYMPHYLODORA* SP.

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GAMETOGENESIS, FERTILIZATION AND CLEAVAGE IN *ASYMPHYLODORA* SP.

By Om Parkash Dhingra, *Late Panjab University Research Scholar, Department of Zoology, Hoshiarpur*

Introduction

It was considered necessary to investigate the processes of gametogenesis and fertilization in *Asymphylodora* sp.* on account of its remarkable sperm, which has a globular form. On the contrary, the sperm in all the trematode forms studied so far has been described as spindle-shaped or thread-like.

The reserve food material which is met with in the cytoplasm of the trematode oocytes, as described by Markell (1943), Willey and Koulish (1950) and Dhingra (1954*b*), has been interpreted differently by many previous workers. Schubmann (1905) and Schellenberg (1911) in *Fasciola hepatica* and Anderson (1935) in *Proterometra* observed globules and interpreted them as the degenerated oogonial cells, ingested by the normal oocytes. Pennypacker (1940) observed small granules in the developing oocytes of a frog lung fluke, whose nature was not clear to him.

The polar bodies, which on their formation are set free into the nutritive material, have been described by Markell (1943) and Dhingra (1954*a*) to take away a little of the cytoplasm with them, but Jones *et al.* (1945) described the absence of the cytoplasm around the polar bodies, and Willey and Koulish (1950) were uncertain. Willey and Godman (1951), however, stated that the polar bodies on their formation leave behind most or all the cytoplasm.

The male and female pronuclei, which attain the same size, have been described by Halkin (1902), Anderson (1935), Chen (1937), Rees (1939), Markell (1943), Willmott (1950), Willey and Godman (1951) and Dhingra (1954*a*) in the resting stage prior to karyogamy, but Jones *et al.* (1945) described them in the prophase with no resting stage. Willey and Koulish (1950), however, described both the conditions. Some have described a direct fusion of the two pronuclei; others have described the formation of fusion nucleus from the chromosomes of the two pronuclei; and still others have reported a direct cleavage from the two pronuclei.

The early segregation of the germ cells in the egg cleavage, as suggested by Ishii (1934) and Chen (1937), and supported by Rees (1939), Willey and Koulish (1950) and Dhingra (1954*a*), has been questioned by Willey and

* Prof. H. R. Mehra of the Allahabad University, who kindly examined my slides, writes in a personal communication that the species is a new one and should be described.

Godman (1951). The illustrations of Jones *et al.* (1945) also appear to be against such a belief.

Ten specimens were fixed in Bouin's fluid. Sections were cut at 10–12 μ and were stained with 0·5% iron haematoxylin.

Acknowledgments

I wish to express my thanks to Dr. Vishwa Nath, Professor and the Head of the Zoology Department, Panjab University, for his kind guidance in the present investigations, made in the Zoology Laboratories of the Panjab University. I am also indebted to him for correcting the manuscript and giving valuable suggestions in preparing this paper for the press. My thanks are also due to Dr. H. R. Mehra, Professor and the Head of the Zoology Department, Allahabad University, who very kindly identified the trematode for me.

Observations

Specimens of the trematode, *Asymphylodora* sp., range from 0·8 mm. to 1·0 mm. in length. There is a single testis lying towards the posterior side. A single ovary lies anterior to the testis in close contact with it. The testis and the ovary are enclosed in thin tunics. I was able to secure a large number of metaphase plates of the mitotic divisions in the testis, ovary and the subcutaneous cells. The somatic cells proved quite satisfactory for determining the diploid number. A few excellent segmentation stages were also met with, which were useful in the study of the morphology of the chromosomes. The diploid number of this species was found to be 18. This count was obtained from segmenting eggs, metaphase plates of subcutaneous tissue and spermatogonial and oogonial mitoses. The study of the distinctive morphology of the chromosome pairs in the metaphase plates of subcutaneous and spermatogonial cells was somewhat hazardous, but their number and the form appear to be the same.

As seen in the first cleavage there are two pairs of metacentric chromosomes about 4·0 μ in length, one pair of small metacentric chromosomes about 3·0 μ, and the rest are straight rod-like chromosomes ranging from 1·0 μ to 2·0 μ in length.

Primordial germ cells and Spermatogonia

The testicular wall is not well demonstrated. The germ cells show some orientation in the testis. The primordial germ cells and the spermatogonia lie close to the testicular wall, while the spermatocytes and the spermatids are more towards the centre. The primordial germ cells, which are seen adhering to the testicular wall, are not plentiful in the mature specimens. Immature worms were, however, not obtained for the study. The primordial germ cells measure each about 6 μ. These cells consist mainly of nuclei. They are ovoid with the nucleus having two nucleoli (Pl. I, fig. 1). The earliest spermatogonia are the largest cells in the whole of germ line except for the primary spermatocytes. They measure each about 10 μ in diameter. In shape they are rounded with distinct cell

membranes. The nucleus contains one or two nucleoli (Pl. I, fig. 2). In spite of the small size of the chromosomes 18 diploid chromosomes can be counted in the polar view of spermatogonial metaphases (Pl. I, fig. 3). In the side view of the equatorial plate the chromosomes are overlapping (Pl. I, fig. 4). As the chromosomes move towards the poles, it is not possible to distinguish them. At the poles they are seen as compact masses, one on either side (Pl. I, fig. 5). They are then reorganized to form the resting nuclei of the secondary spermatogonia.

The secondary spermatogonia remain connected, and each nucleus has generally two nucleoli (Pl. I, fig. 6). A full grown secondary spermatogonium measures about 7·5 μ in diameter and is spherical in shape. In the metaphases of these cells, the chromosomes are rather clumped together and thus the count could not be made definitely in these cells (Pl. I, fig. 7). The secondary spermatogonia on division form a cluster of four cells, the tertiary spermatogonia, which remain connected with each other at a point.

Each tertiary spermatogonium has one nucleolus in the nucleus. In form they are oval, and the nucleus in each is often excentrically placed. A full grown spermatogonium measures about 5·5 μ (Pl. I, fig. 8). Metaphase plates of these cells were very few and I could not count the full diploid number. As a rule the chromosomes are short and condensed (Pl. I, fig. 9). When the daughter chromosomes segregate, a mass of chromatin is seen at each pole (Pl. I, fig. 10). Furrows appear on one side of each cell and divide them to form a cluster of 8 cells, the primary spermatocytes, which remain connected with each other. Centrosomes are rarely seen in the spermatogonial divisions, but the spindles are often met with.

Spermatocytes

Each primary spermatocyte measures about 11 μ in diameter and its nucleus about 7 μ (Pl. I, fig. 11). Sometimes the primary spermatocytes show a centrosomal granule near the nucleus (Pl. I, fig. 12). The chromatin of the nucleus does not pick up the stain at this stage, but gradually the chromosome threads become more distinct and the leptotene stage is reached (Pl. I, fig. 13). The process of pairing then begins, but it is rather difficult to follow the course of the threads. In certain cells, however, it is noticed that the threads shorten and they stain more deeply. The number of visible threads is also reduced. In this stage, which may be called the zygotene, the threads are not polarized (Pl. I, fig. 14). Later on definite loops appear to form the pachytene stage. The ends of the bivalents seem to lie at one side of the nucleus (Pl. I, fig. 15). It was difficult to make a definite count of the loops due to their overlapping and crossing over. At any rate the number of loops was not found to be more than the haploid number of 9. The contraction of the pairs continues, and during this phase of diplotene the pairs open out slightly but are held together at chiasmata. At this stage the bivalents lose their orientation, and they are seen scattered in the nucleus (Pl. I, fig. 16). With the end of diakinesis, the nuclear membrane disappears and the chromosomes are liberated into the cytoplasm (Pl. I, fig. 17). In the polar view of the equatorial plate 9 bivalent chromosomes are very easily made out (Pl. I, figs. 17 and 18).

Centrosomes are not very frequently seen, but in some cases they are evident. In one cell two small granules were seen at each pole of the spindle (Pl. I, fig. 19). The bivalent chromosomes, lying at the equator, segregate and are then drawn towards the poles. At the telophase stage the identity of the chromosomes cannot be made out (Pl. I, fig. 20). As a compact mass of chromosomes is formed at each pole, a cleavage furrow starts from one side in each cell of the cluster (Pl. I, fig. 21). The furrows deepen more and more, and the cells of primary spermatocyte cluster, which show simultaneous divisions, form a 16-celled secondary spermatocyte cluster (Pl. I, fig. 22).

Soon after the formation of the secondary spermatocytes, the nuclei undergo reorganization, but an interkinetic resting stage is not met with. At the 2nd meiotic division a haploid number of chromosomes can be made out in the metaphase plates, but it is difficult to determine the details of the individual chromosomes due to their small size (Pl. I, fig. 23). At the anaphase stage, when the chromosomes divide and are drawn out towards the poles, the groups do not reveal the same number as seen at the metaphase stages (Pl. I, fig. 24). This is due to clumping together of some of the chromosomes. When the telophase stage is reached, the division of the secondary spermatocytes is completed. This results in the formation of a cluster of 32 cells, the spermatids (Pl. I, fig. 25).

Spermateleosis

In the spermatid cluster, the outlines of the spermatids can be made out, but their inner boundaries are rather indistinct. The spermatid nucleus is at first rounded and very much oxyphilic (Pl. I, fig. 26). It then becomes pointed towards the outer free border of the cell, but still looks faint (Pl. II, figs. 27 and 28). The nucleus then loses its ovoid form and becomes somewhat irregular with a reticular appearance (Pl. II, figs. 29 and 30). The nucleus becomes more and more basiphilic and stains deeply (Pl. II, fig. 31). The mature sperms appear globular in shape (Pl. II, fig. 32).

Oogenesis, Fertilization and Cleavage

A single ovary lies anterior to the testis in close contact with it. The ovary contains the oogonia and the primary oocytes. The oogonia are peripheral, while the oocytes are central. The oocytes, when fully developed, come out of the ovary from one side.

The oogonia are quite small and measure about 9-10 μ each in diameter. They are generally packed together in the peripheral region of the ovary and are often in a resting stage. The oogonia have very little of cytoplasm, but their nuclei contain one or two prominent nucleoli (Pl. II, fig. 33). The nucleus then undergoes some necessary changes, and at the end of prophase the nuclear wall breaks down and the chromosomes are set as the equatorial plate. In the metaphases, which are not commonly met with in my preparations, the diploid number cannot be easily counted (Pl. II, fig. 34). The chromosomes then divide equationally and move towards

the respective poles (Pl. II, figs. 35 and 36). Before the oogonium divides, it elongates slightly (Pl. II, fig. 37).

Oocytes

The young primary oocytes are found inner to the peripheral zone of the oogonia. The oocytes measure each about 11 μ to 13 μ in diameter. As they grow, they are pushed forward to one side, from where the oviduct arises. The young oocytes are well packed together, but the older ones lie loose

As the oocytes grow, two or three darkly staining bodies appear in the cytoplasm, which sometimes coalesce (Pl II, figs. 38 and 39). At first these bodies are quite small, but as the growth of the oocytes proceeds these bodies also become bigger.

The nuclear changes in the primary oocyte are concurrent with its growth. At first a faint network of chromatin is seen within the nucleus (Pl. II, fig. 40). It then becomes coarse and stains well. The threads at this stage appear thicker (Pl. II, fig. 41). Later the threads are seen polarized with their ends turned towards the nucleolus (Pl. II, fig. 42). The process of synapsis is probably completed at this stage. Following this the nucleus enters into a diffuse stage, when it stains very faintly (Pl. II, figs. 43, 44 and 45). The oocyte then becomes elongated and is ready to leave the ovary. The haematoxylin-staining bodies in the cytoplasm are present at this stage (Pl. II, fig. 46).

On leaving the ovary, the oocyte enters the oviduct, where a sperm enters the oocyte. A shell is then secreted round the oocyte (Pl. II, fig. 47). The oocyte nucleus is once again activated, and thick darkly staining threads begin to appear in it. As the condensation of the chromatin is going on, the haematoxylin-staining bodies in the cytoplasm start decreasing in size (Pl. II, figs. 48, 49 and 50). Further decrease of these bodies is continued, and they completely disappear before the nuclear wall of the oocyte breaks down (Pl. II, figs. 51 and 52). As the nuclear wall disappears, 9 well-stained bivalent chromosomes are released in the cytoplasm. The sperm, which is a deeply staining body, keeps away from the oocyte chromosomes (Pl. II, figs. 53 and 54). The meiotic chromosomes resemble those of the primary spermatocytes. The chromosomes then segregate, and the first polar body is cut off from the oocyte with a little of cytoplasm.

The oocyte soon undergoes the second meiotic division. The chromosomes of the second meiotic division are very small. The sperm, which was till now a deeply-staining rounded body, lying quiescent in the cytoplasm of the oocyte, becomes reticular and irregular in outline (Pl. II, fig. 55; and Pl. III, fig. 56). Division of the chromosomes in meiosis II then follows, but the individual chromosomes in each set are not distinguishable (Pl. III, figs. 57 and 58). Meanwhile the 1st polar body liberated into the nutritive material elongates and divides into two. For some time the two bodies remain associated together, but one of these two bodies is separated and absorbed in the nutritive material (Pl. III, figs. 59–62).

When meiosis II is completed, the second polar body is also cut off from the mother oocyte. It also carries a little of cytoplasm and is found close to the first polar body (Pl. III, fig. 63).

The male and female pronuclei then undergo reorganization. At first they are seen as compact masses (Pl. III, fig. 64), but soon the nuclear membranes appear. The chromatin appears granular and stains faintly. The nucleoli are not seen at this stage (Pl. III, fig. 65). The male pronucleus is at first smaller than the female, but soon the two are seen to be of the same size. The chromatin of the two pronuclei now stains more lightly, and a nucleolus appears within each pronucleus (Pl. III, fig. 66). At this stage a centrosome is also seen lying between the two pronuclei. The two pronuclei, which grow to 7 μ each, show a little decrease in size, and the centrosomal granule divides into two (Pl. III, fig. 67). The fusion of the two pronuclei then starts. At first the two faintly stained nuclei, each with a small nucleolus, are enclosed in a common sheath (Pl. III, fig. 68). The two pronuclei then fuse to form one well-stained nucleus having two nucleoli (Pl. III, fig. 69). The zygote at this stage measures about 14 μ in diameter.

After undergoing some necessary changes the nuclear wall of the fertilized ovum breaks down and the diploid chromosomes are set free. These chromosomes are the best for the diploid count (18) and morphological study (Pl. III, fig. 70). The metaphase chromosomes then undergo equational division. In the anaphase, where a faint spindle has been observed, the chromosomes look very small (Pl. III, figs. 71 and 72). At the telophase stage the chromosomes form compact irregular masses, one at each pole (Pl. III, fig. 73 and Pl. IV, fig. 74). The egg cleavage then follows and results in the formation of a two-celled embryo, in which one cell is bigger than the other (Pl. IV, fig. 75). The smaller cell with two distinct nucleoli remains quiescent. It has often been called the 'propagatory cell'. The second cleavage affects only the bigger cell. In the metaphase stage of the bigger cell a full diploid number is seen (Pl. IV, fig. 76). Division of the bigger cell, which results in the formation of the three-celled stage, has been followed. This division is also unequal (Pl. IV, figs. 77, 78 and 79). In a three-celled stage the propagatory cell is one of the two smaller cells, which is recognized by the presence of two distinct nucleoli. In the three-celled embryo, the bigger cell again divides (Pl. IV, fig. 80), and results in the formation of a four-celled stage in which there are two small cells, one propagatory cell of medium size with two distinct nucleoli, and a bigger cell (Pl. IV, fig. 81). In the embryo of a 4-celled stage one of the two smaller cells divides (Pl. IV, fig. 82), but in the subsequent stage the bigger cell divides (Pl. IV, figs. 83 and 84). At the 7- or 8-cell stage the propagatory cell, which had remained undivided, appears to be the biggest cell in the embryo (Pl. IV, fig. 85). On further division the stem cells become smaller and smaller, but the propagatory cell retains the same size, as it does not seem to divide at all (Pl. IV, figs. 86 to 88). As the division of the embryonic cells is continued the outlines of the cells become more and more indistinct, so much so that they appear only as nuclear masses embedded in a common matrix (Pl. IV, fig. 89).

It is evident from the above account that the sperm is globular instead of the usual spindle-shaped or thread-like form reported by all the previous workers. Some additional support for the presence of such a form is provided by the fact that it looks globular even in the seminal vesicle or on its penetration in the primary oocyte. The globular form of the sperm is unique in the digenetic trematodes.

My observations on the haematoxylin-staining bodies, which appear, grow and then degenerate in the cytoplasm of the primary oocytes, are in agreement with those of Markell (1943), Willey and Koulish (1950) and Dhingra (1954*b*). My conclusions support my previous observations in *Phyllodistomum spatula* (1954*b*) regarding the nourishing function of these bodies. The haematoxylin-staining masses in the present form develop for some time till the oocyte attains its maximum size, and then start degenerating when the oocyte is about to leave the ovary. The degeneration is continued within the egg shell. Markell (1943) and Willey and Koulish (1950) observed the degeneration within the ovary. Dhingra (1954*b*) recorded the degeneration to start in the three-celled stage. The variation in the stage of degeneration might well be regarded as a generic behaviour. There is, however, no reason to believe that the globules of Schubmann (1905) and Schellenberg (1911) in *Fasciola hepatica* and Anderson (1935) in *Proterometra* in the oocytes could be the degenerated ingested oogonial cells. The small globules observed by Pennypacker (1940) in *Pneumonoeces similiplexus* similarly could not be considered as nuclear products.

My observations on the presence of cytoplasm around the polar nuclei are in accord with those of Markell (1943) in *Probolitrema californiense* and Dhingra (1954*a*) in *Isoparorchis eurytremum*, and differ from those of Jones *et al.* (1945), who described the absence of any cytoplasm around the polar nuclei. Willey and Koulish (1950), however, failed to decide the issue, and Willey and Godman (1951) stated that the polar bodies on their formation leave behind most or all the cytoplasm. The first polar body elongates and divides, as also observed by me in *Isoparorchis eurytremum* (1954*a*). Jones *et al.* (1945), however, stated that the first polar body elongates but does not divide.

The male and female pronuclei are in the resting stage prior to their fusion, and both attain the same size. Halkin (1902), Anderson (1935), Chen (1937), Rees (1939), Markell (1943), Willmott (1950), Willey and Godman (1951) have found a similar condition, but Jones *et al.* (1945) denied the resting state and described the two pronuclei in prophase. Willey and Koulish (1950), however, described both the conditions.

Karyogamy is effected by the direct fusion of the two pronuclei. The zygote nucleus is characterized by the presence of two nucleoli, and the pronuclei have one each. Chen (1937), and Willmott (1950) also made similar observations. Jones *et al.* (1945) and Willey and Godman (1951), however, described the absence of the initial resting stage in the zygote nucleus; but Markell (1943) and Willey and Koulish (1950) could not establish any criteria for distinguishing the zygote nucleus.

It is possible to conclude on the evidence furnished in the present investigation that the germ cell is segregated at the first cleavage stage, as reported by Ishii (1934) and Chen (1937). The first cleavage division is unequal and results in the formation of a small 'propagatory cell' and a bigger 'ectodermal cell' of Ishii and Chen. In a number of subsequent divisions the small cell remains unaffected. Rees (1939), who observed the cleavage only up to a three-celled stage, reported the first division as unequal and the second affecting the bigger cell alone. Willey and Godman (1941) in *Zygocotyle lunata*, Willey and Koulish (1950) in *Gorgoderina attenuata*, Willmott (1950) in *Gigantocotyle bathycotyle* and Dhingra (1964*a*) in *Isoparorchis eurytremum* from their studies on the early cleavage stages concluded similarly. Jones *et al.* (1945) in *Macravestibulum kepneri* has, however, drawn a two-celled stage with equal nuclei; and Willey and Godman (1951) in *Zygocotyle lunata* reported that subsequent to the second division the evidence in support of early segregation of the germ cells is unconvincing.

Summary

1. The processes of spermatogenesis, maturation of the ovum, karyogamy and early cleavages have been worked out in detail in *Asymphylodora* sp.

2. The haploid number of chromosomes is 9 and the diploid 18. The chromosomes have been counted in mitotic and meiotic divisions of germ cells and the cleavage divisions of the egg.

3. The sperm is globular contrary to the usual thread-like or spindle-shaped form reported in many trematodes.

4. The centrosomes and spindles are met with. A centrosomal granule of the first cleavage division is clearly seen, when the male and female pronuclei lie close together.

5. Some reserve food material appears in the cytoplasm of the primary oocyte when it is still in the ovary, but it disappears when the oocyte leaves the ovary.

6. The male and female pronuclei are at first of unequal size, but later they are of the same size. They enter into a resting stage before actual fusion. The male and female pronuclei fuse to form a fusion nucleus which is characterized by the presence of two nucleoli.

7. The 'propagatory cell' is formed in the first egg cleavage division and remains undivided.

8. The polar bodies are seen extruded from the mother oocyte with a little of cytoplasm. The first polar body when formed is seen to enlarge and then divide.

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Explanation of Figures

Abbreviations:—

Pb—Polar body; Pb_1—First polar body; Pb_2—Second polar body; *Pr*—Propagatory cell; *rf*—Reserve food material; *Sp*—Sperm; *SH*—Shell.

Plate I

Fig. 1. Primordial germ cell.
„ 2. Primary spermatogonium.
„ 3. Metaphase of primary spermatogonium (Polar view).
„ 4. Metaphase of primary spermatogonium (Side view).
„ 5. Telophase of primary spermatogonium.
„ 6. Secondary spermatogonia, in a group of two.
„ 7. Secondary spermatogonia in metaphase.
„ 8. A group of four tertiary spermatogonia.
„ 9. A group of four tertiary spermatogonia in metaphase.
„ 10. Dividing tertiary spermatogonia.
„ 11. Section through a cluster of primary spermatocytes, showing 5 of the 8 cells in a resting stage.
„ 12. Primary spermatocyte, showing a centrosomal granule in the cytoplasm.
„ 13. Primary spermatocyte, showing a leptotene stage.
„ 14. Primary spermatocyte, showing a zygotene stage.

FIG. 15. Primary spermatocyte, showing a pachytene stage.
" 16. Four cells of a primary spermatocyte cluster, showing the diakinesis stage.
FIGS. 17 and 18. Primary spermatocytes in metaphase, showing a haploid number of 9 chromosomes.
FIG. 19. Primary spermatocyte in metaphase, showing the divided centrosomes at the poles.
" 20. Telophase of primary spermatocyte.
" 21. A dividing cluster of primary spermatocytes.
" 22. A section through a cluster of secondary spermatocytes, showing 10 of the 16 cells.
" 23. Secondary spermatocytes in metaphase.
" 24. Secondary spermatocytes, showing metaphases and anaphases.
" 25. A section through a cluster of newly formed spermatids, showing 19 of the 32 cells.
" 26. Spermatids in resting stage.

PLATE II

FIGS. 27–31. Successive stages in the development of the sperm.
FIG. 32. Ripe sperms.
" 33. Oogonium.
" 34. Two oogonia in metaphase.
FIGS. 35 and 36. Early and late telophases of the oogonium.
FIG. 37. Oogonium slightly elongated before dividing.
" 38. Primary oocyte in a resting stage, showing a darkly-stained mass (Reserve food material), which has appeared in the cytoplasm.
" 39. Primary oocyte still in a resting stage with a slightly bigger stained mass.
" 40. Primary oocyte in leptotene, showing a little more increase in the size of the darkly-stained mass.
" 41. Primary oocyte in zygotene with a slightly more increased stained mass in the cytoplasm.
" 42. Primary oocyte, showing a bouquet stage and a well-developed cytoplasmic stained mass.
FIGS. 43, 44 and 45. Successive stages of the diffuse stage in the primary oocyte. The chromatin is very faintly stained and the nucleolus is indistinct.
FIG. 46. Primary oocyte, slightly elongated before leaving the ovary. Cytoplasmic bodies are also present.
" 47. Egg, enclosing the primary oocyte with a sperm and the cytoplasmic mass in the cytoplasm.
FIGS. 48 and 49. Eggs, showing the condensation of chromatin and decrease in size of the cytoplasmic mass.
" 50 and 51. Eggs, showing a further condensation of the chromatin and a still further decrease in the size of the cytoplasmic masses.
FIG. 52. Egg, showing the primary oocyte with complete disappearance of the cytoplasmic mass, before the nuclear wall breaks down.
FIGS. 53 and 54. Eggs, showing primary oocytes in metaphase and the sperms lying quiescent as well-stained bodies.
FIG. 55. Egg, showing the chromosomes of meiosis II, a reticular sperm and the First polar body.

PLATE III

FIG. 56. Egg, showing the haploid chromosomes (9) of meiosis II, a reticular sperm and the first polar body.
" 57. Egg, showing the chromosomes of meiosis II on the equatorial plate, a reticular sperm and the first polar body.
" 58. Egg, showing the division of the meiosis II chromosomes, a reticular sperm and the first polar body.

Fig. 59. Egg, showing the dividing chromosomes of meiosis II, a reticular sperm and an elongted first polar body.

„ 60. Egg, showing the dividing chromosomes of meiosis II, a reticular sperm and the dividing first polar body.

„ 61. Egg, showing the dividing chromosomes of meiosis II at the equator, a sperm and the divided first polar body.

„ 62. Egg, showing the chromatic mass of the ovum, a reticular sperm, newly-formed second polar body and the first polar body.

Figs. 63 and 64. Eggs, showing the ovum with its chromatic mass, the sperm and the two polar bodies enclosed in a little of cytoplasm.

Fig. 65. Egg, showing the ovum with a smaller male pronucleus and the bigger female pronucleus. Only one polar body is seen.

„ 66. Egg, showing the ovum with male and female pronuclei of equal size, a centrosomal granule between the pronuclei and two polar bodies.

„ 67. Egg, showing the male and female pronuclei of equal size, divided centrosome and two polar bodies.

„ 68. Egg, showing the male and female pronuclei enclosed in a common sheath and one polar body.

„ 69. Egg, showing an ovum with a fusion nucleus, characterized by two nucleoli, and two polar bodies.

„ 70. Egg, showing the diploid (18) chromosomes of the fusion nucleus at metaphase. No polar body is seen in this section of the egg.

„ 71. Egg, showing the dividing chromosomes at the equator and two polar bodies.

„ 72. Egg, showing the chromosomes of the first cleavage at anaphase and two polar bodies.

„ 73. Egg, showing the chromosomes of the first cleavage at telophase and two polar bodies.

Plate IV

Fig. 74. Egg, showing the chromosomes of the first cleavage at telophase and two polar bodies.

„ 75. Egg, showing a two-celled stage. One is smaller than the other.

„ 76. Egg, showing a two-celled stage, with the bigger cell in metaphase.

„ 77. Egg, showing a two-celled stage with the bigger cell at telophase.

„ 78. Egg, showing a two-celled stage with the bigger cell about to divide.

„ 79. Egg, showing a three-celled stage with the bigger cell having divided unequally.

„ 80. Egg, showing a three-celled stage, with the bigger cell at metaphase.

„ 81. Egg, showing a four-celled stage. There are two small cells, one propagatory and one bigger.

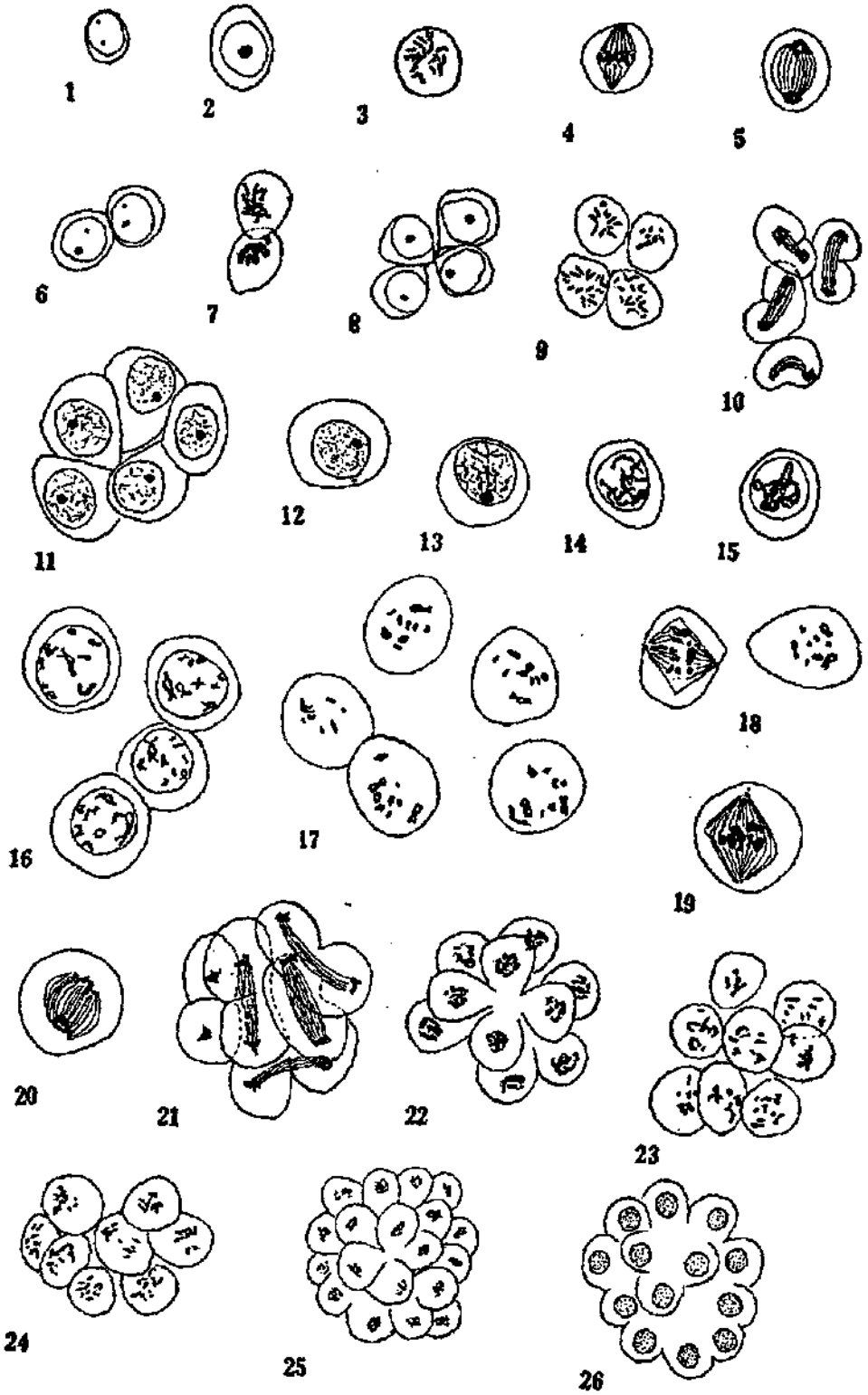
„ 82. Egg, showing a four-celled stage with one of the smaller cells at metaphase.

Figs. 83, 84 and 85. Eggs, showing 5, 6, and 7-celled stages of the embryos with the propagatory cell still not having divided.

„ 86 and 87. Eggs, showing advanced stages of development with the propagatory cell still of the original size.

„ 88 and 89. Eggs, showing further advanced stages of development. The propagatory cell is still distinguishable.

PLATE I.



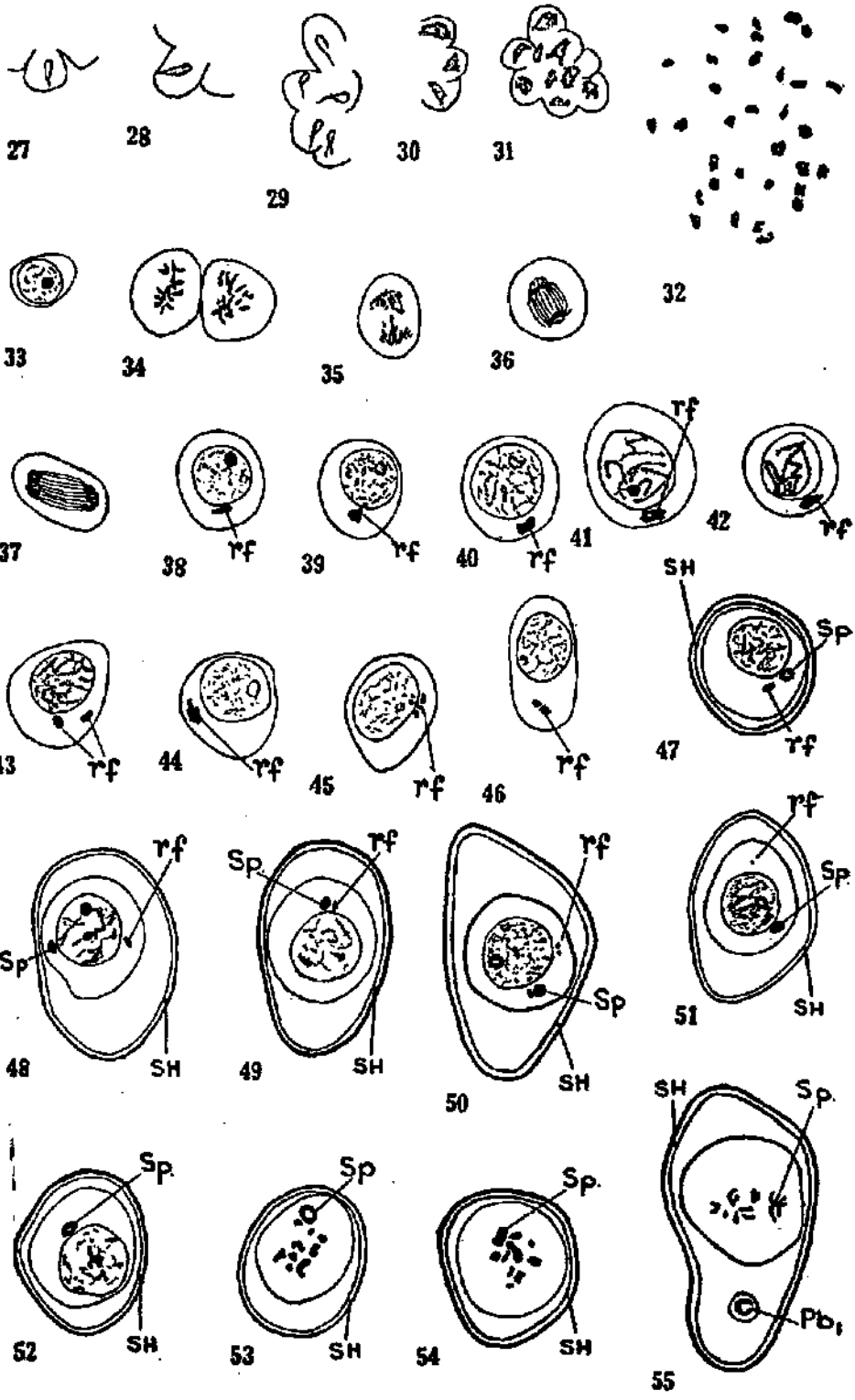

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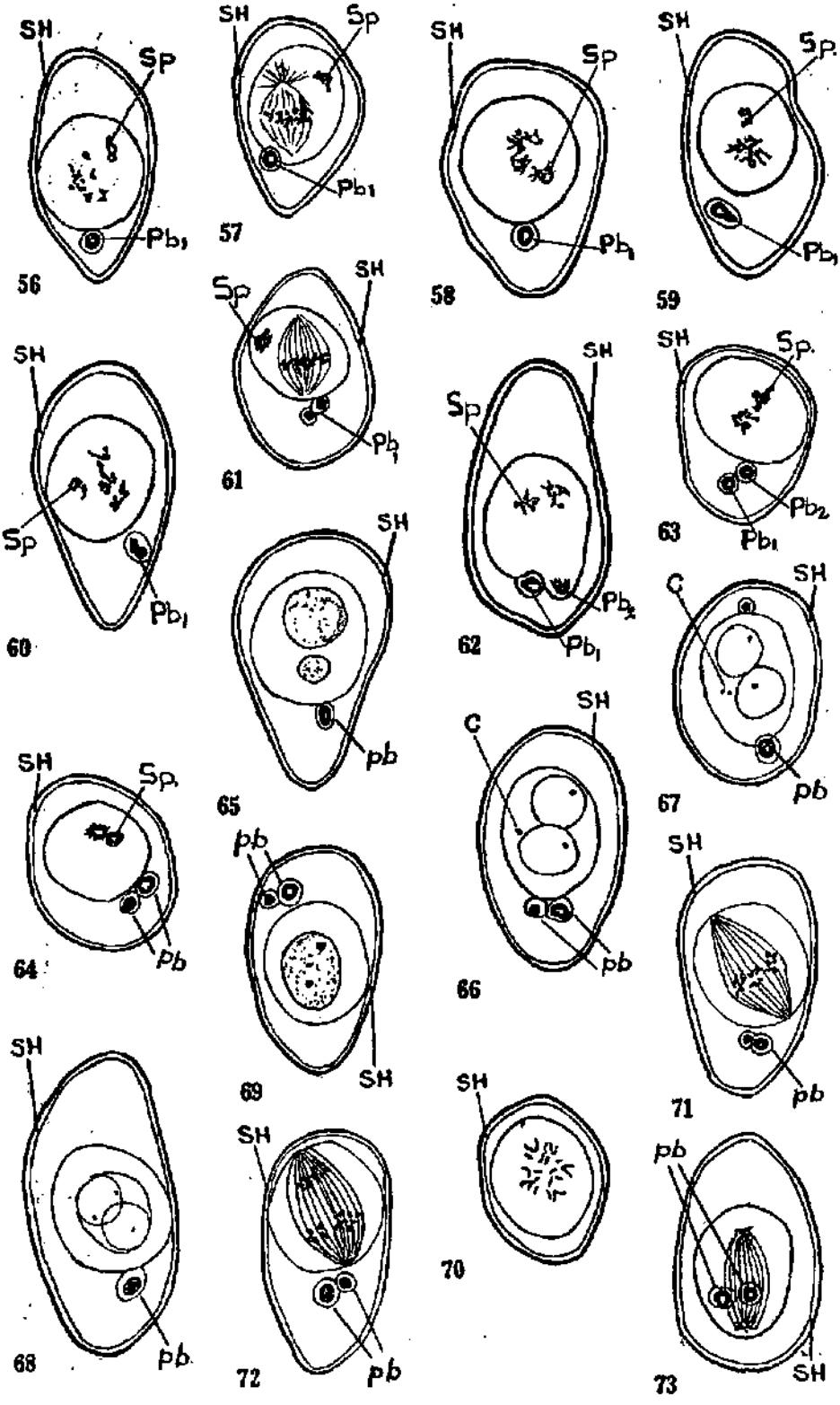
PLATE III.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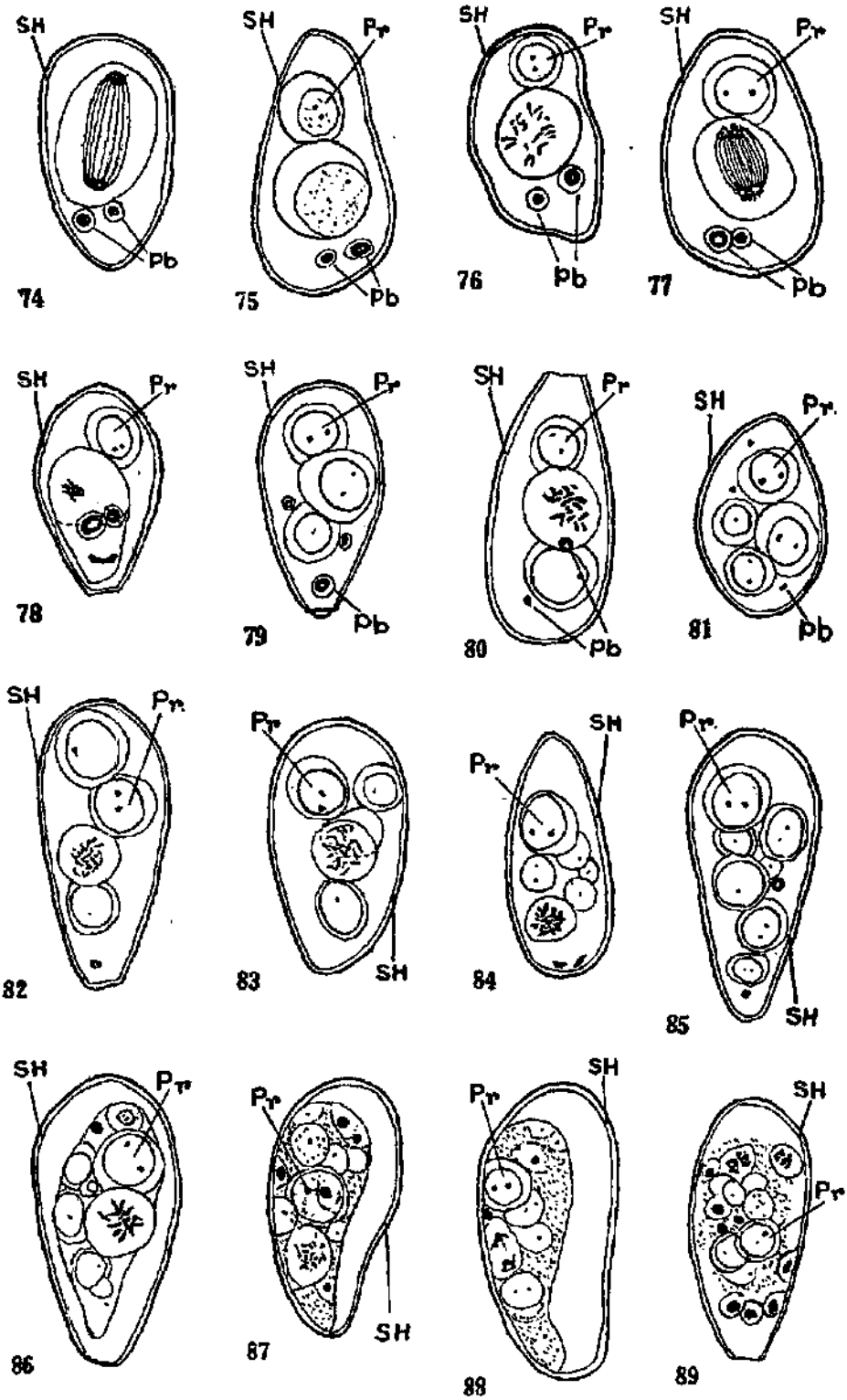

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PARTITIONS IN GENERAL

by

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PARTITIONS IN GENERAL

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§ 1. The general problem in partitions is that of expressing an integer $n > 0$ as a sum of a number of other specified positive integers, with or without restriction. Here, we consider the partitions of n into integers

$$a_0 = 1, a_1, a_2, a_3, \ldots, a_m \quad .. \quad .. \quad .. \quad \text{(A)}$$

where there is no restriction as to the number of times any summand is used.

Without loss of generality, we can assume that

$$a_r < a_s, \quad 0 \leqslant r < s \leqslant m. \quad .. \quad .. \quad .. \quad (1)$$

The generating function giving $P_m(n)$, the number of partitions of n into numbers of set (A), is

$$f_m(x) = \{(1-x)(1-x^{a_1})(1-x^{a_2}) \ldots (1-x^{a_m})\}^{-1}; \quad |x| < 1. \quad (2)$$

In fact, $P_m(n)$ is the coefficient of x^n in the expansion of $f_m(x)$. We take

$$P_m(0) = 1, \; P_m(-j) = 0 \text{ for } j > 0. \quad .. \quad .. \quad (3)$$

In this paper, I prove that

$$\binom{n+m}{m} \leqslant P_m(n) \prod_{t=1}^{m} a_t < \binom{n+\sum_{t=1}^{m} a_t}{m}.$$

The case of partitions into primes has been considered in some detail in the latter half of the paper.

§ 2. Denoting the number of partitions of n into the summands $a_0, a_1, a_2, \ldots, a_r$ of set (A) by $P_r(n)$, we have

$$P_r(n) = P_{r-1}(n) + P_r(n-a_r). \quad .. \quad .. \quad .. \quad (4)$$

This follows readily from the fact that $P_{r-1}(n)$ denotes the number of those partitions of n which employ $a_0, a_1, a_2, \ldots, a_{r-1}$ as summands, while $P_r(n-a_r)$ gives the number of those partitions of n in which a_r occurs at least once.

Moreover,

$$P_r(n) \geqslant P_r(n-1) \geqslant P_r(n-2) \geqslant \ldots, \geqslant P_r(0) = 1, \quad .. \quad (5)$$

because adding unity to each of the $P_r(t)$ partitions of t, we get partitions of $(t+1)$, but these are in general not all the partitions of $(t+1)$ into the first r members of set (A). Replacing n by $n, n-1, n-2, \ldots, n-a_r+1, \ldots$ in succession in (4), we obtain on addition

$$P_r(n) + P_r(n-1) + P_r(n-2) + \ldots + P_r(n-a_r+1) = \sum_{j=0}^{n} P_{r-1}(j). \quad (6)$$

§ 3. Proof of the inequality.

Since $P_0(n) = 1$ for $n \geqslant 0$,

$$P_1(n) = P_0(n)+P_0(n-a_1)+P_0(n-2a_1)+ \ldots +P_0\left(n-\left[\frac{n}{a_1}\right]a_1\right)$$

$$= \left[\frac{n+a_1}{a_1}\right].$$

Let us assume that for $0 \leqslant k \leqslant r-1$,

$$\binom{j+\alpha_k}{k} \leqslant P_k(j) \prod_{t=1}^{k} a_t \leqslant \binom{j+\beta_k}{k} \quad .. \quad .. \quad (7)$$

where α_k and β_k are functions of a's alone.
Then, in view of (5) and (6), we have

$$a_r P_r(n) \geqslant \sum_{j=0}^{n} P_{r-1}(j). \quad .. \quad .. \quad .. \quad (8)$$

and

$$a_r P_r(n-a_r+1) \leqslant \sum_{j=0}^{n} P_{r-1}(j). \quad .. \quad .. \quad .. \quad (9)$$

Hence if $\alpha_{r-1} < r$ as shall be seen to be the case,

$$P_r(n) \prod_{t=1}^{r} a_t \geqslant \sum_{j=0}^{n} \binom{j+\alpha_{r-1}}{r-1} = \binom{n+\alpha_{r-1}+1}{r},$$

so that, we can take

$$\alpha_r = \alpha_{r-1}+1 \text{ or } \alpha_r = r \text{ since } \alpha_1 = 1.$$

Again,

$$P_r(n-a_r+1) \prod_{t=1}^{r} a_t \leqslant \sum_{j=0}^{n} \binom{j+\beta_{r-1}}{r-1} < \binom{n+\beta_{r-1}+1}{r},$$

that is,

$$P_r(n) \prod_{t=1}^{r} a_t < \binom{n+a_r+\beta_{r-1}}{r},$$

so that, we can take

$$\beta_r = a_r+\beta_{r-1} \text{ or } \beta_r = \sum_{t=1}^{r} a_t \text{ because } \beta_1 = a_1.$$

Thus (7) holds for $m \geqslant k \geqslant 0$, with

$$\alpha_k = k \text{ and } \beta_k = \sum_{t=1}^{k} a_t. \quad .. \quad .. \quad .. \quad (10)$$

§ 4. THEOREM.

If $r\beta_r = o(n)$, then

$$P_r(n) \sim \binom{n+r}{r} \prod_{t=1}^{r} a_t^{-1}. \quad .. \quad .. \quad .. \quad (11)$$

Proof. We have

$$\binom{n+\beta_r}{r} \Big/ \binom{n+r}{r} = \frac{n+\beta_r}{n+r} \cdot \frac{n+\beta_r-1}{n+r-1} \cdot \ldots \cdot \frac{n+\beta_r-r+1}{n+1} \cdot \quad .. \quad (12)$$

The value of the right side of (12), lies between

$$\left(1+\frac{\beta_r-r}{n+r}\right)^r \text{ and } \left(1+\frac{\beta_r}{n}\right)^r.$$

Let $n = rq\beta_r$, then the right side of (12), lies between

$$\left\{1+\frac{1-\frac{r}{\beta_r}}{r\left(q+\frac{1}{\beta_r}\right)}\right\}^r \text{ and } \left(1+\frac{1}{qr}\right)^r,$$

that is, between

$$\exp\left(\frac{1-\frac{2r}{\beta_r}}{q+\frac{1}{\beta_r}}\right) \text{ and } \exp\left(\frac{1}{q}\right).$$

But $$\beta_r > \frac{r(r+1)}{2}.$$

Hence, if $r\beta_r = o(n)$, then

$$\binom{n+\beta_r}{r} \sim \binom{n+r}{r}$$

and the result follows.

§ 5. In case, $a_1, a_2, a_3, \ldots, a_m$ are the primes 2, 3, 5, 7, $\ldots, p_m$ the method of partial fractions, which I have employed in the case of ordinary partitions too [1], is particularly useful.

Thus, let

$$\phi_r(x) = \left\{(1-x)(1-x^2)(1-x^3)(1-x^5)\ldots(1-x^{p_r})\right\}^{-1} \prod_{j=1}^{r} p_j, \quad |x|<1;$$

where p_r is the rth prime with $p_1 = 2$.
Then,

$$\left\{1-(1-y)^{p_r}\right\}\phi_r(1-y) = p_r.\phi_{r-1}(1-y),\ 0<y<2. \quad \ldots \quad (13)$$

Suppose

$$y^{t+1}\phi_r(1-y) = \sum_{n=0}^{\infty}\psi_r(n)y^n,$$

then

$$\sum_{j=1}^{p_r}(-1)^{j-1}\binom{p_r}{j}y^{j-1}.\sum_{t=0}^{\infty}\psi_r(t)y^t = p_r.\sum_{t=0}^{\infty}\psi_{r-1}(t)y^t. \quad \ldots \quad (14)$$

Equating the coefficients of y^t on the two sides, we get

$$\psi_r(t)-\tfrac{1}{2}\binom{p_r-1}{1}\psi_r(t-1)+\tfrac{1}{3}\binom{p_r-1}{2}\psi_r(t-2)-\ldots.$$

$$+\frac{(-1)^{p_r-1}}{p_r}\binom{p_r-1}{p_r-1}\psi_r(t-p_r+1) = \psi_{r-1}(t), \quad \ldots \quad \ldots \quad (15)$$

or

$$\psi_r(t)-\psi_0(t)=\sum_{i=1}^{r}\left\{\tfrac{1}{2}\binom{p_i-1}{1}\psi_i(t-1)-\tfrac{1}{3}\binom{p_i-1}{2}\psi_i(t-2)\right.$$
$$\left.+\ldots+\frac{(-1)^{p_i}}{p_i}\binom{p_i-1}{p_i-1}\psi_i(t-p_i+1)\right\}$$

where $$\psi_0(0)=1,\ \psi_0(t)=0 \text{ for } |t|>0. \qquad (16)$$

Thus

$$\psi_r(0)=1,$$
$$\psi_r(1)=\tfrac{1}{2}\sum_{i=1}^{r}(p_i-1),$$
$$\psi_r(2)=\tfrac{1}{2}\sum_{i=1}^{r}\{(p_i-1)\sum_{j=1}^{i}(p_j-1)\}-\tfrac{1}{6}\sum_{i=1}^{r}(p_i-1)(p_i-2),$$

and so on.

We can now write

$$\phi_r(x)=\frac{\psi_r(0)}{(1-x)^{r+1}}+\frac{\psi_r(1)}{(1-x)^r}+\frac{\psi_r(2)}{(1-x)^{r-1}}+\ldots+\frac{\psi_r(r)}{(1-x)}+R_r(x) \qquad (17)$$

where $R_r(x)$ is the sum of the partial fractions corresponding to the factors $\dfrac{1-x^{p_j}}{1-x}$ in the denominator of $\phi_r(x)$; $1\leqslant j\leqslant r$.

The coefficient $C_r(n)$ of x^n in $\phi_r(x)-R_r(x)$ is

$$\sum_{t=0}^{r}\psi_r(t)\binom{n+r-t}{r-t}. \qquad (18)$$

§ 6. The partial fraction corresponding to $\dfrac{1-x^{p_j}}{1-x}$ in the denominator of $\phi_r(x)$ can be computed as follows.

Suppose the partial fraction corresponding to $\dfrac{1-x^{p_j}}{1-x}$ in the denominator of

$$\frac{1}{(1-x^{p_j})(1-x)(1-x^{q_1})(1-x^{q_2})\ldots(1-x^{q_s})}$$

(where the q's are primes different from p_j, not necessarily in any particular order), is

$$\frac{\rho_s(x)}{1-x^{p_j}}$$

where $\rho_s(x)$ is a polynomial in x of degree (p_j-1) with rational coefficients and $\rho_s(1)=0$.

Then, to find the value of $\rho_{s+1}(x)$, we notice that $\rho_{s+1}(x)$ shall have to be such that the numerator of

$$\frac{\rho_s(x)}{(1-x^{p_j})(1-x^{q_{s+1}})}-\frac{\rho_{s+1}(x)}{(1-x^{p_j})}$$

is divisible by $(1-x^{p_j})$ and $\rho_{s+1}(1)=0$.

We proceed to show that these conditions determine $\rho_{s+1}(x)$ uniquely in terms of $\rho_s(x)$.

Let $\rho_s(x) = c_0+c_1x+c_2x^2+\ldots+c_{p_j-1}x^{p_j-1}$

and $\rho_{s+1}(x) = b_0+b_1x+b_2x^2+\ldots+b_{p_j-1}x^{p_j-1}$.

If we write

$$c_i = c_k \text{ and } b_i = b_k, \text{ when } i \equiv k \pmod{p_j};$$

and

$$q_{s+1} \equiv -t \pmod{p_j}, \quad 0 < t < p_j;$$

then the first condition requires that

$$\left.\begin{aligned} b_0-b_t &= c_0, \\ b_t-b_{2t} &= c_t, \\ b_{2t}-b_{3t} &= c_{2t}, \\ &\cdots\cdots\cdots \\ b_{(p_j-1)t}-b_{p_j.t} &= c_{(p_j-1)t}. \end{aligned}\right\} \quad \ldots \quad \ldots \quad \ldots \quad (19)$$

The second condition requires that

$$b_0+b_1+b_2+\ldots+b_{(p_j-1)} = 0,$$

or what is the same thing

$$b_0+b_t+b_{2t}+\ldots+b_{(p_j-1)t} = 0. \quad \ldots \quad \ldots \quad \ldots \quad (20)$$

Solving equations (19) with (20), we get

$$p_jb_0 = (p_j-1)c_0+(p_j-2)c_t+(p_j-3)c_{2t}+\ldots+c_{(p_j-2)t}; \quad \ldots \quad (21)$$

and the other b's are given by

$$\left.\begin{aligned} b_t &= b_0-c_0, \\ b_{2t} &= b_t-c_t, \end{aligned}\right\} \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (22)$$

etc.

If c_k be the numerically greatest of c's, then from (21) and (22), it follows that none of the b's is numerically greater than

$$\tfrac{1}{2}(p_j-1)\,|c_k|. \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (23)$$

Now

$$\frac{1}{(1-x^{p_j})(1-x)} = \frac{A_j}{(1-x)^2}+\frac{B_j}{(1-x)}+\frac{\rho_0(x)}{(1-x^{p_j})}$$

where A_j and B_j are certain constants depending on p_j, and

$$\rho_0(x) = \frac{1}{2p_j}\{(p_j-1)+(p_j-3)x+(p_j-5)x^2+\ldots+(-p_j+1)x^{p_j-1}\}.$$

Thus, none of the coefficients in $\rho_0(x)$ is numerically greater than $\frac{1}{2}$. Hence, none of the coefficients in $\rho_r(x)$ corresponding to $(1-x^{p_j})$ in the denominator of $\phi_r(x)$ is numerically greater than

$$\frac{1}{2^r}(p_j-1)^{r-1}\prod_{i=1}^{r}p_i. \quad \ldots \quad \ldots \quad \ldots \quad (24)$$

The contribution $L_r(n)$ of $R_r(x)$ to the coefficient of x^n in the expansion of $\phi_r(x)$, therefore, does not, in absolute value exceed

$$\frac{1}{2^r}\prod_{i=1}^{r} p_i \cdot \sum_{j=1}^{r} (p_j-1)^{r-1}, \quad \ldots \quad \ldots \quad \ldots \quad (25)$$

which is $$< \frac{1}{2^r}\prod_{i=1}^{r} p_i \cdot \frac{p_r^r}{r}.$$

Hence

$$L_r(n) = O\left(\frac{(r\log r)^{2r}}{2^r \cdot r}\right). \quad \ldots \quad \ldots \quad \ldots \quad (26)$$

because $p_r = O(r\log r)$.

§ 7. The order of $\psi_r(t)$.

We have

$$\psi_r(0) = 1,$$

$$\psi_r(1) = \tfrac{1}{2}\sum_{i=1}^{r}(p_i-1) = O(r^2\log r),$$

Assuming that $\psi_r(t-1) = O(r^2\log r)^{t-1}$,
we have from (16),

$$\psi_r(t)-\psi_0(t) = \sum_{i=1}^{r}\tfrac{1}{2}(p_i-1)\psi_i(t-1) - \sum_{i=1}^{r}\tfrac{1}{3}\binom{p_i-1}{2}\psi_i(t-2)$$

$$+\sum_{i=1}^{r}\left\{\tfrac{1}{4}\binom{p_i-1}{3}\psi_i(t-3) - \ldots + \frac{(-1)^{p_i}}{p_i}\psi_i(t-p_i+1)\right\}$$

$$= O(r^2\log r)^t + O(r^{2t-1}(\log r)^t) + O(r^{2t-1}(\log r)^{t+1})$$

$$= O(r^2\log r)^t.$$

In estimating the last sum, we make use of the fact that the number of terms in the bracket is (p_i-3), and

the j-th term in the bracket $= O((r\log r)^{j+2}(r^2\log r)^{t-j-2})$

$$= O\left(\frac{(r^2\log r)^t}{r^3}\right) = o\left(\frac{(r^2\log r)^t}{r^2\log r}\right).$$

Thus $$\psi_r(t) = O(r^2\log r)^t, \quad t \geqslant 1. \quad \ldots \quad \ldots \quad \ldots \quad (27)$$

Moreover $$\psi_r(1) > \frac{r^2}{3}, \quad r \geqslant 1. \quad \ldots \quad \ldots \quad \ldots \quad (28)$$

§ 8. The coefficient $C_r(n)$ of x^n in $\phi_r(x)-R_r(x)$ has been shown to be

$$= \sum_{t=0}^{r}\psi_r(t)\binom{n+r-t}{r-t}.$$

If $n > kr^3(\log r)^2$, $k > 0$,

and we write

$$C_r(n) = \binom{n+r}{r} + \binom{n+r-1}{r-1}\{\psi_r(1)+B\}.$$

then the $(t-1)$th term in B

$$= \binom{n+r-t}{r-t}\psi_r(t) \Big/ \binom{n+r-1}{r-1}$$

$$= \frac{(r-1)(r-2)\ldots(r-t+1)\psi_r(t)}{(n+r-1)(n+r-2)\ldots(n+r-t+1)}$$

$$= O\left\{\frac{r^t}{n^t}(r^2\log r)^t\right\} = O\left(\frac{r^{3t}(\log r)^t}{k^t r^{3t}(\log r)^{2t}}\right)$$

$$= O\left(\frac{1}{k^t(\log r)^t}\right)$$

Hence

$$|B| = O\left\{\sum_{t=2}^{r}\frac{1}{(k\log r)^t}\right\} = O\left(\frac{1}{(\log r)^2}\right) = o(1) = o(\psi_r(1))$$

so that

$$C_r(n) = \binom{n+r}{r} + \psi_r(1)\binom{n+r-1}{r-1} + o\left\{\psi_r(1)\binom{n+r-1}{r-1}\right\}$$

$$= \binom{n+r}{r} + D + o(D).$$

Finally, since $\psi_r(1) > \frac{r^2}{3}$,

$$D > \frac{r^2}{3}\cdot\frac{n^{r-1}}{r^{r-1}} = \frac{k^{r-1}}{3}\cdot r^{3r-3-r+3}(\log r)^{2r-2}$$

$$= \frac{k^{r-1}(r\log r)^{2r}}{3(\log r)^2}.$$

Therefore,

$$L_r(n) = O\left(\frac{(r\log r)^{2r}}{2^r\cdot r}\right) = o(D).$$

Hence $Q_r(n)$— the number of partitions of n into 1 and the first r primes,

$$= \text{the coefficient of } x^n \text{ in } \phi_r(x) \Big/ \prod_{i=1}^{r} p_i,$$

$$= \{C_r(n)+L_r(n)\} \Big/ \prod_{i=1}^{r} p_i,$$

$$= \left[\binom{n+r}{r} + \left\{\psi_r(1)+o(\psi_r(1))\right\}\binom{n+r-1}{r-1}\right] \Big/ \prod_{i=1}^{r} p_i \quad \ldots \quad (29)$$

for $n > kr^3(\log r)^2$, $k > 0$.

§9. It may be remarked that the method described in §5–§8, would be applicable to any set of integers 1, $a_1, a_2, \ldots, a_m$ every two of which are prime to each other.

In the end, I offer my thanks to Dr. R. P. Bambah for his suggestions and help in the preparation of this paper.

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OOGENESIS OF THE TOAD, *BUFO STOMATICUS* LUTKEN, WITH OBSERVATIONS UNDER THE PHASE CONTRAST MICROSCOPE

by

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OOGENESIS OF THE TOAD, *BUFO STOMATICUS* LUTKEN, WITH OBSERVATIONS UNDER THE PHASE CONTRAST MICROSCOPE

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ABSTRACT

The oocytes of the toad, *Bufo stomaticus* Lutken, have been studied by employing the modern cytological techniques. The living oocytes have been extensively studied under the phase contrast microscope, and microphotographed. The mitochondria form a peripheral ring, in which the albuminous yolk granules appear for the first time. The Golgi bodies, which are essentially granular, begin to stain with Sudan IV when the oocyte measures 0·35 mm. in diameter, and give rise directly to fatty yolk spherules. The Golgi bodies are osmiophilic and argentophilic, and they are not stainable with neutral red. The watery, neutral red stainable vacuome appears for the first time as minute vacuoles in oocytes measuring 0·06 mm. in diameter. They slightly increase in size, but do not condense any yolk in their interior. The nucleolar extrusions, which begin to be extruded into the cytoplasm in very young oocytes, disappear without making any visible contribution to yolk-formation.

INTRODUCTION

In a recent paper Nath and Malhotra (1954) published microphotographs demonstrating the vacuome, Golgi bodies, mitochondria and nucleolar extrusions in the fresh eggs of the frog, *Rana tigrina*, as studied under the phase contrast microscope. Earlier Nath (1931) had demonstrated the vacuome, the Golgi bodies and the mitochondria as independent cytoplasmic components in the fresh eggs of the frog, as studied under the ordinary microscope. But Nath (1931) did not discover any nucleolar extrusions in the eggs of the frogs, which had been all collected during winter hibernation up to the following spring month of March only. Nath and Malhotra (1954) also did not find any nucleolar extrusions in the eggs of the frogs collected during the months of April and May; but oocytes of frogs collected in the month of June showed prominent nucleoli, which are extruded in the cytoplasm. The extrusions, however, quickly disappear and do not make any visible contribution to yolk-formation.

But in the toad, *Bufo stomaticus* Lutken, which forms the subject matter of the present investigation, nucleolar extrusions have been seen in the oocytes of toads collected from the months of March to September. Even a toad collected during the month of November, which is the hibernating month, showed nucleolar extrusions in its oocytes. As in the frog, the nucleolar extrusions disappear without making any visible contribution to yolk-formation.

The toads studied were all collected from Hoshiarpur and were identified as *Bufo stomaticus* Lutken by the Zoological Survey of India,

Calcutta, to whom our thanks are due. Our thanks are also due to Mr. H. S. Vasisht for taking the microphotographs.

Technique

The fresh oocytes of the toad have been very extensively studied under the phase contrast microscope, and microphotographed. The living oocytes have also been studied under the ordinary microscope. Some of the oocytes were also osmicated for short periods and studied fresh in normal saline.

Neutral red was used intravitally and supravitally for the demonstration of the vacuome. Intravitam injections of the dye were given into the body cavity of the toad, and the animals dissected after about two hours to study the oocytes.

Champy fixed preparations were studied, stained with 0·5% iron-haematoxylin.

Kolatchev, Aoyama and Da Fano techniques were also employed. Silver nitrate preparations were also toned in 0·2% gold chloride.

The oocytes stained with Sudan IV or Scharlach R were studied in glycerine.

Bouin's iron-haematoxylin preparations were used as control, and for the study of nucleolar extrusions.

Observations

Golgi Bodies and Fatty Yolk.—The youngest oocyte has a prominent circum-nuclear ring of mitochondrial granules, in which, as a rule, no Golgi bodies can be seen in our Kolatchev and Champy preparations (Pl. VIII, fig. 41 and Pl. V, fig. 24). In slightly older oocytes, however, when the mitochondrial ring has moved away from the nuclear membrane, prominent Golgi bodies can be seen in the mitochondrial ring. In fresh osmicated preparations, the Golgi bodies appear as vesicles with an osmiophilic cortex and an osmiophobic medulla (Pl. II, fig. 7). In Champy iron-haemotoxylin and Aoyama preparations, however, the Golgi bodies appear as granules, not revealing any duplex structure (Pl. V, figs. 25 and 26 and Pl. XI, fig. 56). In still older oocytes the mitochondrial-Golgi ring moves to the periphery of the cell (Pl. II, fig. 8 and Pl. V, fig. 27). The mitochondrial-Golgi ring now begins to break up (Pl. V, fig. 28), and this stage may sometimes be followed by a stage when the Golgi bodies and the mitochondria take up a juxta-nuclear position (Pl. X, fig. 49). Figure 4, Plate I, represents a living oocyte as studied under an ordinary microscope. The Golgi bodies can be seen with the utmost ease as dark refractile granules. Gradually the Golgi bodies begin to disperse in the cytoplasm till they are uniformly spread out (Pl. II, fig. 9; Pl. V, figs. 29 to 32; Pl. IX, fig. 43; and Pl. X, figs. 51 to 53).

When young oocytes are treated with Sudan IV (Kay and Whitehead), and mounted in glycerine, it is noticed that there is nothing in the cells, which is stained. But when the oocyte begins to measure about 0·35 mm.

in diameter, the Golgi bodies begin to stain with Sudan IV. With the growth of the oocyte some of the Golgi bodies also grow in size, and ultimately form the fatty yolk. All Golgi bodies, small and large in size, stain with Sudan IV (Pl. XI, figs. 58 to 60).

In preparations of older oocytes stained with Sudan IV, there is no way to distinguish the fatty yolk from the Golgi body, as both stain red in this stain. But when Kolatchev preparations of oocytes measuring about 1 mm. or more in diameter are decolourized in turpentine, it can be seen at once that, although morphologically alike, the Golgi body and the fatty yolk spherule differ radically in chemical composition. The larger fatty yolk spherules are completely decolourized in turpentine, but the Golgi bodies resist the decolourizing action of turpentine. The completely decolourized fatty yolk spherules appear as clear vacuoles. It is, therefore, not unreasonable to suppose that the Golgi body, which is made up largely of lipoids, directly grows into a fatty yolk spherule containing free fat.

Similarly in Champy unstained preparations the Golgi bodies and the fatty yolk are stained black alike with osmic acid, but the fatty yolk is soon decolourized with turpentine, leaving behind clear vacuoles.

In Da Fano or Aoyama preparations the fatty yolk appears as clear vacuoles, but the Golgi bodies are jet-black. The Golgi bodies are not stainable with neutral red.

Mitochondria and Albuminous Yolk.—The mitochondria of the toad can be best studied in Champy slides stained with 0·5% iron-haematoxylin. In all such preparations the mitochondria appear as grey or blue black granules.

In the youngest oocyte (Pl. V, fig. 24), the mitochondria appear in the form of a circum-nuclear ring, consisting of greyish fine granules. Gradually the mitochondrial ring tears itself off from the nuclear membrane, and takes its position at the periphery of the oocyte (Pl. V, figs. 25 to 27). With the growth of the oocyte the peripheral mitochondrial ring breaks up into numerous islands, which may be connected with each other by strands, consisting of mitochondrial granules (Pl. V, figs. 28 to 32).

Ultimately the mitochondria are uniformly spread out in the cytoplasm, but when the oocyte measures about 0·28 mm. in diameter some of the mitochondria form a prominent ring at the periphery of the oocyte (Pl. II, figs. 9 and 10).

The albuminous yolk granules appear for the first time in this peripheral mitochondrial ring and never in other regions of the oocyte (Pl. III, fig. 14 and Pl. VI, fig. 33). We are convinced that the mitochondrial granules are directly transformed into the albuminous yolk granules. Gradually the albuminous yolk granules at the periphery begin to move inwards (Pl. VI, figs. 34 and 35) till they are more or less uniformly spread out in the entire cytoplasm (Pl. VI, fig. 36). In very advanced oocytes the albuminous yolk bodies have grown into large discs (Pl. VII, figs. 37 to 39).

Nucleolar Extrusions.—The nucleolar extrusions can be best studied in Bouin's preparations stained with 0·5% iron-haematoxylin (Pl. IV,

figs. 17 to 23). Sooner or later the extrusions disappear completely from view without making any visible contribution to the formation of the albuminous yolk.

We have never seen any nuclear irregularity in the living oocytes studied under the ordinary or the phase contrast microscopes, but in fixed preparations the nuclear membrane is very irregular and may show prominent lobes (Pl. IV, figs. 22 and 23). We are, therefore, convinced that nuclear irregularity is an artifact, produced by the action of fixatives, as explained by Chubb (1906) in the oocyte of *Antedon* and by Nath *et al.* (1944) in teleostean fishes.

Vacuome.—When the oocyte measures about 0·06 mm. in diameter small but very clear vacuoles appear throughout the cytoplasm of the oocyte. These vacuoles can be very clearly studied in the living cells under the phase contrast microscope (Pl. I, figs. 1 to 3). The contents of these vacuoles seem to be watery and their reaction acidic, as they are stained brilliantly red with neutral red (Pl. II, figs. 10 to 13). The vacuoles slightly grow in size, but they remain unchanged, and no coagulum, whatsoever, appears in them at any stage (Pl. I, fig. 5).

Observations under the Phase Contrast.—It is possible to see and examine all the cytoplasmic inclusions described above with the utmost ease under the phase contrast microscope. Figures 64 to 66, Plate XII, represent comparatively young oocytes, but they show Golgi bodies, nucleolar extrusions and mitochondria very clearly. The mitochondria are seen in the form of small greyish granules. The Golgi elements are seen in the form of small, highly refractile and dark granules. At places two or three Golgi granules may unite together to form a bigger aggregate, but the component granules can always be seen separately in such an aggregate. The nucleolar extrusions are always much bigger than the Golgi elements, and even the Golgi aggregates. Besides they are much less refractile and less dark as compared with the Golgi granules, so that there can never be any confusion between these two types of cell inclusions. In Figure 65, Plate XII, very small vacuoles also can be very clearly seen in the upper region of the microphotograph.

Figure 71, Plate XII, is a microphotograph of four young oocytes. In two of them a nuclear lobe containing a nucleolus, ready to be expelled into the cytoplasm, can be very clearly seen. Nucleolar extrusions lying in the cytoplasm of these oocytes can also be clearly seen.

Figures 67 to 70, Plate XII, represent parts of oocytes photographed while being examined under the phase contrast. The vacuoles have come up very nicely in these four photographs, especially in the photographs 68 to 70. In these photographs the Golgi bodies, the vacuoles and the nucleolar extrusions can be seen side by side very clearly, the mitochondrial granules forming the background.

Figures 61 to 63, Plate XII, are photographs of still older oocytes. These photographs were taken with a view to bring the peripheral mitochondrial ring into prominence. This ring can be very clearly seen in these photographs.

Discussion

To sum up: The Golgi bodies and the mitochondria form a prominent circum-nuclear ring in young oocytes. This ring soon tears itself away from the nuclear membrane and migrates to the periphery of the oocytes. It then breaks up into fragments till ultimately the Golgi bodies and the mitochondria are uniformly spread out in the cytoplasm of the oocyte. In the course of oogenesis, when the oocyte measures about 1 mm. in diameter, many of the Golgi granules grow into fatty yolk spherules. Some of the mitochondria, on the contrary, form a prominent peripheral ring and directly grow into albuminous yolk bodies.

We are convinced, as a result of our studies under the phase contrast, that the essential form of the Golgi body is granular or spherical. We have never seen Golgi crescents in the living material. We must, therefore, consider the Golgi crescents figured in our Da Fano preparations (Pl. X, figs. 52 and 53) as artifacts. In Figure 53, Plate X, some Golgi 'rods' have also been shown. These again are artifacts due to the aggregations of Golgi granules. We have seen these Golgi aggregations under the phase contrast also.

The nucleolar extrusions in the oocytes of the toad are prominent, but soon they completely disappear from view without making any visible contribution to yolk-formation. These conclusions are identical with those arrived at by the present authors (1954) in *Rana tigrina*.

In comparatively young oocytes watery vacuoles, stainable with neutral red, put in their appearance. Some of these grow in size, but they never show any coagulum inside them and continue to remain as watery vacuoles.

Nath (1931 and 1932), working on the fresh material examined under the ordinary microscope, demonstrated the vacuome, the Golgi bodies, and the mitochondrial granules as independent cytoplasmic inclusions in the oocytes of *Rana tigrina*. Nath described the Golgi bodies as osmiophilic and argentophilic granules, not stainable with neutral red. He also showed that the albuminous yolk is formed directly from the mitochondrial granules, as the fatty yolk spherules are formed directly from the Golgi bodies.

Our conclusions in the toad are exactly identical with the above-mentioned conclusions of Nath, except that the vacuoles of the toad are much smaller than those of *Rana tigrina*.

Nath (1931) did not observe any nucleolar extrusions in the specimens of *Rana tigrina* collected from Lahore during the hibernation period only, but Nath and Malhotra (1954) have described nucleolar extrusions in *Rana tigrina* collected from Hoshiarpur during summer. As in *Rana tigrina* the nucleolar extrusions of the toad disappear without making any visible contribution to yolk-formation.

Explanation of Plates

All figures except figs. 1 to 3 and figs. 61 to 71 were drawn with the Camera lucida at the table level. Diameter of an oocyte is generally given at the bottom of each figure.

Plate I (Figs. 1 to 5)

All the figures of the plate are of fresh oocytes.

Figs. 1 and 2. Young oocytes as studied under the phase contrast microscope and drawn free hand.

Fig. 3. A portion of an oocyte studied under the phase contrast. Some of the vacuoles are seen having come in contact accidentally with the nucleolar extrusions. Free hand.

„ 4. A young oocyte showing a juxta-nuclear mass of Golgi bodies and mitochondria. ×380.

„ 5. Fresh ruptured contents of a 'ripe' oocyte. ×380.

Plate II (Figs. 6 to 13)

Fig. 6. Undifferentiated germinal epithelium osmicated for a short period. ×1000.

Figs. 7 and 8. Young oocytes osmicated for short periods. ×380.

Fig. 9. A portion of an oocyte showing vacuoles, Golgi vesicles and mitochondria. Osmicated. ×380.

Figs. 10 to 13. Contents of oocytes stained with neutral red showing prominent vacuoles, Golgi vesicles and mitochondria. ×380.

Plate III (Figs. 14 to 16)

Bouin's iron-haematoxylin preparations

Fig. 14. A portion of an oocyte showing the first formed albuminous yolk granules embedded in the mitochondrial ring at the periphery. ×380.

„ 15. A magnified view of the peripheral portion of an oocyte showing the origin of albuminous yolk granules from the mitochondria. ×1000.

„ 16. A portion of a comparatively older oocyte showing the inward migration of the albuminous yolk granules. ×380.

Plate IV (Figs. 17 to 23)

Bouin's iron-haematoxylin preparations

Figs. 17 to 20. Young oocytes showing nucleolar extrusions only. ×1000.

Fig. 21. A young oocyte showing prominent nucleolar extrusions. ×380.

„ 22. An oocyte showing a few nucleolar extrusions, and independent radial pockets (caused by the action of the fixative) of the nuclear membrane in a cross-section. ×180.

„ 23. A portion of an oocyte showing irregular nuclear membrane and nucleolar extrusions. ×380.

Plate V (Figs. 24 to 32)

Champy iron-haematoxylin preparations

Fig. 24. The youngest oocyte with a circum-nuclear mitochondrial ring. ×1000.

Figs. 25 to 27. Young oocytes showing the movement of the circum-nuclear ring of mitochondria to the periphery of the cell. ×380.

„ 28 to 32. Oocytes showing the dispersal of the Golgi elements and the mitochondria. Magn. Figs. 28 to 30—×380; Figs. 29 and 32—×1000; and Fig. 31—×260.

Plate VI (Figs. 33 to 36)

Champy iron-haematoxylin preparations

Fig. 33. An older oocyte showing the first appearance of albuminous yolk granules in the peripheral mitochondrial ring. ×380.

Figs. 34 and 35. Portions of oocytes showing the inward migration of albuminous yolk granules. Golgi bodies and vacuoles are also seen. ×1000.

Fig. 36. A portion of an oocyte showing the uniformly dispersed albuminous yolk granules. ×380.

Plate VII (Figs. 37 to 39)

Champy iron-haematoxylin preparations

Fig. 37. A portion of an almost mature oocyte showing the increase in size of the albuminous yolk bodies. Golgi bodies and mitochondria are also seen. ×1000.

Figs. 38 and 39. Contents of 'ripe' oocytes showing the formation of fatty yolk. ×1000.

Plate VIII (Figs. 40 to 42)

Kolatchev preparations

Fig. 40. Undifferentiated germinal epithelium showing the Golgi granules. ×1000.

„ 41. The youngest oocytes showing circum-nuclear rings of mitochondrial granules. ×1000.

„ 42. A portion of an oocyte showing the Golgi bodies, inward migration of albuminous yolk granules and a few vacuoles. ×780.

Plate IX (Figs. 43 to 47)

Kolatchev preparations

Fig. 43. A portion of an early oocyte showing the Golgi bodies, the peripheral aggregations of mitochondria and vacuoles at the periphery. ×1000.

Figs. 44 and 45. Contents of older oocytes showing heavy and optimum impregnations respectively. ×1000.

Fig. 46. A portion of a 'ripe' oocyte showing the formation of fatty yolk. ×1000.

„ 47. A portion of a 'ripe' oocyte treated with turpentine for a fortnight. ×1000.

Plate X (Figs. 48 to 54)

Da Fano preparations

Fig. 48. Undifferentiated germinal epithelium showing the Golgi granules. Toned. ×1000.

„ 49. A young oocyte showing the dispersal of Golgi bodies and mitochondria from a juxta-nuclear position. Toned. ×380.

„ 50. A young oocyte showing the spreading of the Golgi bodies and mitochondria. Toned. ×380.

„ 51. An early oocyte showing Golgi bodies, islands of mitochondrial granules and vacuoles. Toned. ×380.

Figs. 52 and 53. Portions of early oocytes showing the mitochondria, Golgi bodies and vacuoles. Toned. ×1000.

Fig. 54. Contents of an older oocyte. Untoned. ×1000.

Plate XI (Figs. 55 to 60)

Fig. 55. Contents of a Da Fano toned 'ripe' oocyte. The fatty yolk vacuoles have been crushed. ×1000.

„ 56. A young oocyte showing the movement of the circum-nuclear ring of mitochondria and Golgi bodies towards the periphery. Aoyama, untoned. ×1000.

„ 57. Same as figure 55. Aoyama, untoned. ×1000.

„ 58. Contents of an oocyte (measuring 0·35 mm.) in which the Golgi bodies are stained with Sudan IV. ×380.

„ 59. A Portion of an older oocyte stained with Sudan IV showing the increase in size of the Golgi bodies. ×380.

„ 60. Ruptured contents of a 'ripe' oocyte stained with Sudan IV. ×380.

Plate XII (Figs. 61 to 71)

All the figures of the plate are microphotographs of fresh oocytes as studied under *the phase contrast microscope*. Leitz Dialux phase contrast microscope with ×10. Periplanetic Eye-piece and 40 : 1 objective (Apochromatic dry system), giving a magnification of 500 times, and Leica Camera were used.

Figs. 61 to 63. Portions of oocytes showing peripheral yolk-forming ring of mitochondria. About ·3 mm.

„ 64 to 66. Portions of an oocyte under different foci showing nucleolar extrusions, Golgi bodies, vacuoles and mitochondria. 0·12 mm.

„ 67 to 70. Peripheral portions of oocytes showing nucleolar extrusions, Golgi bodies, vacuoles, and mitochondria. 0·204 mm.

Fig. 71. Young oocytes showing nucleolar extrusions.

Lettering

A.Y—Albuminous yolk; *Cpb*—Chromophobic substance of the Golgi element; *FE*—Follicular epithelium; *F.Y*—Fatty yolk; *G*—Golgi element; *M*—Mitochondria; *N*—Nucleus; *NU*—Nucleolus; *N.E*—Nucleolar extrusions; *V*—Vacuoles; *VM*—Vitelline membrane.

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2. Nath, V. (1931). A Demonstration of Vacuome and Golgi Apparatus as Independent Cytoplasmic Components in the fresh Egg of Frog. *Zeit. Zellforsch.* **13**.
3. ——— (1932). Cytological differences between closely Allied Species. *Nature*, 130.
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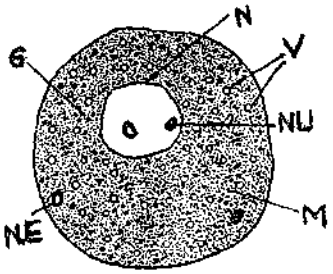

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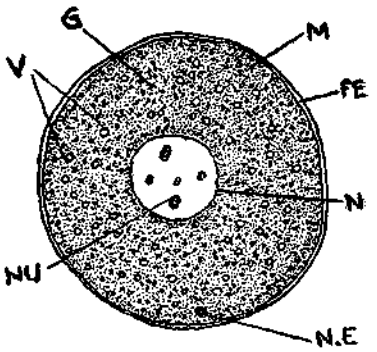

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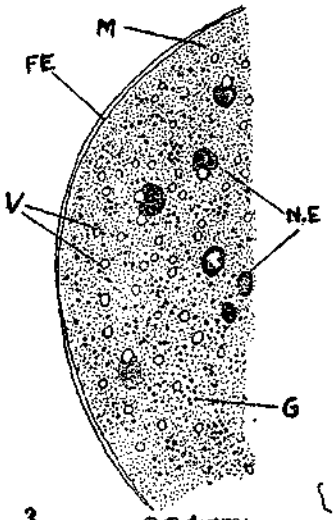

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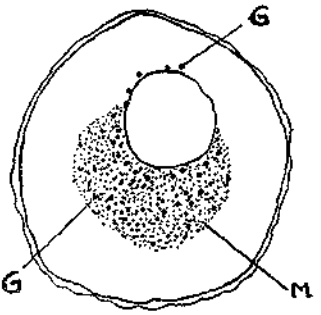

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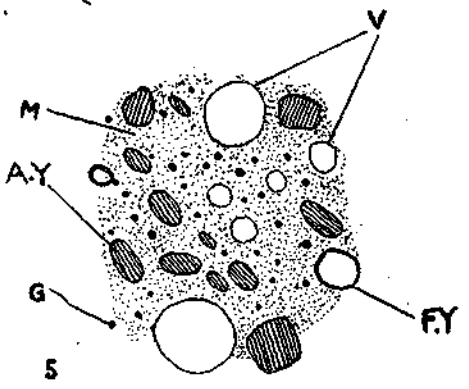

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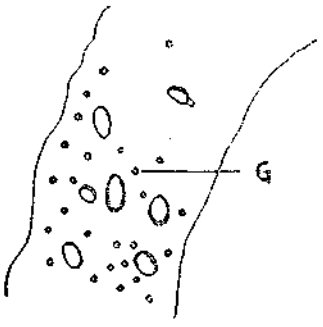

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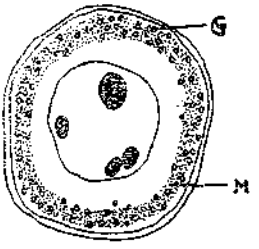

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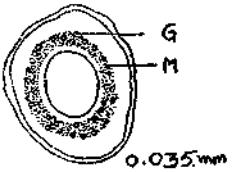

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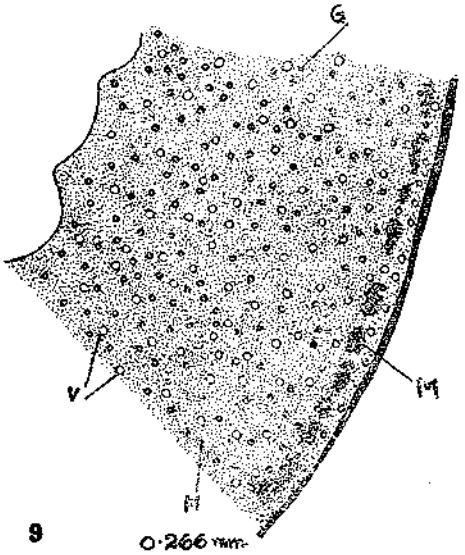

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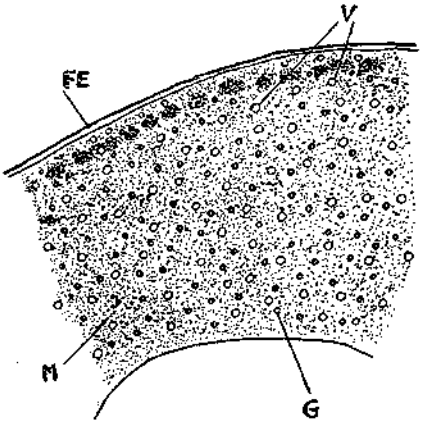

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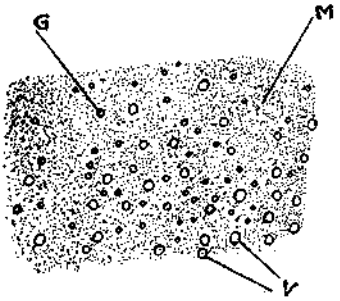

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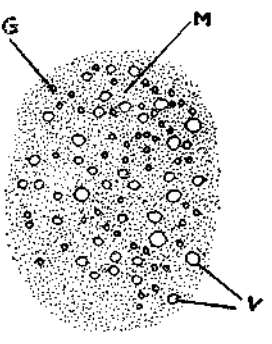

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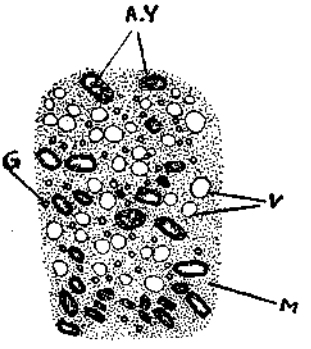

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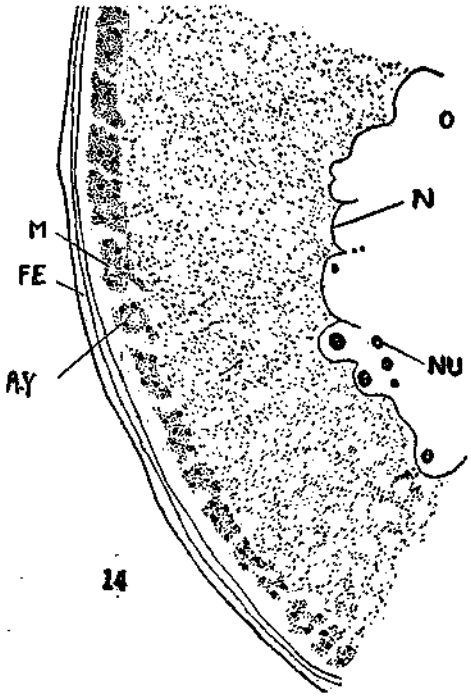

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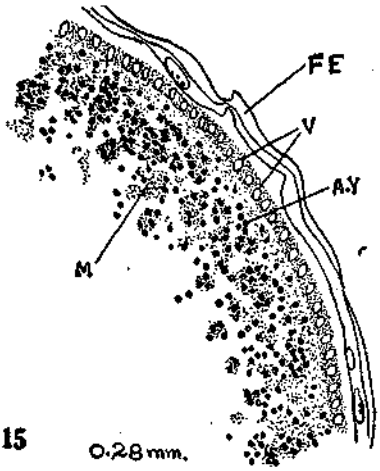

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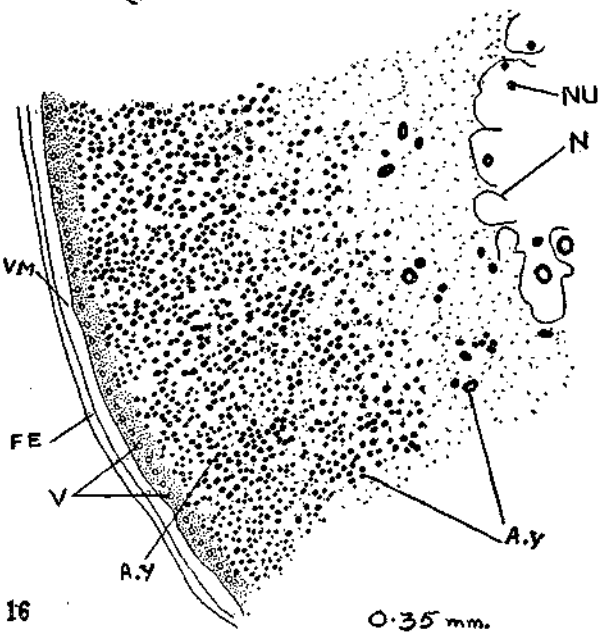

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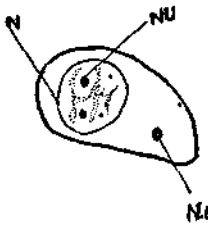

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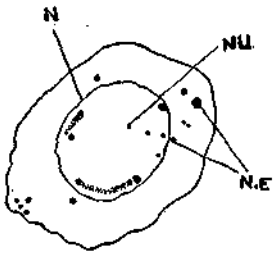

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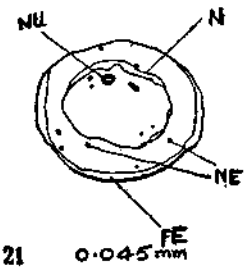

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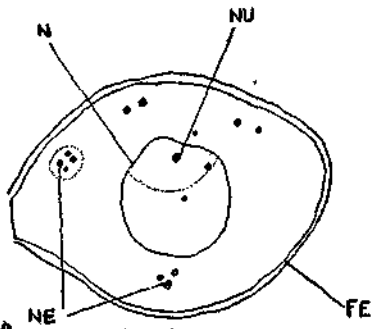

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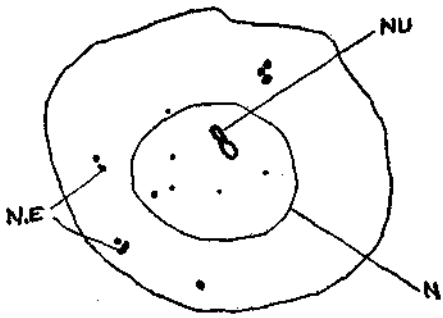

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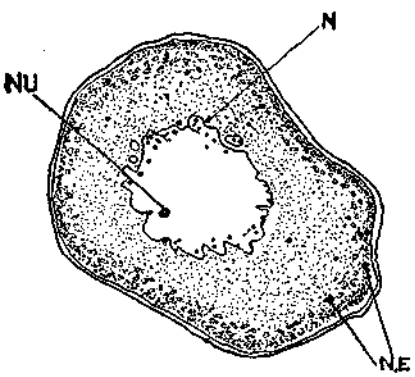

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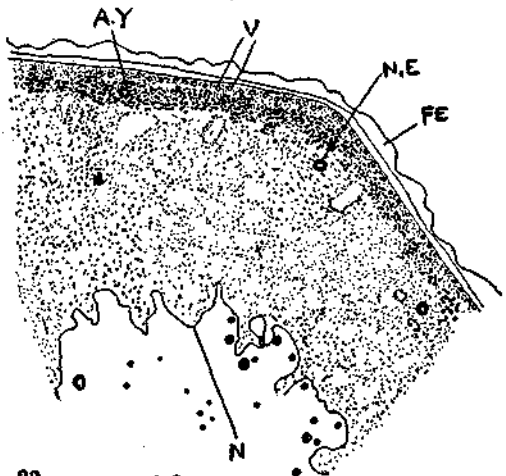

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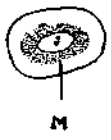

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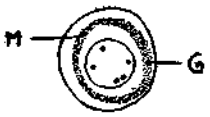

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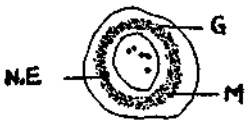

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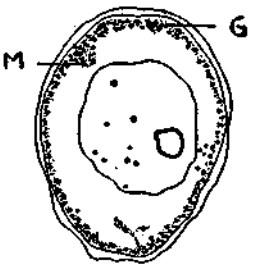

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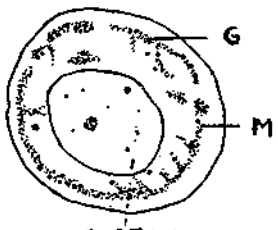

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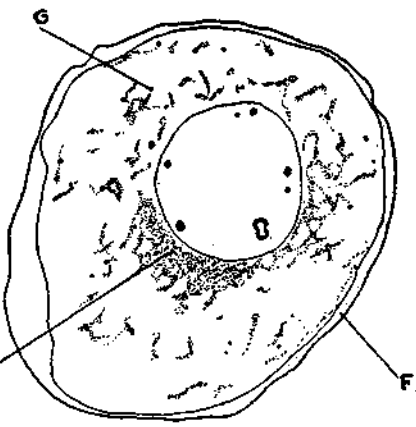
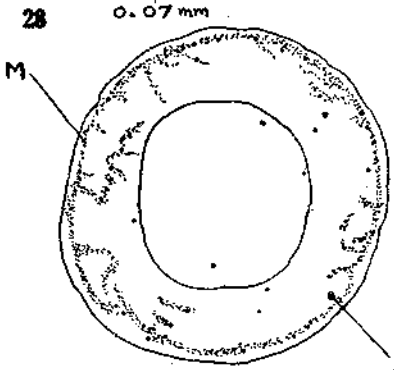
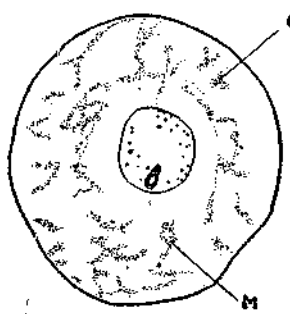


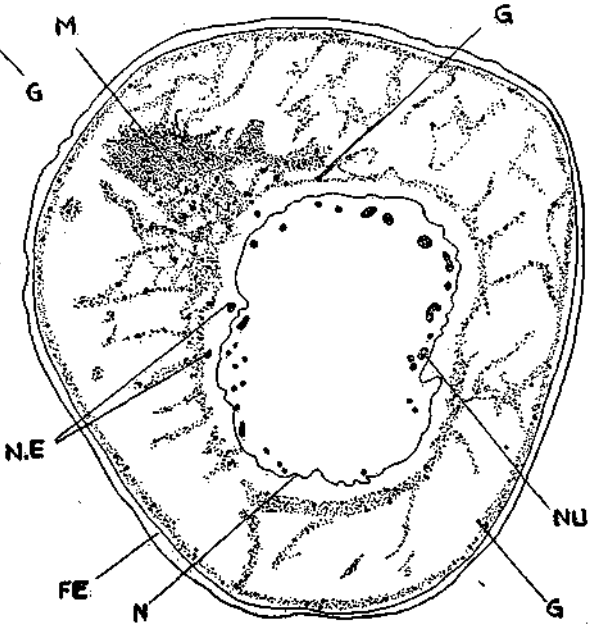
29 0·098 mm.



30 0·112 mm.

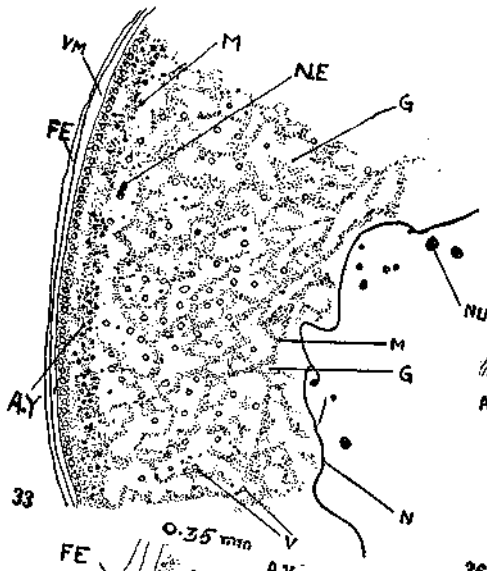


31 0·114 mm.



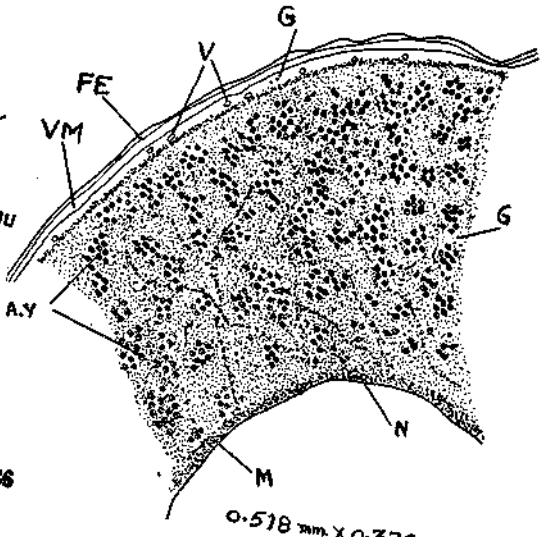
32 0·17 mm.

PLATE VI.



33

0·35 mm.

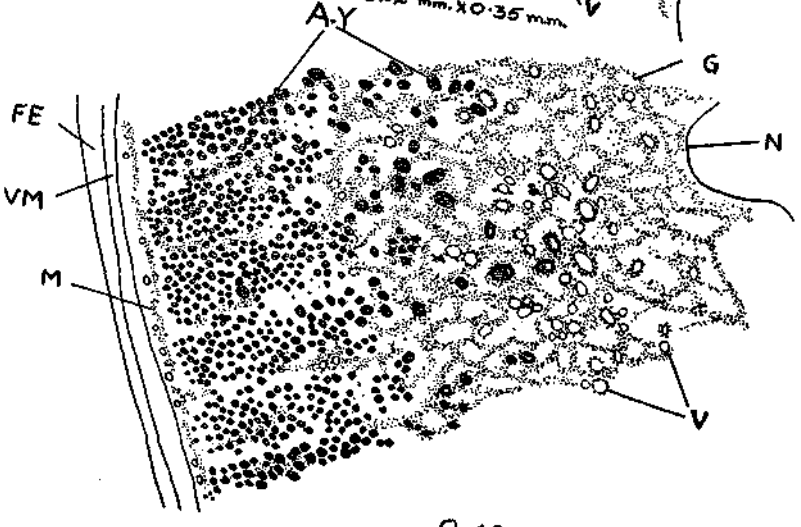


36

0·518 mm. × 0·378 mm.

34

0·322 mm. × 0·35 mm.



35

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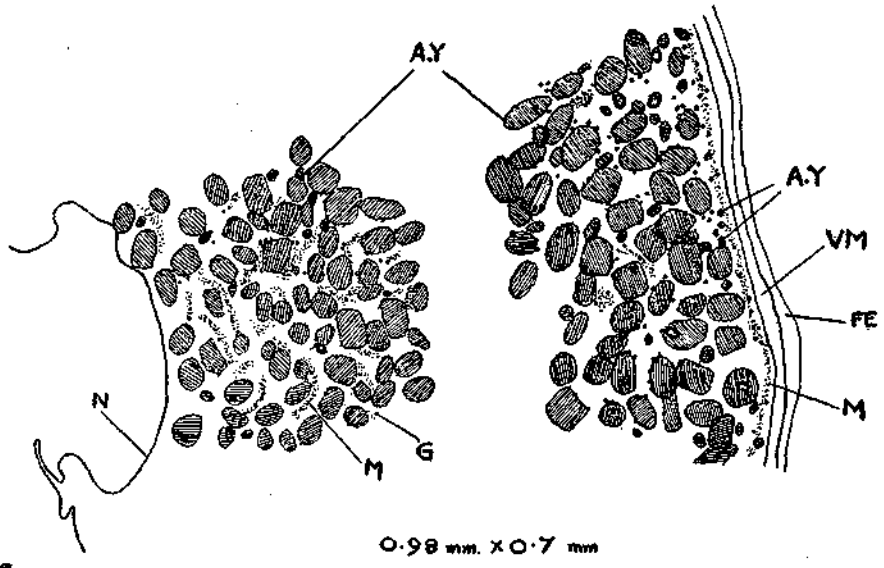
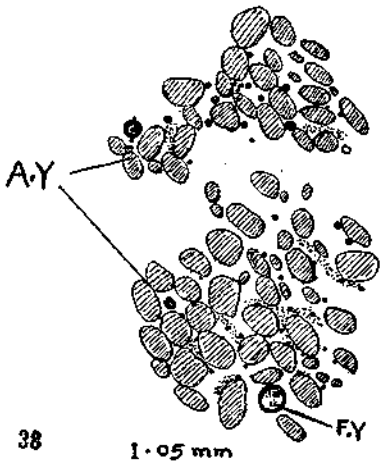
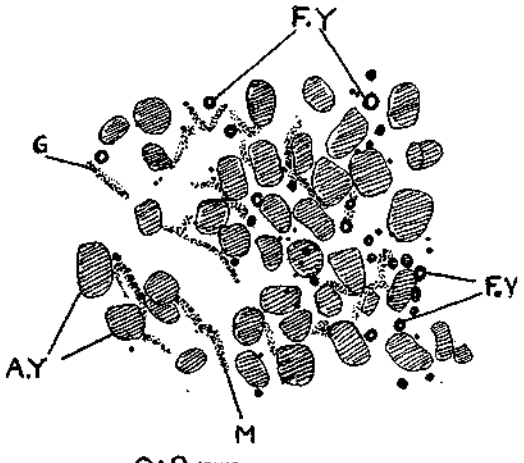


0·98 mm. × 0·7 mm

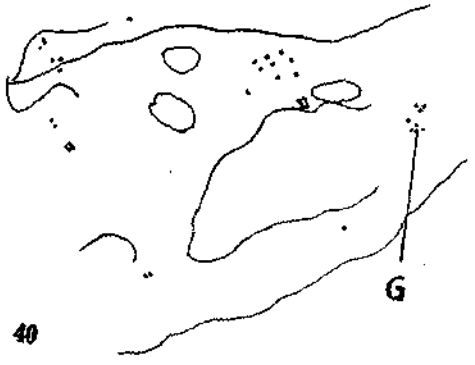
37



38 1·05 mm



39 0·9 mm



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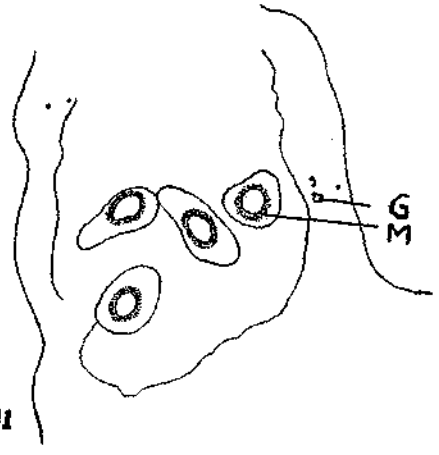

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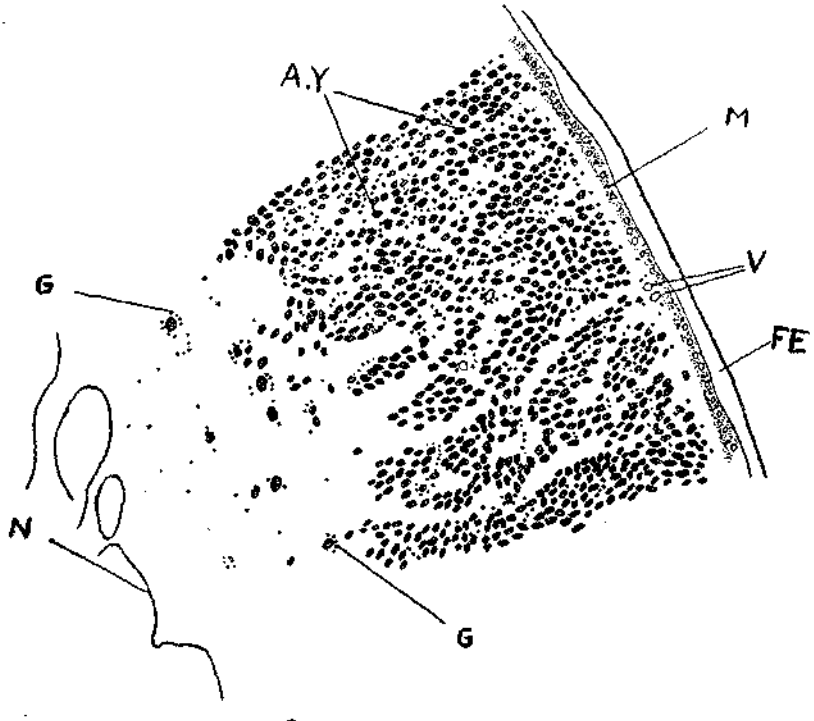
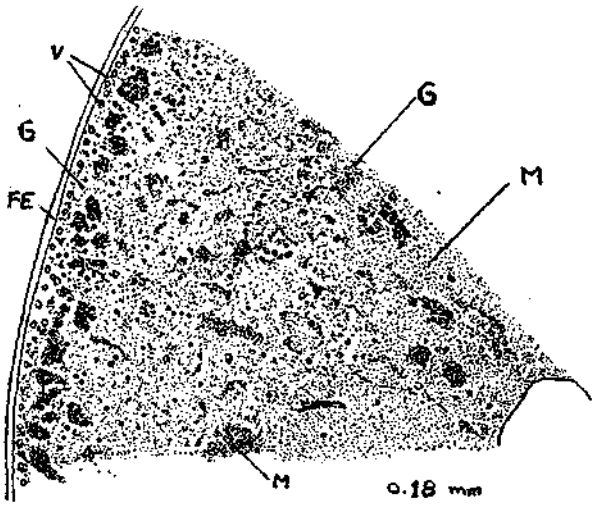


42

0·510 mm × 0·434 mm



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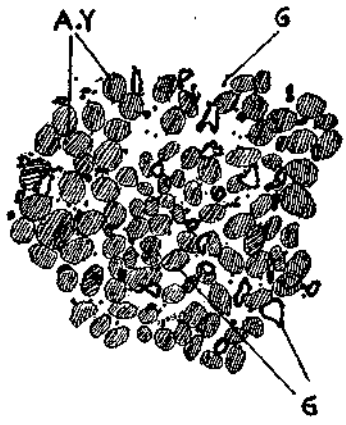
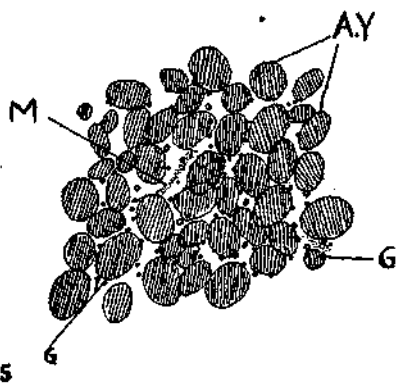


44 0·658 mm



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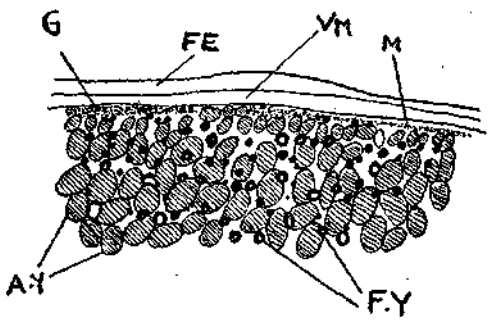

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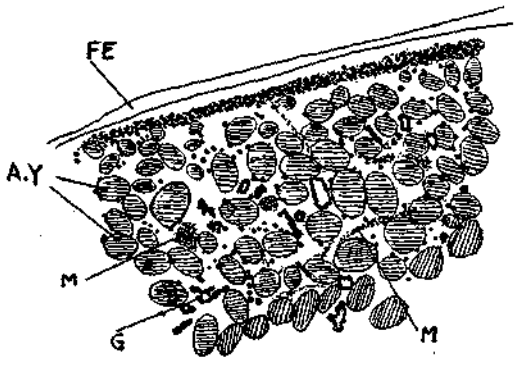
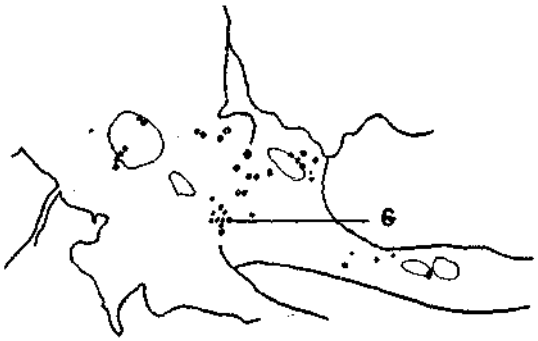


47 1 mm

PLATE X.



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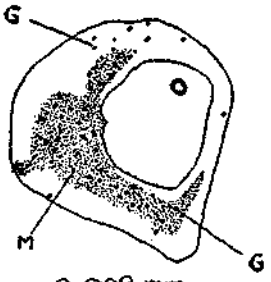
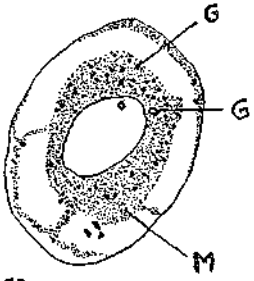


49 0·098 mm



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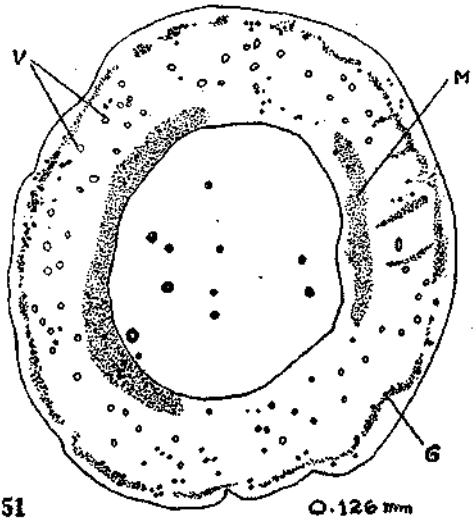
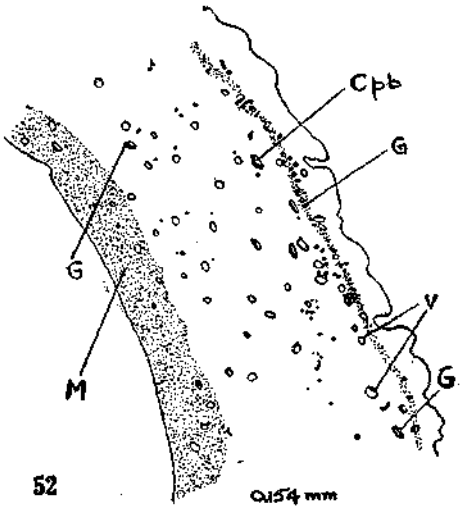
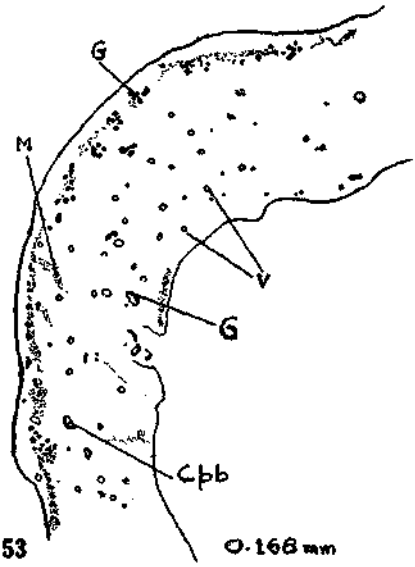


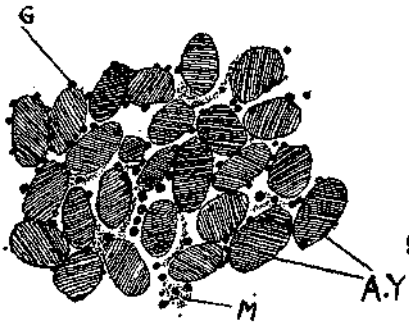
51 0·126 mm



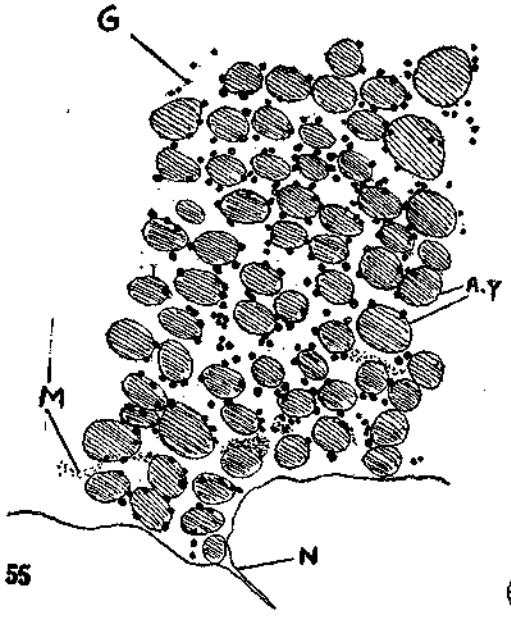
52 0·154 mm



53 0·168 mm



54 0·85 mm



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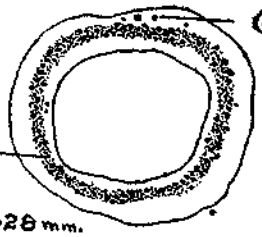
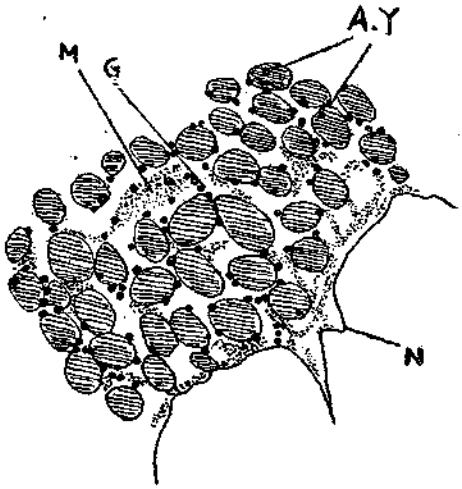
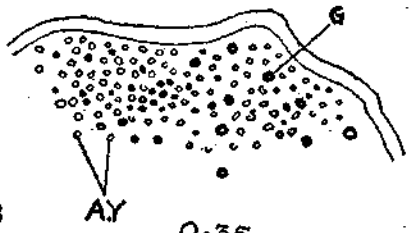


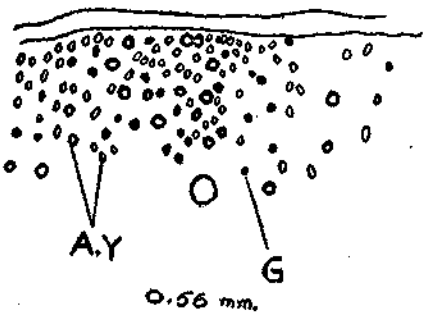
56
0·0·28 mm.



57 0·91 mm. x 0·56 mm.

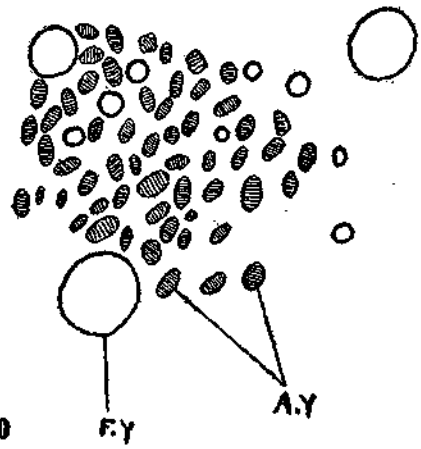


58
0·35 mm.



0·56 mm.

59



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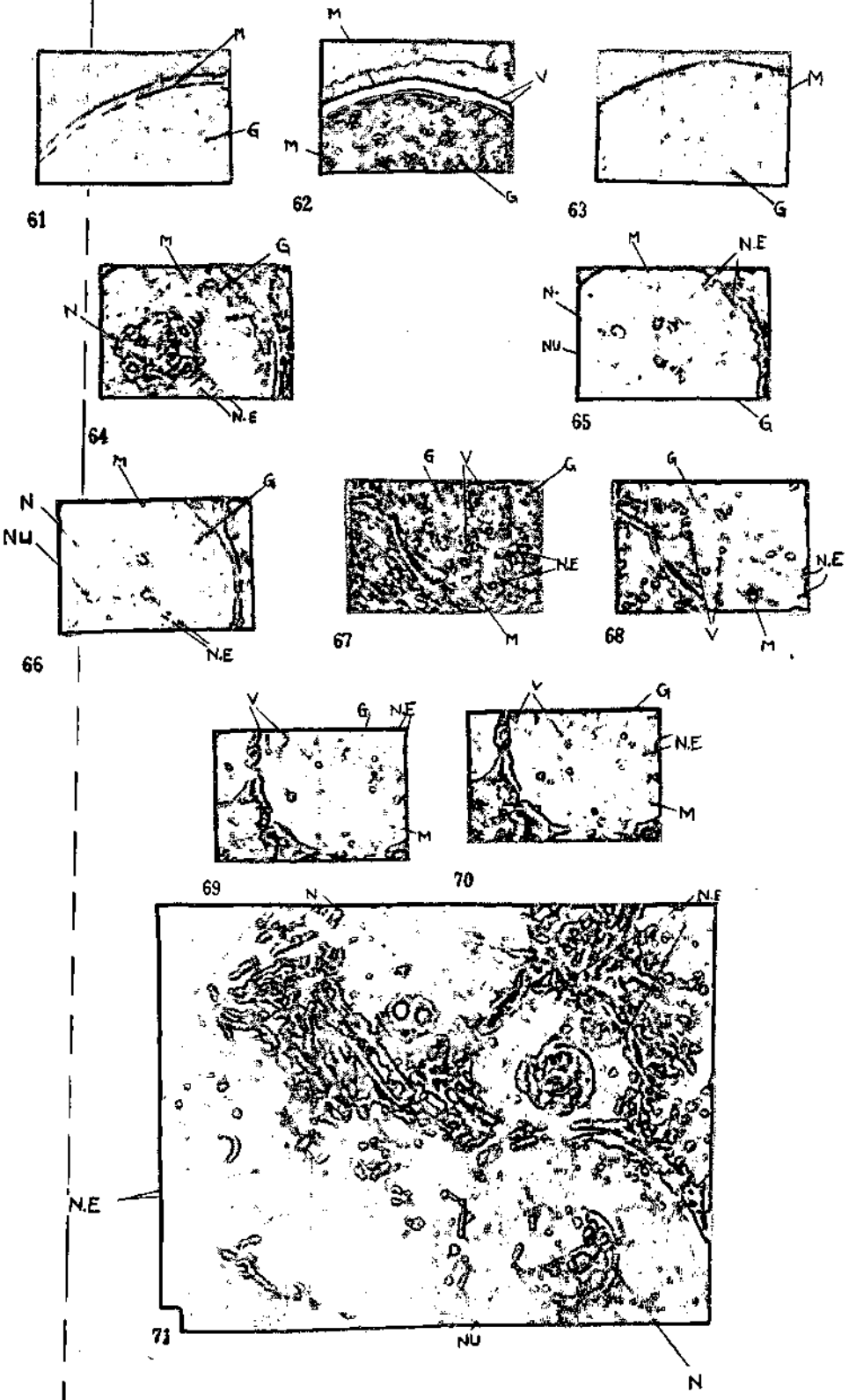

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STUDIES ON INSECT SPERMATOGENESIS
No. III. ORTHOPTERA

Spermatogenesis of *Chrotogonus trachypterus* (Blanch) with observations under the phase contrast microscope

by

SUKHDEV RAJ BAWA

(Department of Zoology, Panjab University, Hoshiarpur)

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SPERMATOGENESIS OF *CHROTOGONUS TRACHYPTERUS* (BLANCH) WITH OBSERVATIONS UNDER THE PHASE CONTRAST MICROSCOPE

By SUKHDEV RAJ BAWA

(Department of Zoology, Panjab University, Hoshiarpur)

ABSTRACT

In the spermatogonium of *Chrotogonus trachypterus* (Blanch) there is no differentiation between the Golgi and mitochondrial elements. In the primary spermatocyte, discrete Golgi granules can be seen embedded in the juxta-nuclear mass of mitochondrial granules. During growth of the spermatocyte, the Golgi granules grow into deeply-staining spheres and the mitochondria tend to clump together. The secondary spermatocyte is definitely smaller than the primary. During late telophase II, the spindle fibres and the mid-body granules have been seen in the living testicular material under the phase contrast microscope. In the spermatid, the mitochondria fuse to form a nebenkern, and the tail-sheath is formed by the attenuating nebenkern. The acrosome is formed under the influence of the Golgi material. The two centrioles in the later process of spermateleosis elongate at the base of the sperm head, and finally form the middle-piece of the sperm.

INTRODUCTION

Bowen (1922), working on *Ceuthophilus* (a tettigonid), described the origin of the acrosome and also traced its later development during late spermateleosis. But he did not study the behaviour of the Golgi and the mitochondrial elements in the spermatocyte and in the early stages of spermateleosis. Regarding the origin of the acrosome in the Acridiidae (*Rhomaleum* and *Dissosteria*) Bowen states: 'It seems probable, however, that from each one (Golgi body) is differentiated its small proportionate share, and by the deposition of many such parts the acrosome is gradually built up'.

Later Chang-Chun Wu (1946) published his paper on the origin of the acrosome and behaviour of the nebenkern in *Diestrammena* sp.

Recently Nath and Bhimber (1953), making use of the phase contrast microscope, gave an exhaustive account of the process of sperm-formation in *Acheta domesticus* Linn. (*Gryllus domesticus*). In this communication, they have emphasized that the real form of the Golgi body is either granular or vesicular.

Earlier Payne (1916) published a paper on the spermatogenesis of *Gryllotalpa borealis* and *G. vulgaris*.

In the present paper, third in the series, I have studied the behaviour of the Golgi and mitochondrial elements during the process of sperm-formation in *Chrotogonus trachypterus* (Acridiidae).

The spindle fibres, till recently considered as coagulation effects due to fixation, have actually been observed in a number of fresh dividing cells studied under the phase contrast microscope (Nath, Bawa and Bhimber, 1954). Moreover, in *Chrotogonus trachypterus* the condensation of the spermatid nucleus is unusually simple inasmuch as it continues to stain lightly till in the final stages of spermateleosis it begins to stain deeply, without showing the usual chromophilic cortex and chromophobic medulla and their subsequent reversal. Another unusual feature of the spermateleosis in the species under discussion is the complete absence of the 'central substance', which usually appears in the attenuating nebenkern of insects.

A thorough study of the fresh testicular material of *Chrotogonus trachypterus* has been made under the phase contrast microscope.

The investigations recorded in this paper were carried out in the Department of Zoology, Panjab University, Hoshiarpur, Panjab (I). I am deeply indebted to Dr. Vishwa Nath, Professor of Zoology, Panjab University, who helped me in preparing the manuscript of this paper. I wish to thank Miss B. K. Dhillon and Brij Gupta for their general assistance.

Material and Methods

The grasshoppers, *Chrotogonus trachypterus*, were collected in large numbers from the grassy lawns adjoining the University Zoological Laboratory. The males can be distinguished easily from the females by the absence of the ovipositor.

The testicular material was fixed in Flemming-without-acetic acid; and twenty-four hours fixation in this fluid gave the best results. The material was washed in running water for the same period so as to remove all traces of chromic acid. Subsequently, it was subjected to the ordinary process of dehydration and clearing, and embedded in paraffin wax. Sections were cut 6 μ thick and stained in 0·5% iron-haematoxylin. Bouin's fluid was used as control.

Observations

Spermatogonia.—Testicular material of *Chrotogonus trachypterus* was examined in the living condition under the phase contrast. The number of spermatogonia varies from two to sixteen in each cyst. Each spermatogonium reveals a juxta-nuclear mass of very fine, highly refractive, dark granules of almost equal size. At present there is no differentiation between the mitochondrial and Golgi elements. In the nucleus a few chromatin granules in addition to a prominent nucleolus can be made out (Pl. III, Fig. 35).

In preparations fixed in F.W.A. and stained with iron-haematoxylin also there is no differentiation between the mitochondria and Golgi elements, there being only one type of granules which stain greyish (Pl. I, Fig. 1).

Primary Spermatocytes.—The primary spermatocytes are definitely larger cells than the spermatogonia (Pl. I, Fig. 2). The cytoplasmic granules of the spermatogonium are now differentiated into two distinct kinds of granules : (1) the mitochondrial granules, which are numerous and stain deeply with haematoxylin and (2) larger, more deeply-staining Golgi granules, which are embedded in the juxta-nuclear mass of mitochondria. Throughout the growth period of the primary spermatocyte, the Golgi bodies also grow in size and appear as homogeneously staining spheres (Pl. I, Figs. 3 to 6). The mitochondrial granules now show a distinct tendency to align themselves (Pl. I, Fig. 4). Later the mitochondrial granules tend to clump together (Pl. I, Figs. 5 and 6). The clumping of the mitochondrial elements proceeds still further, so much so that only one or two compact masses are visible just before the meiotic divisions begin (Pl. I, Fig. 7).

A polar view of metaphase I is shown in Pl. I, Fig. 8. A V-shaped chromosome can be distinguished from the rest. This most probably is the sex-chromosome. During the first meiotic divisions, well-defined spindles can be seen (Pl. I, Figs. 9 to 11). The spindle appears as a fibrillar and fusiform structure with a sharply-staining centriole at each pole (Pl. I, Fig. 9). The Golgi bodies, which are lost to view in early meiotic stages, reappear during the late telophase I. At this stage, they generally appear as spheres, revealing a duplex structure with a chromophilic cortex and a chromophobic medulla. Both the mitochondria and the Golgi bodies are seen to be distributed more or less evenly to the two daughter secondary spermatocytes. The movement of the daughter nuclei to one side of the spindle (telokinesis) is well marked at this stage. Prominent deeply-staining mid-body granules appear at the equator of the spindle (Pl. I, Figs. 10 and 11).

A large number of living primary spermatocytes were thoroughly studied under the phase contrast. The Golgi bodies were always visible as spheres; and various stages of clumping together of the mitochondrial elements were observed and microphotographed (Pl. III, Figs. 36 and 37 ; and Pl. IV, Fig. 50).

Secondary Spermatocytes.—Each resting secondary spermatocyte is smaller than the primary. In Pl. I, Fig. 12 is drawn a newly-formed secondary spermatocyte, showing spindle remains, mitochondria collected near the nucleus, and a few Golgi spheres distributed at random in the cytoplasm. A little later, the spindle fibres disappear from view and the mitochondria tend to aggregate on one side of the nucleus. The nucleus always reveals a sex-chromosome and a nucleolus of irregular shape (Pl. I, Fig. 13).

In late anaphase II the mitochondrial threads place themselves parallel to the spindle and are cut across by the cleavage furrow (Pl. I, Figs. 14 and 15; and Pl. IV, Fig. 51).

A living secondary spermatocyte, in early telophase stage, was microphotographed. Here the chromosomes have reached the poles and the mitochondria can be seen on one side of the spindle (Pl. IV, Fig. 51). In fresh testicular material studied under phase contrast, during the late

telophase II, beautiful spindle fibres, mid-body granules, mitochondrial threads, and the Golgi spheres have been observed (Pl. III, Figs. 38 to 40).

Spermateleosis.—The mitochondrial elements of the early spermatid progressively fuse together (Pl. I, Figs. 16 and 17), and finally form a deeply-staining, compact nebenkern (Pl. I, Figs. 18 and 19). Strange as it would appear, in the fixed preparations, the mitochondrial nebenkern does not reveal any differentiation whatsoever throughout the process of spermateleosis.

But in the fresh testicular material studied under phase contrast, the mitochondrial nebenkern, which is seen as a homogeneous structure in the beginning (Pl. III, Fig. 41), shows differentiation into an outer transparent light area and an inner highly refractile dark core (Pl. IV, Fig. 52). Very often a few strands traversing the light area of the nebenkern can be seen (Pl. III, Fig. 42). Soon the transverse strands disappear from view (Pl. III, Fig. 43). Later on, the emergence of a fine thread-like axial filament from the basal portion of the nucleus initiates the division of the mitochondrial nebenkern into two halves (Pl. III, Fig. 44).

Reverting to the fixed preparations, the two halves of the elongating mitochondrial nebenkern are very often found to enclose a little space in between (Pl. I, Figs. 20 to 22). A little later, this space is obliterated altogether, and the nebenkern, which now appears as a single band at this stage, lengthens out progressively (Pl. II, Figs. 23 to 27). A few bleb-like swellings appear along the length of the attenuating nebenkern, while the intermediate portions between the blebs get much thinned out (Pl. II, Fig. 28). Finally, the bleb-like swellings disappear completely (Pl. II, Figs. 29 to 33). The 'central substance' granules appearing in the chromophobic area of the mitochondrial nebenkern, described earlier by Chang-Chun Wu (1946) and Nath and Bhimber (1953) in other species of Orthoptera, are conspicuous by their absence in *Chrotogonus trachypterus*. Series of fine bleb-like swellings along the entire length of the sperm-tail have been observed in the fresh material under phase contrast (Pl. III, Figs. 45 to 49).

The Golgi bodies in the early stages of spermateleosis generally appear as spheres very similar to those of the secondary spermatocytes (Pl. I, Figs. 16 to 19). When the spermatid elongates, a few Golgi bodies seem to come near each other in the anterior region of the cell in close contact with the nuclear membrane (Pl. I, Fig. 21), but they do not happen to fuse to form a single acroblast. A little later, a small granule makes its appearance in close vicinity of the multiple acroblast (Pl. I, Fig. 22). This is the acrosomal granule. Later on, the acrosomal granule grows in size; and the Golgi bodies (multiple acroblast) are sloughed off (Pl. II, Figs. 23 and 24). The acrosome keeps moving round the nucleus (Pl. II, Figs. 25 to 28) till it gets transformed into a small deeply-staining triangle, lying at the tip of the nucleus (Pl. II, Figs. 29 and 30). Very soon, however, when the nucleus starts elongating, the boundary between the acrosome and the nucleus is obliterated altogether (Pl. II, Figs. 31 to 34).

But in the living testicular material under phase contrast, the acrosome can always be seen as a highly refractile structure lying at the apex of the maturing sperm head. Various stages of acrosome transformation from a

granule to a spine-like structure in the mature sperm have also been studied in the fresh material (Pl. III, Figs. 45 to 49).

Lastly, a brief mention of the nuclear changes and the centriole may be made. The nucleus in the early stages of spermateleosis stains uniformly, except that a few deeply-staining chromatin granules can be seen in its interior (Pl. I, Figs. 16 to 22; and Pl. II, Figs. 23 to 27). As the nucleus draws out into a thick spindle, the chromatin granules disappear completely (Pl. II, Figs. 31 to 33). During spermateleosis, the centriole to begin with is seen as a prominent deeply-staining structure in close contact with the nucleus (Pl. I, Figs. 21 and 22; and Pl. II, Figs. 23 to 29). Later on, the centriole gets divided up into two separate parts (Pl. II, Fig. 30). Subsequently, the two centrioles increase in size and finally lengthen out at the base of the nucleus to form the so-called 'middle-piece' of the sperm (Pl. II, Figs. 31 to 34).

Discussion

Golgi material and the Acrosome.—Throughout the process of sperm-formation in *Chrotogonus trachypterus* Golgi bodies appear as spheres. This has been fully confirmed by my studies of the living material under the phase contrast, and is in harmony with the observations of Nath and Bhimber (1953) on the living testicular material of *Acheta domesticus* Linn. (*Gryllus domesticus*).

During early spermateleosis in *Chrotogonus trachypterus*, a deeply-staining acrosomal granule appears amongst three or four Golgi bodies stuck to the anterior face of the spermatid nucleus. There is, no doubt, that the acrosome is formed under the influence of the Golgi bodies; but the small size of the acrosome, together with the manner in which the Golgi bodies closely invest it, renders observations on the details of its formation difficult.

Chang-Chun Wu (1946), working on *Diestrammena* sp., also describes a few discrete, spherical acroblasts (his Golgi bodies), amongst which an acrosomal granule puts in an appearance. He ascribes the origin of the acrosomal granule to the activity of the chromophobic parts of the Golgi bodies. The acrosome, in his opinion, further reveals differentiation into two portions of chromatic substance, which finally fuse to form the front piece of the sperm.

Nath and Bhimber (1953), on the other hand, describe an 'acroblast' with an acrosomal granule lodged in its interior. Later on, the acrosomal granule is seen deposited on the nucleus. The acrosomal granule further gets differentiated into a triangle, which reveals 'a deeply-staining shelf on which the base of the triangle rests, and a deeply-staining small granule perched at the apex of the triangle'. The differentiation in the acrosome is completely obliterated in the mature sperm.

Mitochondria.—Chang-Chun Wu (1946) described in *Diestrammena* sp. thread-like mitochondria forming a typical insect nebenkern. In his opinion, the tail-sheath of the sperm is formed by the 'central substance' granules, which appear in the chromophobic cavity of the nebenkern. Similar observations were made by Nath and Bhimber (1953) on *Acheta domesticus* Linn. (*Gryllus domesticus*). In the grasshopper, *Chrotogonus*

trachypterus also the tail-sheath is formed by the mitochondrial nebenkern, but there is no 'central substance'.

Nucleus and Centriole.—Although Chang-Chun Wu (1946) has not described the process of condensation of the spermatid nucleus, reference to his figures 17 to 21 would show a clear demarcation of the nucleus into an outer deeply-staining area enclosing a more or less clear area. Later on, however, the differentiation of the nucleus into two areas disappears completely. There is only one centriole to begin with, but as the sperm nucleus has drawn out into a long spindle, it has been shown to divide into two rod-like structures, which finally fuse together to form the middle-piece of the sperm.

Nath and Bhimber (1953) also describe a division of the spermatid nucleus into an outer deeply-staining cortex and an inner lightly-staining medulla. A little later, a small deeply-staining portion of the nucleus is completely cut off from the lightly-staining portion and ultimately degenerates. The centriole in their opinion remains a single granule throughout spermateleosis, and the mature sperm is lacking in a middle-piece.

On the contrary, in *Chrotogonus trachypterus*, the spermatid nucleus does not reveal any demarcation into a deeply-staining cortex, and a feebly-staining medulla. From the beginning of its formation it stains homogeneously, but it shows a few chromatin granules in its interior in early stages of spermateleosis. As the nucleus starts elongating these granules disappear. A similar process of nuclear condensation has been described by me in an earlier communication on Hemiptera (Bawa, 1953). I am, however, in full accord with Chang-Chun Wu as regards the centrosomal origin of the middle-piece.

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Explanation of Lettering in the Plates

A—Acrosome; *A.f*—Axial filament; *A.Ch*—Sex Chromosome; *A.g*—Acrosomal granule; *C*—Centriole; *Ch*—Chromosome; *G*—Golgi body; *M*—Mitochondria; *M.b*—Mid-body; *M.n*—Mitochondrial nebenkern; *N*—Nucleus; N_1—Nucleolus; *S.f*—Spindle fibres.

Explanation of the Plates

All the figures of plates, I and II, have been drawn with camera lucida at the table level with 10 × eye piece and oil immersion objective, giving approximately a magnification of 1,700 times. Unless otherwise mentioned all figures have been selected from sectioned material fixed in Flemming-without-acetic acid.

All the figures of plate III are from the living material studied under the phase contrast microscope.

Figures 50 to 52 (Plate IV) are microphotographs of the fresh testicular material studied under phase contrast microscope.

Plate I

Fig. 1. Spermatogonium showing juxta-nuclear mass of cytoplasmic granules.

Fig. 2. Resting primary spermatocyte showing deeply-staining Golgi granules and lightly-staining mitochondria.

Fig. 3. Primary spermatocyte showing leptotene nucleus, granular mitochondria and a few Golgi spheres.

Fig. 4. Primary spermatocyte showing the alignment of mitochondria and a few Golgi spheres.

Figs. 5 and 6. Primary spermatocytes showing the clumping together of the mitochondria.

Fig. 7. Primary spermatocyte showing the formation of tetrads and two mitochondrial masses in the cytoplasm.

Fig. 8. Polar view metaphase I.

Fig. 9. Side view metaphase I showing two deeply-staining centrioles at each pole of the fibrillar spindle (Bouin).

Figs. 10 and 11. Late telophases II showing deeply-staining mitochondria, spindle fibres and a few Golgi spheres. Mid-body granules can be seen at the equator of the spindle.

Fig. 12. Newly formed secondary spermatocyte.

Fig. 13. Secondary spermatocyte showing aggregated mitochondria and Golgi spheres.

Figs. 14 and 15. Early telophase II.

Figs. 16 and 17. Early spermatids showing a few Golgi spheres and progressive fusion of the mitochondria.

Figs. 18 and 19. Spermatid showing nucleus and deeply-staining mitochondrial nebenkern.

Fig. 20. Spermatid showing up the mitochondrial nebenkern divided into two halves and a prominent centriole (Bouin).

Fig. 21. Spermatid showing multiple acroblast (Golgi bodies) in the vicinity of the nucleus towards the anterior region of the cell. The mitochondrial nebenkern is seen in the form of two elongated bands.

Fig. 22. Spermatid showing the appearance of the acrosomal granule amongst the multiple acroblast.

Plate II

Figs. 23 to 27. Spermatids showing the progressive thinning out of the mitochondrial nebenkern and the movement of the acrosomal granule round the nucleus.

Fig. 28. Late spermatid showing the formation of tail vesicles or blebs.

Fig. 29. Late spermatid showing the acrosome at the apex of the nucleus. The centriole shows sign of duality.

Fig. 30. Late spermatid showing deeply-staining acrosome and two prominent centrioles at the base of the nucleus.

Figs. 31 to 33. Very late spermatids showing progressive stages of elongation of the nucleus.

Fig. 34. A nearly mature sperm showing deeply-staining sperm nucleus and two elongated rod-like centrioles forming the so-called middle-piece.

PLATE III (Phase Contrast)

FIG. 35. A cyst containing four spermatogonia.

FIG. 36. Primary spermatocyte showing Golgi spheres and the alignment of the mitochondrial granules.

FIG. 37. Primary spermatocyte showing clumping together of the mitochondria and a few Golgi spheres revealing duplex structure.

FIGS. 38 and 39. Telophase II showing fibrillar spindle, mid-body granules and the mitochondria are seen at the apex of the spindle. A few Golgi spheres can also be seen.

FIG. 40. Late telophase II showing the bending of the fibrillar spindle.

FIG. 41. Early spermatid showing homogeneously staining mitochondrial nebenkern.

FIGS. 42 to 44. Spermatids showing progressive stages in the differentiation of the mitochondrial nebenkern.

FIGS. 45 to 48. Late spermatids showing gradual elongation of the nucleus. A few bleb-like swellings can be seen on the tail region.

FIG. 49. An immature sperm showing acrosome and two centrioles at the base of the nucleus.

PLATE IV (Microphotographs)

FIG. 50. Primary spermatocyte showing a juxta-nuclear mass of mitochondria. A single Golgi granule can also be seen.

FIG. 51. Telophase II. The chromosomes have reached the poles of the spindle and the mitochondrial threads are seen on one side.

FIG. 52. Spermatid showing a nucleus and mitochondrial nebenkern; the latter reveals a clear outer area and a dark inner core.

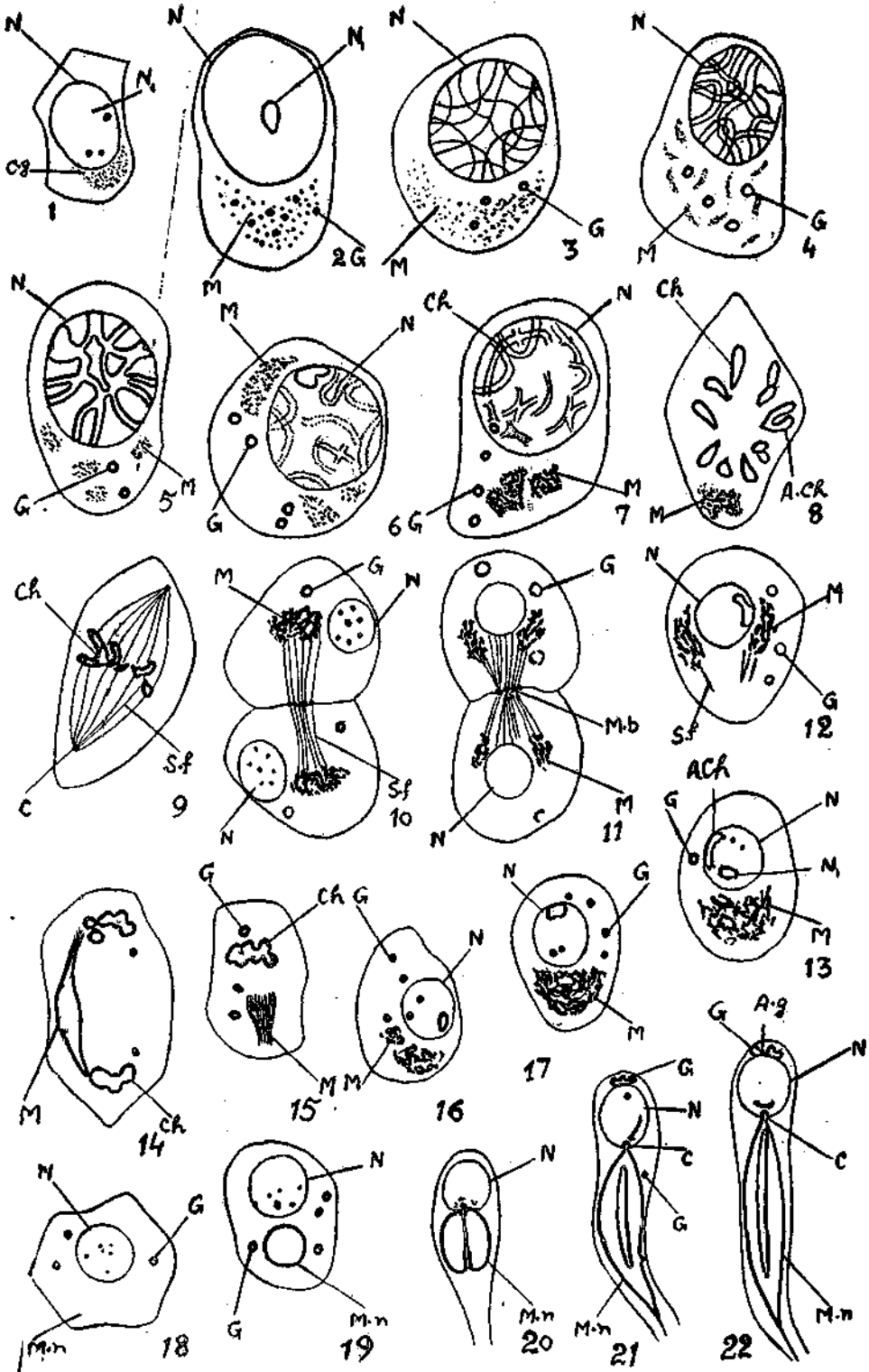
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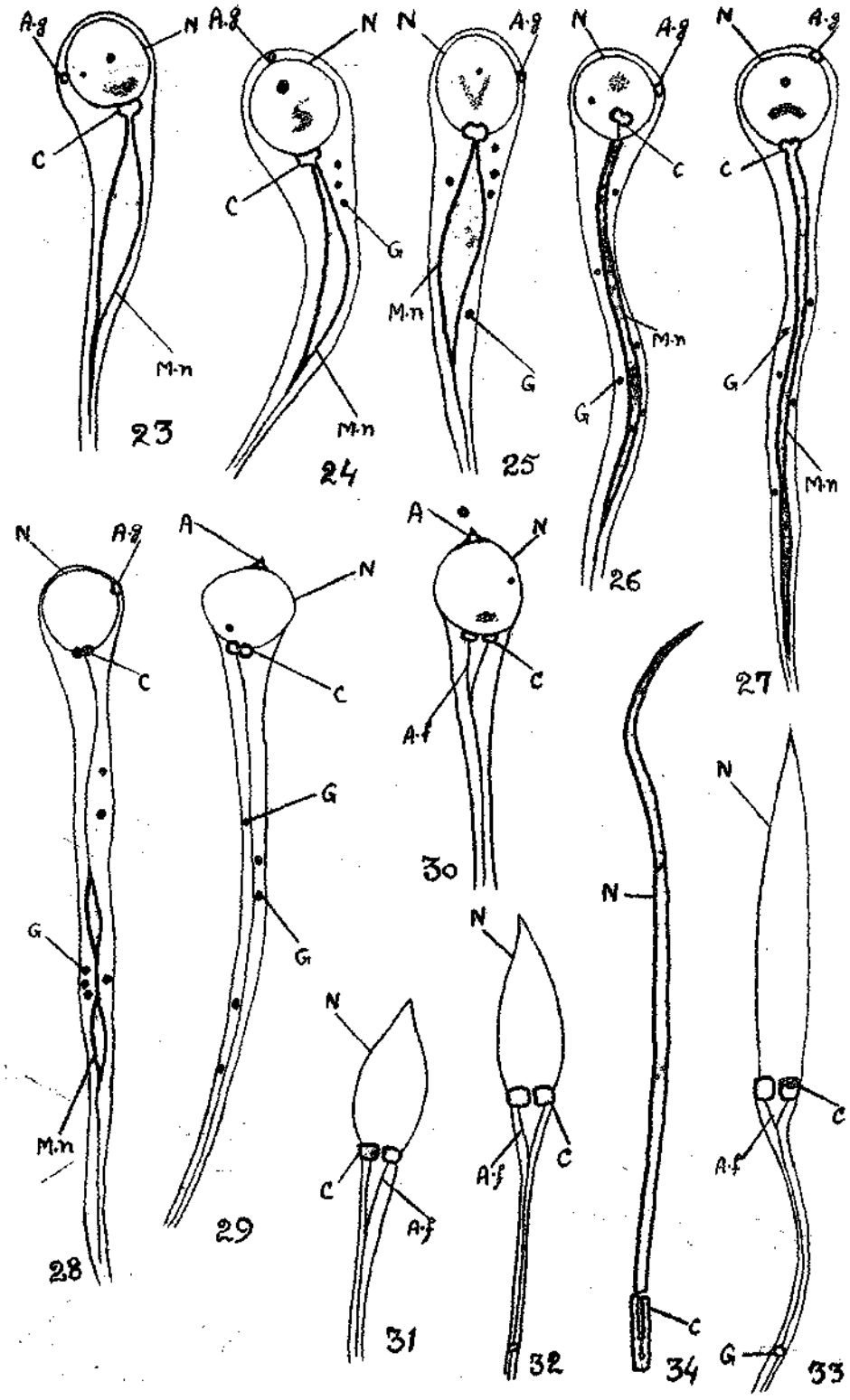
PLATE II.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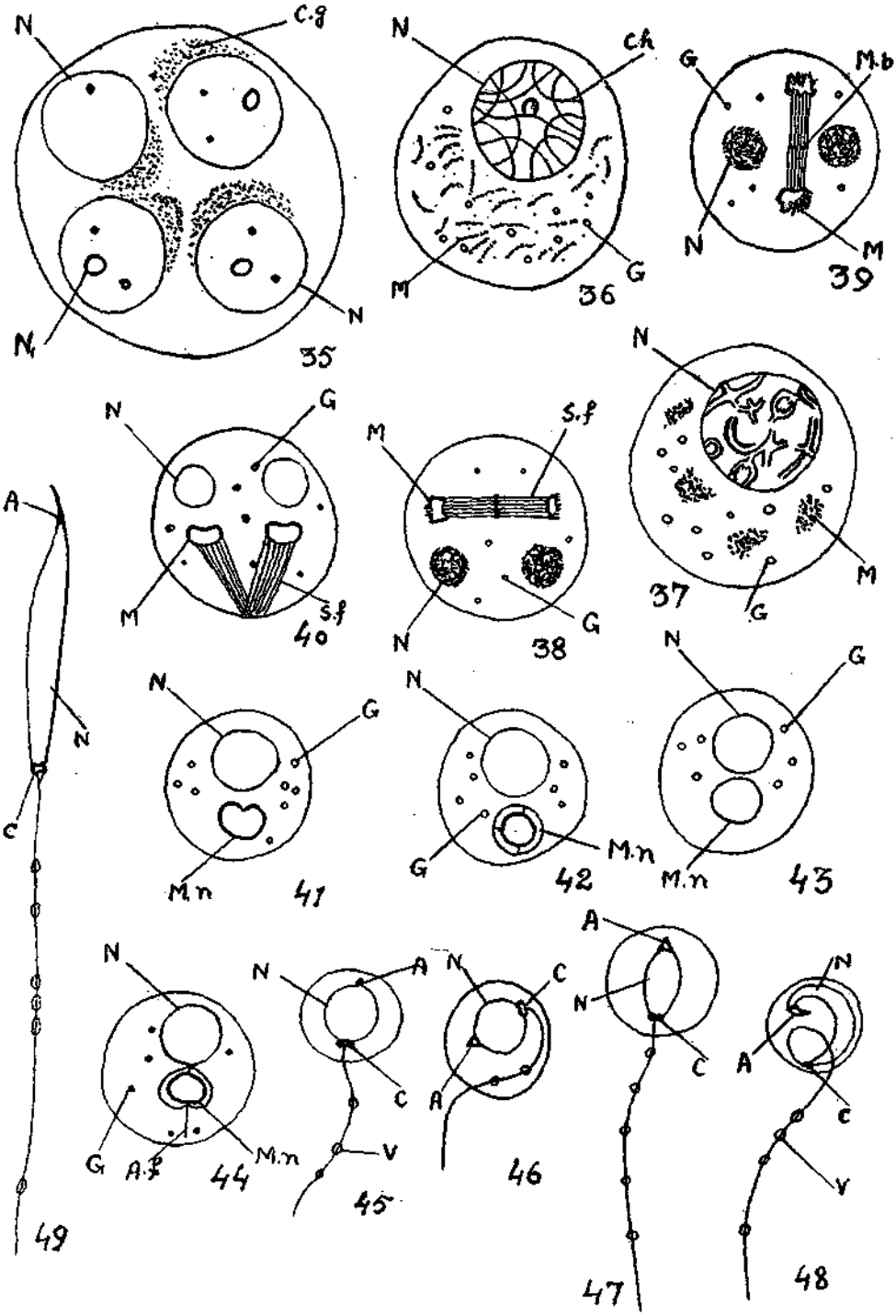
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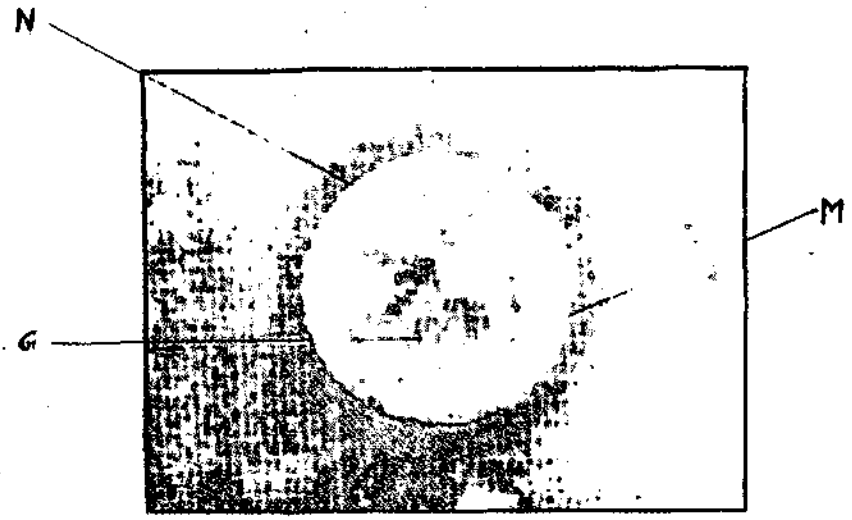

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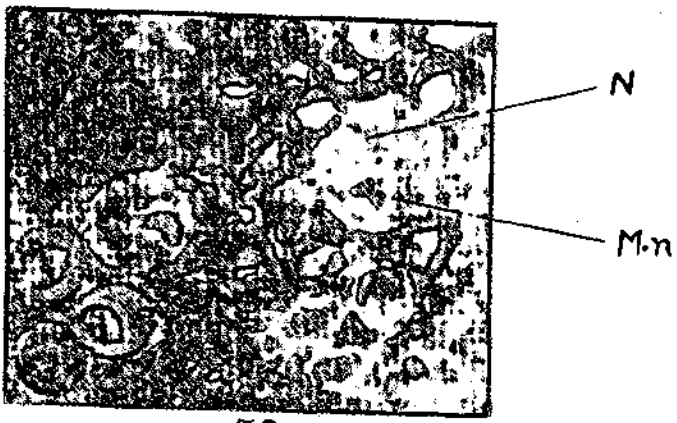

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OOGENESIS OF THE WALL-SPIDER, *CROSSOPRIZA LYONI* (BLACKWALL), AS STUDIED UNDER THE PHASE CONTRAST MICROSCOPE

by

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OOGENESIS OF THE WALL-SPIDER, *CROSSOPRIZA LYONI* (BLACKWALL), AS STUDIED UNDER THE PHASE CONTRAST MICROSCOPE

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(Department of Zoology, Panjab University, Hoshiarpur, India)

Abstract

In this paper the Golgi elements, the mitochondria and the nucleolar extrusions in the oocytes of *Crossopriza lyoni* (Blackwall) have been examined in the living condition under the phase contrast microscope and photographed. The routine laboratory technique of fixing and staining has also been employed. The Golgi element is either a granule or a vesicle with a chromophilic cortex and a chromophobic medulla. The nucleolar extrusions disappear completely long before albuminous yolk puts in its appearance. This yolk seems to arise independently in the cytoplasm. The Golgi vesicles give rise to the fatty yolk spherules.

Introduction

Nath[1] (1928) published a paper on the Oogenesis of the Spider, *Crossopriza lyoni* (Blackwall). In very young oocytes, Nath demonstrated a juxta-nuclear ring of mitochondrial granules and Golgi vacuoles. With the growth of the oocyte the juxta-nuclear mass becomes circumnuclear. Later it fragments into small masses; and ultimately the mitochondrial granules and the Golgi vacuoles are dispersed uniformly throughout the cytoplasm.

Nath studied both these cytoplasmic inclusions in fresh cover-slip preparations, either treated with 2% osmic acid from 10 minutes to half an hour, or with neutral red. Although neutral red does not stain the Golgi elements it improves their visibility, as 'they are embedded in the mitochondrial mass which appears reddish and structureless on account of the precipitation of the stain in the spaces between the mitochondrial elements, which are granular . . .'

Nath obtained very satisfactory results when fresh cover-slip preparations of oocytes treated with 2% osmic acid were studied. With this technique Nath showed that the Golgi vesicles grow into the fatty yolk bodies.

These conclusions were confirmed by the study of Mann-Kopsch preparations. Each Golgi element, unless over-impregnated, was shown to be a vesicle with a chromophilic cortex and a chromophobic medulla. The mitochondrial granules remain uniformly dispersed throughout oogenesis,

[1] *Quart. Journ. Micr. Sci.*, 72.

and do not grow to form the albuminous yolk. Nor did Nath describe any nucleolar extrusions—the albuminous yolk arising independently in the cytoplasm.

In the present paper we have fully confirmed the conclusions of Nath by our studies of the living oocytes under the phase contrast microscope, but we have discovered that in the specimens of the wall-spider (*Crossopriza lyoni* (Blackwall) collected from Hoshiarpur, which is situated at a distance of about 110 miles from Lahore, there is marked nucleolar activity in the oocyte. The nucleolus throws out nucleolar buds in the cytoplasm; but these nucleolar extrusions soon disappear, the albuminous yolk arising from the cytoplasm as claimed by Nath.

With regard to the technique we have examined living oocytes under the phase contrast microscope, and have microphotographed them. We have also examined fresh cover-slip preparations of oocytes treated for a short period with 2% osmic acid. Again we have used neutral red and Sudan IV (Kay and Whitehead). In addition sections of fixed material were cut and examined. Kolatchev's long osmication and Aoyama's silver nitrate methods were used. The material was also fixed in Champy's fluid and stained with ·5% iron-haematoxylin. Bouin's fluid, followed by ·5% iron-haematoxylin, was used as control.

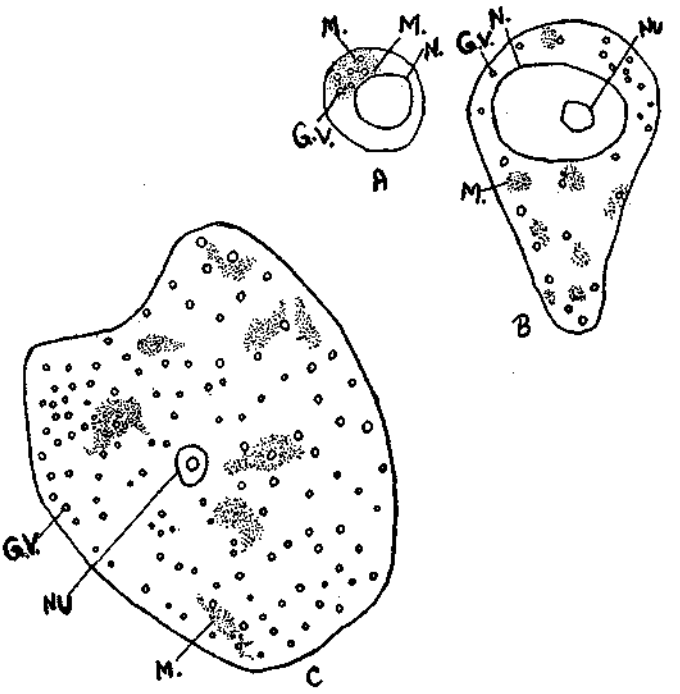
It is a pleasure for us to thank Dr. H. S. Vasisht for taking photographs under the phase contrast microscope.

Observations

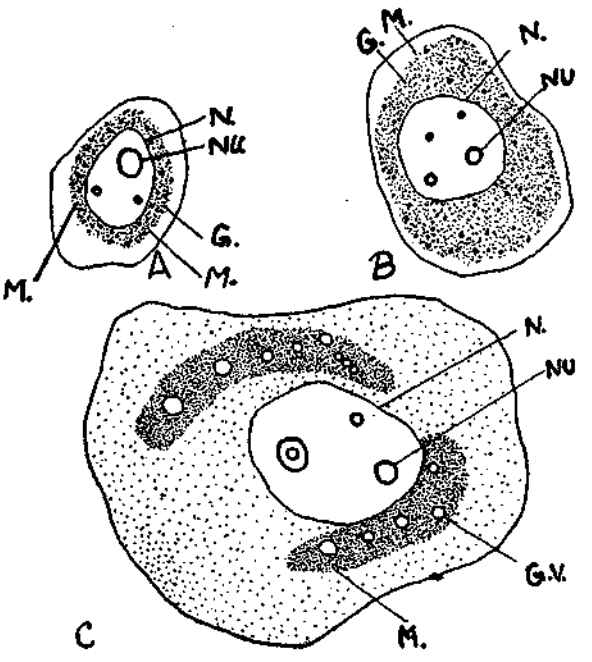
Plate I, figure 1, is a photograph of a living young oocyte, as studied under the phase contrast microscope. The nucleus is surrounded by a circumnuclear ring of mitochondria and Golgi elements. Each Golgi element is in the form of a highly refractile, dark granule, and the mitochondria are in the form of much smaller, much less refractile, and greyish granules. In plate I, figure 2, the oocyte has grown in size, and both the Golgi elements and the mitochondria can be made out in the circum-nuclear ring. Plate I, figure 3 is a microphotograph of a part of a much older oocyte. Both the Golgi granules and mitochondria are now uniformly dispersed. In plate I, figure 4, a number of nucleoli can be detected in the nucleus, and both the Golgi granules and the mitochondria can also be clearly made out. Lastly, figure 5, plate I, illustrates very satisfactorily the extrusions of nucleolar buds in the cytoplasm.

In text-figure 1 three living oocytes as studied under the phase contrast microscope have been drawn. The Golgi vesicles and the mitochondrial granules can be very clearly seen.

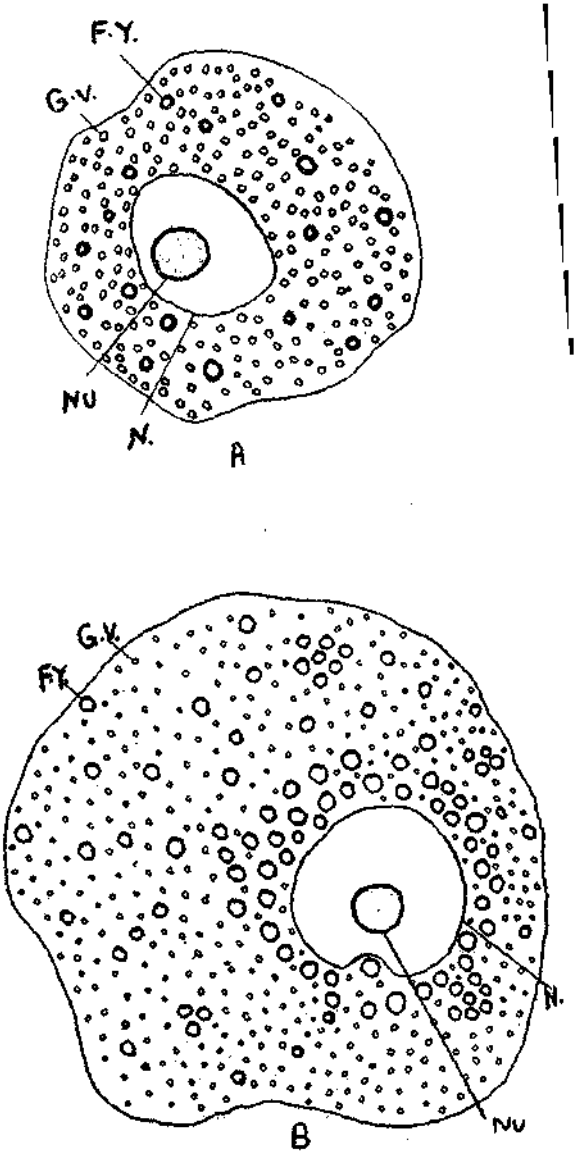
In fresh cover-slip preparations treated with 2% osmic acid the Golgi elements are slightly tinged and may appear as darkened granules (Text-figure 2, *A* and *B*), or as vesicles with osmiophilic rims and osmiophobic cores (Text-figure 2, *C*). In text-figure 3, *A* and *B*, some of the Golgi elements have grown in size and have become more fatty inasmuch as they are blackened with osmic acid much more rapidly than the original Golgi vesicles. These are the fatty yolk spherules.



TEXT-FIG. 1.

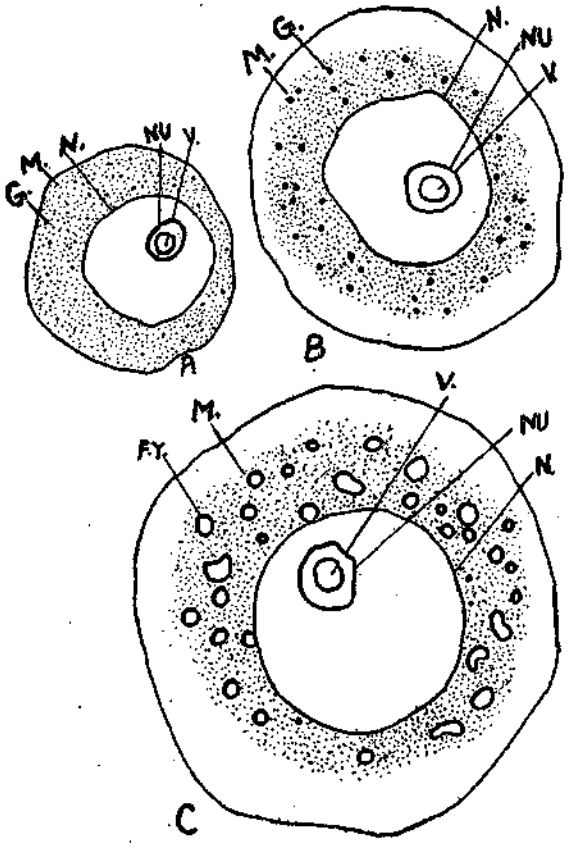


TEXT-FIG. 2.



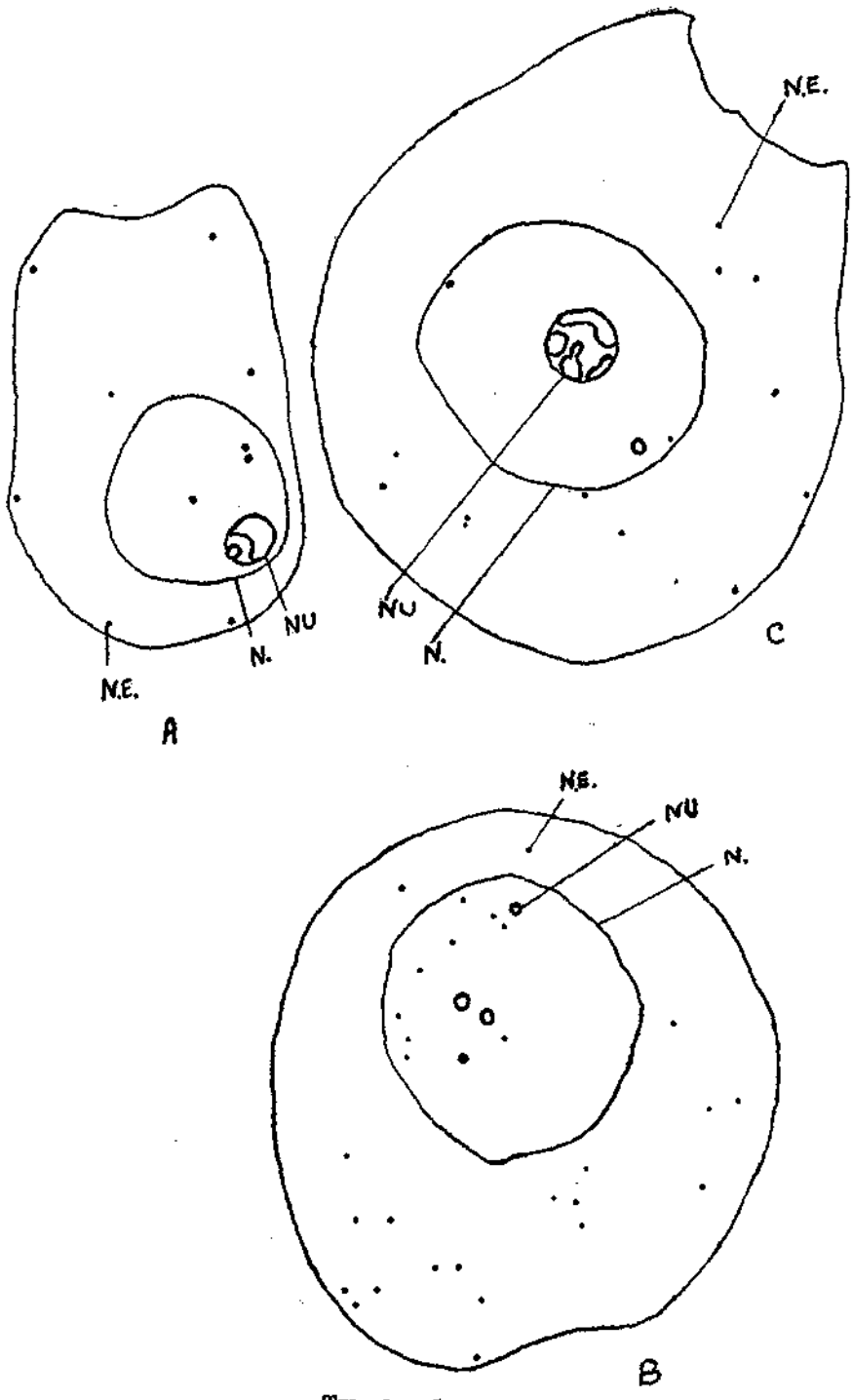
TEXT-FIG. 3.

We have also studied cover-slip preparations stained with Sudan IV (Kay and Whitehead). In text-figure 4, *A* and *B*, the Golgi elements do not stain with Sudan IV, but in text-figure 4, *C*, which represents an older oocyte, many Golgi vesicles have grown into fatty yolk spherules, which stain brilliantly with Sudan IV.



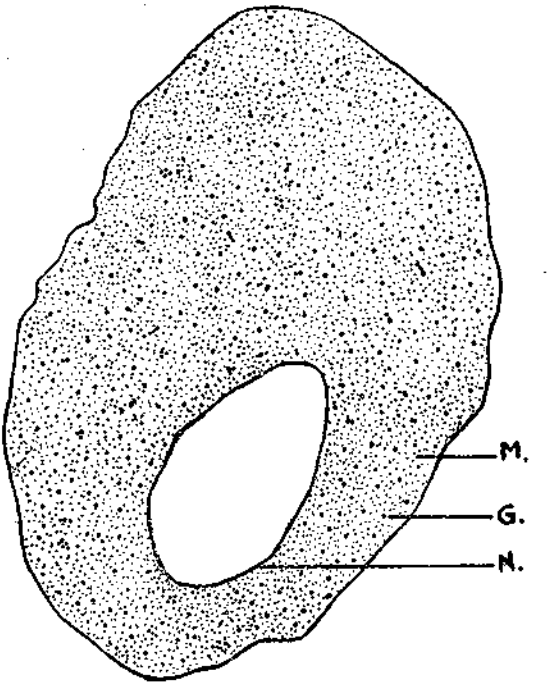
TEXT-FIG. 4.

In preparations fixed with Bouin's fluid and stained with ·5% iron-haematoxylin the Golgi elements and the mitochondria are completely washed out, but the nucleolar extrusions can be demonstrated very satisfactorily (Text-fig. 5, *A*, *B* and *C*).



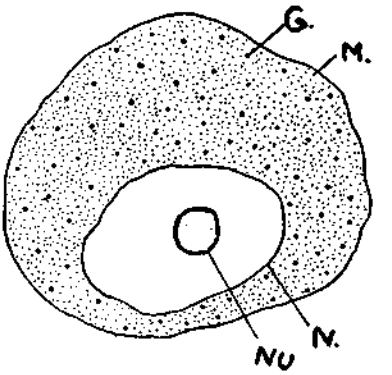
TEXT-FIG. 5.

In Kolatchev unstained preparations of material osmicated for 5 days the Golgi elements appear as jet-black granules, distributed uniformly throughout the cytoplasm. The mitochondrial granules, which are much smaller, more numerous, and brownish in appearance form an excellent background for the Golgi bodies (Text-fig. 6). In Champy preparations



Text-fig. 6.

stained with iron-haematoxylin the Golgi elements are intensely stained and the mitochondria are greyish in appearance (Text-fig. 7).



Text-fig. 7.

Lettering

F.Y.—Fatty yolk; *G.V.*—Golgi vesicle; *G.*—Golgi granule; *M.*—Mitochondria; *N.*—Nucleus; *N.E.*—Nucleolar extrusion; *NU.*—Nucleolus; *V.*—Vacuole.

Explanation of Plate I

All photographs in this plate are of living cells under phase contrast.

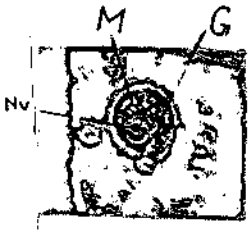
Fig 1. Microphotograph of youngest oocyte, showing circumnuclear ring of Golgi and mitochondrial elements.

Fig. 2. Microphotograph of a young oocyte, showing circum-nuclear ring.

Fig. 3. Microphotograph of a part of an older oocyte, showing a uniform distribution of Golgi and mitochondrial elements.

Fig. 4. Microphotograph of a part of an older oocyte, showing a number of nucleoli in the nucleus and also uniform distribution of Golgi elements and mitochondria in the cytoplasm.

Fig. 5. Microphotograph of an oocyte, illustrating nucleolar buds into the cytoplasm.



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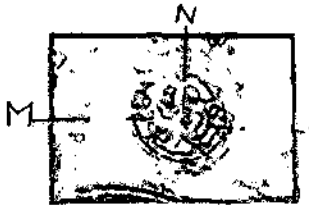

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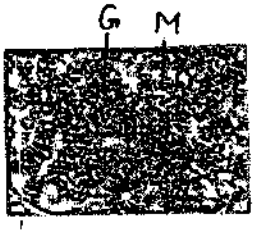

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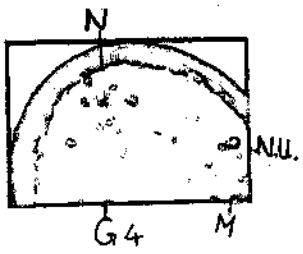

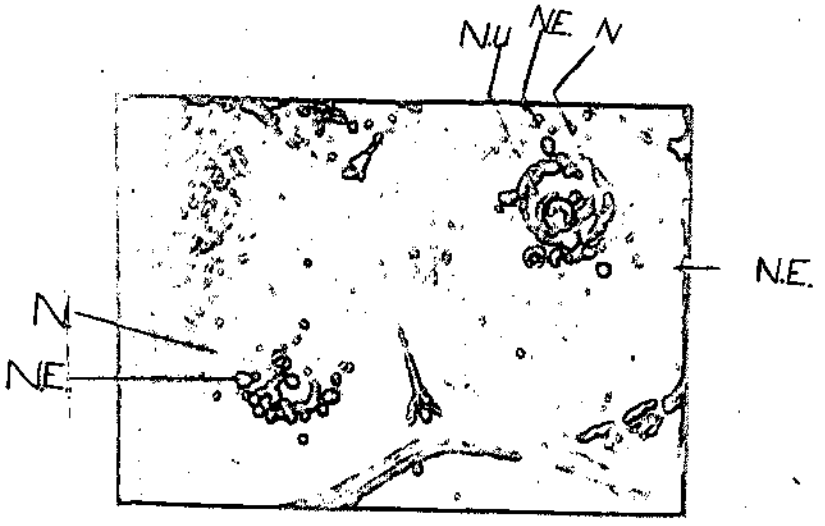

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SODIUM META-VANADATE AS VOLUMETRIC REAGENT

Part III—Diphenylamine Indicator Method

by

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SODIUM META-VANADATE AS VOLUMETRIC REAGENT

Part III—Diphenylamine Indicator Method

By Balwant Singh *and* Ranjit Singh

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Sirokomski and Klimenkov (1938) used ammonium meta-vanadate for the volumetric estimation of iron, molybdenum and copper using phenanthranilic acid as an indicator.

Gopala Rao and co-workers (1943) have shown that sodium vanadate has special advantages over potassium permanganate and dichromate as a volumetric reagent. It can be used for the estimation of ferrous salts in the presence of oxalic acid, citric acid, alcohols and phenols where potassium permanganate, potassium dichromate and even ceric sulphate give too high results. They have used sodium vanadate for the volumetric estimation of ferrocyanide alone and in the presence of hydrochloric acid and oxalic acid, hydroquinone in the presence of some phenolic compounds, uranium, tartaric acid, indigo and indigo carmine, using diphenylbenzidine as an internal indicator.

The authors (1954) have used sodium meta-vanadate as an oxidizing agent in hydrochloric acid medium for the volumetric estimations of potassium iodide, sodium arsenite, mercurous chloride, potassium sulphocyanide, sodium sulphite, sodium bisulphite, sodium thiosulphate, ferrous sulphate and hydrazine sulphate by the iodine monochloride method.

In the present investigation sodium meta-vanadate has been used as a volumetric reagent for the indirect determination of potassium permanganate, potassium dichromate, potassium bromate, potassium meta-periodate, potassium persulphate and hydrogen peroxide, using diphenylamine as an internal indicator.

Sodium meta-vanadate reacts with ferrous ammonium sulphate in presence of an acid :

$$2\ VO_3^- + 2\ Fe^{+2} + 8H^+ = 2\ VO^{+2} + 2\ Fe^{+3} + 4\ H_2O$$

In acid medium ferrous ammonium sulphate reacts with potassium permanganate, potassium dichromate, potassium bromate, potassium meta-periodate, potassium persulphate and hydrogen peroxide according to the following equations:

$$MnO_4^- + 5\ Fe^{+2} + 8\ H^+ = Mn^{+2} + 5\ Fe^{+3} + 4\ H_2O$$

$$Cr_2O_7^{-2} + 6\ Fe^{+2} + 14\ H^+ = 2\ Cr^{+3} + 6\ Fe^{+3} + 7\ H_2O$$

$$BrO_3^- + 6\ Fe^{+2} + 6\ H^+ = Br^- + 6\ Fe^{+3} + 3.H_2O$$

$$IO_4^- + 2\,Fe^{+2} + 2\,H^+ = IO_3^- + 2\,Fe^{+3} + H_2O$$
$$S_2O_8^{-2} + 2\,Fe^{+2} + 2\,H^+ = 2\,Fe^{+3} + 2\,HSO_4^-$$
$$H_2O_2 + 2\,Fe^{+2} + 2\,H^+ = 2\,Fe^{+3} + 2\,H_2O$$

Experimental

A known weight of each substance was taken in a conical flask and about 20 ml. of water, 20 ml. of sulphuric acid and a known excess of standard ferrous ammonium sulphate solution were added to it. The conical flask was stoppered and thoroughly shaken. The solution was diluted with water to bring its acid concentration to just above 1*N*. About 10 ml. of phosphoric acid and a few drops of diphenylamine were added to the solution which was titrated against *N*/10 sodium meta-vanadate to the permanent appearance of bluish-violet colour.

Several titrations were performed in each case. From the volume of sodium meta-vanadate used, corresponding to the end-point in each titration, the amount of each substance was calculated. The results are given in Tables I to VI. These results show that sodium meta-vanadate can be used as volumetric reagent.

Table I

Potassium Permanganate

$KMnO_4$ taken	*N*/10 $FeSO_4(NH_4)_2SO_4.6H_2O$ added	*N*/10 $NaVO_3$ used for excess $FeSO_4(NH_4)_2SO_4.6H_2O$	*N*/10 $FeSO_4(NH_4)_2SO_4.6H_2O$ used by $KMnO_4$	$KMnO_4$ found
gm.	ml.	ml.	ml.	gm.
0·0221	15·00	8·05	6·95	0·0219
0·0316	20·00	10·00	10·00	0·0316
0·0442	25·00	11·00	14·00	0·0442
0·0632	30·00	10·00	20·00	0·0632
0·0790	38·00	13·00	25·00	0·0790

Table II

Potassium Dichromate

$K_2Cr_2O_7$ taken	*N*/10 $FeSO_4(NH_4)_2SO_4.6H_2O$ added	*N*/10 $NaVO_3$ used for excess $FeSO_4(NH_4)_2SO_4.6H_2O$	*N*/10 $FeSO_4(NH_4)_2SO_4.6H_2O$ used by $K_2Cr_2O_7$	$K_2Cr_2O_7$ found
gm.	ml.	ml.	ml.	gm.
0·0490	20·00	10·00	10·00	0·0490
0·0686	25·00	11·00	14·00	0·0686
0·0833	30·00	13·00	17·00	0·0833
0·0980	33·00	13·00	20·00	0·0980
0·1225	40·00	15·00	25·00	0·1225

TABLE III

Potassium Bromate

$KBrO_3$ taken	$N/10$ $FeSO_4(NH_4)_2SO_4.6H_2O$ added	$N/10$ $NaVO_3$ used for excess $FeSO_4(NH_4)_2SO_4.6H_2O$	$N/10$ $FeSO_4(NH_4)_2SO_4.6H_2O$ used by $KBrO_3$	$KBrO_3$ found
gm.	ml.	ml.	ml.	gm.
0·0223	15·00	7·00	8·00	0·0223
0·0278	20·00	10·00	10·00	0·0278
0·0362	25·00	12·00	13·00	0·0362
0·0473	30·00	13·00	17·00	0·0473
0·0640	35·00	12·00	23·00	0·0640

TABLE IV

Potassium Meta-periodate

KIO_4 taken	$N/10$ $FeSO_4(NH_4)_2SO_4.6H_2O$ added	$N/10$ $NaVO_3$ used for excess $FeSO_4(NH_4)_2SO_4.6H_2O$	$N/10$ $FeSO_4(NH_4)_2SO_4.6H_2O$ used by KIO_4	KIO_4 found
gm.	ml.	ml.	ml.	gm.
0·0805	15·00	8·00	7·00	0·0805
0·1150	20·00	9·90	10·10	0·1162
0·1380	25·00	13·00	12·00	0·1380
0·2070	30·00	11·90	18·10	0·2082
0·2300	35·00	14·95	20·05	0·2306

TABLE V

Potassium Persulphate

$K_2S_2O_8$ taken	$N/10$ $FeSO_4(NH_4)_2SO_4.6H_2O$ added	$N/10$ $NaVO_3$ used for excess $FeSO_4(NH_4)_2SO_4.6H_2O$	$N/10$ $FeSO_4(NH_4)_2SO_4.6H_2O$ used by $K_2S_2O_8$	$K_2S_2O_8$ found
gm.	ml.	ml.	ml.	gm.
0·1215	17·00	8·00	9·00	0·1215
0·1755	22·00	9·00	13·00	0·1755
0·1890	25·00	10·95	14·05	0·1897
0·2430	30·00	12·00	18·00	0·2430
0·2700	36·00	16·00	20·00	0·2700

TABLE VI

Hydrogen Peroxide

H_2O_2 taken	$N/10$ $FeSO_4(NH_4)_2SO_4.6H_2O$ added	$N/10$ $NaVO_3$ used for excess $FeSO_4(NH_4)_2SO_4.6H_2O$	$N/10$ $FeSO_4(NH_4)_2SO_4.6H_2O$ used by H_2O_2	H_2O_2 found
gm.	ml.	ml.	ml.	gm.
0·0136	17·00	9·00	8·00	0·0136
0·0221	23·00	10·00	13·00	0·0221
0·0306	30·00	12·00	18·00	0·0306
0·0323	35·00	15·90	19·10	0·0324
0·0408	40·00	15·95	24·05	0·0409

SUMMARY

Sodium meta-vanadate has been used as a volumetric reagent to determine indirectly potassium permanganate, potassium dichromate, potassium bromate, potassium meta-periodate, potassium persulphate and hydrogen peroxide. An excess of ferrous ammonium sulphate added to each of the substances in 1*N* sulphuric acid medium is titrated back with a standard solution of sodium meta-vanadate in presence of phosphoric acid, using diphenylamine as an internal indicator.

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THE MORPHOLOGY OF CHROMOSOMES IN *LACCOTREPHES MACULATUS* FABR. (HEMIPTERA-HETEROPTERA)

by

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THE MORPHOLOGY OF CHROMOSOMES IN *LACCOTREPHES MACULATUS* FABR. (HEMIPTERA-HETEROPTERA)

By G. P. Sharma *and* Ram Parshad,
Department of Zoology, Panjab University, Hoshiarpur

Introduction

Dass (1951) has described the chromosome complement of *Laccotrephes maculata* as $38A+X_1X_2X_3X_4Y$ but Bawa (1953) in his 'Studies on Insect Spermatogenesis' has observed that the X-chromosome in *Laccotrephes maculatus* consists of only two elements with a single Y as the homologue and that the confused stage, so widespread in Hemiptera, is absent. However, the presence of 22 chromosomes in the polar views of both the first and the second meiotic divisions, as illustrated in Bawa's figures 9 and 14, Plate I, clearly suggests their abnormal behaviour. The present investigation has, therefore, been undertaken to analyse the chromosomes of *L. maculatus* so as to throw some light on the above contradiction. The insects were first identified in the Entomological Section of the Indian Agricultural Research Institute, New Delhi, and this identification was subsequently confirmed by Dr. G. L. Arora, M.Sc., Ph.D. (London), Reader in Entomology, Panjab University, to whom we are very thankful.

Material and Technique

The adult insects were collected from the local ponds where they creep sluggishly on the mud at the bottom. The testes from the freshly collected males were removed in normal saline and fixed in Carnoy's and Sanfelice's fluids. Sections varying from 5 to 10 μ in thickness were cut and stained in iron-hæmatoxylin and Feulgen.

Observations

Spermatogonial mitosis.—The resting spermatogonia, which occupy the extreme free ends of the testicular tubules, are small rounded cells, each with two darkly stained bodies in its nucleus (Fig. 1). One of these bodies is Feulgen-positive and represents the fused sex-chromosomes, referred to by the early workers as the chromatin nucleolus, while the other which is Feulgen-negative is the nucleolus. During the early prophase the nucleolus becomes diffused and divides into two or more elements to which the ends of the differentiating chromosomes can be seen attached (Fig. 2). In this respect it appears to behave just as it does in mosquito (Whiting, 1917), in Orthoptera (Carothers, 1913) and in another hemipteran, *Halys dentatus*, recently examined by us. It, thus, seems to contribute its substance to the differentiating chromosomes. In the late prophase the

chromosomes appear as small bodies either in the form of rods or dots without exhibiting any differentiation between the autosomes and the sex-chromosomes (Fig. 3).

During metaphase the chromosomes arrange themselves at the equator of the spindle (Fig. 4). Invariably there are present 41 elements, all of them being in the form of small rods or dots. These chromosomes of the diploid set do not, however, lie in a single plane, some of them always overlapping the others. This is simply due to their large number in a comparatively small cell. Even at this stage no differentiation between the autosomes and the sex-chromosomes can be made.

During anaphase the chromosomes move to the opposite poles and when the telophase stage is reached they become smaller in size as compared with those at the metaphase and they are now aggregated more closely (Fig. 5). The cell divides by a simple constriction to complete its mitotic cycle.

Meiosis

Meiosis I.—The pre-meiotic resting stage (Fig. 6) differs in no respect from the mitotic one and is distinguished from the latter only by its relative position in the testis. During the leptotene stage (Fig. 7) the granular autosomal threads show a distinct polarization, though not resembling exactly a bouquet, while the sex-chromosomes condense into one or two darkly-stained heteropycnotic bodies.

The synaptotene stage reveals a certain amount of contraction of the autosomes which form an undifferentiated mass lying on one side of the nucleus; the heteropycnotic sex-chromatin mass usually lies free on the opposite side (Fig. 8).

During the Zygotene stage the autosomal bivalents show polarization, forming complete loops with their respective ends pointing towards the periphery of the nucleus (Fig. 9). This polarization is maintained till the cell enters the confused stage. Just as in the other heteroptera the confused or the diffused stage is well marked with the 'resting' condition of the nucleus in which a deeply staining nucleolus can be seen lying in close association with the heteropycnotic sex-chromatin mass (Fig. 10).

The diplotene bivalents (Fig. 11) are slender and have the normal forms of rods, rings or double crosses which are spread throughout the substance of the nucleus. The sex-chromosomes, at this stage, disassociate and lie close together as dark bodies. Three such bodies can be easily made out in complete nuclei. During the diakinetic stage (Fig. 12) the bivalents undergo a good deal of contraction and arrange themselves along the periphery of the nucleus. In addition to these changes the chiasmata, practically in all the bivalents, become terminalized, with the result that we have now either rods or rings showing one and two chiasmata respectively. At this stage one can easily count, in the nucleus, 22 components out of which two or three are very small. No clear differentiation can, however, be made between the sex-chromosomes and the autosomes.

At metaphase I the chromosome elements arrange themselves at the equator of the spindle in a single plane so that all the 22 components

visible at one and the same time (Fig. 13). In the polar view the bivalents appear as dots which are, however, rod-like in some cases, being slightly elongated. There is still no distinction between the autosomes and the sex-chromosomes. Dass (1951), on the other hand, has described the five smaller elements in the centre of the metaphase plate as the sex-chromosomes. He further states that the Y-chromosome is slightly larger than the *X*'s. In our preparations, however, there is no marked difference between the individual members of the sex-complex even at metaphase II when the sex-chromosomes are well marked out from the autosomes by their differential behaviour. Further the bivalents display no definite group pattern and they are scattered haphazardly at the equator of the spindle.

During anaphase I the homologous chromosomes are simply pulled towards the opposite poles without any differential behaviour of the sex-chromosomes. The anaphase movement of the chromosomes parallel to the spindle fibres, at right angles to the equator, suggests that they have a localized terminal centromere. The same is true of some other heteroptera (Sharma and Parshad, 1955 and Schrader, 1940). By the time the chromosomes reach their respective poles each of them appears to be cleft, the cleavage appearing in the form of an indentation at the two ends of each autosome. Under high magnification, therefore, each autosome appears as a fused body (Fig. 14). The cleavage represents the plane along which the daughter chromosomes will separate and move to the opposite poles during anaphase II. In the polar view of such a stage the autosomal diads can be seen arranged in the form of a ring enclosing three univalents which are most probably the sex-chromosomes (Fig. 14).

Meiosis II.—Like the other heteroptera the second meiotic division directly follows the first, so that there is no resting stage of the secondary spermatocyte and the telophase I chromosomes are directly transferred to prometaphase II. During prometaphase II (Fig. 15) the chromosomes lie among the spindle fibres as small diads. From amongst the autosomal diads can also be distinguished, though with great difficulty because of very small size, a pseudotrivalent formed by the sex-chromosomes which become very prominent during metaphase II.

The chromosomes at metaphase II are certainly bigger than those in telophase I and prometaphase II. Of the 22 elements of the primary spermatocyte, 19 are represented by the autosomal diads, while the three univalent elements associate together so as to form a pseudotrivalent with two of its chromosomes facing one pole and the remaining one facing the opposite as their homologue (Fig. 16). These are certainly the sex-chromosomes, the two on one side being the *X*'s and the one on the other being the *Y*. Even a superficial observation of these elements leads to the conclusion that they are definitely bigger than at least the smaller autosomal diads and this is the reason why we cannot distinguish them morphologically from the autosomes during the spermatogonial mitosis and the first maturation division. In the polar view this pseudotrivalent lies in the centre of the autosomal diads which are scattered irregularly on the equatorial plate. Two kinds of metaphase plates can be distinguished with respect to the pseudotrivalent, one with two *X*'s seen at the same focus as that of the

autosomes, with Y at a different one (Fig. 17) and the other with a reverse arrangement (Fig. 18).

During anaphase II the chromosomes move to the opposite poles, the two X's going to one pole and the single Y to the opposite. At telophase when the chromosomes have reached their respective poles the cell divides by a simple constriction (Fig. 19) to give rise to two spermatids, each with a haploid set of chromosomes.

Spermatid.—In the resting nucleus of the spermatid the chromosomes are represented by small heteropycnotic dots which have a tendency to come together. In the early stages these chromosome granules are arranged in the form of a compact ring (Fig. 20) which, however, breaks up subsequently. The chromosomes now start moving to one side of the nucleus (Fig. 21) finally forming an undifferentiated contracted mass (Fig. 22).

Chiasma frequency and terminalization coefficient.—Dass (1951) in his study of *Laccotrephes maculata* has stated that the chiasma frequency per bivalent at diplotene is at least two while the mean chiasma frequeny per bivalent is 2·23, 2·0 and 2·0 during diplotene, diakinesis and metaphase respectively. We have, on the other hand, observed that there is present a variable number of rod or double-cross bivalents, at the diplotene, with a single terminal or non-terminal chiasma. It is, however, difficult to make an accurate count of the chiasmata at this stage on account of the rational coiling which still persists. The chiasma frequency per bivalent seems to vary between one and two.

The chiasma frequency and terminalization coefficient during diakinesis and metaphase are given in the table below:—

Frequency distribution of chiasmata, chiasma frequency per nucleus and the terminalization coefficient.

Stages of Meiosis	No. of Cells studied	Cells with Xta.								Total No. of Xta.	Total No. of terminal Xta.	Chiasma frequency per nucleus	Terminalization coefficient
		19	20	21	22	23	24	25	26				
Diakinesis	25	..	3	9	4	3	4	1	1	553	553	22·12 ± 1·641	1
Metaphase	25	4	9	9	3	..	..	..	..	511	511	20·44 ± 0·916	1

Discussion

The Sex-chromosomes.—A critical study of the chromosomes in *Laccotrephes maculatus* reveals that the difference in the observations of Dass (1951) and Bawa (1953) simply lies in the number of sex-chromosomes. According to Dass the chromosome formula for the male is $38A + X_1X_2X_3X_4Y$, while, according to Bawa, it is $38A + X_1X_2Y$ (Bawa did not, however, observe the spermatogonial metaphase and this diploid number has been constructed by us from the number mentioned by him in metaphase I).

Though no distinction between the autosomes and the sex-chromosomes can be made during the spermatogonial metaphase and the first maturation division, metaphase II clearly reveals that the sex-chromosome formula for *L. maculatus* is certainly X_1X_2Y, when the three sex-elements form a pseudotrivalent with the two X's facing one pole of the spindle and a single Y the opposite one as the homologue. Further in the polar view the pseudotrivalent is very clear, lying in the centre of the equatorial plate in such a way that either the two X's are visible at the focus of the autosomes or a single Y. However, by a little change of focus we can very clearly make out all the 22 elements of the primary spermatocyte.

The presence of 41 chromosomes during the spermatogonial metaphase and of 22 at metaphase I leads to the conclusion that the chromosome formula in this insect is $38A+X_1X_2Y$, since the sex-chromosomes divide equationally during the first meiotic division as in the other heteropterans so far known. If we accept the formula $38A+X_1X_2X_3X_4Y$ as given by Dass (1951), the primary spermatocyte should reveal 24 elements at the metaphase. This number has not, however, been observed even in a single cell, the number 22 being constant.

If Dass is correct in his study it will be evident that the change in the number of sex-chromosomes, though of great importance in the chromosome morphology, is insignificant from the evolutionary point of view, since it simply concerns the breaking up or otherwise of the sex-chromosomes into four elements without any disturbance in the genotype of the organism. However, a simple break in the chromosome resulting into two independently functional chromosomes cannot be assumed unless we accept the polycentric theory which is in itself not so convincing. Further, since the evolutionary transformation simply concerns the number of sex-chromosomes, without any change in the autosomal number, we cannot regard the higher number of sex-chromosomes to be evolved directly from the autosomes. This very clearly shows that the evolution of the sex-chromosomes in the aquatic heteroptera involves a very complex phenomenon which is not known so far.

Summary

1. The diploid number of chromosomes in the male *Laccotrephes maculatus* is $38A+X_1X_2Y$.

2. The meiosis follows the normal course, differing in no respect from the other heteropterans. The synaptotene stage reveals synizesis. A well-marked confused stage is present. A secondary spermatocyte with the resting condition of the nucleus is absent.

3. The sex-chromosomes are indistinguishable from the autosomes during the spermatogonial mitosis and the first maturation division. They, however, divide equationally during meiosis I and reductionally during the second when they form a pseudotrivalent which occupies the centre of the equatorial plate surrounded by the autosomes.

4. Chiasma frequency per bivalent during diplotene, diakinesis and metaphase varies between one and two.

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Explanation of Lettering in the Plate

Ch.n—Heteropycnotic sex-chromatin mass (chromatin nucleolus); *N*—Nucleolus; n_1, n_2 —Nucleolar fragments; X_1, X_2 —X-chromosomes; *Y*—Y-chromosome.

Explanation of Figures

All figures have been drawn from the sectioned material with a camera lucida at the table level with 15*X* eye-piece and spencer oil immersion objective, giving an approximate magnification of 2,550 times.

The cell outline has been drawn only in figures 4, 5 and 12 to 19. All the remaining figures depict only the nuclei. In figures 2, 3, 11, 15 and 16 only a part of the chromosome complement has been shown so as to avoid overcrowding.

Fig. 1. Resting spermatogonium.
Fig. 2. Spermatogonial prophase, early.
Fig. 3. Spermatogonial prophase, late.
Fig. 4. Spermatogonial metaphase (polar view).
Fig. 5. Spermatogonial telophase (side view).
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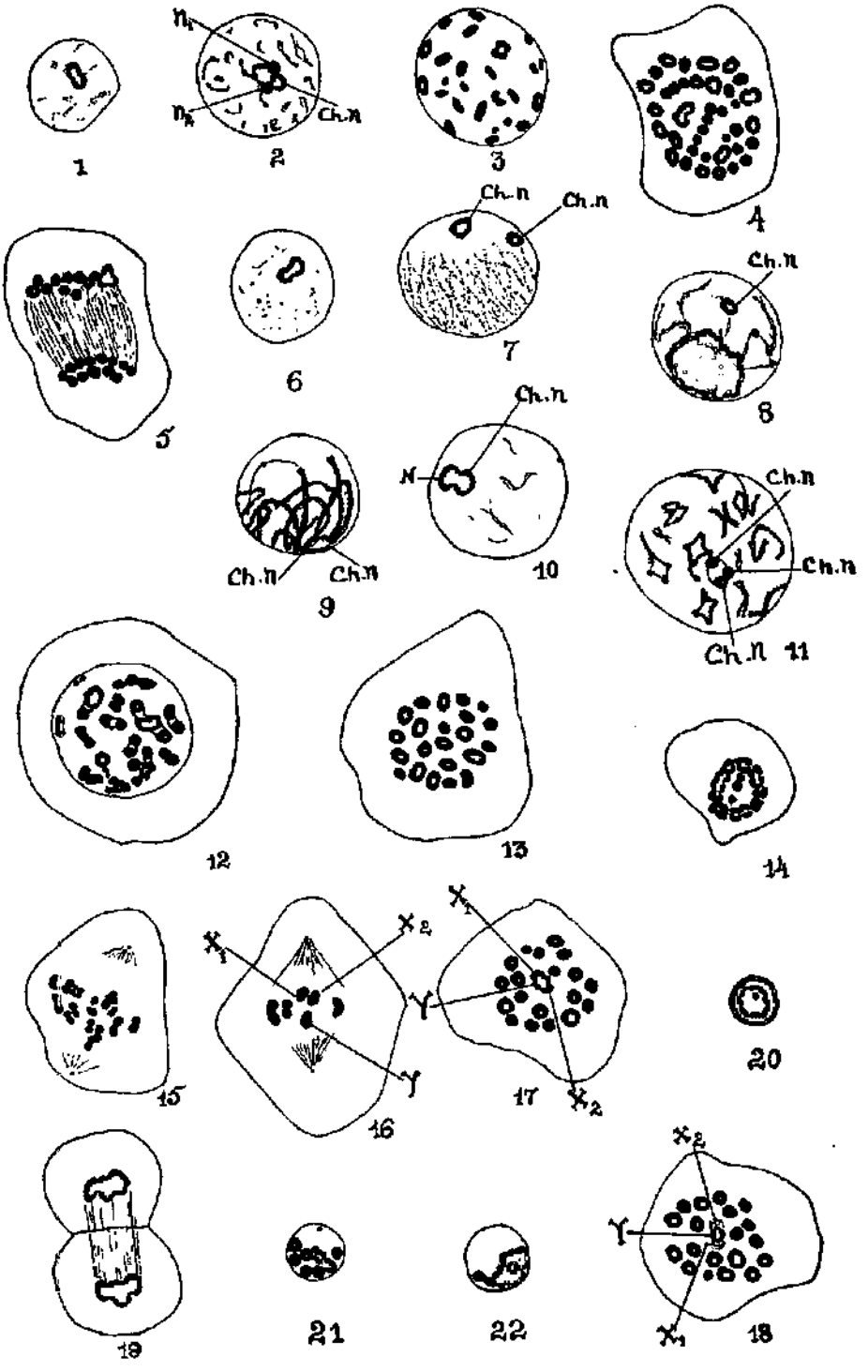

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by

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POTENTIOMETRIC DETERMINATIONS OF ORGANIC COMPOUNDS BY IODINE MONOBROMIDE IN NON-AQUEOUS SOLVENT

By Balwant Singh *and* Mohan Singh

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O. Tomicek and J. Dolezal (1949) and O. Tomicek and Heyrovsky (1950) have potentiometrically titrated organic compounds with bromine solution in glacial acetic acid, using platinum as an indicator electrode coupled with a normal calomel electrode.

O. Tomicek, A. Blazek and Z. Roubal (1950) have titrated some unsaturated organic compounds with bromine solution in glacial acetic acid by a potentiometric method using chloranil reference electrode.

Militzer (1938) found that in carbon disulphide and acetic acid, iodine monobromide functions only as a brominating agent. Even in nitrobenzene in which iodine monobromide forms conducting solutions (Burner and Galecki, 1913), its reaction with phenol and salicylic acid is still one of bromination.

According to F. W. Bennett and A. G. Sharpe (1950) in a solution of a phenol and iodine monobromide in nitrobenzene, free bromine and iodine cations compete for the phenol. Since the bromination of a phenol by bromine is a very fast reaction whereas iodination by the iodine cations is relatively slow (Lambourne and Robertson, 1947), the resultant reaction is iodine—catalyzed bromination and iodine may be recovered at the end of the reaction.

In the present investigation iodine monobromide has been used for the potentiometric estimation of phenol, hydroquinone, resorcinol, pyrogallol, phloroglucinol, thymol, α-naphthol, β-naphthol, *o*-cresol, *p*-cresol, *o*-nitrophenol, *p*-nitrophenol, aniline, *o*-toluidine, *m*-toluidine, *p*-toluidine, α-naphthylamine, acetanilide, salicylic acid, anthranilic acid, *p*-amino benzoic acid and naphthalene in acetic acid medium.

These substances react with iodine monobromide according to the following equations:—

1. *Phenol.*
   $C_6H_5OH + 3\ IBr = C_6H_2Br_3OH + 3\ HI$
2. *Hydroquinone.*
   $C_6H_4(OH)_2 + 3\ IBr = C_6HBr_3(OH)_2 + 3\ HI$
3. *Resorcinol.*
   $C_6H_4(OH)_2 + 3\ IBr = C_6HBr_3(OH)_2 + 3\ HI$
4. *Pyrogallol.*
   $C_6H_3(OH)_3 + 3\ IBr = C_6Br_3(OH)_3 + 3\ HI$

5. *Phloroglucinol.*
$C_6H_3(OH)_3+3\ IBr = C_6Br_3(OH)_3+3\ HI$

6. *Thymol.*
$C_{10}H_{13}OH+2\ IBr = C_{10}H_{11}Br_2OH+2\ HI$

7. *α-Naphthol.*
$C_{10}H_7OH+2\ IBr = C_{10}H_5Br_2OH+2\ HI$

8. *β-Naphthol.*
$C_{10}H_7OH+3\ IBr = C_{10}H_4Br_3OH+3\ HI$

9. *o-Cresol.*
$CH_3C_6H_4OH+3\ IBr = CH_3C_6HBr_3OH+3\ HI$

10. *p-Cresol.*
$CH_3C_6H_4OH+3\ IBr = CH_3C_6HBr_3OH+3\ HI$

11. *o-Nitrophenol.*
$C_6H_4(NO_2)OH+2\ IBr = C_6H_2Br_2(NO_2)OH+2\ HI$

12. *p-Nitrophenol.*
$C_6H_4(NO_2)OH+2\ IBr = C_6H_2Br_2(NO_2)OH+2\ HI$

13. *Aniline.*
$C_6H_5NH_2+3\ IBr = C_6H_2Br_3NH_2+3\ HI$

14. *o-Toluidine.*
$CH_3C_6H_4NH_2+2\ IBr = CH_3C_6H_2Br_2NH_2+2HI$

15. *m-Toluidine.*
$CH_3C_6H_4NH_2+3\ IBr = CH_3C_6HBr_3NH_2+3\ HI$

16. *p-Toluidine.*
$CH_3C_6H_4NH_2+2\ IBr = CH_3C_6H_2Br_2NH_2+2\ HI$

17. *α-Naphthylamine.*
$C_{10}H_7NH_2+IBr = C_{10}H_6BrNH_2+HI$

18. *Acetanilide.*
$C_6H_5NHCOCH_3+IBr = C_6H_4(Br)NHCOCH_3+HI$

19. *Salicylic Acid.*
$C_6H_4(OH)COOH+3\ IBr = C_6H_2Br_3OH+CO_2+3\ HI$

20. *Anthranilic Acid.*
$C_6H_4(NH_2)COOH+3\ IBr = C_6H_2Br_3NH_2+CO_2+3\ HI$

21. *p-Amino Benzoic Acid.*
$C_6H_4(NH_2)COOH+3\ IBr = C_6H_2Br_3NH_2+CO_2+3\ HI$

22. *Naphthalene.*
$C_{10}H_8+IBr = C_{10}H_7Br+HI$

Experimental

Standardization of iodine monobromide solution.—On adding excess of potassium iodide to a measured volume of iodine bromide solution, iodine was liberated.

$$IBr+KI = KBr+I_2$$

The iodine was titrated against a standard solution of sodium thiosulphate

$$I_2 + 2\ Na_2S_2O_3 = Na_2S_4O_6 + 2\ NaI$$

The standard iodine monobromide solution was kept in dark.

Potentiometric titrations.—A known weight of each substance was dissolved in acetic acid and titrated against the standard IBr solution.

The reaction mixture was kept stirred by means of a mechanical stirrer and the progress of the reaction was studied potentiometrically. A bright platinum foil was used as an oxidation-reduction electrode and this was coupled with a saturated calomel electrode through an agar-agar potassium chloride bridge to form a cell. The cell was placed in a water bath, the temperature of which was kept constant.

In all these titrations a solution of sodium acetate in glacial acetic acid was used as a buffer. Sodium acetate removed the hydroiodic acid formed during the reaction.

A series of potentiometric titrations were performed with different amounts of each substance. One titration for each substance, as typical of that set, is recorded in the following tables:—

TABLE I

Titration of 0·0470 gm. of phenol mixed with 15 c.c. of acetic acid, against 0·1000 M IBr. *(Temp. 30°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
1·00	0·7000				180
		13	15·05	0·7110	
3·00	0·7262				162
		9	15·10	0·7191	
5·00	0·7448				11
		5	15·20	0·7202	
7·00	0·7538				10
		4	15·50	0·7233	
9·00	0·7618				7
		3	16·00	0·7267	
11·00	0·7679				6
		3	17·00	0·7327	
13·00	0·7628				6
		6	19·00	0·7450	
14·00	0·7569				4
		10	21·00	0·7535	
14·50	0·7521				5
		26	24·00	0·7670	
14·80	0·7441				3
		50	27·00	0·7766	
14·90	0·7391				1
		60	30·00	0·7800	
14·95	0·7361				
		322 (Maximum)			
15·00	0·7200				

TABLE II

Titration of 0·0550 gm. of hydroquinone mixed with 15 c.c. of acetic acid, against 0·1005 M IBr. *(Temp. 55°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
1·00	0·5806				380 (Maximum)
		12			
3·00	0·6062		14·95	0·5882	
		11			94
5·00	0·6280		15·00	0·5835	
		3			13
7·00	0·6330		15·20	0·5810	
		2			9
9·00	0·6372		15·50	0·5784	
		2			8
11·00	0·6412		16·00	0·5825	
		2			7
13·00	0·6380		17·00	0·5899	
		4			8
14·00	0·6345		19·00	0·6059	
		19			7
14·50	0·6250		21·00	0·6203	
		20			4
14·80	0·6190		24·00	0·6333	
		80			4
14·85	0·6150		27·00	0·6451	
		156			3
14·90	0·6072		30·00	0·6529	

TABLE III

Titration of 0·0367 gm. of resorcinol mixed with 10 c.c. of acetic acid, against 0·1000 M IBr. *(Temp. 30°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
0·00	0·5191				112 (Maximum)
		18	10·00	0·5030	
1·00	0·5007				80
		10	10·05	0·4990	
3·00	0·5210				40
		5	10·10	0·5010	
5·00	0·5316				33
		2	10·20	0·5043	
7·00	0·5356				22
		3	10·50	0·5110	
8·00	0·5324				17
		7	11·00	0·5195	
8·50	0·5289				12
		10	12·00	0·5310	
9·00	0·5240				9
		14	13·00	0·5400	
9·50	0·5170				8
		16	15·00	0·5562	
9·80	0·5122				7
		22	17·00	0·5691	
9·90	0·5100				6
		28	19·00	0·5800	
9·95	0·5086				3
			20·00	0·5831	

TABLE IV

Titration of 0·0630 gm. of pyrogallol mixed with 15 c.c. of acetic acid, against 0·1108 M IBr. *(Temp. 30°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
1·00	0·5188				88
		20	13·60	0·5624	
3·00	0·5590				76
		9	13·70	0·5700	
5·00	0·5776				36
		8	14·00	0·5810	
7·00	0·5932				28
		6	14·50	0·5950	
9·00	0·6053				17
		4	15·00	0·6034	
11·00	0·5975				9
		7	16·00	0·6128	
13·00	0·5841				6
		16	18·00	0·6253	
13·30	0·5792				3
		26	20·00	0·6319	
13·40	0·5766				4
		52	23·00	0·6452	
13·45	0·5740				4
		74	26·00	0·6558	
13·50	0·5703				2
		246 (Maximum)	30·00	0·6638	
13·55	0·5580				

TABLE V

Titration of 0·1134 gm. of phloroglucinol mixed with 27 c.c. of acetic acid, against 0·1108 M IBr. *(Temp. 60°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
1·00	0·4162				24
		10	24·50	0·4965	
3·00	0·4363				18
		9	24·70	0·5000	
6·00	0·4630				13
		3	25·00	0·5038	
10·00	0·4749				7
		3	26·00	0·5111	
14·00	0·4877				9
		3	28·00	0·5289	
18·00	0·4994				5
		1	31·00	0·5448	
22·00	0·5038				4
		3	34·00	0·5582	
24·00	0·5100				4
		10	37·00	0·5682	
24·30	0·5070				2
		84	40·00	0·5730	
24·35	0·5028				2
		176 (Maximum)	43·00	0·5788	
					2
24·40	0·4939		45·00	0·5824	
		28			
24·45	0·4953				

TABLE VI

Titration of 0·1998 gm. of thymol mixed with 27 c.c. of acetic acid, against 0·1000 M IBr. *(Temp. 30°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
0·00	0·5700				56
		20	26·95	0·5673	
1·00	0·5900				176 (Maximum)
		5	27·00	0·5761	
3·00	0·5994				124
		3	27·05	0·5823	
6·00	0·6092				114
		2	27·10	0·5880	
10·00	0·6143				70
		2	27·20	0·5952	
14·00	0·6229				24
		3	27·50	0·6022	
18·00	0·6122				13
		3	28·00	0·6089	
22·00	0·6000				9
		4	31·00	0·6353	
26·00	0·5832				5
		7	34·00	0·6492	
26·50	0·5797				4
		23	37·00	0·6611	
26·80	0·5728				3
		27	41·00	0·6732	
26·90	0·5701				2
			45·00	0·6819	

TABLE VII

Titration of 0·1944 gm. of α-naphthol mixed with 27 c.c. of acetic acid, against 0·0990 M IBr. *(Temp. 40°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
1·00	0·5200				308 (Maximum)
		8	27·30	0·5032	
3·00	0·5362				116
		5	27·35	0·4974	
6·00	0·5498				48
		3	27·40	0·4950	
10·00	0·5620				42
		3	27·50	0·4992	
14·00	0·5742				19
		1	28·00	0·5085	
18·00	0·5795				9
		2	29·00	0·5170	
22·00	0·5730				8
		3	31·00	0·5334	
25·00	0·5632				7
		11	34·00	0·5550	
26·00	0·5522				6
		14	37·00	0·5738	
27·10	0·5372				5
		60	41·00	0·5920	
27·20	0·5312				5
		252	45·00	0·6110	
27·25	0·5186				

TABLE VIII

Titration of 0·1584 gm. of β-naphthol mixed with 33 c.c. of acetic acid, against 0·1000 M IBr. *(Temp. 30°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
1·00	0·5610				58
		6	32·95	0·5760	
3·00	0·5732				136 (Maximum)
		2	33·00	0·5692	
6·00	0·5799				96
		2	33·05	0·5644	
10·00	0·5880				40
		2	33·10	0·5624	
15·00	0·5972				32
		1	33·20	0·5656	
20·00	0·6029				15
		1	33·50	0·5700	
25·00	0·6075				8
		2	34·00	0·5740	
30·00	0·6000				5
		3	37·00	0·5899	
32·00	0·5947				4
		14	40·00	0·6025	
32·50	0·5876				4
		19	43·00	0·6139	
32·80	0·5819				3
		30	46·00	0·6214	
32·90	0·5789				2
			50·00	0·6286	

TABLE IX

Titration of 0·0792 gm. of o-cresol mixed with 22 c.c. of acetic acid, against 0·0970 M IBr. *(Temp. 40°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
1·00	0·6400				52
		16	22·65	0·6754	
3·00	0·6712				238 (Maximum)
		9	22·70	0·6610	
6·00	0·6992				80
		4	22·75	0·6570	
9·00	0·7112				30
		3	22·80	0·6585	
12·00	0·7200				10
		2	23·00	0·6605	
15·00	0·7255				7
		2	23·50	0·6640	
18·00	0·7182				6
		3	24·00	0·6668	
21·00	0·7100				6
		10	26·00	0·6780	
22·00	0·7000				5
		33	29·00	0·6932	
22·30	0·6900				5
		37	32·00	0·7077	
22·50	0·6826				5
		46	36·00	0·7216	
22·60	0·6780				3
			40·00	0·7311	

TABLE X

Titration of 0·0792 gm. of p-cresol mixed with 22 c.c. of acetic acid, against 0·0970 M IBr. *(Temp. 40°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
1·00	0·6663				270 (Maximum)
		8			
3·00	0·6849		22·70	0·6642	
		6			70
6·00	0·7042		22·75	0·6607	
		6			54
9·00	0·7230		22·80	0·6580	
		4			14
12·00	0·7358		23·00	0·6608	
		2			14
15·00	0·7427		23·20	0·6635	
		8			14
18·00	0·7191		23·50	0·6676	
		6			12
21·00	0·7000		24·00	0·6735	
		7			9
22·00	0·6930		26·00	0·6916	
		17			7
22·30	0·6878		29·00	0·7128	
		21			7
22·50	0·6836		32·00	0·7328	
		36			3
22·60	0·6800		36·00	0·7459	
		46			3
22·65	0·6777		40·00	0·7560	

TABLE XI

Titration of 0·1529 gm. of o-nitrophenol mixed with 22 c.c. of acetic acid, against 0·0970 M I Br. *(Temp. 60°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
1·00	0·6892				50
		30	22·65	0·8324	
3·00	0·7492				328 (Maximum)
		17	22·70	0·8160	
6·00	0·8000				90
		8	22·75	0·8115	
9·00	0·8226				50
		4	22·80	0·8090	
12·00	0·8352				50
		4	23·00	0·8190	
15·00	0·8470				26
		3	23·50	0·8320	
18·00	0·8560				18
		2	24·00	0·8410	
21·00	0·8510				3
		5	26·00	0·8470	
22·00	0·8460				3
		13	29·00	0·8552	
22·30	0·8421				3
		18	32·00	0·8650	
22·50	0·8386				1
		37	36·00	0·8700	
22·60	0·8349				1
			40·00	0·8752	

TABLE XII

Titration of 0·1529 gm. of p-nitrophenol mixed with 22 c.c. of acetic acid, against 0·1005 M IBr. *(Temp. 60°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
1·00	0·7200				124
		57	21·95	0·8226	
3·00	0·8332				52
		11	22·00	0·8200	
6·00	0·8675				8
		3	22·20	0·8216	
9·00	0·8770				7
		2	22·50	0·8237	
12·00	0·8715				6
		2	23·00	0·8266	
15·00	0·8666				4
		1	24·00	0·8301	
18·00	0·8630				3
		1	26·00	0·8365	
21·00	0·8595				2
		6	29·00	0·8424	
21·50	0·8565				2
		25	32·00	0·8488	
21·80	0·8488				2
		60	36·00	0·8548	
21·85	0·8458				2
		340 (Maximum)	40·00	0·2616	
21·90	0·8288				

TABLE XIII

Titration of 0·0682 gm. of aniline mixed with 22 c.c. of acetic acid, against 0·0990 M IBr. *(Temp. 40°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
1·00	0·4140				36
		27	22·15	0·6302	
3·00	0·4672				60
		11	22·20	0·6272	
6·00	0·5012				380 (Maximum)
		11	22·25	0·6082	
9·00	0·5332				44
		10	22·30	0·6104	
12·00	0·5620				31
		7	22·50	0·6166	
15·00	0·5835				12
		7	23·00	0·6226	
18·00	0·6036				11
		12	24·00	0·6339	
21·00	0·6380				11
		13	26·00	0·6549	
21·50	0·6445				9
		16	29·00	0·6828	
21·80	0·6398				9
		24	32·00	0·7102	
22·00	0·6350				7
		30	36·00	0·7380	
22·10	0·6320				5
			40·00	0·7489	

TABLE XIV

Titration of 0·1444 gm. of o-toluidine mixed with 27 c.c. of acetic acid, against 0·0990 M IBr. *(Temp. 40°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
1·00	0·4816				70
		13	27·25	0·5616	
3·00	0·5080				152 (Maximum)
		4	27·30	0·5540	
6·00	0·5190				60
		2	27·35	0·5510	
10·00	0·5283				42
		2	27·40	0·5489	
14·00	0·5370				38
		4	27·50	0·5451	
18·00	0·5520				25
		5	27·70	0·5501	
22·00	0·5701				18
		3	28·00	0·5555	
26·00	0·5817				11
		11	31·00	0·5871	
26·50	0·5761				5
		13	34·00	0·6014	
26·80	0·5736				3
		17	37·00	0·6099	
27·00	0·5703				4
		26	41·00	0·6248	
27·20	0·5651				4
			45·00	0·6400	

TABLE XV

Titration of 0·0535 gm. of m-toluidine mixed with 15 c.c. of acetic acid, against 0·0990 M IBr. *(Temp. 40°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
0·00	0·3978				50
		52	15·05	0·5621	
1·00	0·4500				58
		19	15·10	0·5592	
3·00	0·4888				312 (Maximum)
		12	15·15	0·5436	
5·00	0·5128				192
		10	15·20	0·5340	
7·00	0·5334				31
		8	15·50	0·5434	
9·00	0·5498				22
		7	16·00	0·5544	
11·00	0·5642				14
		8	17·00	0·5685	
13·00	0·5800				14
		8	19·00	0·5965	
14·00	0·5880				12
		16	21·00	0·6205	
14·50	0·5802				11
		·28	24·00	0·6539	
14·80	0·5718				11
		36	27·00	0·6860	
15·00	0·5646				10
			30·00	0·7153	

TABLE XVI

Titration of 0·1444 gm. of p-toluidine mixed with 27 c.c. of acetic acid, against 0·1000 M IBr. *(Temp. 30°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
1·00	0·5703				124 (Maximum)
		9			
3·00	0·5883		27·00	0·6241	
		3			82
6·00	0·5985		27·05	0·6200	
		3			24
10·00	0·6100		27·10	0·6188	
		3			21
14·00	0·6211		27·20	0·6167	
		3			10
18·00	0·6320		27·50	0·6198	
		3			7
22·00	0·6453		28·00	0·6234	
		1			7
26·00	0·6500		29·00	0·6305	
		10			8
26·50	0·6448		31·00	0·6460	
		26			8
26·80	0·6370		34·00	0·6700	
		44			7
26·90	0·6326		37·00	0·6910	
		43			6
26·95	0·6303		41·00	0·7160	
					3
			45·00	0·7289	

TABLE XVII

Titration of 0·3861 gm. of α-naphthylamine mixed with 27 c.c. of acetic acid, against 0·1000 M IBr. *(Temp. 30°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
0·00	0·3151				46
		18	26·95	0·4180	
1·00	0·3333				158 (Maximum)
		8	27·00	0·4101	
3·00	0·3497				98
		5	27·05	0·4150	
6·00	0·3650				58
		4	27·10	0·4179	
10·00	0·3815				44
		4	27·20	0·4223	
14·00	0·3954				22
		3	27·50	0·4290	
18·00	0·4082				8
		3	28·00	0·4332	
22·00	0·4197				5
		7	31·00	0·4470	
26·00	0·4472				5
		24	34·00	0·4631	
26·50	0·4350				5
		36	37·00	0·4790	
26·80	0·4241				5
		38	41·00	0·5000	
26·90	0·4203				5
			45·00	0·5210	

TABLE XVIII

Titration of 0·4455 gm. of acetanilide mixed with 33 c.c. of acetic acid, against 0·0970 M IBr. *(Temp. 60°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
1·00	0·7380				362 (Maximum)
		6			
5·00	0·7629		34·05	0·7153	
		6			36
10·00	0·7913		34·10	0·7135	
		3			34
15·00	0·8064		34·20	0·7169	
		2			26
21·00	0·8178		34·50	0·7248	
		5			13
27·00	0·7873		35·00	0·7315	
		6			8
33·00	0·7515		36·00	0·7392	
		15			10
33·50	0·7442		38·00	0·7589	
		17			6
38·80	0·7391		41·00	0·7777	
		21			2
33·90	0·7370		44·00	0·7849	
		38			2
33·95	0·7351		47·00	0·7911	
		44			2
34·00	0·7329		50·00	0·7960	

TABLE XIX

Titration of 0·1012 gm. of salicylic acid mixed with 22 c.c. of acetic acid, against 0·1005 M IBr. *(Temp. 60°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
1·00	0·7598				162
		18	21·95	0·7952	
3·00	0·7948				96
		10	22·00	0·7904	
6·00	0·8240				25
		4	22·20	0·7854	
9·00	0·8368				20
		2	22·50	0·7794	
12·00	0·8430				15
		1	23·00	0·7870	
15·00	0·8470				8
		2	24·00	0·7952	
18·00	0·8530				8
		3	26·00	0·8110	
21·00	0·8350				8
		15	29·00	0·8350	
21·50	0·8273				4
		19	32·00	0·8461	
21·80	0·8215				2
		52	36·00	0·8532	
21·85	0·8189				2
		312 (Maximum)	40·00	0·8600	
21·90	0·8033				

TABLE XX

Titration of 0·1233 gm. of anthranilic acid mixed with 27 c.c. of acetic acid, against 0·1005 M IBr. *(Temp. 60°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
1·00	0·5590				304 (Maximum)
		9			
3·00	0·5771		26·90	0·5716	
		4			32
6·00	0·5880		26·95	0·5732	
		2			28
10·00	0·5973		27·00	0·5746	
		1			19
14·00	0·6026		27·20	0·5783	
		1			17
18·00	0·6064		27·50	0·5834	
		1			8
22·00	0·6116		28·00	0·5873	
		2			6
25·00	0·6081		29·00	0·5935	
		2			6
26·00	0·6040		31·00	0·6054	
		8			6
26·50	0·6000		34·00	0·6234	
		25			6
26·80	0·5924		37·00	0·6410	
		112			5
26·85	0·5868		41·00	0·6598	
					6
			45·00	0·6832	

TABLE XXI

Titration of 0·1005 gm. of p-amino benzoic acid mixed with 22 c.c. of acetic acid, against 0·1108 M IBr. *(Temp. 60°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
1·00	0·5592				314 (Maximum)
		12			
3·00	0·5332		19·85	0·6258	
		7			100
6·00	0·6032		19·90	0·6208	
		6			28
9·00	0·6200		20·00	0·6236	
		9			12
12·00	0·6460		20·20	0·6260	
		7			13
15·00	0·6671		20·50	0·6300	
		2			10
18·00	0·6600		21·00	0·6351	
		5			8
18·00	0·6555		24·00	0·6589	
		11			6
19·50	0·6500		27·00	0·6777	
		19			2
19·70	0·6462		30·00	0·6849	
		44			2
19·75	0·6440		33·00	0·6919	
		50			1
19·80	0·6415		36·00	0·6960	
					1
			40·00	0·6998	

TABLE XXII

Titration of 0·2816 gm. of naphthalene mixed with 22 c.c. of acetic acid, against 0·1108 M IBr. *(Temp. 50°C).*

IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)	IBr. (c.c.)	E.M.F. (volts)	*E/C* (m. volts/c.c.)
1·00	0·7195				32
		35	19·95	0·8285	
3·00	0·7893				30
		8	20·00	0·8270	
6·00	0·8128				15
		8	20·30	0·8314	
9·00	0·8359				28
		4	20·50	0·8370	
12·00	0·8489				12
		2	21·00	0·8429	
15·00	0·8546				5
		1	22·00	0·8480	
18·00	0·8509				4
		3	24·00	0·8565	
19·00	0·8480				2
		6	27·00	0·8629	
19·50	0·8452				1
		17	30·00	0·8666	
19·80	0·8401				1
		36	33·00	0·8701	
19·85	0·8383				1
		164 (Maximum)	36·00	0·8740	
					1
19·90	0·8301				
			40·00	0·8788	

DISCUSSION

It is evident from the foregoing tables that there was a sharp break in E.M.F. at the equivalence point in each potentiometric titration. From the amount of iodine monobromide required in each titration, corresponding to the equivalence point, the amount of the substance was calculated. In the following tables, the values obtained are compared with the amounts of the substances taken for the titrations.

TABLE I

Phenol.			*Hydroquinone.*		
Phenol taken. (gm.)	IBr. used. (gm.)	Phenol found. (gm.)	Hydro-quinone taken. (gm.)	IBr. used. (gm.)	Hydro-quinone found. (gm.)
0·0219	0·14552	0·0218	0·0257	0·14478	0·0257
0·0313	0·20653	0·0312	0·0367	0·20656	0·0366
0·0470	0·31000	0·0469	0·0550	0·31013	0·0549
0·0589	0·45486	0·0589	0·0807	0·45508	0·0807
0·0845	0·55816	0·0845	0·0990	0·55845	0·0990
0·1034	0·68234	0·1033	0·1210	0·68234	0·1209

TABLE II

Resorcinol.			*Pyrogallol.*		
Resorcinol taken. (gm.)	IBr. used. (gm.)	Resorcinol found. (gm.)	Pyrogallol taken. (gm.)	IBr. used. (gm.)	Pyrogallol found. (gm.)
0·0257	0·1450	0·0257	0·0420	0·20649	0·0419
0·0367	0·2066	0·0366	0·0630	0·31000	0·0629
0·0550	0·3105	0·0550	0·0924	0·45538	0·0925
0·0807	0·4547	0·0806	0·1134	0·55879	0·1135
0·0990	0·5588	0·0990	0·1386	0·68272	0·1386
0·1210	0·6823	0·1209	0·1680	0·82664	0·1679

TABLE III

Phloroglucinol.			*Thymol.*		
Phloroglucinol taken. (gm.)	IBr. used. (gm.)	Phloroglucinol found. (gm.)	Thymol taken. (gm.)	IBr. used. (gm.)	Thymol found. (gm.)
0·0420	0·20667	0·0419	0·0518	0·14447	0·0517
0·0630	0·31006	0·0629	0·0740	0·20653	0·0739
0·0924	0·45526	0·0924	0·1110	0·31000	0·1109
0·1134	0·55835	0·1134	0·1628	0·45537	0·1629
0·1386	0·68272	0·1386	0·1998	0·55814	0·1997
0·1680	0·82692	0·1629	0·2442	0·68222	0·2441

TABLE IV

α-Naphthol.			*β-Naphthol.*		
α-Naphthol taken. (gm.)	IBr. used. (gm.)	α-Naphthol found. (gm.)	β-Naphthol taken. (gm.)	IBr. used. (gm.)	β-Naphthol found. (gm.)
0·0504	0·14463	0·0503	0·0336	0·1450	0·0336
0·0720	0·20653	0·0719	0·0480	0·2064	0·0479
0·1080	0·31000	0·1079	0·0720	0·3098	0·0719
0·1584	0·45498	0·1584	0·1056	0·4554	0·1057
0·1944	0·55833	0·1944	0·1296	0·5581	0·1295
0·2376	0·68256	0·2376	0·1584	0·6822	0·1583

TABLE V

o-Cresol.			*p-Cresol.*		
o-Cresol taken. (gm.)	IBr. used. (gm.)	*o*-Cresol found. (gm.)	*p*-Cresol taken. (gm.)	IBr. used. (gm.)	*p*-Cresol found. (gm.)
0·0252	0·14477	0·0251	0·0252	0·14490	0·0252
0·0360	0·20677	0·0359	0·0360	0·20685	0·0360
0·0540	0·31020	0·0540	0·0540	0·31018	0·0539
0·0792	0·45504	0·0792	0·0792	0·45504	0·0792
0·0972	0·55843	0·0972	0·0972	0·55846	0·0972
0·1188	0·68266	0·1188	0·1188	0·68262	0·1188

TABLE VI

o-Nitrophenol.			*p-Nitrophenol.*		
o-Nitro-phenol taken. (gm.)	IBr. used. (gm.)	*o*-Nitro-phenol found. (gm.)	*p*-Nitro-phenol taken. (gm.)	IBr. used. (gm.)	*p*-Nitro-phenol found. (gm.)
0·0486	0·14491	0·0486	0·0486	0·14472	0·0486
0·0695	0·20690	0·0695	0·0695	0·20659	0·0694
0·1042	0·31026	0·1042	0·1042	0·31021	0·1042
0·1529	0·45508	0·1529	0·1529	0·45491	0·1528
0·1876	0·55828	0·1875	0·1876	0·55847	0·1876
0·2293	0·68258	0·2293	0·2293	0·68239	0·2292

TABLE VII

Aniline.			*o-Toluidine.*		
Aniline taken. (gm.)	IBr. used. (gm.)	Aniline found. (gm.)	*o*-Toluidine taken. (gm.)	IBr. used. (gm.)	*o*-Toluidine found. (gm.)
0·0310	0·20653	0·3094	0·0374	0·14475	0·0374
0·0465	0·31000	0·0464	0·0535	0·20650	0·0534
0·0682	0·45500	0·0682	0·0802	0·31000	0·0801
0·0837	0·55827	0·0837	0·1177	0·45500	0·1177
0·1023	0·68259	0·1023	0·1444	0·55850	0·1444
0·1240	0·82607	0·1238	0·1765	0·68256	0·1765

TABLE VIII

m-Toluidine.			*p-Toluidine.*		
m-Toluidine taken. (gm.)	IBr. used. (gm.)	*m*-Toluidine found. (gm.)	*p*-Toluidine taken. (gm.)	IBr. used. (gm.)	*p*-Toluidine found. (gm.)
0·0249	0·14477	0·0249	0·0374	0·14510	0·0375
0·0356	0·20653	0·0356	0·0535	0·20710	0·0535
0·0536	0·31000	0·0534	0·0802	0·31000	0·0801
0·0784	0·45500	0·0784	0·1177	0·45480	0·1176
0·0963	0·55840	0·0962	0·1444	0·55810	0·1443
0·1177	0·68230	0·1176	0·1765	0·68224	0·1764

TABLE IX

α-Naphthylamine			*Acetanilide.*		
α-Naphthyl-amine taken. (gm.)	IBr. used. (gm.)	α-Naphthyl-amine found. (gm.)	Acetanilide taken. (gm.)	IBr. used. (gm.)	Acetanilide found. (gm.)
0·1001	0·14450	0·0999	0·0945	0·14465	0·0944
0·1430	0·20651	0·1428	0·1350	0·20688	0·1350
0·2145	0·30993	0·2143	0·2025	0·31016	0·2024
0·3146	0·45472	0·3145	0·2970	0·45508	0·2970
0·3861	0·55814	0·3859	0·3645	0·55819	0·3644
0·4719	0·68283	0·4720	0·4455	0·68260	0·4455

TABLE X

Salicylic Acid			*Anthranilic Acid.*		
Salicylic Acid taken. (gm.)	IBr. used. (gm.)	Salicylic Acid found. (gm.)	Anthranilic Acid taken. (gm.)	IBr. used. (gm.)	Anthranilic Acid found. (gm.)
0·0322	0·14486	0·0322	0·0319	0·14500	0·0320
0·0460	0·20646	0·0459	0·0456	0·20642	0·0455
0·0690	0·31023	0·0690	0·0685	0·31021	0·0684
0·1012	0·45600	0·1012	0·1005	0·45491	0·1004
0·1242	0·55845	0·1242	0·1233	0·55835	0·1233
0·1518	0·68260	0·1518	0·1507	0·68245	0·1506

TABLE XI

p-Aminobenzoic Acid.			*Naphthalene.*		
p-Amino-benzoic Acid taken. (gm.)	IBr. used. (gm.)	*p*-Amino-benzoic Acid found. (gm.)	Naphthalene taken. (gm.)	IBr. used. (gm.)	Naphthalene found. (gm.)
0·0685	0·30983	0·0684	0·1280	0·20681	0·1279
0·1005	0·45455	0·1004	0·1920	0·30990	0·1918
0·1233	0·55830	0·1233	0·2816	0·45545	0·2818
0·1507	0·68272	0·1507	0·3456	0·55855	0·3456
0·1827	0·82700	0·1826	0·4224	0·68261	0·4224

SUMMARY

Phenol, hydroquinone, resorcinol, pyrogallol, phloroglucinol, thymol, α-naphthol, β-naphthol, *o*-cresol, *p*-cresol, *o*-nitrophenol, *p*-nitrophenol, aniline, *o*-toluidine, *m*-toluidine, *p*-toluidine, α-naphthylamine, acetanilide, salicylic acid, anthranilic acid, *p*-aminobenzoic acid and naphthalene have been determined quantitatively by the potentiometric method, using iodine monobromide as a brominating agent in glacial acetic acid containing sodium acetate.

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ON THE ORIGIN OF THE GOLGI DICTYOSOMES FROM THE MITOCHONDRIA AND ACROSOME FORMATION IN THE SPERMATOGENESIS OF THE SLUG *ANADENUS ALTIVAGUS* (THEOBALD)

by

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ON THE ORIGIN OF THE GOLGI DICTYOSOMES FROM THE MITOCHONDRIA AND ACROSOME FORMATION IN THE SPERMATOGENESIS OF THE SLUG *ANADENUS ALTIVAGUS* (THEOBALD)

By Vishwa Nath *and* Harish Chander Chopra (*Department of Zoology, Panjab University, Hoshiarpur*)

Abstract

In this paper the spermatogenesis of *Anadenus altivagus* has been worked out in detail. The material was fixed in Flemming-without-acetic acid and stained with 0·5% iron-haematoxylin. Living material has also been studied under the phase contrast microscope. Some of the most important conclusions of this investigation are (1) the origin of Golgi dictyosomes from the mitochondrial granules by alignment (cf. Hirsch, 1939), (2) the secretion of a large number of minute acrosomal granules in the chromophobic vesicles of the Golgi dictyosomes, (3) the subsequent deposition of these granules in front of the spermatid nucleus, (4) their fusion to form the acrosome, (5) the subsequent sloughing off of the Golgi apparatus, and (6) the formation of a comparatively long mitochondrial middle-piece in the sperm.

Introduction

One of us (H. C. Chopra) collected specimens of the slug *Anadenus altivagus* (Theobald) from Simla (Punjab, India) during the summer (July to September) of 1953. Some specimens were again collected in 1954 summer for the study of the living cells of the ovo-testis of the slug under the phase contrast. We were successful in microphotographing the living maturing spermatids studied under the phase contrast; and we are publishing in this paper three such photographs (Pl. II, figs. 29–31). As the slugs were killed in a couple of days in the excessive heat of Hoshiarpur, we were not successful in microphotographing the earlier stages of spermatogenesis. We hope to publish the photographs of these earlier stages at some future date.

We have worked exclusively with Flemming-without-acetic acid followed by 0·5% iron-haematoxylin and have obtained excellent preparations with this technique. All preparations were made by one of us (H.C.C.), and examined by both of us. Both of us again examined the living material.

Elsewhere one of us (Nath, 1955) has laid stress on the importance of using F.W.A. *exclusively* in spermatogenesis and avoiding silver nitrate and long osmication techniques, which admittedly produce serious artefacts.

Flemming-without-acetic acid followed by 0·5% iron-haematoxylin, on the contrary, introduces no artefacts whatsoever, and brings up in the

finished slides all the cytoplasmic inclusions and the nuclear contents in an admirable fashion. For this reason we are publishing here photographs of all stages of sperm-forming cells fixed in F.W.A. for comparison with camera lucida drawings. The latter appear in the form of text-figures.

The ovo-testis of *Anadenus altivagus* has unexpectedly turned out to be an excellent material to demonstrate (1) the origin of the Golgi dictyosomes from the mitochondrial granules by alignment; (2) the complete disappearance of the Golgi dictyosomes during meiosis I and II; (3) their reappearance during the interkinetic stage and late telophases II; and (4) the origin of the acrosome from numerous granules, which appear in the chromophobic vesicles of the dictyosomes.

Previous Work

Gatenby (1917), in his paper on the ovo-testis of *Helix aspersa*, reviews the earlier publications of Ancel (1902), Demoll (1913) and Fauré-Frémiet (1909) on the subject. Gatenby also reviews the papers of Schitz (1916) on the pectinibranchiate *Collumbella* and of Terni (1914) on *Geotriton*.

A little later Gatenby (1918) published another paper on the ovo-testis of two species of *Limax*, two of *Arion*, three of *Helix* and one of *Testacella*.

Still later (1919) Gatenby published a paper on the gametogenesis and early development of *Limnaea stagnalis*.

Hickman (1931) published a comprehensive paper on the spermatogenesis of the pulmonate *Succinea ovalis* with special reference to the components of the sperm.

Baker (1945 and 1949) discusses the morphology and histochemistry of the Golgi dictyosomes of the snail, *Helix aspersa*.

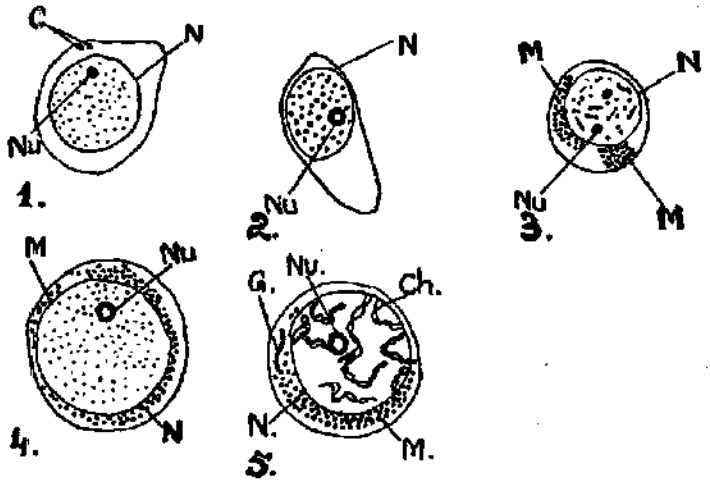
Finally, Watts (1952) examined the spermatogenesis in the slug, *Arion subfuscus*. This paper is of particular value, as the author examined living material under the phase contrast microscope.

Observations

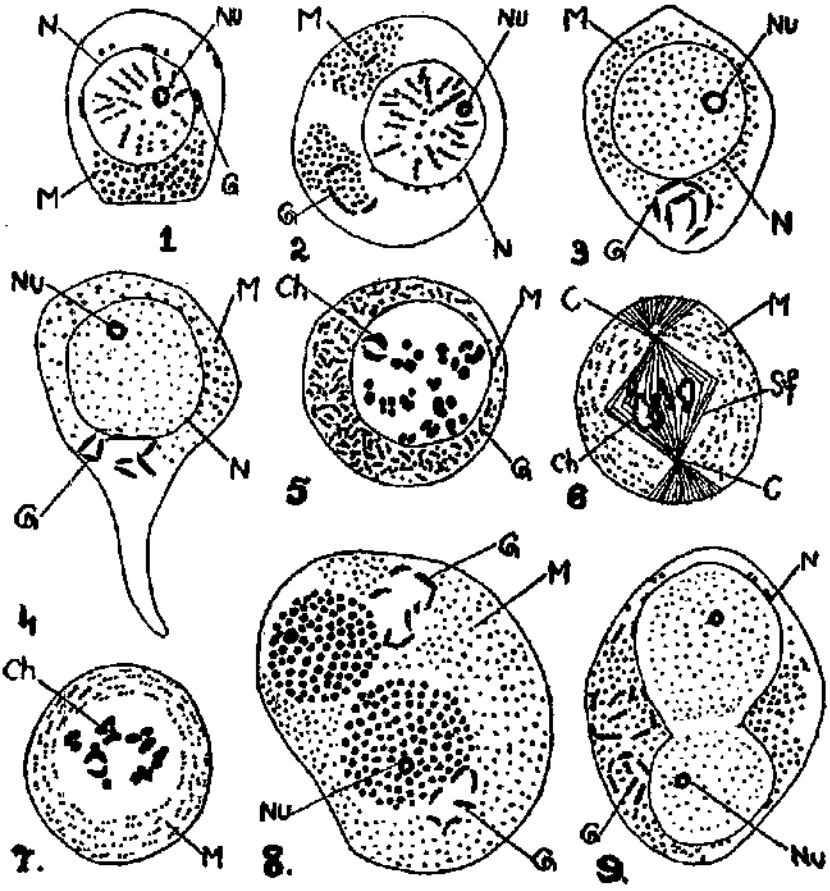
Spermatogonia.—Text-figs. *A*, 1 and 2 represent spermatogonia of a very young slug. In text-fig. *A*, 1 two centrioles can be easily seen, but there are no traces of either mitochondria or Golgi elements at this stage. The microphotographs (Pl. I, figs. 1 and 2) of these cells also show no traces of the mitochondria or the Golgi elements.

Text-figs. *A*, 3, 4 and 5 represent spermatogonia from a mature slug. It will be noticed that the mitochondria have now appeared in the cell in the form of prominent granules, which stain deeply with haematoxylin. Pl. I, figs. 3, 4 and 5 represent the corresponding microphotographs of these cells. In text-fig. *A*, 5—some mitochondrial granules have aligned themselves to form a Golgi rod, but this rod has not come up well in the microphotograph (Pl. I, fig. 5).

Primary Spermatocytes.—But the process of alignment of the mitochondrial granules to form the Golgi dictyosomes really begins in early prophases of the primary spermatocytes (Text-figs. *B*, 1 and 2). In these



TEXT-FIG. *A*. 1-2—earliest spermatogonia; 3–5—later spermatogonia. Further explanation in the text.



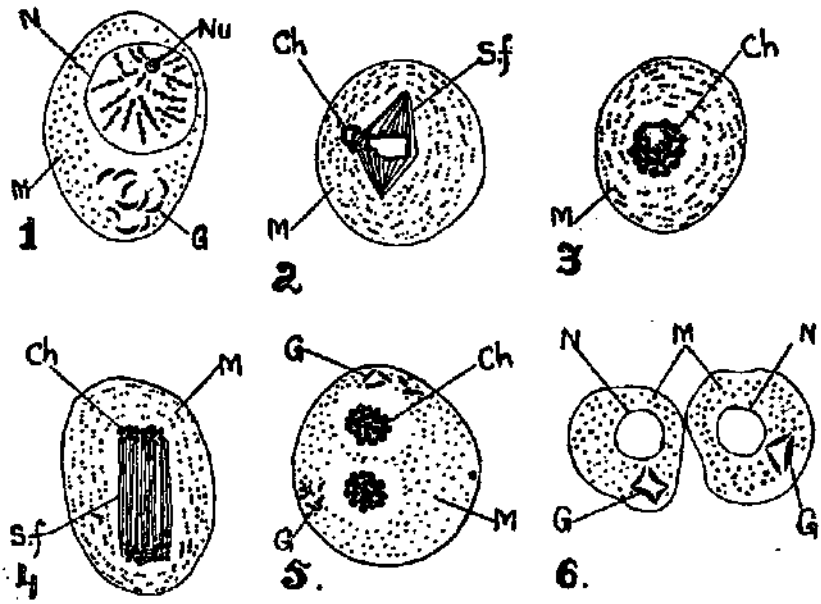
TEXT-FIG. *B*. 1–4—early primary spermatocytes; 5—diakinesis; 6 and 7—metaphase I; 8 and 9—late telophases I. Further explanation in the text.

figs. and in the corresponding microphotographs (Pl. I, figs. 6 and 7) it will be clearly seen that the dictyosomes are being formed by the alignment of granules. Soon, however, the Golgi dictyosomes lose their crenated appearance and their contours become smooth (Text-figs. *B*, 3 and 4; Pl. I, figs. 8 and 9). At the same time the dictyosomes tend to collect at one spot in the neighbourhood of the nucleus. This localized condition is well marked in Text-fig. *B*, 3 and fig. 8, Pl. I. Each dictyosome is thick in the middle and tapers at each end.

During diakinesis the dictyosomes begin to break up into granules (Text-fig. *B*, 5; Pl. I, fig. 10); and during the following metaphase I, all the dictyosomes have merged into the mitochondrial granules (Text-figs. *B*, 6 and 7; Pl. I, figs. 11 and 12). The mitochondrial granules are now arranged in chains, which run parallel to the longitudinal axis of the spindle.

But during late telophase I, the dictyosomes are once more formed by the alignment of mitochondrial granules (Text-figs. *B*, 8 and 9; Pl. I, figs. 13 and 14). In all these figs. the dictyosomes have a crenated appearance, but they again assume uniform contours during the interkinetic stage (Text-fig. *C*, 1; Pl. I, fig. 15).

Secondary Spermatocytes.—During meiosis II, the Golgi dictyosomes once more break up into granules, which merge into the mitochondrial granules (Text-figs. *C*, 2 to 4; Pl. I, fig. 16 and Pl. II, figs. 17 and 18). During meiosis II, the mitochondrial granules are again arranged in chains running parallel to the longitudinal axis of the spindle.

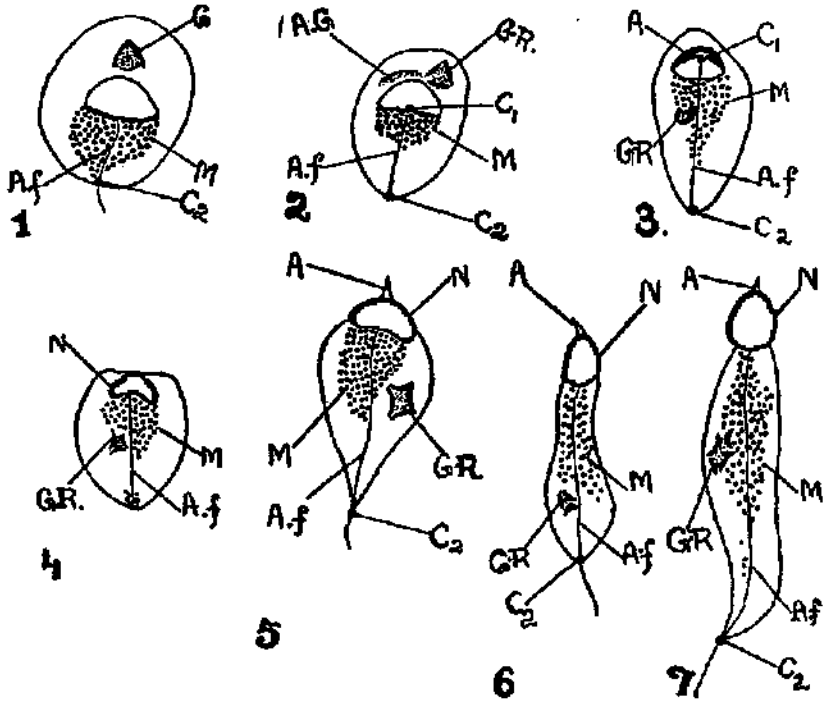


TEXT-FIG. *C*. 1—secondary spermatocyte; 2 and 3—metaphase II; 4 and 5—telophases II; 6—early spermatids. Further explanation in the text.

As towards the close of meiosis I, so towards the close of meiosis II, the Golgi dictyosomes are formed *de novo* from the mitochondrial granules

by alignment (Text-fig. *C*, 5; Pl. II, fig. 19). In these figs. again the dictyosomes have a crenated appearance, but soon, in earliest spermatids, their contours become smooth, and the dictyosomes arrange themselves in the form of triangles or rhomboids (Text-fig. *C*, 6; Pl. II, fig. 20).

Spermateleosis.—Two important changes now take place simultaneously at the very commencement of the process of spermateleosis. One of these changes is the migration of the Golgi dictyosomes to the anterior aspect of the spermatid nucleus. In this site the dictyosomes generally arrange themselves in the form of an open U with its concavity facing towards the nuclear membrane; and for the first time in spermateleosis distinct chromophobic material can be seen surrounded by the chromophilic U (Text-fig. *D*, 1; Pl. II, fig. 21). The other change consists of the movement of practically all the mitochondrial granules to the posterior aspect of the spermatid nucleus, where they press against the nuclear membrane making it flat posteriorly. At this stage the mitochondrial granules have grown in size; and they stain much more deeply with haematoxylin, also becoming more resistant to the action of acetic acid in Bouin's fluid. The axial filament has also appeared at this stage.



TEXT-FIG. *D*. 1–7—early stages of spermateleosis. Further explanation in the text.

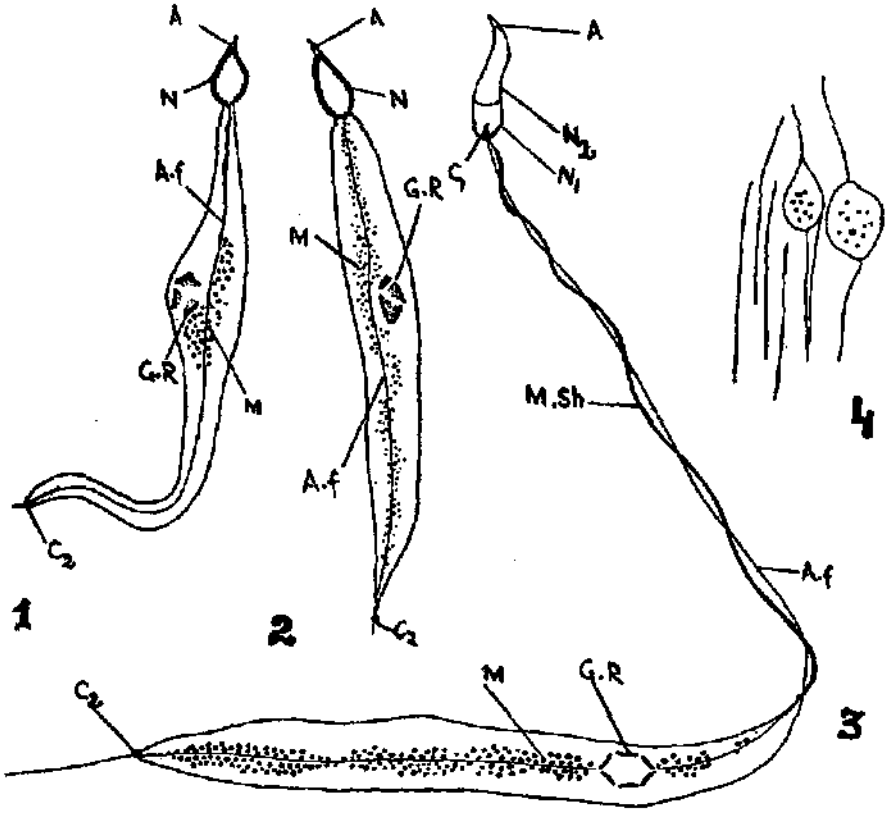
In the next stage the dictyosomes (acroblasts) have deposited a cap of very fine granules (the acrosomal granules) secreted by them in their chromophobic vesicles; and the acroblasts are once more arranged in the form of a triangle (Text-fig. *D*, 2; Pl. II, figs. 22 and 23). The last two figs. are microphotographs of the same cell. It will be noticed that in fig. 22

the cap of acrosomal granules has come up very well, and in fig. 23 three dictyosomes can be clearly made out. The proximal and distal centrioles can also be clearly made out in Text-fig. *D*, 2.

In the next stage of spermateleosis the acrosomal granules have fused to form a deeply-staining acrosomal cap, which reminds one of a similar cap in the mammalian sperm, and the dictyosomes have shifted back behind the nucleus. The spermatid nucleus, however, at this stage stains very poorly (Text-fig. *D*, 3; Pl. II, fig. 24). The proximal centriole has shifted forward and beneath the nucleus.

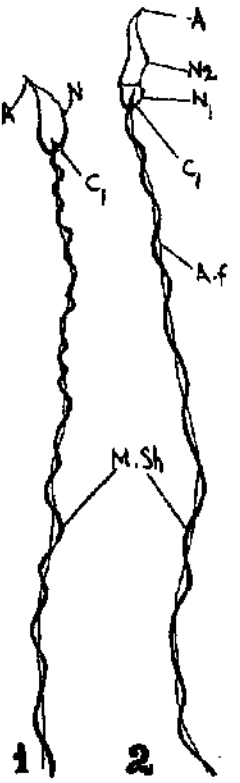
In the next stage the entire sperm head stains deeply, with the result that no line of demarcation can be made out between the nucleus and the acrosome (Text-fig. *D*, 4; Pl. II, fig. 25). The sperm head has become definitely arched, simulating the head of a mushroom.

The spermatid now begins to elongate, and a small, conical, very poorly-staining acrosome stands out prominently in front of the deeply-staining nucleus (Text-figs. *D*, 5 to 7; Pl. II, 26 to 28). The Golgi remnant is still prominent at this stage, but soon it begins to stain poorly, and is ultimately sloughed off. The mitochondria gather round that part of the axial filament whose anterior and posterior boundaries are determined by the proximal and distal centrioles respectively.



TEXT-FIG. *E*. 1–4—late spermateleosis. Further explanation in the text.

The spermatid now rapidly elongates, and the residual cytoplasm containing the mitochondria and the Golgi remnant is progressively carried posteriorward (Text-figs. *E*, 1, 2 and 3). In the part of the spermatid tail in front of the residual cytoplasm (Text-fig. *E*, 3), the mitochondria have already spun out to form a spiral sheath round the axial filament. The residual cytoplasm gets progressively attenuated (Text-fig. *E*, 4), till it completely disappears (Text-figs. *F*, 1 and 2).



Text-fig. *F*. 1 and 2—maturing sperms. Further explanation in the text.

In the living sperm, as studied under the phase contrast, the spiral mitochondrial sheath extends between the proximal centriole in front and the distal centriole behind. Thus is formed a very long middle-piece. Behind this is a short end-piece, which seems to be naked.

Plate II, figs. 29 to 31 are microphotographs of maturing living sperms studied under the phase contrast. The spiral mitochondrial sheath has come up very well in figs. 29 and 31.

Up to the stage represented in Text-fig. *E*, 2 and Plate II, figs. 29 and 30 the sperm nucleus is round or oval in form and stains deeply with haematoxylin, but the small cone-like acrosome stains very poorly. Soon the sperm nucleus gets elongated and twisted (Text-fig. *E*, 3 and Text-figs. *F*, 1 and 2; Pl. II, fig. 31). At the same time an important differentiation is set up in the nucleus. The whole nucleus at this stage stains feebly, but

its basal portion stains little more deeply, with the result that two regions in the nucleus can be easily recognized (Text-figs. *E*, 3; *F*, 2).

The proximal centriole gets elongated and lies underneath the sperm nucleus (Text-figs. *E*, 3; *F*, 1 and 2; Pl. II, figs. 29 and 30).

Discussion

It will be recalled that in the earliest spermatogonia of *Anadenus* there is no indication of any granulation, the cytoplasm staining uniformly with iron-haematoxylin after F.W.A. fixation. In late spermatogonia mitochondria appear for the first time in the form of granules, but there is no indication whatsoever of the Golgi dictyosomes.

It is in the earliest primary spermatocytes that mitochondria begin to align themselves and thus form the Golgi dictyosomes. To begin with, the dictyosomes have a crenated appearance, but soon they acquire a uniform contour. Nor is there any chromophobic vesicle attached to a dictyosome.

In the fully grown primary spermatocyte the dictyosomes all collect together and there is only one such collection to each cell.

During metaphase I, however, when the spindle, the centrioles, the chromosomes and the mitochondrial granules are all brought up very clearly in the finished sections, there is no trace of the Golgi dictyosomes left. Just previous to this, during the preceding diakinesis, the dictyosomes can be clearly seen breaking up into granules, which merge into the mitochondrial granules.

During late telophases I, the dictyosomes are again formed by the alignment of mitochondrial granules in exactly the same manner as at the commencement of the growth period of the primary spermatocytes.

But again, during metaphases II and anaphases II, the dictyosomes break up and merge into the mitochondrial granules, to reappear again by the alignment of mitochondrial granules during late telophases II.

In the earliest spermatid there is a characteristic Golgi apparatus formed, consisting of dictyosomes with even contour arranged in the form of a triangle or a rhomboid. In the course of spermateleosis and before the deposition of the acrosome, each Golgi dictyosome develops in close association with it a chromophobic vesicle, corresponding to the 'internum' of Hirsch (1939), the chromophilic dictyosome being the 'externum'.

The Golgi apparatus shifts to the anterior aspect of the spermatid nucleus; and a large number of minute acrosomal granules, first seen in the chromophobic vesicles, are deposited in front of the nucleus. These granules subsequently form the acrosome, and the Golgi apparatus, having performed its function of secreting the acrosome, is sloughed off.

Hirsch (1939) derives the Golgi pre-substance, i.e., the Golgi granules, from the mitochondria, as quoted by Bourne (1951). In our opinion stronger evidence than *Anadenus* in favour of Hirsch's view could not be wished for, as the Golgi dictyosomes in the spermatogenesis of *Anadenus* can be shown not only to arise from the mitochondria but also to merge into them during metaphases I and II.

We also strongly support the view of Hirsch that the secretion or the product arises in the internum of the Golgi complex.

One of us (Nath, 1942), while working on the spermatogenesis of 34 species of Decapod Crustacea, observed that in many species the earliest spermatogonia reveal only mitochondrial granules, the Golgi granules arising a little later amongst them.

In support of our contention that the Golgi dictyosomes in *Anadenus* originate from the mitochondrial granules, so that to begin with the dictyosomes have a crenated appearance, reference may be made to fig. 24, Pl. 32 of Gatenby (1917) and to text-figs. 4 and 5 of Watts (1952), in which the dictyosomes with a jagged contour have been figured.

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Explanation of Plates

Plate I, figs. 1–16 and Plate II, figs. 17–28 are microphotographs of the cells fixed in Flemming-without-acetic acid and stained with 0·5% iron-haematoxylin. The same cells have been drawn in the form of text-figures. All these microphotographs were taken under the Leitz apochromatic 2 mm. oil immersion with 12× eye-piece giving a total magnification of 1,350 times. They were further enlarged 1½ times.

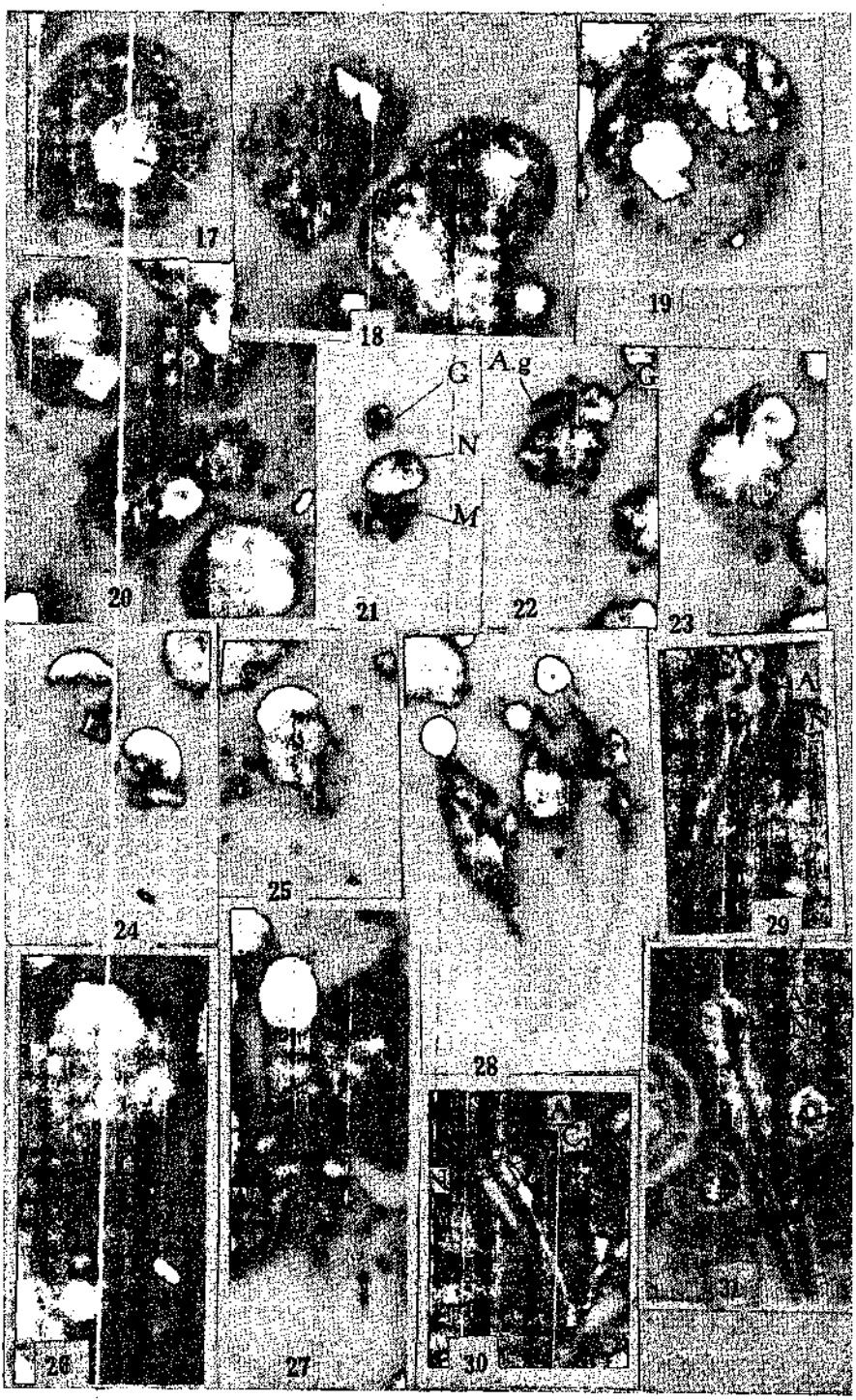
The microphotographs 29–31, Plate II, are of living maturing sperms as studied under Leitz Dialux phase contrast microscope with 10× Periplanetic eye-piece and 40 : 1 objective (apochromatic dry system), giving a magnification of 500 times. Leica camera was used. They were subsequently enlarged $1\frac{1}{2}$ times.

Lettering of figs. in plates same as in text-figs.

Explanation of Lettering in Text-figures

A—acrosome; *A.G*—acrosomal granules; *A.F*—axial filament; *C*—centriole; C_1 and C_2—proximal and distal centrioles; *Ch*—chromosomes; *G*—Golgi dictyosome; *G.R*—Golgi remnant; *M*—mitochondria; *M.Sh*—mitochondrial sheath; *N*—Nucleus; N_1 and N_2—distal and proximal parts of nucleus; *Nu*—nucleolus; *S.f*—spindle fibres.

PLATE II.



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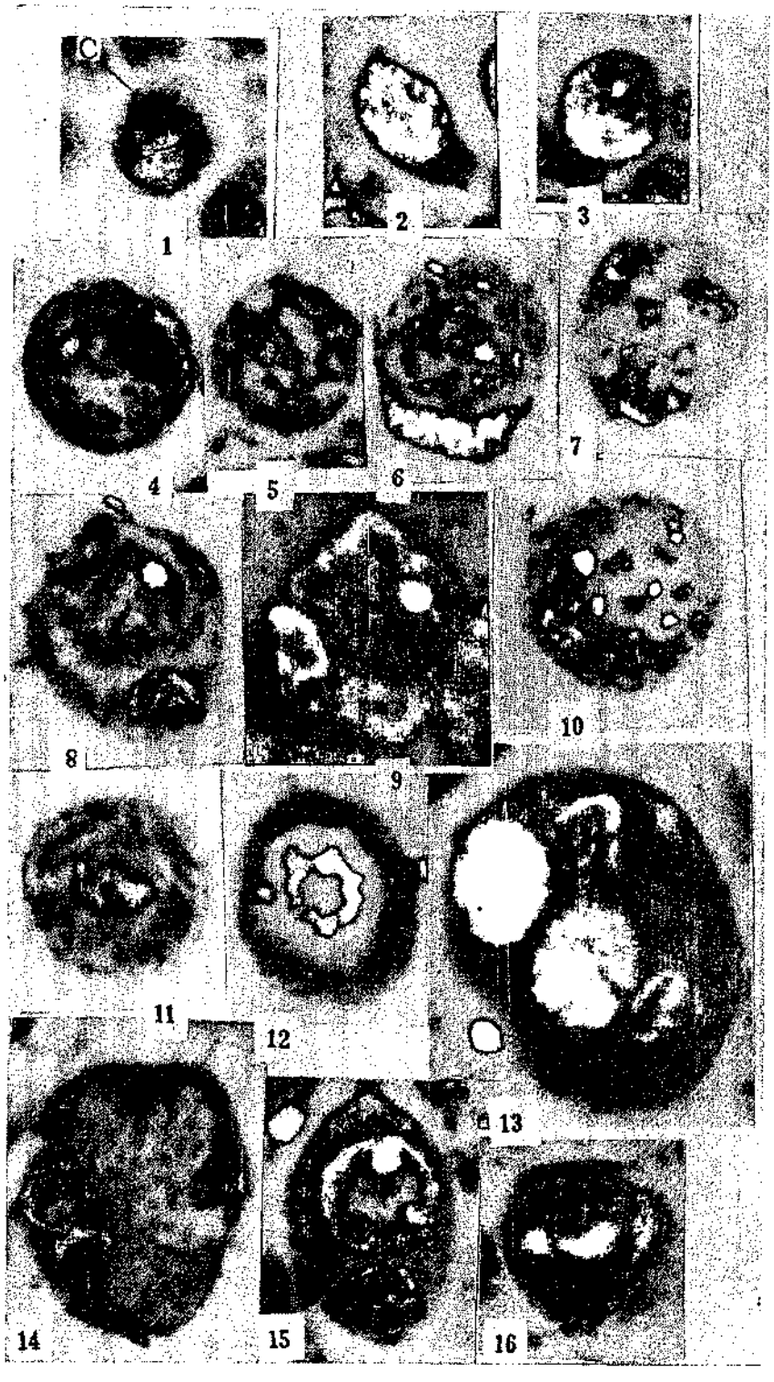
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RESEARCH BULLETIN
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STUDIES IN REPTILIAN SPERMATOGENESIS

I. Spermatogenesis of the Chequered Water Snake, *Natrix p. piscator* Schneider with some Observations on the Spermatids of the Krait and the Cobra

by

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STUDIES IN REPTILIAN SPERMATOGENESIS

I. Spermatogenesis of the Chequered Water Snake, *Natrix p. piscator* Schneider with some Observations on the Spermatids of the Krait and the Cobra

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Abstract

The detailed investigations on the spermatogenesis of *Natrix p. piscator* Schneider and some observations on the spermatids of the Krait and the Cobra have been made from the fixed preparations and the living material under the phase-contrast microscope. Some of the most important conclusions are as follows: (*a*) The fine mitochondrial granules grow considerably and are finally converted into large, yellow spheres but during late spermateleosis the entire mitochondrial material is cast off without forming any sperm component. (*b*) An unusual form of the Golgi material has been observed. In the spermatocytes the Golgi material is seen in the form of osmiophil or sudanophil granules, each associated with a hyaline vacuole of glue-like consistency. The vacuoles during the spermatocyte stages grow steadily at the expense of the osmiophil granules till ultimately the granules disappear. The vacuoles run together and form a single, completely hyaline vacuole, the pro-acrosome. The pro-acrosome is bodily converted into the acrosome. (*c*) The chromatoid body appears and disappears twice during spermatogenesis. There are reasons to believe that it is an independent structure having a *de novo* origin.

Introduction

The cytoplasmic inclusions of the male germ-cells of all the major groups of vertebrates except reptiles have been the subject of investigation. In the case of reptiles, except for a solitary paper by Risley (1936) on the germ-cells of turtles and an abstract by Zia-ud-Din (1946) on the spermatogenesis of tortoise, practically nothing has been reported so far regarding the cytoplasmic structures in the male germ-cells of this group, which is highly diversified from the morphological standpoint. The reason, perhaps, is that on the whole the testicular material of reptiles is highly refractory, and also the size of the cells is quite small.

To fill up this gap in our knowledge of reptilian spermatogenesis the study of male germ-cells was undertaken in various reptiles; and it is intended to publish a series of papers on this subject. I have been able to obtain good fixations of the testicular material of the various representatives of this class, and thus it has been possible to study the cytoplasmic inclusions

even in the stages of spermatogenesis, where the size of the cells happens to be very small.

The investigations recorded in this paper were undertaken in the male germ-cells of the water snake, *Natrix p. piscator* Schneider. In this snake the changes, which the cytoplasmic components undergo during successive stages of spermatogenesis, and the nuclear behaviour during spermateleosis have been carefully studied in the fixed preparations and the living material under the phase-contrast microscope. These observations have revealed unique and interesting phenomena with regard to the Golgi material, the mitochondria, the chromatoid bodies and the spermatid nucleus. The most important of these phenomena is that the entire mitochondrial material is cast off during spermateleosis.

All the previous workers on the spermatogenesis of snakes confined themselves to the study of the nucleus and the chromosomes and paid no attention to the cytoplasmic structures, which play an important rôle in spermatogenesis in general. Notable among these previous workers are Nakamura (1927 and 1928), Thatcher (1922) and Schreiber (1946 and 1947). Ballowitz and Retzius, as quoted by Wilson (1925), have described the structure of the sperm of the snake, *Coluber*. In the absence of any previous work on the cytoplasmic inclusions in the ophidian spermatogenesis the present paper is the first attempt to study these structures in this sub-order of reptiles.

Some spermatids from the F.W.A. iron-hæmatoxylin preparations of the Indian Cobra, *Naia tripudians*, var. typica Merrem, have also been included in the present paper for the sake of comparison.

Some cells in the spermateleosis of the common Krait, *Bungarus cæruleus* Schneider, have been examined and photomicrographed in the living condition under the phase-contrast microscope.

Acknowledgments

I wish to express my gratitude to Professor Vishwa Nath for having suggested to me this line of research, for encouraging me throughout the progress of this work, and also for correcting the manuscript for the press. I am thankful to Dr. B. S. Chauhan, the officiating Director, Zoological Survey of India, for allowing me to consult the library and to Miss Mira B. Kirpalani of the Reptile section of the same department for the identification of the snake. My thanks are also due to Mr. Som Datt Sharma for the translation of some French publications.

Material and Methods

The specimens of the snake, *Natrix p. piscator* Schneider, were collected from the surface wells, which are common in Hoshiarpur. Capturing this snake alive is no easy task, as they run with lightning speed. The author is indebted to Mr. Munsha Singh, a local farmer, for collecting the material.

Live specimens of this snake were kept in the laboratory in an aquarium tank containing a small quantity of water. The specimens refused to accept any type of food during captivity.

The animal was killed either with a sharp blow on the head or with chloroform used as a light anaesthetic. Chloroform did not have any bad effect on the testicular tissue; and, strange as it will appear, the material fixed from the snake made unconscious by chloroform was rather better fixed than the material from the pithed snake. After chloroforming or pithing a cut was made on the ventral side, extending from the anal opening to a little beyond the kidneys, and the testes were removed.

The testes are a pair of white rather elongated structures placed asymmetrically, the right reaching a little further forward than the left. Each testis is provided with a coiled vas deferens, which springs up from its inner border and opens posteriorly into the cloacal chamber.

The testes were quickly removed from the body of the animal, and, after washing off the blood, pieces were cut by a fine pair of scissors and put into watch glasses containing different fixatives. These pieces were immediately teased with needles in the fixing fluids to separate the convoluted tubules of the testes. This procedure insured rapid and complete fixation of this highly refractory material. The teased material was then transferred to glass capsules containing fresh fixatives. The fixatives tried were F.W.A. (Flemming-without-acetic), F.W.A. diluted with an equal amount of water, Flemming with reduced quantity of acetic acid, Champy, Altmann, Kolatchev, Aoyama and Bouin. Among all the osmic acid fixatives tried F.W.A. for 48 hours and Kolatchev for 24 hours proved to be the best. The material was washed in running water for the same period. After dehydration the material was cleared in cedar wood oil and embedded in paraffin wax. Sections were cut 5–8 micra thick. They were mordanted with 5% iron-alum and stained with 0·5% hæmatoxylin. F.W.A. fixed sections were also treated according to Kull's modification and stained with acid fuchsin.

Kolatchev preparations were counterstained with iron-hæmatoxylin after bleaching the sections by potassium permanganate and oxalic acid. Material fixed in Helly's fluid and post-chromed at 37°C. was stained with Sudan black (Thomas, 1948), for the study of Golgi material. Bouin's preparations proved most useful as control. For smears F.W.A. was diluted with an equal quantity of distilled water.

It was felt necessary to study the Golgi material with artificial light as the daylight did not bring out the detailed structure.

Fresh material, teased out in a drop of Ringer's solution for cold-blooded animals, was studied under the phase-contrast microscope and some of the important stages of spermateleosis were photomicrographed.

Observations

A. *Fixed preparations*

(i) *Spermatogonia*

The resting spermatogonium is a rounded or an ovoid cell with a very large nucleus in comparison to the size of its cytoplasm. The nucleus occupies, generally, a central position, and contains a network of lightly-staining, fine chromatin granules in which are embedded a few deeply-

staining, coarse granules (Pl. I, Fig. 1). The thin layer of cytoplasm shows on one side a deeply-staining, granular centriole and on the other a small juxta-nuclear mass of tiny greyish granules, which have been interpreted as the mitochondria. There are no traces of the Golgi bodies at this stage.

(ii) *Primary Spermatocytes*

The earliest primary spermatocyte is almost of the same size as the spermatogonium. It grows considerably and when it attains the maximum size it appears to be the largest cell in the sequence of spermatogenesis. During the growth period the cytoplasm increases more in size than the nucleus.

The resting nucleus contains either one or two nucleoli and in addition there may also be present fine chromatin granules (Pl. I, Figs. 2 to 6). Most of the cytoplasm is crowded with fine mitochondrial granules, which are generally arranged in the form of a juxta-nuclear or even a circum-nuclear mass.

The chromatoid body makes its appearance in the early primary spermatocyte (Pl. I, Fig. 2). It is a large structure, round or ovoid in form (Pl. I, Figs. 2 to 6). In some cells a smaller chromatoid body is seen in addition to the large chromatoid body (Pl. I, Figs. 3 and 5). The chromatoid body is highly resistant to Bouin's fluid (Pl. I, Fig. 6). It takes up a deep blue stain in F.W.A. iron-hæmatoxylin and Bouin iron-hæmatoxylin preparations, but in Kolatchev and Aoyama preparations with optimum impregnation it appears as a yellow structure. In Altmann's acid fuchsin preparations it takes up a deep red stain.

The Golgi material appears for the first time in the primary spermatocyte after the chromatoid body has made its appearance. It appears as osmiophil or sudanophil granules associated with distinct hyaline vacuoles with glue-like contents (Pl. I, Figs. 3 to 5). The Golgi material is embedded amongst the mitochondrial granules.

As the hyaline vacuoles increase in size a corresponding decrease in the size of the Golgi granules takes place. Thus the Golgi granules appear to be gradually consumed in the formation of these strongly chromophobic Golgi vacuoles. The Golgi granules as well as the hyaline vacuoles are dissolved in Bouin's fluid (Pl. I, Fig. 6).

It is difficult to distinguish the centriole from the Golgi granules in the resting primary spermatocyte, but in the material fixed in Bouin's fluid for long periods, in which the Golgi material and the mitochondria are washed out, a granular centriole can be made out (Pl. I, Fig. 6).

(iii) *Secondary Spermatocytes*

The secondary spermatocyte is a smaller cell than the primary spermatocyte (Pl. I, Figs. 7 to 9). Each resting secondary spermatocyte has an ex-centric nucleus with one or two nucleoli. The mitochondria are in the form of fine granules scattered almost throughout the cytoplasm. The Golgi material is mostly represented by chromophobic vacuoles with glue-like contents (Pl. I, Figs. 7 to 9). Only in the early secondary spermatocytes small osmiophilic or sudanophilic thickenings, representing the remains

of the Golgi granules, are seen adhering to the chromophobic Golgi vacuoles (Pl. I, Figs. 7 and 8). The chromophobic vacuoles run together and form bigger and denser vacuoles (Pl. I, Figs. 8 and 9). The chromatoid material at this stage has completely disappeared, to re-appear during spermateleosis.

(iv) *Spermateleosis*

The earliest spermatid is a very small cell with an ex-centric nucleus. The nucleus is a clear, rounded structure, containing two or more nucleoli (Pl. I, Figs. 10 and 11). A portion of the cytoplasm is packed with a very large number of fine mitochondrial granules. The Golgi material is in the form of a single completely chromophobic vacuole with glue-like consistency, situated between the nucleus and the mitochondrial mass. This is the pro-acrosome. The pro-acrosome, like the Golgi material of spermatocytes, is dissolved out in Bouin's fluid.

In the Indian Cobra, *Naia tripudians*, var. typica, the pro-acrosome is similar to that of *Natrix*, but it is formed during spermateleosis. In the early spermatids two or three completely hyaline vacuoles with glue-like consistency are seen. During spermateleosis these vacuoles run together to form a single large vacuole whose contents seem to go denser (Pl. III, Figs. 71 to 75). This is the pro-acrosome.

The pro-acrosome in *Natrix* grows into a clear rounded or oval vesicle with glue-like consistency, and it remains firmly stuck to the nucleus of the spermatid (Pl. I, Figs. 11 to 13). The pro-acrosome exerts considerable pressure on the nuclear membrane and causes a marked depression on the surface of the nucleus. This depression gradually broadens out (Pl. I, Figs. 14, 15, 18, 19 and 20).

Hitherto the nucleus stains very lightly and the pro-acrosome is completely hyaline, but now onwards condensation of both these cell structures takes place, and both of them stain deeply. By the condensation of the pro-acrosome the acrosome is formed. This last is now pushed out of the depression at the anterior pole of the nucleus, the depression thus disappearing completely. At this stage, the nucleus is a darkly-stained, cup-shaped structure over which lies a darkly-stained dome-shaped acrosome (Pl. I, Fig. 21). Later, the nucleus becomes cylindrical and the acrosome at its anterior pole becomes cone-shaped, but both these structures continue to stain darkly (Pl. I, Figs. 22 to 24). The acrosome again loses its staining capacity, except for its extreme anterior point, where a deeply-staining granule can still be observed. But even this granule disappears when the nucleus has also lost its staining capacity and has become club-shaped in form (Pl. I, Figs. 25, 26; Pl. II, Figs. 28 to 32, 38 and 39). After remaining for some time in this condition, the acrosome again becomes chromatic and is drawn out into a fine thread (Pl. II, Figs. 40, 43 and 44).

Now the spermatid nucleus shrinks in size and assumes a rhomboidal or a pyriform shape, but it still remains chromophobic (Pl. II, Figs. 47, 49 and 50; Pl. III, Figs. 52 to 54, 56 and 57).

During the final ripening of the spermatozoon the nucleus further condenses, becomes highly chromatic, and assumes an ovoid form (Pl. III,

Figs. 58 and 59). The boundary of the nucleus and the acrosome, which has now shortened, can still be very easily made out.

The large number of fine mitochondrial granules, which in the earliest spermatid are packed together in a portion of the cytoplasm, are gradually dispersed haphazardly in the cytoplasm (Pl. I, Figs. 10 to 13). These fine mitochondrial granules now grow into large, darkly-staining granules; they are generally localized in a portion of the cytoplasm near the cell membrane, but they may even be scattered throughout the cytoplasm (Pl. I, Figs. 18 to 20). When the condensation of the nucleus and the pro-acrosome has taken place, the mitochondria are arranged at the anterior pole of the spermatid (Pl. I, Figs. 21 and 22). Later, they grow more in size and are dispersed in the cytoplasm (Pl. I, Figs. 23 to 26; Pl. II, Fig. 28).

When the nucleus becomes a clear, club-shaped structure, the darkly-staining granular mitochondria grow into spheres, which are arranged haphazardly in the cytoplasm (Pl. II, Figs. 29 to 32, 38 and 39). The spherical mitochondria now appear yellow in colour, and do not have a cortex and a medulla. They appear to have been ceratinized. They grow still more in size. By the time the acrosome is drawn out into a filament and the nucleus is still club-shaped, the mitochondrial spheres arrange themselves in vertical rows to form a tube, extending from a little below the nucleus up to the basal portion of the acrosome (Pl. II, Figs. 40, 43 and 44). Figures 41 and 42, Plate II, are cross-sections of the spermatids at this stage, and in such sections the spherical mitochondria are seen in the process of forming a ring around the nucleus. The mitochondrial spheres continue to grow in size.

When the mitochondrial spheres are fully grown, they are no longer arranged in the form of a tube around the nucleus. Gradually they are discarded from the sides or the anterior end of the spermatid into the lumen of the follicle (Pl. II, Figs. 49, 50 and Pl. III, Fig. 52). The mitochondria in this material do not migrate into the tail region to form a tail-sheath. The mitochondrial spheres are sometimes so big that they remind one of yolk spheres (Pl. II, Fig. 48). Even in one of the earlier spermatids large and yellow mitochondrial spheres have been found, but this is rare (Pl. III, Fig. 60).

Strange as it would appear, I have not seen the cytoplasm of the spermatid forming a sheath round the axial filament in the tail region in my fixed preparations, but in the living material studied under the phase-contrast I have not only seen it but I have photomicrographed it also (see Plate IV).

The centriole in the early stages of spermateleosis is a deeply-staining granule, situated very near the nucleus and in some cases almost touching the nuclear membrane (Pl. I, Figs. 10 and 17). When the condensation of the pro-acrosome and the nucleus takes place, the centriole comes to lie a little below the posterior pole of the nucleus (Pl. I, Figs. 21 and 22). The axial filament springs up rather late from the centriole. It is destined to form the tail of the spermatozoon (Pl. II, Figs. 28 to 33, 35 and 36).

Just before the final ripening of the spermatozoon a deeply-staining, ring-shaped structure is found attached to the posterior pole of the nucleus

on the inside of the nuclear membrane. In the side view of the maturing spermatozoon this structure generally appears as a deeply-staining thickening at the posterior pole of the nucleus, but sometime the real ring-like character of this structure is revealed (Pl. III, Figs. 53 to 55). In the fully ripe sperm, when the sperm nucleus stains deeply, this ring-shaped structure as well as the granular centriole cannot be made out (Pl. III, Figs. 58 and 59).

A refractile body with a darkly-staining rim makes its appearance inside the developing sperm nucleus at the stage when the spherical mitochondria are still arranged in the form of a tube and the nucleus is still lightly-staining. This body, however, is not visible in all such cells. Perhaps, it is seen in those cells only in which the section passes in the region of this body. Figure 48, Plate II and Figure 51, Plate III, illustrate this refractile body in the centre of the spermatid nucleus. During the final ripening of the spermatozoon, when the nucleus stains deeply, the refractile body cannot be made out.

After the primary spermatocyte stage the chromatoid body reappears during spermateleosis. It makes its reappearance just after the condensation of the nucleus and the pro-acrosome, but in the living condition under phase-contrast it is also seen in some earlier spermatids. To begin with, it is a dumb-bell-shaped, deeply-staining body, but later it appears as a single large, spherical body (Pl. I, Figs. 21 to 26). In subsequent stages two or three additional small chromatoid bodies make their appearance in the cytoplasm. These small chromatoid bodies may possibly have originated as fragments from the large chromatoid body. The small chromatoid bodies are difficult to distinguish from the darkly-staining mitochondria in Flemming-without-acetic preparations, but in Bouin's preparations in which the mitochondria are completely dissolved out, they can be clearly made out (Pl. II, Figs. 27 and 33 to 36). Before the mitochondrial spheres start arranging themselves in vertical rows to form a tube, extending from a little below the nucleus to the basal portion of the acrosome, the chromatoid bodies move forward towards the anterior end and disappear (Pl. II, Figs. 28 to 39).

The fully ripe spermatozoon is of a very simple type (Pl. III, Figs. 58 and 59). It consists of an oval, deeply-staining nucleus, with a cone-shaped, lightly-staining acrosome at its anterior end and an axial filament forming the tail at its posterior end. The centrosome cannot be distinguished clearly at this stage. The refractile body found in the centre of the nucleus and the darkly-staining, ring-shaped structure situated at the base of the nucleus of the developing sperm likewise cannot be made out.

In addition to the normal type of sperm described above, sometimes sperms with thin narrow heads are seen (Pl. III, Fig. 69). The head consists of a deeply-staining posterior part, in front of which there is a clear space. In front of this space is a pointed deeply-staining structure, which may possibly be the acrosome. From the posterior end of the darkly-staining head arises the axial filament, forming the tail. Such sperms are formed from a different category of spermatids, which are peculiar in having correspondingly very small nuclei (Pl. III, Figs. 67 and 68). Such

spermatozoa, however, are not of common occurrence in this material, and they appear to be abnormal.

Here and there in the follicles of the testis are found masses of cytoplasm containing from two to eight spermatid nuclei, embedded in a single cytoplasmic mass. These syncytial masses are probably formed by the failure of the cytoplasm to divide during cell-division. It is interesting to note that in these syncytial masses spermateleosis proceeds normally, and results in the production of the normal type of spermatozoa (Pl. III, Figs. 61 to 66).

In addition to the syncytial masses where spermateleosis proceeds normally, some syncytial masses show degeneration or pycnosis. In such cases the nuclei are condensed to form chromatic masses. The outer limits of these syncytial masses are not well defined, and generally each mass is surrounded by a hollow space (Pl. III, Fig. 70). Pycnosis has also been observed in a few individual cells.

B. *Living material under the phase-contrast microscope*

Practically all the stages of spermateleosis were studied in the living condition under the phase-contrast microscope and some of them were photomicrographed.

In all the early spermatids the hyaline pro-acrosome of glue-like consistency is seen either just sticking to the nuclear membrane or partly sunk into the nucleus (Pl. IV, Photos. 1 and 2*a*).

Also, in the common Krait, *Bungarus cæruleus*, the hyaline pro-acrosome of glue-like consistency has been photomicrographed partly or even completely sunk into the spermatid nucleus (Pl. IV, Photos. 9 and 10).

The chromatoid body appears faint in the living material under phase-contrast. With regard to this cell structure the only point of difference from the fixed preparations is that it was observed in a few spermatids even before the condensation of the nucleus and the pro-acrosome (Pl. IV, Photos. 1 and 2*b*).

The mitochondria appear as dark grey granules in early stages but in late stages of spermateleosis they are in the form of large, yellow spheres. The mitochondrial spheres in late stages arrange themselves in vertical rows to form a tube, extending from a little below the nucleus to the basal portion of the acrosome (Pl. IV, Photo. 3). The nucleus and the proximal portion of the acrosome are only faintly seen through the spaces between the mitochondrial spheres. From the anterior end of the mitochondrial tube projects the distal part of the acrosome and from its posterior end the axial filament is seen coming out. The arrangement of the mitochondrial spheres in the form of a tube ultimately breaks down and they are totally discarded from the sides or the anterior end. Photomicrograph 4, Plate IV, shows a spermatid from which most of the mitochondrial spheres have been cast out and the remaining few are seen being sloughed off from the anterior end. The mitochondrial spheres do not migrate into the tail region.

In the fixed preparations the cytoplasm is never seen migrating to the tail region, but in the living condition, usually after the formation of the

mitochondrial tube, the cytoplasm is seen moving along the axial filament in the form of blebs (Pl. IV, Photos. 4 to 6). These cytoplasmic blebs are completely empty and do not contain mitochondria or the chromatoid elements. The axial filament possesses only the cytoplasmic sheath. The cytoplasmic sheath extends to a considerable length of the axial filament. The part of the axial filament surrounded by the cytoplasmic sheath forms the main-piece of the tail, and the rest of the axial filament forms the end-piece (Pl. IV, Photos. 7 and 8).

Discussion

Mitochondria

The behaviour and fate of the mitochondria is unique and is undoubtedly the most remarkable feature of the spermatogenesis of *Natrix p. piscator*. The mitochondria in the spermatogonia are a few fine granules. In the spermatocytes they appear as numerous fine granules, forming a dense mass in the cytoplasm.

During the early stages of spermateleosis the mitochondria become large and darkly-staining. But in the late stages of spermateleosis the mitochondrial granules grow into yellow, ceratinized spheres. They grow further and become more ceratinized. Later, they arrange themselves in vertical rows to form a tube, extending from a little below the nucleus to the basal portion of the acrosome. During the final ripening of the spermatozoon the arrangement of these mitochondrial spheres in the form of a tube breaks down, and the entire mitochondrial material is cast out into the lumen of the follicle, making no contribution whatsoever to the sperm formation.

In all cases of spermatogenesis, with a few exceptions, the mitochondria, whether they form a nebenkern or remain separate, enter into the final make-up of the sperm. In the flagellate sperm all or most of the mitochondrial material forms a sheath round either a part or nearly the whole length of the axial filament. In the non-flagellate sperms of the Decapod Crustacea (Nath, 1932, 1937 and 1942) and the millipede (Nath and Sharma, 1952), the mitochondria fuse together to form a vesicle, which fits closely into the nuclear cup.

But in the snake under investigation the mitochondria do not make any contribution whatsoever to the final make-up of the sperm. These observations of mine are in sharp contrast with those of Ballowitz and Retzius (quoted by Wilson, 1925), who have described a mitochondrial sheath for the middle-piece of the sperm of the snake, *Coluber*.

There are a few well-authenticated accounts of the complete sloughing off of the mitochondrial material in spermatogenesis. Such a case was first reported by Dingler (1910) in the digenetic trematode, *Dicrocoelium lanceolatus*, where the mitochondria are cast out without forming any sperm component. Dhingra (1954, 1955 and 1955*a*) in three digenetic trematodes, *Isoparorchis eurytremum*, *Cotylophoron elongatum* and *Gastrothylax crumenifer* has also shown that during spermateleosis the mitochondria are left behind in the residual cytoplasmic mass and do not contribute towards the formation of any part of the ripe sperm. Montgomery (1912)

in *Peripatus* has shown a complete sloughing off of the mitochondria during the late stages of sperm formation.

Gatenby (1931) has denied the presence of any mitochondria in the middle-piece of *Desmognathus fusca*. Seshachar (1943) states that the mitochondria are generally absent from the adult urodele sperm.

Hughes-Schrader (1946) has also described a complete sloughing off of the mitochondria in the mature sperm of iceryine coccids.

Sharma (1950) in the spermatogenesis of the spider, *Plexippus paykulli*, has demonstrated the rejection of the entire mitochondrial contents. The sperm consisting of an acrosome, a nucleus and an axial filament just wriggles out of the cytoplasm of the cell, which contains the whole of the mitochondrial material.

Nath and Bhatia (1953) described the complete fading away of the poorly developed and abnormally situated mitochondrial nebenkern in the spermatid of *Lepisma domestica*.

Nath and Rishi (1953) in the dragon-fly, *Sympetrum hypomelas*, have described the complete absence of the mitochondrial material from the earliest spermatogonia to the ripe sperm. This is an extreme case, and suggests that mitochondria are not essential in spermatogenesis and in the final make-up of the sperm.

Golgi Material

The Golgi material was not observed in the spermatogonia and the early primary spermatocytes of *Natrix*. It is seen for the first time during the growth of the primary spermatocyte, as osmiophil or sudanophil granules embedded in the mitochondrial mass. In association with these Golgi granules are seen distinct hyaline vacuoles with glue-like contents. Only rarely a Golgi granule is seen without a hyaline vacuole. During the primary and the secondary spermatocyte stages the hyaline vacuoles develop at the expense of the Golgi granules. In this process the Golgi granules completely disappear, and the Golgi material is represented by these hyaline vacuoles alone. The hyaline vacuoles from the very outset show a tendency to run together and by the spermatid stage they all fuse to form a single hyaline vacuole, whose contents seem to have gone denser. This is the pro-acrosome, which is completely chromophobic and is destined to form the acrosome.

Thus in the spermatogenesis of this snake the Golgi material is either seen as dark granules associated with hyaline vacuoles with glue-like contents, or as vacuoles alone. The different techniques tried (F.W.A. iron-hæmatoxylin, Kolatchev, Kolatchev iron-hæmatoxylin and Sudan black) demonstrate the Golgi material admirably. The Golgi material in the granular form associated with a hyaline vacuole of glue-like consistency is rather unusual.

Acrosome

The acrosome in the present material is formed directly from the single completely hyaline pro-acrosome with glue-like consistency, which makes its appearance in the earliest spermatid. The entire Golgi material is

used up in the formation of this pro-acrosome, which in turn is bodily converted into the acrosome. Thus the whole of the Golgi material is consumed in the formation of the acrosome and the Golgi remnants are completely absent.

In *Naia tripudians*, studied by the present author, the early spermatids reveal in their cytoplasm two or three completely hyaline vacuoles with glue-like contents, which later fuse to form a single pro-acrosome in each spermatid. Thus in this snake the pro-acrosome is formed a little later than in the case of *Natrix*.

In the freshwater turtle, *Lissemys punctata*, also studied by the present author, a similar type of pro-acrosome is formed. The process of formation of this pro-acrosome is also practically the same.

Risley (1936) in the early stages of the spermatogenesis of turtles describes clear hyaline spaces or vacuoles associated with Golgi granules and in his figures he illustrates them surrounding the Golgi granules. In the spermatid two large vacuoles are present in close association with the long centriole, and Risley is of the opinion that these are formed by the enlargement of spaces or vacuoles present in association with the Golgi granules. The texture and shape of these vacuoles appear to be identical with the hyaline pro-acrosome of glue-like consistency, which forms the acrosome in *Natrix*. At this stage when these two vacuoles appear, Risley does not show any Golgi granules, and from this it can be inferred that they are used up in the formation of these two vacuoles. According to Risley 'one of these, usually the larger, migrates around the nucleus and assumes a position at the opposite or anterior end of the nucleus as the latter begins to elongate' and is destined to form the acrosome. The other vacuole remains at the base of the nucleus and Risley is inclined to the view that from this vacuole of Golgi origin is formed the manchette of the turtle sperm.

Chromatoid Body

The chromatoid body appears for the first time in the primary spermatocyte. It is seen before the Golgi material makes its appearance. It is of large size and is either ovoid or spherical in form. In some primary spermatocytes an additional smaller chromatoid body may also be present. The chromatoid body is not seen in the secondary spermatocytes and the early spermatids. It reappears during spermateleosis just after the condensation of the nucleus and the pro-acrosome, but in the living condition under phase-contrast it appears also in a few earlier spermatids. When it reappears in spermateleosis it is dumb-bell-shaped, but later it becomes spherical. Two or three additional very small chromatoid bodies also appear, perhaps as fragments from the main body. During the late stages of spermateleosis the chromatoid elements move towards the anterior end of the spermatid, and finally disappear.

The chromatoid body stains deeply in F.W.A. iron-hæmatoxylin and Bouin iron-hæmatoxylin preparations. But in Kolatchev preparations stained with iron-hæmatoxylin after bleaching with potassium permanganate and oxalic acid it does not pick up hæmatoxylin stain. It appears

yellow, whereas the chromosomes, chromatin and nucleoli take up a deep blue stain.

The chromatoid body does not appear to be Golgi in nature because it does not stain in Kolatchev, Aoyama, Sudan black and F.W.A. unstained preparations. Also, unlike the Golgi material, it is highly resistant to Bouin's fluid and appears very faint in the living condition under phase-contrast. Moreover, it makes its first appearance before the Golgi material is seen in the primary spermatocyte, and again it reappears in the spermatid after the whole of the Golgi material is consumed in the formation of the pro-acrosome. Nor is the chromatoid body associated in any way with the mitochondrial material.

Hence from its reactions to all these fixing and staining media coupled with the study of the living material under phase-contrast the chromatoid body appears to be an independent cell structure having a *de novo* origin. The single 'Golgi body' described by Srivastava (1953) appears to me to be such a chromatoid body.

Nucleus

A peculiar feature of the spermatid nucleus is the sudden appearance of a refractile body with a darkly-staining rim, in its centre. In the ripe spermatozoon, however, this refractile body is not visible.

Bowen (1922) describes a similar structure with an intensely-staining peripheral ring enclosing a clear or lightly-stained medulla in the spermatid nucleus of a pentatomid hemipteran, *Murgantia*, and calls it the intra-nuclear body. The origin and fate of this body is not clear, but due to its almost similar structure to that of the plasmosome of the spermatocyte in the growth period, Bowen is inclined to the view that it most probably represents a true nucleolus or plasmosome and is not of chromatin origin.

A similar structure has also been found by Gatenby and Beams (1935) in the developing and developed head of the majority of spermatozoa in Man and they call it the 'vacuole' or the 'head cavity'. This vacuole in some cases stains like a nucleolus, but the authors consider it to be only doubtfully derived from a nucleolus. They are also doubtful regarding the function of this structure, but they think that it may be some kind of a hydrostatic organ or even a respiratory vacuole. Retzius (1909) has also figured this space or vacuole, as quoted by Gatenby and Beams.

In my view the darkly-staining rim of the refractile body seen in the nucleus of the late spermatids of *Natrix* is nothing but the portion of the chromatin which has taken a lead in becoming intensely basophilic. The clear area enclosed by the dark rim is the oxyphilic medullary substance of the nucleus of the developing sperm. Ultimately in the ripe sperm the whole of the sperm nucleus becomes a solid and homogeneous mass of basichromatin.

Chromatin structure similar in nature to that of the darkly-staining rim of the refractile body but in the form of an intra-nuclear rod has been observed by Nath and Rishi (1953) in the dragon fly, *Sympetrum hypomelas*. This intra-nuclear rod is at first kinky but eventually straightens up to form a straight axial core of the sperm nucleus.

Another darkly-staining ring-shaped structure attached to the posterior pole of the late spermatid nucleus of *Natrix* on the inside of the nuclear membrane is also in all probability the result of concentration of the chromatin. This structure lies close to the centriole and in a fully ripe spermatozoon cannot be made out.

Bowen (1922) in Hemiptera also describes during spermateleosis, on the inside of the nuclear membrane close to the centrioles, a temporary concentration of chromatin which in this case is of ovoid form. This structure in subsequent stages gradually disappears. Bowen names this body as the pseudoblepharoplast because of its constant confusion with the true centrioles by the previous workers.

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Explanation of Lettering in Plates

A—Acrosome; *A.f*—Axial filament; *C*—Centriole; *C.b*—Chromatoid body; *C.g*—Chromatin granules; *Cy*—Cytoplasmic bleb; E.P—End-piece; *G*—Golgi material; *M*—Mitochondria; *M.P*—Main-piece; *n*—Nucleolus; *N*—Nucleus; *Pa*—Pro-acrosome; *R.b*—Refractile body; *R*—Ring-shaped structure; *S*—Hollow space.

Explanation of Figures

All figures have been drawn at table level with a Beck camera lucida, 2 mm. immersion objective and 10×eyepiece giving a magnification of approximately 1,370 times.

Unless otherwise mentioned, all figures have been selected from sectioned material fixed in Flemming-without-acetic acid and stained with 0·5% iron-hæmatoxylin.

Plate I (*Natrix p. piscator*)

Fig. 1. Spermatogonium, showing a granular centriole and juxta-nuclear mass of tiny mitochondrial granules.
Fig. 2. Early spermatocyte with juxta-nuclear mitochondrial mass and a chromatoid body.
Fig. 3. Primary spermatocyte with a dense circum-nuclear mitochondrial mass, two chromatoid bodies and Golgi material in the form of dark granules, mostly associated with hyaline vacuoles with glue-like consistency.
Fig. 4. Primary spermatocyte, showing mitochondria, chromatoid body and Golgi granules associated with hyaline vacuoles (Kolatchev iron-hæmatoxylin).
Fig. 5. Primary spermatocyte, showing mitochondria, two chromatoid bodies and Golgi granules associated with hyaline vacuoles (Kolatchev iron-hæmatoxylin).
Fig. 6. Primary spermatocyte with one chromatoid body and a granular centriole (Bouin iron-hæmatoxylin).
Fig. 7. Secondary spermatocyte, showing sudanophil Golgi granules associated with hyaline vacuoles (Sudan black—Thomas, 1948).
Fig. 8. Secondary spermatocyte with Golgi material in the form of granules associated with hyaline vacuoles and hyaline vacuoles alone (Kolatchev).
Fig. 9. Secondary spermatocyte with mitochondria and the Golgi material represented by two large completely hyaline vacuoles formed by coming together of the smaller hyaline vacuoles (Kolatchev iron-hæmatoxylin).

FIG. 10. Earliest spermatid, showing the hyaline pro-acrosome with glue-like texture, the mitochondrial mass and the centriole.

FIG. 11. Early spermatid, showing the pro-acrosome and the mitochondrial mass.

FIG. 12. Early spermatid with mitochondria and the pro-acrosome. A few mitochondrial granules are seen adhering to the pro-acrosome.

FIG. 13. Spermatid, showing fine mitochondrial granules—scattered unevenly in the cytoplasm—and the pro-acrosome.

FIG. 14. Spermatid with the pro-acrosome pressing on the anterior portion of the nucleus. Mitochondria have become more prominent.

FIG. 15. Spermatid with mitochondria still more prominent and the pro-acrosome fitting into the nucleus.

FIG. 16. Polar view of the spermatid with the pro-acrosome sunk into the nucleus.

FIG. 17. Spermatid, showing the nucleus with a slight depression at its anterior end formed by the pro-acrosome and a granular centriole near its posterior pole (Bouin iron-hæmatoxylin).

FIGS. 18 to 20. Spermatids with prominent darkly-staining mitochondria and the pro-acrosome fitting into the anterior portion of the nucleus.

FIG. 21. Spermatid, showing the condensation of the nucleus and the pro-acrosome and a dumb-bell-shaped chromatoid body.

FIG. 22. A later spermatid with a dumb-bell-shaped chromatoid body, a cylindrical nucleus and a cone-shaped acrosome. Mitochondria are aggregated at the anterior pole of the cell.

FIGS. 23 and 24. Spermatids, each with a spherical chromatoid body.

FIGS. 25 and 26. Spermatids, each with a dark cylindrical nucleus and a faintly-stained acrosome having a dark granule at its anterior tip.

PLATE II (*Natrix p. piscator*)

FIG. 27. Spermatid with two additional small chromatoid elements (Bouin iron-hæmatoxylin).

FIG. 28. Spermatid with lightly-stained nucleus and partly condensed acrosome.

FIGS. 29 to 32. Spermatids, each with faintly-stained nucleus and acrosome. Mitochondrial granules converted into yellow spheres are seen growing gradually.

FIGS. 33 to 36. Spermatids, each with one large and two or three additional small chromatoid bodies gradually moving to the anterior pole of the cell (Bouin iron-hæmatoxylin). In these Bouin fixed stages the nuclei stain darkly but the corresponding stages in Flemming-without-acetic acid preparations show lightly-stained nuclei.

FIG. 37. Spermatids arranged at the periphery of a follicle, showing the chromatoid bodies moving towards the anterior pole of the cells (Bouin iron-hæmatoxylin).

FIGS. 38 and 39. Spermatids from which the chromatoid bodies have disappeared and the mitochondria are being arranged.

FIG. 40. Mitochondrial spheres have formed a tube extending from the centriole to the basal portion of the thread-like, dark acrosome but some mitochondrial spheres are still lying outside the tube.

FIGS. 41 and 42. Cross-sections of the spermatids, each showing mitochondrial spheres arranging themselves in the form of a ring around the nucleus.

FIGS. 43 to 47. Spermatids, showing mitochondrial spheres arranged in vertical rows to form a tube extending from a little below the nucleus to the basal portion of the acrosome. The spermatid nucleus is of various shapes.

FIG. 48. Late spermatid with the refractile body in the centre of the nucleus. Mitochondria are still arranged in vertical rows and some of them have grown into very large spheres.

FIGS. 49 and 50. Late spermatids, showing the mitochondrial spheres being discarded.

Plate III

Figs. 51 to 70—*Natrix p. piscator;* Figs. 71 to 75—*Naia tripudians*, var. typica.

Fig. 51. Late spermatid with the refractile body in the centre of the nucleus.

Fig. 52. Late spermatid with discarded mitochondrial spheres.

Fig. 53. Very late spermatid, showing the ring-shaped structure at the posterior pole of the nucleus.

Fig. 54. Very late spermatid with a thickening at the posterior pole of the nucleus representing the darkly-staining ring-shaped structure.

Fig. 55. Darkly-staining ring-shaped structure situated at the posterior pole of the very late spermatid.

Fig. 56. Very late spermatid, showing a chromophilic acrosome, a chromophobic nucleus, a centriole and the axial filament.

Fig. 57. A still later spermatid.

Figs. 58 and 59. Fully ripe spermatozoa, each showing acrosome, nucleus and a portion of the tail.

Fig. 60. Spermatid, showing precocious development of mitochondria.

Fig. 61. Syncytial mass, showing two spermatid nuclei, two mitochondrial masses and two pro-acrosomes.

Fig. 62. Syncytial mass, showing two spermatid nuclei, each having a pro-acrosome in its concavity.

Fig. 63. Syncytial mass of eight spermatid nuclei, each having a pro-acrosome in its concavity.

Fig. 64. Syncytial mass with two darkly-staining cylindrical spermatid nuclei, darkly-staining acrosomes and two spherical chromatoid bodies.

Fig. 65. Syncytial mass, showing two club-shaped spermatid nuclei with acrosomes and two large and two small chromatoid elements.

Fig. 66. Syncytial mass with two club-shaped spermatid nuclei, each having an acrosome, a centriole and an axial filament. The chromatoid elements are in the form of two large and three small bodies.

Figs. 67 and 68. Spermatids of abnormal sperms, each showing the acrosome and the nucleus (Bouin iron-hæmatoxylin).

Fig. 69. Abnormal sperm (Bouin iron-hæmatoxylin).

Fig. 70. Syncytial mass with degenerating pycnotic nuclei (Bouin iron-hæmatoxylin).

Fig. 71. Spermatid with three completely hyaline vacuoles with glue-like consistency representing the Golgi material.

Fig. 72. Spermatid with three completely hyaline Golgi vacuoles. Two of these vacuoles lie near each other.

Fig. 73. Spermatid with one large and one small hyaline Golgi vacuole.

Fig. 74. Spermatid, showing the running together of the hyaline Golgi vacuoles to form the pro-acrosome.

Fig. 75. Spermatid, showing the pro-acrosome sticking to the nucleus.

Explanation of Photomicrographs

All photomicrographs are of the living material as studied under Leitz Diulux phase-contrast microscope with 10×Periplanetic eyepiece and 40 : 1 objective (Apochromatic dry system), giving a magnification of 500 times. Leica camera was used. All photomicrographs are further enlarged four times and are untouched.

Plate IV

Photos. 1 to 8. *Natrix p. piscator.*
Photos. 9 and 10. *Bungarus cæruleus.*

Photo. 1. Spermatid, showing the pro-acrosome partly sunk into the nucleus. The chromatoid body has also reappeared.

Photo. 2. Cell '*a*' is the spermatid with the pro-acrosome sticking to the nuclear membrane.
Cell '*b*' is the spermatid, showing the chromatoid body, cylindrical nucleus and the axial filament without any cytoplasmic bleb. The mitochondrial spheres which are not in focus lie scattered in the cytoplasm.

Photo. 3. Late spermatid, showing the mitochondrial spheres arranged in vertical rows to form a tube, extending from a little below the nucleus to the basal portion of the acrosome. The axial filament and the distal portion of the acrosome are seen coming out of the mitochondrial tube.

Photo. 4. Late spermatid from which most of the mitochondrial spheres have been discarded and the remaining few are being sloughed off from the anterior end. An empty cytoplasmic bleb is seen on the axial filament.

Photos. 5 and 6. Late spermatid, showing empty cytoplasmic blebs on the axial filament.

Photo. 7. Maturing sperm, showing the acrosome, the nucleus and the main-piece of the tail.

Photo. 8. A portion of the main-piece and the complete end-piece of the maturing sperm shown in photomicrograph 7.

Photo. 9. Syncytial mass, showing a pro-acrosome partly sunk in each spermatid nucleus.

Photo. 10. Syncytial mass, showing only one spermatid nucleus in focus. The pro-acrosome is completely sunk into this nucleus.

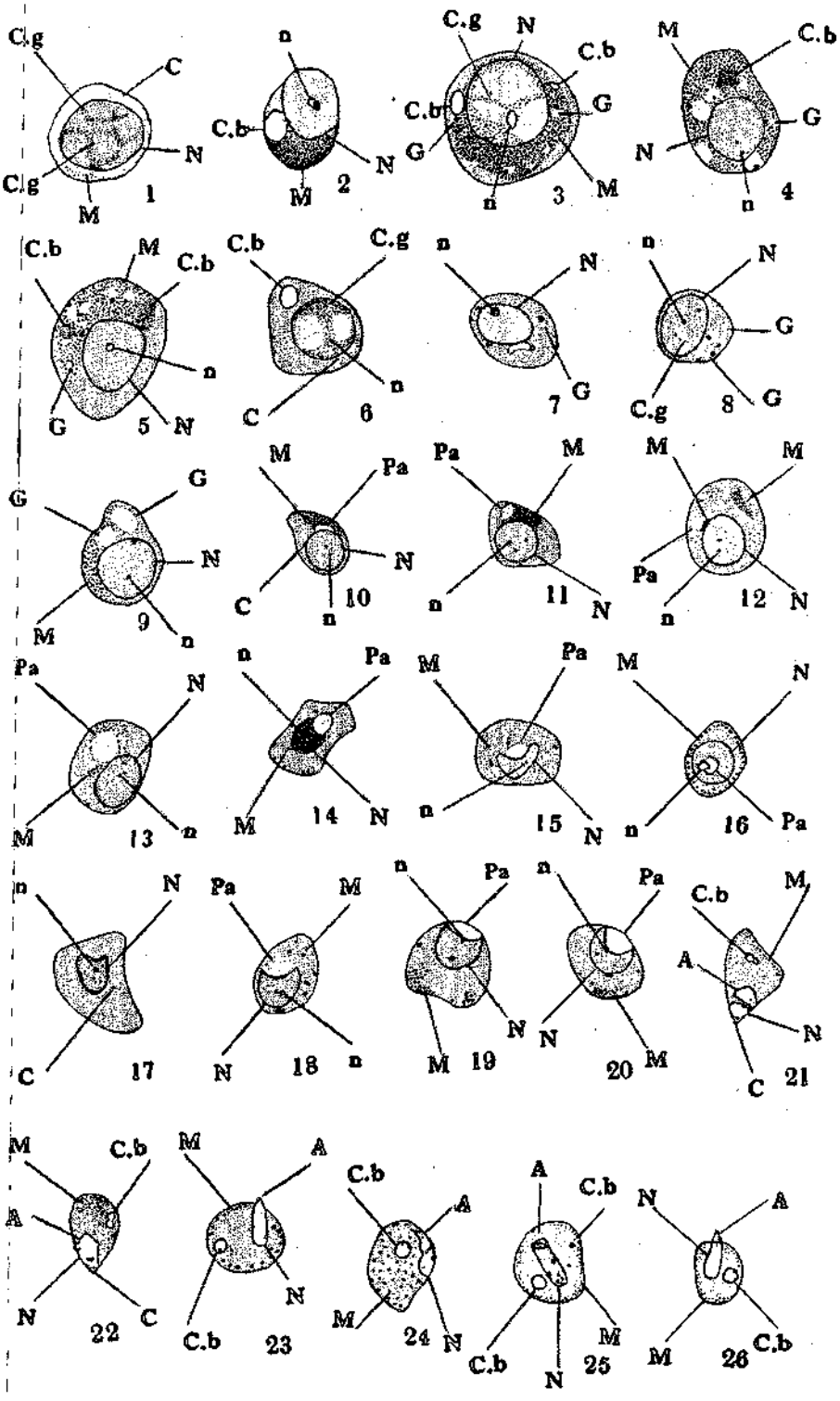
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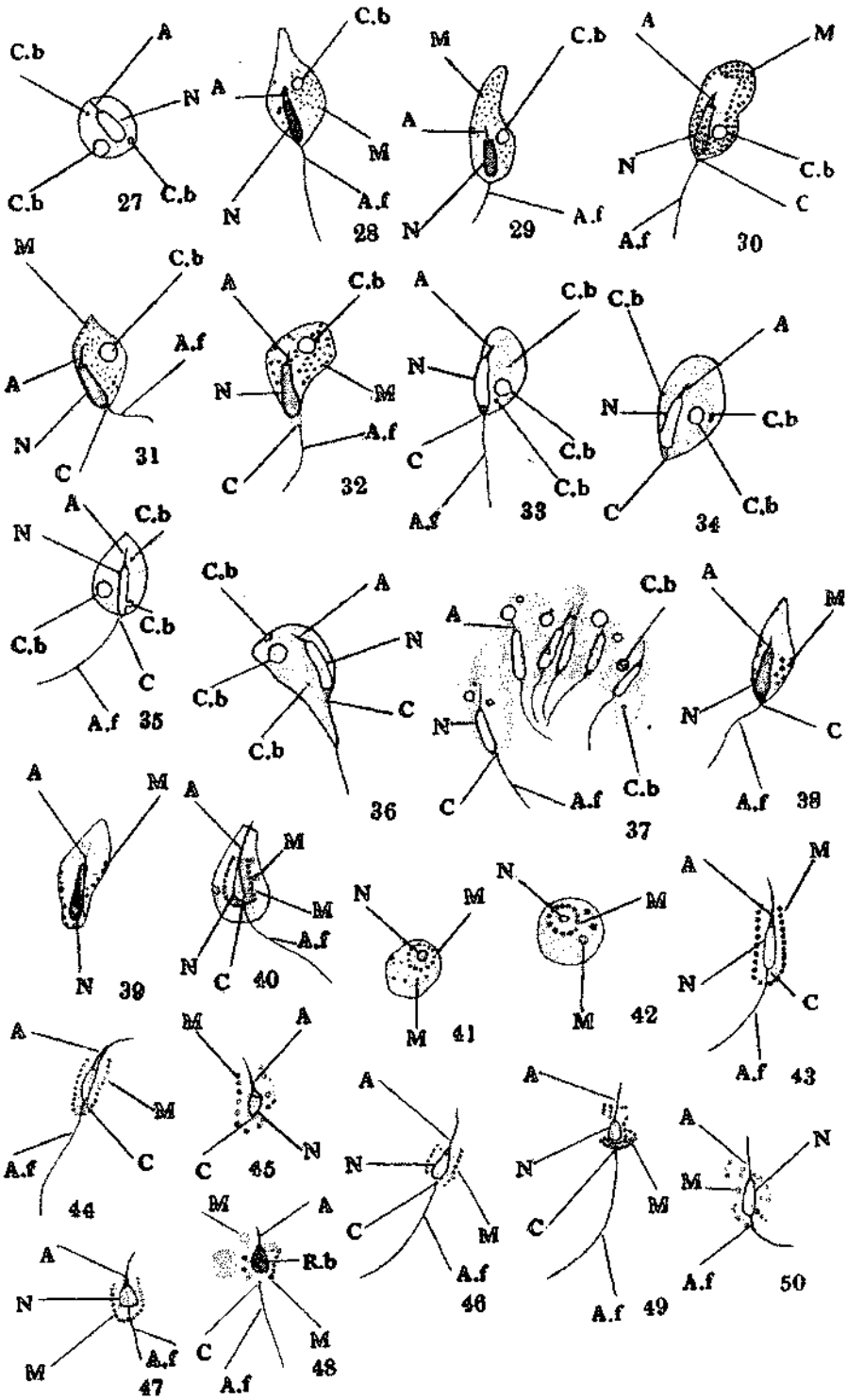
PLATE II.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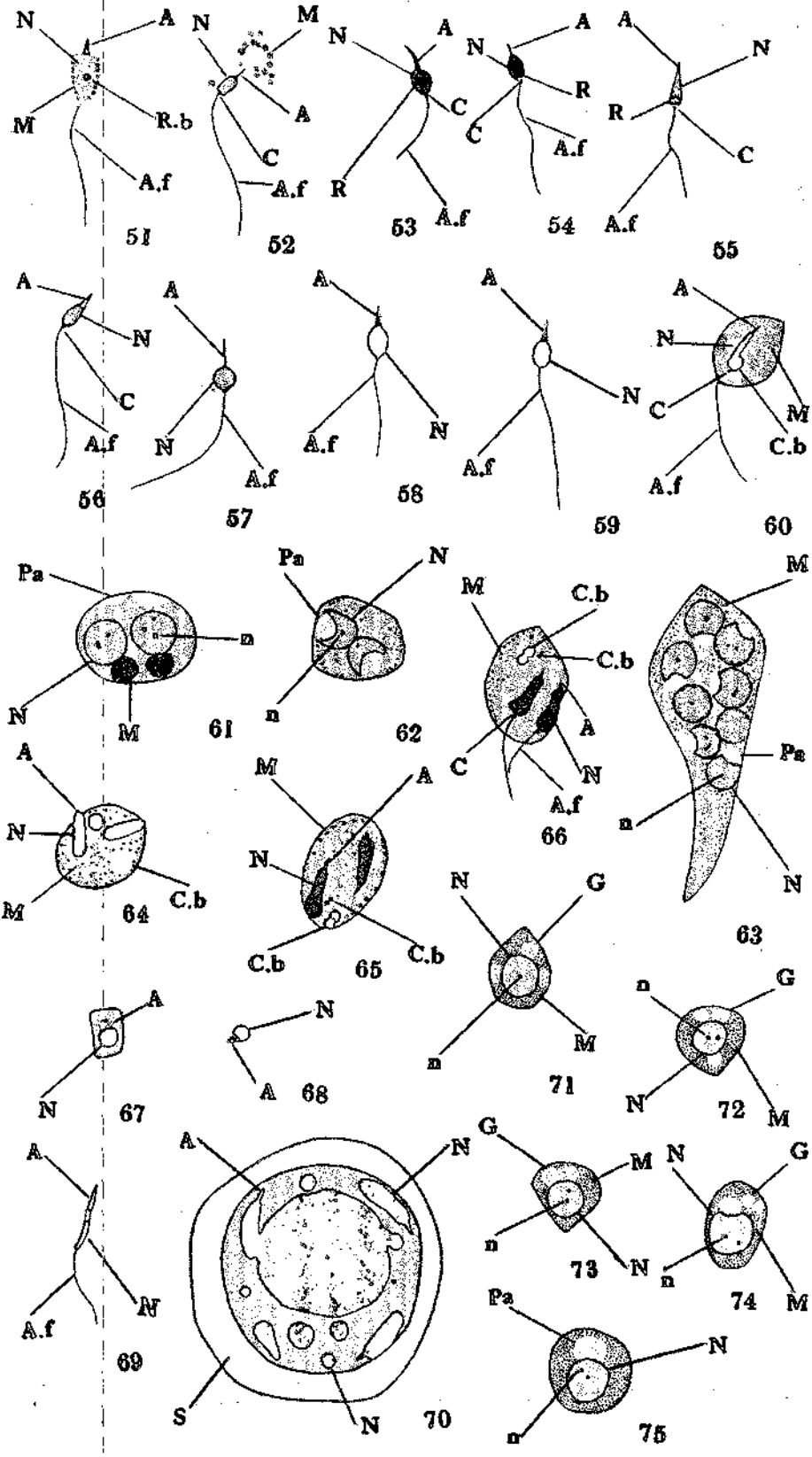
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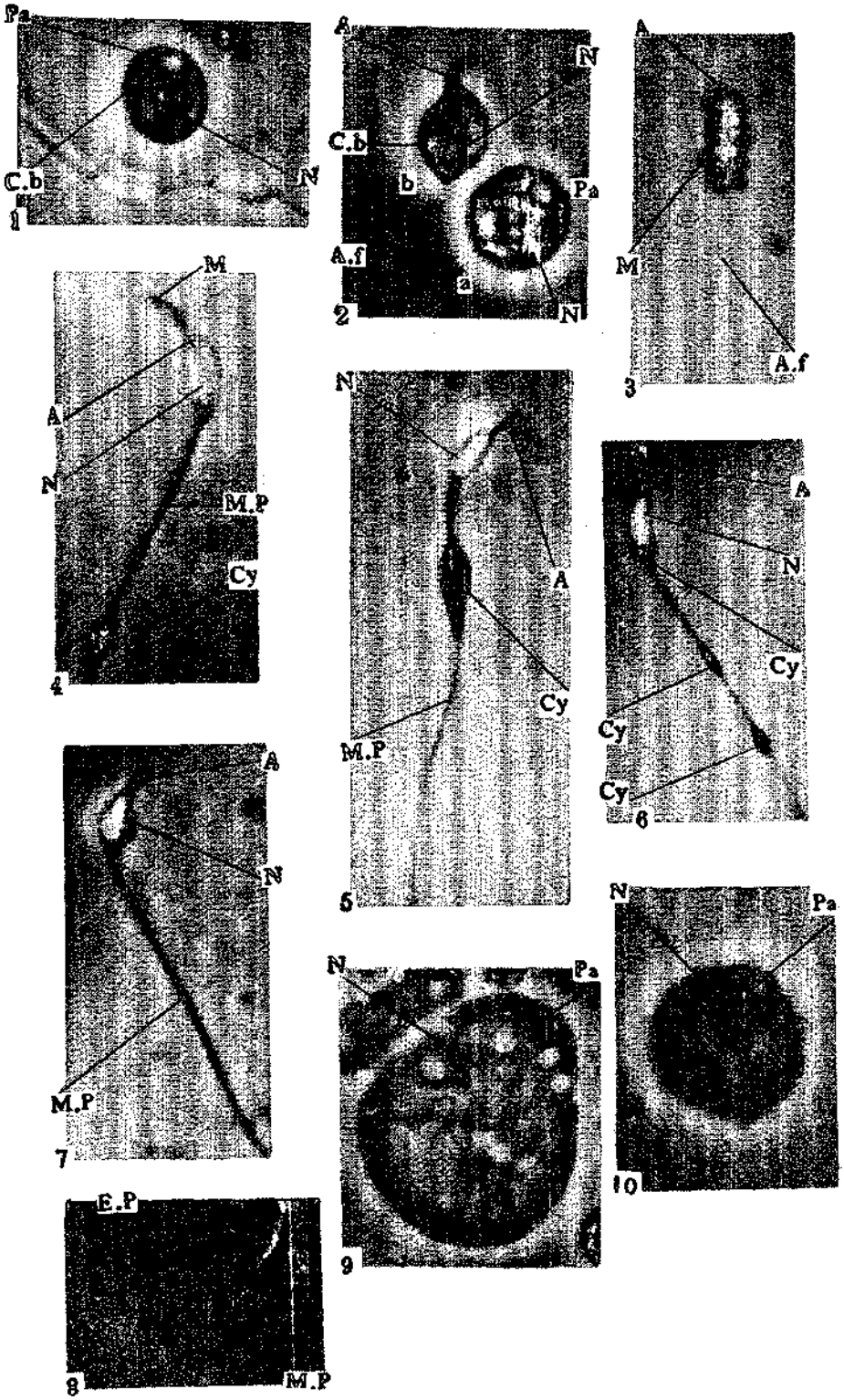
PLATE IV.



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SPERMATOGENESIS OF WHITE RAT WITH OBSERVATIONS UNDER PHASE-CONTRAST MICROSCOPE

by

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SPERMATOGENESIS OF WHITE RAT WITH OBSERVATIONS UNDER PHASE-CONTRAST MICROSCOPE

By (Miss) Balbir Kaur Dhillon

(Department of Zoology, Panjab University, Hoshiarpur)

Abstract

In this paper spermatogenesis of the white rat has been worked out exclusively with Flemming-without-acetic and 0·5% iron-hæmatoxylin. The Golgi elements are granular. Sometimes some Golgi vesicles are also seen. The accessory bodies are some of the extra-idiosomic Golgi granules. Neither the nuclear ring nor the post-nuclear cap of other authors has been observed during this study.

Introduction

In a previous communication from this department Sharma *et al.* (1953) gave a detailed account of the spermatogenesis in the guinea-pig. According to them, the mitochondria remain scattered throughout the cytoplasm in the form of fine, lightly-staining granules right up to the late spermatid stage. During the final stages of sperm-formation, however, the mitochondrial granules, which are included in the 'manchette', become darkly staining. These, by a process of fusion, form bigger and prominent mitochondrial granules, which ultimately arrange themselves around the axial filament to form the middle-piece of the ripe sperm. The rest of the mitochondria are sloughed off with the residual cytoplasm.

The Golgi elements in the early spermatogonia of the guinea-pig exist in the form of a juxta-nuclear mass of darkly-staining granules, which are, however, definitely bigger than the mitochondria. In the fully grown spermatogonia, these become spread out throughout the cytoplasm. In the primary spermatocyte they begin to show a distinct tendency to come together to form bigger granules. Some of these big Golgi granules now collect together, and are seen in the 'idiosome'. This latter structure is very prominent in the secondary spermatocytes and the spermatids.

During the division stages all the Golgi elements, like the mitochondria, become dispersed throughout the cytoplasm. Both the mitochondria and the Golgi elements are thus sorted out, more or less evenly, to the daughter cells.

As the process of spermateleosis begins, the idiosome becomes closely applied to the nucleus. Most of the Golgi granules in it now begin to fuse together gradually till ultimately a single big granule is formed. This is situated almost in the centre of the idiosome. Soon after its formation it becomes surrounded by a clear vacuole, which increases in size as the idiosome moves towards one side. The single Golgi granule now moves

through the vacuole and comes in intimate contact with the nuclear membrane to form the acrosome. To begin with the acrosome is a small structure, but gradually it increases in size and also becomes differentiated into two regions—an inner darkly-staining and an outer lightly-staining. The acrosome grows in size, the regions of the idiosome with or without the Golgi remnants drift back to the posterior side of the nucleus, to be finally sloughed off with the residual cytoplasm.

The so-called 'chromatoid body' and the 'accessory bodies', according to Sharma *et al.* (1953), are merely the Golgi elements, which do not participate in the formation of the acrosome.

The 'post-nuclear granules' were not observed by Sharma *et al.* (1953) at any stage of spermatogenesis. In some of their Da Fano smears, however, deposit of silver was noticed on the posterior part of the nucleus of the ripe sperm. The post-nuclear body of Gatenby and Wigoder (1929) is, according to Sharma *et al.* (1953), the dense posterior region of the nucleus itself and not a separate body. This was confirmed by them by employing Feulgen's reagent on smears as well as on sections.

Sharma *et al.* (1953) have also described a single protoplasmic bead containing a few Golgi elements on the middle-piece of the ripe sperm. This, however, is lost while the sperm is within the epididymis.

The proximal and distal centrosomes do not divide. The proximal centrosome remains granular, while the distal becomes ring-like; after travelling down the axial filament the distal centrosome marks the posterior end of the middle-piece of the ripe sperm.

The 'manchette', according to these authors, is a purely cytoplasmic structure in origin, marking the lateral boundaries of the middle-piece.

Gresson and Zlotnik (1945), working on the spermatogenesis of certain mammals, have recorded the following conclusions regarding the white rat.

The mitochondria are granular, and are scattered throughout the cytoplasm in the spermatogonia. In the primary spermatocytes they are assembled round the localized Golgi material and part of the nucleus. Later, they spread out throughout the cytoplasm. The majority of these mitochondria take part in the formation of the sheath of the middle-piece of the sperm, the remaining being sloughed off along with the residual cytoplasm.

The localized Golgi material of the primary spermatocyte and the spermatid, according to these authors, consists of rods and granules. It breaks up during the division stages and is distributed equally to the daughter cells.

In the spermatid, after forming the pro-acrosome, which appears in the archoplasmic vacuole, the Golgi material moves to the posterior part of the cell, where it divides into two parts. The smaller part forms the argentophil granules of the protoplasmic bead and the larger part of the Golgi material is eliminated with the residual cytoplasm. The acrosome is formed from the pro-acrosome and the material in the archoplasmic vacuole.

According to Gresson and Zlotnik (1945), the so-called 'accessory bodies' arise from the Golgi material of the primary spermatocyte and the spermatids. One of these accessory bodies gets attached in the neck region

of the sperm. A 'residual body' is present in the cytoplasm of the spermatid, which is lost in the residual cytoplasm.

These authors have shown a nuclear ring in the spermatid, which makes its appearance first on the nuclear wall on the rim of the depression caused by the archoplasmic vacuole on the nucleus.

They also show a post-nuclear cap, which, according to them, is small and is restricted to a narrow region adjacent to the sperm neck.

The fat globules, which are not formed from the Golgi material, are present in the early stages of spermateleosis. After the elongation of the spermatid, vacuoles appear in the residual cytoplasm.

The ripe sperm possesses two centrioles, a proximal rounded one and a distal ring-like. The 'manchette' encloses the cytoplasm, which forms the middle-piece of the sperm. When the sperm reaches the epididymis the protoplasmic bead moves down the middle-piece and is eliminated.

Gresson (1950 and 1950*a*) studied the living material of the mouse and rat under the phase-contrast microscope. In the rat the Golgi material in the primary and secondary spermatocytes is made up of thin dark rods and filaments surrounding a lighter area lying against the nucleus. In the mouse the Golgi material in secondary spermatocytes is clearer and is more commonly seen than in the primary spermatocytes. In the smears of the testes of the mouse the mitochondria are scattered throughout the cell, but the Golgi elements are not identified.

During the stages of the metamorphosis of the spermatid of the rat and mouse, Gresson (1950) has observed the archoplasmic vacuole, the pro-acrosome, the developing acrosome, the proximal and distal centrioles and the axial filament. The 'manchette' is sometimes faintly visible in the late spermatids of the rat.

Gresson has also described two or three spherical bodies, each with a thin dark rim in the secondary spermatocytes of the mouse and one such body in the vicinity of the distal pole of the nucleus of the spermatids of the mouse and rat. These bodies, according to him, are most probably the accessory bodies.

The living spermatozoon under phase-contrast microscope reveals the presence of the acrosome, the post-nuclear cap, the ring centriole, the mitochondrial sheath and the protoplasmic bead. Gresson is not sure whether the granule present in the neck of the sperm is the proximal centriole or the accessory body.

Material and Technique

In this paper I have recorded my observations on the spermatogenesis of the white rat, *Rattus norvegicus* Berkenhout, obtained from a dealer in Amritsar (Panjab-India). I have used exclusively the technique of fixing the material with Flemming-without-acetic acid, followed by 0·5% iron-haematoxylin; I have avoided the techniques of silver and osmium impregnation, which admittedly form artefacts. I have also examined the living cells under phase-contrast microscope and obtained some very satisfactory photomicrographs published in this paper.

I am grateful to Dr. Vishwa Nath for suggesting this problem and correcting the manuscript of this paper. I am also thankful to him and to Dr. G. P. Sharma for examining my preparations and for studying the living material along with me. My thanks are also due to Mr. Brij L. Gupta for taking the photomicrographs.

Observations

Fixed and Stained material.

The spermatogonia in the white rat are small cells, lying near the periphery of the follicles (Pl. I, fig. 1). The nucleus of the spermatogonium is vesicular and occupies most of the space in the cell. The mitochondria are very fine, lightly-staining granules, which are uniformly distributed throughout the cytoplasm. A very few Golgi granules, bigger than the mitochondria, are generally met with. These lie close to the nucleus and stain deeply in iron-hæmatoxylin. Spermatogonial divisions were not observed by me.

The primary spermatocytes are definitely bigger cells than the spermatogonia (Pl. I, figs. 2 and 3). The mitochondria, just like those of the spermatogonia, are scattered throughout the cytoplasm. They are very fine and lightly-staining granules. The Golgi elements have now increased in number. Most of them appear in the form of deeply-staining granules; but sometimes it is possible to see a vesicle with a chromophilic cortex and a chromophobic medulla (Pl. I, fig. 2).

In the fully grown primary spermatocytes (Pl. I, fig. 3) a small chromophobic area, the idiosome, makes its appearance in the vicinity of the nucleus. Some of the Golgi granules get embedded in the idiosome and thus is formed the characteristic Golgi-idiosome complex of the mammalian sperm-forming cells. No division stages of the primary spermatocytes were seen by me, except that some polar views of metaphase I were observed (Pl. I, fig. 4).

The secondary spermatocytes are comparatively smaller cells than the primary spermatocytes (Pl. I, figs. 5 and 6). By this time most of the Golgi granules are embedded in the idiosome, but a few Golgi granules and spheres are always found scattered in the cytoplasm. The latter will be referred to hereafter as the extra-idiosomic Golgi granules. The mitochondria continue to remain distributed uniformly throughout the cytoplasm.

It has been noticed that during the second meiotic division the nucleus of the cell divides in the usual way, but the division of the cytoplasm may be delayed. Nevertheless, the development of the cell-constituents is carried on normally (Pl. I, fig. 20).

The earliest spermatid is a small cell with a smaller and condensed nucleus. Most of the Golgi elements are assembled in the idiosome, which lies at a little distance from the nucleus (Pl. I, fig. 7). The mitochondria are still inactive and remain spread out uniformly throughout the cytoplasm.

With the start of spermateleosis the idiosome, with its Golgi granules, comes to lie nearer the nuclear membrane (Pl. I, fig. 8). Some of the extra-

idiosomic Golgi granules also shift nearer the idiosome. The Golgi granules of the idiosome now move towards its periphery, and there they fuse together to form a uniformly thick, deeply-staining cortex round the chromophobic part of the Golgi-idiosome complex. *One* of the Golgi granules, however, does not shift to the periphery, but remains embedded in the centre of the chromophobic idiosomic sphere (Pl. I, fig. 9). This single Golgi granule is the pro-acrosome. Soon the Golgi-idiosomic sphere is seen closely sticking to the nuclear membrane, so much so, that the lower boundary of the sphere thins out and gets flattened (Pl. I, fig. 10). Meanwhile, the extra-idiosomic Golgi granules start drifting away from the vicinity of the idiosome to the posterior side of the nucleus. Later, the chromophilic wall of the almost spherical Golgi-idiosome sphere breaks down at one point; the sphere now closely resembles the sign of interrogation (Pl. I, fig. 11).

Now the single pro-acrosomic granule, which hitherto occupied the centre of the Golgi-idiosomic sphere, moves out of the sphere, and as it does so, it seems to steal, so to say, a part of the chromophobic substance of the sphere. The pro-acrosomic granule with its surrounding chromophobe substance now stands out prominently as a structure, distinct from the original Golgi-idiosomic mass, from which it took its origin (Pl. I, figs. 12 and 13).

The chromophobic substance round the pro-acrosomal granule soon appears to be converted into a transparent vacuole in the centre of which is suspended the pro-acrosomal granule (Pl. I, figs. 14 and 15). The vacuole now spreads out, increases in size and exerts pressure on the nucleus. Consequently a depression is formed on the nuclear membrane, and the vacuole along with its contained pro-acrosomal granule fits into this depression (Pl. I, figs. 16 to 19). When the vacuole has increased sufficiently in size, the pro-acrosomal granule travels down and touches the nuclear membrane (Pl. I, fig. 21). As soon as the pro-acrosomal granule touches the nucleus, the latter starts growing underneath the vacuole in the form of a conical projection with the granule attached at its tip (Pl. I, fig. 22).

The Golgi-idiosomic remnant lying on the vacuole now starts drifting back, and is ultimately sloughed off. The vacuole at its lower margin seems to be limited by a definite membrane, which is clearly visible in the form of a faintly staining streak all around the conical projection of the nucleus (Pl. II, fig. 23).

As the transformation of the spermatid into the ripe sperm proceeds, the nucleus, with the pro-acrosome and the vacuole attached at its beak, begins to elongate (Pl. II, figs. 24 to 27).

The vacuole now spreads out to cover the anterior region of the elongating nucleus. The pro-acrosomal granule now disappears from view; at the same time the contents of the vacuole, which hitherto seemed to be watery, now become dense and firm. Consequently the vacuole begins to stain slightly and forms the acrosome (Pl. II, figs. 28 and 30 to 40). The acrosome spreads out considerably and covers almost the whole of the hook-shaped nucleus except a small posterior region. Simultaneously with these changes, the nucleus begins to elongate and stain homogeneously

(Pl. II, fig. 28). With further elongation the nuclear contents seem to get more and more condensed, with the result that the nucleus stains deeply and homogeneously in the later stages of spermateleosis.

The extra-idiosomic Golgi granules in the later stages of spermateleosis become highly stainable and resistant to the action of acetic acid (Pl. II, figs. 24 to 28). Along with these extra-idiosomic Golgi granules the Golgi-idiosomic remnant, now staining very poorly, is also present.

I have seen the axial filament originating from a granular centriole (lying on the surface of the nucleus) in an early spermatid (Pl. I, fig. 9). Later on, both the proximal and distal centrioles, which are granular, make their appearance. The distal centriole then becomes ring-like and travels down the axial filament (Pl. II, figs. 29, 32 and 34). It marks the posterior extremity of the middle-piece.

A clearer and denser area of the cytoplasm is differentiated behind the elongated and condensed nucleus. This is the 'manchette' (Pl. II, fig. 31), which marks the lateral boundaries of the middle-piece.

The mitochondria are fine and lightly-staining granules. They remain uniformly scattered throughout the cytoplasm up to quite a late stage in spermatogenesis, when they start gathering around the axial filament in the region of the middle-piece. As these granules lie very close together in the middle-piece, they appear to be bigger in size. The anterior and posterior ends of the middle-piece are limited by the proximal and distal centrioles respectively (Pl. II, figs. 32 to 37).

Quite early in spermateleosis the nucleus of the spermatid gets elongated and condensed, and some clear vacuoles make their appearance in the cytoplasm (Pl. II, figs. 30 and 31). These vacuoles, later on, coalesce and form bigger vacuoles, which are ultimately sloughed off along with the idiosome remnant and the extra-idiosomic Golgi granules.

The residual cytoplasm, when it is to be sloughed off, becomes divided into two prominent blebs. One of these blebs is sloughed off from the anterior end of the sperm head and the second from the posterior end along the axial filament. This process of sloughing off has been seen by me in the fixed as well as in the living material studied under the phase-contrast microscope (Pl. II, figs. 38 and 39).

The mature sperm of the white rat has an elongated and hook-shaped head, a long tail and a very small neck in between them. The head consists of a nucleus and an acrosome. The acrosome, in the form of a thin sheath, covers almost the whole of the nucleus except a little portion at the posterior end. The neck is a very small portion of the cytoplasm connecting the head with the middle-piece. The middle-piece is followed by a short main-piece in which region the axial filament seems to be covered by a very thin cytoplasmic sheath. Lastly there is the end-piece, where the axial filament seems to be naked.

Living material under the Phase-contrast Microscope.

The early primary spermatocytes are small cells (Pl. III, photo. I). The Golgi elements are granular and are scattered throughout the cytoplasm. They appear as dark, refractile granules. The mitochondria normally

appear to be very fine granules but, in this cell, they are exceptionally bigger in size. Under the phase-contrast they appear much less refractile than the Golgi elements. Plate I, figure 2 represents the corresponding stage of the primary spermatocyte in the fixed material.

In the secondary spermatocyte the chromophobic idiosome, with the Golgi granules embedded in it, is observed near the membrane of the nucleus. Some of the extra-idiosomic Golgi granules can also be observed. The mitochondria are uniformly distributed. They appear to be slightly brownish in colour under the phase-contrast microscope (Pl. III, photo. 2).

In an early spermatid (Pl. III, photo. 4 corresponding to fig. 8, Pl. I) the nucleus is small and condensed. It lies towards one side of the cell. The Golgi granules are embedded in the idiosome, which lies close to the nucleus. The Golgi granules in the idiosome are very clearly seen in Plate III, photo. 3. The mitochondria are distributed uniformly throughout the cell.

In a later stage of spermateleosis (Pl. III, photo. 5 corresponding to fig. 22, Pl. I) the vacuole and the pro-acrosomal granule were clearly seen under the phase-contrast microscope. The margin of the vacuole together with the nuclear protrusion have come up as a dark line in the photomicrograph. The vacuole itself and the pro-acrosomal granule are out of focus. The idiosomic remnant (I.r) is present above the vacuole and is very prominent. The extra-idiosomic Golgi granules are seen at E.G. The mitochondria are uniformly distributed.

In a still later spermatid the nucleus, with the pro-acrosomal granule and the vacuole attached at its anterior end, was seen protruding out. The part of the vacuole protruding out has come out very well in the photomicrograph (Pl. III, photo. 6). The lower part of the vacuole and the nuclear protrusion are together represented by the thick dark line in the photomicrograph. The Golgi-idiosomic remnant is also seen.

The residual cytoplasm, before it is sloughed off, becomes divided into two blebs. One of these blebs is sloughed off from the anterior end of the sperm head and the second from the posterior side along the axial filament. The anterior bleb contains only a few of the extra-idiosomic Golgi granules. The posterior bleb contains the vacuoles, idiosomic remnants and the rest of the extra-idiosomic Golgi granules (Pl. III, photos. 7–10 corresponding to figs. 38 and 39, Pl. II).

Bundles of sperms were seen actively moving under the phase-contrast microscope. The parts of a mature sperm have been described earlier (Pl. II, fig. 40). Plate III, photos. 11 and 12 represent the head and a part of the middle-piece.

Discussion

Form of Golgi Elements and Acrosome Formation

The Golgi elements in the testicular material of the white rat are invariably in the form of granules. But sometimes a few Golgi vesicles, each with a chromophilic cortex and a chromophobic medulla, are also seen in the primary and secondary spermatocytes. In the later stages of

primary spermatocytes a chromophobic area, the idiosome, with some of the Golgi granules embedded in it, makes its appearance. This is the Golgi-idiosome complex.

In late spermateleosis the Golgi-idiosomic complex comes to lie near the nucleus. Now the Golgi granules in the idiosomic sphere move towards its periphery and form a thick homogeneous cortex. One of the Golgi granules, however, does not shift to the periphery, but remains lying in the chromophobic mass of the idiosomic sphere. This is the pro-acrosomal granule. The lower wall of the sphere, close to the nucleus, thins out and ultimately breaks down, with the result that the whole structure assumes the form of an interrogation mark. Later the pro-acrosomal granule with a small part of the chromophobic mass comes out of the idiosomic sphere and lies near the nucleus. This chromophobic fragment surrounding the pro-acrosomal granule is soon converted into a transparent vacuole, and grows considerably in size. The pro-acrosomal granule remains suspended in this transparent vacuole for some time. As the vacuole grows it depresses the nuclear membrane. Later the pro-acrosomal granule travels down and touches the nuclear membrane. As soon as it touches the nuclear membrane the latter grows underneath the vacuole with the pro-acrosomal granule at its tip. Later when the pro-acrosomal granule disappears completely, the contents of the vacuole begin to condense; consequently the vacuole now begins to take the stain. Ultimately the vacuole forms the acrosome.

Gresson (1942) and Gresson and Zlotnik (1945), working on the spermatogenesis of the mouse and the white rat respectively, have described the Golgi elements as rods and granules lying at the periphery of the idiosome (their archoplasm). Gresson (1942) concludes that 'the Golgi elements are, however, small and closely packed together around the archoplasm, making the observations of their shape extremely difficult'. Gresson (1950, 1950*a*), studying the living material of the mouse and the rat under the phase-contrast microscope, has also described the shape of the Golgi elements as thin dark rods and filaments surrounding a lighter area lying against the nucleus.

Sharma *et al.* (1953) have, however, clearly described in the guinea-pig that 'the real form of the Golgi apparatus is just granular'.

In the white rat also I have never come across, either in the living cells or in the fixed preparations, any Golgi rods or filaments. The form of the Golgi elements is always granular in the idiosomic region, although vesicles may sometimes be seen in the general cytoplasm.

Accessory bodies.—Gresson (1942) describes a Golgi granule in the neck region of the very late spermatids and in nearly ripe spermatozoa of the mouse. He states that 'not only was this substance traced from its origin, from the Golgi material which had moved away from the growing acrosome, but its appearance and reactions in Champy-kull, Aoyama and Kolatchev sections leave little doubt that it is composed of Golgi substance'. Gresson and Zlotnik (1945) also describe accessory bodies in the white rat, and state that they originate from the localized Golgi material of the spermatocyte, and spermatids.

Sharma *et al.* (1953) have interpreted the accessory bodies as those Golgi elements which do not participate in the formation of the pro-acrosome. I agree.

Nuclear Ring.—Gresson and Zlotnik (1945) have shown in the white rat the presence of a nuclear ring. They state that 'the nuclear ring arises on the nuclear membrane at the junction between the outer lower margin of the archoplasmic vacuole and the nucleus' and is argentophilic. I am unable to confirm these observations.

During my observations of the living as well as fixed material of the white rat I have never seen any nuclear ring. However, a faint margin of the vacuole around the nuclear protrusion is visible. Afterwards when the vacuole spreads over the nucleus this margin cannot be seen.

Post-nuclear Cap.—The so-called post-nuclear cap is in reality the posterior condensed region of the nucleus, as suggested by Friend (1936). This worker describes in the sperms of British Muridae an asymmetrical deeply-staining area in the posterior region of the sperm nucleus. He tried Feulgen's reagent and found that this posterior dense area gave a positive reaction. He names this area as the dense posterior region and compares its position with the post-nuclear cap of Gatenby.

Sharma *et al.* (1953), during their study of the guinea-pig sperm, have employed Feulgen's reaction on the smears and have concluded that 'the post-nuclear cap is nothing but a part of the nucleus, probably containing most of the dense chromatin material'. They have also studied Da Fano smears and they claim that 'silver was deposited on the posterior part of the nucleus of the sperm'.

Centrosomes.—I agree with Gresson and Zlotnik (1945) and Sharma *et al.* (1953) that there are two centrosomes, a proximal and a distal. They are granular in appearance in the beginning. But, later on, the distal centrosome becomes ring-like and shifts to the posterior side, where it forms the posterior boundary of the middle-piece.

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Explanation of Lettering in Plates

A—Acrosome; *A.f*—Axial filament; C_1—Proximal centriole; C_2—Distal centriole; *C.b*—Cytoplasmic bleb; $C.i_1$—Chromophobic mass of the idiosome; $C.i_2$—Chromophilic mass of the idiosome; *C.v*—Cytoplasmic vacuoles; *Ch*—Chromosomes; *E.G.*—Extra-idiosomic Golgi granules; G—Golgi elements; *I*—Idiosome; *I.r*—Idiosomic remnant; *M*—Mitochondria; *Ma*—Manchette; *M.p*—Middle-piece; *N*—Nucleus; *P.g*—Pro-acrosomal granule; *R.c*—Residual cytoplasm; *V*—Vacuole; *?*—Most probably C_2 dislocated during the section-cutting process.

Explanation of Plates I and II

All the figures in Plates I and II except Fig. 40 are drawn from the sectioned material of the testes of the white rat, which was fixed in Flemming-without-acetic acid and stained with 0·5% iron-hæmatoxylin. Fig. 40 is drawn from a smear fixed in F.W.A. diluted with an equal quantity of distilled water. All figures have been drawn with camera lucida at the table level using Beck 10× eye-piece and 2 mm. oil immersion objective, giving a magnification of 1,370 times.

Plate I

Fig. 1. An early spermatogonium.

Fig. 2. An early primary spermatocyte.

Fig. 3. Fully grown primary spermatocyte, showing the chromophobic idiosome with the Golgi granules embedded in it.

Fig. 4. Metaphase I (polar view).

Fig. 5. Early secondary spermatocyte, showing idiosome with the Golgi granules, extra-idiosomic Golgi granules and vesicles.

Fig. 6. Late secondary spermatocyte with almost all the Golgi granules embedded in the idiosome.

Fig. 7. An early spermatid.

Fig. 8. Spermatid with the idiosome very close to the nuclear membrane.

Fig. 9. Spermatid, showing the fused Golgi granules of the idiosome forming the deeply-staining and homogeneous cortex of the idiosomic sphere. A single Golgi granule is seen in the centre of the chromophobic medulla of the sphere. Some extra-idiosomic Golgi granules along with the lightly-staining and uniformly distributed mitochondria are also seen. Proximal centriole with the axial filament is lying over the nucleus.

Fig. 10. Spermatid with the idiosomic sphere attached to the nuclear membrane. The wall of the sphere close to the nucleus is thin and flattened.

Fig. 11. Spermatid, showing the breaking of the cortex of the idiosomic sphere. It resembles an interrogation sign (?).

Figs. 12 and 13. Spermatids, representing the separation of some portion of the chromophobic medulla with the pro-acrosomal granule from the rest of the idiosomic sphere.

Figs. 14 to 19. Spermatids, showing the transformation and growth of the spermatid chromophobic mass into a transparent vacuole. The pro-acrosomal granule is seen lying suspended in the vacuole. The vacuole grows and comes to lie in the depression of the nucleus (Figs. 16, 17 and 18). Fig. 19 is the polar view and shows the idiosomic remnant, vacuole and pro-acrosomal granule lying on the top.

Fig. 20. An undivided secondary spermatocyte, showing an advanced stage of the formation of the vacuoles attached in front of the two nuclei.

Fig. 21. Spermatid with the pro-acrosomal granule lying near the nuclear membrane in the vacuole. The idiosomic remnant lies on the side of the vacuole and the extra-idiosomic Golgi granules lie little away from the idiosomic remnant.

Fig. 22. Spermatid, showing the beginning of the cone-like projection of the nucleus into the vacuole with pro-acrosomal granule at its tip.

Plate II

Fig. 23. Spermatid, showing an advanced stage of the protrusion of the nucleus with the pro-acrosomal granule at its tip. The margin of the vacuole is also seen around the nuclear protrusion.

Figs. 24 to 26. Spermatids, showing the elongation of the nucleus with the pro-acrosomal granule and the vacuole at the tip. The axial filament with the proximal centriole is seen in figures 24 and 26. The Golgi remnants are seen in figures 25 and 26. The extra-idiosomic Golgi granules are present in all figures from 24 to 26.

Fig. 27. Spermatid, showing curvature in the nucleus. The vacuole and pro-acrosomal granule attached to the tip of the nucleus. The proximal centriole, axial filament, extra-idiosomic Golgi granules and idiosomic remnants are also seen.

Fig. 28. Spermatid, showing elongated nucleus with acrosome. The nucleus takes up light and homogeneous stain. It also shows proximal and distal granular centrioles with the axial filament, idiosomic remnant and extra-idiosomic Golgi granules.

Fig. 29. Spermatid, showing elongated and deeply-staining nucleus, proximal rounded and distal ring-like centriole and axial filament.

Fig. 30. Spermatid, showing sheath-like acrosome and a big vacuole.

Fig. 31. Spermatid with elongated, curved and dense nucleus, acrosome, manchette and vacuoles.

Figs. 32 to 34. Spermatids, each showing the elongation of the cytoplasm of the cell. In stages 33 and 34 mitochondria are gathering near the axial filament.

Figs. 35 to 37. Spermatids, each showing hook-shaped nucleus, acrosome, middle-piece and residual cytoplasm with a vacuole and extra-idiosomic Golgi granules. In Fig. 37 axial filament at the end of the middle-piece is also visible.

Fig. 38. Sperm, showing two blebs, one being sloughed off from the anterior end and small one on the middle-piece.

Fig. 39. Sperm with anterior bleb about to be sloughed off.

Fig. 40. Mature sperm.

Explanation of Photomicrographs

All the photomicrographs were taken with a Leica camera and Leitz Dialux phase-contrast microscope, either under 2 mm./1·32 oil immersion or a 40 : 1 (Apochromatic dry system) objective and 10 × Periplanatic eye-piece, giving a magnification of 1,125 times or 500 times respectively. All the photomicrographs are further enlarged four times and are untouched.

Plate III

Photomicrograph 1. Early primary spermatocyte, showing the Golgi granules distributed throughout the cytoplasm. The mitochondria are exceptionally bigger in size.

„ 2. Secondary spermatocyte, representing the coming together of the Golgi granules into the chromophobic mass near the nuclear membrane.

Photomicrographs 3 and 4. Spermatids, showing the idiosome with the Golgi granules. They are very clear in photo. 3. In photo. 4 idiosomic complex is touching the nuclear membrane.

Photomicrograph 5. Spermatid, showing idiosome remnant, extra-idiosomic Golgi granules and margin of the vacuole. The nuclear protrusion with pro-acrosomal granule and the vacuole are not in focus.

„ 6. Spermatid, showing the nucleus with the vacuole and pro-acrosomal granule. Vacuole is projecting forward and appears shaded due to the different focus. The idiosomic remnant is lying behind the nucleus.

Photomicrographs 7 to 10. Photomicrographs 7 and 9 show two blebs on each of the sperms. The anterior bleb shows some extra-idiosomic Golgi granules. The posterior bleb of photomicrograph 7 shows idiosomic remnant, extra-idiosomic Golgi granules and vacuoles. Photomicrograph 9 shows a small bleb with idiosomic remnant on the middle-piece. Photomicrograph 8 represents single posterior bleb with very prominent idiosomic remnant and vacuole. Photomicrograph 10 showing anterior bleb only.

Photomicrograph 11. Enlarged head of the sperm with very clear acrosome.

" 12. Mature sperm showing nucleus, acrosome, neck and middle-piece.

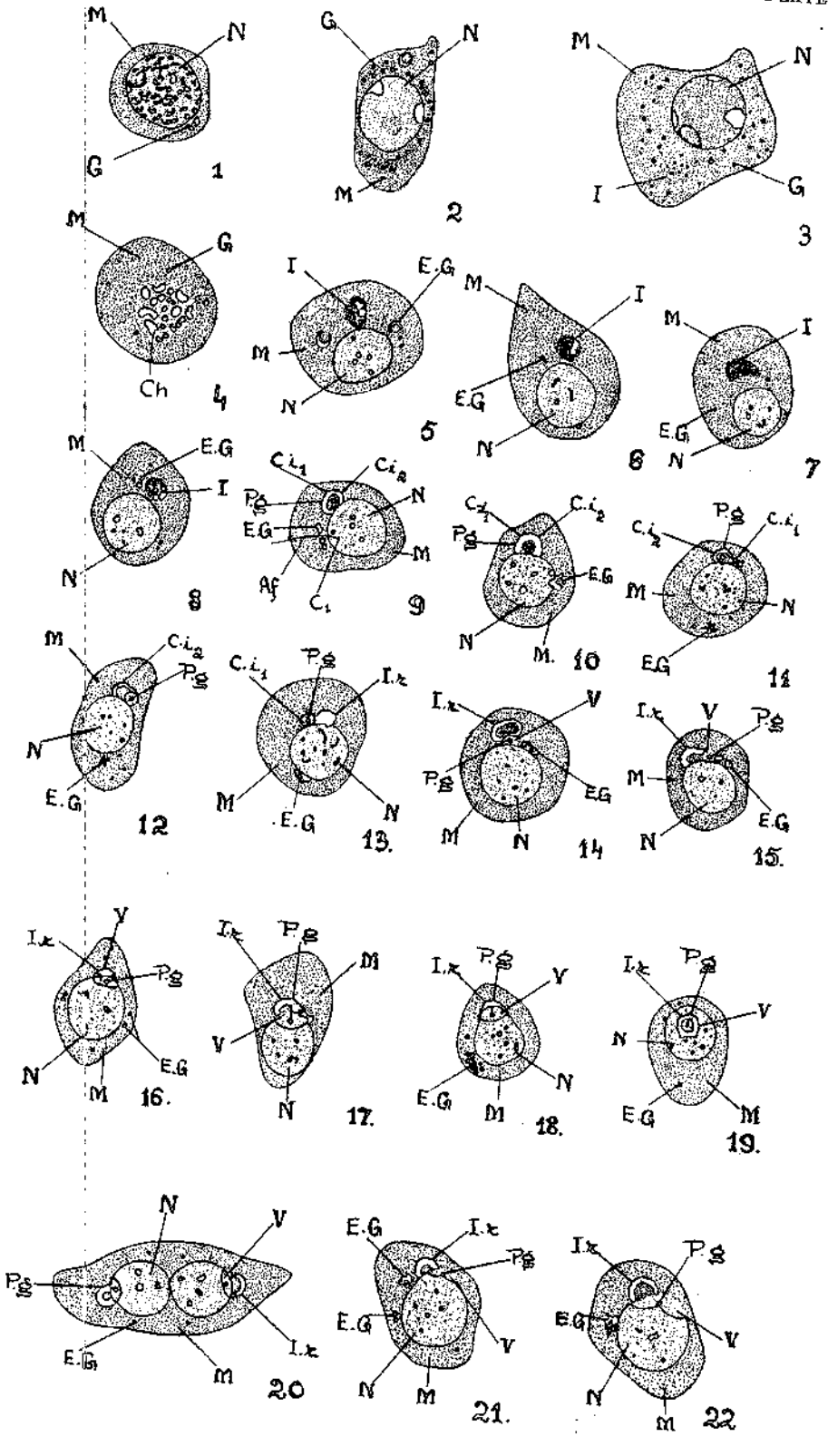
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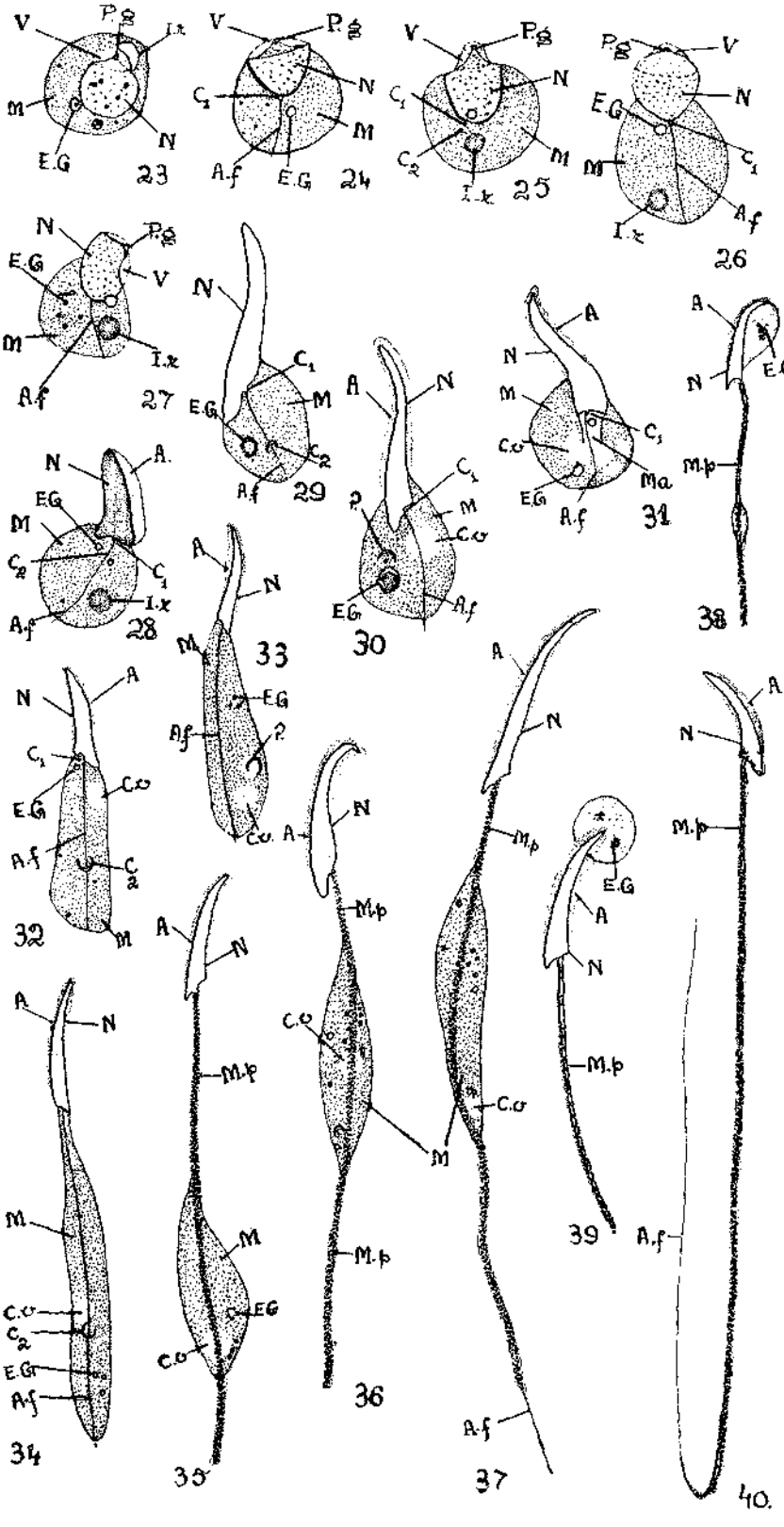
PLATE II.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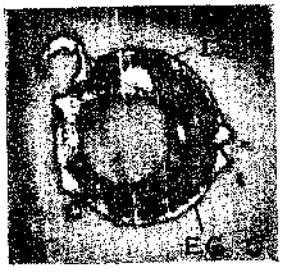

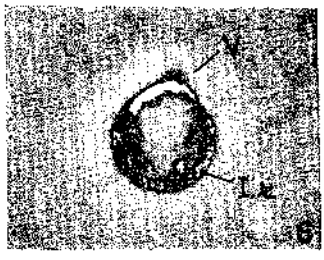

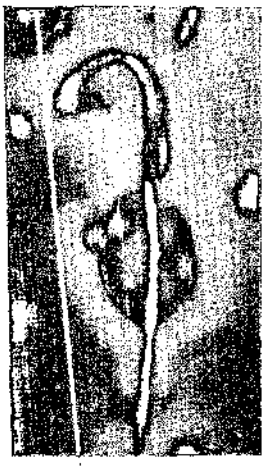

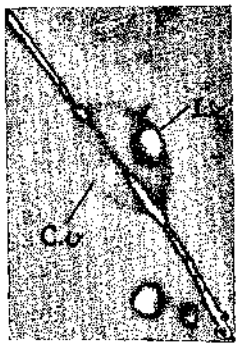

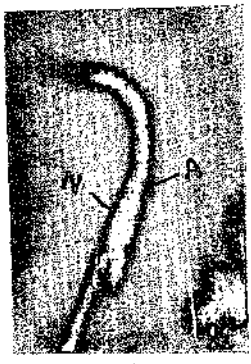

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SPERMATOGENESIS OF THE DOMESTIC DUCK WITH OBSERVATIONS ON LIVING MATERIAL UNDER THE PHASE-CONTRAST MICROSCOPE

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Abstract

The spermatogenesis of the domestic duck has been studied in the fixed material, and the living material under the phase-contrast microscope. Some of the important conclusions are: (*a*) the acrosome is formed directly by the hyaline pro-acrosome, which, in turn, is formed by the direct fusion of all the Golgi granules and spheroids in the earlier stages; (*b*) the Golgi elements differentiate, for the first time, from the mitochondria in the secondary spermatogonium; (*c*) a chromatoid body is present in all the stages of spermatogenesis but it is sloughed off in spermateleosis without contributing anything to the mature sperm.

Introduction

Very few cytologists appear to have paid any attention to the avian spermatogenesis. Although Brunn (1884), Guyer (1909, *a* and *b*), and Miller (1938) have studied the process of sperm formation in birds, none of them has paid any special attention to the cytoplasmic components of these cells. Zlotnik (1947), who worked out the spermatogenesis of domestic fowl, seems to be the only worker to have devoted himself to the study of the cytoplasmic inclusions in avian sperm cells.

In this paper Zlotnik describes the Golgi material in the form of 'a localized body, composed of rods and granules which lie on the surface of the archoplasm'. In the spermatid 'the Golgi material moves away from the nuclear membrane, revealing a clear area—the archoplasmic vacuole which is closely applied to the anterior pole of the nucleus'. The formation of the conical acrosome is described from the pro-acrosome which, in turn, originates 'within the archoplasmic vacuole inside the Golgi material of the spermatid'. The Golgi material, after the acrosome formation, migrates to the residual cytoplasm and is eliminated as the Golgi remnant.

Zlotnik also describes one or more 'accessory bodies' taking a deep stain in chrome-osmium preparations and believes that they originate from the Golgi material. In the spermatids of Aoyama-fixed preparations he describes one or more argentophil granules, homologous with 'accessory bodies'. He has named them as 'Golgi-X'; but he could not identify them in chrome-osmium preparations. One of the 'Golgi-X' is shown to take its position at the base of the nucleus of the mature sperm.

The present paper on the spermatogenesis of the domestic duck is the first of the series which the author intends publishing on the subject of avian spermatogenesis.

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Material and Technique

The testes of the domestic duck were dissected out from the animal killed by chopping off its head, and were placed in Ringer's fluid. Very small pieces of the testicular tissue were fixed in various fixatives like Flemming-without-acetic (both normal strength and diluted), Champy, Bouin and Sanfelice, the last two being used as control. Kolatchev method of osmication, with preliminary fixation in Champy and post-osmication in 2% osmic acid solution for three to six days at 37°C, sudan black method (Thomas, 1948) and Aoyama's silver nitrate techniques were used for the specific study of the Golgi material. Champy-kull's acid-fuchsin technique for mitochondria and the Feulgen's reaction for desoxyribose nucleic acids (D.N.A.) were also tried.

Paraffin wax was employed for embedding the tissue and sections were cut at 5 to 8 micra in thickness. Heidenhain's iron-haematoxylin was used for staining the sections.

Champy-haematoxylin, Kolatchev, sudan black and Feulgen gave the most satisfactory results. My observations are based mostly on Champy-haematoxylin preparations, as they present a clear picture of all the cell inclusions without introducing any artefacts.

Special attention was paid to the study of the living material under the phase-contrast microscope. Ringer's fluid (Darlington and La Cour, 1947) was used as the liquid medium for vital studies.

Observations

Spermatogonia.—There are two categories of spermatogonia in the domestic duck, primary and secondary. The primary spermatogonium is a comparatively small cell with a nucleus showing fine chromatin granules and a few large chromatin nucleoli in the nucleoplasm (Pl. I, fig. 1). The Cytoplasm reveals a few greyish granules, the mitochondria, and quite a large chromophilic body, the chromatoid body, generally surrounded by a clear space. The chromatoid body is highly resistant to acetic acid and takes up a very deep haematoxylin stain in Sanfelice and Bouin preparations (Pl. I, fig. 2). It is completely osmiophobic in Kolatchev and Champy unstained preparations. Nor does it stain with sudan black. It can very easily be made out in the living cells under the ordinary microscope. Under the phase-contrast it appears as a pale body in sharp contrast with the dark Golgi bodies, which appear at a later stage (Pl. III, fig. 60).

The secondary spermatogonium is a larger cell, with a larger vesicular nucleus (Pl. I, fig. 3). In addition to the chromatoid body the cytoplasm now shows quite a prominent juxta-nuclear mass of mitochondrial granules with a few larger dark granules, the Golgi elements, lying amongst them. The Golgi elements appear to be differentiated for the first time from the mitochondria of the secondary spermatogonium. Both the Golgi and the mitochondrial granules increase in number and become circumnuclear in their disposition at a later gonial stage (Pl. I, fig. 4).

The fine mitochondrial granules become evenly distributed in the cytoplasm during the final gonial division, with the Golgi elements scattered amongst them (Pl. I, fig. 5). A faintly staining, fibrillar spindle appears between the two granular centrosomes (Pl. I, fig. 6). The single large chromatoid body is seen generally lying away from the spindle area, which is also devoid of Golgi bodies and the mitochondria. Judging from the fact that the two daughter cells resulting from a gonial division always possess each a chromatoid body, the chromatoid body seems to divide into two during every gonial division; but the actual division has not been observed by me.

Spermatocytes.—The earliest primary spermatocyte is about the same size as the secondary spermatogonium. Both the Golgi elements and the mitochondria form a dense juxta-nuclear mass at this stage (Pl. I, fig. 7); also a large round or oval chromatoid body is always present. With the onset of the meiotic prophase, however, the Golgi elements and the mitochondria begin to disperse; one Golgi granule has already separated from the juxta-nuclear mass at the synizesis stage (Pl. I, fig. 8).

During the growth period, which follows the synizesis stage, the cytoplasm of the cell grows considerably in volume. The mitochondria slowly disperse, and ultimately become evenly distributed throughout the cytoplasm of the pachytene spermatocyte (Pl. I, figs. 9, 10, 13 and 14). The Golgi granules increase in number and also become dispersed in the cytoplasm. Some of the Golgi granules appear to grow into spheroids, which may now show a duplex structure with an osmiophilic externum and an osmiophobic internum in both the Kolatchev and Champy preparations (Pl. I, fig. 12).

In the living spermatocytes, under the phase-contrast microscope, also the Golgi elements are in the form of discrete granules and spheroids, appearing dark and shining against the dull evenly distributed mitochondria. Some of the bigger spheroids occasionally show a double structure, with a dark cortex surrounding a light medulla.

The chromatoid body may show a slight degree of fragmentation in some of the cells in the diplotene stage as figured in fig. 15, Pl. I.

With the advent of metaphase I the nuclear membrane is dissolved and a comparatively small, fibrillar spindle appears between the two centrosomes (Pl. I, fig. 16). The mitochondria and the Golgi elements remain dispersed throughout the cytoplasm in all the later stages of meiosis I (Pl. I, figs. 16 to 18). The single chromatoid body of the earlier spermatocytes divides into two and each of them is seen lying on either side of the cell (Pl. I, fig. 17). During telophase one of the chromatoid bodies moves

to the other pole of the cell, and consequently each of the two secondary spermatocytes receives a single chromatoid body (Pl. I, fig. 18).

Some syncytial cytoplasmic masses can generally be seen with two or four nuclei in the various stages of meiosis (Pl. II, fig. 19). These stages do not appear to be abnormal in any way, and lead to the formation of normal spermatozoa.

The secondary spermatocyte is a much smaller cell than the fully grown primary spermatocyte, with a smaller ex-centric nucleus revealing a number of chromatin nucleoli at the resting stage (Pl. II, figs. 20 to 22). The mitochondria, which have a tendency to come together at the earlier stage (Pl. II, fig. 20), become evenly distributed in the cytoplasm of the late secondary spermatocytes (Pl. II, figs. 21 and 22). The Golgi granules now show a tendency of coming together and forming larger spheroids, some of which may show a duplex structure (Pl. II, fig. 20), and consequently become fewer in the later stages. They, however, show no localization, whatsoever, up to the late spermatid stages. The chromatoid body may be single (Pl. II, fig. 21), or it may consist of two smaller bodies formed by the division of the originally single body (Pl. II, fig. 22).

The distribution of the cytoplasmic components in meiosis II is similar to meiosis II (Pl. I, fig. 23).

Spermateleosis.—The spermatid is a comparatively much smaller cell with a spherical nucleus (Pl. II, fig. 24). In the earlier stages the nucleus reveals a number of fine chromatin nucleoli in the nucleoplasm (Pl. II, figs. 24 to 41; Pl. III, fig. 61), but after the acrosome has been formed the nucleus becomes cylindrical and shows lightly staining chromatin bands (Pl. III, fig. 44). With further advance in the process of spermateleosis the nucleus becomes more chromatic and it also becomes elongated. But, as the cytoplasm does not grow correspondingly, the nucleus becomes coiled up and shows various configurations (Pl. III, figs. 45 to 49). Gradually the cytoplasm also elongates and the deeply staining, coiled nucleus becomes straightened up, bearing a conical acrosome at its tip (Pl. III, figs. 49 and 50). The cytoplasm now begins to move downwards along the nucleus and is later sloughed off. The nucleus contracts slightly during the middle-piece formation and also loses its staining capacity (Pl. III, figs. 56 to 58).

The Golgi elements of the earliest spermatid are in the form of discrete deeply staining granules of varying size lying amongst the mitochondria (Pl. II, fig. 24). These Golgi granules show a distinct tendency of coming together and forming larger granules or spheroids, the latter revealing a duplex structure in both Champy and Kolatchev preparations (Pl. II, figs. 28 and 29). A little later these Golgi granules and spheroids become localized on one side of the nucleus, presumably the anterior side (Pl. II, fig. 30), and gradually fuse together to form a large round or oval chromophilic body—the pro-acrosome (Pl. II, fig. 31). It appears that all the Golgi elements take part in the formation of the pro-acrosome, as no Golgi remnants could be located in any of the late spermatid stages studied in Kolatchev, Champy unstained and sudan black preparations. In the living material under the phase-contrast microscope also, no Golgi remnants could be detected.

This single large pro-acrosome, which is chromophilic to begin with, attaches itself to one side of the nucleus, and is now seen to have a prominent chromophilic cortex and a chromophobic medulla (Pl. II, figs. 32 and 33). Gradually the pro-acrosome is transformed into a completely chromophobic, hyaline vacuole having glue-like consistency (Pl. II, fig. 38). It will thus appear that in this process of transformation of the pro-acrosome into the vacuole the chromophobic substance appears to grow at the expense of the chromophilic externum, which, becoming less and less stainable, ultimately disappears completely (Pl. II, figs. 34 and 35).

The hyaline pro-acrosome now begins to grow and becomes quite large (Pl. II, figs. 35 to 39). It is very prominent and appears like a vacuole in the living spermatids under the phase-contrast microscope (Pl. III, fig. 61). During all these stages the pro-acrosome remains applied to the nuclear membrane at its anterior aspect. In later stages it exerts a pressure upon the nuclear membrane, which it depresses slightly (Pl. II, fig. 40).

The chromatoid body may be seen lying quite close to the pro-acrosome in some spermatids, but this is purely accidental.

A little later the hyaline pro-acrosome becomes triangular and contracts in size to form the acrosome directly (Pl. II, fig. 41; Pl. III, figs. 42 and 43). A deeply-staining granule now appears at the apex of the acrosome triangle. During the earlier stages of the elongation of the nucleus, the acrosome is quite short and faint, but becomes conical and pointed later on (Pl. III, figs. 45 to 48). When the nucleus is straightened up the condensation of the acrosome starts from the dark granule at the tip of the acrosome and proceeds downwards (Pl. III, figs. 51 to 55). The acrosome of the mature sperm becomes cylindrical and takes up a deep stain (Pl. III, fig. 59); it is hardly distinguishable from the nucleus in the living sperm (Pl. III, fig. 62).

In the early spermatids the mitochondria are in the form of fine granules distributed evenly in the cytoplasm (Pl. II, fig. 24). They generally remain uniformly distributed till the nucleus begins to elongate but sometimes they become aggregated (Pl. II, figs. 25, 38 and 39).

When the nucleus of the spermatid elongates, the mitochondria show an increase in the size of the individual granules. Judging from the appreciable decrease in the number of mitochondrial granules, this seems to be brought about by a process of fusion of smaller granules (Pl. III, figs. 47 to 53). In some cases, however, the fusion of mitochondrial granules is precocious (Pl. II, figs. 31, 35 and 40; Pl. III, fig. 43).

As the mitochondrial granules are growing larger, a clear space appears in the cytoplasm around the axial-filament, just at the base of the elongated nucleus (Pl. III, fig. 54). This space, which is triangular to begin with, becomes elongated and narrow, and surrounds almost the whole of the intra-cellular portion of the axial filament (Pl. III, figs. 55 and 56). This space marks the boundaries of the future middle-piece. A little later, when the nucleus loses its chromaticity, the mitochondrial granules bodily enter this clear space around the axial filament and form the middle-piece of the mature sperm (Pl. III, figs. 57 and 58). All the mitochondria take part in the formation of the middle-piece, as no remnants have been seen in the

residual cytoplasm. The middle-piece of the mature sperm is comparatively small, and is mulberry-shaped (Pl. III, figs. 59 and 62).

The single centrosome of the early spermatid divides into two at a later stage, but being granular they become lost to view amongst the mitochondria. But, once, the axial filament has appeared, the two centrosomes can be easily seen (Pl. II, fig. 27). A little later the proximal centrosome takes up its position just below the nucleus (Pl. II, fig. 41) and, with the elongation of the latter, becomes embedded in its posterior end. It cannot be made out now as the nucleus takes up a deep stain; but later when the nucleus loses its staining capacity, the proximal centrosome can be seen at the posterior tip of the nucleus (Pl. III, fig. 56). The distal centrosome, which also retains its granular form, gives rise to a thin and long extracellular axial filament, which is in continuation with the intra-cellular axial filament of the earlier spermatid (Pl. II, fig. 41). The distal centrosome forms the lower limit of the middle-piece in later stages (Pl. III, figs. 57 to 59).

The nucleus of the mature spermatozoon is narrow and cylindrical and stains lightly in sectioned Champy-fixed material, but stains deeply and homogeneously with Feulgen. It is also shorter in length than the nucleus of the earlier spermatids. The acrosome is short and cylindrical, takes a deep-stain in fixed material, but is hardly distinguishable from the nucleus in the living material under the phase-contrast. The middle-piece is small, mulberry-shaped, and tapers towards the posterior end. The axial filament is thin and long (Pl. III, figs. 59 and 62).

Discussion

(a) Golgi elements.—The Golgi elements are in the form of discrete sudanophil and osmiophil granules in the secondary spermatogonia, the spermatocytes and the early spermatids of the domestic duck. In later stages these Golgi granules show a tendency of coming together and forming larger granules or spheroids, some of which may show a duplex structure with chromophilic cortex and chromophobic medulla in both the fixed and the living material. In the spermatids these Golgi granules and spheroids fuse together to form a single chromophilic round or oval pro-acrosome. This attaches itself to the anterior aspect of the nucleus and is gradually transformed into a completely chromophobic hyaline vacuole, which later directly forms the acrosome.

Zlotnik (1947), using mainly Aoyama's silver nitrate technique, describes the Golgi elements during the spermatogenesis of the domestic fowl 'in the form of a localized body, composed of rods and granules which lie on the surface of the archoplasm'. This localized Golgi material, according to Zlotnik, becomes dispersed into a number of rods and granules during the maturation divisions but again becomes localized after each such division.

It has been repeatedly pointed out by Nath (1944, 1955) and Baker (1949, 1953) that the silver nitrate and long osmication methods for demonstrating the Golgi elements tend to introduce artefacts and do not

present a life-like picture of the various cell constituents. My observations with Champy-hæmatoxylin, sudan black and even Kolatchev methods, always revealed the Golgi elements in the form of discrete granules and spheroids, the latter occasionally showing a duplex structure. The Golgi material invariably appears in this form in the living material under the phase-contrast microscope. Thus it appears that the dense, localized form of the Golgi material, as described by Zlotnik, may be due to the excessive deposition of silver and osmium hydroxide in the cells.

Zlotnik (1947) also describes in the domestic fowl a more or less mammalian type of acrosome formation. The pro-acrosome in the domestic fowl originates, according to Zlotnik, 'within the archoplasmic vacuole inside the Golgi material of the spermatid', presumably as a secretion product. This pro-acrosome along with the vacuole forms the acrosome, while the Golgi material is eliminated.

In the domestic duck, on the contrary, it will be recalled that the vacuole that appears during the process of acrosome formation, unlike the archoplasmic vacuole of Zlotnik, is formed directly from the large pro-acrosome, which in turn has been earlier formed by the direct fusion of the Golgi elements. Consequently there are no Golgi remnants.

The details of acrosome formation in the domestic duck are very interesting. The hyaline vacuole-like pro-acrosome first becomes triangular. A dark granule now appears at the tip of the acrosomal triangle before the nucleus begins to elongate. The condensation of the acrosome in the later stages starts from this granule and travels downwards. This granule, however, cannot be homologized with the pro-acrosome of the fowl; it merely represents a feature in the ripening of the pro-acrosome into the acrosome.

Regarding the origin of the Golgi elements in the present material, it seems that the Golgi granules, which appear for the first time in the secondary spermatogonium, are differentiated, like the 'Presubstance' of Hirsch (1939), from the mitochondria. Some of these Golgi granules fuse to form spheroids which, like the 'Golgi system' of Hirsch (1939), show each a chromophilic 'externum' and a chromophobic 'internum'. The chromophilic part of the Golgi elements, however, is also transformed into the chromophobic substance of the pro-acrosomal vacuoles during the acrosome formation.

(*b*) *Chromatoid body.*—A single large, round or oval body, which stains deeply in Champy-hæmatoxylin preparations, is seen in the spermatogonia, the spermatocytes and the spermatids of the domestic duck. This body divides into two at the time of the cell divisions and consequently a single body is always passed on to a daughter cell. It does not contribute anything to the structure of the mature sperm, as it is sloughed off with the residual cytoplasm during the concluding stages of the spermateleosis.

The chromatoid body stains deeply in Champy-hæmatoxylin preparations, is highly resistant to acetic acid, and takes up a deep acid fuchsin stain in Champy-kull preparations. It does not go black in Aoyama, Kolatchev and Champy unstained preparations; remains unstained in sudan black; appears pale in the living cells under the phase-contrast; and finally

it is Feulgen negative. Thus it is strictly homologous with the so-called 'Golgi body' described by Srivastava (1953) in the slug, *Vaginula*, and discussed by Nath and Chopra (1955), and by Sud (1955) in the snake, *Natrix p. piscator*.

(c) *Mitochondria and Centrosomes.*—The general behaviour of the mitochondria, nucleus and the centrosomes is similar to their behaviour in the domestic fowl as described by Zlotnik (1947). The mitochondria, however, appear as fine granules up to the late spermatid stages of duck and are not large and spherical as described by Zlotnik in fowl.

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Explanation of Lettering in Plates

A—Acrosome; *A.f*—Axial-filament; *C*—Centrosome; C_1—Proximal centrosome; C_2—Distal centrosome; *Ch*—Chromosomes; *C.b*—Chromatoid body; *G*—Golgi elements; *M*—Mitochondria; *M.P*—Middle-piece; *m.p*—Main-piece; *N*—Nucleus; *n*—Nucleolus; *Pa*—Pro-acrosome.

Explanation of Figures and Plates

The figures 1 to 59 have been drawn from Champy-hæmatoxylin preparations, except where otherwise mentioned; with a Spencer 1·8 oil immersion objective, 10× ocular and Beck Camera lucida. The figures have been drawn at table level, giving a total magnification of approximately 1,750 times.

Figures 60 to 62 are the photomicrographs of the living material taken by a Leica camera under Leitz Dialux phase-contrast microscope, with a 40 : 1 (Apochromatic dry system) objective and 10× Periplanatic eye-piece, giving a magnification of 500 times. The photomicrographs were further enlarged 4 times and are untouched.

Plate I

FIG. 1. Primary Spermatogonium showing a large chromatoid body and fine mitochondria.

FIG. 2. Primary spermatogonium showing the chromatoid body (Sanfelice-hæmatoxylin).

FIGS. 3. and 4. Secondary spermatogonia showing Golgi elements, mitochondria and the chromatoid body.

FIG. 5. Polar view of gonial metaphase.

FIG. 6. Side-view of gonial telophase.

FIG. 7. Earliest primary spermatocyte showing the juxta-nuclear mass of the mitochondria and the Golgi elements; and the chromatoid body.

FIG. 8. Primary spermatocyte during synizesis stage.

FIGS. 9, 10 and 13. Primary spermatocytes during the growth period, showing the growth and dispersal of the Golgi elements and the mitochondria.

FIG. 11. Primary spermatocyte showing the chromatoid body (Bouin-hæmatoxylin).

FIG. 12. Primary spermatocyte from Kolatchev preparation, showing the Golgi granules and spheroid and osmiophobic chromatoid body.

FIG. 14. Fully grown primary spermatocyte in pachytene stage.

FIG. 15. Primary spermatocyte in diplotene stage, showing the fragmentation of the chromatoid body (Sanfelice-hæmatoxylin).

FIG. 16. Metaphase I, side-view.

FIG. 17. Metaphase I, side-view showing two chromatoid bodies.

FIG. 18. Side-view of the telophase I, showing one of the chromatoid bodies moving to the other pole.

Plate II

FIG. 19. Syncytium showing four primary spermatocyte nuclei in leptotene stage.

FIG. 20. Early secondary spermatocyte showing partially aggregated mitochondria.

FIG. 21. Late secondary spermatocyte showing fully dispersed mitochondria and the Golgi elements, and the chromatoid body.

FIG. 22. Late secondary spermatocyte with two chromatoid bodies.

FIG. 23. Side-view of the metaphase II.

FIGS. 24 and 25. Early spermatids.

FIG. 26. Spermatid showing the chromatoid body (Sanfelice-hæmatoxylin).

FIG. 27. Spermatid showing the proximal and the distal centrosomes with an intracellular axial-filament, and the Golgi granules coming together.

FIG. 28. Spermatid showing a large Golgi spheroid with a duplex structure, and a few smaller Golgi granules.

FIG. 29. Spermatid from the Kolatchev preparation showing osmiophobic chromatoid body.

FIG. 30. Spermatid from the Kolatchev preparation showing all the Golgi elements coming together to form the pro-acrosome.

FIG. 31. Spermatid showing a large ovoid chromophilic pro-acrosome.

FIG. 32. Spermatid with the pro-acrosome applied to the nucleus and revealing a duplex structure.

FIG. 33. Same as in fig. 32, from a Kolatchev preparation.

FIGS. 34 to 39. Spermatids showing the growth of the hyaline pro-acrosome.

FIG. 40. Spermatid showing the hyaline pro-acrosome pressing upon the nuclear membrane.

FIG. 41. Late spermatid showing the triangular pro-acrosome, two centrosomes and the axial-filament.

Plate III

FIGS. 42 and 43. Late spermatids showing the pro-acrosome forming the acrosome.

FIG. 44. Late spermatid with a cylindrical nucleus bearing a lightly staining acrosome at its tip.

FIGS. 45 and 46. Late spermatids showing the elongation and coiling of the nucleus and the partially condensed triangular acrosome.

FIGS. 47 to 50. Late spermatids showing straightening of the nucleus and the elongation of the cytoplasm. Axial-filaments are also clear.

FIGS. 51 to 53. Late spermatids with the cytoplasm drifting downwards along the nucleus.

FIG. 54. Late spermatid showing a clear triangular space appearing below the nucleus.

FIG. 55. The clear space has elongated.

FIG. 56. Very late spermatid showing the fully condensed acrosome; the nucleus loses its chromaticity.

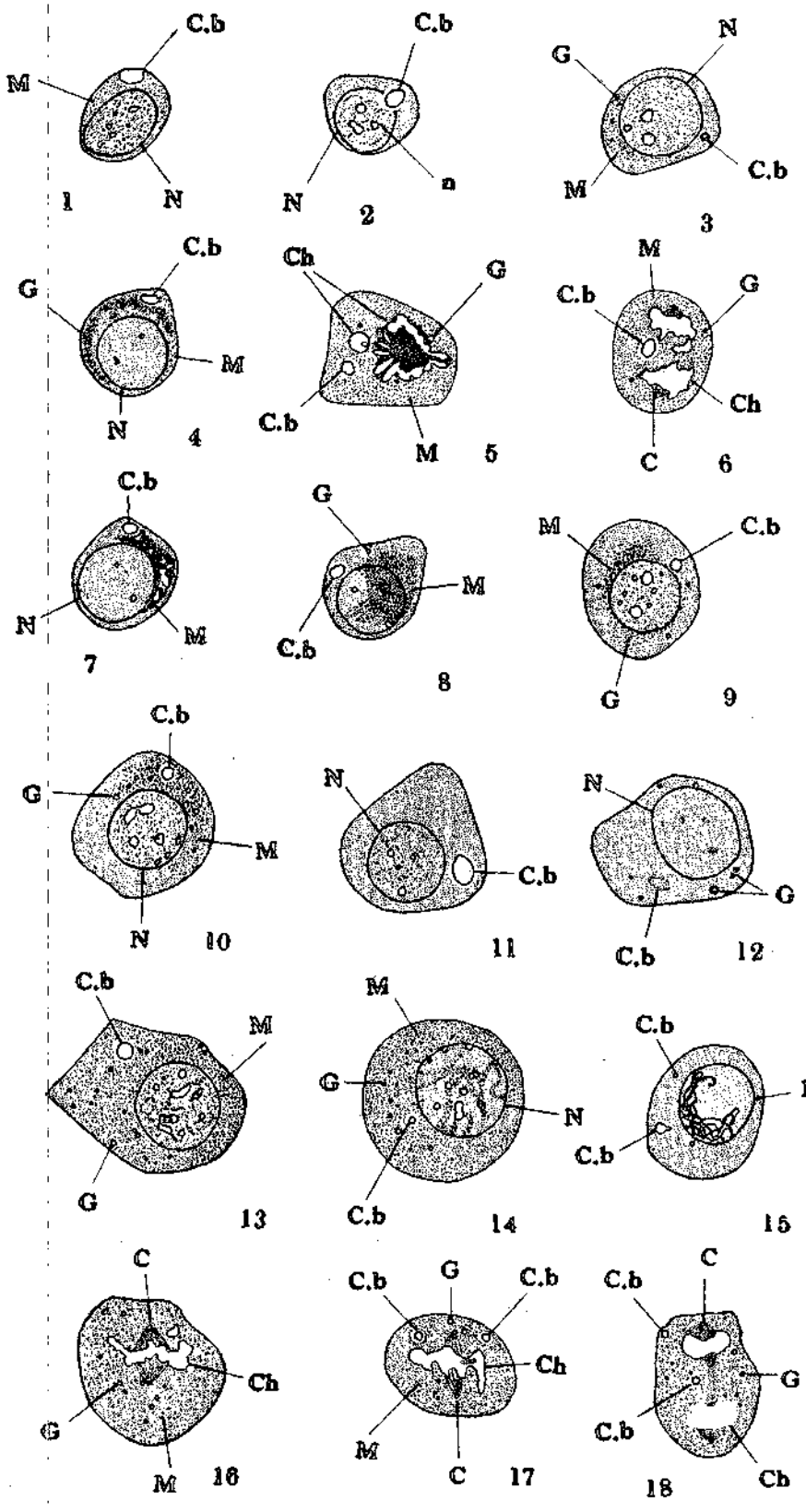
FIGS. 57 and 58. Very late spermatids showing the mitochondria entering the clear space to form the middle-piece. Chromatoid body remains in the cytoplasm.

FIG. 59. Fully mature spermatozoon.

FIG. 60. Photomicrograph of the living primary spermatogonium showing the chromatoid body.

FIG. 61. Photomicrograph of the living spermatid showing the hyaline pro-acrosome, mitochondrial granules and the chromatoid body.

FIG. 62. Photomicrograph of the living sperm.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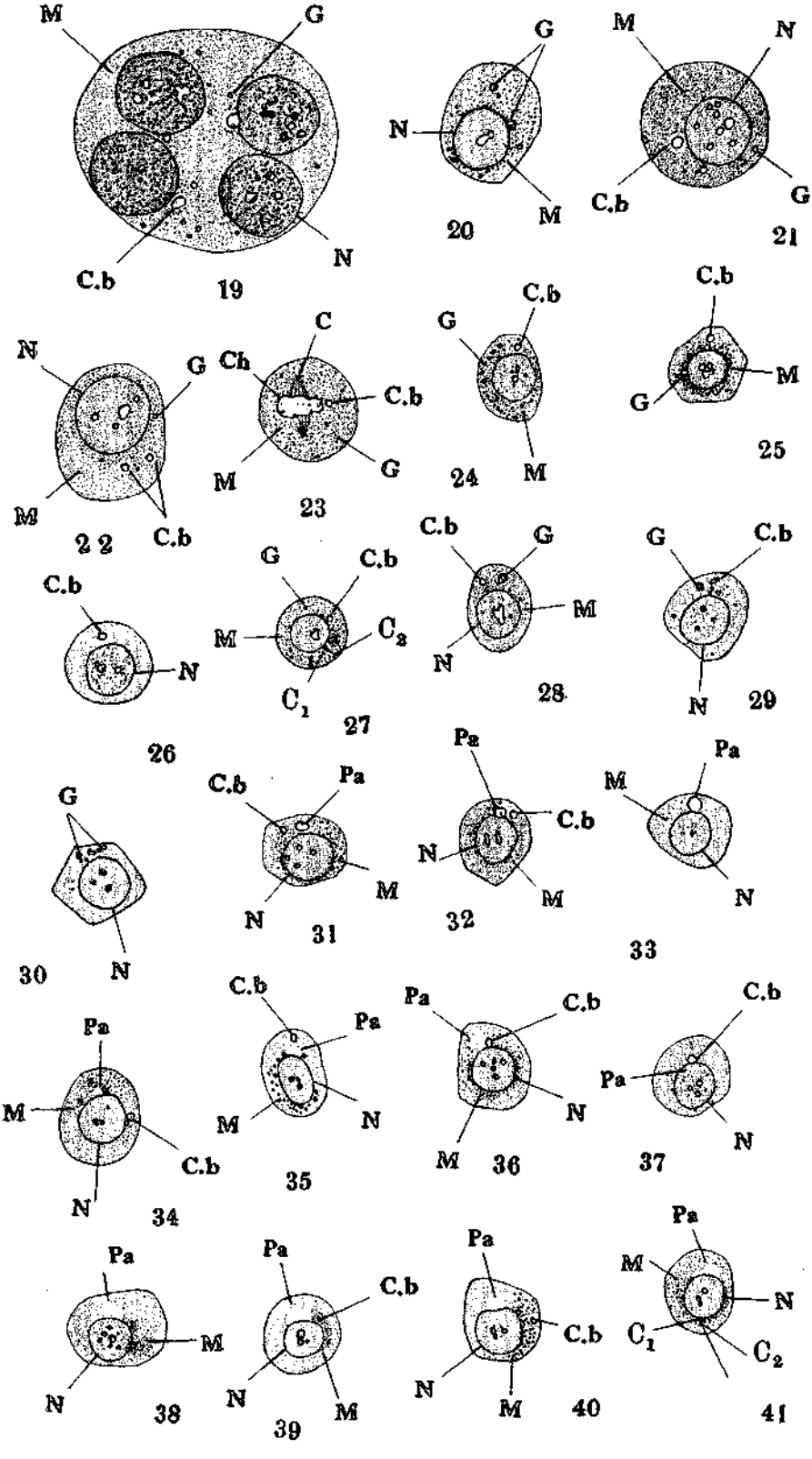
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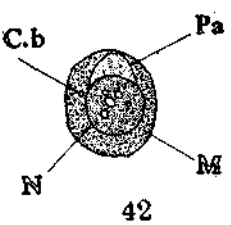

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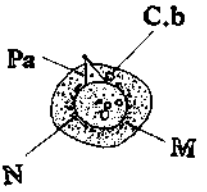

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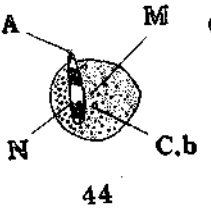

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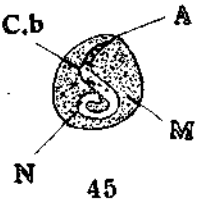

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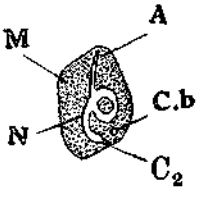

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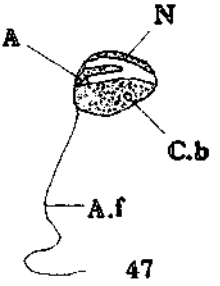

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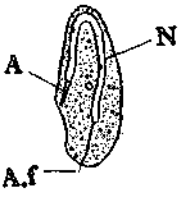

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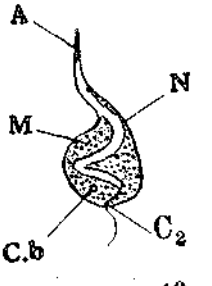

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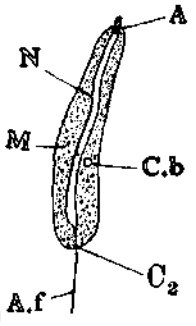

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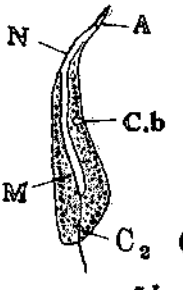

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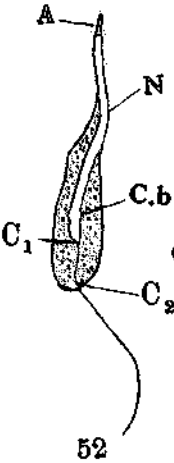

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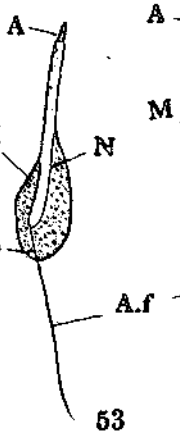

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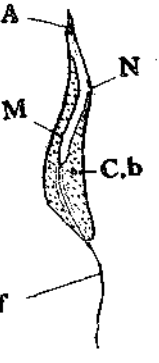

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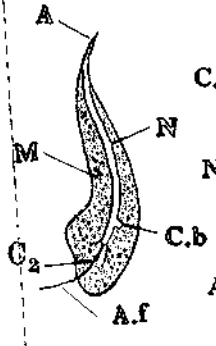

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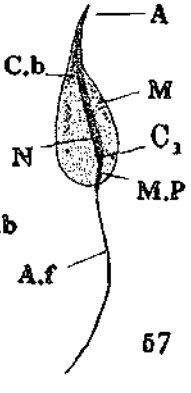

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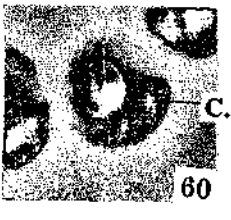

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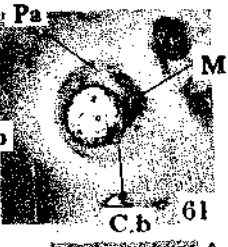

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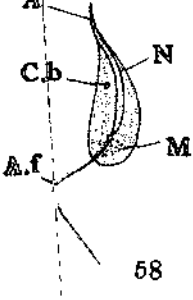

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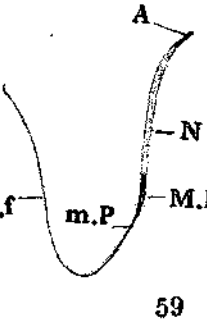

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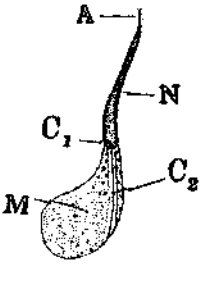

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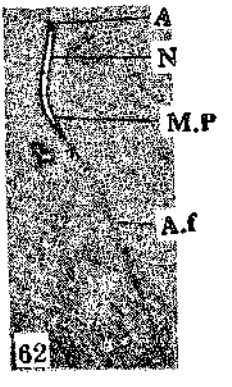

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VOLUMETRIC STUDIES IN OXIDATION-REDUCTION REACTIONS

VI. Oxidation with Chloramine-T Iodine Cyanide Method

by

BALWANT SINGH and KRISHAN CHANDER SOOD

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VOLUMETRIC STUDIES IN OXIDATION-REDUCTION REACTIONS

VI. Oxidation with Chloramine-T Iodine Cyanide Method

By Balwant Singh *and* Krishan Chander Sood

(*Department of Chemistry, Panjab University, Hoshiarpur, Panjab, India*)

Lang's iodine cyanide method (1922) is based on titration with iodate in the presence of hydrogen cyanide, the iodate being quantitatively converted into iodine cyanide. By using iodine monochloride as a preoxidizer and catalyst, it is possible to replace the iodate by any other oxidizing agent which rapidly and completely causes the formation of iodine cyanide in the volumetric estimation.

In the present investigation an attempt has been made to use chloramine-T as an oxidizing agent for the volumetric determination of potassium iodide, hydrazine sulphate, arsenious oxide, stannous chloride, mercurous chloride, potassium thiocyanate and ferrous ammonium sulphate by the iodine cyanide method using starch as an indicator. These substances react with chloramine-T in the presence of iodine monochloride, hydrogen cyanide and hydrochloric acid in accordance with the following equations:—

$$KI + CH_3C_6H_4SO_2N(Na)Cl + ICl + 2\ HCN = KCl + 2\ ICN + NaCl + CH_3C_6H_4SO_2NH_2$$

$$N_2H_4 + 2\ CH_3C_6H_4SO_2N(Na)Cl + ICl + HCN = HCl + ICN + N_2 + 2\ NaCl + 2\ CH_3C_6H_4SO_2NH_2$$

$$As_2O_3 + 2\ CH_3C_6H_4SO_2N(Na)Cl + 2\ H_2O + ICl + HCN = As_2O_5 + ICN + HCl + 2\ NaCl + 2\ CH_3C_6H_4SO_2NH_2$$

$$SnCl_2 + CH_3C_6H_4SO_2N(Na)Cl + HCl + ICl + HCN = SnCl_4 + ICN + NaCl + CH_3C_6H_4SO_2NH_2$$

$$Hg_2Cl_2 + CH_3C_6H_4SO_2N(Na)Cl + HCl + ICl + HCN = 2\ HgCl_2 + ICN + NaCl + CH_3C_6H_4SO_2NH_2$$

$$KCNS + 3\ CH_3C_6H_4SO_2N(Na)Cl + 4\ H_2O + ICl = KHSO_4 + ICN + HCl + 3\ NaCl + 3\ CH_3C_6H_4SO_2NH_2$$

$$6\ FeSO_4 + 3\ CH_3C_6H_4SO_2N(Na)Cl + 5\ HCl + ICl + HCN = 2\ Fe_2(SO_4)_3 + 2\ FeCl_3 + ICN + 3\ NaCl + 3\ CH_3C_6H_4SO_2NH_2$$

Experimental

A known amount of each substance was transferred to a conical flask and 5 ml. of 0·5 M iodine monochloride, 12 ml. of 0·5 M potassium cyanide solution, a few drops of starch solution and sufficient hydrochloric acid to

keep the normality at about 1*N* were added to it. The mixture was titrated against standard chloramine-T solution. The reagent was added from a burette. The titration was carried to the discharge of the iodine-starch blue colour, which appeared at the beginning of titration. Several titrations were performed in each case. From the volume of the chloramine-T solution used, corresponding to the end point in each titration, the amount of the substance was calculated. The results are recorded in Tables I to VII. From these results it is concluded that potassium iodide, hydrazine sulphate, arsenious oxide, stannous chloride, mercurous chloride, potassium thiocyanate and ferrous ammonium sulphate can be determined volumetrically by the iodine cyanide method using chloramine-T as an oxidizing agent in presence of iodine monochloride.

TABLE I

Potassium Iodide

KI taken.	0·10*N* chloramine-T used.	KI found.
g.	ml.	g.
0·1246	15·00	0·1245
0·1496	18·00	0·1494
0·2074	25·00	0·2075
0·2656	32·00	0·2656
0·3322	40·00	0·3320

TABLE II

Hydrazine Sulphate

$N_2H_4.H_2SO_4$ taken.	0·10*N* chloramine-T used.	$N_2H_4.H_2SO_4$ found.
g.	ml.	g.
0·0326	10·00	0·0325
0·0586	18·00	0·0586
0·0878	27·00	0·0878
0·1043	32·10	0·1044
0·1206	37·00	0·1204

TABLE III

Arsenious Oxide

As_2O_3 taken.	0·10*N* chloramine-T used.	As_2O_3 found.
g.	ml.	g.
0·0741	15·00	0·0742
0·0893	18·10	0·0895
0·1238	25·00	0·1236
0·1590	32·10	0·1588
0·1980	40·00	0·1978

TABLE IV

Stannous Chloride

$SnCl_2.2H_2O$ taken.	0·10*N* chloramine-T used.	$SnCl_2.2H_2O$ found.
g.	ml.	g.
0·1130	10·00	0·1128
0·2032	18·00	0·2031
0·3048	27·00	0·3046
0·4068	36·00	0·4062
0·4740	42·00	0·4739

TABLE V

Mercurous Chloride

Hg_2Cl_2 taken.	0·10*N* chloramine-T used.	Hg_2Cl_2 found.
g.	ml.	g.
0·3543	15·00	0·3541
0·4255	18·00	0·4249
0·6376	27·00	0·6374
0·7560	32·00	0·7554
0·9449	40·00	0·9443

TABLE VI

Potassium Thiocyanate

KCNS taken.	0·10*N* chloramine-T used.	KCNS found.
g.	ml.	g.
0·0162	10·00	0·0162
0·0290	18·00	0·0292
0·0424	26·00	0·0421
0·0585	36·00	0·0583
0·0731	45·00	0·0729

TABLE VII

Ferrous Ammonium Sulphate

$FeSO_4.(NH_4)_2SO_4.6H_2O$ taken.	0·10*N* chloramine-T used.	$FeSO_4.(NH_4)_2SO_4.6H_2O$ found.
g.	ml.	g.
0·2748	7·00	0·2745
0·4708	12·00	0·4706
0·6670	17·00	0·6667
0·7880	20·10	0·7882
0·9806	25·00	0·9804

Summary

Chloramine-T has been used as an oxidizing agent for the volumetric determination of potassium iodide, hydrazine sulphate, arsenious oxide, stannous chloride, mercurous chloride, potassium thiocyanate and ferrous ammonium sulphate by the iodine cyanide method using starch as an indicator. Iodine monochloride was used as a preoxidizer and catalyst in these volumetric estimations.

Literature cited

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AMPHIBIAN SPERMATOGENESIS

I. The Sperm of Frog

by

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Department of Zoology, Panjab University,
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AMPHIBIAN SPERMATOGENESIS

I. The Sperm of Frog

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Introduction

The male germ cells of amphibians have been the subject of investigation since perhaps the beginning of cytological research. But, strange as it would appear, no attention has so far been paid to the detailed study of spermatogenesis in the common frog, *Rana tigrina*, which is considered to be the most convenient principal type all over India. In order, therefore, to fill up this gap one of us (K.K.S.) prepared some slides of the testicular material of this species and these were studied independently as well as jointly by both of us. The present paper is an account of these studies.

Previous Work

In the present century Retzius (1906) seems to be the first worker who described two deeply staining basal bodies—the centrioles, lying side by side in the neck region of the sperm of *Rana esculenta*. In *Rana temporaria* Broman, as stated by Wilson (1947), finds a distinct middle-piece, containing a distal and a proximal centriole, and in *Rana mugiens* this has a spiral envelope. The mitochondrial sheath is quite conspicuous in *Rana fusca* (Broman, 1907) while it is small and rounded in *Rana nigromaculata* (Morita, 1928).

The most outstanding work on the family Ranidæ is that of Champy (1913) who has given a very exhaustive account of the spermatogenesis of practically all the groups of amphibia. Unfortunately the cytologists at that time were under the influence of the erroneous interpretation of the Golgi apparatus as the Golgi-Holmgren canals and Champy, being no exception to that, has actually described it as such. In spite of this Champy's work has been considered as epoch-making with respect to amphibia.

Recently Galiano (1948) has also worked on *Rana esculenta* but the observations made by this author are confined to the spermatids and the immature spermatozoa only.

Material and Technique

The frog, *Rana tigrina*, is very common all over India. The specimens for this investigation were collected from the ponds and water reservoirs

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situated near the Panjab University College, Hoshiarpur. The males can be easily distinguished from the females as they are characterized by the presence of highly developed thumb pads and vocal sacs, especially during the breeding season, i.e. from June to August. It is, however, quite difficult to obtain frogs from November to March, when they undergo hibernation.

The animal was pithed alive and the testes were dissected out immediately. The testes of frog are a pair of white ovoid bodies. Each of these is about half an inch long, lying immediately ventral to the anterior end of the kidney to which it is attached by a fold of peritoneum. It was very commonly observed that one of the testes was much larger than the other.

Small pieces of the testicular material were instantaneously transferred to the following fixing fluids:—(i) Bouin, (ii) Flemming, (iii) Flemming-without acetic, (iv) Champy, (v) Champy-kull, (vi) Altmann, (vii) Kolatchew with Nassonow's modification and (viii) Aoyama. The best preparations were, however, obtained with Bouin (3–6 hours), Champy (20 hours) and Altmann (24 hours).

Paraffin method of embedding was employed in all the cases and the sections were cut 4–6 microns thick. These were mostly stained with 0·5% iron hæmatoxylin.

For the detection of mitochondria, staining with Altmann's hot acid fuchsin technique gave good results.

Smears proved very helpful for the study of mature sperms as sections hardly revealed the isolated sperms distinctly, the sperms by the time they attained maturity being always clumped together in bundles and attached to the sertoli cells.

Double staining with acid fuchsin and methyl green was also tried for distinguishing the structure of the nucleus and the middle-piece in the ripe sperm but these stains were found to be temporary, quickly vanishing after being mounted in canada balsam.

It will not be out of place to mention here that many a time the material was found to be tinged with the colour of the fixing reagents in spite of overwashing. This caused a great handicap for successful staining. However, by resorting to bleaching with 5% oxalic acid and 1% potassium permanganate, very encouraging results were obtained.

Observations

Primordial germ cells.—The primordial germ cells, from which the spermatogonia are differentiated, are very small, somewhat rounded cells, situated on the periphery of the seminiferous tubules (Plate I, Fig. 1). In each of them there is a vesicular nucleus showing two or more deeply staining nucleoli. In their cytoplasm can be frequently found a very small juxta-nuclear mass of fine granules which appear greyish after the iron hæmatoxylin stain. These are most probably the mitochondria. Amongst these one can also easily distinguish two or more deeply staining bigger granules which are undoubtedly the Golgi elements.

Primary spermatogonia.—In figure 2 (Plate I) is shown a very early primary spermatogonium which possesses all the characteristics of the cell just described except that it is slightly bigger in size.

Polymorphism.—The late primary spermatogonia surpass in size all the other cells in the testis, frequently attaining a diameter of 30μ (Plate I, Figs. 3 to 10). In contour they are nearly spherical but in sections they exhibit the polygonal outline presumably due to the pressure of the adjoining cells. The character which first arrests attention in these cells is the form of the nucleus. It is sometimes spherical but is commonly very irregular, being frequently crescentic, reniform or dumb-bell-shaped. In some cases it is in the form of two or more lobes reaching a climax when the major portion of the cell is occupied by a very large multilobed nucleus. Such nuclei are the 'polymorphic forms' described by a number of earlier workers in the amphibian testis (Plate I, Figs. 3 to 10).

The late primary spermatogonia, on account of their large size, stand out in sharp contrast with the surrounding elements. Most of them, as we have just stated, show the polymorphic type of nucleus, the internal structure of which is more or less the same irrespective of the character of its surface. In all cases, the nuclear membrane is distinct as it takes up the hæmatoxylin stain deeply. Internally the nucleus reveals a somewhat ill-defined linin reticulum, suspended in which there is a varying number of small masses of chromatin. This, of course, depends upon the nature of the fixatives employed, appearing somewhat homogeneous with Champy and Aoyama but in Bouin's fluid it is precipitated in the form of a very fine network. The nucleoli are the only elements which are sufficiently constant under the most diverse reactions. Their number is quite variable and it seems to depend upon the degree of polymorphism. One can say as a general rule that there is a large nucleolus in each lobe of the nucleus.

A very interesting feature regarding the structure of the nucleolus is that in most of the cases two very small nucleoli are attached to the principal nucleolus with the result that this feature usually becomes diagnostic for the primary spermatogonia (Plate I, Fig. 3).

One also frequently observes the nucleoli dividing. The nucleolus is first drawn out like a dumb-bell and the two portions, sometimes unequal, often remain joined by a thick filament of viscous appearance (Plate I, Fig. 4). This filament ends by being broken and disappearing subsequently.

It is noticed that in those primary spermatogonia in which the nucleus is bilobed or kidney-shaped (Plate I, Fig. 5) the cytoplasm enters like the finger of a glove right into the middle of the nucleus. Consequently a sort of canal is formed which is termed as the 'internuclear canaliculus' by the earlier workers. Very often there is a small nucleolus pressed against the blind extremity of this canal. What exactly is the function of this, is very difficult to say, but it has been suggested that it serves for the establishment of a relation between the cytoplasm and the nucleus whereby an active exchange of substances can easily take place between the two.

The mitochondria and the Golgi elements which generally remain scattered uniformly throughout the cytoplasm (Plate I, Figs. 3 and 4) may become aggregated in one or more groups near the nucleus when it becomes polymorphic (Plate I, Figs. 5 and 6). The Golgi bodies are stained jet-black in iron hæmatoxylin whereas the mitochondria either do not stain at all or

become just greyish. The Golgi elements are essentially granular, more often forming the major portion of the juxta-nuclear mass. Very rarely a big Golgi granule may reveal a duplex structure with a chromophilic cortex and a chromophobic core (Plate I, Fig. 4). The mitochondria are of varying sizes, ranging from dust-like granules to bigger ones (Plate I, Fig. 6).

Most of the earlier workers have interpreted the polymorphic form of the nucleus as a result of amitosis. We have, on the other hand, observed that the two lobes of the nucleus are very often quite unequal (Plate I, Fig. 11). These are gradually separated from each other (Plate I, Fig. 12) and it is sometimes seen that the smaller one gets disorganized to be subsequently absorbed in the general cytoplasm (Plate I, Fig. 13).

Several gradations of the separation of the equal lobes of the nucleus are also met with (Plate I, Figs. 14 and 15). It is, however, quite likely that these equal lobes may in reality be the nuclei themselves of different generations of spermatogonia, which are formed after a normal process of mitosis with this difference only that the division of the cytoplasm has been delayed due to some reason or the other. In figure 16 (Plate II) are shown two daughter cells (secondary spermatogonia), just formed. The intervening cell membrane seems to cut through the nucleus and it may be interpreted as a sort of amitotic division but a glance at figure 21 (Plate II) will leave no doubt that it is simply an illusion. We are, therefore, of the opinion that the separation of the nuclear lobes simply leads to their degeneration and that no amitosis in the real sense of the word takes place in the primary spermatogonia.

The rounded form of the nucleus seems to be a prerequisite for the formation of the prophase chromosomes as the latter make their appearance only in those cells which have regular spherical nuclei (Plate II, Fig. 17). The Golgi elements and the mitochondria in them take up a very faint stain and are scattered throughout the cytoplasm. In figure 18 (Plate II) is shown the prometaphase stage of a primary spermatogonium in which the chromosomes are seen arranging themselves at the equator of the spindle. At each pole of the spindle is a small deeply staining granule—the centrosome. A polar view of the metaphase is represented in figure 19 (Plate II). The chromosomes here are very large and of various shapes. In the telophase (Plate II, Fig. 20) the two groups of chromosomes have reached their respective poles. After this, the two daughter nuclei are reconstituted and separated by a cell membrane (Plate II, Fig. 21). The resulting cells are the secondary spermatogonia.

Secondary and later spermatogonia.—The secondary and later spermatogonia are usually grouped in cysts each of which contains the descendants of perhaps only one primary spermatogonium. As the account given already for the primary spermatogonia equally applies to these also, it will be sufficient to give here only the points of difference. In the first place the secondary and later spermatogonia never show polymorphic nuclei and secondly they are much smaller. Their size, of course, varies according to the number of generations by which they are removed from the primary ones.

In form, they are usually angular on account of compression. Roughly speaking they may have a conical or a pyramidal shape, the base of the cone or pyramid facing outwards towards the cyst wall (Plate II, Figs. 22 and 23).

The Golgi elements are localized on one side of the nucleus in the form of a cap. They have the same characteristics as in the primary spermatogonia.

The mitochondria are small greyish granules of varying sizes. They are interspersed not only in the juxta-nuclear mass formed by the Golgi elements but also uniformly throughout the cytoplasm. Here and there the mitochondrial granules align themselves to form small filaments which, in extreme cases, even anastomose with one another. Due to this aggregation these take up more stain (Plate II, Fig. 22).

Primary spermatocyte.—After the period of multiplication has come to an end the cysts are closely packed with small cells in a condition of rest and these now, without any further division, become transformed into much larger cells—the primary spermatocytes. The period during which this change takes place is the 'growth period'.

A very interesting feature which makes itself felt is that there is a seriation of stages and the different stages succeed one another in regular groups. The whole cyst is in a particular state and the others in some other state. This synchronism is presumably due to the fact that all the elements of spermatogenesis in a particular cyst evolve at the same time.

The primary spermatocyte resembles in all essential characteristics the secondary spermatogonia described already excepting the difference in size of the nucleus and that of the cytoplasm (Plate II, Figs. 24 and 25). The nucleus in them is quite big and vesicular and contains two or more nucleoli scattered in a somewhat granular background.

Most of the Golgi elements in the primary spermatocyte occur as a crescentic mass closely applied to the nuclear membrane while the mitochondria appear in the form of granules scattered all over the cytoplasm. However, even the mitochondria show a greater concentration, very often in the form of filaments, in the region of the Golgi mass (Plate II, Fig. 24). In extreme cases the mitochondrial filaments, formed, of course, by the alignment of mitochondrial granules, assume the form of an anastomosing mesh-work which completely envelops the Golgi mass (Plate II, Fig. 25).

Even after the advent of the prophase changes in the nucleus for the first meiotic division (Plate II, Figs. 25 to 27) the juxta-nuclear mass of the mitochondria and the Golgi elements remains as such till the end of the zygotene stage (Plate II, Fig. 28) when the nucleus reveals a typical bouquet. During the pachytene stage (Plate II, Figs. 29 and 30) the juxta-nuclear mass breaks up and its constituents, the mitochondria and the Golgi elements, gradually start becoming circum-nuclear till by the diakinetic stage (Plate II, Fig. 31) they are all uniformly spread throughout the cytoplasm. In figure 32 (Plate II) is shown the side view of the first metaphase stage in which the chromosomes are arranged on the equator of the spindle. They lie so closely crowded together that their exact number and forms are hardly revealed. During the telophase stage (Plate II, Fig. 33), when the process of nuclear reconstruction is going on, a few deeply staining

granules appear at the equator of the dividing cell. These granules constitute the so-called mid-body.

Secondary spermatocyte.—The secondary spermatocyte which is formed as a result of the first meiotic division is much smaller in size than the primary. It is characterized by a homogeneously and lightly stained nucleus, showing no visible internal structure (Plate II, Fig. 34). In the cytoplasm the granules do not repose in a definite mass but are scattered all over, usually in a circum-nuclear position. The Golgi elements are in the form of deeply staining granules while the mitochondria, taking up the usual greyish hue, are just tiny particles.

After a short period of interkinesis the nucleus undergoes the usual changes and at metaphase II (Plate II, Fig. 35) the chromosomes can be seen arranged at the equator of the achromatic figure. No definite count of these chromosomes can, however, be made as they are somewhat fused with each other. The metaphase II differs from the first not only in the size of the cell but also in that of the chromosomes which are distinctly smaller in the former.

Later on the two groups of chromosomes move apart and after reaching the corresponding poles of the spindle get resolved into two distinct nuclei (Plate II, Fig. 36) and the two daughter cells, thus formed, are undoubtedly the spermatids.

Spermatid and its metamorphosis.—The earliest spermatid is a small more or less rounded cell with a faint, homogeneously stained nucleus (Plate II, Fig. 37). The cytoplasmic components, at this stage, are never localized to form a juxta-nuclear mass. Of these the tiny mitochondrial granules take up the usual greyish tinge and are spread uniformly throughout the cytoplasm. Distributed haphazardly amongst these are a few small deeply staining Golgi elements. This is, so to say, a resting stage for the spermatid but as there are very few cysts having cells in this condition it seems to last for a very short time.

With the initiation of the period of transformation of the spermatid into the spermatozoon the Golgi elements start showing a distinct tendency towards close grouping (Plate II, Fig. 38). As a result of this are formed bigger Golgi granules which in their turn fuse to form still bigger Golgi granules (Plate II, Fig. 39 and Plate III, Figs. 40 and 41). Even these big Golgi granules do not show any differentiation into the externum and the internum. One of these big Golgi granules now comes towards the nucleus and before it starts moving anteriorly one can also observe near it two more fine and sharply staining granules (Plate II, Fig. 39). As an axial filament can be clearly seen originating from them there can be no doubt that these are the centrosomes. They mark the posterior end of the cell.

The big Golgi granule which gradually moves away from the centrosomes (Plate II, Fig. 39 and Plate III, Figs. 40 to 42) ultimately takes up its position at the anterior end of the nucleus diametrically opposite the centrosomes (Plate III, Figs. 44 and 45). This Golgi granule may be termed as the proacrosome.

In the meantime one of the two centrosomes also moves towards the nucleus. This is the proximal centrosome. As it fuses completely with

the posterior end of the nucleus (Plate III, Fig. 42) it becomes increasingly difficult to trace accurately its later history. It, however, seems to grow to form a rod-like structure (Plate III, Figs. 43 to 45). The other centrosome which is now the distal also grows much bigger in size. It finally takes up the form of a ring which in sections very often appears as a crescent (Plate III, Figs. 42 to 52).

During all this time the mitochondria do not undergo any visible change and remain scattered evenly throughout the cell.

The proacrosome now begins to press against the nuclear membrane (Plate III, Fig. 45) and thus it seems to lie in a depression at the anterior end of the nucleus (Plate III, Fig. 46). Up to this stage the proacrosome has been taking up an intense stain but now it becomes differentiated into an outer chromophilic cortex and an inner chromophobic core. Generally the proacrosome is very intimately connected with the anterior end of the nucleus (Plate III, Fig. 48) but sometimes it may even lie free in the cytoplasm (Plate III, Fig. 47). The latter condition is, however, rare.

The proacrosome at the stage shown in figure 48 (Plate III) appears to be in the form of a vacuole with glue-like contents under the phase contrast microscope.

From now onwards the nucleus begins to stain intensely but the proacrosome can still be clearly differentiated from it (Plate III, Fig. 49). This proacrosome is then directly transformed into a triangular acrosome the base of which is completely fused with the anterior end of the nucleus (Plate III, Figs. 50 to 53 and Plate IV, Figs. 54 to 56). At the apex of the triangular acrosome can also be made out a deeply staining acrosomal granule (Plate III, Figs. 51 and 52 and Plate IV, Figs. 55 and 56).

Simultaneously when these changes are going on some of the mitochondria reveal a distinct tendency to collect together around the axial filament between the ring-like distal centrosome and the rod-like proximal centrosome which, as we have stated already, fuses with the posterior end of the nucleus. Thus, is formed a very small faintly staining middle-piece intercalated between the distal centrosome and the nucleus (Plate III, Figs. 52 and 53 and Plate IV, Figs. 54 to 56).

The deeply staining nucleus now begins to elongate in the anteroposterior direction and the cytoplasm consequently recedes gradually (Plate IV, Figs. 54 to 62). This retreating cytoplasm contains all those mitochondria and the Golgi elements which have not been consumed in the formation of the middle-piece and the acrosome respectively. Large vacuoles probably of some oily nature can also be clearly observed in the residual cytoplasm which moves slowly down the axial filament to be finally cast off in the lumen of the testis.

During the process of elongation of the nucleus the staining capacity of the acrosome, the nucleus and the middle-piece increases so much that they all take up a homogeneously dark stain (Plate IV, Figs. 57 to 62). It is, therefore, impossible to make out these structures separately unless, of course, these cells are destained heavily.

Ripe sperm.—A fully mature sperm is a long structure composed of a deeply staining head and a vibratile tail. Normally there is no demarcation

of the acrosome and the middle-piece from the nucleus as all these parts of the sperm take up a homogeneously dark stain but when the sperm is thoroughly destained or is studied alive under the phase contrast microscope it reveals a slightly elongated triangular acrosome with a deeply staining acrosomal granule at its tip, situated at the anterior end of the nucleus and a small middle-piece just behind the nucleus. The anterior boundary of this short middle-piece is marked by the rod-like proximal centrosome and the posterior by the ring-like distal centrosome (Plate IV, Fig. 63).

The sperms are usually clumped together in bundles forming parachute-like structures.

Discussion

Golgi elements.—In the primordial germ cells and the early primary spermatogonia of the common frog, *Rana tigrina*, the Golgi elements are seen as two or more deeply staining granules in the juxta-nuclear mass of fine, dust-like mitochondria. Afterwards, however, these small Golgi elements grow bigger in size and their number also increases considerably. Some of the fully grown Golgi elements at this stage may also reveal a duplex structure with a chromophilic cortex and a chromophobic central core. Though the Golgi elements may sometimes be seen distributed uniformly throughout the cytoplasm of the growing primary spermatogonia yet they show a distinct tendency to collect near the nuclear lobes.

In the secondary and later spermatogonia and the primary spermatocytes the Golgi elements are not only fewer in number but also smaller in size and they occupy the major portion of the juxta-nuclear mass. In the late prophase of the first maturation division they seem to break up into still smaller bodies which are distinguished from the fine mitochondria only with great difficulty. These are presumably sorted out more or less evenly to the two daughter cells.

In the secondary spermatocytes the Golgi elements which are now circum-nuclear in position are distinctly smaller bodies. During the second maturation division they are again distributed, more or less evenly, to the two spermatids, thus formed.

It will, therefore, be seen that the Golgi elements in the sperm-forming cells of *Rana tigrina* are essentially granular but with the growth and differentiation of the cells some of them at least may reveal a duplex structure with a chromophilic cortex and a chromophobic central core. The Golgi apparatus, when originally discovered by Camillo Golgi (1898), was described in the form of a close network. During the early stages of this discovery the subject was confused by the claim of Holmgren (1902) that the apparatus was identical with a system of clear canals 'trophospongium' which he had observed in many cells. Cajal (1908) added still further to this confusion by referring to the Golgi nets as the Golgi-Holmgren canals.

Champy (1913), while working out the spermatogenesis of a large number of amphibians, was completely misled by this erroneous homology of Cajal. His attention was drawn towards such networks and canals over the nucleus but on account of the faulty technique he could not get a real picture and so made only a passing reference to these. He, thus, evaded the wider issues involving the real structure and the important rôle

which the Golgi apparatus plays in the course of spermatogenesis and this is the only loophole in his otherwise marvellous and epoch-making work in the field of amphibia. As has been shown earlier by Sharma *et al* (1953) the Golgi-nets, -crescents, -dictyosomes, -batonettes and -rods, etc., are all artifacts formed as a result of the faulty technique and by the alignment of the granular Golgi elements which are overcrowded in the juxta-nuclear mass of fine mitochondria.

Acrosome.—It will be recalled that we described the Golgi elements in the earliest spermatid of *Rana tigrina* as a few small, deeply staining granules, scattered here and there in the cytoplasm. Soon they reveal a distinct tendency to come together and thus by a process of coalescence a big Golgi granule called the proacrosome is formed. This now begins to travel forward in close relation with the nucleus and ultimately takes up its position at its anterior end. The final position of the proacrosome is, therefore, diametrically opposite the centrosomes which retain their original position at the posterior end of the nucleus. McGregor (1899), however, feels convinced that in *Amphiuma* it is the acrosomal vesicle which remains in its original position while the centrosomes migrate from the anterior to the posterior end of the nucleus.

The proacrosome which is, to begin with, stained very deeply gradually becomes differentiated into an outer chromophilic cortex and an inner chromophobic core. In the living cells at this stage it can be seen in the form of a clear vacuole with glue-like contents intimately attached to the anterior end of the nucleus. During the process of spermateleosis the proacrosome is directly transformed into a triangular acrosome and a deeply staining acrosomal granule is also differentiated at its apex. Even in the ripe sperm this acrosomal granule can be easily made out at the tip of the triangular acrosome which is now slightly elongated. Those Golgi elements which do not take part in the formation of the acrosome are sloughed off with the residual cytoplasm.

Champy (1913) asserts that the acrosome in all the amphibians is formed by the anterior group of central corpuscles. A glance at our figures will, however, clearly show that Champy was led to this erroneous conclusion on account of his faulty technique and in view of the fact that the centrosomes are also situated nearby when the proacrosome is just formed.

In the case of *Rana esculenta* Champy figures a triangular structure at the anterior end of the nucleus. This is in reality the proacrosome which is being directly transformed into the acrosome proper.

Gatenby (1931) states that the acrosome in *Desmognathus* does not appear, in the fresh condition, to contain a central more solid bead, as described for *Triton* by Hirschler (1928) or for *Amphiuma* by McGregor (1899). According to Gatenby the acrosome is rather a limpid vesicular structure of an extremely delicate nature. The acrosome of *Plethodon* also is drawn as a vesicle without a central granule by Bowen (1922). Similarly Terni (1914) does not figure a central granule in earlier stages of acrosome formation in *Geotriton fusca*.

Mitochondria.—In the primordial germ cells and the primary spermatogonia of *Rana tigrina* the mitochondria are minute, lightly staining granules,

scattered throughout the cytoplasm but they show a distinct tendency to concentrate in the juxta-nuclear mass of Golgi granules.

In the secondary and later spermatogonia and the primary spermatocytes the small mitochondrial granules which are interspersed in between the Golgi elements align themselves to form filaments. In the late prophase, however, these filaments break up into small granules which are all distributed approximately equally to the daughter cells.

In the secondary spermatocytes and the early spermatids the mitochondria are essentially in the form of small granules which are spread uniformly all over the cytoplasm. During the late stages of spermateleosis, however, some of the mitochondria which are situated at the base of the nucleus and around the axial filament in between the two centrosomes start coming together. They, thus, contribute to the formation of a very small middle-piece which afterwards takes up a light homogeneous stain. The rest of the mitochondria which constitute about 90% of the whole travel down with the receding cytoplasm and are ultimately sloughed off with it.

As the middle-piece in the ripe sperm takes up a very homogeneous stain which is closely comparable with that of the nucleus it becomes increasingly difficult to distinguish it externally unless, of course, the sperm is destained to a great extent.

It will, thus, be seen from the above account that, in *Rana tigrina*, the behaviour of the mitochondria in general conforms to that observed in most of the other animals studied so far.

According to Champy (1913) all the granules in the cytoplasm of the spermatogonia or spermatocytes, excepting one or more pyrenoid bodies, are mitochondrial in nature. These mitochondria are said to exist in the form of chondriomites or threads of grains, chondrioconts or smooth filaments and chondriochondres or isolated granules. The isolated granules often group themselves into the mitochondrial bodies. In the spermatid stage some of the mitochondria aggregate to form one or two homogeneous bodies around the distal ring centrosome and these subsequently form the middle-piece.

It will be clear from this account that Champy had included even the Golgi elements in his description of the mitochondria. Later on, however, he did not hesitate to admit that in Kolatchew preparations not only the pyrenoid bodies but also some big mitochondrial granules were blackened. From this it follows that Champy was labouring under misapprehension and thus had confused the Golgi elements with the mitochondria.

Morita (1928), while working out the spermatogenesis of *Rana nigromaculata*, has shown that the mitochondria exist in the ripe sperm, in the form of granules dotted over both the nucleus and the tail but they do not repose directly upon a true middle-piece as is seen in so many other animals.

Terni (1914) has given a very good account of the mitochondria in *Geotriton fusca*. According to him the mitochondria may though seem to be stripped off or absent from the ripe sperm yet they can be shown to be present by keeping the sperms in 7% NaCl for three days, followed by fixation in chrome-osmium and staining in Altmann's fuchsin. By this treatment the mitochondria have been seen to occupy a region from the top

of the head centrosome to well up the nucleus. Gatenby (1931), however, failed to confirm the above account of Terni in spite of the fact that he used several strengths of salt solution as well as distilled water.

Centrosomes and the Axial Filament

In the cytoplasm of the spermatogonia and the spermatocytes of *Rana tigrina* a fine, deeply staining granule is sometimes seen to be surrounded by a clear area. This is most probably the centrosome. During the division stages, however, it is very distinct at each pole of the spindle. In the spermatids when the proacrosome is just formed two deeply staining, but fine, granules are observed lying nearby. As an axial filament can also be seen growing out from them there can be no doubt that these are the centrosomes. One of these now moves towards the nucleus. This is the proximal centrosome. As it fuses completely with the posterior end of the nucleus it becomes increasingly difficult to trace accurately its later history. It, however, seems to grow to form a rod-like structure. The other centrosome which is now the distal also grows much bigger in size. It finally takes up the form of a ring which in sections very often appears as a crescent. This forms the posterior boundary of the middle-piece.

Champy (1913) states that the proximal centrosome in the case of *Rana esculenta* first enters the base of the nucleus and later on inflates itself to a great extent. Some similar observations have also been made by a number of earlier workers in a large variety of cases. But our own observations on *Rana tigrina* have thoroughly convinced us that it is not so. We have clearly shown that the rod-like proximal centrosome lies just at the base of the nucleus in close apposition to it. Similarly Gatenby (1931) has observed in *Desmognathus* that the proximal centriole assumes the shape of a long and thick rod which simply touches the posterior extremity of the nucleus.

We have also not been able to make out in *Rana tigrina* any undulating membrane which, according to Champy (1913), is present in a rudimentary state on the axial filament of the ripe sperm of *Rana esculenta*. There is, however, no denying the fact that the tail in the ripe sperm of *Rana tigrina* is quite thick.

Summary

1. The spermatogenesis of the common Indian frog, *Rana tigrina*, has been completely worked out in this paper.

2. The primary spermatogonia surpass in size all the other cells in the testis and exhibit polymorphism of the nucleus.

3. Both the mitochondria and the Golgi elements are in the form of granules but the latter are appreciably bigger than the former.

4. The granular mitochondria align themselves in the secondary and later spermatogonia and the primary spermatocytes to form short filaments which later on again break up into small granules.

5. In the spermatid stage the Golgi elements have a distinct tendency to come together to form bigger granules. One of these big Golgi granules is directly transformed into the acrosome of the ripe sperm.

6. Only a very small portion of the mitochondria contributes to the formation of the middle-piece.

7. In the early spermatid there are present two centrosomes from which a long axial filament can be seen growing out. The proximal of these moves towards the nucleus and after becoming rod-like fuses with the base of it. The distal centrosome assumes the shape of a ring and marks the posterior boundary of the middle-piece.

8. In the ripe sperm there is no clear differentiation of the middle-piece and the acrosome from the nucleus as all these structures take up a homogeneously dark stain.

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Explanation of Lettering in Plates

A—Acrosome. *A.f*—Axial filament. *A.g*—Acrosomal granule. *C*—Centrosome. C_1—Proximal centrosome. C_2—Distal centrosome. *Ch*—Chromosomes. *C.g*—Chromatin granules. *G*—Golgi element. *'G*—Golgi remnant. *H*—Head. *I.c*—Internuclear canaliculus. *M*—Mitochondria. *M.b*—Mid-body. *M.P*—Middle-piece. *N*—Nucleus. *n*—Nucleolus. *Pa*—Proacrosome. *R.c*—Residual cytoplasm. *S.f*—Spindle fibres. T—Tail.

Explanation of Plates

All figures have been drawn with a camera lucida at the table level with Spencer 10× eye-piece and 1·25 oil immersion objective giving a total magnification of 1,750 times.

All figures except figures 59 to 62 (Plate IV) have been selected from the sectioned material fixed in Champy's fixative followed by 0·5% iron haematoxylin. Figures 59 to 62 are from smears.

Plate I

Fig. 1.—Primordial germ cell.
Fig. 2.—Earliest primary spermatogonium.
Fig. 3.—Primary spermatogonium with nucleoli in their typical position. Mitochondria and the Golgi elements are circum-nuclear.
Fig. 4.—Primary spermatogonium showing division of the nucleolus. One of the Golgi elements presents a duplex structure.
Fig. 5.—Primary spermatogonium with a kidney-shaped nucleus showing an internuclear canaliculus.
Fig. 6.—Primary spermatogonium with a dumb-bell-shaped nucleus. Mitochondria and the Golgi elements are grouped in definite masses.
Fig. 7.—Primary spermatogonium with three lobes of the nucleus.
Fig. 8.—Primary spermatogonium with four lobes of the nucleus.
Fig. 9.—Primary spermatogonium showing extreme polymorphism of the nucleus.
Fig. 10.—Fully grown primary spermatogonium.
Fig. 11.—Primary spermatogonium with two unequal lobes of the nucleus.
Fig. 12.—Primary spermatogonium showing the separation of the lobes of the nucleus.
Fig. 13.—Primary spermatogonium in which one of the nuclear lobes is seen degenerating.
Fig. 14.—Primary spermatogonium with two big almost equal lobes of the nucleus.
Fig. 15.—Primary spermatogonium with two small almost equal lobes of the nucleus.

Plate II

Fig. 16. Two daughter secondary spermatogonia just formed.
Fig. 17.—Primary spermatogonium—Prophase.
Fig. 18.—Primary spermatogonium—Pro-metaphase.
Fig. 19.—Primary spermatogonium—Metaphase (polar view).
Fig. 20.—Primary spermatogonium—Telophase.
Fig. 21.—Two secondary spermatogonia just formed.
Fig. 22.—Secondary spermatogonium.
Fig. 23.—Secondary spermatogonium.
Fig. 24.—Early primary spermatocyte.
Fig. 25.—Primary spermatocyte—Early leptotene.
Fig. 26.—Primary spermatocyte—Late leptotene.
Fig. 27.—Primary spermatocyte—Early zygotene.
Fig. 28.—Primary spermatocyte—Late zygotene.
Fig. 29.—Primary spermatocyte—Pachytene.
Fig. 30.—Primary spermatocyte—Pachytene.
Fig. 31.—Primary spermatocyte—Diakinesis.
Fig. 32.—Metaphase I (side view).
Fig. 33.—Telophase I (side view).
Fig. 34.—Secondary spermatocyte—Resting stage.
Fig. 35.—Metaphase II (side view).
Fig. 36.—Late Telophase II.
Fig. 37.—Earliest spermatid.
Fig. 38.—Spermatid in which the Golgi elements can be seen coming together to form bigger granules.
Fig. 39.—Spermatid showing the proacrosome, centrosomes and the axial filament.

Plate III

Fig. 40.—Spermatid in which the proacrosome has started moving anteriorly.

Fig. 41.—Spermatid showing a little later stage.

Fig. 42.—Spermatid in which the proximal centrosome has come in contact with the base of the nucleus. The distal centrosome is becoming ring-like.

Fig. 43.—Spermatid in which the proximal centrosome has assumed the rod-like form.

Fig. 44.—Spermatid in which the proacrosome has taken up its position at the anterior end of the nucleus diametrically opposite the proximal centrosome.

Fig. 45.—Spermatid in which the proacrosome is seen pressing the anterior end of the nucleus.

Fig. 46.—Spermatid in which the proacrosome is seen lying in a depression at the anterior end of the nucleus.

Fig. 47.—Spermatid showing the differentiation of the proacrosome into an outer chromophilic rim and an inner chromophobic core.

Fig. 48.—Spermatid in which the proacrosome is in the form of a vacuole with glue-like contents. It is now in intimate contact with the anterior end of the nucleus. The distal centrosome is definitely ring-like.

Figs. 49 to 51.—Spermatids showing the direct transformation of the proacrosome into a triangular acrosome. A deeply staining acrosomal granule can also be made out at the apex of the triangle in figure 51.

Fig. 52.—Spermatid showing the aggregation of a few mitochondria at the base of the nucleus to form a small middle-piece.

Fig. 53.—An early elongating spermatid.

Plate IV

Figs. 54 to 56.—Spermatids showing the elongation of the nucleus. The acrosome and the middle-piece can be clearly made out.

Figs. 57 and 58.—Late spermatids with the head taking up a deep and uniform stain. The residual cytoplasm can be seen receding.

Figs. 59 to 62.—Maturing sperms. The residual cytoplasm is being sloughed off.

Fig. 63.—A fully ripe sperm.

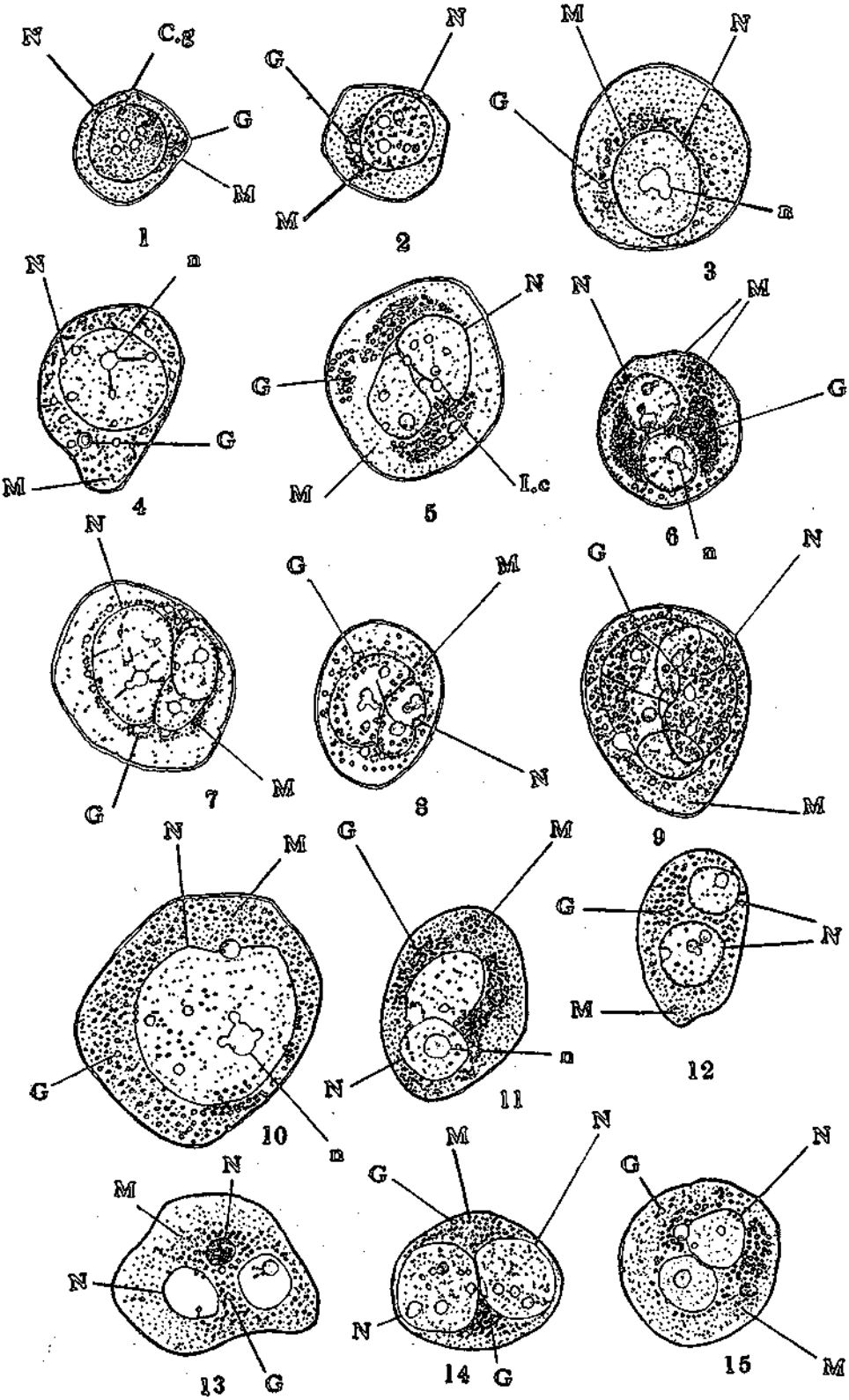
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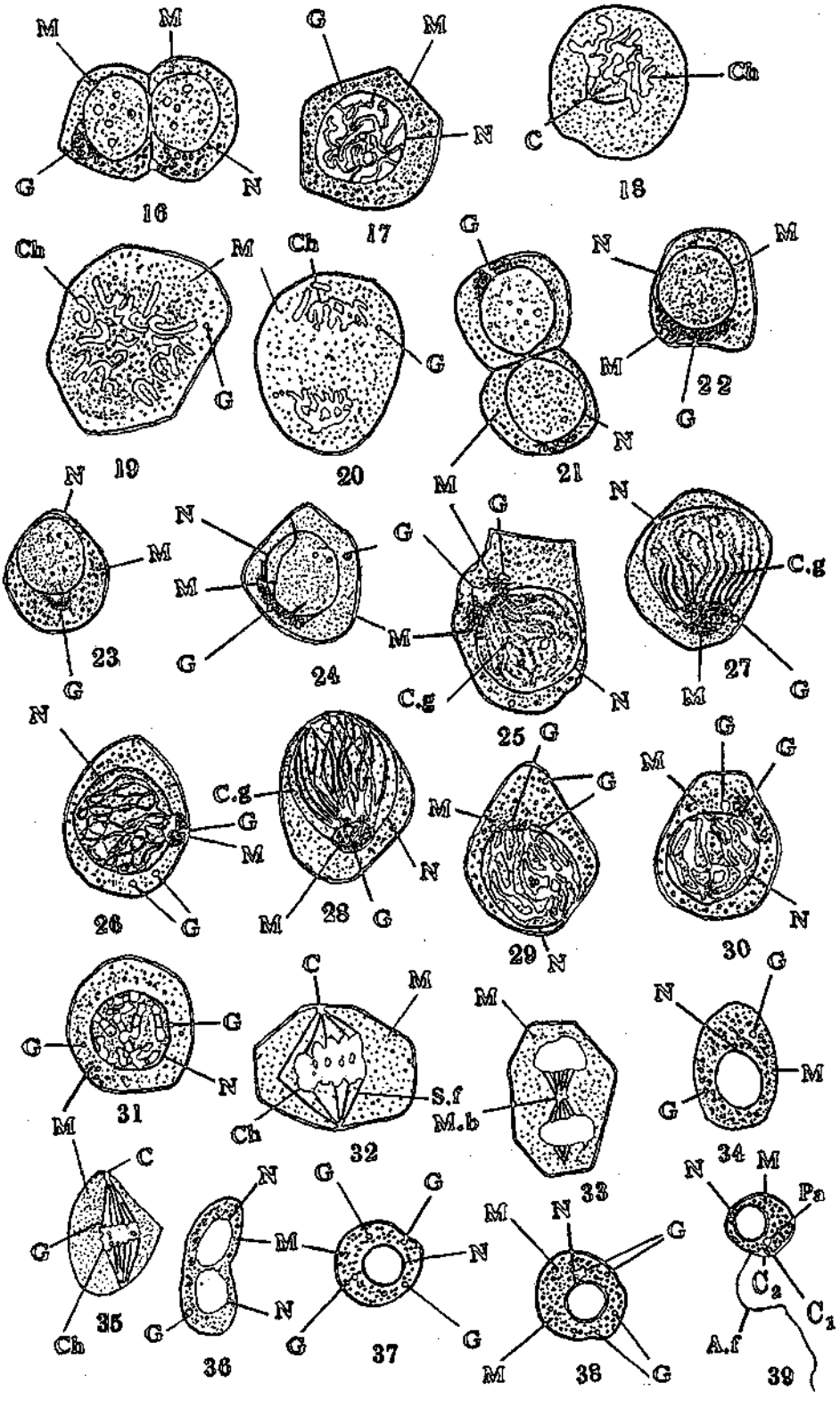
PLATE II.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S.f
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C2
C1
A.f
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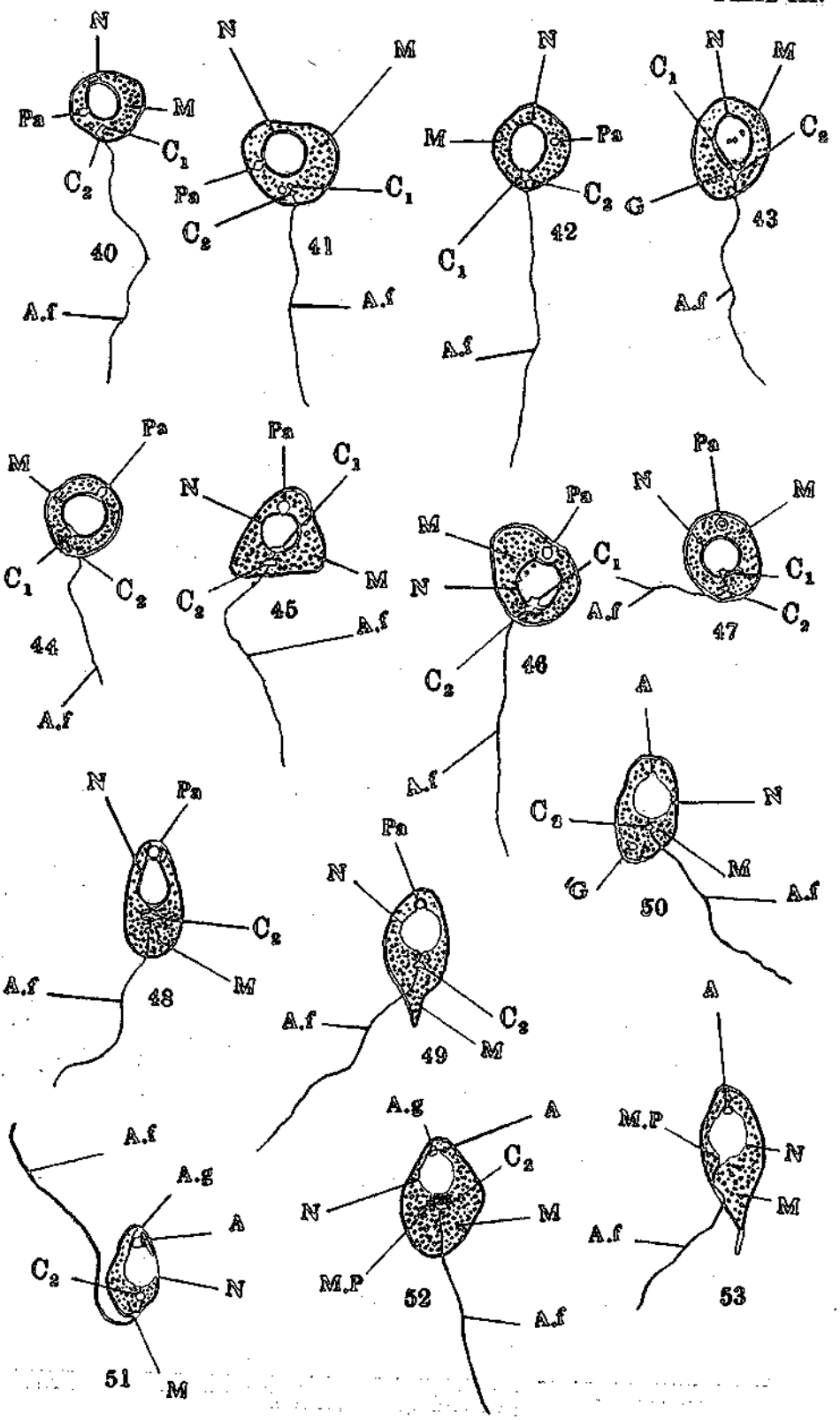
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42
A.f
N
M
C1
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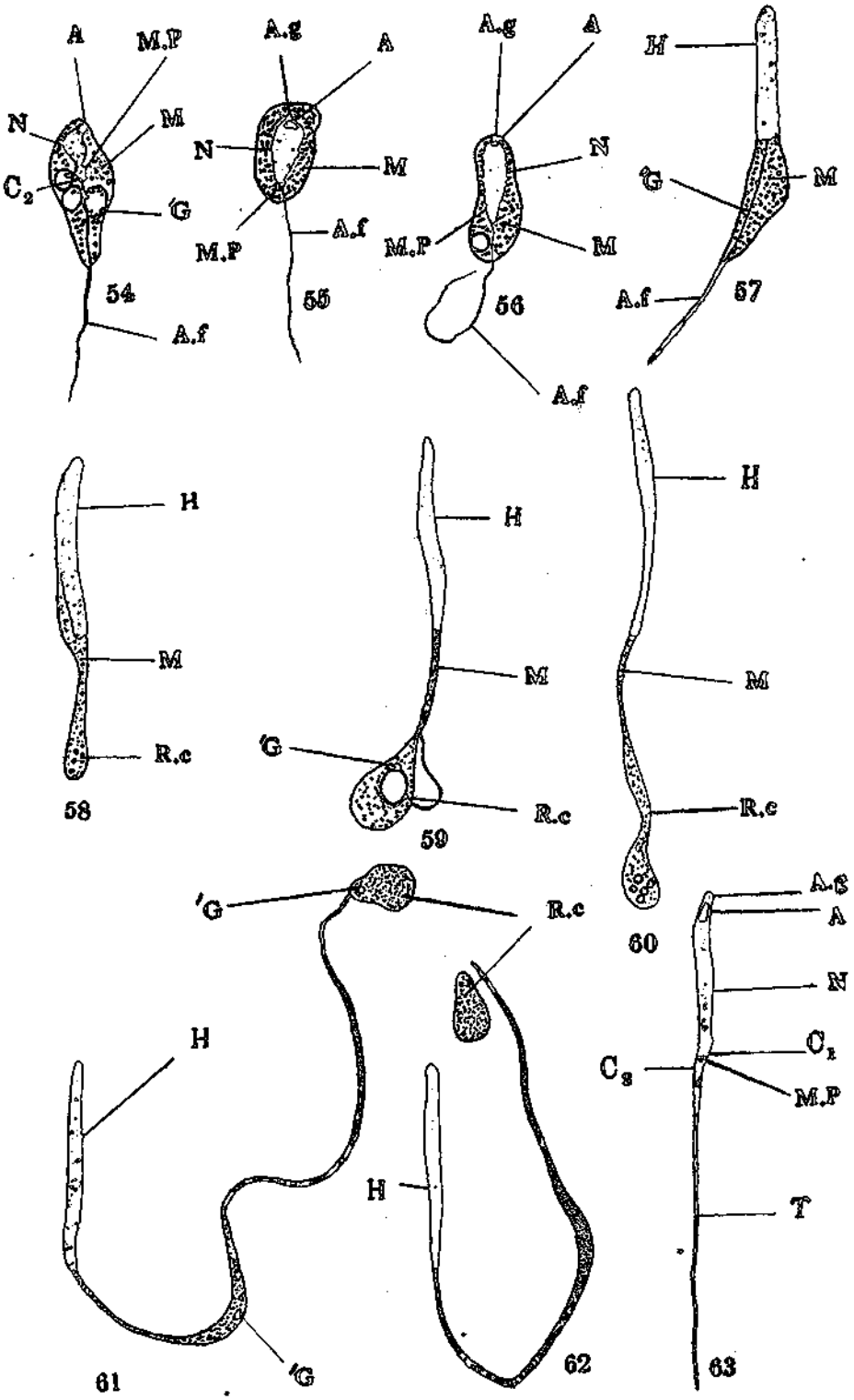
PLATE IV.



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CYTOLOGY OF INDIAN MEDICINAL PLANTS

II. Aconites, Valerians and Senna

by

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CYTOLOGY OF INDIAN MEDICINAL PLANTS

II. Aconites, Valerians and Senna

By P. N. Mehra *and* S. N. Sobti

In a previous investigation (Mehra and Sobti, in press) a cytological study of the Solanaceous drugs was undertaken. In the present paper Aconites and a few other medicinal plants are presented.

Genus Aconitum

Aconitum is a large genus belonging to the family Ranunculaceae. It comprises more than 300 species distributed over the Northern temperate regions of the globe with its greatest diversity in central Asian mountains (Index Kewensis, 1895–1953). The latest systematic work on Indian Aconites is by Stapf (1905), who reports some 24 species from India.

Aconites are highly poisonous plants of great medicinal value. The poisonous properties are due to the alkaloids which fall into two different groups, Aconitines and Atisines.

No work has been done on the cytology of Indian Aconites so far. Out of 24 species of Aconites found in India only six are found in Kashmir in the Alpine zone from 11,000–13,000 ft. Some of these also extend eastwards. The remaining 18 species are met from Kumaon to Eastern Himalayas in Sikkim and Assam. All the six species, namely *Aconitum chasmanthum* Stapf Ex Holmes, *A. violaceum* Jacquem, *A. deinorrhizum* Stapf, *A. heterophyllum* Wall, *A. kashmiricum* Stapf Ex Coventry and *A. laeve* Royle, found in Kashmir have been worked out cytologically in the present investigation.

Aconitum napellus L. is official in the Pharmacopœias of various countries. It does not occur in India. *A. chasmanthum* is found to be a good substitute and is included in Indian Pharmacopœial list. According to Chopra (1933) it contains seven times more alkaloids than *A. napellus* but the Indaconitine met with in the former is less potent than Aconitine from *A. napellus*. *A. deinorrhizum* contains alkaloid Pseudoaconitine which is more potent than Aconitine from *A. napellus*. *A. heterophyllum* contains alkaloid Atisine and is used as tonic in the indigenous system of medicine. The other three species, *A. violaceum*, *A. kashmiricum* and *A. laeve*, are considered poisonous but nothing is known about their chemistry and pharmacology.

Taxonomy of the genus Aconitum :—

The genus is taxonomically divided into three groups on the basis of the nature of underground portion. The first group *Gymnaconitum* Stapf

is an annual and contains only one species *A. gymnandrum* Stapf. The second group *Lycoctonum* D.C. has perennial rhizome which gives rise to a new flowering stem each year. The third group is comprised of the large section *Eu-Aconitum* C. A. Meyer and the small section *Anthora* D.C. and has tubers. The individual tuber is monocarpic.

Group *Gymnaconitum* is not represented in India. Of the six investigated species only *A. laeve* belongs to the group *Lycoctonum* while the remaining five species are of Eu-Aconite group.

Material :—

The species have been collected from the following places in Kashmir :

	Name of the species		*Place*
1.	*Aconitum laeve*	..	Toshmaidan, 22 miles from Gulmarg.
2.	*A. chasmanthum*	..	Hapat Khud, 4 miles from Gulmarg.
3.	*A. violaceum*	..	Toshmaidan.
4.	*A. deinorrhizum*	..	Padari, 8 miles from Bhaderwa Eastern District of Kashmir.
5.	*A. heterophyllum*	..	Drang Forest, 5 miles from Gulmarg.
6.	*A. kashmiricum*	..	Khillenmarg, 3 miles from Gulmarg.

The pressed specimens of all the six species of Aconites, along with three species of Valerians and one species of Cassia mentioned hereafter, have been deposited in the herbarium of Panjab University Department of Botany.

Methods

The flower buds of the five species except *A. violaceum* were fixed in acetic alcohol for 24 hours, and thereafter stored in 70% alcohol until use. The smears of the pollen mother cells were prepared by the usual aceto-carmine method and the slides made permanent by Maclintock's technique. In the case of *A. violaceum* flower buds were fixed in Craf and were sectioned at 12μ thickness by the usual paraffin technique. Sections were stained by Newton's iodin-crystal violet method.

Karyotype study has been undertaken only of *A. chasmanthum* and *A. violaceum* from the secondary roots formed when the tuberous primary roots were planted in pots at Srinagar. The root-tips were pretreated with 0·01% colchicine solution for two hours (from 11 a.m. to 1 p.m.) and then fixed in acetic alcohol and squashed in acetocarmine.

Drawings have been made with 1·5 (100×) Ziess apochromatic objective and (12·5×) Ocular with the aid of Spencer's camera lucida giving a magnification of roughly 2,300 times.

Cytological observations :—

Fig. 1 shows 16 somatic chromosomes at metaphase in a root-tip cell of *A. chasmanthum*. The 16 chromosomes form 8 homologous pairs. Two of these, the largest and the smallest pair, have a median centromere. The

next larger pair possesses a sub-median centromere with the smaller arm 2½ times less than the larger. The remaining five pairs which are hardly distinguishable from one another possess a sub-terminal centromere.

Figs. 2 and 3 show two pollen mother cells each having 8 bivalents of the same species. There are two exceptionally larger bivalents than the rest. Each of these two large bivalents possesses two chiasmata. The smallest bivalent is with a terminal chiasma. The nucleolus is absent in this species at diakinesis. There is no secondary association and meiosis is normal.

The karyotype of *A. violaceum* is shown in Fig. 4. There are 16 somatic chromosomes forming 8 homologous pairs. It will be noticed that they are smaller in size than those of *A. chasmanthum*. In this species there are three pairs with a median centromere, a largest, another middling in size, and a third which is the smallest pair of complement. The second largest pair has a sub-median centromere. The remaining four pairs have sub-terminal centromere. These four pairs are similar in size, excepting that one can be distinguished by the presence of a satellite in the shorter arm. The largest pair of the complement corresponds to the second largest pair of *A. chasmanthum* in matter of size.

Figs. 5 and 6 show first metaphase plates of meiosis in pollen mother cells of the same species. The eight bivalents can be clearly counted in each case. There are two bivalents which possess two chiasmata each. The chromosomes separate normally at first anaphase and the largest chromosomes are last to separate (Figs. 7 and 8). No secondary association is visible at the first and second metaphases in this species. Meiosis is normal.

Figs. 9 and 10 show two pollen mother cells of *A. deinorrhizum*. Sixteen bivalents are counted in each case. There are four large bivalents, each of which possesses two chiasmata. There are two small bivalents each with a single terminal chiasma. Meiosis is regular with the formation of normal pollen grains. Nucleolus is present at diakinesis.

Two pollen mother cells of *A. heterophyllum*, each with 8 clear bivalents, are shown in Figs. 11 and 12. This agrees with the findings of Schafer and Lacour (1934) who reported $2n = 16$. There are observed a few probable tetraploid mother cells scattered among the diploid ones. The exact number of chromosomes could not be counted in these but the evidence from the mass of the chromosomes as also from the size of the mother cells is indicative of their tetraploid nature. It seems possible that a small percentage of abnormal diploid pollen may also be formed in nature in this species. The smallest bivalent possesses a terminal chiasma. Meiosis is normal. Nucleolus is present at diakinesis.

In Figs. 13 and 14 are shown two pollen mother cells of *A. kashmiricum* each with 8 bivalents at diakinesis. There are present two large bivalents as usual, each with two chiasmata. The smallest bivalent is with a terminal chiasma. Meiosis is normal with the formation of apparently viable pollen. There is present a nucleolus at diakinesis.

Fig. 15 shows 8 bivalents at diakinesis in pollen mother cell of *A. laeve*. There are present two large bivalents. The chromosomes separate regularly

All Figs. ×2300. Dotted circles represent nucleoli.

FIG. 1. *Aconitum chasmanthum*, $2n = 16$, root squash.

FIGS. 2 AND 3. *A. chasmanthum*, $n = 8$, diakinesis.

FIG. 4. *A. violaceum*, $2n = 16$, root squash.

FIGS. 5 AND 6. *A. violaceum*, $n = 8$, first metaphase.

FIG. 7. *A. violaceum*, early anaphase in which three bivalents marked D have disjointed.

FIG. 8. *A. violaceum*, late anaphase. Seven can be seen at each pole while the eighth is eclipsed underneath.

FIG. 9. *A. deinorrhizum*, $n = 16$, diakinesis.

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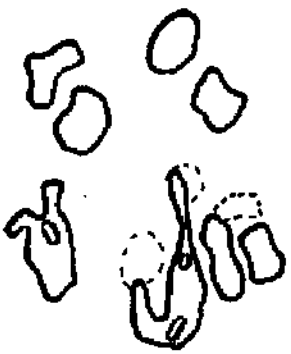


14

16

15

FIG. 10. *Aconitum deinorrhizum*, $n = 16$, diakinesis.
FIGS. 11 AND 12. *A. heterophyllum*, $n = 8$, diakinesis.
FIGS. 13 AND 14. *A. kashmiricum*, $n = 8$, diakinesis.
FIG. 15. *A. laeve*, $n = 8$, diakinesis.
FIG. 16. *A. laeve*, first anaphase, $n = 8$.

at first anaphase and are much condensed (Fig. 16). A large nucleolus is present at diakinesis. Meiosis is normal.

The chiasmata are randomized in all the six species of *Aconitum* studied in the present investigation.

It may also be remarked that there is a regular sequence in the development of stamens in a flower. The outermost stamens may have formed the pollen grains while in the innermost stamens around the carpels the mother cells may not yet have rounded up.

Discussion

Family Ranunculaceae is divided into two sections on the basis of chromosome size (Lewitsky, 1931). One section includes genera like *Delphinium*, *Ranunculus* and *Helleborus*, and the other includes genera like *Thalictrum* and *Aquilegia* with small chromosomes. Genus *Aconitum* possesses large chromosomes and thus belongs to the former section.

According to Lewitsky (*vide* Stebbins, 1950) a karyotype consisting of chromosomes essentially similar to each other in size and with median or sub-median centromeres is termed symmetrical. Asymmetrical karyotype is the one which has many chromosomes with sub-terminal centromeres or where there is great variation in size between the largest and the smallest chromosomes, or in which both the characters are present. According to Lewitsky (1931) the primitive genus like *Helleborus* possesses symmetrical karyotype as compared to the genus *Delphinium* which possesses asymmetrical karyotype. He concludes that the asymmetrical karyotype is advanced compared to the symmetrical one. *Aconitum* which is an advanced genus of the same family on morphological grounds has got a very asymmetrical karyotype and corroborates Lewitsky's view within the compass of this family. Chromosomes in this genus vary in size from 3μ to 12μ and in form with a few V but mostly J shaped chromosomes (Figs. 1 and 4).

Below is given the resume of the findings of various workers * in regard to the chromosome numbers of the genus:—

	Name of the species	Chromosome number		Author
		n	2*n*	
	Section Lycoctonum			
1.	*A. vulparia* Rechb.	..	16	Langlet
2.	*A. orientale* Boiss	..	16	Schafer and Lacour
3.	*A. thyriacum* Blocki ..	..	16	,,
4.	*A. luridum* Hook fil, et Thomas	..	16	Afify
5.	*A. septentrionale*	..	16	Langlet
6.	*A. laeve* Royle	8	..	Authors
	Section Anthora			
7.	*A. anthora* Linn.	..	32	Langlet

* Compiled from Darlington and Janaki Ammal, 1945, and Schafer and Lacour, 1934.

	Name of the species	Chromosome number		Author
		n	2*n*	
	Section Eu-Aconitum : Diploid			
8.	*A. forestii* Stapf		16	Schafer and Lacour
9.	*A. transectum* Diels		16	,,
10.	*A. hemsleyanum* Pritzel		16	,,
11.	*A. variegatum*		16	,,
12.	*A. barbatum*		16	Skoloveskeji and Strelkove
13.	*A. excelsum*		16	Langlet
14.	*A. kamtschatinum*		16	,,
15.	*A. uncinatum*		16	Longacre
16.	*A. chasmanthum* Stapf Ex Holmes ..		16	Schafer and Lacour
		8	16	Authors
17.	*A. violaceum* Jacquem ..	8	16	,,
18.	*A. heterophyllum*		16	Schafer and Lacour
		8	..	Authors
19.	*A. kashmiricum* Stapf Ex Coventry	8	..	,,
	Triploid			
20.	*A. stoerkianum* Rechb.		24	Schafer and Lacour
	Tetraploid			
21.	*A. chinense* Sieb		32	Schafer and Lacour
22.	*A. delavayi*		32	Langlet
23.	*A. kuznetzoffii*		32	,,
24.	*A. paniculatum*		32	,,
25.	*A. volubile*		32	Schafer and Lacour
26.	*A. spicatum*		32	,,
27.	*A. napellus* Linn.		32	Langlet
28.	*A. anglicum*		32	Schafer and Lacour
29.	*A. altaicum* Stienb		32	Skoloveskeji and Strelkove
30.	*A. deinorrhizum* Stapf ..	16	..	Authors
	Hexaploid			
31.	*A. palmatum*		48	Schafer and Lacour
	Octaploid			
32.	*A. wilsoni*		62	Schafer and Lacour

It will be observed that the base number in the genus is strictly 8. Incidentally this number is the most prevalent and the lowest. In the present case out of six species investigated this is present in five. Jensen (1950) while studying meiosis in an unusual form of *A. uncinatum* which showed some characteristics of *A. reclinatum* observed secondary association among its bivalents at metaphase. He concluded that 4 is the base number instead of 8 observed by Langlet (1932), Afify (1933), Schafer and Lacour (1934), Darlington and Janaki Ammal (1945). Meiosis studied in the six species during the present work has not revealed any secondary association by which it could be concluded that 4 is the base number. In the other related genera like *Delphinium*, *Ranunculus*, *Anemone* and *Helleborus*, the base number is also 8. Darlington (1932) observes that secondary association can arise in the diploids having parts of their chromosomes reduplicated and, therefore, having internal relationships not unlike those of polyploids. In the light of Jensen's statement that the plant under his study was an abnormal one, it is quite likely that the secondary association observed by him was due to the duplicated material in the chromosomes. In any case his conclusion is open to question since no other investigator has observed any evidence of secondary association in the genus.

The present evidence indicates that all the species belonging to section Lycoctonum are diploids ($n = 8$). On the other hand in the section Eu-Aconitum higher numbers are present and they appear to have arisen from the base number 8 by polyploidy. Out of twenty-five species worked out so far twelve are diploids and ten are tetraploids. The triploid number ($2n = 24$) has been reported in *A. stoerkianum* (Schafer and Lacour, 1934). Both on taxonomic and cytologic grounds this is a hybrid between diploid *A. variegatum* and tetraploid *A. napellus*. Three more triploid plants have been reported by Schafer and Lacour (1934) which, too, seem to owe their origin to hybridization between diploid and tetraploid species under cultivation. Only one hexaploid species $2n = 48$ is known. *A. wilsoni* is the only octaploid species with $2n = 64$ reported so far.

It is obvious that polyploidy is the only means by which numerical change has operated in genus *Aconitum* to give rise to new species. There is no indication of Aneuploidy.

Schafer and Lacour (1934) found morphological odd chromosomes amongst the homologous pairs in the nuclei of some plants and attributed this fact to structural hybridity. No evidence of structural hybridity has been found in the six species studied in the present investigation.

II. Valerians

Valeriana is a large genus of family Valerianaceae. It includes more than two hundred species, distributed in cool temperate regions, except Australia and S. Africa. The chief centre of development of this genus is in the Mediterranean region and it is scarcely represented south of the Equator in the old world. In the new world there is a strong development of this genus on the Pacific side, especially in the Chilean Andes, and extends towards south.

In India about 13 species are represented (Hooker, 1882). Of these 10 are found in the Himalayas extending from Kashmir in the west to Assam in the east. Three species are found in the south.

Valerians are prized for an essential oil which is present in the rhizome, and is used medicinally as tonic, antispasmodic, diaphoretic and anti-epileptic. *Valeriana officinalis* is official in most of the European countries. In India *V. wallichii* is an excellent substitute. The rhizome of *V. hardwickii* is also known to possess strong odour of ethereal oil but its commercial value has not been investigated.

Material and methods :—

Three species *V. hardwickii*, *V. wallichii* and *V. stracheyi* have been investigated.

The flower buds of *V. hardwickii* were collected from Toshmaidan (Kashmir) at 13,000 ft. where it grows abundantly in the forest under fir trees. The flower buds were prefixed in Carnoy for a minute and then in Craf for 24 hours. The rhizome of *V. wallichii* and *V. stracheyi* were collected in July from Gulmarg and Ninglinalla (Kashmir) respectively where they grow abundantly. These were planted in pots and new roots and shoots were produced after a fortnight. The root-tips were fixed in Craf to which a pinch of maltose was added.

Microtome sections of flower buds and root-tips were cut 10–12μ thick with the usual paraffin method. The slides were stained by Newton's iodin-crystal violet.

Cytological observations :—

Valeriana hardwickii Wall.

The chromosomes are counted from the first meiotic division. Clear 28 bivalents are present at metaphase (Figs. 17 and 18). There appear to be some secondary associations which it has not been possible to analyse. Meiosis is perfectly normal. Pollen grains appear viable.

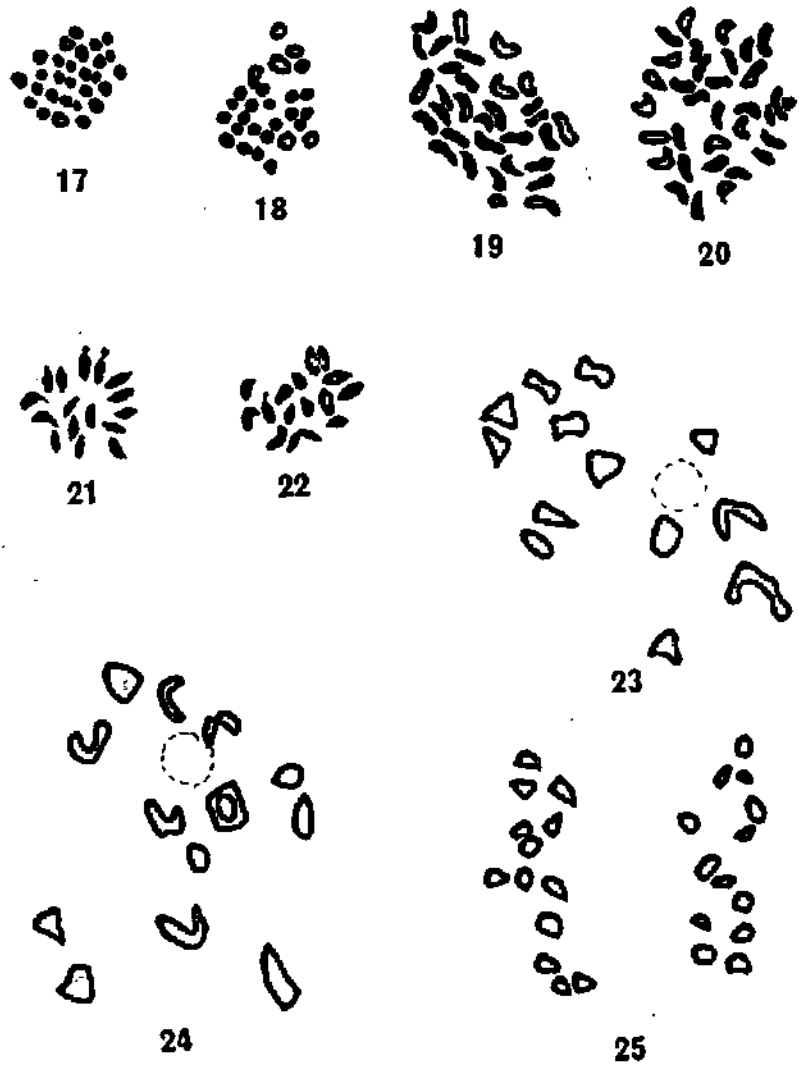
V. wallichii D.C.

Root-tip cells show 32 chromosomes at metaphase (Figs. 19 and 20). The chromosomes are small and karyotype details are hard to analyse.

V. stracheyi C.B. Clarke

The root-tip cells at metaphase show 16 chromosomes (Figs. 21 and 22). These are smaller than those of *V. wallichii* (Figs. 19 and 21). There are two chromosomes with satellites. The homologous chromosomes lie side by side at metaphase indicative of somatic pairing (Fig. 21).

Resume of the cytological work on the genus.



FIGS. 17 AND 18. *Valeriana hardwickii*, $n = 28$, first metaphase.
FIGS. 19 AND 20. *V. wallichii*, $2n = 32$, from root-tip metaphase.
FIGS. 21 AND 22. *V. stracheyi*, $2n = 16$, from root-tip metaphase.
FIGS. 23 AND 24. *Cassia angustifolia*, $n = 13$, diakinesis.
FIG. 25. *C. angustifolia*, first anaphase, $n = 13$.

	Name of species	Chromosome number		Author
		n	*2n*	
1.	*Valeriana altaica*	..	14	Ranquist
2.	*V. officinalis*	..	14	,,
		..	28	Meurman*
		..	28, 56	Skalinka*
3.	*V. capitata*	..	56	Skoloveskeji and Strelkove*
4.	*V. Sambucifolia*	..	56	Meurman*
	as *excelsa*	..	14, 56	Ranquist
5.	*V. salina*	..	56	,,
6.	*V. dioica*	..	16	Meurman*
7.	*V. flaccidissma*	..	16	Matsura and Suto*
8.	*V. wallichii*	14	..	Kishore
		..	32	Authors
9.	*V. hardwickii*	28	..	,,
10.	*V. stracheyi*	..	16	,,

Discussion

The base chromosome numbers in the genus *Valeriana* are 7 and 8. Both these numbers are equally prevalent. It is difficult to say at present which number gave rise to the other. Out of the 10 species worked out so far the number 7 or its multiple is found in six species and 8 or its multiple in four species.

Polyploidy is quite common in this genus and has also been found in the same taxonomic species *V. officinalis*. It is evident that polyploidy has played a significant rôle in the evolution of the genus both at specific and sub-specific levels.

The present evidence reveals that *V. wallichii* has two races with both 7 and 8 base numbers. The writers' observations indicate that the species is a tetraploid of 8 series while according to Kishore (1952) it is a tetraploid of 7 series. If this is the correct position, then a close comparative study of the species from various sources should reveal as to which number is original and which derived. From that angle the evolutionary interest of this species is very great indeed. It is also not unlikely that *V. wallichii* as worked by the writers and Kishore may actually be two different taxonomic forms.

III. Senna

The dried leaflets of *Cassia angustifolia* Vahl are sold in commerce under the name of Tinnevelly Senna. The plant is indigenous to Arabia, Somaliland, Sind and W. Panjab. In India it is abundantly cultivated in Tinnevelly in South India.

---

* From Darlington and Janaki Ammal, 1945.

Senna is useful purgative either for habitual constipation or occasional use. It contains anthraquinone derivatives, aloe-emodin, rhein, and few glycosides (Trease, 1949).

The flower buds of the species were secured from the plants cultivated at Drug Research Laboratory experimental farm, Jammu, and fixed in acetic alcohol for 12 hours. Aceto-carmine smears of the pollen mother cells were prepared in the usual way.

Observations

There are found 13 bivalents mostly with terminal chiasmata at diakinesis and metaphase (Figs. 23 and 24). These bivalents separate regularly at anaphase (Fig. 25). Meiosis is normal and cytokinesis simultaneous. Pollen grains are all alike and apparently viable.

Three base numbers are reported in the genus *Cassia* 6, 7 and 13 (Darlington and Janaki Ammal, 1945). The base number 13 has perhaps arisen by amphidiploidy between species with base numbers 6 and 7. *Cassia angustifolia* has got 13 bivalents and therefore belongs to the third group.

Summary

Chromosome counts have been made of six Indian species of *Aconitum* from the pollen mother cells. *A. laeve*, *A. chasmanthum*, *A. violaceum*, *A. heterophyllum*, and *A. kashmiricum* are diploids with $n = 8$ while one species *A. dienorrhizum* is tetraploid with $n = 16$. Karyotype of two of the above species, namely *A. chasmanthum* and *A. violaceum*, is studied from the root-tip squashes. It is of the asymmetrical type. Section Lycoctonum is uniformly diploid while the Eu-Aconites show various grades of ploidy.

Meiosis is normal in *Valeriana hardwickii* with $n = 28$. In the other two species *V. stracheyi* and *V. wallichii* the $2n$ number determined from root-tips is 16 and 32 respectively. Observation in respect of the latter is at variance from that of Kishore who reports $n = 14$ in *V. wallichii*. There are two base numbers in the genus. Polyploidy seems to play a significant rôle in evolution at specific as well as sub-specific levels.

In *Cassia angustifolia* chromosome number $n = 13$ has been found from pollen mother cells.

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DIVIDED CELLS

by

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DIVIDED CELLS

By R. P. Bambah, *Department of Mathematics, Panjab University, Hoshiarpur, Panjab, India*

1. Let Λ be an inhomogeneous lattice of determinant Δ in the $x-y$ plane. In vector notation Λ is the set of points $P+A$, $A \in \mathfrak{L}$, $P \notin \mathfrak{L}$, where $\mathfrak{L}$ is a homogeneous lattice. If O, A, $A+B$, B are the vertices of a fundamental parallelogram of $\mathfrak{L}$, the parallelogram with vertices P, $P+A$, $P+A+B$, $P+B$ is called a *cell* of Λ. A cell is said to be *divided* if it has one vertex in each of the four quadrants. The theorem on divided cells given below is important for applications to the inhomogeneous minima of binary quadratic forms. (See, e.g., Barnes and Swinnerton-Dyer [1], especially §1).

Theorem: *If Λ is a two-dimensional inhomogeneous lattice having no point on either of the coordinate axes $x = 0$, $y = 0$, then Λ has at least one divided cell.*

A proof has been given by Delauney [2]. (For an English version, see §2 of [1]). The alternative proof given in §2 below may be of interest.

2. It will be enough to show that there exists a convex quadrilateral $ABCD$ whose vertices are points of Λ lying in different quadrants and which contains no point of Λ other than the vertices A, B, C and D. For, then, since each of the triangles ABC, ABD, BCD and ACD has area $\frac{1}{2}\Delta$ (because none of these triangles contains a point of Λ other than the vertices), it would follow that $ABCD$ is a parallelogram of area Δ and hence a cell of Λ.

We define a *configuration* $ABCD$ to be the convex cover of four points A, B, C and D of Λ lying in different quadrants. Since Λ has points in each quadrant, it follows that 'configurations' do exist.

Since no straight line can have points in all the four quadrants, it follows that every 'configuration' is either a convex quadrilateral or a triangle with points of Λ as its vertices. Since the areas of such figures are multiples of $\frac{1}{2}\Delta$, it follows that there exists a 'configuration' $B_1B_2B_3B_4$, say, where B_i is in the i-th quadrant, whose area is minimal for all 'configurations'. Also $B_1B_2B_3B_4$ is either a convex quadrilateral or a triangle.

If $B_1B_2B_3B_4$ is a quadrilateral then it cannot contain a point of Λ other than the vertices. If not, let E be one such point. Then, since E does not lie on the axes, it is in some quadrant, say the i-th. The 'configuration', obtained from B_1, B_2, B_3, B_4 replacing B_i by E, can be easily seen to have area less than the 'configuration' $B_1B_2B_3B_4$, which is impossible. Therefore $B_1B_2B_3B_4$ is the required divided cell and we have nothing to prove.

We can now suppose the 'configuration' $B_1B_2B_3B_4$ is a triangle. Without loss of generality we can suppose it is the triangle $B_1B_3B_4$ i.e.

B_1, B_3, B_4 are the vertices while B_2 lies in the closed triangle $B_1B_3B_4$. The part of $B_1B_3B_4$ outside the second quadrant cannot contain a point of Λ other than B_1, B_3 or B_4. If not, suppose E is a point of Λ different from B_i in the triangle in the i-th quadrant. (E must lie in some quadrant.) Then the configuration obtained by replacing B_i by E can be seen to have smaller area than $B_1B_3B_4$, which is a contradiction. Since the triangle $B_1B_3B_4$ contains a finite number of points of Λ in the second quadrant and since at least one such point does exist, we can select a point C_2 of Λ in the second quadrant part of $B_1B_3B_4$ such that

(i) B_1C_2 makes the least angle θ with B_1O.

(ii) of all points of Λ in the second quadrant that make angle θ with B_1O, C_2 is the nearest to B_1.

Then the triangle $B_1C_2B_4$ does not contain any point of Λ other than the vertices, and therefore has area $\frac{1}{2}\Delta$. Since the slopes of OB_1 and B_3B_1 are positive, that of C_2B_1 is also positive. Therefore the line through B_4 parallel to B_1C_2 has an infinite length included in the third quadrant. Since the line l through B_4 parallel to B_1C_2 has infinity of points of Λ in the third quadrant and at least one in the fourth, we can select on l points C_3 and C_4 of Λ lying in the third and fourth quadrants respectively such that the segment C_3C_4 does not contain any other point of Λ. The parallelogram $B_1C_2C_3C_4$ is a divided cell of Λ.

3. If we assign each of the four semi-axes to one of the two quadrants separated by it, then the restriction that Λ has no points on the axes can be removed. If we assign the origin O to one particular quadrant, say the first, and then the semi-axes bounding the first quadrant also to the first, while we assign each of the other semi-axes to one of the two quadrants separated by it, the restriction that Λ is inhomogeneous can also be removed.

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AMPHIBIAN SPERMATOGENESIS

II. The Sperm of Toad

by

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AMPHIBIAN SPERMATOGENESIS

II. The Sperm of Toad

By G. P. Sharma *and* K. S. Dhindsa*

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Introduction

In the first paper of this series Sharma and Sekhri (1955) described in detail the process of spermatogenesis in the common Indian frog, *Rana tigrina*. Encouraged by the interesting and far-reaching results obtained by them, one of us (K. S. D.) prepared some slides of the testicular material of the common toad, *Bufo stomaticus* Lütken,† and these were studied independently as well as jointly by both of us. The present paper is an account of these studies.

Previous Work

King (1901) seems to be the first worker in this century to figure and describe the mature sperm of *Bufo lentiginosus*. According to her only the head (nucleus) of this sperm stains black with iron hæmatoxylin while the apex (acrosome), the middle-piece and the tail remain unstained. She further states that there is no evidence that a centrosome is contained in any part of this sperm. In a later paper, however, King (1907), while giving an exhaustive account of spermatogenesis in this very species of toad, clearly describes a centrosome surrounded by a granular attraction sphere in the resting stages of the earlier germ cells. In the newly formed spermatid the centrosome, according to her, is enclosed in a clear, round or oval vesicle which is sharply marked off from the surrounding cytoplasm. Soon this centrosome divides and as the two products of division move apart the vesicle enclosing them elongates to form the middle-piece of the ripe sperm. One of the centrosomes which remains at the anterior end of the middle-piece becomes embedded in the deeply staining substance of the sperm head, and the other which remains at the posterior end of the middle-piece soon becomes disc-shaped and later flattens itself considerably.

King (1907) also describes an acroblast which divides in each of the spermatogonial mitoses and also in both the meiotic divisions. One acroblast is, therefore, always present in the cytoplasm of every early spermatid where it undergoes a final division into two parts. One of these migrates

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† The material was very kindly identified by the Director, Zoological Survey of India, Calcutta, and we are very grateful to him for this.

to the anterior end of the spermatid to form the acrosome while the other remains in the posterior region of the spermatid to finally disappear. King (1907) believes that this latter part has something to do with the formation of the marginal filament or the tail membrane while the axial filament itself grows out of the outer centrosome situated at the posterior extremity of the middle-piece.

Stohler (1928) and Saez *et al.* (1936) have also worked on the various species of toads but their work is mainly concerned with the chromosomes. According to Stohler (1928), however, there is only one filament in the tail.

Material and Technique

The toad, *Bufo stomaticus* Lütken, is very common in Hoshiarpur during the rainy season, i.e. from June to September, which is also the breeding season for them. They can be easily distinguished from the frogs as their skin is comparatively dry and is covered over with glandular warts. Moreover, the hind-limbs in them are proportionally shorter and there are no teeth. It is also very easy to distinguish a male toad from the female as the former is somewhat yellowish in colour while the latter is slightly darkish. The mature male toads, in addition, possess the nuptial or thumb pads which are, however, absent in the females.

The material for the present study was collected mostly during night time from near the light posts on the roads, the toads being found in abundance there, preying upon the insects.

The animals were pithed alive on the dissecting board and the testes dissected out immediately. The testes of the toads are much smaller than those found in the frogs. They are a pair of ovoid bodies, each situated in close connection with the ventral surface of the anterior tip of a kidney. One of the two testes was frequently found to be bigger in size.

The testes were cut into very small pieces which ensured rapid fixation. The following fixatives were used: Bouin, Flemming without acetic acid, Champy and Kolatchew with Nassonow's modification. The best results were, however, obtained with Bouin (4–6 hours), F.W.A. (20 hours) and Champy (18 hours).

In all the cases, paraffin method of embedding, using the paraffin wax of 60°C., was employed and the sections were cut mostly five microns in thickness. The staining method applied was 4% iron alum followed by 0·5% hæmatoxylin.

The study of mature sperms was made from the smears which were fixed in F.W.A. diluted with an equal quantity of distilled water and stained in the usual way.

Observations

Primordial germ cells.—The primordial germ cells, from which the spermatogonia are differentiated, are small, rounded or ovoid cells, generally arranged on the periphery of the seminiferous tubules (Plate I, Fig. 1). Each of them possesses a large nucleus revealing inside it two or more deeply staining nucleoli besides the numerous fine chromatin granules.

Surrounding the nucleus is a thin layer of cytoplasm in which a small, lightly staining granule can also be clearly made out. This seems to be the Golgi element. There is, however, no trace of the mitochondria in these cells.

Primary spermatogonia.—The earliest primary spermatogonium (Plate I, Fig. 2) resembles the cell just described except that it is slightly bigger in size and its cytoplasmic inclusions have become more distinct. They seem to be formed by the growth of the primordial germ cells and in shape they may be rounded or ovoid. The nucleus, as usual, reveals two or more deeply staining nucleoli besides the fine chromatin granules. In the cytoplasm are clearly visible two or three deeply staining granules which *are the Golgi elements. The mitochondria put in their appearance for the* first time as fine, lightly staining, dust-like particles and these are distributed throughout the cytoplasm.

The above-mentioned cells now grow considerably, thus forming huge cells which surpass in size all the other cells in the testis (Plate I, Figs. 3, 8 and 11). They are generally spherical in contour, each containing a large nucleus (Plate I, Fig. 3) in which are visible a few big and deeply staining bodies of different sizes scattered amongst the fine chromatin granules. These are the nucleoli. The cytoplasmic inclusions are well marked out at this stage. Of these, the mitochondria appear, as usual, in the form of fine, lightly staining, dust-like particles, distributed uniformly throughout the cytoplasm, but the Golgi elements can now be recognized as deeply staining granules of varying sizes. One of these is very large, yet it does not reveal any duplex structure (Plate I, Fig. 3).

Polymorphism.—The nucleus of these fully grown primary spermatogonia exhibits a peculiar phenomenon of polymorphism resulting in the formation of several nuclear lobes by a process of simple constriction. Figure 4 (Plate I) shows the beginning of this process. In this particular cell the nucleus has elongated and an invagination in the form of a blind canal is visible on one side of it. The interior of this canal is occupied by a core of cytoplasm in which can be distinguished some lightly staining, dust-like mitochondria and one or two deeply staining big Golgi elements. This blind canal penetrating the nucleus is homologous to the 'inter-nuclear canaliculus' of the earlier workers. The nucleus itself reveals inside it fine chromatin granules along with two big nucleoli, each contained in one lobe of the nucleus. Another small nucleolus may often be seen lying in close contact with the bigger one, in one of the lobes of the nucleus.

Further penetration of the 'inter-nuclear canaliculus' separates the two lobes of the nucleus, each containing a nucleolus of its own (Plate I, Fig. 5). *In the cytoplasm of the cell are visible the mitochondria, distributed uni*formly as usual.

In Figure 6 (Plate I) is shown the complete separation of the two lobes of the nucleus but one of them further reveals a constriction into two, each overlapping the other. The nuclear contents appear as usual in each lobe but the tiny mitochondria are now aggregated in the form of a juxta-nuclear mass. Two big Golgi elements can also be clearly made out, each lying near a nuclear lobe.

As more lobes of the nucleus are constricted off, the size of the cell increases considerably. Two such large primary spermatogonia are illustrated in Figures 7 and 8 (Plate I). In these cells the originally single nucleus has given rise to a number of lobes.

The nucleus in some of the primary spermatogonia very often appears in the form of a rosette showing many unconstricted lobes (Plate I, Fig. 9). In Figure 10 (Plate I) is shown another rosette-shaped nucleus in which some of the lobes are constricted off but they overlap the others.

An extreme case of polymorphism is depicted in Figure 11 (Plate I) where some of the nuclear lobes which have already been constricted off are seen overlapping the partly constricted rosette-shaped nucleus. The cell at this stage attains its maximum size.

It will not be out of place to mention here that the several lobes of the nucleus, thus formed, are the result of a simple constriction of the nucleus. A process like 'amitosis' does not exist at all, as at no place the constriction of the nucleus is seen to be followed by a cytoplasmic division.

Mitosis.—Just before the process of mitosis begins the nucleus regains its original spherical form. Soon the nuclear membrane disappears and the rod-like chromosomes make their appearance (Plate I, Fig. 12). Scattered in the cytoplasm can also be seen some tiny granules which are most probably the mitochondria. These are, however, very few in number.

In the side view of the prometaphase stage (Plate I, Fig. 13) the chromosomes are seen arranging themselves at the equator of the spindle, each pole of which is occupied by a sharply staining centrosome. The Golgi elements at this stage are represented by two deeply staining granules, one of them being quite big, while the mitochondria are seen in the form of a cluster on one side of the cell.

An early telophase stage is shown in Figure 14 (Plate I) where the two groups of chromosomes have already reached their respective poles. The Golgi elements are, however, still placed at the equator of the spindle.

The chromosomes now collect together and consequently lose their identity as such (Plate I, Fig. 15). Simultaneously there appear at the equator of the spindle a few deeply staining granules which constitute the so-called mid-body. The Golgi elements and the mitochondria seem to be distributed almost evenly to the two daughter cells.

In Figure 16 (Plate II) are shown two daughter cells (secondary spermatogonia) just formed but the remains of the spindle fibres connecting them still persist. In some other cells, however, even after the nuclei of the daughter cells have been completely formed the cytoplasmic division does not take place (Plate II, Figs. 17 and 18).

Secondary and later spermatogonia.—The secondary and later spermatogonia are much smaller cells than the primary, their size, of course, varies according to the number of generations by which they are removed from the primary. They also differ from the primary spermatogonia in the fact that they never show polymorphism of the nucleus.

Figure 19 (Plate II) represents a secondary spermatogonium in the resting condition. Besides the chromatin granules, there are also present in its nucleus a few deeply staining nucleoli. In its cytoplasm can also be

observed a deeply staining, granular Golgi element and a few lightly staining, dust-like mitochondria.

Primary spermatocytes.—The daughter cells formed after the last gonial division rest for a short while and then they grow to form cells which are slightly larger than the secondary spermatogonia. These are the primary spermatocytes (Plate II, Fig. 20). Their cytoplasmic inclusions are very well marked, the mitochondria having grown fairly big in size. A big, deeply staining, Golgi granule can also be clearly seen on one side of the nucleus.

After a short period of rest the primary spermatocytes start behaving differently. To begin with, a small process is given out on one side of the cell (Plate II, Fig. 21). This is full of the mitochondrial granules amongst which can also be distinguished one or two Golgi elements. In Figure 22 (Plate II) the mitochondria appear as usual but the single Golgi element reveals a duplex structure with an outer chromophilic cortex and an inner chromophobic core.

An extreme case of the formation of the horn-like processes by the cell cytoplasm is represented in Figure 23 (Plate II) where the entire cell appears to be an Amoeba-like organism. The nucleus is placed on one side, showing the usual chromatin granules inside it. The mitochondria, besides being distributed uniformly throughout the cytoplasm, also aggregate to form small masses in each of the horn-like processes of the cell and near the nucleus. A big Golgi element, showing a duplex structure, can also be clearly made out in the juxta-nuclear mass of the mitochondria and another granular Golgi element lies close to it.

Meiosis I.—As is well known the nucleus of the primary spermatocyte undergoes many changes just before the maturation divisions. These changes are, however, very difficult to be marked out in F.W.A. or Champy preparations. Figure 24 (Plate II) represents a primary spermatocyte with the nucleus in the early leptotene stage. The cytoplasm in this particular cell clearly reveals a big spherical Golgi element. In the polar view of the prometaphase stage (Plate II, Fig. 25), when the chromosomes are arranging themselves to form the equatorial plate, the mitochondria are seen distributed throughout the cytoplasm. Two Golgi granules can also be clearly observed lying side by side just near the chromosomes. The side view of the metaphase I is shown in Figure 26 (Plate II). Here each pole of the spindle is occupied by a sharply staining centrosome. Besides the mitochondria which are uniformly distributed throughout the cytoplasm, two Golgi elements can also be clearly seen, each going towards its respective pole. These may have been formed as a result of the division of the single spherical Golgi element seen earlier in the leptotene stage (Plate II, Fig. 24), but we have never observed the actual division. A sub-polar view of an early anaphase stage is illustrated in Figure 27 (Plate II) in which the chromosomes have just started moving apart. In Figure 28 (Plate II) is shown an early telophase stage where the two groups of chromosomes have almost reached their respective poles. The two centrosomes can, however, be still clearly made out. Quite a late telophase stage is depicted in Figure 29 (Plate II) in which though the nuclei of the two daughter cells

have been completely formed yet the faint line of demarcation between them is visible only on one side. During this division also both the mitochondria and the Golgi elements are sorted out almost equally to the two daughter cells.

Secondary spermatocytes.—The daughter cells, formed as a result of the division of the primary, are the secondary spermatocytes. These are small cells, their nuclei also being smaller than those of the primary (Plate II, Fig. 30 and Plate III, Figs. 31 and 32). The mitochondria remain distributed throughout their cytoplasm and the single, deeply staining Golgi element may sometime reveal a duplex structure with an outer chromophilic cortex and an inner chromophobic core (Plate III, Fig. 32).

Meiosis II.—After a short period of interkinesis the nucleus of the secondary spermatocyte undergoes the usual changes. In Figure 33 (Plate III) is shown a late prophase stage where the chromosomes have been completely formed. These will now arrange themselves at the equator of the spindle, each pole of which is occupied by a sharply staining centrosome (Plate III, Fig. 34). Besides the mitochondria which are uniformly distributed throughout the cytoplasm, two deeply staining Golgi elements can also be clearly made out, each going towards its respective pole. Figure 35 (Plate III) reveals a late anaphase stage in which the two groups of chromosomes have gone quite apart. In the subsequent telophase stage (Plate III, Fig. 36) these groups of chromosomes are seen occupying their respective poles. Figure 37 (Plate III) represents a late telophase stage in which the nuclei of the two daughter cells have been completely formed but the cytoplasmic membrane to separate the two has not yet made its appearance. Almost a similar cell is illustrated in Figure 38 (Plate III) in which even the process of spermateleosis has also begun without the complete separation of the two daughter spermatids. The distribution of the mitochondria and the Golgi elements is again almost equal to the two daughter cells formed after meiosis II.

Spermatids.—The spermatids are small, more or less, rounded cells, each having a vesicular nucleus which reveals inside it fine chromatin granules (Plate III, Fig. 39). Uniformly spread throughout the cytoplasm are the mitochondria and the Golgi elements—both in the form of well stained granules. As the Golgi elements at this stage do not show any duplex structure, they are distinguished from the mitochondria only by their larger size.

Spermateleosis.—With the beginning of the process of spermateleosis some of the mitochondria collect together on one side of the nucleus between it and the cell membrane (Plate III, Figs. 40 to 47). The rest of them, however, still remain scattered throughout the cell cytoplasm. As an axial filament can also be seen growing out of this mass of mitochondria, they definitely determine the future posterior end of the spermatid nucleus. It is, however, unfortunate that due to this close aggregation of the mitochondria no such structures as the centrosomes could be made out at this stage.

Concomitant with these changes the Golgi elements also reveal a distinct tendency to come together (Plate III, Figs. 42 and 43). This results in the formation of bigger granules which in their turn form still bigger granules by fusion (Plate III, Fig. 44). One of these big Golgi granules now comes in close contact with that end of the nucleus which is opposite the axial filament (Plate III, Figs. 45 to 47). This may be termed as the proacrosome. The other Golgi elements which are not so used up in the formation of the proacrosome move backwards to be ultimately sloughed off with the residual cytoplasm.

To begin with, the proacrosome stains deeply and uniformly (Plate III, Figs. 45 to 47). But, as it grows gradually, it becomes differentiated into an outer chromophilic cortex and an inner chromophobic core (Plate III, Fig. 48). A careful study of the fresh material under the phase-contrast microscope clearly reveals that the proacrosome at this stage is in the form of a distinct vacuole with glue-like contents. The presence of this vacuole-like proacrosome is also indicated in such cells from fixed preparations as illustrated in Figures 40 and 41 (Plate III).

As the process of spermateleosis continues the nucleus begins to elongate in an antero-posterior direction which is already so well determined by the cluster of mitochondria at its posterior end and the subsequent fixation of the proacrosome at its anterior end (Plate III, Figs. 48 to 52). Simultaneously with the elongation of the nucleus its staining capacity also increases and consequently it becomes increasingly difficult to differentiate it from the equally deep staining proacrosome which has, in the meanwhile, directly transformed itself into a triangular acrosome (Plate III, *Fig. 53 and Plate IV, Figs. 54 to 58*).

The nucleus, in all these elongating spermatids, is seen surrounded by a thin layer of cytoplasm containing mostly those mitochondria which do not form a cluster at its posterior end. Sometimes even the Golgi elements which have not been used up in the formation of the acrosome can also be seen in this attenuated layer of cytoplasm (Plate IV, Figs. 54 to 58).

At this stage the mitochondria which had earlier collected in the form of a cluster at the posterior end of the nucleus come closer together and form a small, deeply staining middle-piece (Plate III, Figs. 59 to 61). This, after it has fixed itself at the posterior end of the nucleus, becomes one with it and so cannot be easily differentiated in an overstained preparation.

The attenuating layer of cytoplasm which has been receding gradually is now seen in the form of a bead in the middle-piece region of the maturing sperms (Plate IV, Figs. 59 to 61). The granules contained inside it are mostly the mitochondria which have not been used up in the formation of the middle-piece but some of the bigger ones may also be the Golgi remnants.

It must have been noticed that in the cells illustrated in Figures 41 to 53 (Plate III) and in Figures 54 to 61 (Plate IV) only one axial filament has been shown, though in some of them at least it is quite thick. It is significant that all these cells have been selected from the sectioned material fixed in Champy or F.W.A. On the other hand, an examination of the

smear preparations, fixed in diluted F.W.A., has clearly shown the tail to be formed by two axial filaments coiled round each other (Plate IV, Figs. 62 to 68). This is further proved by a study of the fresh material under the phase-contrast microscope.

Now the single axial filament seen in the sectioned material may either be due to a closer packing of the two axial filaments under the action of some fixing reagent or it is, in reality, single during the earlier stages, the second axial filament appearing suddenly at a later stage. We are, however, inclined to believe the latter alternative.

Ripe sperm.—The ripe sperm of the toad possesses a deeply staining head formed by a cylindrical nucleus with a pointed needle-like acrosome fixed at its anterior tip. Immediately behind the head and in intimate contact with it is a small and equally deep staining middle-piece which is followed by a long tail formed by two axial filaments coiled round each other (Plate IV, Figs. 67 and 68). Since the two axial filaments do not easily separate it appears that they are joined together by some sort of undulating membrane which is, however, very rudimentary.

Each axial filament arises from a granular centrosome but as both the centrosomes lie side by side at the base of the deeply staining middle-piece they cannot be easily made out. Both of these centrosomes were, however, clearly observed, each with its own axial filament, when, during the study of fresh material under the phase-contrast microscope, the head along with the middle-piece of a particular sperm was, by the application of a certain pressure on the cover slip, completely separated from the tail which was still moving like the broken tail of a lizard, probably under the influence of the two centrosomes, each governing the movement of its own axial filament.

We are, therefore, inclined to believe that these two centrosomes are formed by the division of the originally single centrosome which could not, however, be observed on account of the aggregation of some of the mitochondrial granules at a very early stage of spermateleosis for the formation of the middle-piece in the ripe sperm.

The bead of residual cytoplasm containing mostly the mitochondria which was seen earlier in the middle-piece region of the maturing sperms gradually shrinks and moves down the axial filaments to be finally sloughed off (Plate IV, Figs. 59 to 66). The fully mature sperms are, therefore, completely devoid of it (Plate IV, Figs. 67 and 68).

Coiling during spermateleosis.—A frequent coiling of the cells is observed in the late elongating spermatids and the maturing sperms (Plate IV, Figs. 69 to 73). The possible cause for this may be the lack of space in the testis.

Discussion

Golgi elements and the acrosome.—It will be recalled that we described a single, lightly staining, granular Golgi element in the otherwise clear cytoplasm of the primordial germ cells of the toad, *Bufo stomaticus*. In the early primary spermatogonia, however, there are two or three deeply staining Golgi granules which grow in size still further with the all round growth of cells to form huge primary spermatogonia. During the

subsequent mitotic division the Golgi elements seem to be distributed almost equally to the two daughter cells. In the secondary spermatogonia and the early primary spermatocytes there is generally seen only one big spherical Golgi element which may later on reveal a duplex structure with a chromophilic cortex and a chromophobic core when the cytoplasm of the latter is produced into horn-like processes. Another deeply staining granular Golgi element may be seen lying near it in these Amœba-like primary spermatocytes. During meiosis I the granular Golgi elements are again sorted out almost equally to the two daughter cells. In the cytoplasm of the secondary spermatocytes there is usually seen a deeply staining granular Golgi element which may sometime be differentiated to show a duplex structure. The distribution of the Golgi elements during meiosis II is again almost equal to the two daughter cells.

In the earliest spermatid there are a few deeply staining Golgi granules spread uniformly throughout the cytoplasm. Soon these granules reveal a distinct tendency to come together to form bigger granules which in their turn form still bigger granules by fusion. One of these big Golgi granules now comes in intimate contact with the anterior end of the nucleus and this is the proacrosome. As it gradually grows in size it becomes differentiated into an outer chromophilic cortex and an inner chromophobic core. In the fresh material studied under the phase-contrast microscope the proacrosome, at this stage, appears to be in the form of a clear vacuole filled with glue-like contents. This proacrosome is then directly transformed into a triangular acrosome which after slight elongation appears needle-like in the ripe sperm.

These observations are strongly supported by the recent work of Sharma and Sekhri (1955) on the sperm of the frog, *Rana tigrina*, and the earlier work of King (1907) on the toad itself. According to her there is only one acroblast (our Golgi element) in the primary spermatogonium. This divides in each of the spermatogonial mitoses and in both the meiotic divisions. One acroblast is, therefore, always present in the cytoplasm of every early spermatid where it undergoes a final division into two parts. One of these now migrates to the anterior end of the spermatid to form the acrosome while the other remains in the posterior region of the spermatid to finally disappear.

It appears to us that she could not make out the other Golgi elements in her preparations on account of the strong Flemming's solution which she used as a fixative for her material.

Mitochondria and the middle-piece.—During the present investigation on the testicular material of the toad, *Bufo stomaticus*, we could not make out in the cytoplasm of the primordial germ cells any granules which could be labelled as the mitochondria. They seem to put in their appearance for the first time only in the primary spermatogonia in the form of minute, dust-like particles distributed uniformly throughout the cytoplasm. As, with the growth of the primary spermatogonia, the nucleus becomes polymorphic, these mitochondria may be seen forming a juxta-nuclear mass. During the subsequent spermatogonial divisions they are distributed almost evenly to the two daughter cells.

In the secondary spermatogonia and the primary spermatocytes the mitochondria are spread uniformly throughout the cytoplasm but in the latter they appear, on account of their growth, in the form of very prominent granules. These are again roughly sorted out, almost equally, to the two daughter cells during both the meiotic divisions.

In the earliest spermatid the mitochondria are uniformly spread throughout the cytoplasm but as soon as the process of spermateleosis begins some of them at least definitely collect together at the base of the nucleus while the others still remain distributed all over. During the end stages of spermateleosis those mitochondria which had earlier formed a cluster come still closer together and thus directly form a small, deeply staining middle-piece intimately attached to the posterior end of the now cylindrical nucleus of the ripe sperm. The remaining mitochondria are gradually sloughed off with the residual cytoplasm which is generally seen in the form of a bead in the middle-piece region of the maturing sperms.

Most of these observations are strongly supported by the earlier work of Sharma and Sekhri (1955) on the sperm of the frog, *Rana tigrina*. But King (1907), working on the testicular material of the toad, *Bufo lentiginosus*, did not describe or figure any mitochondria at any stage of spermatogenesis. This is not surprising at all in view of the fact that she used strong Flemming's solution for fixing her material. The clear vesicle, described by her in the early spermatids, the nature of which she could not determine and from which she derived the middle-piece of the ripe sperm, seems to us to be formed by the dissolution of those mitochondria which collect together at this stage in the form of a cluster at the base of the nucleus.

Centrosomes and the axial filaments.—In the present investigation the centrosomes were seen clearly only during the division stages when they appeared in the form of sharply staining distinct granules, each situated at its respective pole of the spindle. During the resting stages a centrosome can be distinguished from the granular mitochondria only if it is surrounded by a clear area. One such resting secondary spermatocyte is illustrated in Figure 32 (Plate III).

Besides figuring a deeply staining centrosome at each pole of the spindle during all the division stages, King (1907) also describes a centrosome surrounded by a granular attraction sphere in the resting stages of the earlier germ cells. In the newly formed spermatid, the centrosome, according to her, is surrounded by a clear vesicle which, as we have shown earlier, is probably formed by the dissolution of the mitochondria collected there in the form of a cluster.

King (1907) further states that the single centrosome of the early spermatid soon divides into two. One of these which she calls as 'inner' moves towards the nucleus and is finally embedded in the deeply staining substance of the sperm head. The other centrosome called 'outer' by her, while still maintaining its connection with the 'inner', moves to the posterior end of the clear vesicle where it remains even after the middle-piece has been formed from it. The axial filament of the tail grows out from the 'outer' centrosome while the marginal filament (our second axial filament)

or the tail membrane is possibly formed by that part of the acroblast which is left in the posterior region of the spermatid.

We have, however, clearly shown that in the ripe sperm of *Bufo stomaticus* each axial filament has its own centrosome but as both of these centrosomes remain surrounded by the cluster of mitochondria in the earlier stages and lie side by side at the base of the deeply staining middle-piece in the later stages of spermateleosis they cannot always be clearly made out. The two axial filaments remain joined together by a rudimentary undulating membrane.

Summary

1. The spermatogenesis of the toad, *Bufo stomaticus* Lütken, has been completely worked out in this paper.

2. The fully grown primary spermatogonium is the biggest cell in the testis and exhibits nuclear polymorphism.

3. The cytoplasm of the primary spermatocytes is often produced into horn-like processes.

4. Both the mitochondria and the Golgi elements are essentially granular in form. The latter, however, may sometime reveal a duplex structure with an outer chromophilic cortex and an inner chromophobic core.

5. The mitochondria seem to put in their appearance for the first time only in the spermatogonia but the Golgi material in the form of a single, lightly staining granule may be present in the cytoplasm of even the primordial germ cells.

6. Some of the mitochondria in the earliest spermatid collect together at the base of the nucleus and these come still closer together during the later stages of spermateleosis to form directly a small, deeply staining middle-piece.

7. The Golgi elements also reveal a distinct tendency to come together in the earliest spermatid and this results in the formation of bigger and bigger granules. One of these big Golgi granules is then directly transformed into the acrosome.

8. The ripe sperm of the toad possesses a deeply staining head formed by a cylindrical nucleus with a pointed needle-like acrosome situated at its anterior tip. Immediately behind the head and in intimate contact with it is a small and equally deep staining middle-piece which is followed by a long tail formed by two axial filaments coiled round each other. These, however, remain joined together by a rudimentary undulating membrane.

9. Each axial filament has its own centrosome but as both the centrosomes lie side by side at the base of the deeply staining middle-piece they cannot always be clearly made out.

10. The receding residual cytoplasm forms a conspicuous bead in the middle-piece region of the maturing sperms. Gradually this moves down the axial filaments and is finally sloughed off.

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Explanation of Lettering in Plates

A—Acrosome; *A.f*—Axial filament; *C*—Centrosome; *Ch*—Chromosomes; *C.G*—Chromatin granules; *G*—Golgi element; *'G*—Golgi remnant; *Inc*—Internuclear canaliculus; *M*—Mitochondria; *m.b*—mid-body; *m.p*—middle-piece; *N*—Nucleus; N_1—Nucleolus; *p*—Pocket or lobe of the nucleus; *Pa*—Proacrosome; *Pr*—Process of the cell; *P.N*—Polymorphic nucleus; *R. Cy*—Residual cytoplasm; *S.f*—Spindle fibres.

Explanation of Plates

All figures have been drawn with a camera lucida at the table level with Beck 10× eyepiece and an oil immersion objective giving an approximate magnification of 1,350 times.

All figures, except Figures 62 to 68 (Plate IV) which are from smears, have been selected from sectioned material fixed either in F.W.A. or Champy followed by 0·5% *iron hematoxylin*.

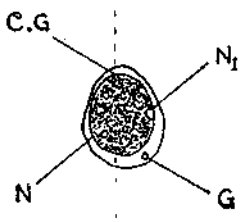
Plate I

Fig. 1. Primordial germ cell.
Fig. 2. Earliest primary spermatogonium.
Fig. 3. Late primary spermatogonium.
Fig. 4. Primary spermatogonium showing the beginning of the process of nuclear polymorphism.
Fig. 5. Primary spermatogonium with two lobes of the nucleus.
Figs. 6 to 8. Primary spermatogonia showing the formation of more lobes of the nucleus.
Fig. 9. Primary spermatogonium with rosette-shaped polymorphic nucleus.
Fig. 10. Primary spermatogonium in which some of the lobes of the rosette-shaped nucleus have been separated but they are still overlapping the others.
Fig. 11. Primary spermatogonium showing an extreme case of nuclear polymorphism.
Fig. 12. Primary spermatogonium—Prometaphase (Polar view).
Fig. 13. Primary spermatogonium—Prometaphase (Side view).
Fig. 14. Primary spermatogonium—Early telophase.
Fig. 15. Primary spermatogonium—Late telophase.

Plate II

Fig. 16. Primary spermatogonium showing the mid-body and spindle remains which are still connecting the two daughter cells.
Figs. 17 and 18. Primary spermatogonia, each with two fully formed nuclei of the daughter cells which are not yet completely separated.
Fig. 19. Secondary spermatogonium.

PLATE I.



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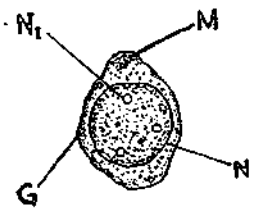

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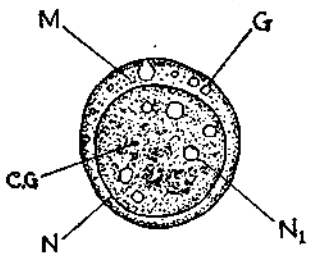

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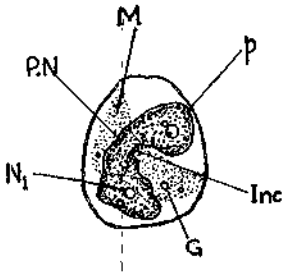

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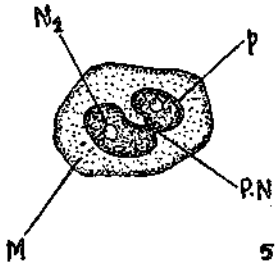

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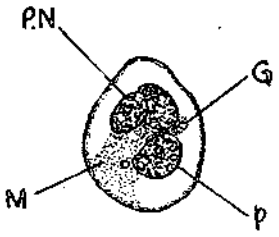

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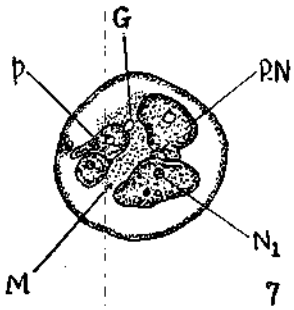

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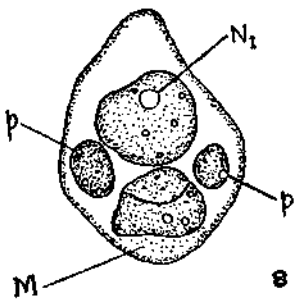

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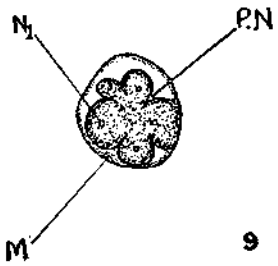

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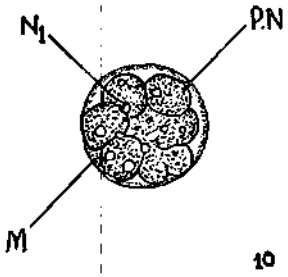

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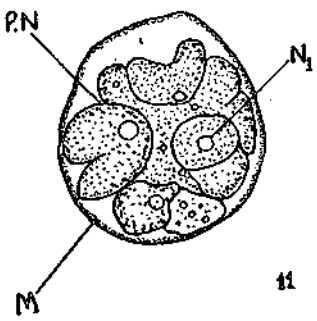

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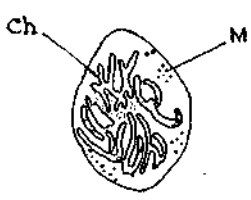

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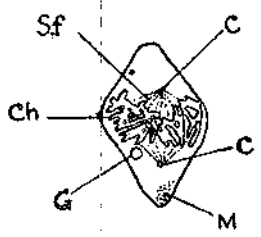

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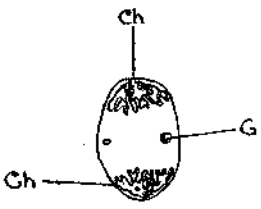

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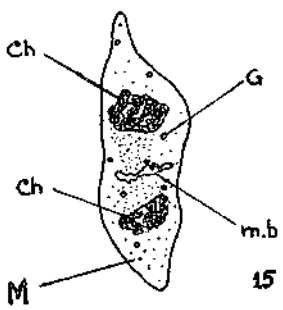

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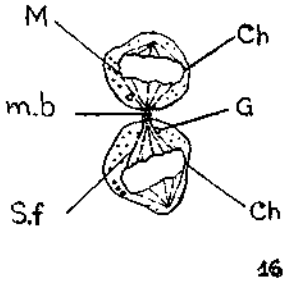

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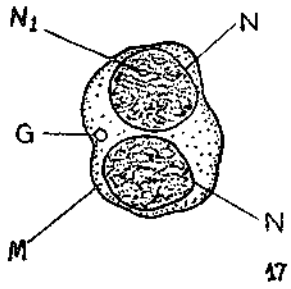

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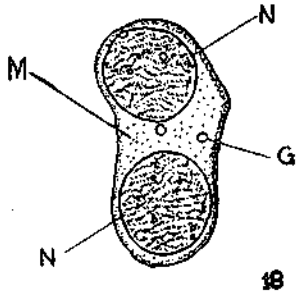

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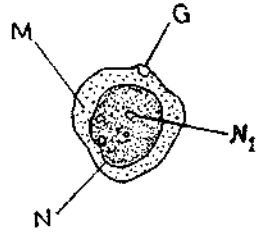

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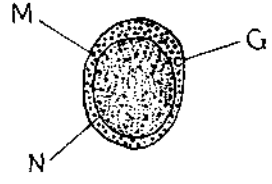

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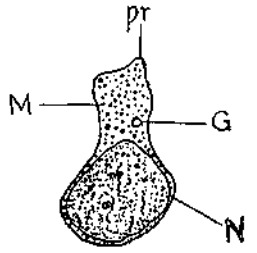

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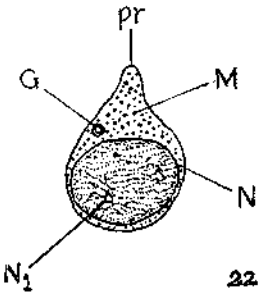

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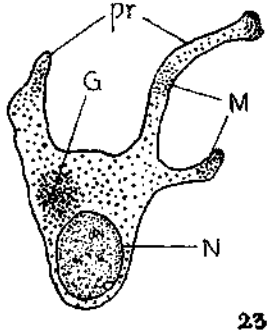

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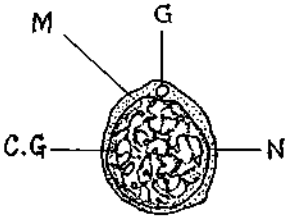

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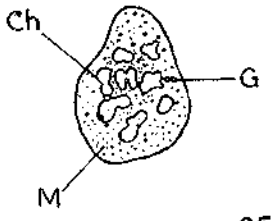

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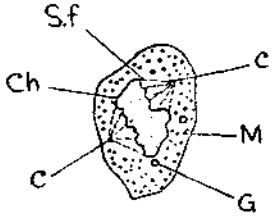

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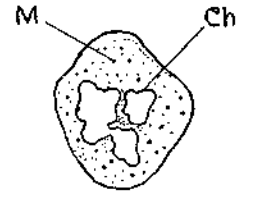

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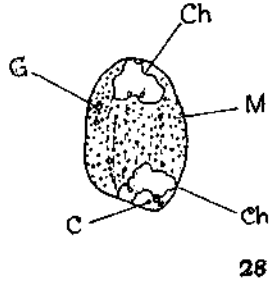

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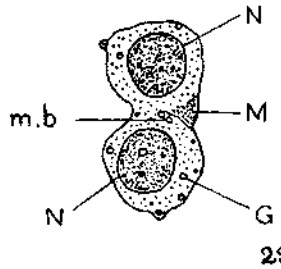

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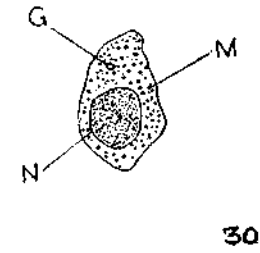

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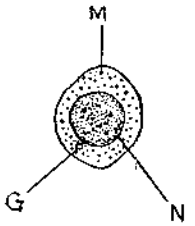

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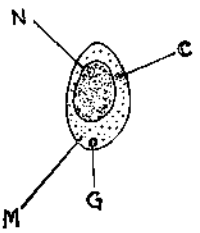

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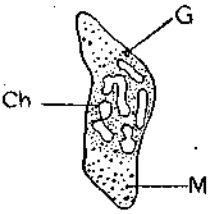

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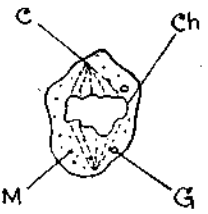

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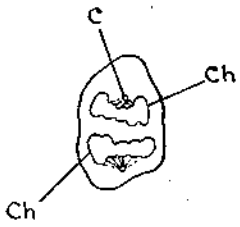

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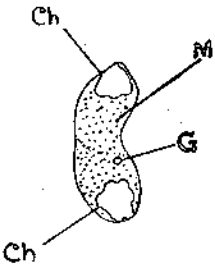

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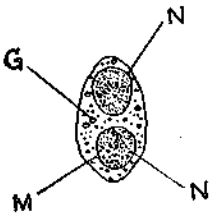

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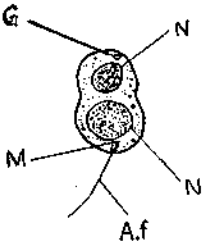

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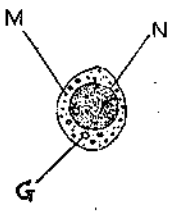

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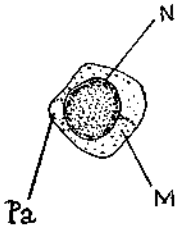

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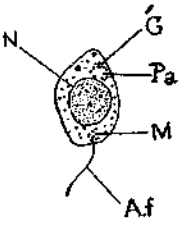

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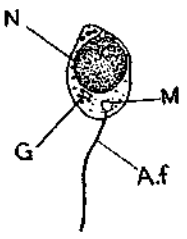

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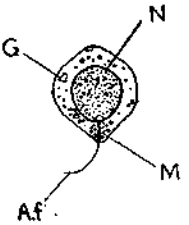

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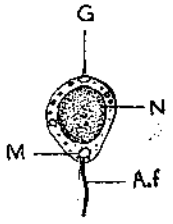

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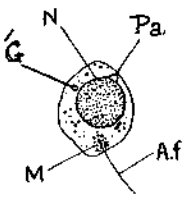

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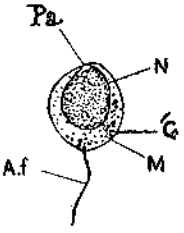

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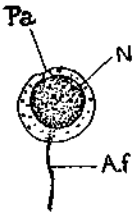

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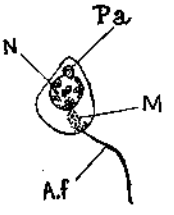

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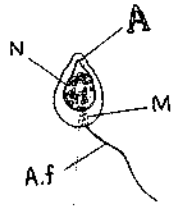

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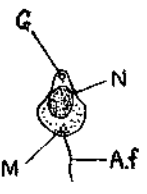

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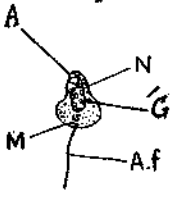

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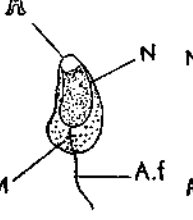

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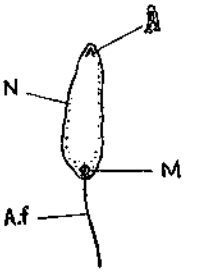

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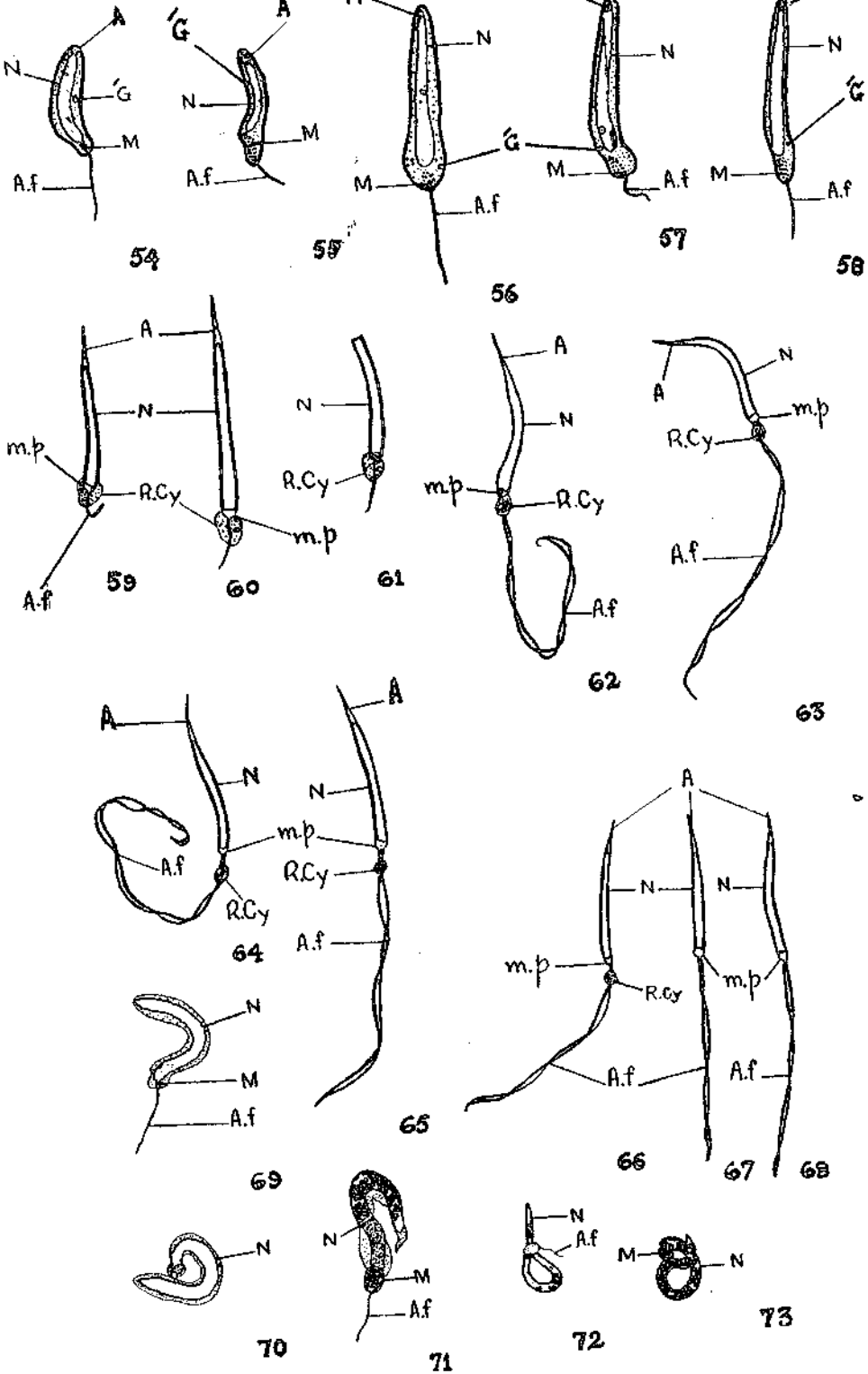
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FIG. 20. Primary spermatocyte.
FIG. 21. Primary spermatocyte with a cytoplasmic process.
FIG. 22. Primary spermatocyte with the single Golgi element revealing a duplex structure.
FIG. 23. Primary spermatocyte with many horn-like processes.
FIG. 24. Primary spermatocyte with the nucleus in the early leptotene stage.
FIG. 25. Prometaphase I (Polar view).
FIG. 26. Metaphase I (Side view).
FIG. 27. Anaphase I (Sub-polar view).
FIG. 28. Early telophase I.
FIG. 29. Late telophase I.
FIG. 30. Secondary spermatocyte.

Plate III

FIG. 31. Secondary spermatocyte.
FIG. 32. Secondary spermatocyte showing a centrosome. The Golgi element reveals a duplex structure.
FIG. 33. Late prophase II.
FIG. 34. Metaphase II (Side view).
FIG. 35. Late anaphase II.
FIG. 36. Early telophase II.
FIG. 37. Late telophase II.
Fig. 38. Late telophase II showing even the beginning of the process of spermateleosis in one of the daughter cells which have not yet separated completely.
FIG. 39. Earliest spermatid.
FIG. 40. Spermatid in which some of the mitochondria are seen collecting on one side of the nucleus. Diametrically opposite to this cluster of mitochondria is the vacuole-like proacrosome.
FIG. 41. Spermatid in which an axial filament can be seen growing out of the cluster of mitochondria. Slight indications of a vacuole-like proacrosome are also available on the other side of the nucleus.
FIGS. 42 and 43. Spermatids in which the Golgi elements are seen coming together to form bigger granules.
FIG. 44. Spermatid in which one of the big Golgi granules is seen coming near the anterior end of the nucleus.
FIGS. 45 to 47. Spermatids in which one of the big Golgi granules has come in intimate contact with the anterior end of the nucleus. This is the proacrosome and the Golgi remnants are seen moving backwards.
FIG. 48. Spermatid in which the proacrosome is being differentiated into an outer chromophilic cortex and an inner chromophobic core.
FIGS. 49 to 53. Elongating spermatids. The proacrosome is being directly transformed into a triangular acrosome.

Plate IV

FIGS. 54 to 58. Elongating spermatids. The mitochondria at the base of the nucleus can be seen coming closer together to form the middle-piece.
FIGS. 59 to 61. Maturing sperms with the bead of residual cytoplasm still sticking to the fully formed middle-piece.
FIGS. 62 to 66. Nearly mature sperms. The bead of residual cytoplasm is seen moving down the axial filaments to be finally sloughed off.
FIGS. 67 and 68. Ripe sperms.
FIGS. 69 to 73. Late elongating spermatids and maturing sperms showing the coiling of the cells.

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February, 1956

CYTOLOGICAL STUDIES ON THE MALE GERM CELLS OF THE SPIDER, *PARDOSA* SP., WITH OBSERVATIONS UNDER THE PHASE CONTRAST MICROSCOPE

by

G. P. SHARMA and BRIJ L. GUPTA

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By G. P. Sharma *and* Brij L. Gupta

Department of Zoology, Panjab University, Hoshiarpur

Abstract

The spermatogenesis of the spider, *Pardosa* sp., has been worked out completely and the following important conclusions arrived at:—(1) The mitochondria, which appear as granules in the fixed material, are actually in the form of fibres with a granule at each tip, in the living cells under the phase contrast microscope. They do not take any part in the formation of the mature sperm. (2) The Golgi elements are in the form of solid g- ules throughout spermatogenesis. During spermateleosis they all unite to form the acrosome directly. They are highly sudanophil, take up neutral red and give a very high phase-change under the positive phase contrast. (3) A single enigmatic chromatoid body has also been described in the spermatocytes. This takes up a deep stain in chrom-osmium preparations but is not stained at all with sudan black and neutral red. (4) The diploid number of chromosomes in this species is 28, with XX-O type of sex-determining mechanism. All the chromosomes are of the simple rod-type and acrocentric.

Introduction

Sokolska (1924), working on the male germ cells of the spider, *Tegenaria*, has described a single large Golgi body which goes to only one of the two secondary spermatocytes, during the first maturation division. This single body is fragmented during the second maturation division and the dictyosomes, thus formed, are evenly sorted out to the two spermatids. According to this author, therefore, only half of the spermatids receive the Golgi elements while the others remain without them.

Sharma (1950), on the other hand, has described in the spider, *Plexippus paykulli*, a perfectly normal behaviour of the granular Golgi elements, some of which fuse together in the spermatid to form directly the triangular acrosome of the mature sperm. He has, however, pointed out that such important cell components as the mitochondria are not represented at all in the mature sperm of this spider.

As no other worker seems to have paid any attention to the cytoplasmic inclusions of the male germ cells of the spiders, the present work was undertaken to throw some more light on the conflicting views regarding the behaviour of the Golgi elements and the mitochondria in this ancient group. Special attention was paid to the study of the living material with vital dyes and under the phase contrast microscope.

Besides the cytoplasmic inclusions the behaviour of the chromosomes during spermatogenesis was also studied. The chromosome studies in the family Lycosidae had been made earlier by Montgomery (1905) and Painter (1914). They were followed by Hard (1939) who was the first investigator to report on the chromosomes of *Schizocosa crassipes*, using modern techniques. Subsequently the chromosomes of 16 more species of this family were worked out by Hackman (1948), and of five species by Suzuki (1954). In this country Bole-Gowda (1953) published an abstract of his work on seven species of *Lycosid* spiders. All the species so far studied in this family show close resemblance in their chromosomes, the two compound X's being always present. The haploid number of chromosomes ranges from 12 to 15 and the most common diploid number is 28. The two X's are generally acrocentric and of equal size, although some clear size difference between the two X's has been reported by Hackman (1948) and Suzuki (1954) in some species. Bole-Gowda (1953) has given the diploid number as 28 in the two species of the genus *Pardosa*, with two sex-chromosomes of equal size.

Material and Technique

The specimens of *Pardosa* sp. were found to be very common in the college lawns near the water channels. The animals were collected during the months of February to September, 1953. The males can be easily differentiated from the dull brown females by their black colour with a brown area in the centre of the cephalothorax.

The testes were dissected out in 0·7% saline solution, to every 100 c.c. of which 0·2 c.c. of 10% calcium chloride solution was added (Baker, 1944). The same physiological solution was also employed for the study of the living cells. The testicular tubes were fixed for varying periods in the following fixatives: Flemming-without-acetic, Champy, Bouin, Allen's modification of Bouin, Flemming (strong), Flemming (weak), Sanfelice and Carnoy. The material fixed in Champy, Sanfelice and Flemming (strong), however, gave the most satisfactory results.

For the specific study of the Golgi elements sudan black technique of Thomas (1948) was employed. Cain's (1948) technique of staining the mitochondria was also used but did not prove very useful.

Paraffin wax was employed for embedding and sections were generally stained with 0·5% iron hæmatoxylin.

Considerable attention was paid to the living studies with vital dyes like the neutral red chloride (B.D.H.) and Janus green B (Harleco—U.S.A.). Both the dyes were used in the final concentration of 0·01%.

Carl Zeiss stand 'W', phase contrast microscope was employed for the living study under the positive phase contrast.

Observations

Spermatogonia.—The spermatogonia, which were not many in our preparations, are generally found in the various active stages of the mitotic division.

The earliest spermatogonium is a large, more or less, rounded cell, with the nucleus showing a coarse network of fine chromatin granules (Plate I, fig. 1). The thin film of cytoplasm at this stage reveals a number of lightly stained fine granules lying on one side of the nucleus in chrom-osmium preparations. These granules take up a light blue and homogeneous stain in sudan black, indicating the presence of diffused lipides. They are most probably the mitochondria. Besides them the cytoplasm also shows one or two deeply staining larger granules which have a great affinity for neutral red and are sudanophil (Plate I, fig. 1). They may be assigned to the category of the Golgi elements. Both types of these inclusions can also be made out clearly in the living cells under the phase contrast microscope.

With the advent of the prophase the chromatin granules of the resting gonial nucleus arrange themselves in small granular chromosomal structures, each showing a distinct longitudinal split even at this early stage. These can generally be counted as twenty-eight in number which is the diploid number of chromosomes in this species (Plate IV, fig. 51). In the late prophase of the spermatogonium the chromosomes are rod-like and are distributed irregularly in the whole of the nucleus (Plate IV, fig. 52).

It was not possible to find these stages in the chrom-osmium preparations. In the living cells under the phase contrast the cytoplasm revealed an increased number of the mitochondrial granules, some of which were arranged linearly to form moniliform threads.

In the prometaphase stage (Plate I, fig. 2) the chromosomes can be seen arranging themselves at the equator of the spindle, each pole of which is occupied by a granular centrosome. Besides the mitochondria which are uniformly spread throughout the cytoplasm, one or two Golgi granules can also be clearly made out.

It may be pointed out here that from this stage onward the mitochondria appear as long fibrillae with a dark granule at each end in the living material under the phase contrast. They retain this form in the dividing cells also, till they are left finally in the cytoplasmic remnants. Their granular form in the fixed preparations seems to be due not only to their poor preservation in the fixatives employed but also to their extremely delicate nature.

The chromosomes in the gonial metaphase are distinctly shorter than those in the prophase stages and do not show any longitudinal split (Plate I, fig. 3 and Plate IV, fig. 53). It has been concluded from a number of the metaphase polar views that the diploid chromosome number in this species is invariably twenty-eight. All the chromosomes are essentially stout and of simple rod-type, with one end always pointed, towards which is situated the centromere. They show a gradual seriation in their size. Most of the chromosomes are quite short but generally six to eight larger chromosomes of almost the same size can be recognized. The chromosomes are always arranged on the equatorial plate in a radial manner with their tapering ends pointing inwards, thus reflecting their acrocentric nature. They are generally seen lying in pairs, each member of a pair being almost similar in size and shape to the other. These most probably represent the homo-

logous pairs of chromosomes. It has not been possible to spot any of the sex-chromosomes at this stage as they present no differential behaviour or structure during mitotic division. But it can be concluded from their later behaviour that they belong to the category of the larger chromosomes.

During the gonial anaphase all the chromosomes divide longitudinally and the products of division move almost simultaneously towards their respective poles, each lying parallel to the spindle fibres, with its tapering end pointing towards the centrosome (Plate IV, fig. 54).

In the telophase (Plate IV, fig. 55) the chromosomes at each pole of the spindle can very often be seen arranged in the form of a rosette around the granular centrosome. They do not, however, lose their staining capacity in any of the telophase stages.

Primary Spermatocytes.—A typical resting stage characterized by a vesicular nucleus does not occur in the primary spermatocytes of this material. Consequently, the spermatocyte, as soon as it is formed, directly enters the prophase stage of meiosis.

The primary spermatocyte is definitely a larger cell than the spermatogonium, but possesses a comparatively smaller nucleus (Plate I, figs. 4 to 6).

It is generally not possible to identify the various prophase stages in the slides prepared from the F.W.A.-fixed material, as in them the chromatin of the nucleus does not take up any dark stain. But the sex-chromosomes, which show a definite positive heteropycnosis during all the later stages, are always stained darkly even in these preparations.

In the cytoplasm of the early primary spermatocytes the mitochondria form a dense, juxta-nuclear mass and among them can be seen a few larger darkly staining and sudanophil granules—the Golgi elements (Plate I, fig. 4). As the spermatocyte, however, grows, the mitochondria and the Golgi elements gradually become circumnuclear in arrangement (Plate I, figs. 5 and 6).

In the living cells seen under the positive phase contrast the Golgi elements present a very high phase-change but the mitochondrial filaments *are not resolved easily although their tip granules are very prominent.* The Golgi elements are stained with neutral red whereas the mitochondria take up Janus green B.

Besides the cytoplasmic inclusions mentioned above a large deeply staining and round body is also generally seen in these stages in chromosmium preparations (Plate I, figs. 4 to 6). As it does not stain in sudan black and gives a very small phase-change in the living cells, it may be the chromatoid body.

Just after the gonial telophase the nucleus of the primary spermatocyte reveals inside it widely distributed chromatin masses (Plate IV, fig. 56), which are granular and show a clear longitudinal split in majority of cases. But soon they resolve themselves into fine autosomal threads, filling up the whole of the leptotene nucleus (Plate IV, fig. 57). The sex-chromosomes, however, remain in the form of two highly condensed, darkly staining heteropycnotic rods, closely applied to each other. Occasionally they unite to form a single dark body. A little later the autosomal

threads become completely polarized and show dark heteropycnotic granules at their free ends which are very clearly seen in the living cells under the positive phase contrast (Plate IV, fig. 58). The two heteropycnotic X's lie close to the nuclear membrane towards the polarized end. These present a very high phase-change and are the most prominent objects in the prophase nucleus.

The zygotene stage appears to be a very short-lived one in this material. However, the association of the chromosome threads appears to be complete. The homologous pairs, thus formed, detach themselves from the nuclear membrane (Plate IV, fig. 59) and seem to enter a marked contraction or synizesis stage in the fixed material (Plate IV, fig. 60). Such a stage, however, was not seen in the living cells and the areas of leptotene cells were followed by pachytene stages in a longitudinally crushed testicular tube.

In the pachytene stage which follows the synizesis the autosomal threads show a double linear alignment of granules revealing their longitudinally double nature. These threads are in a slightly confused state in the early stages (Plate IV, fig. 61), but later on they become shorter and completely polarized (Plate IV, fig. 62). They can be counted as 13, which represent the haploid number (Plate IV, fig. 62). One end of each pachytene thread is in close association with the nuclear membrane while the other, lying free in the nuclear cavity, bears a dark positively heteropycnotic granule on it. The double and granular nature of these threads is also clearly visible in the living state where even the heteropycnotic tip granules are also seen as double (Plate V, fig. 76).

The sex-chromosomes are in the form of two dark and short rods, lying separately or closely applied to each other, near the nuclear membrane on the polarized end (Plate IV, figs. 61 and 62 and Plate V, fig. 75). In some cases, however, each of the two X's may show a distinct longitudinal split at this stage.

The polar orientation of the autosomal bivalents breaks up gradually with the advent of the diplotene stages. In the early diplotene (Plate IV, fig. 63) the synaptic mates become longitudinally coiled round each other as represented in the strepsitene stage of Wilson (1925). With the advancement of the diplotene stage, however, they lose their twists and come to occupy the whole of the nuclear vesicle. The autosomal bivalents generally show a single chiasma and they are all of double-cross type (Plate IV, figs. 64 and 65). No ring-tetrad was ever identified. The sex-chromosomes retain their positive heteropycnocy throughout these stages.

During the diakinetic stage (Plate IV, figs. 66 and 67) the autosomal bivalents assume a typical double-cross shape and show a peripheral disposition in the nucleus. In the subsequent prometaphase stages the autosomal bivalents lie scattered in the cytoplasm whereas the two X's occupy a peripheral position (Plate I, figs. 7 and 8 and Plate IV, fig. 68). In the side view two prominent centrosomes can also be seen clearly, each occupying one pole of the fibrillar spindle (Plate I, fig. 8).

During the metaphase stage, when the autosomal bivalents arrange themselves radially at the equator of the spindle, the two X's always form

a separate plate. Both of them are attached, each by a separate spindle fibre, to the same centrosome towards which they later on proceed together (Plate IV, figs. 69 to 72). In the polar views the V-shaped or dumb-bell-shaped autosomal bivalents are invariably thirteen in number, which is the haploid number of chromosomes in this species.

During all these stages the mitochondria are homogeneously distributed throughout the cytoplasm.

In the living cells the mitochondrial fibres surround the spindle longitudinally but the spindle area is completely free from them and thus appears quite clear. No spindle fibres could be resolved in the living cells (Plate V, fig. 77).

During anaphase the sex-chromosomes always precede the autosomal diads but, as has been stated earlier, they go only to one of the two poles, though the time of their departure may vary slightly (Plate I, figs. 9 and 10; Plate IV, fig. 73 and Plate V, fig. 78). The course of movement of these sex-chromosomes is always peripheral along the spindle. In some favourable views each of the two X's may show a longitudinal split (Plate I, fig. 9).

The autosomal bivalents separate into the expected V-shaped diads at the anaphase; the two limbs of the V's later on joining together to form rods (Plate IV, fig. 74).

In F.W.A. preparations the autosomal diads take up a very light stain excepting the two heterochromatic tip granules in each (Plate I, fig. 10). The two X's, however, stain deeply.

As a result of the reductional division of the two sex-chromosomes are produced two types of secondary spermatocytes, one with only thirteen autosomal diads (Plate I, fig. 12) and the other with two deeply staining X's besides the thirteen autosomal diads (Plate I, fig. 11).

In the late telophase stage the cell becomes elongated and divides by a constriction in the middle. Some darkly staining granules appear at the equator of the spindle at this stage and they constitute the so-called 'mid-body' (Plate I, fig. 13). These granules later on fuse together to form a conspicuous darkly staining ring around the remains of the spindle fibres (Plate I, fig. 14).

The process of reduction of the sex-chromosomes, the movement of the chromosomes, the formation of the mid-body and the constriction of the cell into two, followed by the nuclear reconstruction, have all been clearly studied in a single living cell under the phase contrast (Plate V, figs. 78 to 85). As the chromosomes reach their respective poles the cell elongates slightly and two constrictions appear at the equator, one on either side. Immediately afterwards a few granules—the mid-body granules—which present a slightly higher phase-change than the cytoplasm, make their appearance at the equator. These granules seem to arise *de novo* and appear within a period of five minutes. They surround the constricted portion of the cell, unite to form a homogeneous ring around it and seem to strangle the cell into two. The whole process of cell constriction from the early telophase to the process of nuclear reconstruction takes about fifty minutes.

For the process of nuclear reconstruction the chromosomes become massed at each pole and take up an intensely dark stain in fixed cells (Plate I, fig. 13). They, however, soon lose their density and present, gradually, a lower phase-change under the positive phase contrast. They ultimately become very faint and the two heteropycnotic X's now become prominent in one of the two daughter cells.

With the formation of the nuclear membrane, which takes a comparatively long time, the nucleus loses its staining capacity excepting the two sex-chromosomes (Plate I, figs. 14 and 15). The mitochondria and the Golgi elements seem to be sorted out almost equally between the two daughter cells during meiosis I. The Golgi elements become quite easily visible during late telophase stages in the living cells. The mid-body and the spindle fibre remnants persist for some time in the form of a bridge between the two daughter cells. But ultimately they are all discarded in the lumen of the testis.

Secondary Spermatocytes.—Just after the first meiotic division the secondary spermatocyte appears as a smaller cell than the primary. The mitochondria in it are generally aggregated on one side of the cytoplasm and are fewer in number. The Golgi elements remain in the form of deeply staining granules, which are sudanophil and also take up neutral red (Plate I, fig. 15).

The cell now shows a marked growth of the cytoplasm till, ultimately, its size becomes almost equal to that of the primary. Simultaneously an increase in the number of the mitochondria also takes place and these now spread around the nucleus, finally forming a horse-shoe-shaped mass. The Golgi elements also seem to increase in number during these stages (Plate I, figs. 16 and 17 and Plate II, figs. 20 and 21). The chromatoid body is also generally seen in some of these cells, and it takes up a deep stain.

The nucleus of the secondary spermatocyte, however, does not show any perceptible growth in its size. It takes up a light stain revealing a few dark chromatin granules in it (Plate II, fig. 21). The sex-chromosomes, present only in half of the cells, remain positively heteropycnotic.

A clear prophase stage of meiosis II has not been found. Two types of polar views of the metaphase plates of meiosis II have been recovered. One of them shows fifteen and the other only thirteen chromosomal elements (Plate I, figs. 18 and 19). The sex-chromosomes are indistinguishable from the autosomes during this division.

Besides the mitochondria, which are uniformly distributed throughout the cytoplasm, a few large darkly staining Golgi elements can also be clearly seen in these stages (Plate I, fig. 19 and Plate II, figs. 22 and 23). The spindle is a fibrillar structure, each of its poles being occupied by a centrosome (Plate II, figs. 22 and 23). It is quite interesting to note that in this division also the chromosomes, excepting the single heterochromatic granule situated at the tip of each, lose their staining capacity at the anaphase stage in F.W.A. preparations. The sex-chromosomes show no differential behaviour at all during meiosis II (Plate II, fig. 22). During anaphase the chromosomes are rod-shaped and move longitudinally towards their respective poles (Plate II, fig. 23).

In the telophase the chromosomes form a darkly staining mass at each pole of the spindle (Plate II, fig. 24). The subsequent process of cell division, which takes place by the appearance of a mid-body, is exactly the same as has been described earlier for meiosis I (Plate II, figs. 24 to 27). The ring-shaped mid-body may persist along with the spindle fibre remnants till quite late in the form of a cytoplasmic bridge between the two spermatids. During meiosis II also the Golgi elements and the mitochondria seem to be equally sorted out between the two daughter cells (Plate II, figs. 24 and 25).

Spermateleosis.—As a result of the heterogametic nature of the males, the spermatids can be distinguished into two distinct types: one showing the two X's in the heteropycnotic form while the other is without any sex-chromosome at all (Plate II, fig. 31). Both these types of spermatids are formed from their corresponding secondary spermatocytes.

The nucleus of the early spermatid is in the form of a vesicle which reveals inside it some fine chromatin granules (Plate II, fig. 25). The sex-chromosomes, when present, appear either in the form of a dark rounded body or as two closely applied rods (Plate II, figs. 25 and 31). The cytoplasm shows irregularly distributed mitochondria and among them can also be seen some prominent, deeply staining Golgi elements. The latter are sudanophil and reveal a distinct tendency for coming together to form larger granules (Plate II, fig. 25).

It is quite interesting to note that the process of sperm formation may proceed normally without the complete separation of the two spermatids after meiosis II (Plate II, fig. 28).

The mitochondria generally become crowded in one half of the cytoplasm during the early stages of spermateleosis (Plate II, figs. 29 to 31). In these stages it is not always possible to demonstrate the Golgi elements as they are probably lost in the overcrowded mitochondrial mass. In the later stages the mitochondria appear as fine granules in the fixed preparations and these are now arranged just below the cell membrane (Plate II, figs. 32 and later). They are finally sloughed off with the cytoplasm from which the mature sperm just wriggles out. Thus, they do not take any morphological part in the formation of the mature sperm (Plate III, figs. 47 and 48).

In the living cells the mitochondria always appeared as filaments with a dark granule at each tip. They generally lie close to the cell membrane. In the late sperm-forming stages, it has been found that sometimes a portion of the cytoplasm containing the whole lot of the mitochondria buds off from the spermatids and degenerates in the testicular lumen. Such cytoplasmic masses containing the mitochondrial filaments are commonly met with during the living study.

The centrosome which is single in the early stages of spermateleosis becomes divided into a proximal and a distal centrosome (Plate II, fig. 29). The proximal one takes up its position on the nuclear membrane at a very early stage, but the distal remains in the cytoplasm (Plate II, fig. 30). Soon, however, as the proximal centrosome moves forward upon the nuclear membrane the distal too moves towards it till it touches the nucleus

(Plate II, fig. 32). In the meanwhile the two centrosomes become connected by an intra-cellular axial filament. The extra-cellular axial filament bearing the bleb-like swellings can also be seen arising from the distal centrosome (Plate II, figs. 30 and 32).

The acrosome in this spider arises from the Golgi elements, all of which fuse to form a single large deeply staining granule (Plate II, fig. 32), which takes up its position at the anterior end of the nucleus (Plate II, fig. 33). This granule is ultimately transformed directly into the acrosome of the mature sperm.

The nucleus, which becomes oval to begin with, takes up the shape of a pear in the later stages of development (Plate II, figs. 34 to 37). During this process its staining capacity increases and consequently it becomes very deeply stained. For the same reason it is no longer possible to differentiate the two centrosomes and the intra-cellular axial filament in these stages. The acrosome can, however, be clearly seen at the anterior end of the nucleus in the form of a lightly staining triangular area bearing a dark granule at its tip (Plate II, figs. 35 and 36). With further progress in the process of spermateleosis, the nucleus, which is now in the form of a deep cup, loses its staining capacity. Since, however, the margins of the nuclear cup are folded they seem to take up a much deeper stain than the rest of it (Plate II, fig. 38 and Plate III, fig. 39). At a still later stage the acrosome becomes distinctly triangular. It is now perfectly clear but at its tip can still be seen a large deeply staining granule. The centrosomes, however, cannot be differentiated at all at this stage (Plate III, fig. 40).

Gradually the staining capacity of the nucleus again increases and its lateral folds now meet to enclose a clear space in the centre (Plate III, figs. 41 and 42). This can be clearly seen in the transverse section of the nucleus at such a stage (Plate III, fig. 50).

The nucleus now stains heavily and shows a deep curve at the anterior end because of its sinuous nature. The posterior end of the nucleus, in the meanwhile, becomes attenuated and curved (Plate III, fig. 42). The intra-cellular axial filament at this stage can be seen arising from the proximal centrosome, which has moved forward along the nuclear membrane to lie near the acrosome. Posteriorly it joins the distal centrosome which is situated at the attenuated tip of the nucleus.

With further differentiation the nucleus becomes more slender and the curves become less marked. The axial-filament detaches itself from the posterior tip of the nucleus and thus comes to lie away from the distal centrosome. It can now be traced directly to the proximal centrosome which can be detected with some difficulty near the anterior margin of the nucleus (Plate III, figs. 44 to 47).

The nucleus with the acrosome at its tip now leaves its central position in the cell and gradually wriggles out of the cytoplasm containing the whole lot of the mitochondria (Plate III, fig. 47 and Plate V, fig. 86). The ripe sperm, therefore, does not have any morphological trace of the mitochondria (Plate III, fig. 48).

The acrosome reveals various configurations during these later stages of development. It becomes cylindrical and darkly staining, but later on

it becomes clear again, showing a dark ring or plate and a granule at its tip (Plate III, figs. 43 to 45). Ultimately, however, the acrosome becomes clear and triangular and is continuous with the sinuous, deeply staining nucleus (Plate III, figs. 46 to 48).

The mature spermatozoon, consisting of a dark nucleus, a clear triangular acrosome at its tip and a long axial filament (Plate III, fig. 48), coils up like a watch spring in the lumen of the testis. In the early process of coiling the acrosome can be seen lying in the innermost region and the axial filament is wound around the nucleus (Plate III, fig. 49). But in the fully coiled up sperm, it is not possible to make out the various parts of the spermatozoon.

Discussion

(*a*) *Mitochondria.*—The most important point in the process of sperm formation in *Pardosa* sp. is, undoubtedly, the absence of any mitochondrial material in the mature sperm. This conclusion is in full accord with Sharma (1950), who has carefully described in the spider, *Plexippus paykulli*, the wriggling out of the mature sperm from the spermatid cytoplasm, in which the whole lot of the mitochondrial material is left. The process of wriggling out of the mature sperm was also described by earlier workers like Bösenberg (1905) in *Lycosa* and Wallace (1909) in *Agalena naevia*, who, however, made no mention of the cytoplasmic inclusions as such.

The absence of the mitochondrial material from the mature sperm has been recorded in other animals too by the various workers. For example, Nath and Bhatia (1953) described a complete paling off of the mitochondrial nebenkern in the later stages of sperm formation in the insect, *Lepisma domestica*. According to Dhingra (1954), the mitochondria in the trematode, *Isoparorchis eurytremum*, 'are left behind in the residual cytoplasmic mass and do not contribute towards the formation of any part of the ripe sperm'. Similarly Sud (1955) and Hughes-Schrader (1946) have also described the absence of the mitochondrial material in the ripe sperms of the snake, *Natrix* and iceryine coccids respectively. The climax is reached in the dragon-fly, *Sympetrum hypomelas*, in which, according to Nath and Rishi (1953), the mitochondrial material is never differentiated at all in the male germ cells.

Thus the complete absence of the mitochondria from the sperm of *Pardosa* sp. and the above-stated observations of other workers lend full support to the conclusions of Nath and Rishi (1953) that the mitochondria are not essential at all in the final make up of the sperm.

The form of the mitochondria in *Pardosa* sp. appears as granular in the fixed preparations. Sharma (1950) has also figured mitochondria as fine granules in *Plexippus paykulli*. But the living study of *Pardosa* germ cells under the positive phase contrast has revealed their filamentous nature very clearly. A single mitochondrion is a fine long filament, just at the limit of the microscopical resolution, with one dark granule at each tip. These filaments, however, seem to be formed by the linear alignment of the granular mitochondria of the spermatogonia. Their appearance as granules in the fixed material is attributed to their extremely delicate nature and poor preservation in the fixatives employed.

(*b*) *Golgi elements and the chromatoid body.*—The present work confirms fully the conclusions reached earlier by Nath (1944), Sharma (1950) and Sharma *et al.* (1953 *a* and *b*) that the Golgi elements are essentially in the form of discrete granules which may grow to form vesicles or spheres in later stages. Our extensive study of the living material under the phase contrast and with neutral red revealed to us nothing but granular Golgi elements, which take up neutral red and are strongly sudanophil. This indicates that they contain or are lipides with acidic reaction. In their form and staining behaviour the Golgi elements of this spider are homologous with the lipochondria in the neurones of *Locusta* (Shafiq, 1953 and 1954) and *Helix* (Roque, 1954).

We also fully support the view of Sharma (1950) that the acrosome in the spiders is directly formed from the Golgi elements of the spermatid. The acrosome of the mature sperm of the spider, *Plexippus paykulli*, was described by him as triangular with a deeply staining granule at its apex which was connected to the nucleus by means of a lightly staining fibre. Almost a similar description was given for the 'apical body' by Bösenberg (1905) in *Lycosa* and by Wallace (1909) in *Agalena naevia*. This deeply staining granule is visible at the tip of the cylindrical acrosome in the early stages of our spider also, but later on it disappears, leaving a clear triangular acrosome of the mature sperm. The lightly staining fibre traversing the acrosome, as seen by earlier workers, could not, however, be detected in any stage of the acrosome formation. All the Golgi elements of the spermatid are consumed in the formation of the acrosome and consequently there are no Golgi remnants.

Sokolska (1924) gave a very interesting and abnormal description of the behaviour of the Golgi elements during meiosis in the spider, *Tegenaria*. According to this author, a single large Golgi body, which goes only to one of the two secondary spermatocytes during the first meiosis, is fragmented into dictyosomes during the second maturation division. These dictyosomes are then evenly sorted out to the two spermatids. Thus, only half of the spermatids, according to Sokolska, receive the Golgi elements.

But this is probably the solitary example of such a distribution of the Golgi material in the whole of the animal kingdom. Our observations on *Pardosa* sp. as well as those of Sharma (1950) on *Plexippus paykulli* clearly show that the granular Golgi elements are distributed almost equally to the two daughter cells in all the divisions.

In the opinion of the authors, therefore, Sokolska (1924) has mistaken the single chromatoid body of the primary spermatocytes with the Golgi elements. The single chromatoid body of the spider, *Pardosa* sp., however, is not identical with the Golgi elements as it is not sudanophil, does not take up neutral red and gives a very small phase-change under the positive phase contrast in the living cells. It seems to go only to half of the secondary spermatocytes after the first maturation division as it is visible only in some of these cells. The authors, however, have not seen the fragmentation of this body into dictyosomes at any stage. Such a chromatoid body has also been described in the male germ cells of the snake,

Natrix and the turtle, *Lissemys* by Sud (1955 and 1956) and of the domestic duck by Gupta (1955).

(*c*) *Chromosomes.*—The diploid number of chromosomes is 28 in the spider, *Pardosa* sp., which presents an XX-O type of sex-determining mechanism, since the haploid number is 15 and 13 in this species. The chromosomes are all rod-like and acrocentric and the autosomal bivalents are all of double-cross type at meiosis I. Bole-Gowda (1953) has also stated that the diploid number of chromosomes in the two species of the spider, *Pardosa*, worked out by him is 28, out of which two are the sex-chromosomes. Both the sex-chromosomes are of equal length and similar shape and they always precede the autosomes in meiosis I. In meiosis II, however, they are indistinguishable. All the previous workers on the family Lycosidae like Hard (1939), Hackman (1948), Bole-Gowda (1953) and Suzuki (1954), etc., etc. have described a similar behaviour of the two X's.

A marked synizesis stage, wherein all the autosomes contract to form a deeply staining knot on one side of the nuclear vesicle, has been observed only in the fixed preparations of this material. But as the extensive studies of the living material under the phase contrast never revealed such a stage, it appears to us that it is formed as a result of fixation.

(*d*) *Mid-body.*—A clear mid-body is seen in the fixed preparations, in *the form of a dark ring surrounding the spindle fibre remains between the* two daughter cells, in both the first and the second maturation divisions. This ring is formed by the fusion of some dark granules which make their appearance at the equator of the spindle during the early telophase stage of these divisions. The appearance of these mid-body granules has also been clearly observed in a single living cell under the phase contrast. These granules seem to arise *de novo* and their appearance does not take more than five minutes. The whole process of cell constriction is completed in nearly fifty minutes and the mid-body-ring seems to perform a strangling act upon the constricting cell. Such a mid-body has also been figured by the previous workers on spider spermatogenesis like Bösenberg (1905), Wallace (1909) and Sharma (1950).

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Explanation of Plates

All the figures on Plates I to IV have been drawn at table level, using a Spencer 10× ocular and 95/1·25 oil immersion objective and a Beck Camera Lucida, giving a total magnification of 1,700 times. The figures on Plates I to III have been selected from Champy or F.W.A.—Iron hæmatoxylin preparations whereas those on Plate IV

are from Sanfelice—Iron hæmatoxylin slides. Plate V shows the photomicrographs of the living cells under the Phase Contrast Microscope, taken with a Contax 35 mm. Camera, Carl Zeiss Micro-reflex Camera attachment, K 8× ocular and 100/1·25 oil immersion objective. All the photomicrographs are further enlarged four times, giving a total magnification of 1,600 times and are untouched.

Plate I

FIG. 1. Resting spermatogonium showing fine mitochondrial granules and two large Golgi granules.

FIG. 2. Gonial prometaphase, side view.

FIG. 3. Gonial metaphase, polar view.

FIGS. 4 to 6. Primary spermatocytes in prophase stages showing very fine mitochondria, larger and darker Golgi granules and a still larger, deeply staining chromatoid body.

FIG. 7. Meiosis I; prometaphase, polar view.

FIG. 8. Meiosis I; prometaphase, side view.

FIGS. 9 and 10. Meiosis I; anaphase, side views.

FIGS. 11 and 12. Meiosis I; telophase, polar views showing 15 and 13 chromosomal elements respectively.

FIG. 13. Meiosis I; late telophase, side view showing mid-body granules.

FIGS. 14 and 15. Early secondary spermatocytes showing mitochondria and the Golgi granules *in the cytoplasm and the heteropycnotic* X's *in the nuclei*. Mid-body is also visible.

FIGS. 16 and 17. Secondary spermatocytes, resting.

FIGS. 18 and 19. Meiosis II; metaphase, polar views.

Plate II

FIGS. 20 and 21. Secondary spermatocytes showing the circum-nuclear mitochondria and Golgi elements. Deeply staining large chromatoid body is also visible.

FIGS. 22 and 23. Meiosis II; anaphase, side views.

FIG. 24. Meiosis II; telophase, side view showing mid-body granules.

FIG. 25. Early spermatids showing mid-body.

FIG. 26. Spermatids still not separated, showing mitochondria and Golgi granules.

FIGS. 27 and 28. Spermatids showing precocious development without separation.

FIG. 29. Spermatid showing mitochondria, Golgi granules and two closely applied centrosomes lying partially on the nuclear membrane.

FIG. 30. Spermatid showing an intra-cellular axial filament between the two centrosomes continuous with an extra-cellular axial filament with two blebs. Four large Golgi granules are also visible.

FIG. 31. Two spermatids with and without heteropycnotic X's.

FIG. 32. Spermatid showing both the centrosomes on the nuclear membrane and all the Golgi granules fused into a single large sphere.

FIG. 33. Golgi sphere is attached to the anterior end of the precociously condensed nucleus.

FIG. 34. Spermatid with oval nucleus showing the acrosome at the anterior end and the proximal centrosome on the side of the nuclear membrane giving rise to the axial filament.

FIGS. 35 to 37. Deeply staining pear-shaped nucleus with a triangular acrosome at the anterior tip.

FIG. 38. Spermatid showing folded nucleus taking a light stain.

Plate III

FIGS. 39 to 41. The nuclear folds of the spermatid meeting to enclose a space.

FIG. 42. Spermatid showing deeply staining sinuous nucleus, a clear triangular acrosome with a dark granule at the tip and the axial filament arising from the proximal centrosome lying near the anterior end of the nucleus and passing through the distal centrosome at the posterior tip.

FIGS. 43 to 47. Late stages of metamorphosis of the spermatid. Acrosome shows various developmental configurations.

FIG. 48. Mature sperm.

FIG. 49. Coiling of the mature sperm like a watch spring.

FIG. 50. Transverse section through a spermatid as shown in fig. 42, showing the concentration of the chromatin on the margins of the nucleus.

Plate IV

FIG. 51. Spermatogonium showing 28 chromosomal masses.

FIG. 52. Gonial prophase.

FIG. 53. Gonial metaphase, polar view with 28 chromosomes.

FIG. 54. Gonial anaphase, very late, side view.

FIG. 55. Gonial telophase, side view.

FIG. 56. Early primary spermatocyte showing chromatin masses, each with a longitudinal split.

FIGS. 57 and 58. Prophase I; leptotene.

FIG. 59. Prophase I; zygotene.

FIG. 60. Prophase I; synizesis.

FIGS. 61 and 62. Prophase I; early and late pachytenes.

FIG. 63. Prophase I; strepsitene.

FIGS. 64 and 65. Prophase I; diplotene.

FIGS. 66 and 67. Prophase I; sections of the same cell at diakinesis.

FIG. 68. Prometaphase I; polar view.

FIGS. 69 to 71. Metaphase I; polar views showing 2 X's and 13 bivalents.

FIG. 72. Metaphase I; side view.

FIG. 73. Anaphase I; side view.

FIG. 74. Showing the separation of a double-cross bivalent during anaphase I.

Plate V

FIG. 75. Primary spermatocytes showing heteropycnotic X's.

FIG. 76. Primary spermatocyte, syncytium with two nuclei showing Pachytene chromosomes with heterochromatic ends.

FIG. 77. Anaphase I, side view, showing fibrillar mitochondria surrounding the spindle.

FIG. 78. Anaphase I, side view, showing the two X chromosomes preceding the autosomes.

FIGS. 79 to 85. Serial photomicrographs of a single cell from late anaphase I to very late telophase I showing the formation of the mid-body and the process of cell division.

FIG. 86. Very late spermatids showing sinuous nucleus, triangular acrosome and an axial filament in each.

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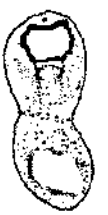

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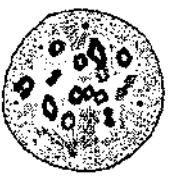

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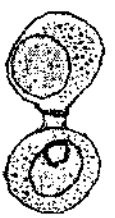

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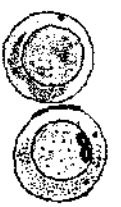

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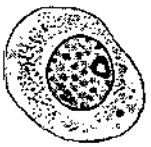

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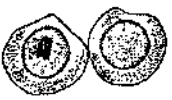

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PLATE IV.

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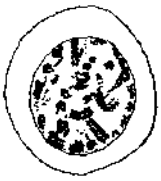

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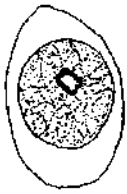

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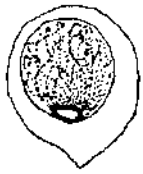

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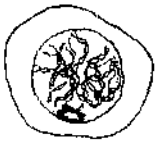

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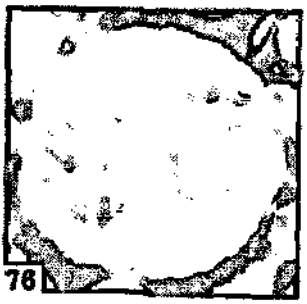
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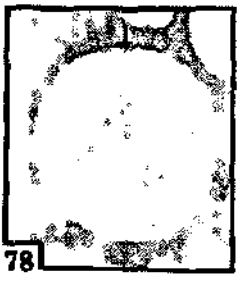
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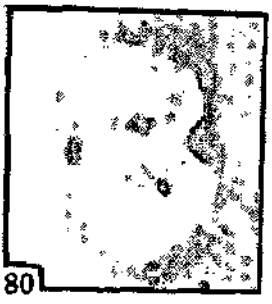
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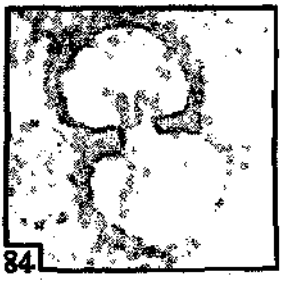
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February, 1956

A PRELIMINARY LIST OF THE MOSSES OF MUSSOORIE (HIMALAYAS)

by

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A PRELIMINARY LIST OF THE MOSSES OF MUSSOORIE (HIMALAYAS)

By R. S. Chopra, B. K. Abrol *and* M. S. Banga

(*Botany Department, Panjab University, Amritsar, Panjab, India*)

The stimulus provided by the publication of 'Liverworts of the Western Himalayas and the Panjab Plains' by the late Prof. S. R. Kashyap impressed upon the senior author the need of a sister work on the mosses of this area. A study of the mosses, which had been collected by the late Prof. S. R. Kashyap and Mr. R. L. Badhwar and had been identified by late Mr. H. N. Dixon, was started in 1942. A good deal of work had been put in but the events following the partition of the Panjab in 1947 put an end to this study. A start was again made in 1950 by collecting mosses from Mussoorie. Collections have been made as under:—

Chopra and Abrol, 1950; Abrol, 1951; Chopra and Banga, 1952; and Banga, 1953.

To begin with mosses were sent for identification to the British Museum of Natural History, Dr. W. C. Steere, and Dr. A. Nagouchi. Further specimens were later sent only to the British Museum which has the advantage of having a collection of Indian mosses determined by late Mr. H. N. Dixon. Dr. Steere passed on the lot of mosses sent to him to Mr. A. H. Crum, who has identified this lot jointly with Mr. E. B. Bartram. Sincerest thanks are due to the authorities of the British Museum of Natural History, Mr. A. H. Norket, Dr. A. Nagouchi, Dr. W. C. Steere, Messrs. H. A. Crum and E. B. Bartram for the determination of mosses and to Prof. P. N. Mehra for suggestions and providing facilities, which have enabled us to carry out this work.

Pending a full account of the 'Mosses of Mussoorie', it is thought desirable that a preliminary list may be published.

POLYTRICHALES

Polytrichaceae

Pogonatum Palis

P. microstomum (R. Br.) Brid.	.. ..	2 *, 144.
P. perichaetiale (Mont.) Jaeg.	.. ..	1, 143.
P. stevensii Ren. & Card.	.. ..	3, 145, 198, 276, 298.

* These figures indicate the Herbarium numbers of various mosses in the departmental Herbarium.

Atrichum * P. Beauv.

A. obtusulum C.M. .. .. .. 5, 147, 219.
A. pallidum Ren. & Card. .. .. 4, 146, 255.

FISSIDENTALES

Fissidentaceae

Fissidens Hedw.

F. cristatus Mitt. .. .. .. 299.
F. grandifrons Brid. (C. & B. det.) .. 201, 383.
F. obscurus Mitt. .. .. .. 8, 10, 150, 271.
F. schmidii C. Mull. .. .. .. 282, 324, 325.
F. sylvaticus Griffith. .. .. .. 9, 300.
F. taxifolius Hedw. f. Bartr. (C. & B. det.) .. N.R. 412.
F. zippelianus Doz. & Molk. (Nagouchi det.) .. 202, 251.

According to Dixon (Mosses of Celebes: Annals Bryologicii, Vol. VII, p. 23 (1934), *F. sylvaticus* Griff. is the synonym of *F. zippelianus*.

DICRANALES

Dicranaceae

Ditrichum Timm.

D. duthiei Broth. .. .. .. 315.
D. heteromallum (Hedw.) Britt. (Nagouchi det.) N.R. 199.
D. tortile (schrad.) Lindb. .. .. 315, 319.

Mr. Norket does not see very much difference between *D. duthiei* and *D. tortile*.

Trematodon Michx.

T. capillifolius C.M. (C. & B. det.) .. .. 423.

Dicranella Schimp.

D. heteromalla (Hedw.) Schimp. .. .. 6, 148.

Campylopus Brid.

C. piriformis (Schulz.) Brid. .. .. N.R. 7, 149.

Dicranodontium B. & S.

D. caespitosum (Mitt.) Par. .. .. mixed in 185.
D. denudatum (Brid.) Hag. .. .. 200.

---

* The names given in this list are those given by the persons determining these mosses although some of these are synonyms and should be replaced by others. For example, *Atrichum* Palis., Prodr., p. 42 (1805), should be discarded in favour of *Catharinaea* Ehrb. in *Hanov. Mag.*, p. 933:1780. This point will be taken up later on.

Brothera C. Mull.

B. leana (Sull.) C. Mull. (Nagouchi det.) .. 200.

Ceratodon Brid.

C. purpureus (Hedw.) Brid. .. .. 153 (mixed with another).

POTTIALES

Pottiaceae

Molendoa Lindb.

M. roylei (Mitt.) Broth. .. .. 12, 151.
M. sendtneriana (B. & S.) Limp. .. .. 300.
M. yunanensis Broth. (Nagouchi det.) .. 204.

Gymnostomum Hedw.

G. aurantiacum (Mitt.) Par. (Nagouchi det.) .. N.R. 205.
G. calcareum Nees & Horn. .. .. 294, 310, 312.
G. recurvirostrum Hedw. .. .. N.R. 73, 74, 266, 272, 287.

Hyophila Brid.

H. involuta (Hook.) Jaeg. .. .. 13, 79, 269, 408, 415, 420.

Barbula Hedw.

* *B. amplexifolia* Mitt. .. .. .. 14, 153.
* *B. constricta* Mitt. .. .. .. 17, 304.
B. ehrenbergii (Lor.) Fl. (C. & B. det.) .. N.R. 394, 410.
B. horricomis C. Mull. .. .. .. 16, 155.
B. leucodontoides C. Mull. .. .. N.R. 15, 154.

Hydrogonium (C. Mull.) Jaeg. & Sauerb.

H. amplexifolium (Mitt.) Chen. .. .. 284.
H. commosum (Doz. & Molk.) Chen. .. 302.
H. gracilentum (Mitt.) Chen. .. .. 211, 279.

Reimersia Chen.

R. inconspicua (Griff.) Chen. .. .. 72, 205, 300.

Bryoerythrophyllum Nom. Nov. Chen.

B. atrorubens (Besch.) Chen. .. .. 303.

Timmiella (De Not.) Limp.

T. anomala (B. & S.) Schimp. .. .. 19, 157, 239, 246, 285, 313, 390.
T. dimunata (C. Mull.) Chen. .. .. N.R. 18, 156.

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* Mr. Norket is of the opinion that specimens originally named as *Barbula amplexifolia* Mitt. are instead *B. constricta* Mitt.

Hymenostylium Brid.

H. curvirostre (Ehrh.) Lindb. .. .. 20, 158, 397.

Weisia Hedw.

W. viridula Hedw. .. .. .. 306, 307, 318.

Hymenostomum R. Brown.

H. tortile (Schw.) B. & S. .. .. 21, 159.

Anoectangium (Hedw.) Bry. Eur.

A. bicolor Ren. & Card. .. .. 23, 267.
A. stracheyanum Mitt. .. .. .. 160, 209, 277, 293.
A. thomsoni Mitt. (C. & B. det.) .. .. 403.

FUNARIALES

FUNARIACEAE

Funaria Schreb.

F. calvescens Schw. (C. & B. det.) .. .. N.R. 402.
F. hygrometrica Hedw. .. .. 24, 161.
F. wallichii (Mitt.) Broth. Collected by Prof. S. R. Kashyap, Herb. H. N. Dixon, Ref. No. 12 *b*, British Museum of Natural History. *

Physcomitrium (Brid.) Furnr.

Ph. japonicum (Hedw.) Mitt. (C. & B. det.) .. 424.

SPLACHNACEAE

Splachnobryum C. Mull.

S. sp. .. .. 316.

EUBRYALES

BRYACEAE

Pohlia Hedw. = Webra Hedw.

P. elongata Hedw. .. .. .. 25, 162.
P. flexuosa Hook. .. .. .. 26, 163.
P. rigidifolia Dixon & Badhwar .. .. 289, 320.

Brachymenium Schw.

B. exile (Doz. & Molk.) Bry. Jav. .. .. 28, 165.
B. nepalense Hook. .. .. .. 27, 164, 256.

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* This and a few other specimens were obtained by the kindness of the authorities of the British Museum of Natural History. Thanks are due to them.

LEUCODONTACEAE

Leucodon Schw.

L. secundus (Harv.) Mitt. .. .. 45, 177.

TRACHYPODACEAE

Diaphanodon Ren. & Card.

D. blandus (Harv.) Ren. & Card. W. Dudgeon collection. Herb. H. N. Dixon, Ref. No. 3, British Museum of Natural History.

Trachypus Reinw. & Hornsch.

T. bicolor Reinw. & Hornsch. (C. & B. det.) .. 375.

Trachypodopsis Fleisch.

T. crispatula (Hook.) Fleisch. .. .. 50, 180, 326.
T. declinata (Wils.) Fleisch (C. & B. det.) .. 377.

Duthiella C. Mull.

D. mussooriensis Reimers. .. .. N.R. 81, 258.

PTEROBRYACEAE

Pterobryopsis Fleisch.

P. orientalis (C. Mull.) Fleisch. .. .. 47, 178.

METEORIACEAE

Meteorium Doz. & Molk.

M. buchanani (Brid.) Broth. (C. & B. det.) .. 389.

Floribundaria C. Mull.

F. floribunda (Doz. & Molk.) Fleisch. .. 241, 242, 288.

Meteoriopsis Fleisch.

M. reclinata (Mull.) Fleisch. H. N. Dixon considered it to be identical with *M. ancistrodes* (C. Mull.) Broth. also reported from Garhwal. 49, 179, 281.

NECKERACEAE

Cryptoleptodon Ren. & Card.

C. flexuosus (Harv.) Ren. & Card. .. .. 88, 243, 265, 296, 396.

Homalia (Brid.) Bry. Eur.

H. exigua Bosch & Lacoste .. .. N.R. 51, 259.

Thamnium Bry. Eur.

Th. latifolium (Bry. Jav.) Paris (Nagouchi det.) 222.
Th. subseriatum (Hook.) Doz. & Molk. (C. & B. det.) 427.

HOOKERIALES

Hypopterygiaceae

Hypopterygium Brid.

H. flavolimbatum C. Mull. .. .. 223, 311.

Cyathophorella (Broth.) Fleisch.

C. tonkiensis Broth. et Par. (Nagouchi det.) .. N.R. 52, 254.

HYPNOBRYALES

Fabroniaceae

Fabronia Raddi

F. minuta Mitt. W. Dudgeon, Herb. H. N. Dixon, British Museum of Natural History.

Leskeaceae

Lindbergia Kindb.

L. duthiei Broth. .. .. .. 62, 187.

Thuidiaceae

Anomodon Hook. & Tayl.

A. minor (P. Beauv.) Furnr. .. .. 61, 186.
According to H. N. Dixon (Ann. Bry., III, p. 64) this species is identical with *A. integerrimus* Mitt.

A. planatus Mitt. .. .. .. 280.

Herpetineuron (C. Mull.) Card.

H. toccoae (Sull. & Lesq.) Card. .. .. 85, 228, 241, 292, 308, 388.

Claopodium (Lesq. & James) Ren. & Card.

C. strepsiphyllum Dixon .. .. 87, 227, 263.

Haplocladium (C. Mull.) C. Mull.

H. capillatum (Mitt.) Broth. W. Dudgeon, H. N. Dixon herbarium, Ref. No. 63, British Museum of Natural History.

H. microphyllum (Sw.) Broth. .. .. 206, 278, 391.

Thuidium Bry. Eur.

Th. cymbifolium (Doz. & Molk.) Bry. Jav. .. 64, 189.
Th. haplohymenium (Harv.) Jaeg. (C. & B. det.) 395.
Th. sparsifolium (Mitt.) Jaeg. .. .. 305.
Th. squarrosulum Ren. & Card. W. Dudgeon, Herb. H. N. Dixon, Ref. No. 132, British Museum of Natural History.
Th. tamariscellum (C. Mull.) Bry. Jav. (Nagouchi det.) .. .. 226.
Th. thamnicladum (C. Mull.) Par. N.R. Gollan, Herb. H. N. Dixon, Ref. No. 79, British Museum of Natural History.

AMBLYSTEGIACEAE

Cratoneuron (Sull.) Roth.

C. filicinum (Hedw.) Roth. .. .. N.R. 65, 89, 91, 190, 253, 268.

Leptodictyum (Schimp.) Warnst.

L. riparium (L.) Warnst. (Nagouchi det.) .. 238.

Hygrohypnum Lindb.

H. luridum (Hedw.) Jennigs .. .. N.R. 83, 90, 261.

Platyhypnidium Fleisch.

P. longirameum (C. Mull.) Fleisch. (Nagouchi det.) N.R. 229.
P. riparioides (Hedw.) Dixon (C. & B. det.) N.R. 386.

BRACHYTHECIACEAE

Brachythecium Bry. Eur.

B. buchanani (Hook.) Jaeg. .. .. 68, 193, 232, 297, 404.
B. emodi-glareosum Broth. Mrs. Bremner, Herb. H. N. Dixon, Ref. No. 125, British Museum of Natural History.
B. latifrons C. Mull. in edit. .. .. 69, 194.

Rhynchostegium Bry. Eur.

Rh. duthiei C. Mull. ex Dixon .. .. 71, 196.
Rh. planiusculum (Mitt.) Jaeg. (C. & B. det.) .. 377.
Rh. percomplanatum (C. Mull.) Broth. Mrs. Bremner, Herb. H. N. Dixon, British Museum of Natural History.

Rhynchostegiella (Bry. Eur.) Limp.

Rh. scabriseta (Schw.) Broth. .. .. 70, 195.
Rh. schwetschkeoides Dixon in edit. Mixed with the above species in the same numbers.

Oxyrrhynchium (Bry. Eur.) Warnst.

O. sp. .. .. 233.

Nagouchi considers it to be a new species.

ENTODONTACEAE

Entodon C. Mull.

E. luridus (Griff.) Jaeg. .. .. 376.
E. myurus (Hook.) Jaeg. .. .. 56, 184, 392.
E. prorepens (Mitt.) Jaeg. .. .. 55, 57, 59, 183, 264.
E. plicatus C. Mull. .. .. .. 54, 182.
*E. sp.** .. .. 224.
*E. sp.** .. .. 225.

* Nagouchi considers them to be new species.

Levierella C. Mull.

L. fabroniacea C. Mull. .. .. 53, 181.

PLAGIOTHECIACEAE

Stereophyllum Mitt.

S. anceps (Doz. & Molk.) Broth. (C. & B. det.) 418.
S. wightii (Mitt.) Jaeg. .. .. 84, 223, 242, 262, 291.
S. sp. .. .. 387.

Crum and Bartram consider it to be a new species.

SEMATOPHYLLACEAE

Glossadelphus Fleisch.

G. sp. .. .. 234.

Nagouchi considers it to be a new species.

HYPNACEAE

Pylaisia B. & S.

P. aurea (Hook.) Broth. .. .. 60, 185, 379.

Homomalium (Schimp.) Loesk.

H. simlaense (Mitt.) Broth. (Nagouchi det.) .. 235.

Ectropothecium Mitt.

E. rostellatus (Mitt.) Jaeg. (C. & B. det.) .. 398, 400.
E. sikkimensis Ren. & Card. .. .. 66, 191.

Isopterygium Mitt.

I. distichaceum (Mitt.) Jaeg. (C. & B. det.) .. 409.

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STUDIES IN REPTILIAN SPERMATOGENESIS

II. Spermatogenesis of the Freshwater Turtle, *Lissemys punctata punctata* Bonnaterre, with Observations on the Living Material under the Phase-Contrast Microscope

by

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Abstract

A comprehensive study of the spermatogenesis of the local freshwater turtle, *Lissemys punctata punctata* Bonnaterre, has been made by the latest cytological methods. The technique of phase-contrast microscopy has been used as far as possible for the study of the vitally stained and unstained living material. The important conclusions are as follows: (*a*) The Golgi material shows three distinct phases of growth during spermatogenesis. To begin with it is in the form of chromophilic, lipoidal granules which later on develop in their association chromophobic vacuoles with acidic and watery contents. The chromophobic vacuoles grow and coalesce and simultaneously with this the lipoidal granules associated with these vacuoles disappear and the Golgi material is represented by chromophobic vacuoles alone. Ultimately a single, completely hyaline vacuole with firm consistency is formed. This is the pro-acrosome which is bodily converted into the acrosome. The Golgi remnants are completely absent. (*b*) The entire mitochondria form the sheath of the middle-piece. (*c*) An enigmatic chromatoid body appears and disappears without any apparent function. (*d*) The granular centriole of the early spermatid stages gets firmly stuck to the posterior pole of the condensed and elongating nucleus, forming the proximal centriole which gives off the axial filament. A ring-shaped distal centriole is budded off from the proximal centriole and it migrates downwards along the axial filament to form the posterior limit of the middle-piece. (*e*) The manchette together with the plasma membrane of that region forms the lateral boundaries of the cytoplasm of the future middle-piece.

Introduction

In the first paper of this series (Sud, 1955), the spermatogenesis of the chequered water snake, *Natrix p. piscator* Schneider, was studied in detail. It was found that to begin with the Golgi material is in the form of chromophilic granules, practically each associated with a hyaline vacuole. The hyaline vacuoles develop at the expense of the Golgi granules till the latter completely disappear. These vacuoles gradually run together and a single completely hyaline vacuole with denser contents is formed. This is the

pro-acrosome which is bodily converted into the acrosome. The mitochondria grow gradually from tiny, greyish granules to large, yellow spheres but during the late stages of spermatogenesis the entire mitochondrial material is cast off without forming any sperm component. The chromatoid body appears and disappears twice during spermatogenesis and it appears to be an independent structure having a *de novo* origin.

In this, the second of the series, the spermatogenesis of the local freshwater turtle, *Lissemys punctata punctata* Bonnaterre, has been worked out by the study of the living material under the phase-contrast microscope and other latest cytological techniques. In this turtle the Golgi material shows three distinct phases during its growth. To start with, the entire Golgi material is in the form of chromophilic granules which are later associated with hyaline vacuoles and finally the Golgi material is represented by vacuoles alone. The acrosome formation is practically on the same lines as that in the snake, *Natrix p. piscator*. The mitochondria form a definite middle-piece, whereas in *Natrix* the entire mitochondrial material is sloughed off and the middle-piece is completely absent. Thus in the turtle, *Lissemys punctata punctata*, and the snake, *Natrix piscator piscator*, the structure of the sperm is different and this may be correlated with the diverse morphology of these two reptiles.

All the previous workers on the chelonian male germ-cells except Risley (1936) and Zia-ud-Din (1946) devoted their attention to the nucleus (Yasuzumi *et al.*, 1951), the chromosomes (Pkhakadze, 1939; Oguma, 1937; Nakamura, 1935 and Jordan, 1914), the seasonal changes in the testis (Risley, 1938), the origin and evolution of gonocytes (Allen, 1911 and Dustin, 1910) and the activity of the germinal epithelium (Risley, 1934). Risley (1936) deals mainly with the centrioles and the manchette but he also makes some casual observations on the Golgi material and the mitochondria. Most of his study, however, is based on Bouin fixed material. Zia-ud-Din (1946) published an abstract on the spermatogenesis of tortoise, using Flemming-without-acetic and Champy followed by 0·5 per cent. iron-hæmatoxylin but, so far, he has not published the detailed account of his study. Retzius, as quoted by Nath (1925), has described the structure of the sperm of the land tortoise, *Testudo*.

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Material and Methods

Lissemys punctata punctata Bonnaterre is the most common turtle of Hoshiarpur. The specimens were collected from the local ponds and the testicular material was fixed and was also studied in the living condition, under the phase-contrast microscope, practically during all the months of

the year. In the laboratory the live specimens can be kept in damp sand for a pretty long period.

This turtle is extremely timid and can easily be dissected alive or after using chloroform as a light anaesthetic. The easiest way to open this turtle is to make a cut extending from the nuchal between the costals and the plastron in the anterior half and between the costals and the marginals in the posterior half of the animal.

The testes are a pair of large, elongated, white structures which appear greyish because of a thin, dark, membranous envelope. Each testis is attached to the ventral face of the corresponding kidney. The vas deferens is greatly coiled and communicates to the outside through the cloaca from the median solid penis developed from the ventral wall of the latter.

For the fixation of the testicular material of this turtle the same procedure was adopted as in the case of the snake, *Natrix p. piscator* (Sud, 1955). The following techniques were employed:

1. *Phase-contrast microscopy.* The fresh testicular material was teased and examined in a drop of either 0·7 per cent. saline solution, to every 100 c.c. of which 0·2 c.c. of 10 per cent. calcium chloride solution was added (Baker, 1944) or Ringer solution for cold-blooded animals (Darlington and La Cour, 1947), by positive phase-contrast microscopy. Some of the important stages were photomicrographed.

2. *Vital staining.* Supravital staining was carried out with 0·01 per cent. solution of neutral red chloride (B.D.H.) prepared in the above-mentioned physiological solution or Ringer solution.

3. *Sudan dyes studies.*

(*a*) Paraffin sections of the material fixed in Helly's fluid followed by post-chroming at 37°C. were stained in a saturated solution of Sudan black B in 70 per cent. alcohol and mounted in Farrants's medium (Thomas, 1948).

(*b*) Paraffin sections of material fixed in Aoyama fluid (no silver) for four hours, washed in running water for one hour, dehydrated and cleared in xylol, were stained in a saturated solution of Sudan black B in 70 per cent. alcohol and mounted in Farrants's medium (Gatenby and Moussa, 1949).

(*c*) Paraffin sections of Helly post-chromed material were also stained in Sudan IV and mounted in Farrants's medium for triglycerides.

4. *Chrom-osmium techniques.*

(*a*) Flemming-without-acetic acid for 56 hours, Flemming with reduced quantity of acetic acid for 11 hours and Champy for 24 hours proved to be the best. The material was washed in running water for at least 24 hours in each case. Sections were cut $2\frac{1}{2}$ to $7\frac{1}{2}$ microns thick, mordanted in 5 per cent. iron-alum and stained in 0·5 per cent. hæmatoxylin.

(*b*) Champy Kull's acid fuchsin technique was tried.

(*c*) Smears of the contents of the vas deferens were made in Flemming-without-acetic acid and Champy, each diluted with an equal quantity of distilled water. The smears were stained in 0·5 per cent. iron-hæmatoxylin.

5. *Golgi techniques* (*impregnation methods*).

(*a*) Formal-osmication. The material fixed in formal saline was osmicated at 37°C. in 1 per cent. solution of osmium tetroxide in 0·7 per cent. sodium chloride solution. Osmication for 20 hours proved to be the best. The material was embedded by Peterfi's methyl-benzoate-celloidin method (celloidin and paraffin). The sections were mounted unstained in balsam (Baker, 1944). Sections were also counterstained in iron-hæmatoxylin after bleaching with potassium permanganate and oxalic acid for the study of the chromatoid body.

(*b*) Aoyama's method. The material fixed in Aoyama's fixative was treated for varying periods in 1·5 per cent. silver nitrate solution and the reducing mixture.

6. Bouin and Carnoy fixed material stained with 0·5 per cent. iron-hæmatoxylin was used as control.

Observations

(i) *Spermatogonia*

Three categories of spermatogonia, the primary, secondary and tertiary, were made out. Each resting primary spermatogonium is a small cell with a large nucleus and very scanty cytoplasm (Pl. I, fig. 1). The nucleus is a spherical structure, containing a large number of very fine chromatin granules and a few small nucleoli in its nucleoplasm. The cytoplasm reveals greyish, amorphous mitochondria, distributed evenly in the cytoplasm.

A secondary resting spermatogonium is a larger cell than a primary resting spermatogonium (Pl. I, fig. 2). Its nucleus contains a number of large nucleoli of varied sizes. To begin with the cytoplasm contains uniformly distributed greyish, amorphous mitochondria and a juxta-nuclear mass of sudanophil, osmiophil and argentophil Golgi granules. Just near this mass of Golgi granules is seen a chromatoid body which can be made out from the Golgi granules in chrom-osmium iron-hæmatoxylin preparations from its bigger size and deeper stain. The chromatoid body appears yellow in unstained, freshly mounted Champy and Flemming-without-acetic preparations. It is not blackened in silver and long osmication techniques and is strongly non-sudanophil. It remains yellow in formal-osmication preparations bleached with potassium permanganate and oxalic acid and counterstained in iron-hæmatoxylin. It takes up a deep red acid fuchsin stain in Champy-Kull preparations. It is highly resistant to Bouin and Carnoy fixations and takes up deep blue hæmatoxylin stain in these preparations (Pl. I, fig. 4). In the living material, under positive phase-contrast, it gives a very low phase-change.

During the growth of the secondary spermatogonium the amorphous mitochondria grow into fine, greyish granules and most of them arrange themselves in the form of a horse-shoe-shaped mass around the nucleus. The Golgi granules spread out and are seen embedded in the mitochondrial mass around the nucleus. The chromatoid body is also seen in the mitochondrial mass (Pl. I, fig. 3).

In the living material, under positive phase-contrast, the secondary spermatogonium shows Golgi material in the form of prominent granules, giving a very high phase-change, aggregated first in the form of a juxta-nuclear mass and later in the form of a loose circum-nuclear mass (Pl. V, figs. 49 and 50). The mitochondria in these cells appear in the form of dust-like particles, giving a low phase-change, distributed almost evenly in the cytoplasm. The nucleoli in the early secondary spermatogonia are small but in the late secondary spermatogonia they appear as large spheres of very dark contrast (Pl. V, figs. 49 and 51).

The tertiary spermatogonium is bigger in size than the secondary spermatogonium. Each resting tertiary spermatogonium has a large nucleus with fine chromatin granules and a single large nucleolus in its nucleoplasm (Pl. I, figs. 5 and 6). The cytoplasm has increased in size and it reveals a horse-shoe-shaped mass of dark grey mitochondrial granules in which are embedded deeply-staining Golgi granules. Some mitochondrial granules may be seen scattered outside the horse-shoe-shaped mitochondrial mass. A chromatoid body is also seen in or near the mitochondrial mass (Pl. I, fig. 6).

In the fresh tertiary spermatogonia, studied under positive phase-contrast, the mitochondrial granules of greyish contrast and Golgi granules of dark contrast are found in a compact crescent-shaped mass, very closely applied to the nucleus (Pl. V, fig. 52).

(ii) *Primary Spermatocytes*

The resting primary spermatocyte is almost of the same size as that of the resting tertiary spermatogonium but its nucleus is little smaller than the latter (Pl. I, figs. 7 to 10). The resting primary spermatocyte nucleus is generally seen ex-centric in position and has a single nucleolus. During the growth of the primary spermatocyte the mitochondrial granules and the Golgi material spread out uniformly in the cytoplasm.

At this stage the chromophilic Golgi granules develop each a clear, hyaline vacuole with the Golgi granule lying at its periphery (Pl. I, fig. 7). Sometimes two or even three Golgi granules may be seen associated with a larger hyaline vacuole (Pl. I, figs. 9 and 10). Such larger vacuoles are formed by running together of smaller vacuoles. There may also be seen very large hyaline vacuoles without any trace of the Golgi granules associated with them (Pl. I, fig. 8). These are the fully formed vacuoles. The Golgi granules are either used up in the formation of these large vacuoles or they degenerate when the vacuoles are fully formed. As opposed to the Golgi granules the hyaline vacuoles neither reduce osmium and silver nor are stained in Sudan black but they segregate the basic vital dye, neutral

red. Sudan IV test for triglycerides gave negative results when Helly post-chromed paraffin sections were stained with this dye.

In the fresh material, under positive phase-contrast, the primary spermatocyte shows Golgi material in the form of granules of dark contrast, mostly associated with small hyaline vacuoles (Pl. V, figs. 53 and 54). When a neutral red preparation is examined under positive phase-contrast, at first the vacuoles alone are stained with neutral red and the Golgi granules of dark contrast are seen associated with them. Later, the Golgi granules are not seen and the neutral red staining bodies appear homogeneous.

The chromatoid body in the primary spermatocyte is a large spherical structure and in addition to the main chromatoid body one or two additional chromatoid elements may also be present (Pl. I, figs. 7 and 9).

In meiosis I and II no demonstrable Golgi material and the chromatoid bodies are present but fine mitochondrial granules are seen uniformly dispersed in the cytoplasm (Pl. II, fig. 11). A single, large, granular centriole is seen at each pole of the spindle in these division stages.

(iii) *Secondary Spermatocytes*

The secondary spermatocyte is a smaller cell than the primary spermatocyte (Pl. II, figs. 12 to 15). The resting nucleus mostly contains a single nucleolus in its nucleoplasm. The mitochondria are generally uniformly dispersed throughout the cytoplasm but in some cells they are seen in one or two dense clusters (Pl. II, fig. 14). The Golgi material is distributed throughout the cytoplasm and is in the form of one or two chromophilic granules associated with a clear, hyaline vacuole. The vacuoles tend to coalesce to form larger and denser vacuoles.

A single, large, spherical chromatoid body is present in the early stages of the secondary spermatocyte but, later, it is not seen in most of the cells in the fixed preparations (Pl. II, figs. 12 to 14).

In the fresh material, under positive phase-contrast, the secondary spermatocyte shows Golgi material generally in the form of granules associated with vacuoles (Pl. V, figs. 55 and 56), but sometimes completely chromophobic Golgi vacuoles are also seen (Pl. V, fig. 57). The chromatoid body is invariably seen in the fresh secondary spermatocytes. It is a large, homogeneous, faint, brownish structure. Sometimes the chromatoid body is seen to possess a dark refractile cortex (Pl. V, fig. 57). This dark cortex is most probably an optical 'edge effect'.

(iv) *Spermateleosis*

The early spermatids are smaller cells than even the primary spermatogonia (Pl. II, figs. 16 to 22). Each spermatid nucleus contains either one or two nucleoli. In these cells the mitochondria are seen in the form of fine granules, generally evenly distributed in the cytoplasm. The Golgi material is mostly in the form of granules associated with hyaline vacuoles but, sometimes it is also in the form of vacuoles not associated with granules. An appreciable increase in the size of the Golgi vacuoles is noticed in these early stages, especially in the chrom-osmium iron-hæmatoxylin and Helly-

Sudan black preparations (Pl. II, figs. 16 to 19). The chromatoid body is seen only in a few early spermatids (Pl. II, figs. 21 and 22).

During spermateleosis the chromophilic part of the Golgi material (granules) gradually disappear from view and the vacuoles progressively coalesce till a single, large, clear, hyaline vacuole with firm consistency is formed (Pl. III, figs. 23 to 25). This is the pro-acrosome. The pro-acrosome, even before it is completely formed, gets stuck to the anterior aspect of the nucleus and starts depressing the nuclear membrane. This has been observed in the fixed preparations (Pl. III, fig. 24) and also in the living material under positive phase-contrast (Pl. V, figs. 58 and 59).

When the pro-acrosome is fully formed it is of very large size and is almost three-fourth the size of the nucleus (Pl. III, fig. 25). The spermatid stage with a single hyaline pro-acrosome is short-lived and there are, therefore, relatively few such spermatids at any given time.

The pro-acrosome later on makes the depression on the nuclear membrane little deeper and wider, and simultaneously with this the condensation of the nucleus and the pro-acrosome takes place. The study of the living material, under positive phase-contrast, revealed that the pro-acrosome gains a lead over the nucleus in the process of condensation. The condensed pro-acrosome appears yellowish in the living condition.

By the condensation of the pro-acrosome the acrosome is formed. When the pro-acrosome condenses into the acrosome it is pushed out of the nuclear depression, and a clear space appears between the acrosome and the nucleus (Pl. III, figs. 26 to 29). When both the pro-acrosome and the nucleus have condensed they appear as two hemispherical structures of equal sizes with their flat surfaces facing each other, and the clear space is seen between them (Pl. III, figs. 26 to 28). The condensed pro-acrosome (acrosome) and the nucleus take up a deep hæmatoxylin stain in chrom-osmium, Bouin and Carnoy preparations. At this stage the acrosome cannot be distinguished from the nucleus due to their similarity in shape, size and staining capacity.

As the spermatid grows further the nucleus gradually elongates and assumes a long cylindrical form and the acrosome, lying at its anterior end, assumes a conical shape (Pl. III, figs. 29 to 33). During these changes the clear space between the nucleus and the acrosome completely disappears. The boundary between the acrosome and the nucleus can still be made out by the difference in the width or the staining capacities of these two structures.

In the matter of elongation the cytoplasm lags behind the nucleus with the result that the nucleus generally appears curved (Pl. III, figs. 32 and 33). Later, the cytoplasm elongates and the nucleus becomes straightened up, but still remains slightly curved (Pl. III, figs. 34 and 35; Pl. IV, figs. 36 and 37). The cytoplasm now begins to contract and move downwards along the acrosome and the nucleus and ultimately the cell membrane appears to be closely applied to these cell structures (Pl. III, figs. 34 and 35; Pl. IV, figs. 36, and 38 to 40).

The mitochondria in the early stages of spermateleosis are in the form of fine granules, distributed evenly in the cytoplasm (Pl. II, figs. 16 and 18).

In some cells they are localized in certain regions of the cytoplasm and appear much more prominent (Pl. II, fig. 17; Pl. III, figs. 25 and 26). In some spermatids, after the condensation of the nucleus and the pro-acrosome, the mitochondria show a marked increase in the size of the individual granules and they appear aggregated together (Pl. III, figs. 27 and 33). In these spermatids there is a corresponding decrease in the number of the mitochondrial granules and from this it is evident that the larger mitochondria are formed by the fusion of the fine granules. This development of the mitochondria in these cells appears to be rather precocious because, generally, the mitochondria remain in the form of fine granules till very late stages of spermateleosis (Pl. III, figs. 26, 32, 34 and 35; Pl. IV, figs. 36, 38 and 39).

In the late stages of spermateleosis a cylindrical membranous structure, the manchette, is seen in the cytoplasm arising from the posterior pole of the condensed and elongated nucleus (Pl. III, fig. 33; Pl. IV, figs. 36, 38 and 39). The manchette is easily seen in chrom-osmium preparations stained with iron-hæmatoxylin but in the living material, under positive phase-contrast, this fine membrane-like structure is not clearly seen. The manchette encloses the denser part of the cytoplasm which surrounds the intra-cellular part of the axial filament. As the cytoplasm contracts and travels downwards the manchette, together with the plasma membrane of that region, forms the lateral boundaries of the cytoplasm of the future middle-piece. It is, generally, during this process that the fine mitochondria appear to fuse to form prominent, large, darkly-staining granules. The entire mitochondrial granules migrate into the middle-piece of the maturing sperm and arrange themselves around the axial filament to give a mulberry-like appearance (Pl. IV, figs. 37 and 40). As the sperm further matures the middle-piece appears to elongate slightly and the mitochondria gradually fade away to form the mitochondrial sheath of the middle-piece (Pl. IV, figs. 41 and 42).

The chromatoid body is seen in early spermatids mostly after the formation of the pro-acrosome (Pl. III, figs. 25 to 35; Pl. IV, figs. 36 to 39). In some spermatids two or even more than two chromatoid bodies are seen (Pl. III, figs. 27, 29, 32 and 34; Pl. IV, figs. 36 and 39). All the chromatoid elements, however, disappear during the formation of the mitochondrial middle-piece (Pl. IV, fig. 40).

The centriole in the early stages of spermateleosis is in the form of a small granule. During these stages it is difficult to make out the centriole from the fine mitochondrial granules. After the condensation of the nucleus and the pro-acrosome, when in some of the cells the mitochondria show a marked increase in the size of the individual granules, the fine granular centriole may be made out (Pl. III, fig. 27). Later, the centriole becomes applied to the posterior pole of the condensed and elongated nucleus and it is termed the proximal centriole (Pl. III, fig. 35). It is so firmly applied to the nucleus that it is generally lost to view. The axial filament arises rather late from the proximal centriole and is destined to form the tail of the ripe sperm (Pl. III, figs. 31 and 33). Now a distal centriole makes its appearance in the form of a ring which in sections very often appears

as a crescent, and encircles the axial filament (Pl. III, fig. 35). The distal centriole is presumably budded off from the proximal centriole during the elongation of the spermatid. It travels downwards along the axial filament and finally takes up its position at the posterior limit of the middle-piece (Pl. IV, figs. 36 to 42).

In the living material, under positive phase-contrast, the proximal centriole is seen with difficulty but the distal centriole is clearly seen as a ring-like structure of dark contrast (Pl. V, fig. 60).

The ripe spermatozoon is composed of the head, the middle-piece and the tail (Pl. IV, figs. 40 to 42; Pl. V, figs. 60 to 62). The head consists of a homogeneously-stained, elongated, curved or wavy nucleus, carrying a comparatively lightly-stained, short, conical acrosome at its anterior end. In the living sperm, under positive phase-contrast, the nucleus does not appear homogeneous but shows light medullary portion and dark cortex along the nuclear membrane (Pl. V, figs. 61 and 62). The boundary of the nucleus and the acrosome can easily be made out both in the fixed and the living material, under positive phase-contrast.

The head is followed by a well defined middle-piece which is limited by the proximal centriole in front and the distal ring centriole behind. The proximal centriole cannot be made out clearly but the distal ring-shaped centriole appears prominent in the fixed (Pl. IV, figs. 40 to 42) and the living sperms, under positive phase-contrast (Pl. V, fig. 60). The middle-piece is traversed through its centre by the axial filament which arises from the proximal centriole. In this region the axial filament is surrounded by the mitochondrial sheath (Pl. IV, figs. 40 to 42; Pl. V, figs. 61 and 62). The axial filament of the middle-piece is prolonged posteriorly into the vibratile tail. The axial filament of the tail appears naked in the fixed preparations (Pl. IV, figs. 40 to 42) but in the living sperm, under positive phase-contrast, it is seen to be surrounded by a thin cytoplasmic sheath (Pl. V, figs. 60 to 62). This cytoplasmic sheath is broadest at the base of the middle-piece, and it gradually tapers to the tip of the tail. There appears to be no end-piece of the tail.

Certain abnormal spermatids were noticed during spermateleosis (Pl. IV, figs. 43 to 48). They appear to result from the unequal division of the nucleus and the cytoplasm of the cells. In these cells the process of spermateleosis appears to be identical to that of normal spermatids.

Syncytial masses, resulting from the failure of the cytoplasm to divide during cell division, are present but they are not of very common occurrence.

Discussion

Golgi Material

The Golgi material is not seen in the primary spermatogonia but in the later gonial stages it appears in the form of prominent sudanophil, osmiophil and argentophil granules. In the living condition, under positive phase-contrast, these Golgi granules give a very high phase-change and appear as homogeneous bodies of dark contrast. During the spermatocyte stages hyaline vacuoles develop in association with the Golgi granules and

generally a single Golgi granule is seen touching such a vacuole at its periphery. The hyaline vacuoles show a distinct tendency to run together and thus two or even three Golgi granules are seen associated with larger hyaline vacuoles. Whereas the Golgi granules appear as highly refringent, dark structures, the vacuoles are hyaline. These vacuoles are not impregnated with silver or osmium under optimal conditions of impregnation but are stainable with the basic vital dye, neutral red. These reactions show that whereas the granular part of the Golgi body is lipoidal, the vacuolar is acidic and watery (vacuome of Parat).

Neutral red-staining Golgi vacuoles have also been observed by the present author in the spermatocytes and spermatids of the lizard, *Calotes versicolor*.

A granule associated with a vacuole represents the binary structure of the Golgi material but in this case the cortical component (granule) does not completely encircle the medulla (vacuole) but forms a partial investment only.

When a vacuole has reached its full development the granule attached to it disappears and the Golgi material is now represented by the hyaline vacuole alone. Such Golgi vacuoles usually appear during spermateleosis but they may also be seen in spermatocytes.

The three types of Golgi material—granule, granule associated with a vacuole and the vacuole alone—are seen in the fixed preparations as well as in the living material, under positive phase-contrast. It is evident that these three types of Golgi material represent different phases in the growth of this cellular constituent.

The Golgi material in the form of a lipoidal granule associated with a hyaline vacuole stainable with neutral red, observed in the male germ-cells of the present material, has also been described in the neurones of *Helix aspersa* and the sympathetic neurones of mouse (Thomas, 1947 and 1948). A similar form of the Golgi material has been described in this laboratory in the neurones of *Laccotrephes rubra* by Malhotra (1955). Sud (1955) has also described Golgi material in the form of a chromophilic granule associated with a hyaline vacuole in the male germ-cells of the snake, *Natrix p. piscator*.

Risley (1936) in the testicular material of turtles describes deeply-staining bodies associated with vacuoles. He also interprets these deeply-staining bodies together with the vacuoles as representing some phase of the Golgi material in the cell. The vacuoles in his figures are shown to surround the darkly-staining bodies, whereas in the present material the vacuoles lie on the side of the darkly-staining granules in organic contact with the latter.

Acrosome

The entire Golgi material is converted into a single acrosomal vacuole, the pro-acrosome, by the gradual coming together of the Golgi vacuoles and the simultaneous disappearance of the lipoidal granules associated with them. The pro-acrosome is completely hyaline and has a firm consistency. It depresses the nuclear membrane at its anterior aspect and its contents become more and more firm till it is converted into the acrosome.

During this process there is no trace of any acrosomal granule in the pro-acrosome, nor are there any Golgi remnants.

A similar process of acrosome formation has been observed in the snake, *Natrix p. piscator*, and a similar type of pro-acrosome has been seen in the snakes, *Bungarus cæruleus* and *Naia tripudians* var. *typica*, by Sud (1955).

In the lizards, *Uromastix hardwikii* and *Calotes versicolor*, almost a similar type of pro-acrosome has been observed by the present author in the living material, under positive phase-contrast.

Risley (1936) in turtles (as discussed by Sud, 1955) states that the acrosome is formed from a large, clear vacuole. This vacuole appears to be identical in shape and texture to the pro-acrosome of the present material. Risley in his material describes acrosomal remnant in the form of a darkly-staining body with a clear vacuolar area about it and he presumes that in the mature sperm it is present in the middle-piece. In the present material, however, no acrosomal remnant has been observed.

Mitochondria

The mitochondria generally remain in the form of very fine granules till very late stages of spermateleosis. It is only when they are about to migrate into the middle-piece or when they have actually shifted to the middle-piece that they appear as prominent, large, darkly-staining granules. These large mitochondrial granules are most probably formed by the fusion of the fine mitochondria. The large mitochondrial granules ultimately fade away appreciably to form the sheath of the middle-piece of the mature sperm.

Retzius, as quoted by Nath (1925), has described in the sperm of the tortoise, *Testudo*, a mitochondrial sheath of the middle-piece. Risley (1936) describes a mitochondrial middle-piece in turtles.

In the snake, *Natrix p. piscator* (Sud, 1955), the behaviour and fate of the mitochondria is quite different. The mitochondria grow steadily from fine granules to large yellow spheres but finally the entire mitochondrial material is cast off, making no contribution whatsoever to the sperm formation.

Chromatoid Body

The chromatoid body makes its first appearance in the secondary spermatogonium. It is not seen in most of the early spermatids before the formation of the pro-acrosome. Later, additional chromatoid bodies also appear but during the formation of the middle-piece all the chromatoid elements disappear without contributing anything to the final make-up of the sperm.

Risley (1936) in turtles does not describe any chromatoid body during spermatogenesis but in the opinion of the present author the acrosomal remnant described by him may well be a chromatoid body.

Sud (1955) in the snake, *Natrix p. piscator*, describes a typical chromatoid body. The physical and chemical nature of the chromatoid body

in this snake and the present material is identical. In both these reptiles it is a homogeneous structure and does not contain any vacuole. It is not blackened in silver nitrate and long osmication techniques and is strongly non-sudanophil. In the living material, under positive phase-contrast, it gives a very low phase-change. It is not even slightly corroded in Bouin and Carnoy preparations. The origin and significance of this cell structure is an enigma in both the species but it may be suggested that it appears to be an independent cell structure having a *de novo* origin.

Centrioles

In the meiotic divisions a large granular centriole is seen at each pole of the spindle. In early stages of spermateleosis a single, fine, granular centriole is present which later gets firmly stuck to the posterior pole of the condensed and elongating nucleus and gives off the axial filament. This centriole is termed the proximal centriole. From the proximal centriole a ring-shaped distal centriole appears to be budded off. The distal centriole travels along the axial filament and marks the posterior boundary of the middle-piece.

Risley (1936) in the spermatogenesis of turtles describes rod-shaped centrioles. Each spermatid receives a single rod centriole which in spermateleosis becomes the central axis of the middle-piece of the mature sperm. From the distal end of this long centriole touching the cell membrane arises the flagellum and from the proximal end which touches the nucleus and happens to be the generative end is given off a ring-shaped distal centriole. The distal centriole migrates posteriorly over the long centriole until it meets the cell membrane at the end of the middle-piece and loses its identity.

Manchette

The manchette in *Lissemys punctata punctata* makes its appearance during spermateleosis in the form of a membranous, cylindrical structure, enclosing the denser cytoplasm around the intra-cellular part of the axial filament. With the contraction and downward migration of the cytoplasm, during the final stages of spermateleosis, the manchette together with the plasma membrane of that region forms the lateral boundaries of the cytoplasm of the future middle-piece.

Risley (1936) describes a manchette in the late spermatid stages of turtles. He is inclined to the view that the manchette of the turtles, studied by him, is derived from the Golgi material. But my observations on the present material do not suggest even the remotest chance of such a possibility. On the contrary it may be suggested that the origin of the manchette in the present material may be ascribed to the cytoplasmic differentiation, as pointed out by Meves (1899) and Branca (1924), or the nuclear membrane, as recommended by Schoenfeld (1900) and Van Mollé (1906). Risley further states that the manchette ultimately completely ensheaths the long centriole which forms the central axis of the middle-piece, and the mitochondria fill in the middle-piece between the manchette and the cell membrane. My observations on *Lissemys punctata punctata*, however, show

that during the contraction and the downward migration of the cytoplasm the manchette fuses with the plasma membrane of that region and the entire mitochondria enter the middle-piece where it is surrounded by the fused manchette and plasma membrane, forming the lateral boundaries of the middle-piece.

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Explanation of Lettering in Plates

A—Acrosome; *A.f*—Axial filament; *C*—Centriole; C_1—Proximal centriole; C_2—Distal centriole; *C.b*—Chromatoid body; *C.g*—Chromatin granules; *Ch*—Chromosomes; *G*—Golgi material; *M*—Mitochondria; *n*—Nucleolus; *N*—Nucleus; *Mt*—Manchette; *Mp*—Middle-piece; *Pa*—Pro-acrosome; *Sf*—Spindle fibres; *T*—Tail.

Explanation of Figures

All figures of Plates I, II, III and IV except Fig. 42 have been selected from the sectioned testicular material. Fig. 42 has been selected from a smear preparation of the contents of the vas deferens. All figures in these plates have been drawn at table level with Beck camera lucida, 2 mm. oil immersion objective and 10× ocular. The figures were further enlarged two times, thus giving a total magnification of approximately 2,740 times.

All figures of Plate V are photomicrographs of the fresh, unstained testicular material as studied under the Carl Zeiss stand 'W' phase-contrast microscope, 100/1·25 *oil immersion objective and* K8× *ocular. Photomicrographs were taken with* Carl Zeiss micro-reflex-camera attachment and contax 35 mm. camera. The photomicrographs were further enlarged four times, giving a total magnification of 1,600 times and are untouched.

Plate I

Fig. 1. Primary spermatogonium with chromatin granules and small nucleoli in the nucleoplasm. The cytoplasm has only amorphous mitochondria which are dispersed evenly (Champy iron-hæmatoxylin).

Fig. 2. Early secondary spermatogonium with large nucleoli of varied sizes in the nucleoplasm. The cytoplasm contains a juxta-nuclear mass of chromophilic Golgi granules and the chromatoid body in addition to uniformly dispersed, amorphous mitochondria (Champy iron-hæmatoxylin).

Fig. 3. Late secondary spermatogonium in which the mitochondria have grown into fine granules and are mostly arranged in the form of a horse-shoe-shaped mass around the nucleus. The chromophilic Golgi granules have spread out and are embedded in the horse-shoe-shaped mitochondrial mass along with the chromatoid body (Champy iron-hæmatoxylin).

Fig. 4. Secondary spermatogonium, showing the chromatoid body in the cytoplasm (*Bouin iron-hæmatoxylin*).

Fig. 5. Tertiary spermatogonium, showing the chromatin granules and a single large nucleolus in the nucleoplasm. Most of the mitochondrial granules are arranged in a horse-shoe-shaped mass around the nucleus and the chromophilic Golgi granules are embedded in this mass (Champy iron-hæmatoxylin).

Fig. 6. Tertiary spermatogonium, showing the chromatoid body and the Golgi granules embedded in the mitochondrial mass (Champy iron-hæmatoxylin).

Fig. 7. Primary spermatocyte with chromatin granules and a single nucleolus in the nucleoplasm. The mitochondria and the Golgi material are spread out throughout the cytoplasm. The chromophilic Golgi granules are associated with small, clear, hyaline vacuoles. There is one large and three small chromatoid bodies (Champy iron-hæmatoxylin).

Fig. 8. Primary spermatocyte, showing the Golgi elements in the form of chromophilic granules, each associated with a hyaline vacuole, and also the hyaline vacuoles alone (Flemming-without-acetic and iron-hæmatoxylin).

Fig. 9. Primary spermatocyte, showing the Golgi elements in the form of hyaline vacuoles, each associated with one, two or even three chromophilic granules (Flemming-without-acetic and iron-hæmatoxylin).

Fig. 10. Primary spermatocyte, showing the Golgi material in the form of one or two sudanophil granules associated with a hyaline vacuole (Aoyama-Sudan black).

Plate II

Fig. 11. Metaphase of the meiosis I (side view), showing a granular centriole at each pole of the spindle. Only uniformly dispersed mitochondria are seen in the cytoplasm (Champy iron-hæmatoxylin).

Fig. 12. Secondary spermatocyte with the nucleus containing a single, large nucleolus. In the cytoplasm the mitochondria are almost uniformly dispersed. The Golgi elements are seen in the form of one or two chromophilic granules associated with a hyaline vacuole (Flemming-without-acetic and iron-hæmatoxylin).

Fig. 13. Secondary spermatocyte, showing the nucleus with one large and four small nucleoli. Fine mitochondrial granules and Golgi bodies in the form of one or two chromophilic granules associated with a hyaline vacuole are uniformly dispersed in the cytoplasm (Flemming-without-acetic and iron-hæmatoxylin).

Fig. 14. Secondary spermatocyte with the nucleus containing two nucleoli and chromatin granules. The mitochondria are in the form of two dense clusters. The Golgi elements, in the form of one or two chromophilic granules associated with a hyaline vacuole, are uniformly dispersed in the cytoplasm (Flemming-without-acetic and iron-hæmatoxylin).

Fig. 15. Secondary spermatocyte, showing the Golgi material, in the form of one or two sudanophil granules associated with a hyaline vacuole, evenly distributed in the cytoplasm (Helly-Sudan black).

Fig. 16. Early spermatid, showing the nucleus with two small nucleoli. The mitochondria are evenly distributed and the Golgi material is in the form of one or two chromophilic granules associated with a hyaline vacuole (Flemming-without-acetic and iron-hæmatoxylin).

Fig. 17. Early spermatid, showing the mitochondria aggregated at one pole of the cell. Golgi material is in the form of completely hyaline vacuoles and a hyaline vacuole associated with a chromophilic granule (Flemming with reduced quantity of acetic acid, and iron-hæmatoxylin).

Fig. 18. Early spermatid with the nucleus containing a single nucleolus and the chromatin granules. The mitochondria are evenly distributed and the Golgi material is in the form of one or two chromophilic granules associated with a hyaline vacuole (Flemming-without-acetic and iron-hæmatoxylin).

Fig. 19. Early spermatid, showing the Golgi material in the form of one or two sudanophil granules associated with a hyaline vacuole (Helly-Sudan black).

Fig. 20. Early spermatid, showing the Golgi material in the form of one or two argentophil granules associated with a hyaline vacuole (Aoyama's method).

Fig. 21. Early spermatid, showing the argentophil Golgi granules, each associated with a hyaline vacuole. A golden yellow chromatoid body is also seen (Aoyama's method).

Fig. 22. Early spermatid with Golgi material in the form of one or two osmiophil granules associated with a hyaline vacuole. The chromatoid body appears yellow (Formal-osmication).

Plate III

Fig. 23. Early spermatid, showing the Golgi material in the form of completely hyaline vacuoles. The chromatoid body appears yellow (Formal-osmication).

Fig. 24. Spermatid, showing the coming together of the hyaline vacuoles to form the pro-acrosome. One of the vacuoles which is completely hyaline has pushed the nuclear membrane downwards (Flemming with reduced quantity of acetic acid, and iron-hæmatoxylin).

Fig. 25. Spermatid, showing the pro-acrosome depressing the nuclear membrane at the anterior pole of the nucleus. Mitochondria are aggregated in a portion

of the cytoplasm. A chromatoid body is also seen (Flemming with reduced quantity of acetic acid, and iron-hæmatoxylin).

FIG. 26. Spermatid, showing the acrosome, the condensed nucleus and a clear space between these two structures. The mitochondria are arranged along the periphery of the cell. A chromatoid body is also seen. (Flemming-without-acetic and iron-hæmatoxylin).

FIG. 27. Spermatid, showing the acrosome and the condensed nucleus with a small clear space in between. The mitochondria have become large, spherical granules. A granular centriole and two chromatoid bodies are also seen (Flemming-without-acetic and iron-hæmatoxylin).

FIG. 28. Spermatid, showing the acrosome and the condensed nucleus with a clear space in between. A single chromatoid body is seen (Bouin iron-hæmatoxylin).

FIG. 29. Spermatid with the acrosome and slightly elongated, condensed nucleus. Two chromatoid bodies are seen (Bouin iron-hæmatoxylin).

FIG. 30. Spermatid, showing the elongating, condensed nucleus and cone-shaped acrosome. The acrosome actually appears lighter than the nucleus and the boundaries of the two can easily be made out. A single chromatoid body is seen (Bouin iron-hæmatoxylin).

FIG. 31. Late spermatid, showing the cone-shaped acrosome, elongating nucleus, long axial filament and a single chromatoid body (Bouin iron-hæmatoxylin).

FIG. 32. Late spermatid, with elongated, condensed nucleus and lightly-staining acrosome. There are five chromatoid bodies (Flemming with reduced quantity of acetic acid, and iron-hæmatoxylin).

FIG. 33. Late spermatid with elongated nucleus and prominent acrosome. Aggregated, prominent, large mitochondria and a single chromatoid body are seen. The manchette, surrounding the cytoplasm around the intra-cellular part of the axial filament, has also appeared (Flemming-without-acetic and iron-hæmatoxylin).

FIG. 34. Late spermatid with the elongated nucleus and the cone-shaped acrosome. Three chromatoid bodies are seen (Flemming-without-acetic and iron-hæmatoxylin).

FIG. 35. Late spermatid, showing the distal centriole travelling downwards along the axial filament (Champy iron-hæmatoxylin).

PLATE IV

FIG. 36. Late spermatid, showing the intra-cellular part of the axial filament, the manchette and the distal centriole (Champy iron-hæmatoxylin).

FIG. 37. Late spermatid, showing the manchette getting fused with the plasma membrane of that region and the formation of prominent, large mitochondria (Champy iron-hæmatoxylin).

FIG. 38. Late spermatid with greatly elongated nucleus and the acrosome (Champy iron-hæmatoxylin).

FIG. 39. Late spermatid, showing the cytoplasm contracting and travelling downwards (Flemming-without-acetic and iron-hæmatoxylin).

FIG. 40. Sperm consisting of the acrosome, the nucleus, the middle-piece and the tail. Prominent mitochondria are seen around the axial filament in the middle-piece. (Flemming-without-acetic and iron-hæmatoxylin).

FIG. 41. Mature sperm with the mitochondria in the middle-piece less prominent than in figure 40 (Flemming-without-acetic and iron-hæmatoxylin).

FIG. 42. A still more mature sperm in which the mitochondria in the middle-piece have become very faint (Flemming-without-acetic and iron-hæmatoxylin).

FIG. 43. Small-sized abnormal early spermatid with the nucleus, the mitochondria, and the Golgi material in the form of chromophilic granules, each associated with a hyaline vacuole. Two of the Golgi vacuoles are about to coalesce (Champy iron-hæmatoxylin).

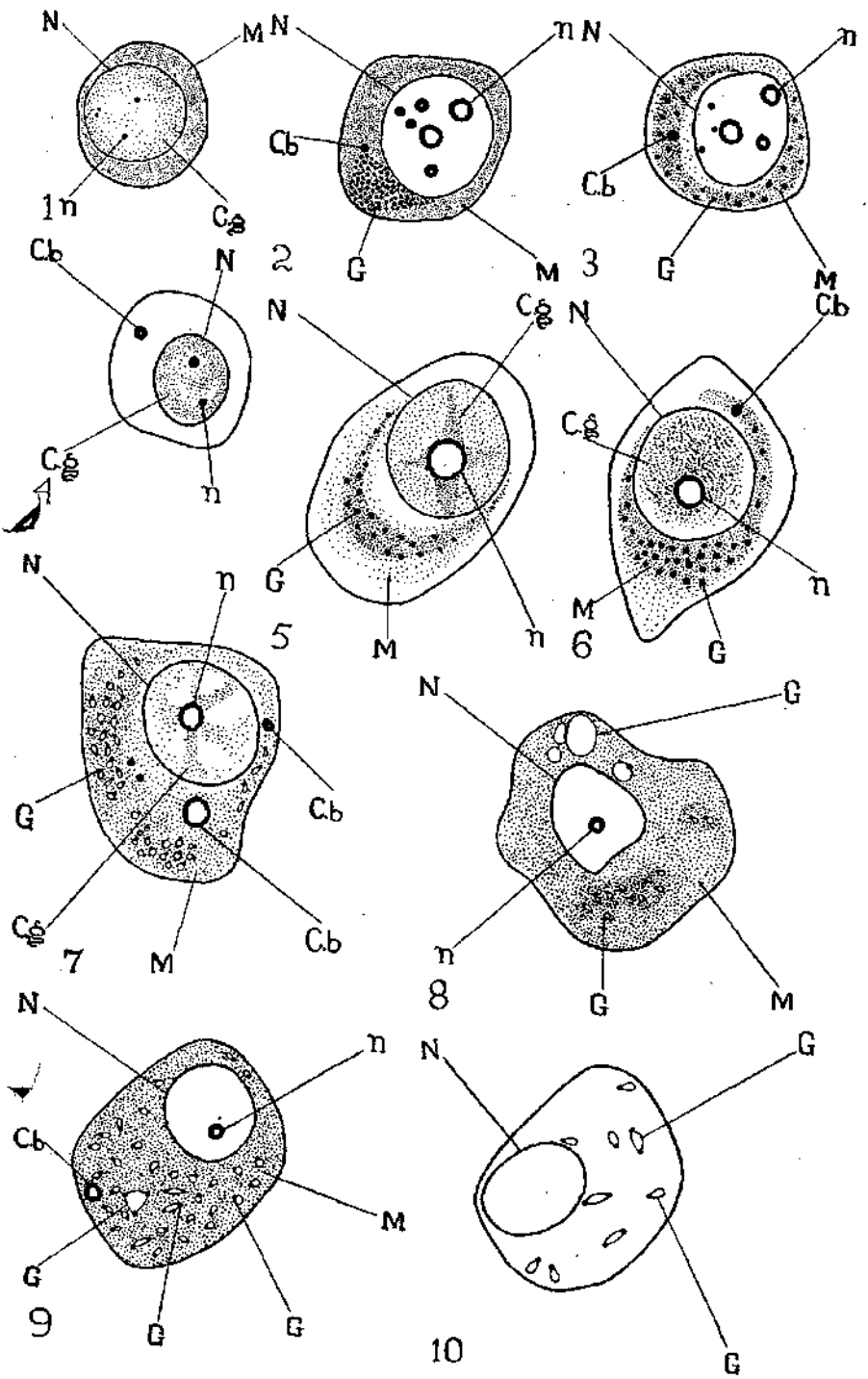
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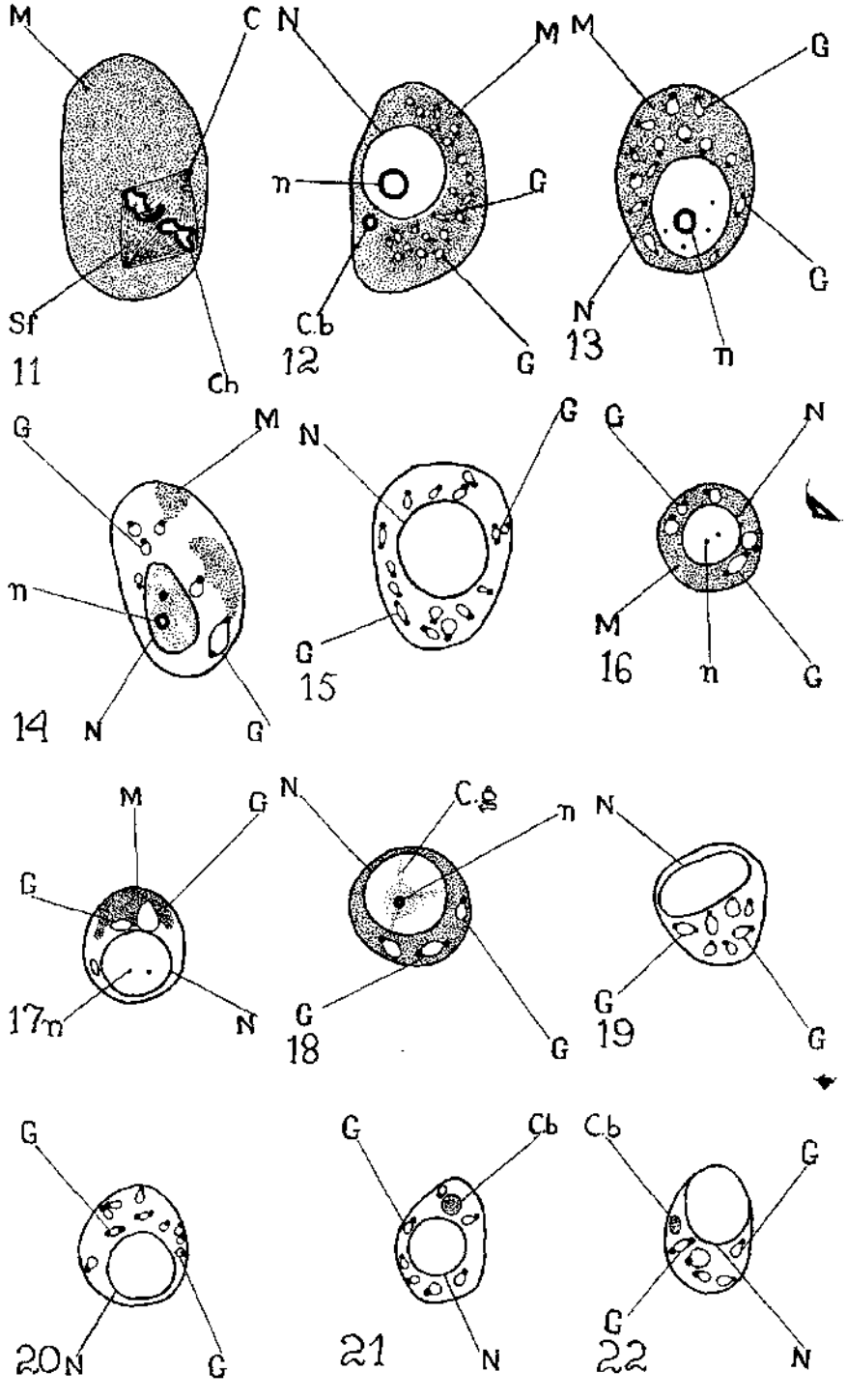
PLATE II.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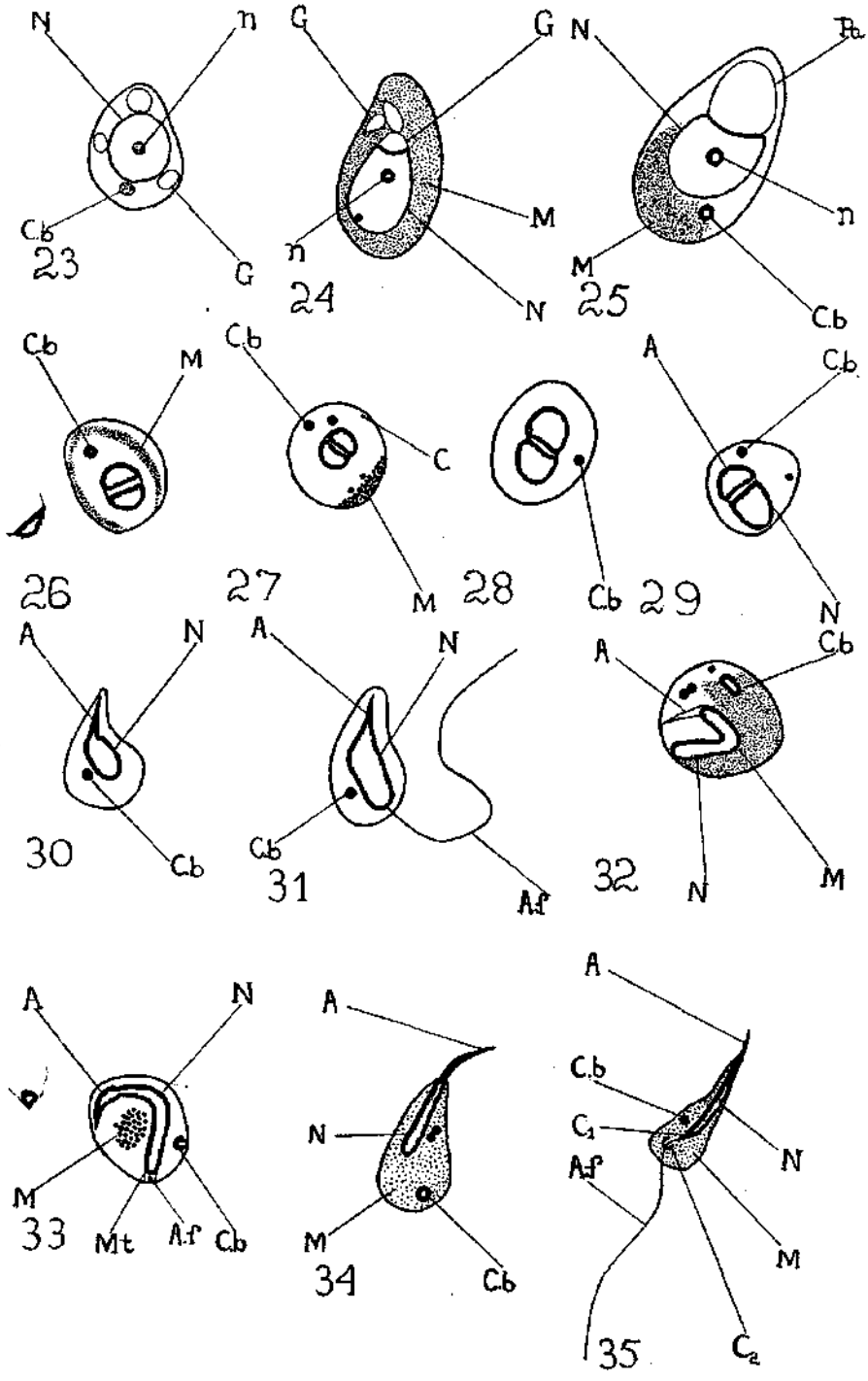
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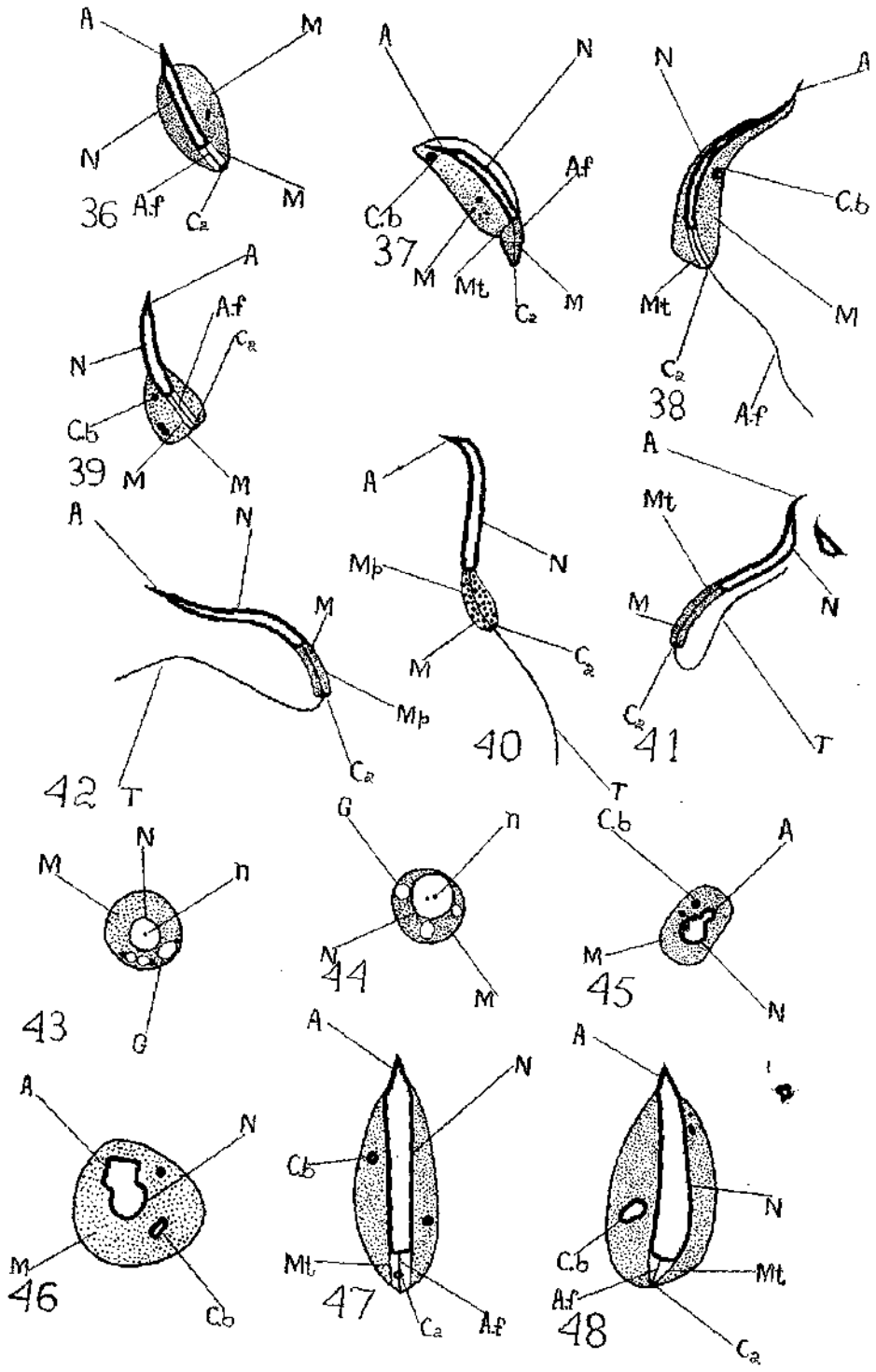
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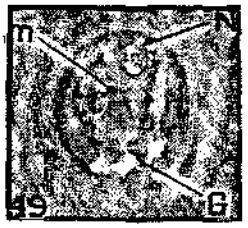
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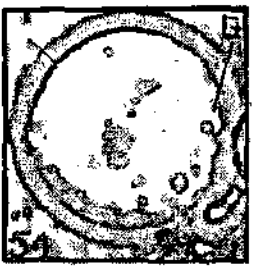
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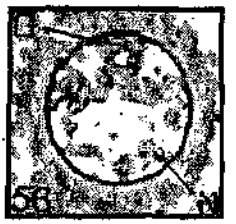
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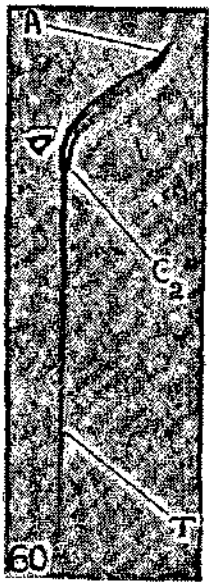
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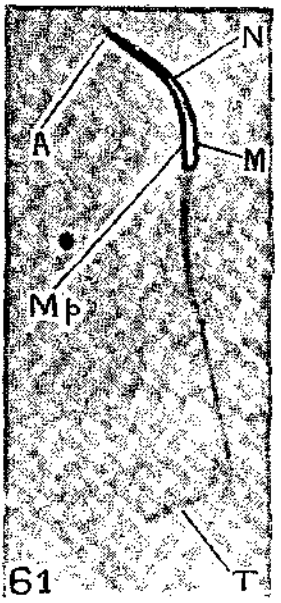
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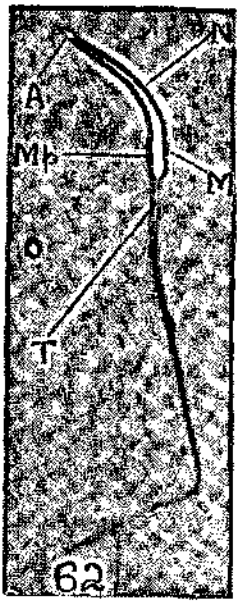
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FIG. 44. Small-sized abnormal early spermatid with the Golgi material in the form of completely hyaline vacuoles (Champy iron-hæmatoxylin).

FIG. 45. Small-sized abnormal spermatid in which the pro-acrosome and the nucleus have condensed. Uniformly dispersed mitochondria and two chromatoid bodies are seen (Flemming with reduced quantity of acetic acid, and iron-hæmatoxylin).

FIG. 46. Large-sized abnormal spermatid, showing the acrosome and the condensed nucleus. The chromatoid bodies and uniformly dispersed mitochondria are also seen (Champy and iron-hæmatoxylin).

FIG. 47. Large-sized abnormal late spermatid, showing thick and long nucleus, the cone-shaped acrosome, the manchette and the distal centriole moving downwards along the intracellular part of the axial filament. Uniformly dispersed mitochondria and the chromatoid bodies are also seen (Flemming-without-acetic and iron-hæmatoxylin).

FIG. 48. Large-sized abnormal late spermatid, showing the distal centriole touching the nuclear membrane (Flemming-without-acetic and iron-hæmatoxylin).

PLATE V

FIG. 49. Photomicrograph of an early secondary spermatogonium, showing the Golgi material in the form of granules of dark contrast arranged in a juxta-nuclear mass. The nucleus contains small nucleoli.

FIG. 50. Photomicrograph of a late secondary spermatogonium, showing the Golgi granules of dark contrast arranged in a loose circum-nuclear mass.

FIG. 51. Photomicrograph of a late secondary spermatogonium, showing in focus the nucleus containing large nucleoli of varied sizes.

FIG. 52. Photomicrograph of a tertiary spermatogonium, showing a crescent-shaped mass of greyish mitochondria and dark Golgi granules closely applied to the nucleus.

FIGS. 53 and 54. Photomicrographs of primary spermatocytes, showing the Golgi material in the form of dark granules, and dark granules associated with hyaline vacuoles. Each hyaline vacuole shows one or two dark granules attached to its periphery.

FIG. 55. Photomicrograph of a secondary spermatocyte, showing in focus two dark Golgi granules associated with a hyaline vacuole.

FIG. 56. Photomicrograph of a secondary spermatocyte, showing in focus a Golgi granule of dark contrast associated with a hyaline vacuole.

FIG. 57. Photomicrograph of a secondary spermatocyte, showing in focus a completely hyaline Golgi vacuole and the chromatoid body. The chromatoid body shows a duplex structure due to 'Edge-effect'.

FIG. 58. Photomicrograph of an early spermatid, showing in focus a completely hyaline Golgi vacuole in the mitochondrial mass sticking to the nuclear membrane.

FIG. 59. Photomicrograph of an early spermatid, showing a completely hyaline vacuole depressing the nuclear membrane. Dark spherical bodies, on the left side of this Golgi vacuole, are some foreign elements.

FIG. 60. Photomicrograph of a sperm, showing in focus the acrosome, the distal centriole and the tail. The tail possesses a thin cytoplasmic sheath.

FIG. 61. Photomicrograph of a complete sperm, showing the acrosome, the nucleus, the middle-piece and the tail. Prominent mitochondrial granules are seen in the middle-piece.

FIG. 62. Photomicrograph of the same sperm as that in figure 61, showing more clearly the thin cytoplasmic sheath around the tail.

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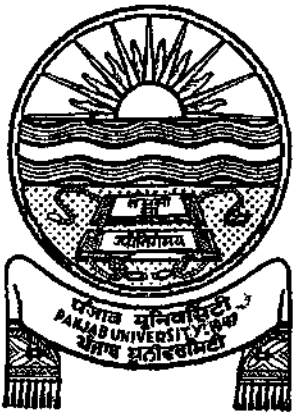

March, 1956

STUDIES IN THIOPEGANS

by

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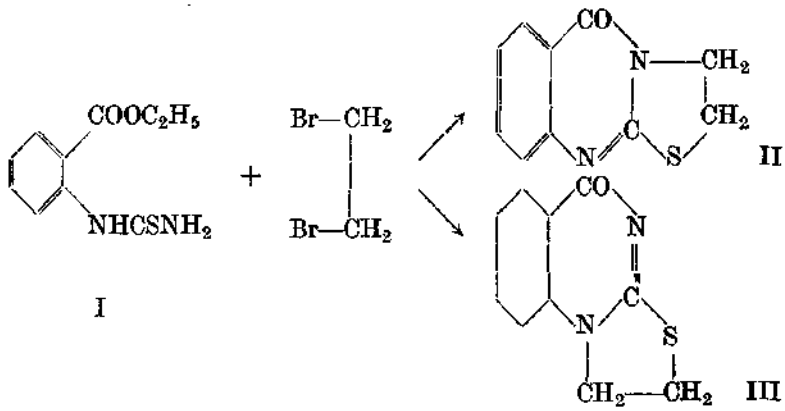
STUDIES IN THIOPEGANS

By G. M. Sharma, I. Sen Gupta *and* K. S. Narang

(*Department of Chemistry, Panjab University, Hoshiarpur, Panjab, India*)

Narang[1] *et al.* reported only one product from the reaction of 2-carbethoxy phenyl thiourea I with ethylene bromide, though theoretically two products II and III are possible.

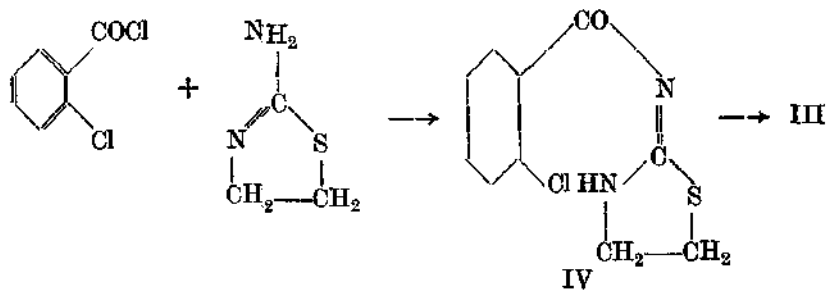


I

Attempts have, therefore, been made to synthesize either of the products II and III by an alternate unambiguous method.

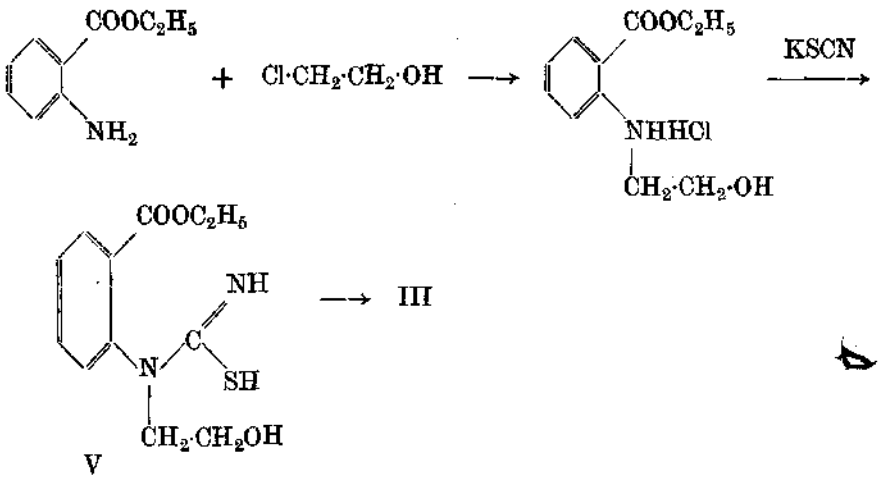
The linear compound II could be synthesized[2] by the condensation of 2-chloro-thiazoline with anthranilic acid. But 2-chloro-thiazoline could not be obtained in pure form from 2-amino-thiazoline according to the method used by Ganapathi[3] *et al.* Attempted synthesis of II by condensation of o-chloro-benzoic acid with 2-amino-thiazoline, even in the presence of catalyst like copper powder at 180°C., did not materialize.

Attempts were then made to synthesize angular compound III when it was thought to condense 2-amino-thiazoline with o-chloro-benzoyl chloride giving the amide IV which on subsequent cyclization would yield the desired product.



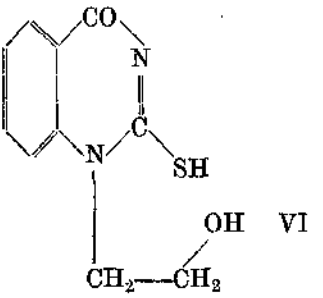
The reaction, however, gave a product the nitrogen and sulphur values of which correspond to di-o-chloro benzoyl derivative.

Another attempt in this direction was made by condensing ethylene chlorohydrin with ethyl anthranilate. It was thought that the hydrochloride of the condensed product on treatment with potassium sulphocyanide would yield N′-β-hydroxy ethyl-N′-2-carbethoxy phenyl thiourea V which on cyclization will give the angular product III.



V

The condensation of ethylene chlorohydrin with anthranilic acid was smooth. The hydrochloride of this product on treatment with potassium sulphocyanide could not give the required thiourea V in pure form. The reaction mixture, however, on refluxing in alcoholic solution for 24 hours yielded a product which is soluble in alkali. On acidification of the alkaline solution the product got precipitated. From this behaviour and from the analytical results of the product, it appears to be the tetrahydro compound VI.



VI

Cyclization of compound VI by heating with phosphorous penta oxide or in presence of sulphuric acid has not materialized so far.

The condensation of ethylene bromide in place of ethylene chlorohydrin with ethyl anthranilate also gave no tangible results. The cyclization of product VI is under investigation.

Experimental

Attempted condensation of o-chloro-benzoic acid with 2-amino-thiazoline :—

O-chloro-benzoic acid (3·13 gm.), 2-amino-thiazoline (2·04 gm.) and copper powder (0·3 gm.) were thoroughly mixed and heated for two hours at 160°C. when a green colour developed. The temperature was raised to 180°C. for 20 minutes. After cooling the reaction mixture was treated with sodium carbonate solution. The residue left behind could not be purified.

In another trial the same amounts of the reactants were heated at 180°C. for six hours without copper powder. The reaction mixture was made alkaline and the precipitate isolated. All attempts to purify it failed.

Condensation of o-chloro-benzoyl chloride with 2-amino-thiazoline :—

O-chloro-benzoyl chloride (3·4 gm.) was added gradually to 2-amino-thiazoline (2 gm.) in dry benzene (20 c.c.). A precipitate appeared at once and the mixture was refluxed on a water bath for an hour. The precipitate was filtered and crystallized from absolute alcohol m.p. 182°C., yield being 0·2 gm.

The reactants when shaken in presence of 10% sodium hydroxide solution also gave the same product.

Found: N, 7·4%, S, 8·0%. $C_{17}H_{12}O_2N_2Cl_2S$ requires N, 7·38%, S, 8·44%.

Condensation of ethylene chlorohydrin with ethyl anthranilate :—

A mixture of ethyl anthranilate (100 g.) and ethylene chlorohydrin (24·3 g.) was heated at 120–130° for twenty hours. It was then decomposed with sodium carbonate solution and extracted with ether. The ether extract was dried over sodium sulphate and distilled under reduced pressure. The first fraction distilled at 120–130°/5 mm., followed by second fraction at 167–180°/5 mm. The second fraction was dissolved in dry ether and the solution saturated with dry hydrochloric acid gas. A white crystalline product separated which was collected and crystallized from acetone, m.p. 113°C.

Found: N, 5·4%. $C_{11}H_{15}O_2N.HCl$ requires N, 5·7%.

Attempted synthesis of 9 : 10-thiopeg-10-ene-4-one :—

Ethyl-(N-β-hydroxy ethyl)-anthranilate hydrochloride (5 g.) was thoroughly mixed with potassium sulphocyanide (3 g.) in presence of water (1 c.c.) and the mixture got liquified. After five minutes, it again got solidified and was allowed to stand for 12 hours. Attempts to crystallize the thiourea failed and, therefore, the reaction mixture was extracted with absolute alcohol. The alcoholic extract was refluxed for 24 hours. After 12 hours' refluxing, some precipitate appeared which increased on further heating. The product was collected on cooling and crystallized from acetic

acid (1·2 g.), m.p. 280°. The compound is soluble in alkali; and it gets precipitated on acidification. It appears to be tetrahydro compound VI.

Found: N, 12·01%, S, 14·3%. $C_{10}H_{10}O_2N_2S$ requires N, 12·1%, S, 14·4%.

Acknowledgment

The authors wish to express their thanks to Dr. S. M. Mukherji, University Professor and Head of the Chemistry Department, Panjab University, Hoshiarpur, for his keen interest in the work.

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STUDIES IN SURFACE COMPLEXES OF CHARCOAL

by

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STUDIES IN SURFACE COMPLEXES OF CHARCOAL

By Balwant Rai Puri, Y. P. Myer *and* Lekh Raj Sharma

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It has been known for some time that surface complexes on charcoal and carbon blacks can be removed on heating at high temperatures *in vacuo* (1, 2, 3, 6, 9, 10, 13). Lowry (10), on evacuating a number of charcoals at different temperatures, found that considerable amounts of CO, CO_2 and water vapour were evolved up to 900° and that increasingly larger quantities of hydrogen were given out between 900° and 1,200°. Anderson and Emmett, using a similar technique, extended these observations to a few commercial charcoals (2) and carbon blacks (3).

The presence of oxygen complexes is known to increase base-adsorption capacity of charcoal (4, 7, 15). Puri *et al.* (11, 12) regard 'base adsorption' by charcoal as a neutralizing reaction due to the presence of CO_2 in the complex. This, however, needs confirmation by measuring the amount of CO_2 actually evolved on high temperature evacuation of charcoal and comparing it with the amount of alkali neutralized by it. This point does not appear to have received adequate attention. Besides, a careful estimation of the gases evolved on evacuating different samples of charcoal at increasing temperatures is also likely to be helpful in elucidating the number, nature, composition and stability of the oxygen complexes. The work described in this paper was undertaken with these objectives in view.

Experimental

Materials.—Three different varieties of charcoal prepared by the carbonization of pure sucrose (by means of pure sulphuric acid followed by exhaustive washings with hot distilled water), coconut shell and pine wood (by heating at about 450° in a limited supply of air) were used in these investigations. Sugar charcoal was almost 'ash-less', while the other two samples were rendered ash free by HF-HCl treatment. All the samples were then exposed to an atmosphere of oxygen for about 24 hours at room temperature (11).

Methods

(*a*) *Neutralization of alkali.*—It has been already shown (11) that charcoal can neutralize different alkalies in equivalent proportions. Therefore, only one alkali, namely barium hydroxide, was used in these experiments. The procedure adopted was essentially the same as described before. Briefly 1·0 g. of each sample was mixed with 100 ml. of 0·2 N $Ba(OH)_2$ and the suspension shaken mechanically for about 48 hours.

The amount of the unused alkali was estimated by titrating an aliquot of the clear supernatant liquid against a standard acid solution.

(*b*) *Evacuation at different temperatures.*—About 2 g. charcoal dried at 150° to constant weight was taken for evacuation at each temperature. The apparatus employed was essentially the same as used by Anderson and Emmett (2). There was, however, one difference in the procedure. Whereas Anderson and Emmett heated one and the same lot of a particular sample to increasing temperatures, we took different lots for evacuations at different temperatures. Thus at each temperature at which evacuation was done, charcoal had its complex in full. The temperature of evacuation varied from 200° to 1,200° at intervals of 100°. The evolved gases were analysed in the following sequence: water was removed in calcium chloride tubes and carbon dioxide in a series of Erlenmeyer flasks containing a known amount of barium hydroxide solution. Hydrogen and carbon monoxide in the rest of the gaseous mixture were estimated by exploding with a known excess of oxygen followed by measurement of contraction on cooling and on introduction of potash solution. This technique was standardized by working with mixtures of CO_2, CO and H_2 of known composition. Replicate determinations were also made at some temperatures and fairly concordant results were obtained.

Results and Discussion

The results of high-temperature evacuation experiments are recorded in Table I. It is interesting to note that each sample on evacuation at 1,200° loses an appreciable amount of its weight—about 41 per cent in the case of sugar, 30 per cent in the case of coconut shell and 24 per cent in the case of pine wood charcoal—in the form of CO, CO_2, H_2O and H_2. As regards the neutralization of barium hydroxide, it was noticed that while before evacuation these samples could remove 7·88, 3·92 and 3·85 milli-quivalents of the alkali per g. respectively, they suffered a systematic decrease in this property on evacuating at progressively increasing temperatures and that the first sample at 800° and the other two at 700° lost this property altogether.

Assuming that the interaction between barium hydroxide and charcoal is entirely due to the presence of CO_2 in the complex, the amount of the latter before and after evacuation at different temperatures can be easily calculated. These values are given in Table II. The amount of CO_2 evolved at the various temperatures are also reproduced from Table I in a separate column for each charcoal. It is highly significant that in the case of all the three samples, *the sum of the amounts of CO_2 evolved and retained up to about 800° is almost the same and invariably close to the amount originally contained in the charcoal before evacuation.* A slight though progressive decrease in the amounts of CO_2 evolved at higher temperatures is probably due to its partial conversion into CO by a secondary reaction as mentioned below. These results confirm the view that interaction of charcoal with alkalies is almost entirely due to the presence of

carbon dioxide in the complex and that when the latter is eliminated, the resulting material loses this property altogether.

TABLE I

Gases evolved on evacuation of charcoals at different temperatures in g./100 g.

Temperature, °C.	CO_2	CO	H_2O	H_2	Total combined oxygen	Total hydrogen
			Sugar Charcoal			
200	2·42	Nil	Nil	Nil	1·76	Nil
300	7·82	Nil	0·71	Nil	6·31	0·08
400	10·68	Nil	1·08	Nil	8·72	0·11
500	15·76	1·59	3·39	0·02	15·37	0·39
600	16·50	3·56	4·80	0·04	18·30	0·57
700	17·18	4·93	5·09	0·08	19·83	0·64
800	17·38	10·25	6·31	0·27	24·11	0·97
900	16·50	13·44	7·97	1·28	26·75	2·16
1,000	14·34	16·14	8·23	1·43	26·96	2·35
1,100	14·15	16·28	8·86	1·44	27·42	2·43
1,200	13·37	17·37	8·97	1·48	27·62	2·48
			Coconut Shell Charcoal			
200	Nil	Nil	Nil	Nil	Nil	Nil
300	1·56	Nil	0·06	Nil	1·19	0·006
400	7·17	Nil	0·81	Nil	5·93	0·09
500	8·44	Nil	1·30	Nil	7·29	0·14
600	8·46	0·81	2·90	0·004	9·19	0·32
700	8·15	1·35	3·80	0·06	10·07	0·48
800	7·93	5·43	5·90	0·22	14·10	0·87
900	7·83	9·75	6·80	0·76	17·29	1·50
1,000	7·70	11·82	7·68	1·01	19·19	1·86
1,100	6·93	13·77	7·79	1·36	19·80	2·22
1,200	6·39	14·60	7·80	1·51	19·92	2·38
			Pine Wood Charcoal			
200	Nil	Nil	0·23	Nil	0·20	0·02
300	1·82	Nil	0·72	Nil	1·96	0·08
400	4·34	Nil	1·01	Nil	4·05	0·11
500	6·98	Nil	1·37	Nil	6·30	0·15
600	8·26	Nil	3·17	Nil	8·82	0·35
700	8·44	Nil	3·33	Nil	9·10	0·37
800	8·40	6·87	4·64	0·08	14·15	0·59
900	8·37	7·65	5·02	0·24	14·93	0·79
1,000	7·69	8·79	5·65	0·32	15·85	0·94
1,100	7·45	9·11	5·79	0·77	15·76	1·37
1,200	7·17	9·61	5·87	0·97	15·92	1·60

It is evident from Table I that while the evolution of CO_2 and H_2O starts at 200° or 300° that of CO and H_2 does not commence till a higher temperature (500° in sugar, 600° in coconut shell and 800° in pine wood charcoal) is attained. This shows that there may be more than one type of complex on charcoal and that the composition of the evolved gases depends on the type and the amount of the complex decomposing at a particular temperature.

TABLE II

*Amounts of carbon dioxide evolved and retained * on evacuating charcoals at different temperatures in g./100 g.*

Evacuation temperature, °C.	Sugar charcoal			Coconut shell charcoal			Pine wood charcoal		
	Amount of CO_2 evolved	Amount of CO_2 retained	Total	Amount of CO_2 evolved	Amount of CO_2 retained	Total	Amount of CO_2 evolved	Amount of CO_2 retained	Total
Before evacuation	Nil	17·33	17·33	Nil	8·60	8·60	Nil	8·47	8·47
200	2·42	14·90	17·32	Nil	8·60	8·60	Nil	8·46	8·46
300	7·82	9·58	17·40	1·56	7·02	8·58	1·82	6·64	8·46
400	10·68	6·63	17·31	7·17	1·38	8·61	4·84	4·14	8·48
500	15·76	1·62	17·38	8·44	0·12	8·56	6·98	1·49	8·47
600	16·50	0·89	17·39	8·46	Nil	8·46	8·26	0·24	8·50
700	17·18	0·21	17·39	8·15	Nil	8·15	8·44	Nil	8·44
800	17·38	Nil	17·38	7·93	Nil	7·93	8·40	Nil	8·40
900	16·50	Nil	16·50	7·83	Nil	7·83	8·37	Nil	8·37
1,000	14·34	Nil	14·34	7·70	Nil	7·70	7·69	Nil	7·69
1,100	14·15	Nil	14·15	6·93	Nil	6·93	7·45	Nil	7·45
1,200	13·37	Nil	13·37	6·39	Nil	6·39	7·17	Nil	7·17

* Calculated from the amounts of barium hydroxide neutralized by the charcoals.

The amounts of oxygen evolved as CO, CO_2 and H_2O and of hydrogen evolved as H_2O and H_2 at the various temperatures are also presented in Table I (columns 6 and 7). It appears that while hydrogen continues to be evolved even up to 1,200°, total oxygen tends to acquire a constant value after 1,000°. This shows that combined oxygen on charcoal gets almost completely eliminated on evacuating above 1,000°.

The fact that the amount of CO_2 evolved decreases on evacuating at temperatures above 800° shows the possibility of its reduction by one or both of the following reactions:—

(i) $CO_2 + C \rightarrow 2CO$

(ii) $CO_2 + H_2 \rightarrow CO + H_2O$.

But since at higher temperatures, the relative increase in the amount of CO evolved is greater than that of H_2O and the latter acquires almost a constant value at 1,000°, the probability of the occurrence of the former reaction is greater.

The results presented above indicate that there are probably two types of surface oxygen complexes, one that reacts with alkalies and starts decomposing at about 200° to 300° giving off CO_2 and the other that is more stable and decomposes at higher temperatures with the evolution of CO. This is in conformity with the view put forward by Smith, Pierce and Joel in a recent publication (14).

According to Long and Sykes (8) and Smith *et al.* (14), the possible atoms or groups chemisorbed on charcoal surface which provide for different types of complexes may be (O), (OH), (O_2), (H), (H_2), etc., depending upon the state of surface carbon atoms and the number of residual bonds available for interaction with other molecules. In the light of this, the first oxygen complex may be considered tentatively to have (O_2) fixed per 'active' carbon atom and to decompose as CO_2 on heating. The second complex may be considered to have (OH) fixed per 'active' carbon atom and to decompose on heating, in the first stage to give H_2O involving two (OH) groups attached to neighbouring carbon atoms, and then at a higher temperature the intermediate product may decompose to give CO. This view receives some support from the fact that the amounts of water and carbon monoxide (when corrected for the amount resulting from reaction (i) above) given out on complete evacuation are nearly in equi-molecular proportions to one another as required.

It can be shown that the amount of CO evolved in terms of moles/g. is greater than that of CO_2, even when allowance is made for the secondary reaction mentioned above. This indicates that the second complex covers a larger portion of charcoal surface than the first.

Surface areas of the charcoals, calculated by Harvey's method (5) from the ethyl alcohol isotherms determined at 25°, are given in Table III. Assuming that each surface carbon atom occupies 2·6 $A^{\circ 2}$ (3) the number of carbon atoms on the surface can be easily obtained. The evolved oxygen and hydrogen can also be computed as atoms and the number of oxygen and hydrogen atoms combined per surface carbon atom can be readily obtained. The results of these calculations are included in Table III.

It is seen that the ratios of oxygen and hydrogen to carbon atoms are of the same order in all the three samples of charcoal. This is quite significant as it indicates that the concentration of surface complexes depends to a large extent on the surface area of the charcoal.

TABLE III

Surface area, total oxygen and hydrogen evolved and ratios of oxygen and hydrogen to surface carbon atoms in different charcoals

Nature of charcoal	Surface area (sq. m./g.)	Amount of total combined oxygen removed on evacuation at 1,200° (g./g.)	Amount of total hydrogen removed on evacuation at 1,200° (g./g.)	Ratios of oxygen and hydrogen atoms to carbon atoms	
				$\frac{O}{C}$	$\frac{H}{C}$
Sugar charcoal ..	205·9	0·2762	0·0248	1·32	1·90
Coconut shell charcoal	161·0	0·2001	0·0238	1·22	2·33
Pine wood charcoal..	126·4	0·1592	0·0160	1·24	1·99

The ratio of oxygen to carbon atoms is seen to vary between 1·22 and 1·32 in the various samples. This appears to offer further support to the tentative formulae assigned to the two complexes, for if both the complexes were present in equal amounts, the ratio should have been 1·5, but since the second complex covers a larger portion of the surface than the first, as mentioned above, the ratio should lie between 1 and 1·5 as is actually the case.

The ratio of hydrogen to carbon atoms is seen to be about 2. This shows that an appreciable amount of chemisorbed hydrogen is held in some form other than (OH) as well.

SUMMARY

Evacuations of three different samples of charcoal at temperatures varying from 200° to 1,200°, analyses of the gases evolved and interactions with barium hydroxide before and after degassing at each temperature have been described. The results indicate that 'base adsorption' of charcoal is almost entirely due to the presence of CO_2 in the complex and that there are perhaps two types of oxygen complexes, one which reacts with alkalies and decomposes to give CO_2 and the other which decomposes in the first stage to give H_2O and in the second stage at a higher temperature to give CO.

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May, 1956

STUDIES ON THE DIGENETIC TREMATODES OF MARINE FISHES FROM THE GULF OF MANAAR (INDIA)

by

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STUDIES ON THE DIGENETIC TREMATODES OF MARINE FISHES FROM THE GULF OF MANAAR (INDIA)

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(*From the Department of Parasitology, London School of Hygiene and Tropical Medicine, London*)

Abstract

The digenetic trematodes of some marine fishes from the Gulf of Manaar (India) have been studied. Six new species, *Stenopera pteroisi*, *Hamacreadium leiperi*, *Hamacreadium krusadaiensis*, *Opecoeloides manaarensis*, *Buckleytrema indica* and *Steringophorus lethrini*, have been described. In two already known forms, *Plagioporus serotinus* Stafford, 1904 and *Mehratrema adlfusi* Srivastava, 1939, some interesting features have been given. One new genus, *Buckleytrema*, has been erected.

Introduction

During the month of July, 1954, prior to my departure for the United Kingdom, I went to South India to visit Krusadai Island and Mandapam Camp (Gulf of Manaar, India) for the collection of research material. At these two places marine fishes belonging to 29 genera were dissected for examination of parasitic worms and a wide collection of trematodes, cestodes and nematodes was made. Nematode infection was comparatively scarce. In this paper I have given an account of eight digenetic trematodes belonging to three different families, Allocreadiidae Stossich, 1903, Monodhelminthidae Dollfus, 1937 and Fellodistomatidae Odhner, 1911. Of these eight trematodes six are new to science and two already known. A new genus has been proposed for one of the new species.

The work on this material was carried out in the Department of Parasitology, London School of Hygiene and Tropical Medicine, London, under the supervision and guidance of Professor J. J. C. Buckley, to whom I am greatly indebted. I am also grateful to Professor R. T. Leiper for the loan of some literature and Dr. Ben Dawes and Mr. S. Prudhoe for their keen interest in the progress of my work. My thanks are also due to the Director of Public Instruction, Panjab (India), for granting me study leave (ex-India) and the Vice-Chancellor, Panjab (India) University, for awarding me the research fellowship for carrying on research at this School.

Family: Allocreadiidae Stossich, 1903.

Genus: *Stenopera* Manter, 1933.

The genus *Stenopera* was erected by Manter (1933) for the species *Stenopera equilata* recovered from the intestine of a marine fish, *Holocentrus*

ascensions, at Tortugas, Florida, U.S.A. *S. equilata* Manter, 1933, is the only species so far known to the genus. In having unipolar filamented eggs, the genus *Stenopera* stands close to the genus *Helicometra* Odhner, 1902 nec. Travassos, 1928. On the basis of the shape and great length of the cirrus sac, far forward position of the ventral sucker and the elongated body with sides nearly parallel, Manter in 1933 differentiated the genus *Stenopera* from *Helicometra*. The writer has been able to obtain two digenetic trematodes having eggs with unipolar filaments and very much elongated cirrus sac. These specimens have been assigned to a new species under the genus *Stenopera*.

Generic diagnosis.—Allocreadiidae Stossich, 1903. Body: elongate. Suckers: ventral sucker far forward. Gut: pharynx small; oesophagus moderately long; caeca narrow, extending to posterior end of body. Excretory system: excretory vesicle extending to level of ovary. Reproductive system: genital pore median or submedian, anterior to intestinal bifurcation near the base of pharynx. Cirrus sac slender, curved, very long, extending posteriorly behind ventral sucker. Testes tandem, median and slightly lobed. Ovary lobed, situated anterior to testes. Vitellaria lateral, confluent behind testes, not extending anterior to intestinal fork; yolk reservoir anterior to ovary; receptaculum seminis either in front or dorsal to ovary. Metraterm present, shorter than cirrus sac. Eggs with unipolar filaments.

Type species: *Stenopera equilata* Manter, 1933.
Parasites of marine fishes.

The generic diagnosis has been modified in respect of the position of the receptaculum seminis, which is dorsal in my specimen, and the vitellaria.

Stenopera pteroisi n.sp. (Fig. 1)

Two specimens of *Stenopera pteroisi* n.sp. were found in the marine fish, *Pterois russelii*, dissected at Mandapam Camp. The mounted specimens are 4·2–4·5 mm. long and 0·32–1·25 mm. broad across the region of the testes. The anterior end is more attenuated than the posterior end. The integument is without spines or papillae. At the anterior end of each specimen are seen some fibrillar projections. The oral sucker is subterminal and it measures 0·09–0·22×0·16–0·22 mm. Prepharynx is absent. The oral sucker is directly followed by the muscular pharynx which is 0·06–0·08 mm. long and 0·06–0·11 mm. broad. The oesophagus is 0·24–0·35 mm. long and 0·01–0·03 mm. broad. It forks into two intestinal caeca at a distance of 0·23 mm. from the anterior border of the ventral sucker. The intestinal caeca run parallel to the lateral sides of the body and terminate a little in front of the posterior end of the body. The ventral sucker lies at a distance of 0·69–0·83 mm. from the anterior end of the body. It is almost circular, i.e. 0·17×0·17 or 0·36×0·36 mm. Its aperture is small. The ratio between the diameters of the oral and ventral

suckers is nearly 1 : 1 in one specimen and 1 : 1·5 in the other. The excretory pore is median and dorsally placed at the posterior end of the body. The excretory vesicle is dilated at first and then becomes narrow and uniform in width.

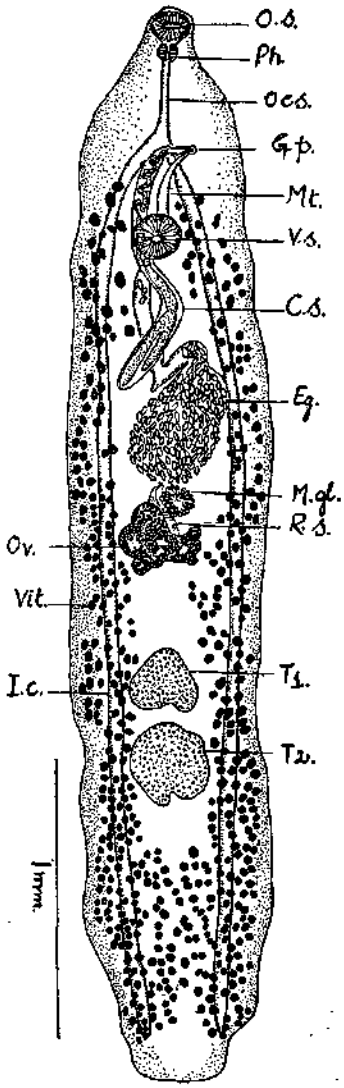

FIG. 1. Ventral view of *Stenopera pteroisi* n.sp.

C.s.—cirrus sac; *Eg.*—egg; *G.p.*—genital pore; *I.c.*—intestinal caecum; *M.gl.*—Mehlis' gland; *Mt.*—metraterm; *O.s.*—oral sucker; *Oes.*—oesophagus; *Ov.*—ovary; *Ph.*—pharynx; *R.s.*—receptaculum seminis; T_1.—anterior testis; T_2.—posterior testis; *V.s.*—ventral sucker; *Vit.*—vitellaria.

The genital pore is to the left of the median line, external to the left intestinal caecum and 0·19 mm. inward from the left body margin. In the second specimen the genital pore is almost median and placed near the point where the bifurcation of intestinal caeca occurs. The cirrus sac is very much elongated and is 0·88–0·92 mm. in length. It extends both in

front and behind the ventral sucker. The seminal vesicle is coiled and fills either one-half or one-third of the cirrus sac. The prostate glands are few in number. The cirrus is very prominent. The vasa efferentia are seen meeting close to the base of the cirrus sac. The testes are irregularly shaped or may be lobed on their posterior aspects only. In the second specimen only one testis is present, the other seems to have atrophied. The anterior testis measures 0·17×0·19 mm. while the posterior measures 0·27–0·28×0·21–0·29 mm.

The ovary is lobed and lies much in front of the anterior testis in the median line. It has three lobes on the left and four on the right. The Mehlis' gland lies in front and close to the ovary. The receptaculum seminis is partly dorsal to the ovary. The uterus is very much coiled but it is confined to the pre-ovarian region. It crosses the obliquely placed basal part of the cirrus sac and then turns to the right and proceeds forward dorsal to the acetabulum and opens at the genital pore through the metraterm which is a straight tube. The vitellaria extend from the level of the region between the intestinal fork and the ventral sucker back to the ends of the intestinal caeca or the posterior end of the body. In the post-testicular region they coalesce in the median line. The eggs are provided with unipolar non-motile filaments and are 0·038–0·041 mm. long and 0·019–0·026 mm. broad.

Relationships:—Stenopera pteroisi n.sp. differs from *S. equilata* Manter, 1933, in having a longer oesophagus, vitellaria extending from the posterior extremity of the body to the level of or a little in front of the ventral sucker; the ovary situated at an appreciable distance from the anterior testis, with seven lobes, three on the left and four on the right; and testes lobed especially on the posterior aspects. In *S. equilata*, the oesophagus is short, the ventral sucker is quite close to the intestinal fork, the vitellaria terminate a little behind the ventral sucker, the ovary is slightly lobed and is placed not far from the anterior testis and the testes are lobed almost on all sides.

The new species is larger than *S. equilata* and has smaller eggs. It has been named after the genus of its host.

Host: *Pterois russelii* (Bennett).
Location: Intestine.
Locality: Gulf of Manaar (India).

Genus: *Plagioporus* Stafford, 1904
Plagioporus serotinus Stafford, 1904 (Fig. 2)

Only one specimen of *Plagioporus serotinus* Stafford, 1904, was found in the intestine of a marine cat-fish caught from the Gulf of Manaar. In this specimen the author finds that the testes are diagonally placed instead of tandem, the ovary is smaller than the testes and lies in front of the posterior testis, the vitellaria are confluent in the post-testicular region and the eggs measure 0·068–0·084×0·038–0·045 mm. There were fifteen eggs seen in the uterus.

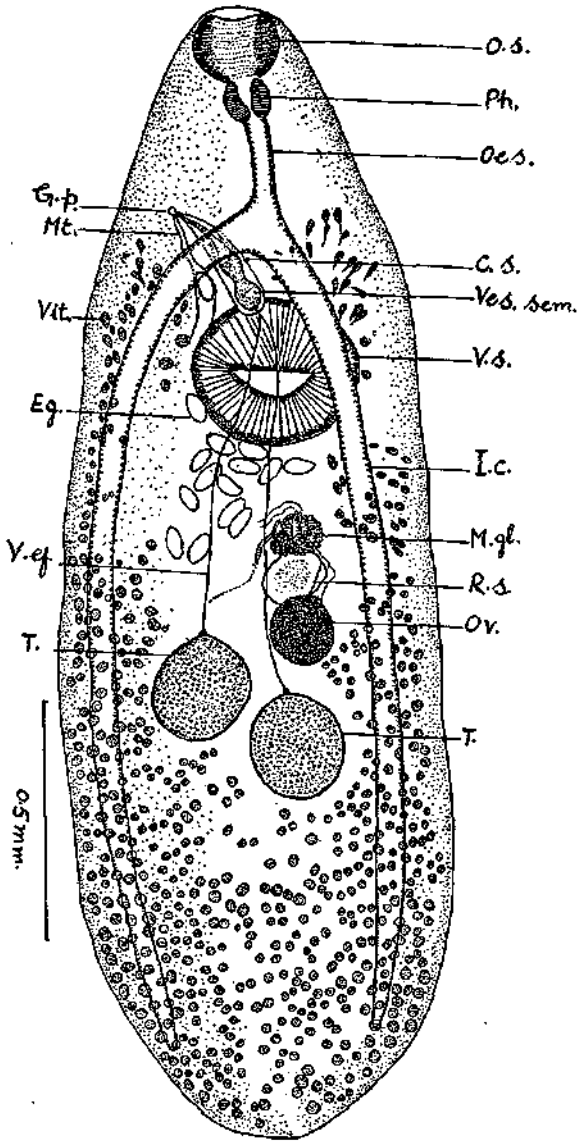

FIG. 2. Dorsal view of *Plagioporus serotinus* Stafford, 1904.

C.s.—cirrus sac; *Eg.*—egg; *G.p.*—genital pore; *I.c.*—intestinal caecum; *M.gl.*—Mehlis' gland; *Mt.*—metraterm; *O.s.*—oral sucker; *Oes.*—oesophagus; *Ov.*—ovary; *Ph.*—pharynx; *R.s.*—receptaculum seminis; *T.*—testis; *V.ef.*—vas efferens; *V.s.*—ventral sucker; *Ves. sem.*—vesicula seminalis; *Vit.*—vitellaria.

Genus: *Hamacreadium* Linton, 1910
Syn.: *Emmettrema* Caballero, 1946

The genus *Hamacreadium* was proposed by Linton in 1910 for four new species, viz. *Hamacreadium mutabile, H. gulella, H. consuetum* and *H. oscitans. H. mutabile* was recovered from the intestines of different marine fishes, namely *Neomaenis griseus, N. apodus, Anisotremus virginicus, Ocyurus chrysurus* and *Pomacanthus arcuatus.* The species *H. gulella*

was found together with *H. mutabile* in the intestine of *Neomaenis griseus*, *H. consuetum* from the digestive tracts of *Haemulon plumieri* and *Haemulon sciurus*, and *H. oscitans* from the same hosts as of *H. consuetum*.

Yamaguti (1934) added two new species to the genus, namely *H. lethrini* from the stomach and intestine of *Lethrinus haematopterus* and *H. epinepheli* from the intestines of *Epinephelus akaara* and *Lethrinus haematopterus*. The hosts were obtained from the Inland Sea and Pacific coast, Japan. Nagaty in 1941 recorded two new species, *H. mehsena* and *H. interruptus*, the former from *Lethrinus mehsena* and the latter from *Lethrinus mehsenoides* of Red Sea. He also reported the occurrence of *H. mutabile* in five new fish hosts. He regarded *H. epinepheli* Yamaguti, 1934, as a synonym of *H. mutabile*. Baz (1946) found a new species, *H. morgani*, from the intestine of *Pagrus vulgaris* caught in the Mediterranean Sea. He considered the interruption of vitellaria as noticed by Nagaty (1941) in his new species as an unstable character. This inconstant feature as he further said might be the result of contraction and relaxation of one side of the parasite more than the other or the result of pressure exerted on it during the process of spreading and fixation.

Caballero in 1946 described a new species, *Emmettrema lariosi*, under the new genus *Emmettrema* from the large intestine of the host 'Mero' (an *unidentified fish) obtained from the Pacific Ocean from Mexico. (This* species has been transferred to the genus *Hamacreadium* Linton, 1910.) Yamaguti (1953) included also in the genus *Hamacreadium* the species *Distomum palleniscum* Shipley and Hornell, 1905, parasitic in *Balistes* sp. He made the genus *Emmettrema* a synonym of *Hamacreadium*.

Hamacreadium leiperi n.sp. (Fig. 3)

Two specimens of *Hamacreadium leiperi* were found in the intestine of a marine cat-fish dissected at Krusadai Island. The live worm is of whitish appearance. The body is flat and elongated and measures 3·5–5·5 mm. in length and 1·48–1·58 mm. in maximum breadth, which occurs across the testicular region. The cuticle is smooth without any spines or armature. The oral sucker is somewhat spherical, subterminal and measures 0·28×0·3–0·33 mm. The prepharynx is quite distinct and is crossed by the commissure of the brain ganglia. The pharynx, 0·16–0·19 mm. long and 0·17–0·22 mm. broad, is a muscular organ and lies behind the prepharynx. The oesophagus is moderately long having a width of 0·08–0·09 mm. and length of 0·2–0·33 mm. The two intestinal caeca run parallel to each other along the lateral sides towards the posterior end of the body where they terminate at about 0·14–0·16 mm. in front of it. The intestinal fork is 0·62–0·72 mm. in front of the ventral sucker. The ventral sucker is larger than the oral sucker and lies in the posterior region of the first half of the body. It measures 0·28–0·54 mm. in longitudinal axis and 0·48–0·54 mm. transversely.

The testes are spherical or ovoid with smooth or slightly indented margins and are situated obliquely in the middle of the post-acetabular region. The anterior testis is to the left of the median line, close to the

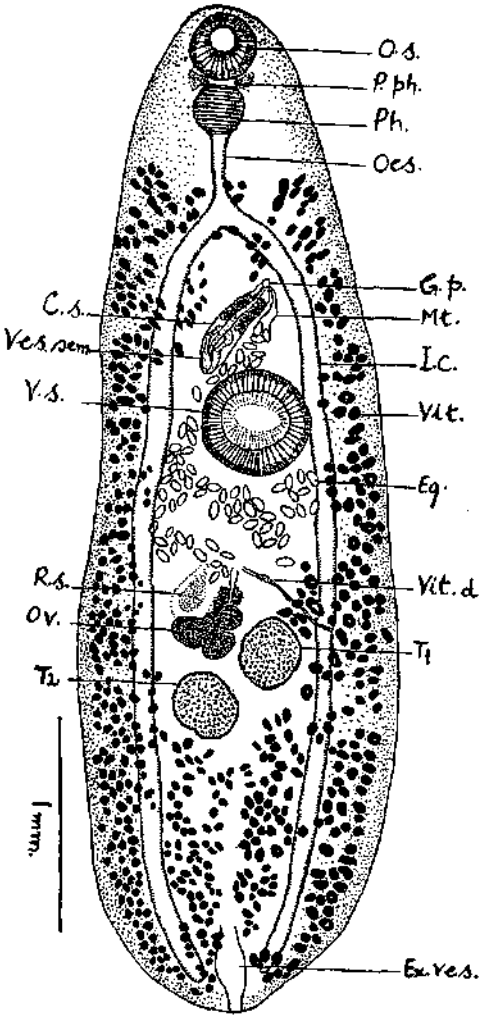

FIG. 3. Ventral view of *Hamacreadium leiperi* n.sp.

C.s.—cirrus sac; *Eg.*—egg; *Ex.ves.*—excretory vesicle; *G.p.*—genital pore; *I.c.*—intestinal caecum; *Mt.*—metraterm; *O.s.*—oral sucker; *Oes.*—oesophagus; *Ov.*—ovary; *P.ph.*—prepharynx; *Ph.*—pharynx; *R.s.*—receptaculum seminis; T_1.—anterior testis; T_2.—posterior testis; *V.s.*—ventral sucker; *Ves.sem.*—vesicula seminalis; *Vit.*—vitellaria; *Vit.d.*—vitelline duct.

left intestinal caecum and measures 0·29–0·51 × 0·24–0·29 mm. The posterior testis is 0·33 × 0·25–0·29 mm. and is adjacent to the right intestinal caecum. From anterior aspect of each testis, the vasa efferentia run forward and meet at the base of the cirrus pouch to form the vas deferens. The cirrus pouch is well developed and lies obliquely in front of the ventral sucker. It measures 0·48–0·57 mm. in length and 0·14–0·16 mm. in maximum breadth. It encloses the coiled seminal vesicle, pars prostatica and the ejaculatory duct. The genital pore lies to the left of the median line much behind the intestinal fork and opens a little inward to the intestinal caecum.

The ovary is trilobed. It measures 0·2×0·3 mm. and is situated in front of the posterior testis and to the right of the anterior testis. All the three organs, namely two testes and the ovary, are arranged in a triangle. The Mehlis' gland complex is just in front of and adjacent to the left lobe of the ovary. The receptaculum seminis is to the right of the Mehlis' gland complex and anterior to the ovary. The vitellaria consist of small follicles which extend from the posterior end of the body to the level of the oesophagus and laterally external to the intestinal caeca. In the post-testicular region the vitellaria of the two sides coalesce medially and are densely congregated. In the pre-acetabular zone a few of the vitellaria invade the intercaecal zone. The uterus is confined to the pre-testicular region. While ascending, it first runs along the left intestinal caecum, then it takes a turn to the right, running along the intestinal caecum. It again bends to the left to run along the cirrus pouch and open at the genital pore through the metraterm. The eggs measure 0·074–0·076×0·041–0·053 mm.

The excretory vesicle is I-shaped and its opening lies in the middle of the posterior end of the body.

Relationships:—In having a trilobed ovary, the new species *Hamacreadium leiperi* differs from *H. mutabile* Linton, 1910 and *H. lariosi* (Caballero, 1946) in which the ovary is much lobed; from *H. gulella* Linton, 1910, *H. consuetum* Linton, 1910, *H. oscitans* Linton, 1910, *H. mehsena* Nagaty, 1941, *H. interruptus* Nagaty, 1941 and *H. morgani* Baz, 1946, in which the ovary is either tetra- or penta- or hexa-lobed or tetra- to penta-lobed or penta- to hexa-lobed, and from *H. lethrini* Yamaguti, 1934 and *H. epinepheli* Yamaguti, 1934, which have irregularly lobed ovaries.

The new species further deviates from *H. gulella*, *H. consuetum* and *H. epinepheli* in which the genital pore is median in position; from *H. mehsena*, *H. oscitans* and *H. lariosi* in which the genital pore lies at the intestinal bifurcation; from *H. lethrini* and *H. morgani* in which the genital pore is ventral to the left intestinal caecum; and from *H. interruptus* which has its genital pore external to the left intestinal caecum. In *H. lethrini* the vitellaria stop short of the intestinal fork, the cirrus pouch extends behind up to the middle of the ventral sucker, in *H. mehsena* and *H. oscitans* the vitellaria extend up to the region of the ventral sucker; therefore, in these respects too, *H. leiperi* n.sp. stands apart from them. In the position of the genital pore, the new species resembles *H. palleniscum* (Shipley and Hornell, 1905) but it is also different from it in the extent of the vitellaria and the cirrus sac and in the shape of the ovary.

The new species has been named after Professor R. T. Leiper.

Host: Marine cat-fish.
Location: Intestine.
Locality: Gulf of Manaar (India).

Hamacreadium krusadaiensis n.sp. (Fig. 4)

One specimen only of *Hamacreadium krusadaiensis* n.sp. was found in the intestine of a marine cat-fish collected from the Gulf of Manaar. It is a

small worm of 1·19 mm. in length and 0·52 mm. in breadth across the region of the testes. The cuticle is non-spinous. The oral sucker, 0·1× 0·12 mm. in size, is subterminally placed. The prepharynx is apparently absent. The muscular pharynx is spherical and measures 0·06×0·064 mm. The oesophagus is 0·034 mm. long and 0·019 mm. broad. The intestinal caeca are densely covered with vitellaria and so their extent in the hinder region is obscured.

The ventral sucker is strongly developed and it measures 0·19×0·27 mm. It is more than double the size of the oral sucker.

The two testes, very close to each other, are somewhat obliquely placed one behind the other in the anterior part of the second half of the body. The anterior testis is 0·1×0·14 mm. in size, while the posterior testis is 0·16×0·17 mm. The cirrus sac is an elongated structure, posteriorly it extends up to the anterior one-third of the ventral sucker. The vesicula seminalis is coiled. The prostate glands are scanty. The

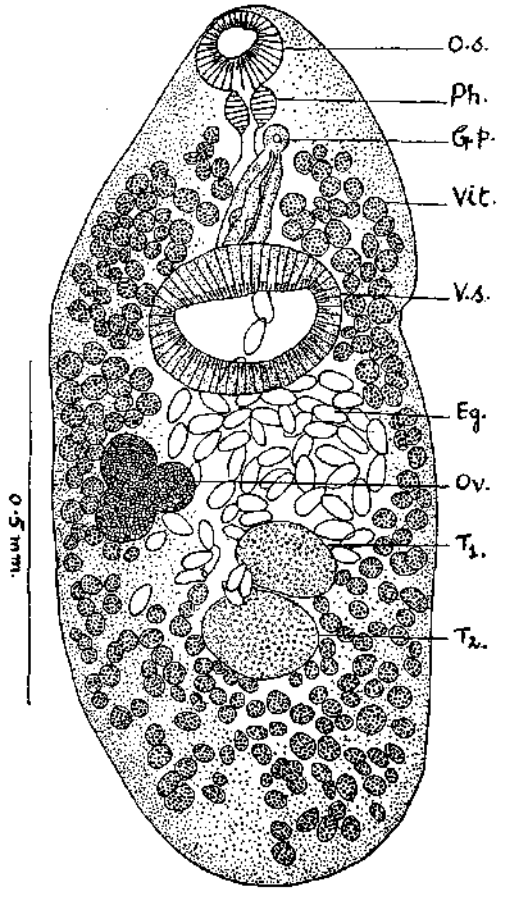

FIG. 4. Ventral view of *Hamacreadium krusadaiensis* n.sp.

Eg.—egg; *G.p.*—genital pore; *O.s.*—oral sucker; *Ov.*—ovary; *Ph.*—pharynx; T_1.—anterior testis; T_2.—posterior testis; *V.s.*—ventral sucker; *Vit.*—vitellaria.

genital pore lies to the left of the median line, close to the pharynx and in front of the intestinal fork.

The ovary is trilobed, the lobes are extended like the petals of a flower. Of the lobes, one is directed to the left and the other two to the right. It is situated to the right of the median line and ventral to the right intestinal caecum. It measures 0·16×0·12 mm. The Mehlis' gland complex and the receptaculum seminis are hidden by the vitellaria. The uterus extends posteriorly up to the second testis, while anteriorly it runs dorsal to the ventral sucker and through metratum it opens at the genital pore. The vitelline follicles extend laterally from the level of the base of the pharynx up to the posterior extremity of the body. Both in the post-acetabular and pre-acetabular regions the vitellaria of both sides become confluent.

The eggs measure 0·053–0·06×0·026–0·038 mm.

Relationships:—The new species *Hamacreadium krusadaiensis* is characterised by having a trilobed ovary. Therefore in this character it differs from the following species, *H. mutabile* Linton, 1910, *H. gulella* Linton, 1910, *H. consuetum* Linton, 1910, *H. oscitans* Linton, 1910, *H. epinepheli* Yamaguti, 1934, *H. lethrini* Yamaguti, 1934, *H. mehsena* Nagaty, 1941, *H. interruptus* Nagaty, 1941, *H. morgani* Baz, 1946 and *H. lariosi* (Caballero, 1946). In this character it resembles *H. palleniscum* (Shipley and Hornell, 1905) and *H. leiperi* n.sp. It, however, stands apart from all of them in the position of the genital pore which in the new species, *H. krusadaiensis*, lies to the left of the median line, in front of the intestinal fork and close to the pharynx. It further deviates from *H. leiperi* in the ratio between the diameters of the oral and ventral suckers, size of the body and the eggs.

The new species has been named after the place from the vicinity of which its host was caught and dissected.

Host: Marine cat-fish.
Location: Intestine.
Locality: Gulf of Manaar (India).

Genus: *Opecoeloides* Odhner, 1928
Syn.: *Cymbephallus* Linton, 1934

The genus *Opecoeloides* was proposed by Odhner in 1928 for *Distomum furcatum* Bremser, 1819 (in Rudolphi, 1819). He differentiated this genus from *Opecoelus* Ozaki, 1925, in the presence of an accessory sucker between the acetabulum and the genital pore, in the absence of a cirrus sac and the caeca entering the excretory vesicle instead of opening to the exterior. In the genera *Anisoporus* Ozaki, 1928, and *Genitocotyle* Park, 1937, the accessory sucker is also met with but in the former genus the intestinal caeca open through a single anus and not into the excretory vesicle. In the latter genus the acetabular papillae are absent. *Pseudopecoeloides* Yamaguti, 1940, has a pedunculated acetabulum and a uroproct but lacks both the accessory sucker and the acetabular papillae.

Von Wicklen (1946) described a new form, *Opecoeloides polynemi*, from the intestine of *Polynemus octonemus* Girard, a thread fin fish, obtained

from the Gulf of Mexico, Galveston, Texas. In the same paper he synonymised the genus *Cymbephallus* Linton, 1934, with the genus *Opecoeloides* Odhner, 1928. The type species of the genus *Cymbephallus*, i.e. *C. vitellosus* (Linton, 1900) Linton, 1934, was also transferred to the genus *Opecoeloides*. As this specimen was restudied by him, he made clear that it has a pre-acetabular accessory sucker without a limiting membrane, the acetabulum when extended would be pedicellate, with three papillae on its anterior lip and two on the posterior lip. The anus is absent. He also considered *O. manteri* (Hunninen and Cable, 1940) Hunninen and Cable, 1941, as a synonym of *O. vitellosus* (Linton, 1900) Von Wicklen, 1946, on the basis of common characters found in them, and *Anisoporus eucinostomi* Manter, 1940, should be *O. eucinostomi* (Manter, 1940) Von Wicklen, 1946.

Read (1947) gave the diagnosis of a new species, *Opecoeloides polyfimbriatus*, from the intestine of *Synodus foetens* obtained from the Gulf of Mexico. He pointed out that the accessory sucker in the new species lies on the right side of the body whereas in other members of the genus it is on the left. Manter (1947) in America added two new species, *O. brachyteleus* and *O. elongatus*, to the genus, the former species from the intestine of *Mulloidichthys martinicus* (Cuv. and Val.), a yellow goat fish, and the latter from the intestine of *Pseudupeneus maculatus* (Bloch), a red goat fish. He also suggested the transference of *Anisoporus thrinopsi*, which was described by him earlier in 1940 from the intestine of *Thrinops pachylepsis* (Gunther), to the genus *Opecoeloides*.

Opecoeloides manaarensis n.sp. (Figs. 5 and 6)

Opecoeloides manaarensis was found in the intestine of a marine fish, *Upeneoides* (Tamil name—Nagrai). It is a thin elongated worm, 3·45 mm. in length and 0·25 mm. in maximum breadth, which is across the level of the anterior testis. The body cuticle is smooth. The oral sucker, 0·072 × 0·072 mm., is placed at the anterior end of the body. Its opening is, however, directed subterminally. It is about half the size of the acetabulum. The prepharynx is absent apparently. The pharynx measures 0·057 × 0·068 mm., and is followed by an oesophagus, 0·13 mm. in length. The two intestinal caeca run along the sides of the body and in the post-testicular region they are densely covered with vitellaria.

The acetabulum is situated on a short pedicle and measures 0·14 × 0·15 mm. It is larger than the oral sucker and is provided with nine well-marked papillae, of which four are placed anteriorly and the remaining five posteriorly. The anterior papillae are covered by a thin membranous fold. A similar fold extends from the body along the posterior border of the acetabulum but it only covers two of the papillae. All these papillae can be seen under different foci. Between the genital pore and the acetabulum there lies a very small and indistinct accessory sucker.

There are two irregularly shaped testes placed one behind the other in the anterior region of the second half of the body. The anterior testis is 0·19 × 0·18 mm., while the posterior is 0·26 × 0·19 mm. in size. The vesicula

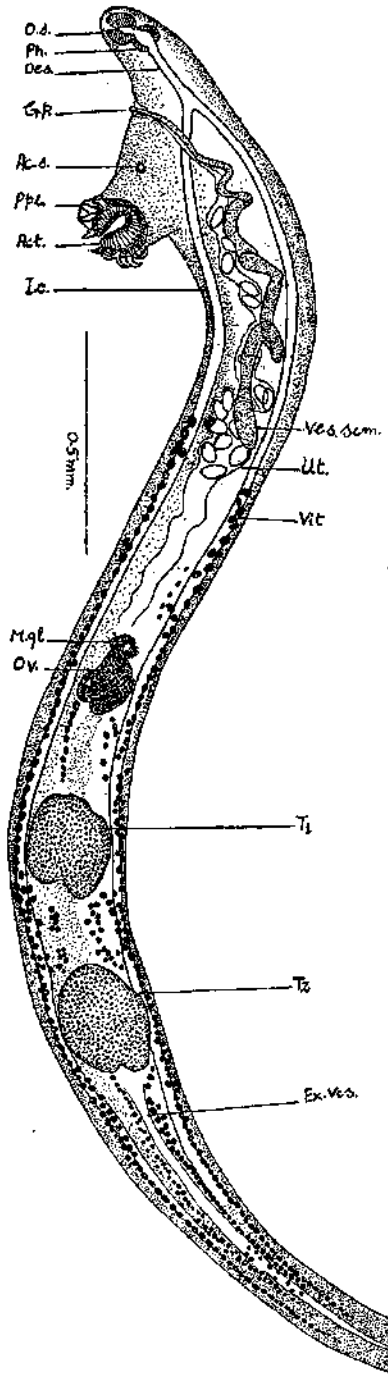

Fig. 5. Ventro-lateral view of *Opecoeloides manaarensis* n.sp.

Ac.s.—accessory sucker; *Act.*—acetabulum; *Ex.ves.*—excretory vesicle; *G.p.*—genital pore; *I.c.*—intestinal caecum; *M.gl.*—Mehlis' gland; *O.s.*—oral sucker; *Oes.*—oesophagus; *Ov.*—ovary; *Ph.*—pharynx; *Ppl.*—papillae; T_1.—anterior testis; T_2.—posterior testis; *Ut.*—uterus; *Ves.sem.*—vesicula seminalis; *Vit.*—vitellaria.

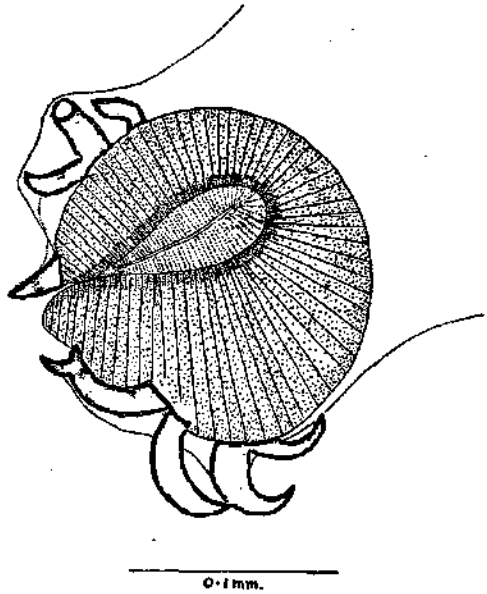

FIG. 6. Acetabulum of *Opecoeloides manaarensis* n.sp. showing papillae.

seminalis is sinuous and extends for about half or even more than half the distance between the acetabulum and the ovary. It is followed by the pars prostatica and the ejaculatory duct. The cirrus is protrusible and opens along the metraterm at the genital opening which is situated on the right lateral body margin at about the middle of the pharynx and the acetabulum.

The ovary is somewhat triangular in shape and lies in front of the testes, in the hinder region of the first half of the body. It measures 0·14 mm. in length and 0·11 mm. in maximum breadth. The Mehlis' gland complex and the yolk reservoir lie in front of the ovary. The vitellaria consist of fine follicles which extend along the lateral sides of the body from the level of the base of the vesicula seminalis back to the posterior extremity of the body. In the post-testicular region, the vitelline follicles are seen extending inwards filling the space between the body wall and the excretory vesicle. The uterus has an ascending limb only, which is both pre-ovarian and intercaecal in position. The metraterm runs alongside the cirrus to open at the genital pore. The eggs are operculate. A few of them seem to have collapsed. They measure 0·049–0·053 × 0·034–0·041 mm.

Relationships:—The genus *Opecoeloides* Odhner, 1928, comprises the species, namely *Opecoeloides furcatus* (Bremser, 1819), *O. vitellosus* (Linton, 1900) Von Wicklen, 1946, *O. eucinostomi* (Manter, 1940) Von Wicklen, 1946, *O. polynemi* Von Wicklen, 1946, *O. polyfimbriatus* Read, 1947, *O. brachyteleus* Manter, 1947, *O. elongatus* Manter, 1947 and *O. thyrinopsi* (Manter, 1940) Manter, 1947. The new species *Opecoeloides manaarensis* is characterised by having nine papillae on its acetabulum, of which four are placed anteriorly and five posteriorly; and by a somewhat triangular ovary. It, therefore, stands apart from all the species already known

except *Opecoeloides vitellosus* with which it resembles closely. Linton (1900) while describing *D. vitellosum* did not make mention of the papillae on the acetabulum. A year later, the same author emended his previous description and stated that the acetabulum of that parasite is notched at its posterior edge so as to form two or three blunt digitate lobes. Four years afterwards he further supplemented the description by describing the genital sucker in one specimen. In 1934, he again modified the earlier description of the same species by stating that the acetabulum is pedicellate and possesses four or five lobes on its posterior aspect and about four on the anterior. In 1940, he reiterated the same account but in the diagrams (Nos. 18 and 19) given on Plate 2, he has shown the presence of three anterior and two posterior papillae.

Von Wicklen (1946) studied the material of this species obtained from the United States National Museum Helm. collection (No. 70760, old number 6351) and noted the following features:

(i) The accessory sucker is quite distinct but without any limiting membrane and has no connection with the genital ducts.

(ii) The acetabulum bears three papillae on its anterior lip and two on the posterior lip.

(iii) No anus could be seen and the ends of the caeca are obscured by the vitellaria.

It appears that No. (ii) character does not tally with the sketch given by Linton (1940). So it is confirmed that the acetabulum of *O. vitellosus* has five papillae only. Thus the new species described in this paper is quite distinct in number of acetabular papillae in addition to the shape of the ovary and measurements of the organs.

Host: *Upeneoides* sp. (Nagrai).
Location: Intestine.
Locality: Gulf of Manaar (India).

Family: Monodhelminthidae Dollfus, 1937
Genus: *Buckleytrema* gen. nov.

Dollfus in 1937 erected a new genus *Monodhelmis* for the new species, *M. torpedinis*, parasitic in the gut of a *Narcacion torpedo*. For the reception of this genus, he suggested the family Monodhelminthidae. Srivastava (1939) described a new species *Mehratrema dollfusi* from the small intestine of *Scatophagus argus* obtained from the Bay of Bengal and Arabian Sea and assigned it to the new genus *Mehratrema* under the family Monodhelmidae (Dollfus, 1937) instead of Monodhelminthidae. He differentiated the genus *Mehratrema* from *Monodhelmis* by the presence of a well developed cirrus sac, a peculiar metraterm, genital sucker and the anterior extent of the vitellaria. Chauhan (1943) gave an account of a new species *Mehratrema polynemusinis* from the intestines of the marine fishes, *Polynemus indicus*, *Muraenesox talabonoides* and *Sciaena* sp. at Bombay.

Yamaguti (1952) reported the occurrence of *Monodhelmis arii* n.sp. in the small intestine of *Arius* sp. A year after, the same author included

two more genera in the family Monodhelminthidae, i.e. *Prosogonarium* Yamaguti, 1952 and *Tandanicola* Johnston, 1927.

So far, the family Monodhelminthidae comprises four genera, namely *Mehratrema, Prosogonarium, Monodhelmis* and *Tandanicola.* In the new species, *Buckleytrema indica,* the testes are obliquely situated one behind the other and they are post-acetabular or the posterior testis alone is post-acetabular while the anterior testis is partly or completely superimposed by the acetabulum; a cirrus sac is present; the vitellaria do not extend behind the testes; the ovary is pre-acetabular; the intestinal caeca do not reach the posterior end of the body. The species with these characters cannot be accommodated in any of the known genera of the family Monodhelminthidae. Since the acetabulum lies between the ovary and the testes, therefore the diagnostic characters of the family have also been emended.

Family diagnosis (emended).

Body: rather plump. Integument: spinous or non-spinous. Suckers: oral sucker well developed; acetabulum simple or complex in structure apart from anterior extremity. Gut: prepharynx and pharynx present; oesophagus distinct; intestinal caeca not reaching the posterior extremity. Reproductive systems: Male—testes two intercaecal, pre- or post-acetabular, obliquely or side by side placed; cirrus pouch present or absent; vesicula seminalis free or enclosed in cirrus pouch; pars prostatica distinct; no cirrus proper; genital atrium median, pre-ovarian with accessory organ directed posteriorly. Female—ovary pre-acetabular and pretesticular or may be partly intertesticular; receptaculum seminis and Laurer's canal present; vitellaria in two lateral groups of follicles; uterus in post-acetabular median field or more extensive. Excretory system: excretory vesicle U- or V-shaped.

Parasites of fishes.

Type genus : *Monodhelmis* Dollfus, 1937.

Generic diagnosis.—Monodhelminthidae Dollfus, 1937 emend. Body: elongate. Integument: Smooth. Suckers: oral sucker distinctly larger than acetabulum which is partially or completely pretesticular. Gut: prepharynx, pharynx and oesophagus present; intestinal caeca terminate blindly in the second half of body but far in front of the posterior extremity. Reproductive systems: Male—testes two, compact, obliquely situated one behind the other, post-acetabular or posterior testis alone post-acetabular and the anterior testis being partially or completely overlapped by the acetabulum; cirrus sac elongated, tubular, encloses the seminal vesicle and pars prostatica. Female—ovary pre-acetabular and pretesticular; vitellaria follicular, lateral, extra—or intercaecal, extending from the level of ovary to testes; uterus post-testicular. Eggs numerous. Accessory or the copulatory organ present. Genital atrium surrounded by a genital sucker-like structure continuous behind into an accessory organ. Excretory bladder U-shaped with long cornua.

Type species : *Buckleytrema indica* n.sp.

Buckleytrema indica n.sp. (Fig. 7)

A few specimens of *Buckleytrema indica* n.sp. were recovered from a marine cat-fish at Krusadai Island. The live worm is of whitish appearance and can be easily detected in the intestinal contents. Some of the specimens were flattened under slight pressure for toto preparations and others after narcotising with a weak solution of alcohol were fixed in Bouin's. Those flattened were also fixed in Bouin's Picro-Formol or hot 70% alcohol. The flattened specimens were stained with carmine stain (Gower's modified method) and sections with Ehrlich's acid haematoxylin and eosin. These trematodes, however, do not pick up stain so readily as other trematodes.

The fluke is elongated and it measures 2·69–4·94 mm. in length and 0·53–1·06 mm. in maximum breadth which occurs at the region of the testes. The body cuticle is smooth. The acetabulum is much smaller than the oral sucker and it lies in between the ovary and the testes. In some specimens it may be overlapped by the latter organ. In this unique parasite, the ovary is pre-acetabular and the testes post-acetabular. The acetabulum measures 0·08–0·13×0·08–0·12 mm., lies behind the ovary and close to the Mehlis' gland complex. It is longer than broad in most of the specimens.

The muscular oral sucker is comparatively much larger than the acetabulum and lies subterminally at the anterior end of the body. It is 0·28–0·4 mm. long and 0·28–0·46 mm. broad. The oral aperture is somewhat oval and is also directed subterminally. The prepharynx measures 0·032–0·048×0·08 mm. The pharynx, 0·08–0·14×0·08–0·14 mm. in size, lies behind the prepharynx. The oesophagus, with a width of 0·064–0·08 mm., extends for 0·17–0·48 mm. It bifurcates into two intestinal caeca which run along the lateral margins of the body and terminate in the second half of it but far in front of its posterior end.

The two somewhat spherical testes lie obliquely one behind the other in the intercaecal zone and behind the acetabulum. Sometimes the anterior testis may be partially covered over by the acetabulum. The anterior testis measures 0·096–0·28×0·096–0·24 mm. while the posterior testis is 0·096–0·28×0·096–0·22 mm. From the anterior aspect of each testis arises a vas efferens, the vasa efferentia meeting to form the vas deferens before penetrating into the cirrus pouch.

The vesicula seminalis is dumb-bell-shaped, its proximal portion completely filling the basal part of the cirrus pouch while the distal part can be made out from the wall of the cirrus pouch. The pars prostatica is sufficiently long and is surrounded by the prostate gland cells. The anterior part of this duct gradually narrows down to open into the genital sinus which is 0·064–0·076 mm. long and 0·064–0·079 mm. wide. It is surrounded by concentric layers of thick muscle fibres and is continued behind into an accessory organ or the copulatory organ. It is a muscular organ of 0·14–0·33×0·16–0·19 mm. in size and contains a prominent muscular papilla. The base of the accessory organ is surrounded by a cluster of gland cells.

The ovary, 0·08–0·17×0·08–0·14 mm., lies in the median line in front

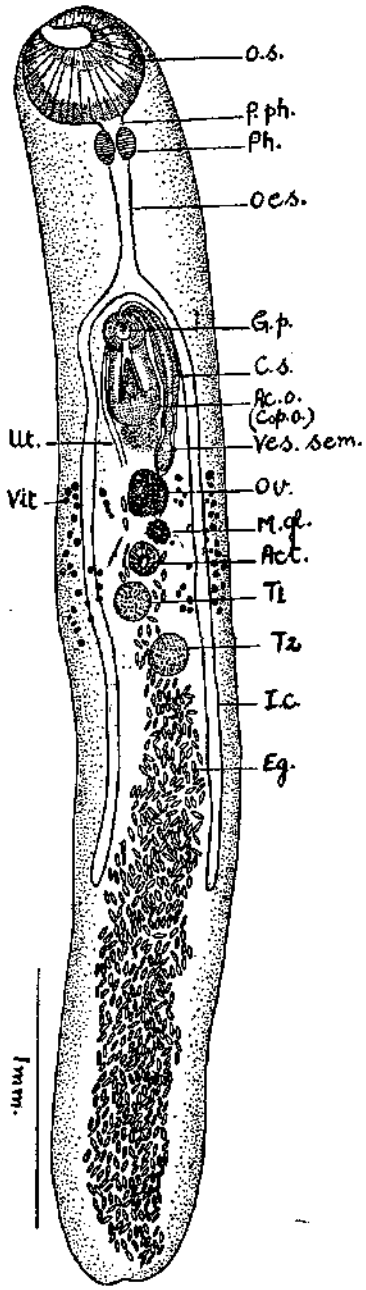

FIG. 7. Ventral view of *Buckleytrema indica* n.sp.

Ac.o.—accessory organ; *Act.*—acetabulum; *C.s.*—cirrus sac; *Cop.o.*—copulatory organ; *Eg.*—egg; *G.p.*—genital pore; *I.c.*—intestinal caecum; *M.gl.*—Mehlis' gland; *O.s.*—oral sucker; *Oes.*—oesophagus; *Ov.*—ovary; *P.ph.*—prepharynx; *Ph.*—pharynx; T_1.—anterior testis; T_2.—posterior testis; *Ut.*—uterus; *Ves.sem.*—vesicula seminalis; *Vit.*—vitellaria.

of the acetabulum. The Mehlis' gland complex is placed posterior to the ovary. The receptaculum seminis is present. The uterus forms descending and ascending limbs, the boundaries of which could not be made out on account of the numerous eggs present in them. In front of the ovary, the uterus runs forward along the accessory organ to open into the genital sinus. The vitellaria are scanty and they are both extra- and intercaecal. They extend from the level of anterior border of the ovary back to the posterior testis but not passing behind it.

The eggs are operculate and measure 0·038–0·057 × 0·019–0·026 mm.

Host: Marine cat-fish.

Location: Intestine.

Locality: Gulf of Manaar (India).

Genus: *Mehratrema* Srivastava, 1939

Mehratrema dollfusi Srivastava, 1939 (Fig. 8)

A few specimens of *Mehratrema dollfusi* were recovered from the intestine of a marine cat-fish dissected at Krusadai Island. These specimens differ from the original description of the parasite in the following features:—

(1) Oral sucker is slightly larger than the acetabulum. Oral sucker, 0·22–0·32 × 0·25–0·33 mm. Acetabulum 0·22 × 0·19–0·2 mm.

(2) Ovary is 0·11–0·4 × 0·14–0·19 mm. in size and trilobed.

(3) Eggs measure 0·045–0·06 × 0·19–0·22 mm.

(4) An internal valve-like structure at the junction of the oesophagus and the intestinal caeca has been noticed.

(5) Copulatory organ is distinct.

Family: Fellodistomatidae Odhner, 1911 emend. Nicoll, 1935

Subfamily: Fellodistomatinae Nicoll, 1909

Genus: *Steringophorus* Odhner, 1905

The genus *Steringophorus* was erected by Odhner, 1905, for the species *Distoma furcigerum* Olsson, 1868, which he also described in detail. Nicoll (1909) gave the account of a new species *Steringophorus cluthensis* which he found in the duodenum and caeca of *Pleuronectes microcephalus* (Lemon dale) from the Firth of Clyde. Odhner (1911) raised the genus *Steringophorus* to the ranks of subfamily Steringophorinae and family Steringophoridae. After having received the interpretation on the issue from C. H. W. Stiles, Stunkard and Nigrelli (1930) pointed out that Nicoll (1909) had already erected the subfamily Fellodistominae for the genera *Fellodistomum* Stafford, 1904 and *Steringophorus* Odhner, 1905, and since the groups are co-extensive, the proposing of the subfamily Steringophorinae was therefore a deliberate renaming of a previously validly named subfamily.

Manter (1934) added two new species to the genus, *Steringophorus magnus* and *Steringophorus profundus*, the former being obtained from the

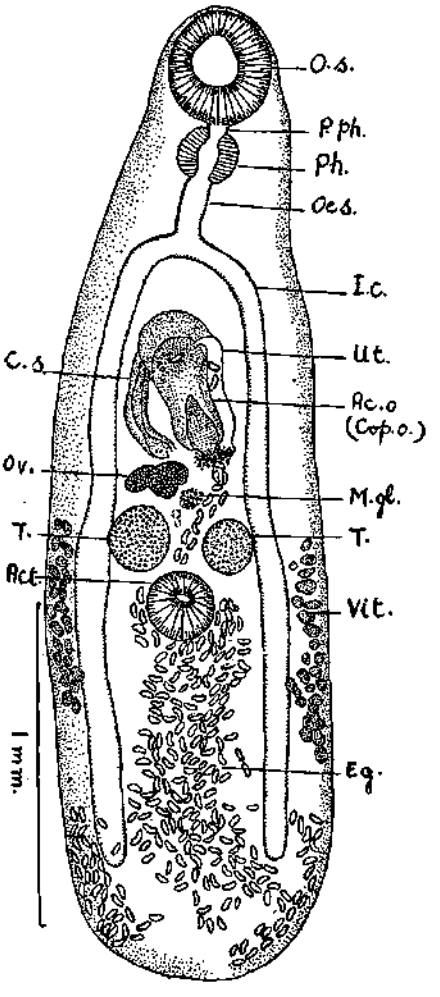

FIG. 8. Ventral view of *Mehratrema dollfusi* Srivastava, 1939.

Ac.o.—accessory organ; *Act.*—acetabulum; *C.s.*—cirrus sac; *Cop.o.*—copulatory organ; *Eg.*—egg; *I.c.*—intestinal caecum; *M.gl.*—Mehlis' gland; *O.s.*—oral sucker; *Oes.*—oesophagus; *Ov.*—ovary; *P.ph.*—prepharynx; *Ph.*—pharynx; *T.*—testis; *Ut.*—uterus; *Vit.*—vitellaria.

intestine of an unidentified eel-like fish and the latter species from *Argentina striata* from Tortugas, Florida. In the same paper, he also reported the occurrence of two unnamed immature forms of *Steringophorus*, one in the lizard fish, the other in *Aleposomos*. Yamaguti (1953) has considered the genus *Steringophorus* as a synonym of the genus *Fellodistomum* Stafford, 1904, without assigning any reason and recognised the genus *Steringotrema* Odhner, 1911. If the former genus be considered synonymous with the latter, the validity of *Steringotrema* is questionable. The author has, therefore, retained the genus *Steringophorus* as a distinct genus.

The author in this paper has also added another new species to the genus which he got from the intestine of *Lethrinus* sp. (Partevelamin).

Steringophorus lethrini n.sp. (Fig. 9)

Four specimens of *Steringophorus lethrini* were recovered from the intestine of a marine fish, Partevelamin (*Lethrinus* sp.), at Krusadai Island. Of these, two were mature specimens and the remaining two immature.

The body of *Steringophorus lethrini* n.sp. varies from 2·2–2·28 mm. in length and 1·14–1·33 mm. in breadth across the testicular region. Both its anterior and posterior ends are rounded. The integument is devoid of cuticular spines. The mouth opening is subterminal and the oral sucker, 0·2–0·24 mm. in length and 0·25–0·35 mm. in breadth, is rounded or spherical or may be more elongated transversely. The pharynx is either situated behind the oral sucker or may be overlapped by the latter organ. The pharynx measures 0·16–0·19×0·24 mm. Following the pharynx there is an oesophagus the length of which varies from 0·064–0·096 mm. The two

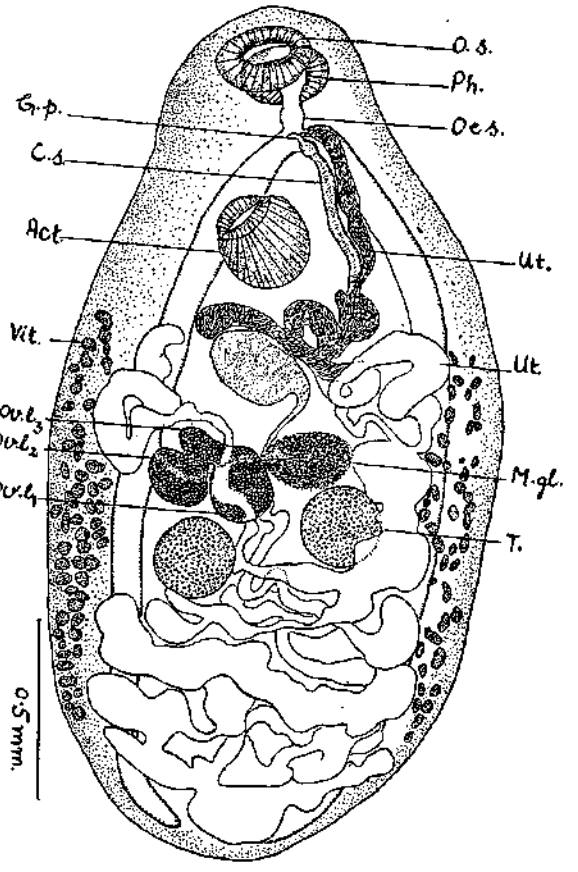

FIG. 9. Ventral view of *Steringophorus lethrini* n.sp.

Act.—acetabulum; *C.s.*—cirrus sac; *G.p.*—genital pore; *M.gl.*—Mehlis' gland; *O.s.*—oral sucker; *Oes.*—oesophagus; *Ov.l.*—ovarian lobe; *Ph.*—pharynx; *T.*—testis; *Ut.*—uterus; *Vit.*—vitellaria.

simple intestinal caeca run posteriorly in the dorsal and lateral regions of the body ending blindly near its posterior extremity.

The acetabulum, 0·24–0·27 mm. in length and 0·22–0·25 mm. in breadth, globular and peculiarly shaped, is situated at a distance of 0·12 mm. behind the intestinal fork and 0·41–0·46 mm. from the anterior end of the body. The shape of the acetabulum seems to be peculiar in these trematodes.

The excretory pore is at the posterior end of the body, leading into a cylindrical vesicle which receives two lateral cornua a little behind the acetabulum.

The two testes are situated far behind the ventral sucker, one on each side and close to the intestinal caecum of the respective sides. The left testis may be a little advanced to the right, and measures 0·22–0·27 × 0·19–0·27 mm. The right testis is 0·22–0·25 × 0·2–0·27 mm. in size. The cirrus sac is very elongated, extending in front, behind and along the lateral side of the acetabulum. The cirrus is prominent. The genital pore is median and ventral to the intestinal fork or may be slightly lateral to the median line. The ovary is trilobed, situated close to the right intestinal caecum and in front of the right testis. The lobes are extraordinarily prominent. The first lobe measures 0·11–0·19 × 0·17; the second lobe 0·14 × 0·16 mm. and the third lobe 0·64 × 0·16 mm. In one specimen, second and third lobes seem to have merged together. Thus they measure collectively 0·12 × 0·16 mm. The Mehlis' gland complex lies in front of the left testis and measures 0·14–0·16 × 0·22–0·28 mm. The receptaculum seminis is placed anterior to the Mehlis' gland complex measuring 0·24 × 0·16 mm. in the type specimen. The vitelline reservoir lies at the anterior edge of the Mehlis' gland complex. The vitelline follicles are extracaecal, extending from the level of the acetabulum to a little in front of the posterior extremity of the body. The uterus is very much coiled and full of eggs. It forms both descending and ascending limbs. The folds of the uterus lying in the post-testicular region are arranged almost transversely. Anteriorly it finally runs along the cirrus sac to open at the genital pore.

The eggs are operculate and measure 0·015–0·019 × 0·007 mm.

Relationships:—The genus *Steringophorus* Odhner, 1905, comprises so far four species, namely *Steringophorus furciger* (Olsson, 1868) Odhner, 1905, *S. cluthensis* Nicoll, 1909, *S. magnus* Manter, 1934 and *S. profundus* anter, 1934. The new species *Steringophorus lethrini* is characterised by the presence of a much elongated cirrus sac, the vitellaria extending from the level of the region a little behind the acetabulum back to a little in front of the posterior extremity and peculiarly shaped acetabulum. Therefore, in these characters, the new species remains distinct from the other species mentioned above.

The new species has been given the name after its host.

Host: *Lethrinus* sp. (Partevelamin).

Location: Intestine.

Locality: Gulf of Manaar (India).

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Abstract

The characters of a generalized Symphytan type have been elucidated by a comparison of 66 characters of the external anatomy of one representative from each of the ten Symphytan families. The condition of each of these characters has been compared with the corresponding condition in the representatives of the Panorpoid complex and the Orthopteroid orders. This comparison reveals clearly that the Symphyta share many features with the Panorpoid insects and far fewer with the Orthopteroids. By excluding those features which the Symphyta share with all primitive Panorpoid types and those which are peculiar, among Endopterygotes, to the Symphyta, an attempt has been made to decide more precisely which Panorpoid orders are most like the Symphyta. This has revealed that the Mecoptera and Diptera show relatively few resemblances to the Symphyta, and that, even though further data are still required, the Neuroptera, out of the remaining Panorpoid orders, are most closely affiliated to the Hymenoptera, the latter having probably arisen from a stock more generalized than the existing Neuropteran groups.

Introduction

The problem of the relationship of the Hymenoptera to the other orders of insects has engaged the attention of entomologists for at least seventy years. The fossil record of the Hymenoptera is rather scanty. The oldest fossil is *Liadoxyela praecox*, founded by Martynov (1937) on the impression of the wings from the Lower Lias of Turkestan. It is comparable to the primitive living Symphytan *Macroxyela*. There is also *Pseudosirex* from the Lias of Siberia and the Upper Jurassic of Solenhofen. This, according to Handlirsch (1908), has the appearance of *Sirex* with its thorax as much evolved as the present-day Siricidae, but with a shorter ovipositor shaft and wings relatively poor in closed cells though with a system of fine longitudinal veins. The presence of these Siricoids together with the other Hymenoptera, such as the Orussoids and Ichneumonoids, according to Carpenter (1930), 'makes it rather obvious that the order had been sometime in existence before that period'—a view also held by Handlirsch (1925). These fossils, however, throw no light on the origin of the group.

There may be said to be three chief views as to the origin and relationship of the Hymenoptera:

1. Tillyard (1924) attempted to derive the order from the Lower Permian Protohymenoptera. This group is now admitted to belong to the Megasecoptera and to have no connection with the Hymenoptera (Martynov, 1930; Carpenter, 1930).
2. Handlirsch (1925) attempted to derive the Hymenoptera from Protoblattoid ancestors, quite independently of the rest of the Endopterygota. Besides the objection to assuming a polyphyletic origin for the specialized type of metamorphosis, there are not, as shown later in the present work, such close anatomical resemblances to the Orthopteroid insects as has usually been supposed.
3. Crampton (1921, 1927) and Carpenter (1930) have drawn attention to the importance of similarities between the Hymenoptera* and the Panorpoid orders. A suggestion of a common ancestry for all these orders (and also the Coleoptera) has the attraction of assuming that the Endopterygota are a monophyletic group. Ross (1936) has shown that the hymenopterous type of wing-venation could well be derived from that of the Megaloptera.

The object of the present study is to compare in detail the external anatomy of the adults of some representative Endopterygota with that of the Orthopteroid orders. This will provide detailed evidence on which to assess the resemblance of the Hymenoptera firstly to the other Endopterygota or to the Orthopteroids, and secondly to any particular order in either main group.

Aims and Methods

It was decided that the first step in attempting to assess the affinities of the Hymenoptera on the basis of their adult skeletal morphology was to obtain a detailed morphological characterization of the generalized members of the order. In practice, this meant the characterization of a generalized Symphytan and to accomplish this end, 10 species, one from each of the 10 larger families of Symphyta, were studied and the resulting data supplemented by the work of Ross (1937) and of a few other authors mentioned later.

From the data obtained by studying the 10 representative Symphyta, it was easy to draw up a detailed morphological characterization of the generalized Symphytan. The next step was to compare the conditions found in the Symphyta with those obtaining in other orders. Representatives of the Panorpoid orders, Coleoptera and of Orthopteroid orders were subjected to a detailed study and compared with the Symphyta point

* As noted by Carpenter, certain features of the Hymenoptera, such as the polynephric malpighian tubes, tend to isolate the order from the other Endopterygota.

by point. The information thus obtained has been supplemented by that given in the literature.

The following insects were studied:—

I. Hymenoptera-Symphyta, Fam. Diprionidae, (1) *Diprion pini* (Linnaeus); Fam. Tenthredinidae, (2) *Selandria serva* (Fabricius); Fam. Cimbicidae, (3) *Cimbex femorata* (Linnaeus); Fam. Argidae, (4) *Arge ochropus* (Gmelin in Linnaeus); Fam. Megalodontidae, (5) *Megalodontes klugii* (Leach) ; Fam. Xyelidae, (6) *Xyela julii* (Brebisson); Fam. Pamphiliidae, (7) *Pamphilius stramineipes* (Hartig); Fam. Cephidae, (8) *Cephus pygmaeus* (Linnaeus); Fam. Xiphydriidae, (9) *Xiphydria prolongata* (Geoffroy in Fourcroy); Fam. Siricidae, (10) *Urocerus gigas* (Linnaeus).

II. Neuroptera-Megaloptera, Fam. Sialidae, (11) *Sialis lutaria* (Linnaeus); Fam. Raphidiidae, (12) *Raphidia notata* Fabricius; Planipennia, Fam. Hemerobiidae, (13) *Hemerobius stigma* Stephens, (14) *Micromus variegatus* (Fabricius).

III. Mecoptera, Fam. Panorpidae, (15) *Panorpa germanica* Linnaeus.

IV. Diptera-Nematocera, Fam. Tipulidae, (16) *Tipula fascipennis* Meigen.

V. Trichoptera, Fam. Limnephilidae, (17) *Limnephilus marmoratus* Curtis.

VI. Lepidoptera-Homoneura, Fam. Hepialidae, (18) *Hepialus humuli* (Linnaeus).

VII. Coleoptera-Adephaga, Fam. Carabidae, (19) *Feronia madida* (Fabricius).

VIII. Dictyoptera, Fam. Blattidae, (20) *Periplaneta americana* (Linnaeus).

IX. Orthoptera, Fam. Acrididae, (21) *Nomadacris septemfasciata* (Audinet-Serville).

X. Plecoptera, Fam. Perlidae, (22) *Chloroperla torrentium* (Pictet).

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THE COMPARATIVE MORPHOLOGY OF THE SYMPHYTA AND ITS RELATIONSHIP WITH OTHER INSECTS

1. *Maxilla*

A. Symphyta: (Pl. I, Figs. 1–9)

An examination of the maxillae in the different families of Symphyta shows a fundamental plan with some interesting modifications. The cardo is always undivided in the Symphyta. It is triangular and extended

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posteriorly in *Diprion*, *Selandria*, *Arge* and *Xyela*. It is elongate with its proximal part curved dorsally forward in *Megalodontes*, *Pamphilius* and *Cephus*. In these cases the cardo stands almost at right angles to the stipes with only a small part of the distal portion visible in the ventral view. In *Xiphydria* and *Urocerus*, it is elongated laterally inward to meet its fellow from the other side on the ventral surface, their cranial articulations, however, remaining separate.

The stipes is undivided and 'the narrow inconspicuous sclerite, the parastipes' mentioned by Ross (1937) in *Macroxyela* and *Arge* has not been observed in any species examined. There is, however, a lateral ridge along the inner margin of the stipes for the attachment of the muscles. The stipes is elongated and arched, more so in *Megalodontes*, *Pamphilius* and *Cephus* than in others. It is broad proximally and narrow distally, the distal portion giving a falsely two-segmented appearance to the galea that it carries terminally. In *Urocerus* a specialized condition is met with, where the two stipes broaden out laterally inward and are fused to form a bridge ventrally across the labium.

The galea is always a terminal, undivided lobe except in *Xyela* in which its distal portion is partially divided into two lateral parts, an external ectogalea and an internal endogalea (Crampton, 1923). The galea amongst the sawflies is quite variable in shape. It is an oval lobe in *Diprion*, *Selandria*, *Cimbex* and *Arge*, flask-shaped in *Pamphilius* with the basal stalk and the distal distended portion, long and blade-like in *Megalodontes*, long and finger-shaped in *Cephus*, a broad crescentic lobe in *Xiphydria* and a large conical structure with strong spines in *Urocerus*.

The lacinia is a membranous lobe, lying inner and dorsal to the galea but at a slightly lower level. It is usually armed with setae and pubescence along its inner margin. It is a soft oval lobe, as large as the galea in *Cimbex*, rounded and smaller than the galea in *Arge*, blade-like in *Diprion*, small and conical in *Selandria*, somewhat quadrangular but smaller than the galea in *Megalodontes*, *Pamphilius*, *Cephus* and *Xyela*, small and stump-like in *Xiphydria*, and missing in *Urocerus*. Roughly correlated to the enlargement of the galea, there is a corresponding reduction in the size of the lacinia, leading to its complete disappearance.

The maxillary palp is, as a rule, six-segmented save in *Xiphydria* and *Urocerus*, in which it is five-segmented and one-segmented respectively. The length of the maxillary palp and the relative proportion of its various segments are subject to variation. The palp is appreciably longer than the maxilla in *Diprion*, *Selandria*, *Arge*, *Pamphilius* and *Xyela*, becoming smaller in *Cimbex*, *Cephus* and *Megalodontes*. A further reduction in size and number of segments is met with in *Xiphydria* and *Urocerus*. In the former, the second segment is exceptionally long but an incomplete dividing line persists, denoting its formation from two segments. *Xyela* is peculiar in having a small stump-like sixth segment.

B. Comparison with other insects: (Pl. I, Figs. 10–13)

All the investigated sawflies agree in possessing (1) an undivided cardo, (2) an elongate, undivided stipes, and (3) a simple lobed galea. If one accepts

Xiphydria and *Urocerus*, one can add another two characters, viz. (4) a simple lacinia without terminal maxadentes and (5) a six-segmented maxillary palp, and as *Urocerus* and *Xiphydria* are usually considered to be somewhat specialized, the five characters may reasonably be regarded as the primitive features of the Symphytan maxilla.

If now one turns to the other non-Symphytan species studied, one finds that the only one to show all the Symphytan characters is *Sialis*, while a 90% similarity is found in *Raphidia*, *Panorpa* and *Limnephilus*, the three species which are identical in respect of the five main characters and differ from the Symphyta and *Sialis* only in the possession of a five-segmented palp. A six-segmented palp is known to be of sporadic occurrence in the Panorpoid orders as shown by Killington (1936) in *Megalomus*, *Boriomyia*, *Wesmaelius* and *Sympherobius* (Hemerobiidae), by Martynov (1914) in *Paduniella uralensis* (Psychomyidae), by Lestage (1926) in Paduniellinae, by Busck and Boving (1914) in Eriocraniidae, and by the author in *Sialis* and *Hemerobius*. It is, therefore, not unlikely that the five features quoted above for Symphyta and *Sialis* are, in fact, generalized Panorpoid characters. The strongly Panorpoid facies of the Symphytan maxilla is therefore clearly demonstrated.

It is generally considered (Tillyard, 1937) that the Neuroptera-Megaloptera and Mecoptera are the more generalized Panorpoids, so the fact that *Hemerobius*, *Tipula* and *Hepialus* differ in certain respects from the *Sialis*-Symphytan pattern does not in any way invalidate the theory of a Panorpoid origin of the Hymenoptera; it merely means that the three species mentioned belong to groups which have, to some extent and in different ways, diverged in maxillary structure more from the primitive Panorpoid type than have the Symphyta.

The second clear fact which emerges from this study is the considerable difference in maxillary structure between the Orthopteroids and the *Sialis*-Symphytan type, the former having a divided cardo, a divided stipes, a divided galea, a lacinia with maxadentes, and a five-segmented maxillary palp. There is thus little evidence here in support of any theory of Orthopteroid ancestry for the Symphyta.

2. *Labium*

A. Symphyta: (Pl. I, Figs. 14–20; Pl. II, Figs. 1, 4, 7)

The labium of *Diprion* (Arora, 1953) represents a generalized condition. Variations in the shape and relative sizes of the component structures are, however, met with in the different families of the group. The postmental region is broad and weakly sclerotized with the small centrally placed, setiferous postmental sclerite, which is pear-shaped in *Diprion*, oval in *Selandria* and *Cephus* and narrowly spear-shaped in *Cimbex*. The postmental sclerite is broad and shield-shaped in *Arge*, *Xiphydria* and *Urocerus*, but occupies the whole of the postmental area in *Xyela* and *Pamphilius*. *Megalodontes* stands alone in having three small, triangular postmental sclerites, arranged in a triangle.

The prementum is a sclerotized plate, showing a faint division into two distally. It is smaller than the postmentum in *Diprion*, *Selandria*,

Cimbex and *Arge*, narrow and equal to the postmentum in *Cephus*, broad and slightly longer than the postmentum in *Xyela*, *Xiphydria* and *Urocerus*, and elongated and considerably longer than the postmentum in *Megalodontes* and *Pamphilius*. The prementum carries the ligular lobes distally and the paired labial palps laterally, one on either side of the former. There are generally three ligular lobes, the median alaglossa formed by the combined glossae, and the two lateral paraglossae, with a varying degree of fusion of their basal parts. The alaglossa and the paraglossae are free and conical in *Diprion*, the former longer and slightly broader than the latter, and bear setae all over with a cluster of small spines at the tip in each. The alaglossa is cylindrical in *Selandria* whereas the paraglossae are crescent-shaped and slightly broader but shorter. These lobes are separate and their basal parts are beset with small, strong setae while the distal half of each lobe is marked into reticulations by transverse and longitudinal striae and carries short, thick pubescence at its extremity. The alaglossa in *Cimbex* is narrow and cylindrical and of nearly half the width of the paraglossa but equal to it in length. There is no fusion between the alaglossa and the paraglossae and they are covered with setae and striations as in *Selandria*. Basal fusion between the alaglossa and the paraglossae is quite marked in *Arge*, in which the thick and bluntly pointed alaglossa is one-and-a-half times as wide as the paraglossa but somewhat shorter. The setae and the striations, borne on the lobes, are similar to those in *Selandria* and *Cimbex*. The alaglossa in *Megalodontes* is long and cylindrical and the paraglossae elongated and blade-like, the former twice as wide as the latter and resembling the tongue of the honey-bee. The lobes are free and provided with setae and striations as above. In *Cephus* the alaglossa is spatulate, broad distally and twice the width of the paraglossae. These lobes do not show any fusion and bear the setae and the striations as in *Selandria* and others. The lobes in *Pamphilius* are more or less rounded, the alaglossa again being twice as wide as the paraglossae. The lobes are united proximally and free distally and bear setae and markings as above. *Xyela* has a distinct, broad and well developed alaglossa, twice the width of the paraglossae which are bent inward. Ross (1937) found the alaglossa to be missing in *Macroxyela ferruginea*, and according to him 'the alaglossa is represented by a membranous area connecting the bases of the paraglossae'. A further fusion of the proximal portions of the lobes is witnessed in *Xiphydria*, in which only the terminal parts of the lobes are distinctly separate, the broad and truncate apical part of the alaglossa projecting slightly beyond the comparatively narrow and bluntly pointed distal parts of the paraglossae. The lobes bear setae and striations as in *Selandria* and others. *Urocerus* represents an extreme case of fusion, where the alaglossa and the paraglossae have formed a composite structure, the 'totaglossa', clothed with long setae. Ross (1937) described a totaglossa in *Incalia* (Loboceridae), *Decameria* (Perreyiidae) and *Urocerus* (Siricidae). The labial palps are four-segmented, all segments carrying setae. In *Xiphydria* and *Urocerus*, the labial palps have been regarded as three-segmented. The broad third segment bears an oval area, described as 'a round, membranous, sensory area' by Ross (1937). A

closer examination of this area reveals that it is possibly a reduced stump-like fourth segment with its basal portion bearing long setae, like the setae borne on the third segment, and a terminal, probably sensory, circular patch. This reduction of the fourth segment would, perhaps, be expected in what are specialized genera in many other respects. Another deviation from the normal was recorded by Ross (1937) in *Macroxyela*, in which the labial palps are three-segmented, though the allied *Megaxyela* was reported by him to possess four-segmented labial palps like those in *Xyela*. The labial palps in most of the sawflies reach well beyond the tips of the lobes, except in *Megalodontes* and *Cephus*, in which they just reach the tips.

A narrow membrane generally stretches between the inner margins of the cardo and the stipes on the one hand and the outer margin of the postmentum on the other, forming a maxillo-labial complex; the prementum ordinarily does not take part in the union except in *Megalodontes*, *Pamphilius* and *Xyela*. In *Megalodontes* and *Pamphilius* the membrane extends as far as the anterior third of the prementum while in *Xyela* the whole of the prementum is thus engaged.

B. Comparison with other insects: (Pl. II, Figs. 2, 3, 5, 6, 8)

From the study of the sawfly labia it will be apparent that the generalized Symphytan labium is characterized by (1) an undivided postmentum, (2) fusion of glossae into alaglossa and (3) presence of a four-segmented labial palp. *Xiphydria* and *Urocerus* are specialized and show a reduction in the number of segments of the labial palps. A varying degree of fusion between the paraglossae and the alaglossa is evident. It is, in fact, possible to trace a series of forms from the condition in *Cimbex* and *Selandria*, where the alaglossa is narrower than the paraglossa, through one in *Diprion*, *Arge*, *Xyela*, *Megalodontes* and *Cephus*, where the alaglossa has grown wider than the paraglossa, leading to a condition of partial fusion of lobes as in *Pamphilius* and *Xiphydria*, and ultimately resulting in a more or less complete fusion of the lobes into a totaglossa as in *Urocerus* and others.

A study of the labia shows that *Sialis* and *Hemerobius* from among the Panorpoids coincide entirely with the generalized Symphytan condition, differing from the latter only in the greater fusion of the ligular components which parallels the condition noted above in some of the sawflies, e.g. *Xiphydria* and *Urocerus*. The Mecoptera and the Diptera do not approach the Symphytan condition, having lost the ligula and specialized in having two-segmented, bulbous palps. *Limnephilus* differs from the generalized Symphytan in having three-segmented labial palps but four-segmented labial palps had been recorded by Martynov (1914) in *Paduniella* and by Lestage (1926) in the sub-family Paduniellinae, corresponding to the four-segmented condition of labial palps in Symphyta and some Neuroptera. It may further be pointed out that the six-segmented maxillary condition is accompanied by a four-segmented labial palp. There thus exists a similarity between the Symphyta and the hypothetical generalized Trichopteran condition also, which suggests that the basal Panorpoid stock may have been closer to the Symphyta than are the recent Mecoptera and Diptera. The

Orthopteroid types studied deviate considerably from the Symphytan condition as they possess the postmentum divided into submentum and mentum, free ligular lobes with the glossae tending to be reduced, and three-segmented labial palps. From the above, one may clearly consider the structure of the labium to indicate that the Symphyta have close affinities with the Neuroptera (especially Sialoidea and Planipennia) and probably also with the basal Panorpoid types. The close resemblance between the Diptera and the Mecoptera and the suggestion of a Panorpoid condition in the Coleoptera are other noteworthy features of the analysis of labial structure. Once again, it is clear that the Orthopteroid condition is far removed from that of the Symphyta.

3. *Hypopharynx*

A. Symphyta: (Pl. I, Figs. 14–18, 20; Pl. II, Figs. 1, 4, 7)

The hypopharynx is a small projection or raised lobe, intimately fused with the oral surface of the labium. According to Snodgrass (1947), the hypopharynx in the Hymenoptera has no independent movement. In the sawflies it lies on the dorsal surface of the prementum at the base of the alaglossa or the totaglossa. It is a large, ovate and weakly sclerotized lobe with short spines in *Selandria*, a narrow sclerotized lobe with spinules in *Diprion* and *Cimbex*, a vase-shaped sclerotized lobe, covered with thickly set long pubescence in *Arge*, and a small oval weakly sclerotized lobe with spinules in *Xyela*. It is a small disc-shaped strongly sclerotized structure covered with spines in *Pamphilius*, *Cephus*, *Xiphydria* and *Urocerus*, while it is large and elongated in *Megalodontes*. According to Ross (1937), the hypopharynx in *Urocerus* is reduced to a small area of setae.

A pair of slender, lateral suspensorial rods support the membranous region on the sides of the hypopharynx and extend posteriorly to the angles of the mouth. Anteriorly these rods run inward and almost meet in the mid-line.

A salivary duct has been seen, in every case, entering the hypopharynx at its base and is traceable to its anterior end. No salivarium is formed and no part of the hypopharynx projects beyond the labium, to the dorsal wall of which it is united.

B. Comparison with other insects: (Pl. II, Figs. 3, 9–13)

There is a uniformity among the Symphyta which agree in having a hypopharynx characterized by (1) a single undivided lobe with no superlinguae, (2) the lobe fused with the labium throughout and (3) the salivary duct opening at the distal end of the hypopharynx.

The Symphytan hypopharynx resembles the hypopharynx in the Neuroptera, the Trichoptera, the Lepidoptera and the Coleoptera. Snodgrass (1947) pointed out the resemblance among the Hymenoptera, the Neuroptera and the Coleoptera in having the hypopharynx intimately fused with the labium. To these three may be added the Trichoptera, the Lepidoptera and some Diptera like *Borborus* (Peterson, 1916), in which the hypopharynx is completely fused with the labium as well. Most of

the Diptera, however, possess a hypopharynx which is an elongated lobe and, according to Snodgrass (1944), without any muscles and probably serving principally for the hypodermic injection of the saliva into the wound made by other stylets. The Mecoptera are the only Endopterygotes, which have departed from the other Endopterygotes and possess a conical, membranous lobe hanging in the preoral cavity with the salivary duct opening at the base of the free lobe.

The Symphyta differ from the Dictyoptera, the Orthoptera and the Plecoptera in the main hypopharyngeal features, the latter groups having the Orthopterous type of hypopharynx with a distal tongue-like lobe, capable of movement, and a proximal fixed part.

The Symphytan type of hypopharynx appears to be almost universal in the Endopterygotes and therefore affords little information on the precise affinities of the Hymenoptera. It does, however, suggest that they are closer to the Neuroptera than to the Mecoptera.

4. *Tentorium*

A. Symphyta: (Pl. II, Figs. 14–22)

A typical Symphytan tentorium is H-shaped and is composed of three pairs of tentorial arms, the anterior, the posterior and the dorsal, with a tentorial bridge or body running across the occipital foramen. The anterior tentorial arms are short, broad and distally expanded in *Diprion*, *Selandria*, *Cimbex* and *Arge*, with a small, blunt, mesally directed process from each. These arms are proximally broad in the middle and narrow again distally in *Megalodontes* and *Pamphilius*, each with a hump of the inner margin. They are almost cylindrical in *Cephus* and *Xiphydria*, the former somewhat broader at their bases due to inwardly directed projections, in which respect they resemble the anterior arms of *Xyela*; the inner projections in *Xyela*, however, are more pronounced. In *Urocerus* the anterior arms are short and thick and have the inner margins of their proximal halves produced into broad truncate lobes. The anterior arms amongst the Symphyta are sclerotized and meet the tentorial bridge, one at either end of the latter at its antero-ventral margin. The mesal processes, which Ross (1937) calls 'mesal shelf-like processes on the pretentoria', do not meet in the mid-line.

The short, sclerotized posterior tentorial arms run inward from the posterior tentorial pits, at the sides of the occipital foramen, to meet in the middle, forming a narrow tentorial bridge across it. Each posterior arm is indistinguishably fused with the thickening on the side of the occipital foramen.

The tentorial bridge is narrow in most of the forms. It is a comparatively broad band in *Pamphilius* and *Megalodontes*. In *Xiphydria* and *Urocerus* the tentorial bridge is exceptionally wide and tubular (with a cavity which is triangular in cross-section) and projects into the head.

The dorsal tentorial arms, though mentioned by Ross (1937) to be 'issuing from the anterior or antero-dorsal margin of the corpotentorium', arise, in fact, from the dorso-lateral margins of the anterior tentorial arms,

either at their bases or further up. They are quite thick in *Diprion, Selandria, Cimbex* and *Arge*, making obtuse angles with the corresponding anterior arms. They are short and finger-like in *Megalodontes, Pamphilius* and *Xiphydria*, with an acute angle between each and the anterior arm from which it arises. They are long and filiform in *Urocerus*, very slender in *Cephus*, and somewhat club-shaped in *Xyela*. The dorsal tentorial arms reach the cranial wall.

B. Comparison with other insects: (Pl. III, Figs. 1–5)

The Symphytan tentorium possesses (1) H-shaped body, (2) anterior tentorial arms with mesal processes, which never meet in the mid-line, (3) dorsal tentorial arms arising from the anterior tentorial arms and (4) the tentorial bridge running across the occipital foramen and dividing the latter into two.

All these Symphytan features are shared by a number of other Endopterygotes, such as *Panorpa, Limnephilus* and *Hepialus*. *Sialis, Hemerobius* and *Feronia* have the first three characteristics in common with the Symphyta and the others, but the tentorium is medianly placed and forwardly removed from the occipital foramen—a condition which, according to Walker (1933) and Hudson (1945), is supposed to be primitive. Though *Tipula* has a reduced tentorium with short anterior tentorial arms and lacking the posterior and the dorsal tentorial arms and the tentorial body, a generalized Dipteran tentorium, as constructed by Peterson (1916), is H-shaped with slender tentorial arms and a short and slender tentorial bridge, e.g. in *Tabanus* and *Simulium*. As such the Symphyta as well as the Mecoptera, the generalized Diptera, the Trichoptera and the Lepidoptera deviate somewhat from the Neuroptera and the Coleoptera. The Symphyta differ from the other four groups in having short and thick anterior tentorial arms (long and narrow in the others), in which respect they are a nearest approach to Neuroptera. There is a good deal of resemblance between the tentorium of Mecoptera and that of the generalized Diptera in so far that both possess very narrow tentorial bridges and that the dorsal tentorial arms are slender, tending to disappear. The presence of a pair of anterior divergent and a pair of posterior convergent processes from the ends of the tentorial bridge in Trichoptera and Lepidoptera bring the two together.

The possession of the X-shaped tentorium, the presence of the frontal plate, and the body of the tentorium bounding the cephalic margin of the occipital foramen are the principal features of the Orthopteran tentorium, which separate it from the tentoria of the Symphyta and the other Endopterygotes. Snodgrass (1935) regards the H-shaped tentorium as primitive. *Chloroperla* agrees with the Orthoptera in having an X-shaped tentorium with a narrow frontal plate, but has the tentorium, like *Sialis*, placed forward. Among other Plecoptera, as has been pointed by Hoke (1924), Walker (1933) and Hudson (1948), the cephalic margin of the occipital foramen is bounded by the body of the tentorium. Thus the Plecoptera seem to be more intimately related to the Orthoptera than to the Endopterygotes.

The general conclusion is that the Symphytan tentorium, which is very uniform in its general features, closely resembles that of the more primitive Panorpoids but that this common type is probably a little more specialized than the Sialoid type. Whether this indicates that the Symphyta arose from basal Panorpoid stock, subsequent to the separation of the latter from a Neuropteran ancestor, is a question which can only be settled after other characters are taken into account.

5. *Mesothoracic and Metathoracic Terga*

A. Symphyta: (Pl. III, Figs. 6–10)

The mesotergum is considerably larger than the metatergum. The mesotergum is designated by Ross (1937) and other taxonomists as the mesonotum. As has been described earlier in *Diprion pini* (Arora, 1953), the mesonotum comprises the prescutum, the scutum and the scutellum, and represents the generalized condition among the Symphyta. The prescutum is a diamond-shaped area in *Diprion, Megalodontes, Pamphilius, Xyela* and *Cephus*, bound laterally by the convergent sutures, which meet posteriorly, and divided into two by a median suture; *Cephus* has a relatively small prescutum. The prescutum in *Selandria, Cimbex, Arge* and *Xiphydria is not completely delimited but is confluent with the scutum because the* median and the convergent sutures do not meet posteriorly. In *Xiphydria* the convergent sutures almost approach the trans-scutal suture, a characteristic of the family. According to Ross (1937), these convergent sutures meet the trans-scutal suture in *Orussus* as well—a condition shown by Snodgrass (1910) in *Eurytoma* (Chalcididae) and other higher Hymenoptera. *Urocerus* is the only one in which the prescutum remains undifferentiated and is fused with the scutum. Snodgrass (1910) described the mesoscutum of *Tremex* without a prescutal division but Ross (1937) in the same species mentioned the presence of faint traces of the prescutal sutures, also shown by Benson (1951) in a Siricid. The scutum in *Diprion, Megalodontes, Pamphilius, Xyela* and *Cephus* consists, in each, of two lateral areas connected by a transverse bridge between the prescutum and the scutellum. The parapsidal furrows in these forms are complete. In *Selandria, Cimbex* and *Arge*, in which the prescutum is continuous with the scutum, the parapsidal sutures are either poorly or partially developed. In *Xiphydria* an additional trans-scutal suture runs across and divides the scutum into an anterior and a posterior part. A pair of transverse ridges arises from the parapsidal sutures, each running posteriorly to divide the parascutellar area of its side into two. *Urocerus*, as has already been stated, is specialized in having its scutum fused with the prescutum; the parapsidal furrows are distinct and a trans-scutal suture is absent. The scutellum is a V-shaped sclerite, with the apex of the V directed forward, in all the Symphyta, except in *Cimbex* and *Urocerus* in which it is semilunar. A scuto-scutellar suture separates the scutellum from the scutum. In *Diprion, Megalodontes, Pamphilius* and *Cephus*, in which the post-tergite is a narrow cord, and in *Xyela* (with relatively broad post-tergite), the scutellum does not cover the postnotum or the metanotum. Though the post-tergite is likewise

narrow in *Xiphydria* and *Urocerus*, the scutellum is large and extends posteriorly to cover the postnotum and a part of the metanotum. The post-tergite is fairly broad in *Selandria*, *Cimbex* and *Arge*, and the scutellum covers only the anterior part of the postnotum. The post-tergite, according to Ross (1937), is absent in all Tenthredinoidea except the Diprionidae and the Tenthredinidae. A small lobe, termed the 'postscutellum' (post-tergite), on the posterior margin of the scutellum was described by Snodgrass (1910) in *Tremex*.

The postnotum is a sclerotized band behind the scutellum with generally a small membranous part in front between it and the latter. It is notched anteriorly and bears a bilobed phragma, which extends posteriorly beneath the metatergum or beyond. The postnotum is quite exposed (not invaginated) in *Megalodontes*, *Pamphilius*, *Xyela* and *Cephus*. Its membranous part is somewhat covered over by the scutellum in *Diprion* and *Selandria*. In *Cimbex*, *Arge*, *Xiphydria* and *Urocerus*, on the other hand, the anterior membranous part is completely obliterated and the postnotum is invaginated, partly in the former two and completely in the others. Ross (1937) mentioned the postnotum in the sawflies, 'most of which is invaginated', but that is really not the case as shown above. The phragma, however, lies invaginated beneath the metatergum, sometimes extending to the first abdominal segment as in *Diprion*, *Cimbex*, *Arge*, *Cephus*, *Xiphydria* and *Urocerus*. Laterally the postnotum runs outward and forward, each end connected with the epimeron of its side. Snodgrass (1910) had also referred to the postnotum in the mesothorax of Tenthredinoidea as 'consisting of an exposed transverse plate behind the scutellum connected laterally with the mesothoracic epimera', adding that 'it is visible externally also in *Tremex* but is less exposed here than in Tenthredinoids'.

The metatergum is much smaller than the mesotergum and is generally separated from the latter by mesopostnotum, except in *Xiphydria* and *Urocerus* in which the mesoscutellum extends posteriorly and covers a part of the metatergum. The latter consists of the notum and the postnotum. The notum comprises the scutum and the scutellum, the prescutum being prominent by its absence. The scutum is formed by two lateral areas connected by a transverse bridge in front of the scutellum. Each lateral area bears a prominent, membranous cenchrus. The parapsidal sutures are faintly indicated in *Diprion* and *Pamphilius*, strongly developed in *Selandria* and *Megalodontes*, incomplete in *Cimbex*, and absent in *Xyela* and *Cephus*. The parapsidal sutures are strongly developed and bifurcate in *Xiphydria* (as in the mesonotum), while in *Urocerus* they are similar to those in *Xiphydria* but are incompletely developed. The scutellum is almost triangular with rounded angles. It is broader than long and is provided with a small post-tergite. The postnotum is sufficiently narrow with a membranous strip in front in *Diprion*, *Selandria* and *Megalodontes*, while in others there is no membrane. The postnotum is invaginated in *Xyela*, *Cephus*, *Xiphydria* and *Urocerus* and is seen only on the sides.

B. Comparison with other insects : (Pl. III, Figs. 11–13; Pl. IV, Figs. 1–3)

The generalized Symphytan pteroterga have (1) mesotergum larger than the metatergum, (2) meso-prescutum completely delimited by convergent sutures and divided into two by a median suture, (3) mesoscutellum triangular, separated from the mesoscutum by the scuto-scutellar suture, (4) a post-tergite, (5) no meta-prescutum and (6) well developed meso- and meta-postnotum.

The above quoted characters are common to most of the Symphyta except some in which the convergent sutures, bounding the prescutum, are not complete (*Selandria*, *Arge*, etc.) or are missing (*Urocerus*). The condition in *Urocerus* is presumably a specialized one, considering that it is specialized in a number of characters. Another variation is the broadening of the scutellum, which assumes a semilunar shape (*Cimbex* and *Urocerus*). This again appears to be a modified condition of the generalized triangular shape, though it is difficult to decide which of the two is primitive.

The Symphyta resemble *Hemerobius* and *Hepialus* the most, differing from them only in the presence of a post-tergite of the scutellum—a specialized feature of the Symphyta (not possessed by any Panorpoid). Their next of kin are the Megaloptera and the Trichoptera, which lack the convergent sutures but have well-differentiated prescutal areas with the median suture. This may mean that *Sialis*, *Raphidia* and *Limnephilus* have lost these convergent sutures secondarily, like some of the sawflies which have likewise lost them partially or completely. The Mecoptera and the Diptera also are not far removed from the Symphyta because the characters in which they differ from the generalized Symphyta (e.g. semilunar mesoscutellum and partial or complete disappearance of convergent sutures) are possessed by some of the sawflies. It follows, therefore, that the Symphyta and the Panorpoids could have descended from a common ancestral stock, varying but little in one character or the other, and that the Symphyta have their closest allies in the Neuroptera and the Lepidoptera, which are more primitive than them in the construction of the meso- and the metaterga.

The Symphyta and the Panorpoids, though resembling the Coleoptera in some of the characters, differ from them in two fundamental characters, viz. (1) the possession by the latter of a mesotergum considerably smaller than the metatergum and (2) the possession of the meta-prescutum.

The Symphyta and the Panorpoids show a complete departure from the Orthopteroid type of pteroterga, the latter possessing a mesotergum as large as or slightly smaller than the metatergum, a narrow, transverse meso- and meta-prescutum, a U- or V-shaped mesoscutellum continuous with the mesoscutum, a reduplicated band of the meso- and metascutellum, and no mesopostnotum. Hence the Symphyta and the Panorpoids cannot be said to be at all closely related to the Orthopteroid insects.

6. *Mesosternum and its relation with Mesopleuron*

The mesothoracic sternum in a generalized Pterygote insect, according to Snodgrass (1935), comprises the presternum, the basisternum, the furcasternum and the spinasternum. These divisions have been demonstrated

to be present in most of the insects examined for this work. Ferris and his collaborators (Ferris and Rees, 1939; Ferris and Pennebaker, 1939; Rees and Ferris, 1939; Ferris, 1940, and Campau, 1940) have proposed a new theoretical interpretation of the mesosternal components and define the sternum as part of the ventral surface between the sternal apophyseal pits, the sclerotized areas lying lateral to them being derived from the pleura. These pleural elements, which have caused the sternum to be greatly reduced and infolded in certain insects, have been named the pre-episterna and have been said, in conjunction with the katepisterna, to occupy the ventral part of the thoracic segment, meeting in the 'discriminal line' in the middle. It is not the purpose of this paper to go into the merits and demerits of Ferris's theory but suffice it to say that Snodgrass's scheme is being adopted.

A. Symphyta: (Pl. IV, Figs. 5–7)

The mesosternum in the Symphyta consists of the four main divisions. The presternum is small and narrow in *Diprion*, *Selandria*, *Cimbex*, *Arge*, *Cephus* and *Megalodontes*. It forms a broad presternal bridge in *Pamphilius* and *Xyela*, which puts the katepisterna into communication with each other. In the case of *Xiphydria* and *Urocerus*, because of the absence of the sternopleural sutures, the presternal bridge is the region in front of the anterior end of the median longitudinal ridge which divides the basisternum (fused with the episterna) into two.

The basisternum presents three conditions:

(1) It is separated from the katepisterna by distinct sternopleural sutures as in *Xyela*, *Pamphilius*, *Megalodontes*, *Arge* and *Cephus*. It is trapezoidal in *Megalodontes* and *Arge*, long and triangular in *Cephus*, and short and triangular in *Xyela* and *Pamphilius*. In every case it is divided into two by a median ridge.

(2) The sternopleural sutures are obsolescent and the basisternum is differentiated as a raised area as in *Xiphydria* and *Urocerus*. The median suture is present but does not reach the anterior limit of the sternum.

(3) The basisternum and the katepisterna are fused to form a composite plate, divided into two by the median suture. This condition is found in *Diprion*, *Selandria* and *Cimbex* and is probably a specialized condition.

The mesopleuron on each side has a distinct pleural suture in most of the sawflies except in *Xiphydria* and *Urocerus*, where it is incomplete. The pleural suture divides the pleuron into the episternum and epimeron. From the antero-dorsal end of the episternum is demarked a more or less triangular piece which has been variously designated, as already mentioned (Arora, 1953), and is the 'prepectus' of the taxonomists. Bird (1926) calls it the 'basalar sclerite' in *Hoplocampa* while Reeks (1937) names it as probably representing 'an epipleurite' in *Diprion*. This piece probably corresponds to the anepisternum. The pleural ridge on the inner side of the pleural suture runs right up to the extremity of this piece, showing thereby that the latter is a part of the episternum. In *Xyela* the suture that divides the episternum into two parts is not deep and the 'anepisternum'

is comparatively larger. In *Megalodontes*, where the 'prepectus' is supposed to be absent (Ross, 1937), a faint incomplete suture dividing the episternum into two parts can still be made out; the anepisternum is nearly as large as the katepisternum. Furthermore, the division of the meta-episterna into anepisterna and katepisterna suggests a similar division of the meso-episterna as well. The epimeron is narrow and divided into the anepimeron and katepimeron in a large number of sawflies, except in *Megalodontes*, *Cephus*, *Xiphydria* and *Urocerus*.

The furcasternum lies behind the basisternum and bears the furcal arms internally in the sawflies. These furcal arms differ in shape and size in the different families. The furcasternum sends a pair of lateral processes backward and outward, each articulating with the inner coxal margin of its side, a little above and posterior to the trochantinal coxal articulation.

The spinasternum is membranous, lying behind the legs and the diverging processes of the furcasternum. It can be better seen when the legs are pulled up and apart. There is, however, no spine.

B. Comparison with other insects: (Pl. IV, Figs. 4, 8–13)

The generalized Symphytan mesosternum with adjacent parts consists of (1) a differentiated presternum, (2) basisternum divided into two by a median suture and separated from the episterna by the pleurosternal sutures, (3) a narrow furcasternum, (4) episternum divided into anepisternum and katepisternum, and (5) is without a pre-episternum.

The Symphyta resemble *Raphidia*, *Panorpa*, *Limnephilus* and *Hepialus* in all the above characters. They differ from *Sialis* and *Hemerobius* in the latter having a faintly indicated presternum which is probably beginning to be lost. *Tipula* has specialized in one of the characters, i.e. the sterno-pleural suture is incomplete like some of the specialized sawflies. It is thus clear that the Symphyta and the Panorpoids have adhered quite closely to the generalized pattern.

The Symphyta differ from the beetle *Feronia* which has an undivided basisternum and a pre-episternum delimited from the episternum—two characters in which the beetle resembles the Orthopteroid insects.

The Symphyta differ from the Orthopteroid insects in so far that the latter possess a pre-episternum, undivided basisternum and episternum, and a furcasternum with broadly separated lobes. It is clear from the above that the Symphyta-Panorpoid complex and the Orthopteroids have followed two divergent lines of evolution and have very little in common.

7. *Coxal Articulations*

A. Symphyta: (Pl. IV, Figs. 5–7)

The coxal articulations present different modifications in the pro- and ptero-thoracic legs. The fore-coxae in the sawflies, in addition to the pleural articulation, have usually a sternal articulation which has been called the secondary sternal articulation by Weber (1933) in *Athalia*. Snodgrass (1910) was of the opinion that the trochantin, when it appears to be absent, as it does in the higher orders, might 'simply

be fused with the sternum in which case the apparent sternal coxal process may be really the trochantinal condyle'. This view is not acceptable because one finds in some sawflies mentioned below that the sternal coxal articulation coexists with the small reduced trochantin in the membrane in front of each coxa. If, as is presumed here, the condition of triple coxal articulation is the most primitive, one can say that most of the sawflies have retained two articulations, the pleural and the sternal, while the third, i.e. the trochantinal articulation, has disappeared with either the reduction of the trochantin to a mere vestige, lying in the precoxal membrane, or its complete disappearance. All the species of Symphyta have the pleural and the sternal articulations in the fore-legs, except *Megalodontes* and *Pamphilius*, in which there is the pleural articulation only. The first trochantin is a small sclerite lying in the membrane in front of each coxa in *Xyela*, *Diprion*, *Selandria* and *Arge*, while it is missing in the others. Snodgrass (1909) thought that a trochantin 'as a distinct sclerite was missing in all three segments' in the Hymenoptera but he mentioned a very small plate in *Arge*, on each side in front of the coxa, which from its position looks like the trochantin seen in the above Symphyta.

The mid- and the hind-coxae have retained all the three coxal articulations, the pleural, the trochantinal and the sternal, in all the sawflies examined. The trochantin in each case, though fused with the sternum for most of its length, can still be recognized. Its internal articular knob, by means of which it articulates with the coxal rim, is quite distinct, lying ventral and anterior to the sternal coxal articulation.

B. Comparison with other insects: (Pl. IV, Figs. 4, 8–13)

The generalized Symphytan shows that (1) the fore-coxa has pleural and sternal articulations, (2) the fore-trochantin, when present, is small and not articulating with the coxa, and (3) the mid- and hind-coxae have triple articulations, viz. pleural, trochantinal and sternal.

The significant fact resulting from this study is the uniformity among the Endopterygotes in possessing three articulations for the mid- and the hind-coxae. This appears to be a primitive condition from which the other conditions have probably been derived. Ferris and Pennebaker (1939) designate the sternal articulation as the ventral articulation, and according to them, 'the presence of this ventral condyle is one of the major features of orders which share the line of development in which the Neuroptera lie near the base'.

The Symphyta have retained two articulations (pleural and sternal) in the first leg, which character is shared by the Coleopteran *Feronia*, but sawflies such as *Megalodontes* and *Pamphilius* have lost the sternal articulation as well and have thus reached a stage which corresponds to that in the Panorpoids (i.e. they retain the pleural articulation only). It may further be pointed out that the Endopterygotes do not possess a trochantinal articulation for the first leg and that the trochantin, when present, is small.

The Symphyta and the other Endopterygotes differ in all respects from the Orthopteroid insects, the latter having the pleural and the

trochantinal articulations for all the legs, but never a sternal articulation. So the Symphyta, with the Endopterygotes, have followed a different line of evolution from that of the Orthopteroid insects.

The nearest allies of the Symphyta, on the basis of coxal articulation, are *Sialis*, *Hemerobius* (Neuroptera), *Panorpa* (Mecoptera), *Limnephilus* (Trichoptera) and *Feronia* (Coleoptera), which, together with some of the Symphyta (e.g. *Cimbex*, *Cephus*, *Xiphydria* and *Urocerus*), have moved a little away from the parent stock on the road to specialization (reduction).

8. *Cenchri*

A. Symphyta: (Pl. III, Figs. 6–10)

The cenchri are a pair of lateral membranous areas on the metascutum, one on either side of the middle line, near its anterior margin. Snodgrass (1909) mentions cenchri in Siricoidea and Tenthredinoidea as two small, oval lobes on the metanotum. They are membranous in *Diprion*, *Selandria* and *Arge*, elongated laterally in the former and egg-shaped in the other two. They are elliptical in *Cimbex* and *Megalodontes* but are elevated slightly in the former and appreciably in the latter. They are oval and raised on hard basal areas in *Pamphilius*, *Xyela*, *Xiphydria* and *Urocerus*. *Cephus* is the only genus which lacks the cenchri.

B. Comparison with other insects: (Pl. III, Figs. 11–13)

All the Symphyta possess lateral membranous cenchri on the metascutum except *Cephus* in which they are absent.

Raphidia and *Hemerobius* (Neuroptera) are the only two forms among the Panorpoids with corresponding membranous areas on the metascutum. *Sialis* possesses a median triangular, membranous area on the metascutum, but whether it has anything to do with cenchri is uncertain; if it has, then the condition in *Sialis* may either be primitive and has led to the paired areas by divergence and splitting, or may be specialized and is the result of the confluence of the two cenchri into a median area. The representatives of the other groups do not show any cenchrial areas. The presence, then, of lateral membranous areas in the Symphyta and some of the Neuroptera again places the Symphyta and the Neuroptera nearer each other.

9. *Tegula*

Snodgrass (1909) defines the tegula as 'the scale-like plate overlapping the front angle of the base of the wing in Lepidoptera, Hymenoptera and Diptera, and its pad-like representative at the base of the wing in the other orders'.

A. Symphyta: (Pl. III, Figs. 6–10)

The tegula is well sclerotized in the Symphyta but its shape and armature vary in the different families. It is like a pad, slightly longer than broad and beset with spines in *Selandria*, *Arge*, *Xyela* and *Pamphilius*. It is quadrant-shaped with its outer angle produced into a sharp point in *Diprion*, *Cephus* and *Megalodontes*, the surface bearing small setae in the first two, and long black spines in the third. The tegula is triangular with

rounded angles and furnished with small setae in *Cimbex* and *Xiphydria*. *Urocerus* is the only one with a weakly sclerotized tegula of the shape of a rounded, elevated lobe, with setae and pubescence.

B. Comparison with other insects: (Pl. III, Figs. 11–13; Pl. IV, Figs. 1–2)

The study of the tegulae in the Symphyta shows the tegula to be a large, strongly sclerotized scale-like structure at the base of the anterior margin of the wing. It is variously shaped in the different sawflies and clothed with spines and pubescence.

The Symphytan tegula resembles closely that in *Hemerobius* (Neuroptera) but differs from the Megalopteran tegulae, which are weakly sclerotized, raised lobes with setae (resembling the one in *Urocerus*). The Symphyta differ from the remaining Panorpoids which possess larger and more highly sclerotized and sculptured tegulae with stronger setae and thickly set long pubescence. One cannot say with certainty if the difference in the degree of sclerotization of the tegula and its armature throw any light on relationships or help us decide as to which condition is more primitive.

The tegulae of the Orthoptera and Dictyoptera on the other hand are distinctly unlike those of the Endopterygotes, being very small and membranous.

10. *Tibia and Tarsus*

A. Symphyta: (Pl. V, Figs. 1–6, 13)

The tibial spurs are spine-like structures, articulating with the distal end of the tibia and as such are called the apical tibial spurs. They may be accompanied by similar spurs, placed at some distance higher up on the tibia, called the preapical tibial spurs.

In the Symphyta, all tibiae possess one or two apical tibial spurs but the preapical tibial spurs are borne on the middle and the hind legs only. Ross (1937) mentions an exception in *Acantholyda* (Pamphiliidae), in which the front tibia is provided with a preapical spur.

There are two similar apical tibial spurs on the front tibiae in *Diprion* and *Cimbex*, two unequal but similar in *Arge* and *Megalodontes*, and two dissimilar spurs in *Selandria*, *Pamphilius* and *Xyela*, one of which is flattened, bifurcate and somewhat sickle-shaped and the other simple. *Cephus*, *Xiphydria* and *Urocerus* have but one modified apical spur only.

The middle and hind tibiae carry two similar apical tibial spurs in *Diprion*, *Selandria*, *Cimbex* and *Xiphydria*, which do not possess any preapical spurs. Two equal apical tibial spurs with two preapical spurs are present in *Xyela* and *Pamphilius*, two unequal apical accompanied by two preapical spurs in *Megalodontes* and *Cephus*, and two unequal apical spurs with one preapical in *Arge*. *Urocerus* is specialized in having a different armature in the middle and the hind tibiae, the former carrying one apical spur and no preapical, and the latter carrying two unequal pointed apical spurs and no preapical spur.

The tarsus is 5-segmented in all the sawflies, the first segment being the longest, the second, third and fourth progressively smaller, and the fifth longer than the second but smaller than the first. The tarsal pulvilli

are present on the first four tarsal segments in *Diprion, Selandria, Cimbex, Arge, Cephus* and *Xiphydria*. The tarsal pulvilli are not fully differentiated and are represented by elevated ridges in *Megalodontes* and *Pamphilius*, while they are absent in *Xyela*. *Urocerus* has no pulvilli but its tarsal segments are distally enlarged.

B. Comparison with other insects: (Pl. V, Figs. 15–17)

The generalized Symphytan possesses (1) articulated tibial spurs, (2) a 5-segmented tarsus, and (3) first tarsal segment (basitarsus) as the longest.

It is interesting to note that there is absolute uniformity among all the Endopterygotes examined in possessing the three above-mentioned characters, except for *Hepialus*, in which the tibial spurs are missing. The spurs in *Hepialus* have probably been secondarily lost as is borne out by the fact that allied Homoneura still possess them, e.g. the Prototheoridae and Anomosetidae (Turner, 1946), and the Micropterygoidea and the Eriocranoidea (Bourgogne in Grassé, 1951).

Among the Panorpoids, preapical tibial spurs on the middle and hind legs, in addition to apical tibial spurs, are present in Limnephilus (Trichoptera) only, recalling a condition in some sawflies, e.g. *Xyela* and *Pamphilius*, etc. Preapical spurs on the front tibiae are present in *Feronia*, corresponding to the condition cited by Ross (1937) in *Acantholyda* (not found in any other sawfly examined). It cannot be ascertained which of the two conditions, with or without the preapical spurs, is primitive, so the precise relationship between the different Endopterygote orders cannot be established.

Flap-like tarsal pulvilli, resembling those of certain Symphyta mentioned earlier, have been noted in *Tipula* (Diptera), while distal tarsal enlargements, corresponding to the distal tarsal enlargements in the sawfly *Urocerus*, are present in the Megaloptera and the Coleopteran *Feronia*. Here again the primitive condition is difficult to determine and the tarsal pulvilli do not afford a clue to relationships.

The absence of articulated apical and preapical tibial spurs in the Orthopteroid insects separates them from the Symphyta and the other Endopterygotes; the former, instead, possess rows of large, flexible, hollow spines, quite different from the tibial spurs. In the matter of the tarsus, though *Periplaneta* shows a resemblance to the Symphyta and its allies, *Nomadacris* and *Chloroperla* differ from them in having a reduced 3-segmented tarsus with the last tarsal segment the longest.

11. *Pretarsus*

A. Symphyta: (Pl. V, Figs. 5–10, 13)

A typical Symphytan pretarsus has already been described in *Diprion pini* (Arora, 1953). Minor differences are noticeable in the different families of the group. All the Symphyta agree in possessing a highly sclerotized and sculptured unguitractor plate. In most of them this plate shows some grooves and ridges, except in *Megalodontes, Pamphilius* and *Xyela*.

The planta is a broad sclerotized plate in all, except *Cimbex*, where it is a narrow band. There are two long spines on the planta in *Xyela*, *Cephus*, *Diprion*, *Pamphilius*, *Cimbex* and *Xiphydria*, and more than two in others.

A sclerite, the orbicula, is present at the base of the arolium dorsally in all the sawflies. It is generally longer than broad and may be shield-shaped as in *Selandria*, *Cimbex*, *Arge* and *Xyela*, more or less triangular with rounded angles in *Diprion*, *Megalodontes* and *Pamphilius*, elongated and rod-like in *Cephus*, and elongated and pointed anteriorly in *Xiphydria* and *Urocerus*.

A pair of basipulvilli (auxilia) is present at the bases of the claws, one on either side of the planta, in every species examined.

The arolium is large and membranous, more or less trumpet-shaped and slightly bilobate, provided distally with a strong, well developed camera in *Diprion*, *Xyela*, *Cephus* and *Xiphydria*, and rather a weak one in others. *Urocerus* is the only genus without an arolium. According to Holway (1935), no arolium is present in *Tremex*.

Ungues or claws are simple in *Xyela* and *Arge*, while in others there is a tooth at the base of each claw.

B. Comparison with other insects: (Pl. V, Figs. 11, 12, 14, 15, 18)

The generalized Symphytan pretarsus is characterized by (1) a large strongly sclerotized planta with spines, (2) the presence of an orbicula, (3) the presence of basipulvilli, and (4) a large membranous arolium with a camera.

Hemerobius is the only Neuropteran in which the pretarsus agrees closely with the Symphytan condition, differing only in having a narrow planta (found also in *Cimbex*). The other Neuroptera and the Panorpoids do not show such close relationship, differing from the Symphyta in more than one character. It is very difficult in the case of the pretarsus to say which condition of each character should be considered primitive, and consequently one is not sure whether the Symphytan pretarsus is primitive or specialized. As pointed out by Holway (1935), the number of spines and the sclerotization of the planta may be helpful in determining affinities. And surely this brings the Symphyta and the Neuroptera much closer than the other Panorpoids with a narrow planta. Also, the weakly sclerotized planta, with one or no spines, and the unsculptured unguitractor plate, found among the Orthoptera and the Dictyoptera, remove these orders from the Symphyta and its allies.

The absence of a planta in the Diptera and the Coleoptera probably shows that these are specialized. The presence of setiferous pulvilli, covering the bases of the claws, sets apart another group of insects, the Diptera, the Trichoptera and the Lepidoptera, as more specialized than their Panorpoid and Symphytan relatives.

Most of the non-Symphytan species possess simple claws like *Xyela* and *Arge*, but in *Raphidia* and *Feronia* each claw has a basal tooth (like most of the sawflies); *Panorpa* and *Osmylus* (Killington, 1936) have claws which are provided with basal teeth forming a pecten. The claws, as

such, do not afford any clues to precise relationships because one cannot say which condition of the claw, the simple or the toothed, is primitive.

The study of the pretarsus thus reveals that the Symphyta are much closer to the Neuroptera than the Mecoptera and other Panorpoids, that grouping within the Panorpoid orders can be obtained on the basis of certain characters, and that the Orthopteroid insects are far removed from the Symphyta.

12. *Wings*

A. Symphyta : (Pl. V, Figs. 19–25; Pl. VI, Figs. 1–4)

Ross (1936) has discussed the different systems of nomenclature for the Hymenopterous wing. It is not intended here to go over the same ground again. Suffice it to say that the interpretations of Ross, based on the derivation of the Hymenoptera from *Sialis*-like Panorpoid, are accepted because they agree with the conclusions arrived at after a study of a much wider range of characters, drawn from different parts of the body.

The description of the wings of *Diprion pini* (Arora, 1953) is based on Ross's system. There are a few venational variations met with in the other families of the Symphyta. Ross (1936) considered a hypothetical wing from which the wing-venation in the different families could easily be derived by atrophy of one or the other vein—presumably what must have happened during the course of evolution.

A pterostigma is present in all the sawflies. The margins bear fine pubescence. The costa runs along the anterior margin of the wing. The subcosta is fused with the radius in most of the sawflies except in *Pamphilius*. Ross (1937) mentions a free subcosta in Xyelidae as well, but this condition does not exist in *Xyela julii*, where the subcosta is fused with the radius for most of its length. Benson (1951) has also stated a similar fusion of the subcosta with the radius in *Xyelinae*. *Rs* is a simple vein in the group, except in *Xyela* in which it divides into two branches. Ross (1937) also describes the branching of *Rs* in *Pleroneura* (Xyelidae). There are two radial cross-veins in most of the sawflies but only one in *Diprion* and *Arge*. *M* and Cu_1 run as a common vein for the basal part. *M* separates from Cu_1 and meets *Rs* before the latter separates from *R* in *Diprion*, *Selandria*, *Arge* and *Cimbex*. *M* meets *Rs* after *Rs* has separated from *R* in the others, except in *Urocerus*, in which *M* does not meet *Rs* at all. The last condition, which is supposed to be primitive (Ross, 1937), is also met with in *Pleroneura*. There are two radio-medial cross-veins in every case, except in *Urocerus* in which there are three. After separating from *Rs*, *M* goes straight to the wing-margin unbranched.

There are two medio-cubital cross-veins in all the sawflies, except again in *Urocerus*, where an additional third stub is present.

Cu_1 is divided into Cu_{1a} and Cu_{1b} in all cases.

Cu_2 is mostly absent except in a reduced condition in the hind-wings of *Xyela*. One cubito-anal cross-vein exists in all the wings.

1A is always present but *2A* and *3A* are variously atrophied. The anal cross-vein is generally present and two anal cells are formed, except

in *Selandria*, in which the anal cross-vein is lost and there is one cell only. *Urocerus*, however, has the bases of all the three anal veins intact.

The hind-wing bears the hook-shaped hamuli on its anterior margin near its middle and a few setae near its base. The hamuli catch on the curved posterior margin of the fore-wing and form a coupling apparatus. There is a reduction in the size and number of veins and cross-veins in the hind-wing. There is no radial cross-vein in any of the species examined, though one has been mentioned by Ross (1936) in *Adelomus*. *Rs* and *M* do not run together as they do in the fore-wing. There is only one medio-cubital cross-vein in all except *Urocerus*, in which there are two. An anal lobe is present.

B. Comparison with other insects : (Pl. VI, Figs. 5–9)

The generalized Symphytan wing is characterized by (1) well developed pterostigma, (2) *SC* fused with *R* (free in Pamphiliidae and Xyelidae), (3) *Rs*, when branched, showing dichotomous branching, (4) *M* and *Cu* confluent, (5) Cu_1 divided into Cu_{1a} and Cu_{1b}, (6) Cu_2, when present, very much reduced (generally absent), (7) variously atrophied anals, (8) two radial cross-veins, (9) 2 radio-medial cross-veins (3 in *Urocerus* and some Xyelidae), (10) 2 medio-cubital cross-veins, (11) a cubito-anal cross-vein, (12) reduction of veins and cross-veins in hind-wing, and (13) hamuli on hind-wing, forming the coupling apparatus.

Of the above characters, the free condition of *SC* (in Pamphiliidae and Xyelidae), the division of *Rs* into two or more branches (Xyelidae), the reduced Cu_2 (Xyelidae), and 3 radio-medial cross-veins as a result of *M* and *Rs* not meeting each other (Siricidae and Xyelidae) are characters which are probably primitive. Most of the Symphyta have specialized in the same direction but these primitive features are retained by some, particularly the family Xyelidae which has been regarded by Hymenopterists as a primitive family of the Symphyta.

Ross (1936) derived the Hymenopterous wing from a *Sialis*-like ancestor on the basis of definite number of cross-veins between the longitudinal veins. According to him, 'a reduction of the longitudinal veins does not necessarily imply a corresponding reduction of the cross-veins'. There is a close similarity between the wings of the Symphyta and the Neuroptera and their allies. In characters such as 3 radial cross-veins, Cu_2 normally developed and normal anals, in which the Symphyta differ from *Sialis* and other Panorpoids, the latter are more primitive. Of these primitive characters, there are a few (e.g. 3 radial cross-veins and normal anals) which are present either in *Sialis* alone or in the Neuroptera. Furthermore, *Sialis* or the Neuroptera do not possess any wing character in which they can be said to be more highly specialized than the Symphyta. It follows, therefore, that the Neuroptera are the most primitive existing representative of the stock from which the Symphyta and the Panorpoids arose.

The Symphyta and the Neuroptera are again more primitive than the Mecoptera and the Diptera in having Cu_1 divided into Cu_{1a} and Cu_{1b} (undivided in others). They are more primitive than Diptera, Trichoptera

and Lepidoptera in having two medio-cubital cross-veins (only one in others). As such the latter groups cannot be ancestral either to the Symphyta or the Neuroptera.

The absence of a pterostigma, the pectinate type of branching of *Rs*, the large number of cross-veins forming a network, Cu_1 with more than two branches, the anals meeting proximally, and the comparatively larger hind-wing with more veins than in the fore-wing are some of the characters in which the Dictyoptera and the Orthoptera differ from the Symphyta and most of the Panorpoids. The Symphyta, thus, have very little in common with the Orthopteroid insects and so the suggestion of an Orthopteroid ancestry for the Hymenoptera is unlikely.

13. *Ovipositor*

A. Symphyta: (Pl. VI, Figs. 10–17)

There is a great uniformity in the structure of the ovipositor in the Symphyta. The ovipositor may be exserted or abbreviated and the valves partly or completely fused in the different families.

The seventh sternum is produced into a subgenital plate, which covers the base of the ovipositor, in most of the sawflies, except *Megalodontes*, *Pamphilius* and *Cephus* in which there is a very slight basal retraction, if any, of the ovipositor.

The eighth sternal region is mostly membranous with a small sclerotization in the middle, between the bases of the first valvulae. The venter of the eighth segment in *Pteronidea*, according to Snodgrass (1933), 'is represented only by the membranous integument forming the anterior wall of the vestibulum containing the genital aperture'.

The ninth sternum is reduced to a membranous strip, connecting the bases of the second valvifers.

The ovipositor shaft in the sawflies is formed by the paired first and second valvulae. The first valvulae are always separate in most of the sawflies and lodged at rest in the ventral concavity of the partly or completely fused second valvulae. *Xyela* is an exception in that the first valvulae are almost fused with the second valvulae and the two pairs form a composite blade.

The second valvulae are fused all along their length in *Megalodontes*, *Pamphilius* and *Arge*. They have their tips free in *Diprion*, *Selandria*, *Xiphydria* and *Urocerus*, while their distal one-third is free in *Cimbex* and *Cephus*.

The third valvulae form the sheath for the other two valvulae, their length depending on the length of the saw.

The ovipositor is short and scarcely projecting beyond the tip of the abdomen in *Megalodontes* and *Pamphilius*. The saws are small and hardly visible from above in *Diprion*, *Selandria* and *Arge*. The valves are slightly exserted in *Cimbex* and *Cephus*, and strongly extruded in *Xyela*, *Xiphydria* and *Urocerus*.

B. Comparison with other insects: (Pl. VII, Figs. 1–6)

The Symphytan ovipositor is characterized by (1) being an appendicular ovipositor, (2) the seventh sternum more or less produced into a subgenital plate, (3) reduced eighth sternum, (4) ninth sternum reduced and occupying area between the bases of the inner ovipositor components, and (5) ovipositor shaft formed by first and second valvulae, the third being sheath-like.

There is a marked uniformity in the structure of the ovipositor in the Symphyta.

The Symphyta resemble the Megalopteran *Raphidia* in all characters except the formation of the ovipositor sheath. Two processes are given off in *Raphidia* from the weakly sclerotized eighth sternum, on the sides and below. They unite posteriorly to form a slender median prolongation, the fused first valvulae, with flattened bases, the first valvifers; the median prolongation (very short in *Agulla* and called the eighth sternite by Ferris and Pennebaker, 1939) lies within and extends almost to the tip of the fused second valvulae. The ninth tergum extends laterally and ventrally and a more or less triangular sclerite, possibly the second valvifer, articulates anteriorly with it on either side. The inner margin of this second valvifer is produced posteriorly into an elongated second valvula; the two valvulae are connected by a membrane throughout their length, except at their tips, where each bears a small lobe beset with strong setae. Grassé (1951) regards these two valves as possibly gonopods. The two valves (i.e. first and second), thus, in both Symphyta and *Raphidia* take part in the formation of the ovipositor shaft, but in *Raphidia* the third valve (sheath-like in Symphyta) is missing unless it be assumed that the small lobes at the end of the shaft are reduced third valvulae. The ovipositor in the other Panorpoids is vestigial and lacks valves, but there exists some sort of an apparatus for oviposition. Reduced sternal lobes or processes have been homologized differently by different authors and it is unsafe to make comparisons.

The Symphyta, though they apparently resemble the Dictyoptera and the Orthoptera in the possession of an appendicular ovipositor, differ fundamentally from them in possessing an ovipositor shaft formed by two valves only; the ovipositor shaft in the others being formed by three valves. Furthermore, the ovipositor in the Dictyoptera is reduced and internal, while in the Orthoptera it is very much specialized with reduced second valvulae. In some of the Orthopteroid forms, even when the ovipositor is typically formed by three pairs of well developed valves, e.g. in Grylloblattidae and Tettigoniidae (Snodgrass, 1935, and Kramer, 1944), the third pair of valves is not sheath-like. From the above study it becomes clear that the Symphyta have their nearest relatives in the Neuroptera and that the Orthopteroid insects have followed a different line.

14. *Male Genitalia*

A. Symphyta: (Pl. VII, Figs. 7–15)

Snodgrass (1941) gave a comprehensive comparative account of the male genitalia of Hymenoptera, devoting a section to the Chalastogastra.

Crampton (1920) and Ross (1937) had likewise dealt with the male genitalia, using a somewhat different terminology. It is not intended here to give detailed accounts of the various component structures, but reference will be made only to the differences presented by the larger families of the group and to characters, which, on close study, appear to offer material for comparison with the other orders of insects. The terminology of Snodgrass has been followed but corresponding names of parts by other authors have in some places been given as well, with a view to understanding homologies.

The male genital apparatus of *Diprion pini* (Arora, 1953) is a generalized one for the Symphyta. Two types of genitalia are met with, viz. the strophandrious (inverted condition), represented by *Diprion, Selandria, Cimbex, Arge* and *Xyela,* and the orthandrious (normal condition) met with in the others. Ross (1937) placed the Xyelidae with the Megalodontidae and treated it as orthandrious, but Snodgrass (1941) found *Xyela minor* and *Pleroneura koebelei* (Xyelidae) as strophandrious like *Xyela julii.* The Family Xyelidae, thus, is peculiar in having both the orthandrious and the strophandrious types.

The ninth sternum in the male forms a subgenital plate, convex externally. In the concavity of this plate lies the phallus, loosely connected to it by membrane. The phallus is a compact structure, with its parameres, volsellae and aedeagus borne on the basal ring.

The parameres are articulated with the parameral plates in all the species studied, except in *Cephus* in which the parameral plates and the parameres form continuous structures without any line of division. It has been shown by Ross (1937) that the parameres (harpes) and parameral plates (gonostipes) in the Cephoidea are 'separated either by an indistinct suture or none at all', whereas Snodgrass (1941) regards the parameres in Cephoidea as well as in Orussidae to be continuous with the parameral plates as in *Cephus.* Cupping disks (gonomaculae of Crampton, 1920) are present at the tips of parameres in *Megalodontes, Pamphilius, Xyela, Xiphydria* and *Urocerus,* and absent in the others. According to Snodgrass (1941), they are limited to some families with articulated parameres.

The volsellae are ventral in orthandrious and secondarily dorsal in the strophandrious species. They generally terminate in a pincer-like structure (formed by the inner digitus and the outer cuspis) in *Diprion, Selandria, Cimbex, Arge, Cephus* and *Xiphydria.* The terminal volsellar part in *Xyela* is provided with corresponding lobe-like digitus and cuspis, while it is represented by a single setiferous digitus in *Megalodontes, Pamphilius* and *Urocerus,* with the cuspis very much reduced.

The penis valves are of various shapes and sizes in the different families.

B. Comparison with other insects: (Pl. VII, Figs. 16–21)

The male genital apparatus in the Symphyta is characterized by (1) ninth sternum forms an exposed subgenital plate, without styli, (2) phallus is loosely connected with the ninth sternum, (3) phallus is compact, with parameral plates, etc. (gonocoxites and gonostyli) and aedeagus borne on the basal ring, and (4) volsellae are present.

Attempts at homologizing parts of the male genitalia throughout the orders of insects have been made by Newell (1918), by Crampton (1920) and by Michener (1944). Snodgrass (1935) believes that certain structures, like the claspers, developed differently in the different groups. He, thus, regards the Hymenopterous outer claspers (parameres) as of phallic origin and the corresponding outer claspers of Diptera, Lepidoptera, etc., as periphallic organs. In 1941, he reviews the whole question of the origin of these claspers and finds the existing evidence inconclusive. According to him, the lobes which, in immature forms, give rise to the claspers in the Hymenoptera, divide incompletely and develop into the outer and the inner claspers, while in Trichoptera and Lepidoptera, as shown by Zander (1901 and 1903), the outer claspers arise from the outer division of such lobes and then migrate to establish their association with the ninth segment. From this it appears that the outer claspers of Hymenoptera, Trichoptera and Lepidoptera (and possibly of others, like Diptera and Mecoptera, etc.) are homologous structures. Mehta (1933), however, claims that the outer claspers of Lepidoptera arise independently, and one is, therefore, not sure of the validity of the homologies. From the comparative morphological study of the genitalia of the Holometabolous insects, it is apparent that there is a uniform structural plan throughout and that Michener's terminology is quite satisfactory.

There is a marked structural uniformity among the Symphyta. They resemble the Panorpoids in three out of the four listed Symphytan characters, differing from them in the loosely connected phallus. While the Mecopteran shows the closest alliance with the Symphyta, the three characters are also shared by one or the other Neuropteran as well, suggesting that the Symphyta, the Mecoptera and the Neuroptera have come from the same stock and that the Symphyta have specialized in having a loosely connected phallus—a character which the others have still retained in the primitive condition. On the basis of this study, one cannot definitely say which one of the groups is ancestral to the Symphyta.

Another interesting observation is the close resemblance between the Symphyta and the Coleoptera in that both possess a phallus loosely connected to the genital chamber.

The Orthopteroid insects, which possess a subgenital plate with styli (e.g. in *Periplaneta*) and a phallus with lobes and sclerites and lacking in the volsellae, do not show sufficient resemblance to merit a close alliance with the Symphyta or the Panorpoids.

15. *Abdominal Terminalia*

A. Symphyta: (Pl. VI, Figs. 10–17)

The abdomen of the Symphyta possesses ten segments, the terminal one bearing the anus. The tenth segment bears a pair of appendages, the cerci (socii), which have been termed pygostyles by Snodgrass (1941). According to him, the eleventh segment in the larvae of Symphyta (Chalastogastra) is absent and the pygopods of the tenth larval segment (as in *Gilpinia polytoma*) ultimately give rise to these pygostyles. Furthermore

he was able to observe a pair of small, similar, setigerous appendages on the everted anal vesicles in the male of the Ichneumonid *Megarhyssa lunator*, in addition to the pygostyles. He, therefore, regards the appendages of the tenth segment (the pygostyles) as different from the true cerci of the eleventh segment. It may be mentioned here that *Megarhyssa lunator* is the only example so far known in which both the structures are supposed to coexist and even then only in the male.

In the absence of more convincing data, one can say that the cerci in the case of the Symphyta are borne on the tenth segment while in others, where there are more than ten segments, they are borne on the eleventh. One cannot easily dismiss the possibility of the eleventh segment having fused with the tenth or vice versa in the case of the Symphyta because this process of reduction seems to have gone still further in the higher Hymenoptera, e.g. *Lariophagus distinguendus* (Chalcididae), in which the terminal segment is apparently ninth but appears to be ninth and tenth fused and that it is this segment which bears the very reduced cerci.

The cerci in the Symphyta are unsegmented processes bearing setae and trichobothria. They are small and pointed in *Diprion*, *Selandria*, *Cephus* and *Xyela*. They are short and conical in *Cimbex*, *Arge*, *Megalodontes* and *Pamphilius*, while they are short and stump-like in *Xiphydria* and *Urocerus*.

B. Comparison with other insects: (Pl. VII, Figs. 1–3, 6, 19, 22)

The Symphytan abdominal terminalia is marked by (1) tenth segment being the terminal one, and (2) cerci being unsegmented and borne on the tenth segment.

As has been pointed out earlier, there is no deviation from the above condition of abdominal terminalia among the Symphyta. The size of the cerci, however, varies, and in forms like *Urocerus* they are like small stumps.

The Symphyta resemble the Diptera*, the Trichoptera and the Lepidoptera in all respects but differ from the Neuroptera because the latter do not possess cerci as such and have, instead, elevated areas with trichobothria on the tenth segment. Similar elevated areas have been designated as cerci by Maki (1936) in *Chauliodes* (Megaloptera) and by Grassé (1951) in *Sialis*. In the Megalopteran *Corydalis* (Grassé, 1951), however, unsegmented cerci have been mentioned. It appears, therefore, that the elevated areas of the Neuroptera are modified cerci. Furthermore, a very similar condition prevails in *Lariophagus distinguendus* in which there is a raised area or protuberance with three trichobothria on the terminal ninth segment, which shows that the elevated areas correspond to the cerci. It is difficult to say which condition of the cerci is more primitive but the one can easily be derived from the other. If this is so, the Symphyta and all the Panorpoids, except Mecoptera, have complete uniformity and as such are closely related. The Mecoptera differ from Symphyta and other Panorpoids in having an 11-segmented abdomen and two-segmented cerci. Whether

* Some Diptera, however, have segmented cerci (e.g. some Stratiomyiidae).

this is a specialized or a primitive condition with them cannot easily be determined. Their resemblance to the Orthopteroids in these characters may purely be a matter of convergence, for they differ from the latter in most of the other characters examined.

The Symphyta and the Panorpoids differ considerably from the Orthopteroid insects because the latter possess eleven abdominal segments and bear the multi-articulated cerci (unsegmented in *Nomadacris*, etc.) behind the tenth segment, in relation to the eleventh segment.

General Conclusions

From the foregoing comparative study it is possible to make a detailed morphological characterization of a generalized Symphytan and this has been compared with seven Panorpoid insects, with the beetle *Feronia*, and with one representative each of the Dictyoptera, Orthoptera and Plecoptera.

The character of the Symphyta is deduced from a study of sixty-six features of their external anatomy, drawn from different parts of the body. These characters are shown in the form of a chart. The generalized Symphytan may be characterized as having the condition (A) of the sixty-six characters (mentioned in column 2 of the chart), while deviations from the generalized condition (A) are represented by condition (B), (C) or (D) as the case may be.

The morphological study has to some extent been supplemented by other published work for purposes of discussion, though the preceding list of characters in the chart is based on the present work alone. By ascribing arbitrary marks to each condition it has been possible to assess the degree of overall resemblance of the Symphyta to the other groups. In this way the following figures were obtained (also shown at the end of the chart):—

Generalized Symphytan	100%
Raphidia notata	79%
Hemerobius stigma	78%
Limnephilus marmoratus	78%
Sialis lutaria	77%
Hepialus humuli	72%
Panorpa germanica	68%
Tipula fascipennis	63%
Feronia madida	51%
Chloroperla torrentium	39%
Periplaneta americana	23%
Nomadacris septemfasciata	22%

This clearly shows how much closer the characters of the Symphyta are to those of the Panorpoid orders than to those of the Orthopteroids.

In order to examine whether the Symphyta are more closely related to one particular group of Panorpoids than to another, it is necessary first to divide the sixty-six characters into four groups. These are:—

(*a*) 18 characters common to the generalized Symphytan and all the Panorpoid types studied:

Nos. 2, 6, 9, 14, 17, 19, 22, 23, 24, 26, 28, 29, 30, 33, 36, 37, 44, 60.

(*b*) 20 characters of the generalized Symphytan which, though not occurring in all the Panorpoid types, are yet found in many of them and agree with what is probably the primitive Panorpoid condition:

Nos. 1, 3, 4, 7, 12, 15, 18, 25, 27, 32, 35, 38, 41, 42, 43, 46, 50, 55, 63, 64.

(*c*) 7 characters found only in the generalized Symphytan:

Nos. 21, 31, 45, 48, 52, 59, 61.

(*d*) 21 characters found in the Symphyta and in some Panorpoids but which are probably not primitive Panorpoid characters or are difficult to evaluate:

Nos. 5, 8, 10, 11, 13, 16, 20, 34, 39, 40, 47, 49, 51, 53, 54, 56, 57, 58, 62, 65, 66.

Characters in groups (*a*) and (*b*) demonstrate the Panorpoid affinities of the Symphyta and those in group (*c*) show the way in which the Symphyta are peculiarly specialized but cannot help in deciding which particular group of Panorpoids the Symphyta most closely resemble. Characters in group (*d*), while possibly including some which ought, if their significance were properly understood, to be transferred to group (*a*) or (*b*), are nevertheless those which have to be considered, in conjunction with characters of group (*b*), in deciding which Panorpoid types resemble the generalized Symphytan most closely. An analysis of these forty-one characters of groups (*b*) and (*d*) shows the following percentage resemblances, taking Symphyta equal to 100%:—

Group of insects	Percentage resemblance		
	Characters of group (*b*)	Characters of group (*d*)	Total % of (*b*) and (*d*)
Generalized Symphytan ..	100%	100%	100%
Sialis	81%	71%	76%
Raphidia	80%	77%	79%
Hemerobius	75%	83%	79%
Generalized Neuropteran	95%	94%	94%
Limnephilus	87%	68%	77%
Panorpa	82%	44%	63%
Hepialus	73%	64%	68%
Tipula	56%	50%	53%

The relatively small resemblance of *Panorpa* and *Tipula* is noteworthy and the Symphyta are closer to the generalized Neuropteran than to the other Panorpoid orders particularly when one finds that out of the forty-one characters, thirty-seven are such as are possessed by one or the other Neuropteran. In the remaining four characters (Nos. 16, 49, 53 and 64), where a decision is possible, the Neuroptera are more primitive than the Symphyta in two characters (Nos. 49 and 53). In the third character (No. 16), the primitive condition cannot be determined, while in the fourth character (No. 64, pertaining to cerci) the Symphyta are probably more

primitive than any of the Neuroptera examined. It may be pointed out that unsegmented cerci, similar to the Symphytan cerci, are met with in the Neuropteran *Corydalis* (Grassé, 1951), as mentioned earlier under abdominal terminalia, so the generalized Neuropteran resembles the generalized Symphytan almost completely. This striking resemblance seems to suggest that the Symphyta and the Neuroptera probably arose from a common stock, more generalized than the existing groups.

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Abbreviations

A—Anal vein; *Ab*—Abdominal segment; *Acr*—Alacristae; *Aed*—Aedeagus; *Aem*—Anepimeron; *Aes*—Anepisternum; *Agl*—Alaglossa; *An*—Anus; *Ar*—Arolium; *Ar. Scl*—Arched sclerite; *Ata*—Anterior tentorial arm; *Atp*—Anterior tentorial pit; *Ax.C*—Axillary cord; *B.p*—Basipulvilli; *B.R*—Basal ring; *B.S*—Basisternum; *B.T*—Body of the tentorium; *C*—Costa; *Ca*—Camera; *Cd*—Cardo; *Ce*—Cercus; *Cen*—Cenchri; *Cl*—Claw; *Cu*—Cubitus; *cua*—Cubito-anal cross-vein; *Cup.d*—Cupping disk; *Cx*—Coxa; *Dta*—Dorsal tentorial arm; *Dte*—Distal tarsal enlargement; *Eg*—Egg-guide; *Ej.d*—Ejaculatory duct; *Epm*—Epimeron; *Epr*—Epiproct; *Eps*—Episternum; *F.P*—Frontal plate; *F.S*—Furcasternum; *Fu*—Furcal arm; *Ga*—Galea; *Gcx*—Gonocoxite; *Gl*—Glossa; *Gsty*—Gonostyle; *Gu*—Gula; *Ham*—Hamuli; *Hau*—Haustellum; *Hyp*—Hypopharynx; *Ju.l*—Jugal lobe; *Kem*—Katepimeron; *Kes*—Katepisternum; *Lac*—Lacinia; *Lig*—Ligula; *Lp*—Labial palp; *L.phlm*—Left phallomere; *M*—Media; *Mb*—Membrane; *Mb.a*—Membranous area; *mcu*—Medio-cubital cross-vein; *Me*—Meron; *Mt*—Mentum; *Mxd*—Maxadentes; *Mxl*—Maxillaria; *Mxp*—Maxillary palp; *Oc.C*—Occipital condyle; *O.F*—Occipital foramen; *Oe.t*—Oesotendon; *Orb*—Orbicula; *Pf*—Palpifer; *Pgl*—Paraglossa; *Phl*—Phallus; *Phlm*—Phallomere; *Phr*—Phragma; *Phlt*—Phallotreme; *Pl*—Planta; *Pl.R*—Pleural ridge; *Pl.S*—Pleural suture; *Pm*—Postmentum; *Pm.p*—Parameral plate; *Pmr*—Paramere; *Pm.Scl*—Postmental sclerite; *P.N*—Postnotum; *Ppr*—Paraproct; *Preps*—Pre-episternum; *Prm*—Prementum; *Pr.N*—Pronotum; *Pr.S*—Presternum; *Pr.S.B.*—Presternal bridge; *Pr.Sc*—Prescutum; *Pr.T*—Pretarsus; *Ps*—Parapsides; *Ps.S*—Parapsidal suture; *Pt*—Post-tergite; *Ptp*—Posterior tentorial pit; *P.T.S*—Preapical tibial spur; *Pts*—Pterostigma; *Pul*—Pulvillus; *P.V*—Penis valve; *R*—Radius; *Rd.B*—Reduplicated band; *Rs*—Radial sector; *r*—Radial cross-vein; *rm*—Radio-medial cross-vein; *r.phlm*—Right phallomere; *S*—Sternum; *S.a*—Sternal articulation; *SC*—Subcosta; *Sc*—Scutum; *Scl*—Scutellum; *Sl.D*—Salivary duct; *Sm*—Submentum; *Sp*—Spiracle; *Sp.R*—Suspensorial rod; *Sq*—Squama; *S.S*—Spinasternum; *St*—Stipes; *Sty*—Stylus; *T*—Tergum; *Ta*—Tarsus; *Ta.d*—Tarsal disc; *Ta.S*—Tarsal segment; *Teg*—Tegula; *Tp*—Tarsal pulvilli; *Tr*—Trochantin; *Tr.a*—Trochantinal articulation; *Trb*—Trichobothria; *T.S*—Tibial spur; *T.Sp*—Tibial spine; *T.Sc.S*—Trans-scutal suture; *T.Sg*—Terminal segment; *Uf*—Unguifer; *Utp*—Unguitractor plate; *V*—Vannals; *Vl*—Valvula; *Vlf*—Valvifer; *Vol*—Volsella; *Vul*—Vulva.

Explanation of Plates

Plate I

Fig. 1. Maxilla of *Selandria serva*.
„ 2. Maxilla of *Cimbex femorata*.
„ 3. Maxilla of *Arge ochropus*.
„ 4. Maxilla of *Megalodontes klugii*.
„ 5. Maxilla of *Pamphilius stramineipes*.
„ 6. Maxilla of *Xyela julii*.
„ 7. Maxilla of *Cephus pygmaeus*.
„ 8. Maxilla of *Xiphydria prolongata*.
„ 9. Maxilla of *Urocerus gigas*.
„ 10. Maxilla of *Raphidia notata*.
„ 11. Maxilla of *Hemerobius stigma*.
„ 12. Maxilla of *Sialis lutaria*.
„ 13. Maxilla of *Nomadacris septemfasciata*.
„ 14. Labium of *Cephus pygmaeus*.
„ 15. Labium of *Selandria serva*.
„ 16. Labium of *Cimbex femorata*.
„ 17. Labium of *Arge ochropus*.
„ 18. Labium of *Megalodontes klugii*.
„ 19. Postmental sclerites of *M. klugii*.
„ 20. Labium of *Xiphydria prolongata*.

CHART—*concld.*

SYMPHYTA								OTHER INSECTS										
Arge ochropus	*Megalodontes klugii*	*Pamphilius stramineipes*	*Xyela julii*	*Cephus pygmaeus*	*Xiphydria prolongata*	*Urocerus gigas*	Gen. Symphytan	*Sialis lutaria*	*Raphidia notata*	*Hemerobius stigma*	*Panorpa germanica*	*Tipula fascipennis*	*Limnephilus marmoratus*	*Hepialus humuli*	*Feronia madida*	*Periplaneta americana*	*Nomadaeris septemfasciata*	*Chloroperla torrentium*
A	A	A	B	A	A	A	A	C	C	C	C	C	C	B		C	C	C
A	A	A	A	A	A	A	A	B	B	B	A	A	A	A	ELYTRA	C	A	B
A	A	A	A	A	A	A	A	A	A	A	A	A	A	B		C	C	A
A	A	A	A	A	A	A	A	B	A	B	B	B	B	B	B	A	A	B
A	A	A	A	A	A	A	A	B	A	A	B	B	B	B	B	A	B	B
A	A	A	A	A	A	A	A	B	A	C	B	B	B	B	B	A	B	B
A	A	A	A	A	A	A	A	B	A	B	B	B	B	B	B	A	A	B
A	A	A	A	A	A	A	A	C	C	C	C	C	C	C	C	B	B	C
A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	C	A	A

CHART

No.	CHARACTER	SYMPHYTA									
		Diprion pini	*Selandria serva*	*Cimbex femorata*	*Arge ochropus*	*Megalodontes klugii*	*Pamphilius stramineipes*	*Xyela julii*	*Cephus pygmaeus*	*Xiphydria prolongata*	*Urocerus gigas*
	I—MAXILLA										
1	*CARDO* Undivided (A) Divided (B)	A	A	A	A	A	A	A	A	A	A
2	*STIPES* Undivided elongate (A) Divided (B)	A	A	A	A	A	A	A	A	A	A
3	*GALEA* Simple lobe (A) Divided (B) Absent (C)	A	A	A	A	A	A	A	A	A	A
4	*LACINIA* Simple, without maxadentes (A) With maxadentes (B) Absent (C)	A	A	A	A	A	A	A	A	A	C
5	*MAXILLARY PALP* 6-segmented (A) 5-segmented (B) Less than five (reduced) (C)	A	A	A	A	A	A	A	A	B	C
	II—LABIUM										
6	*POSTMENTUM* Undivided (A) Divided (B)	A	A	A	A	A	A	A	A	A	A
7	*LIGULA* Lobes fused partially or completely (A) Lobes free (B) Lobes absent (C)	A	A	A	A	A	A	A	A	A	A
8	*LABIAL PALP* 4-segmented (A) 3-segmented (B) Reduced or absent (C)	A	A	A	A	A	A	A	A	B	B
	III—HYPOPHARYNX										
9	*HYPOPHARYNX* Single, undivided lobe (A) Divided into a proximal and distal lobe (B)	A	A	A	A	A	A	A	A	A	A
10	*FUSION WITH LABIUM* Throughout (A) Anteriorly a free lobe (B)	A	A	A	A	A	A	A	A	A	A
11	*SALIVARY DUCT* Opening at the distal margin of hypopharynx; no salivarium (A) Opens at the base of free lobe into a salivarium (B)	A	A	A	A	A	A	A	A	A	A

CHART—*contd.*

SYMPHYTA							OTHER INSECTS									
Gen. Symphytan	*Pamphilius stramineipes*	*Xyela julii*	*Cephus pygmaeus*	*Xiphydria prolongata*	*Urocerus gigas*	Gen. Symphytan	*Stolis lutaria*	*Rophidia notata*	*Hemerobius nigrita*	*Panorpa germanica*	*Tipula fascipennis*	*Limnephilus marmoratus*	*Hepialus humuli*	*Feronia madida*	*Periplaneta americana*	*Nomadacris septemfasciata*	*Chloroperla torrentium*
A	A	A	A	B	C	A	C	C	A	C	B	C	A	A	D	D	A
A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B
A	A	A	A	A	B	A	A	A	A	B	B	A	A	A	C	C	C
A	A	A	A	A	A	A	B	B	B	B	B	B	B	A	C	C	C
A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	A
A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B
A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	A	A
A	A	A	A	A	A	A	B	A	B	A	A	A	A	A	C	C	A
A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B	B
A	A	A	A	B	B	A	A	A	A	A	B	A	A	A	A	A	A
A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B	B

No.	CHARACTER	SYMPHYTA										
		Diprion pini	*Sclandria serva*	*Cimbex femorata*	*Arge ochropus*	*Megalodontes klugii*	*Pamphilius stramineipes*	*Xyela julii*	*Cephus pygmaeus*	*Xiphydria prolongata*	*Urocerus gigas*	Gen. Symphytan
	IX—TEGULA											
35	*TEGULA* Large, strongly sclerotized plate with spines and pubescence (A) Large weakly sclerotized lobe with setae (B) Small membranous pad with or without pubescence (C)	A	A	A	A	A	A	A	A	A	B	A
	X—TIBIA AND TARSUS											
36	*TARSUS* 5-segmented (A) Reduction to less than five (B)	A	A	A	A	A	A	A	A	A	A	
37	*LONGEST SEGMENT* First tarsal segment the longest (A) Last tarsal segment the longest (B)	A	A	A	A	A	A	A	A	A	A	
38	*TIBIA* Apical tibial spurs, with or without preapical spurs (A) Tibial spines in rows all along tibia (B) No tibial spurs (C)	A	A	A	A	A	A	A	A	A	A	
	XI—PRETARSUS											
39	*PLANTA* Large, strongly sclerotized with two or more spines (A) Narrow transverse band with spines (B) Weakly sclerotized with one spine or no spines (C) Not differentiated (D)	A	A	B	A	A	A	A	A	A	A	A
40	*ORBICULA* Present (A) Absent (B)	A	A	A	A	A	A	A	A	A	A	A
41	*BASIPULVILLI* Present, without pulvilli (A) Present with pulvilli (B) Absent (C)	A	A	A	A	A	A	A	A	A	A	A
42	*AROLIUM* Large, membranous with camera (A) Small membranous area between the claws (B) Arolium undifferentiated (C) Large, cushion-shaped, without a camera (D)	A	A	A	A	A	A	A	A	A	C	A
	XII—WINGS											

Plate II

Fig. 1. Labium of *Xyela julii*.
„ 2. Labium of *Sialis lutaria*.
„ 3. Labium of *Hemerobius stigma*.
„ 4. Labium of *Pamphilius stramineipes*.
„ 5. Labium of *Panorpa germanica*.
„ 6. Labium of *Feronia madida*.
„ 7. Labium of *Urocerus gigas*.
„ 8. Labium of *Nomadacris septemfasciata*.
„ 9. Hypopharynx of *Panorpa germanica*.
„ 10. Hypopharynx of *Sialis lutaria*.
„ 11. Hypopharynx of *Limnephilus marmoratus*.
„ 12. Hypopharynx of *Feronia madida*.
„ 13. Hypopharynx of *Periplaneta americana*.
„ 14. Tentorium of *Megalodontes klugii*.
„ 15. Tentorium of *Selandria serva*.
„ 16. Tentorium of *Xyela julii*.
„ 17. Tentorium of *Cephus pygmaeus*.
„ 18. Tentorium of *Xiphydria prolongata*.
„ 19. Tentorium of *Urocerus gigas*.
„ 20. Tentorium of *Cimbex femorata*.
„ 21. Tentorium of *Arge ochropus*.
„ 22. Tentorium of *Pamphilius stramineipes*.

Plate III

Fig. 1. Tentorium of *Sialis lutaria*.
„ 2. Tentorium of *Hemerobius stigma*.
„ 3. Tentorium of *Feronia madida*.
„ 4. Tentorium of *Periplaneta americana*.
„ 5. Tentorium of *Limnephilus marmoratus*.
„ 6. Pteroterga of *Megalodontes klugii*.
„ 7. Pteroterga of *Selandria serva*.
„ 8. Pteroterga of *Xiphydria prolongata*.
„ 9. Pteroterga of *Cephus pygmaeus*.
„ 10. Pteroterga of *Urocerus gigas*.
„ 11. Pteroterga of *Hemerobius stigma*.
„ 12. Pteroterga of *Sialis lutaria*.
„ 13. Pteroterga of *Raphidia notata*.

Plate IV

Fig. 1. Pteroterga of *Nomadacris septemfasciata*.
„ 2. Pteroterga of *Limnephilus marmoratus*.
„ 3. Pteroterga of *Feronia madida*.
„ 4. Mesosternum of *Raphidia notata*.
„ 5. Mesosternum of *Arge ochropus*.
„ 6. Mesosternum of *Cephus pygmaeus*.
„ 7. Mesosternum of *Xyela julii*.
„ 8. Mesosternum of *Sialis lutaria*.
„ 9. Meso- and metasternum of *Feronia madida*.
„ 10. Mesosternum of *Limnephilus marmoratus*.
„ 11. Mesosternum of *Hemerobius stigma*.
„ 12. Mesosternum of *Panorpa germanica*.
„ 13. Mesosternum of *Nomadacris septemfasciata*.

Plate V

Fig. 1. Hind-leg of *Megalodontes klugii*.
„ 2. Fore-leg of *Pamphilius stramineipes*.
„ 3. Fore-leg of *Xyela julii*.
„ 4. Fore-leg of *Cephus pygmaeus*.
„ 5. Pretarsus of *Selandria serva*.
„ 6. Pretarsus of *Xiphydria prolongata*.
„ 7. Pretarsus of *Megalodontes klugii*.
„ 8. Pretarsus of *Xyela julii*.
„ 9. Pretarsus of *Cimbex femorata*.
„ 10. Pretarsus of *Arge ochropus*.
„ 11. Pretarsus of *Hemerobius stigma*.
„ 12. Pretarsus of *Panorpa germanica*.
„ 13. Pretarsus of *Urocerus gigas*.
„ 14. Pretarsus of *Limnephilus marmoratus*.
„ 15. Pretarsus and some tarsal segments of *Sialis lutaria*.
„ 16. Tarsus of *Nomadacris septemfasciata*.
„ 17. Tibial spurs and tarsus of *Feronia madida*.
„ 18. Pretarsus of *Nomadacris septemfasciata*.
„ 19. Fore-wing of *Selandria serva*.
„ 20. Fore-wing of *Arge ochropus*.
„ 21. Fore-wing of *Xiphydria prolongata*.
„ 22. Fore-wing of *Megalodontes klugii*.
„ 23. Fore-wing of *Cimbex femorata*.
„ 24. Fore-wing of *Pamphilius stramineipes*.
„ 25. Fore-wing of *Cephus pygmaeus*.

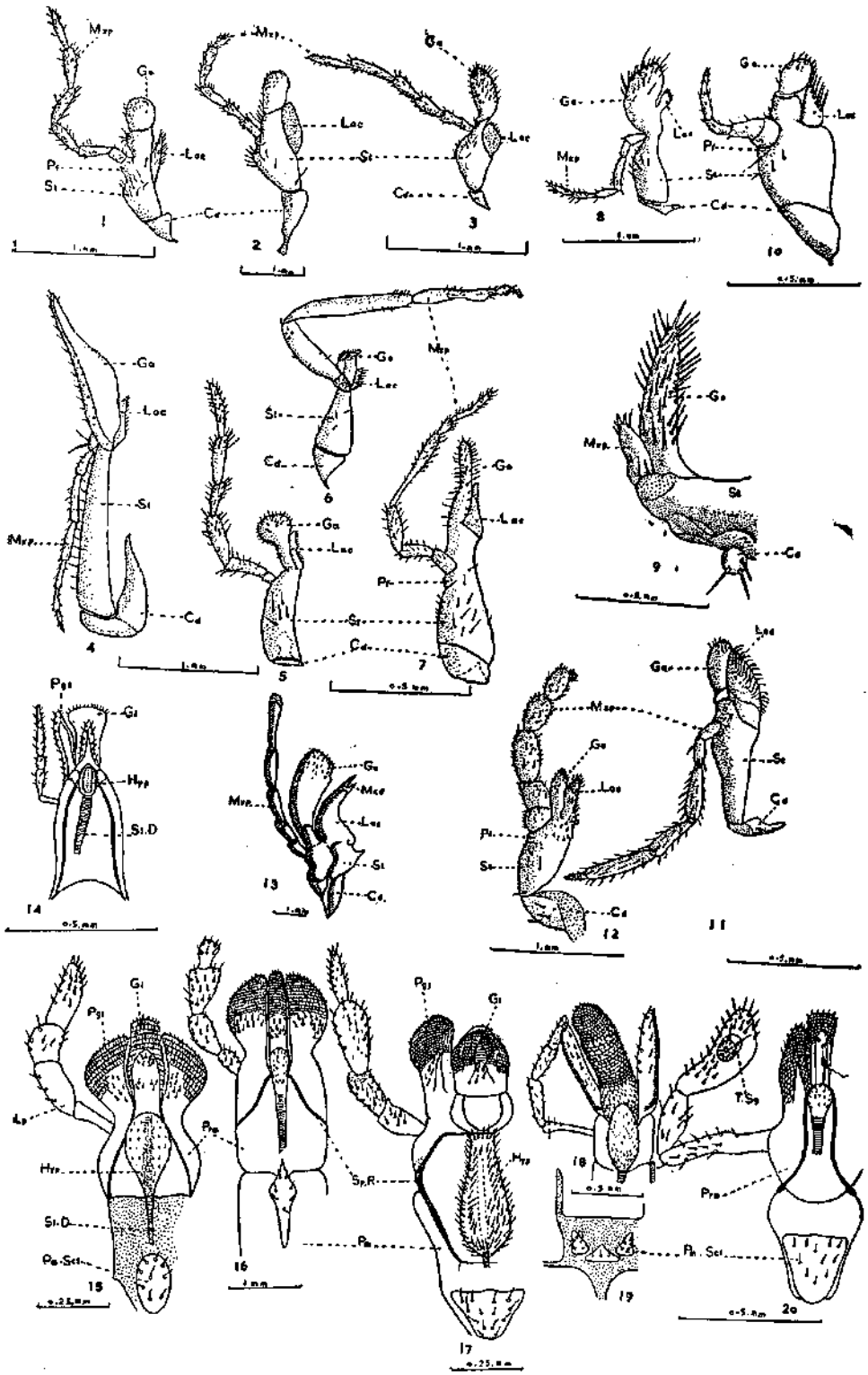
Plate VI

Fig. 1. Fore-wing of *Xyela julii*.
„ 2. Hind-wing of *Xyela julii*.
„ 3. Fore-wing of *Urocerus gigas*.
„ 4. Hind-wing of *Urocerus gigas*.
„ 5. Fore-wing of *Panorpa germanica*.
„ 6. Fore-wing of *Sialis lutaria*.
„ 7. Fore-wing of *Tipula fascipennis*.
„ 8. Fore-wing of *Limnephilus marmoratus*.
„ 9. Fore-wing of *Periplaneta americana*.
„ 10. Genital segments and ovipositor of *Selandria serva*, lateral view.
„ 11. Genital segments and ovipositor of *Urocerus gigas*, lateral view.
„ 12. Genital segments and ovipositor of *Cimbex femorata*, lateral view.
„ 13. Genital segments and ovipositor of *Arge ochropus*, lateral view.
„ 14. Genital segments and ovipositor of *Megalodontes klugii*, lateral view.
„ 15. Genital segments and ovipositor of *Xyela julii*, lateral view.
„ 16. Genital segments and ovipositor of *Cephus pygmaeus*, lateral view.
„ 17. Genital segments and ovipositor of *Xiphydria prolongata*, lateral view.

Plate VII

Fig. 1. Genital apparatus of *Sialis lutaria*, female, lateral view.
„ 2. Genital apparatus of *Tipula fascipennis*, female, lateral view.
„ 3. Terminal abdominal segments of *Panorpa germanica*, female, lateral view.
„ 4. Genital apparatus of *Raphidia notata*, female, ventral view.
„ 5. Terminal part of genital valves of *Raphidia notata*, female, ventral view.
„ 6. Genital segments and ovipositor of *Nomadacris septemfasciata*, lateral vie
„ 7. Phallus of *Arge ochropus*, dorsal view.

FIG. 8. Phallus of *Cimbex femorata*, dorsal view.
„ 9. Phallus of *Selandria serva*, dorsal view.
„ 10. Phallus of *Xyela julii*, dorsal view.
„ 11. Phallus of *Urocerus gigas*, ventral view.
„ 12. Phallus of *Cephus pygmaeus*, ventral view.
„ 13. Phallus of *Pamphilius stramineipes*, ventral view.
„ 14. Phallus of *Xiphydria prolongata*, ventral view.
„ 15. Phallus of *Megalodontes klugii*, ventral view.
„ 16. Phallus of *Panorpa germanica*, ventral view.
„ 17. Phallus and genital segments of *Tipula fascipennis*, ventral view.
„ 18. Genital apparatus of *Raphidia notata*, male, ventral view.
„ 19. Genital apparatus of *Sialis lutaria*, male, lateral view.
„ 20. Phallus of *Sialis lutaria*, dorsal view.
„ 21. Phallus of *Periplaneta americana*, dorsal view.
„ 22. Terminal abdominal segment of *Lariophagus distinguendus*, female, lateral view.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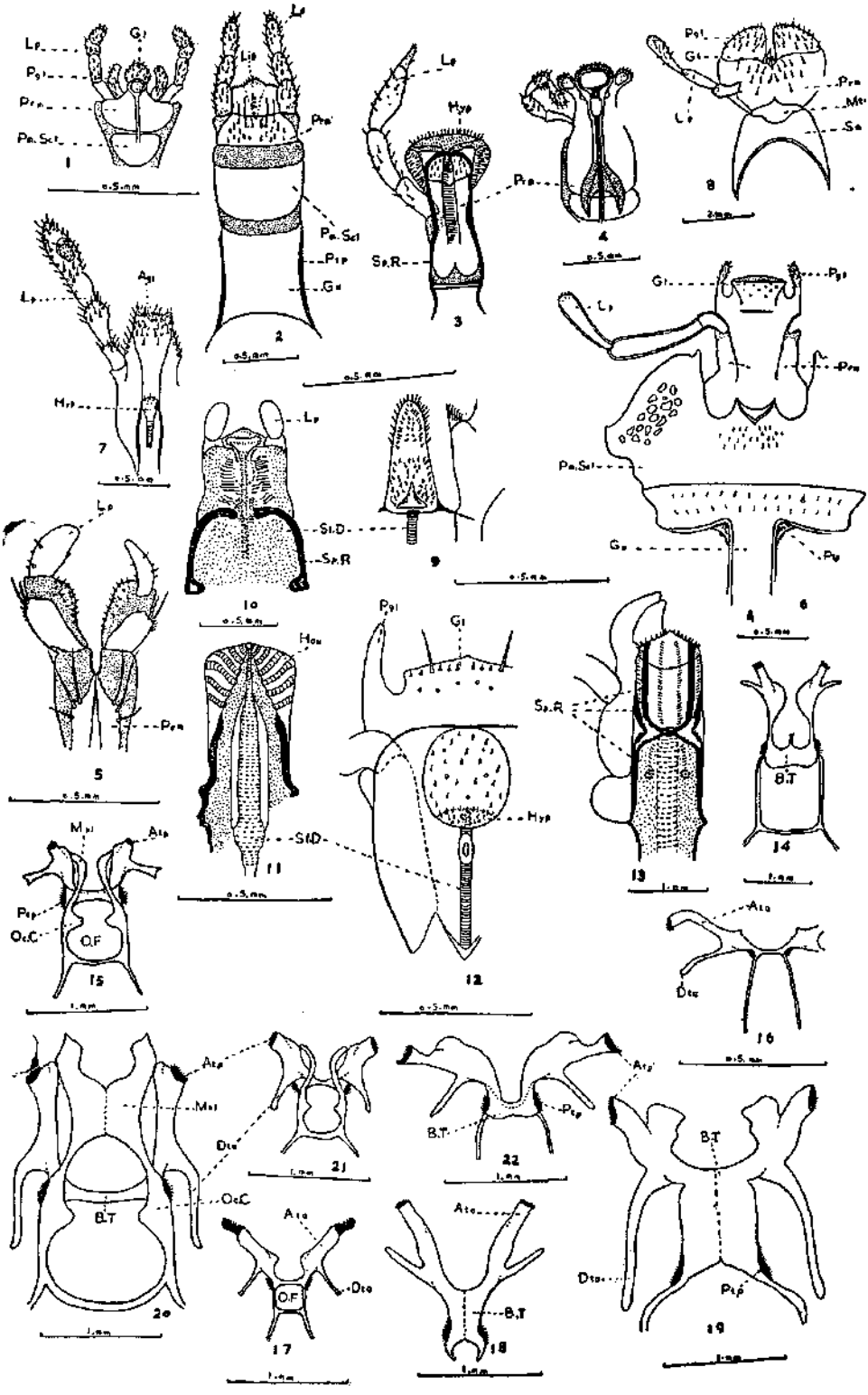
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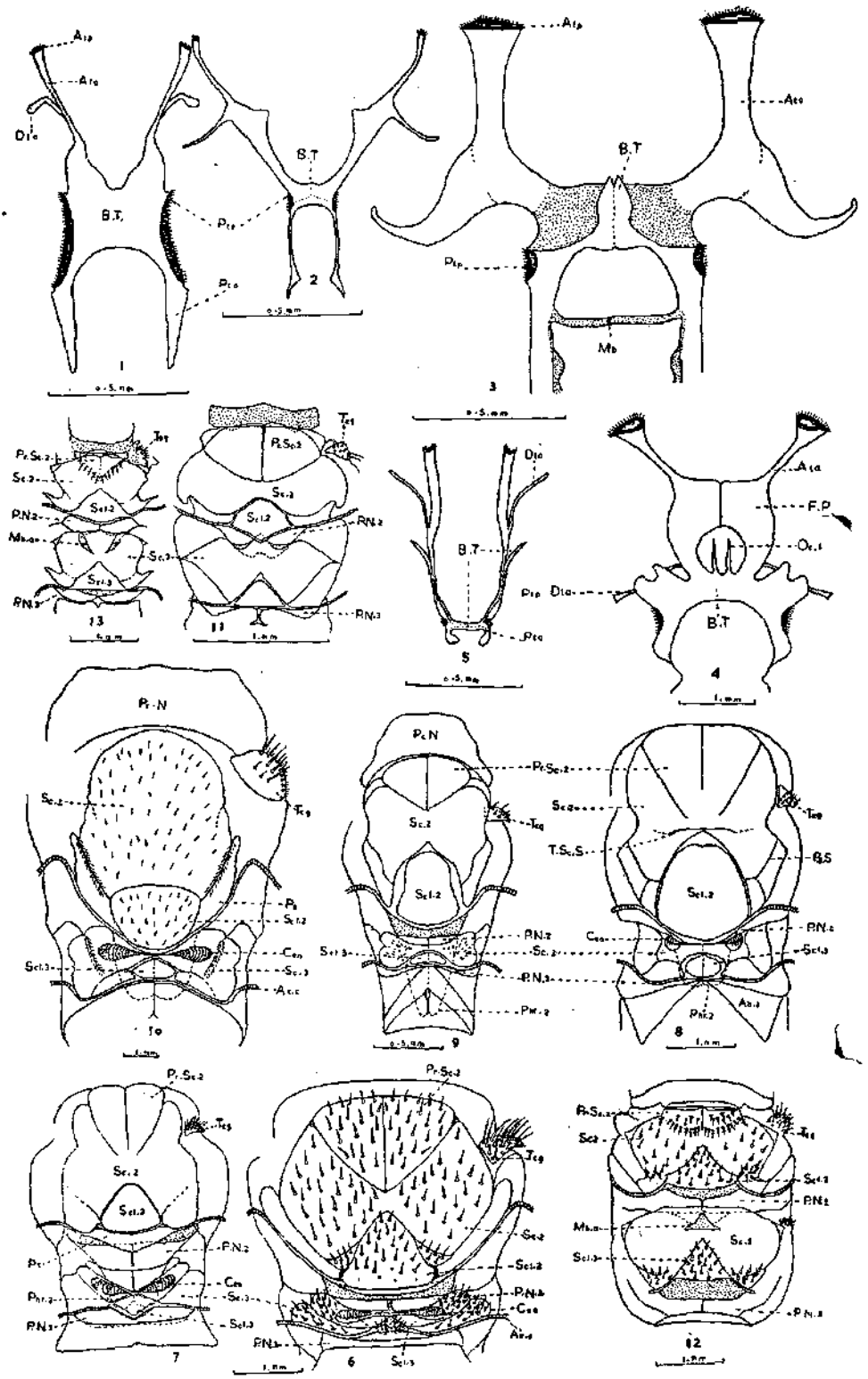
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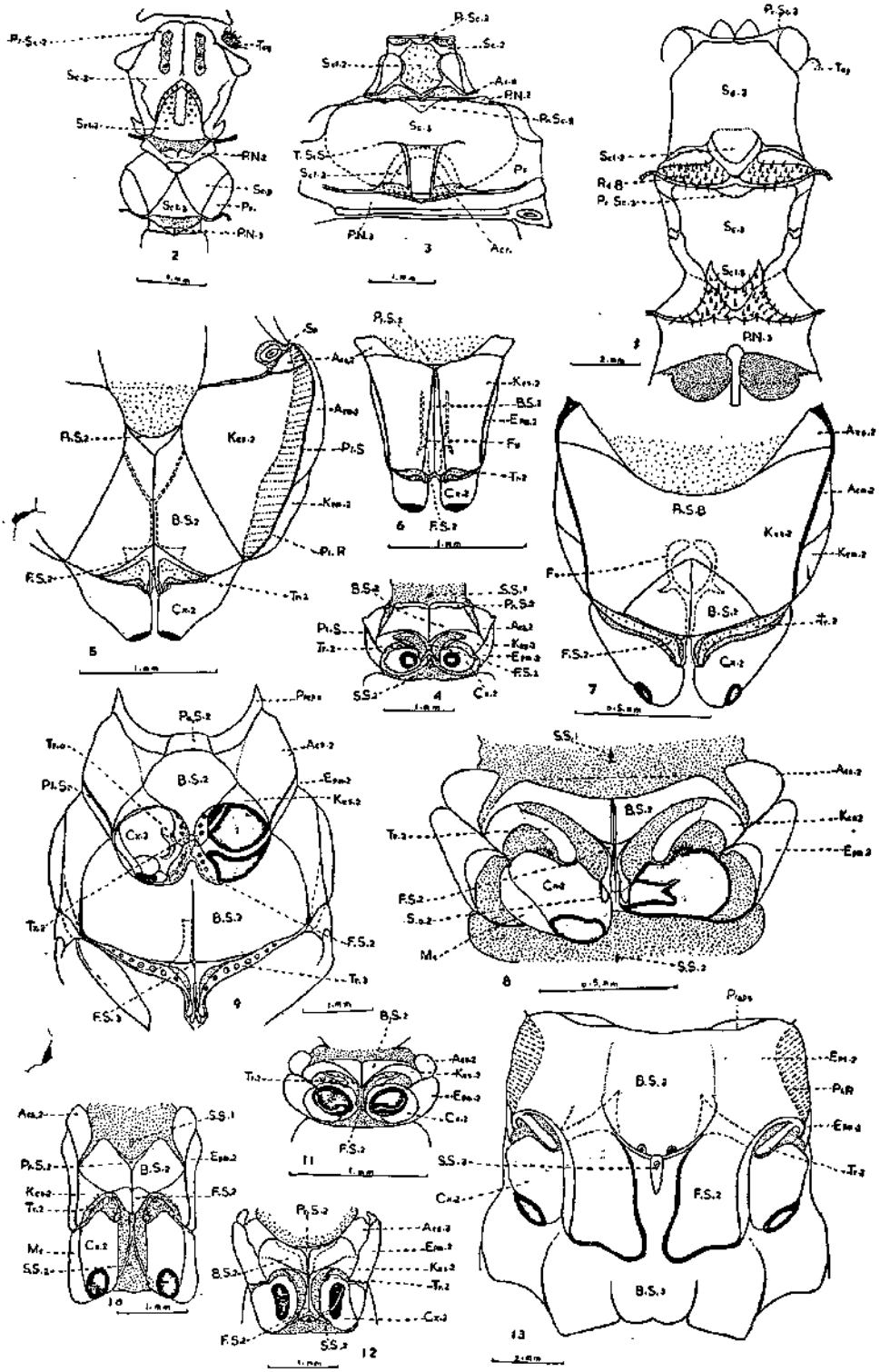

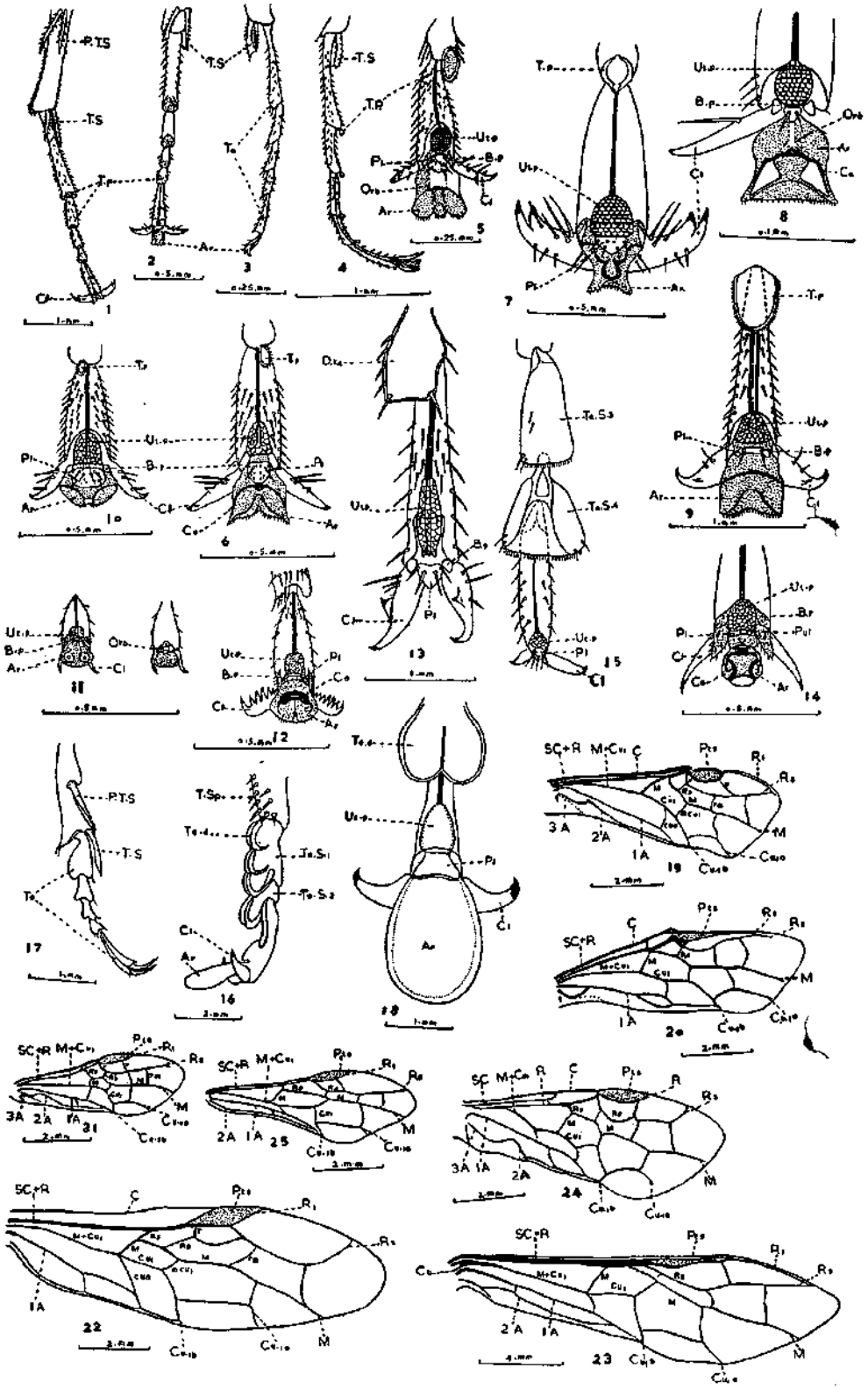
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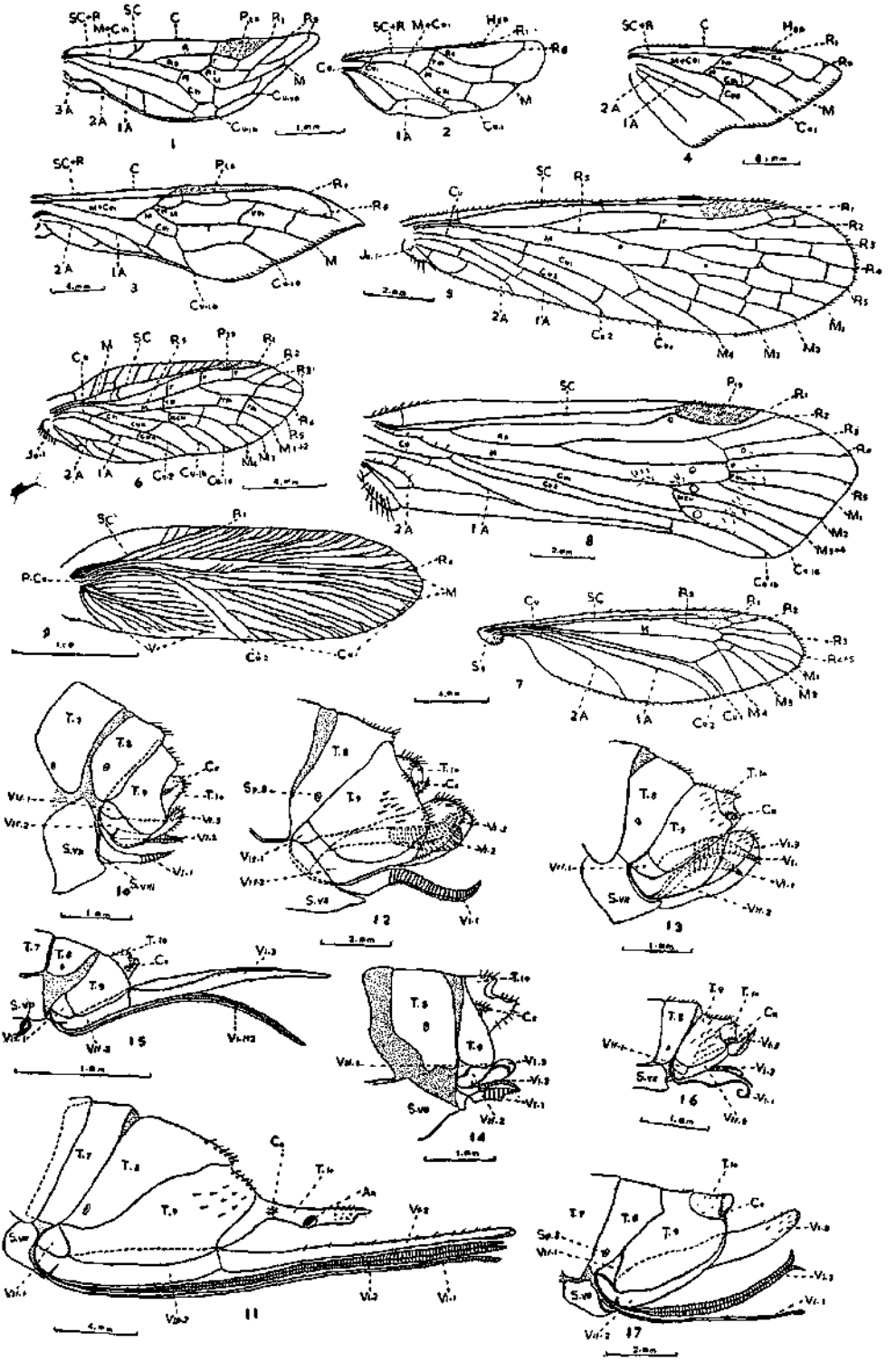
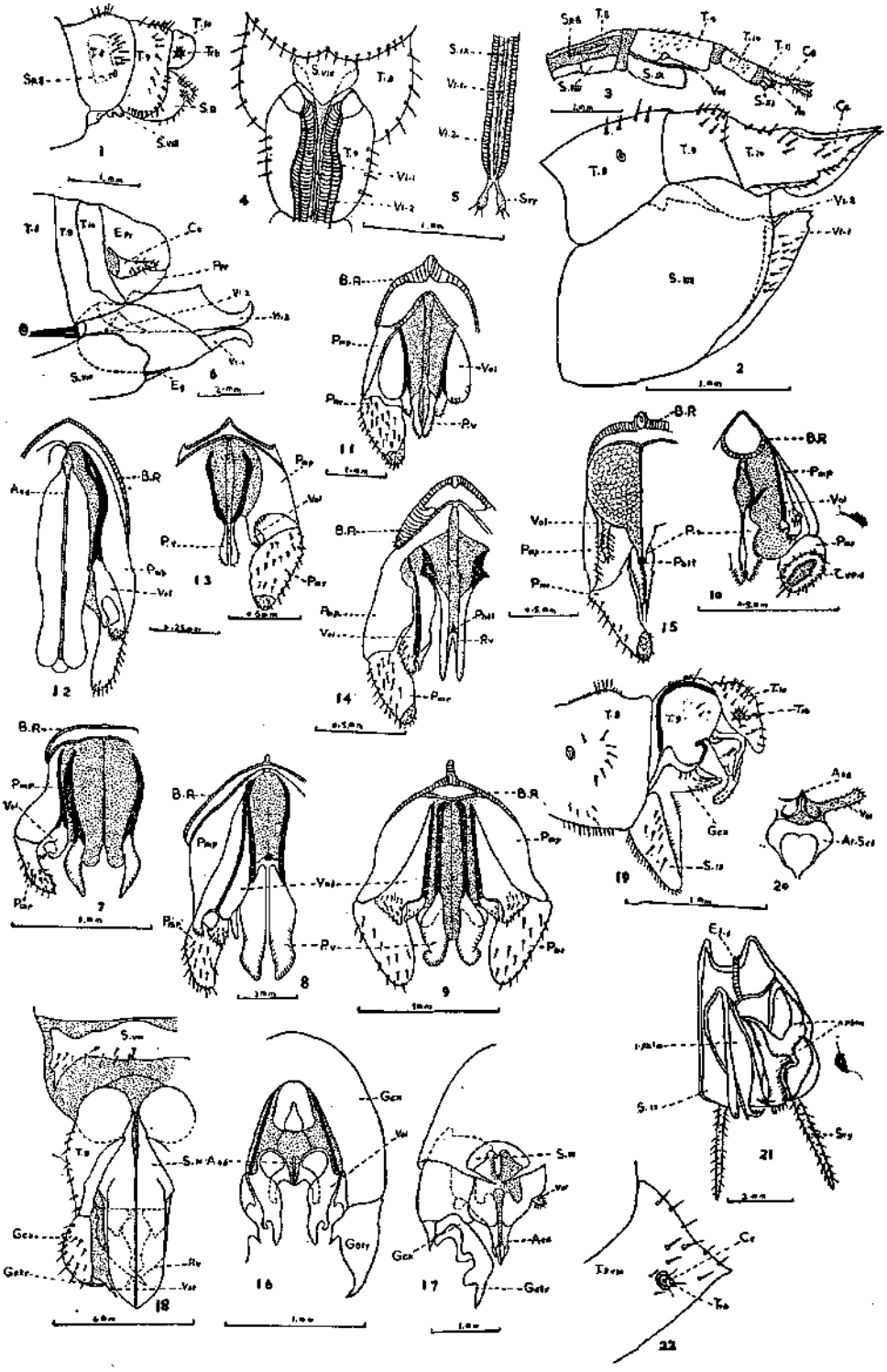
PLATE VI.





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THE NEMATODE SPERM

by

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THE NEMATODE SPERM

By VISHWA NATH *and* SANT SINGH (*Department of Zoology, Panjab University, Hoshiarpur, Panjab, India*)

ABSTRACT

In this paper the authors have dealt with the spermatogenesis of *Ascaris suilla* and *Porrocaecum angusticolle*. It has been shown that the well-known refringent granules, which appear in the growing primary spermatocytes of the nematodes, are formed directly from the Golgi spheroids, and are not secretory products of the latter. In spermateleosis the refringent granules progressively fuse together to form one large refringent body, the acrosome of the ripe sperm. This last is a cone-like cell having a small nucleus surrounded by mitochondria towards the broad end of the cone, the acrosome lying towards the narrow end. In *Porrocaecum* the mitochondria remain granular and, more or less, inactive throughout spermatogenesis; but in *Ascaris suilla* they grow into comparatively large vesicles towards the last phases of spermateleosis. It has been confirmed in both species that the last stages of the maturation of the sperm take place in the uterus of the female.

I. INTRODUCTION

The only paper, so far as the authors are aware, on nematode sperm, which fulfils the requirements of a modern investigation, is by Sturdivant (1934) on the spermatogenesis of *Ascaris megalocephala*, with special reference to the central bodies, Golgi complex and mitochondria. This work was carried out under the supervision of the late Professor E. B. Wilson, and is a comprehensive piece of work on the subject. Sturdivant also gives an excellent review of the previous work on the nematode sperm.

All the workers before Sturdivant (1934) were unanimous in regard to the existence of the 'refringent body' in the ripe sperm; but Sturdivant for the first time showed clearly that this enigmatic body was formed by the union of 'refringent granules', which had been secreted earlier in the chromophobe substance of the Golgi crescents. These granules are, therefore, homologous with the proacrosomal granules of normal spermatogenesis, and the refringent body with the acrosome.

Sturdivant (1934) figures the ripe sperm of *Ascaris megalocephala* as a cone-like cell with a broad base and a narrow apex. The nucleus with most of the mitochondrial vesicles lies towards the broad end, and the acrosome (refringent body) towards the narrow end. There is no Golgi material as such in the ripe sperm.

Collier (1936) does not make any reference to the earlier paper of Sturdivant (1934) on *Ascaris megalocephala*, and she has given no account or figures illustrating the process of spermatogenesis in *A. suilla*. But she says: 'The refringent granules arise in the formation of the sperm as a differentiation product of the Golgi material in a manner comparable to the formation of the acrosome in flagellate sperm. As the granules are formed,

their connection with the Golgi bodies is severed and they collect in the cytoplasm in large numbers. Any relationship which they seem to have with the mitochondria is purely topographical. During the spermatid stage these numerous bodies come together and fuse to form one large refringent body, or, as I shall term it, an acrosome'.

Collier (1936) figures the ripe sperm of *Ascaris suilla* more or less of the same form as in *A. megalocephala*, but she shows a smaller nucleus and granular mitochondria. She also includes a few Golgi elements, contrary to the findings of Sturdivant (1934) in *A. megalocephala*.

Walton (1940) states that 'the spermatozoa of nematodes are described as non-flagellated, frequently amoeboid cells, containing a considerable amount of stored material in the "refringent body", or acrosome. This type of spermatozoon is usually regarded as a simple modification of the fundamental structural plan of a flagellate sperm and has arisen secondarily during the evolution of this phylum'. Walton further states that '*Passalurus ambiguus* (*Oxyuris ambigua*) has spermatozoa that may almost be considered as flagellate (Meves, 1911; Bowen, 1925). Recently Chitwood (1931) has described the spermatozoa from *Trilobus longus* which seem to be of truly flagellate form'. Again, according to Walton, '*Passalurus* (*Oxyuris*) and *Trilobus* have the acrosomal body at the morphologically anterior end of the spermatozoon. It is perhaps to be expected that in *Parascaris*, and in related genera where chromosomal behaviour as well as other criteria point to a high degree of specialization, the acrosome likewise would tend to vary from the normal, as perhaps is shown by its unusual position behind the nucleus'.

In the present paper we have recorded our observations on the spermatogenesis of *Ascaris suilla* from the pig and *Porrocaecum angusticolle* from the vulture. We have employed the techniques of Champy and Bouin followed by 0·5% iron-haematoxylin, and Kolatchev. We have also studied the ripe sperms and spermatids with phase-contrast microscopy.

II. Observations

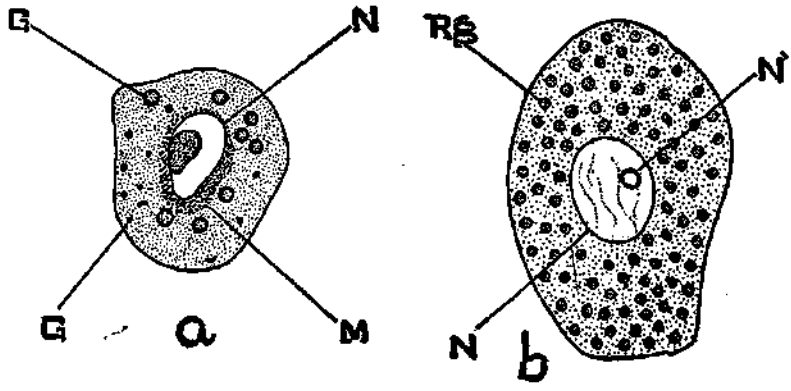
A. *Porrocaecum angusticolle*

We obtained very satisfactory preparations of the testicular material of *Porrocaecum* with Champy followed by 0·5% iron-haematoxylin; but Kolatchev yielded poor results. We have examined primary and secondary spermatocytes, and maturing spermatids, as fixed in Champy and stained with iron-haematoxylin. We have also examined living late spermatids from the seminal vesicles of the male and living ripe sperms from the uterus of the female under the phase-contrast microscope.

(*a*) *Primary Spermatocytes*

Text-fig. 1, *a* represents an early primary spermatocyte from Champy fixed material. The mitochondria are extremely small granules, characteristically grouped round the nucleus. They invariably stain feebly, appearing as ill-defined, greyish granules. But for the fact that

they are collected in a circum-nuclear mass, it would be very difficult to demonstrate them. In the region of the cytoplasm peripheral to the circum-nuclear ring of the mitochondria, are to be found Golgi bodies in the form of (1) granules, which are bigger than the mitochondria and take up a very much darker stain, and (2) Golgi spheroids, which in optical sections appear as rings. Each spheroid has a chromophobic sphere, completely ensheathed by a chromophilic cortex. There does not seem to be any doubt that the Golgi spheroids are evolved from the Golgi granules.



TEXT-FIG. 1. *Porrocaecum angusticolle*; *a*, early primary spermatocyte; *b*, secondary spermatocyte.

Lettering for all Figures

A—acrosome; *Ag*—acrosomal granule; *G*—Golgi elements; *M*—mitochondria; *N*—nucleus; *N'*—nucleolus; *P.L*—plasma lobe; *Rg*—refringent granules.

We have not studied the growth stages of the primary spermatocytes in *Porrocaecum*; but in *Ascaris suilla* we were fortunate enough to have good preparations of the growth stages of the primary spermatocytes, showing the direct metamorphosis of the Golgi spheroids into the well-known refringent granules.

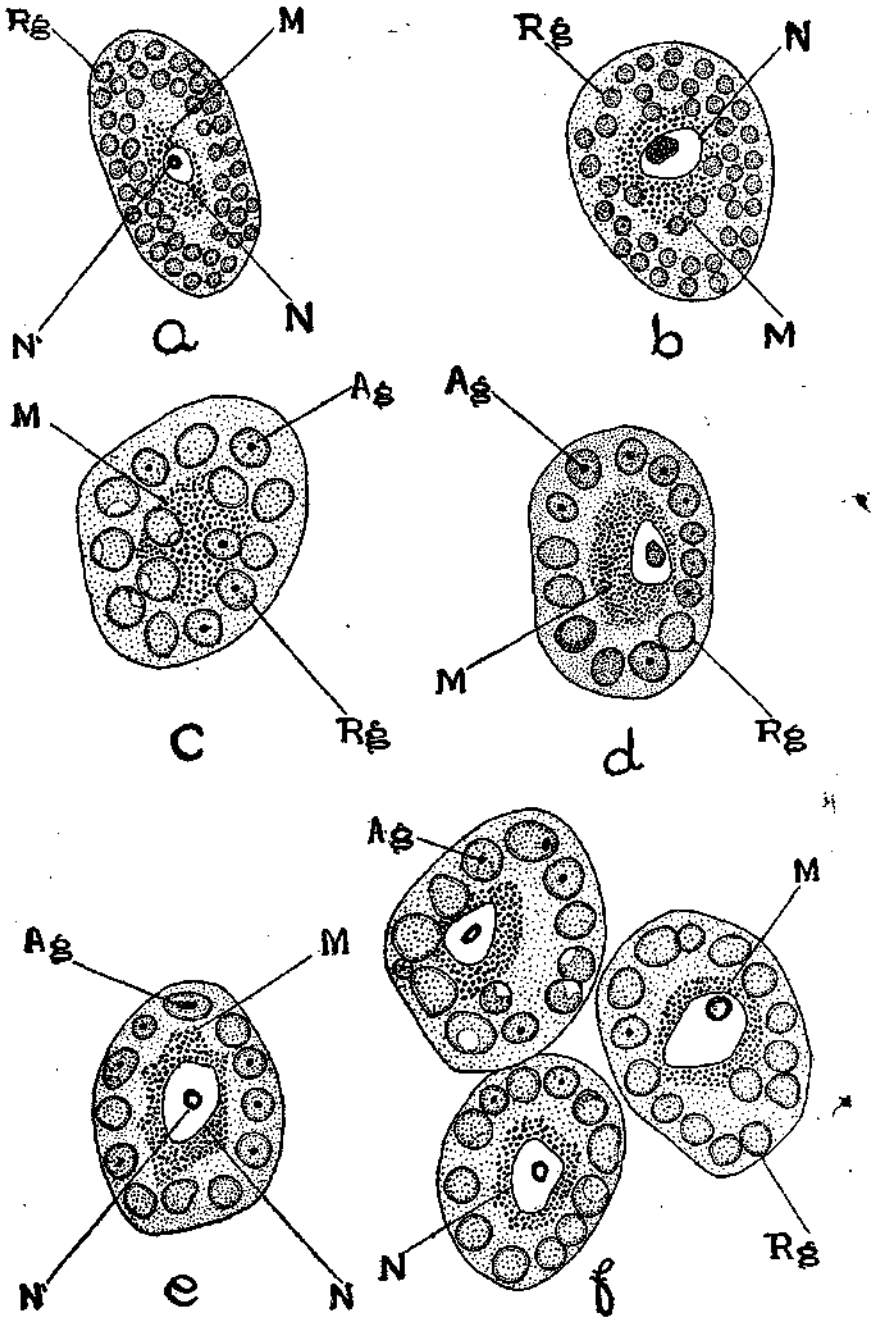
(*b*) *Secondary Spermatocytes*

The secondary spermatocytes (Text-fig. 1, *b*) are always choked with refringent granules. It will thus appear that all the Golgi spheroids have been directly converted into the refringent bodies during the growth stages of the primary spermatocyte. A refringent body is spherical like the Golgi spheroid, but it is completely devoid of a duplex structure. It always appears as a perfectly homogeneous sphere, staining rather feebly as compared with a Golgi spheroid. It is not possible to demonstrate the minute mitochondrial granules in the secondary spermatocytes, as they are uniformly spread out in the cytoplasm.

(*c*) *Spermateleosis*

Text-fig. 2, *a* represents an early spermatid from Champy fixed material. The mitochondria have now slightly grown in size and have

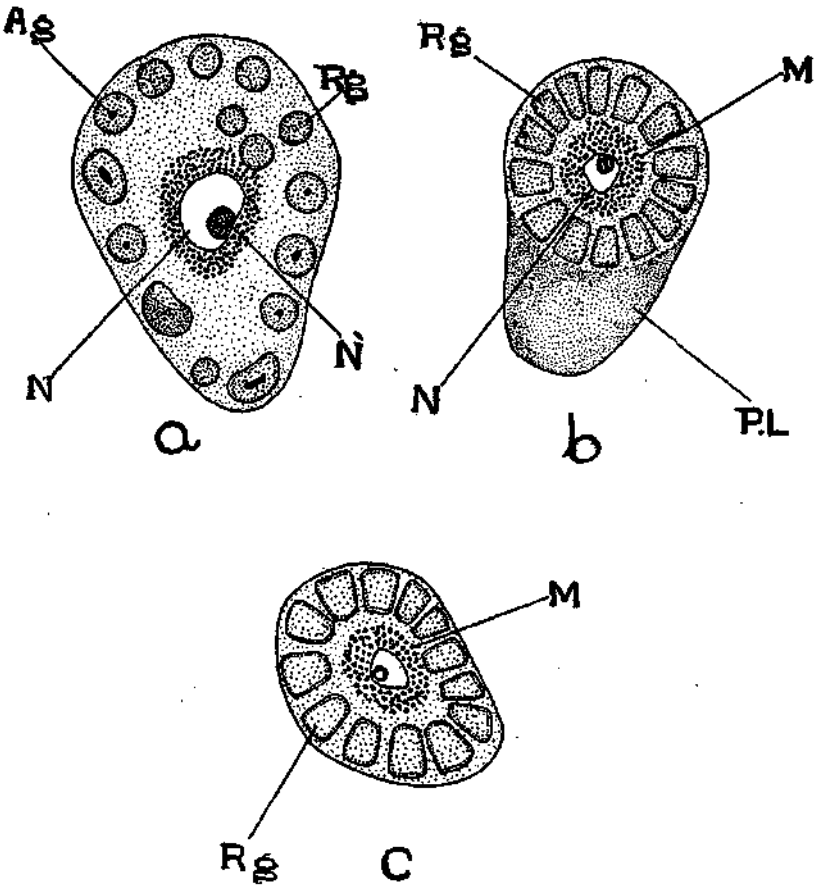
once more become circum-nuclear. The refringent bodies tend to become peripheral in position. The mitochondria continue to remain circum-



TEXT-FIG. 2. *Porrocaecum angusticolle*; *a–f*, spermatids.

nuclear in the spermatids studied from the male genital tract; but the refringent bodies progressively undergo an important process of coalescence to form fewer and bigger masses. At the same time there appears a sharply

staining granule, the acrosomal granule, in each refringent body, now much enlarged (Text-figs. 2, *a–f*; 3, *a*). Sometimes we have observed an acrosomal *rod* instead of a granule in the interior of a refringent body (Text-figs. 2, *e*; 3, *a*).



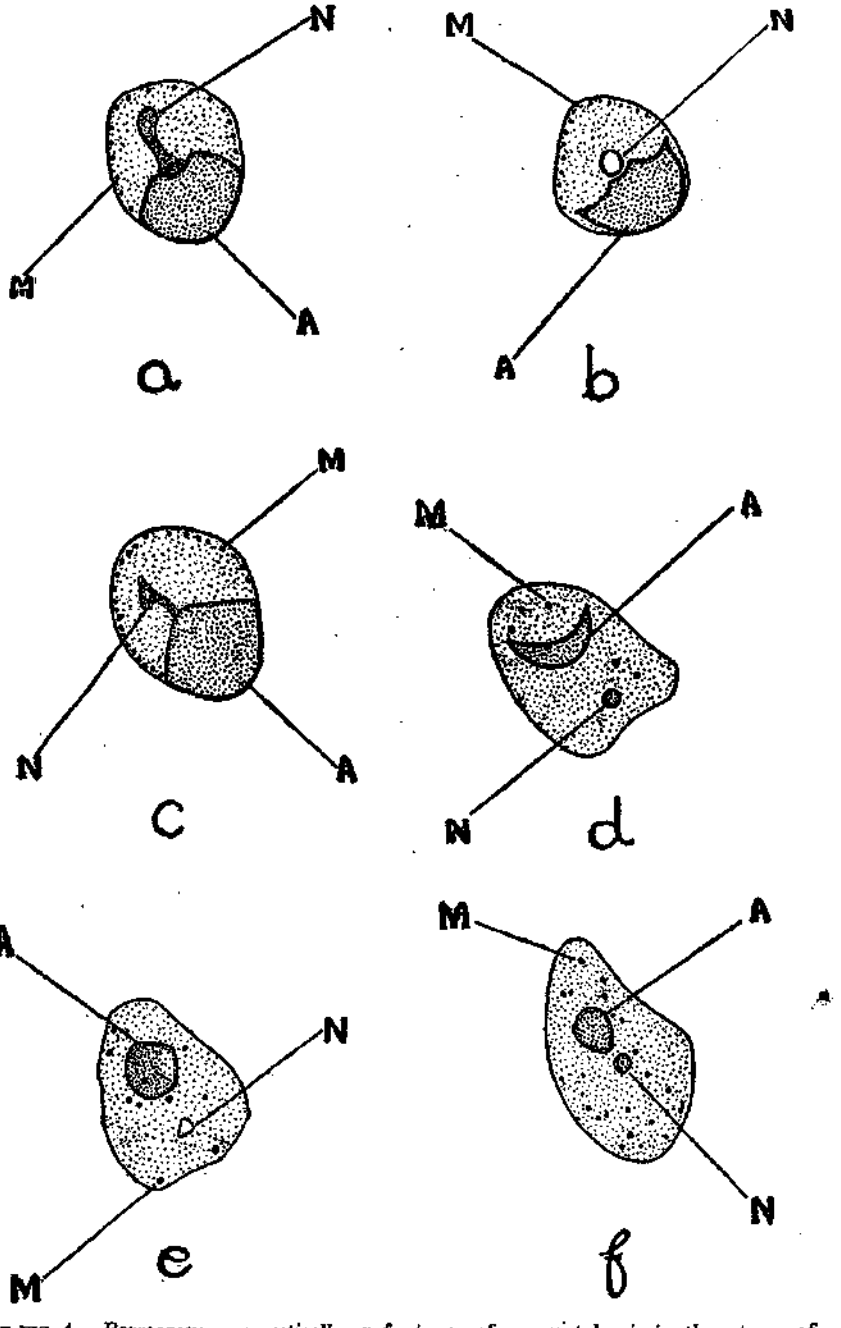
TEXT-FIG. 3. *Porrocaecum angusticolle*; *a–c*, late spermatids.

A remarkable, probably unique, process of cytoplasmic reduction in the spermatid now ensues. The late spermatids put out pseudopodial processes which are cut off (Text-fig. 3, *b*; and Pl. I, figs. 1, 2). Very often the cut off pseudopodial processes can be seen in the neighbourhood of the spermatid from which they have been cut off. The pseudopodial processes may be conveniently described as plasma lobes, which are absolutely devoid of both mitochondria and refringent bodies. The presence of a single refringent body containing an acrosomal granule (Pl. I, fig. 2) must, therefore, be considered accidental.

Simultaneously with this process of cytoplasmic reduction, the refringent bodies get elongated and place themselves at the periphery of the spermatid cytoplasm. Each refringent body has a broad end pointing towards the cell membrane, and a narrow end pointing towards the nucleus.

The transformation of the spermatid into the cone-like sperm stops at the stage represented in Text-fig. 3, *c*, and will continue only after the spermatids have been removed to the uterus of the female after copulation.

The final stages of spermateleosis are met with in the uterus of the female only. Text-fig. 4, *a–f* represent spermatids maturing into the



Text-fig. 4. *Porrocaecum angusticolle*; *a–f*, stages of spermateleosis in the uterus of the female.

ripe sperm, fixed with Champy and stained with 0·5% iron-haematoxylin. The acrosome has been fully formed although it has not yet assumed its final cone-like form. The mitochondria have now grown in size and become much more stainable. The nucleus has considerably shrunk in size. There are no traces of Golgi elements.

But in the ripe living sperm studied under the positive phase-contrast microscope there are always a few granules, which give a much higher phase-change than the mitochondria (Pl. I, fig. 3). These are the Golgi granules. In this photomicrograph there are two Golgi granules lying close together on the cone-like acrosome. The mitochondria are very minute granules. They lie below the base of the acrosome, surrounding the tiny nucleus. Under the positive phase-contrast they appear slightly yellowish and have a very low phase-change. The acrosome invariably appears as a bright bluish cone-like body.

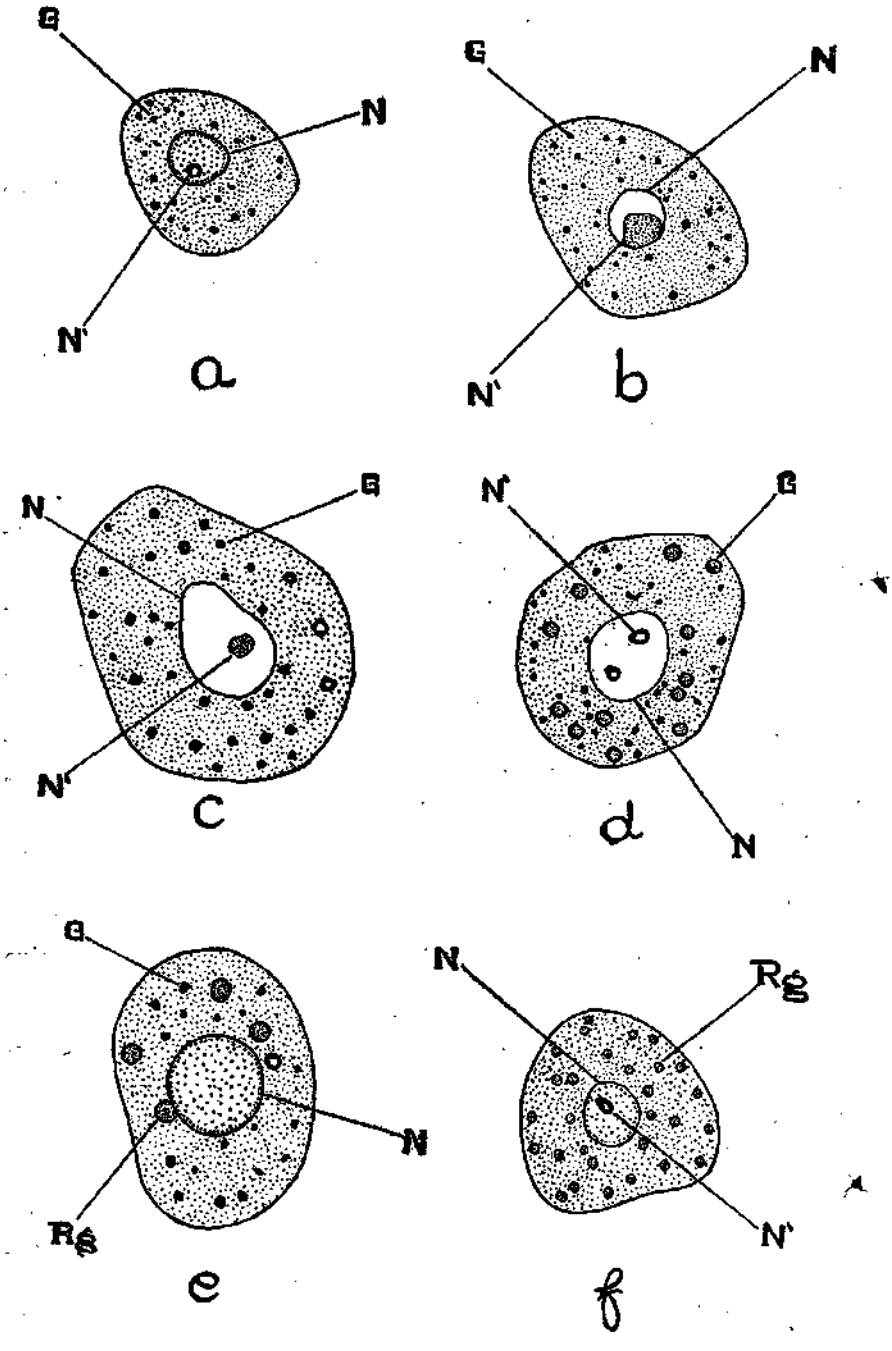
B. *Ascaris suilla*

We obtained very satisfactory preparations of the testicular material of *Ascaris suilla* both with Kolatchev and Champy techniques. We have also examined the ripe sperms from the uterus of the female with phase-contrast microscopy. But we have not been fortunate like Sturdivant (1934) to have examined the meiotic stages when the acrosomal granules or rods appear in the interior of the refringent granules, as these stages were missing from our preparations. On the contrary, we were fortunate enough to have a series of satisfactory preparations to show that the refringent granules are formed directly from the Golgi elements, and are not secretory products of the latter as claimed by Sturdivant.

(*a*) *Primary Spermatocytes*

Text-fig. 5, *a–c* represent three primary spermatocytes in a progressive series of growth fixed in Champy and stained with 0·5% iron-haematoxylin. The Golgi elements are found in the form of deeply staining granules, which grow with the growth of the spermatocyte. But it is very difficult to demonstrate the mitochondria, which seem to exist in the form of very minute granules scattered all over the cytoplasm. Text-fig. 6, *a* represents an early primary spermatocyte fixed in Kolatchev. The Golgi elements appear in the form of jet black granules.

With the growth of the primary spermatocyte the Golgi granules begin to grow directly into the Golgi spheroids, each having a duplex structure with a chromophilic cortex completely investing a chromophobic sphere. This is very clearly brought out in Champy iron-haematoxylin preparations (Text-fig. 5, *d*). Very soon, however, the Golgi spheroids are converted directly into refringent granules. In Champy iron-haematoxylin preparations (Text-fig. 5, *e*) the refringent granules stain very feebly; nor do they go black in Kolatchev (Text-fig. 5, *f*). In the fully grown primary spermatocytes all the Golgi spheroids seem to have been converted into the refringent granules, as is clear from the study of Text-fig. 5, *f*.

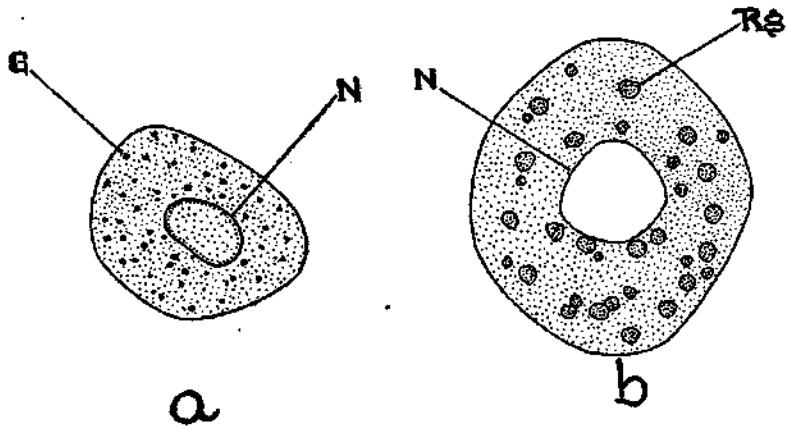


TEXT-FIG. 5. *Ascaris suilla*; *a–f*, primary spermatocytes.

(b) Secondary Spermatocytes

In the secondary spermatocytes the refringent granules seem to grow in size and at the same time they become more chromophobic in both Champy iron-haematoxylin (Text-fig. 6, *b*) and Kolatchev preparations.

It is very difficult to demonstrate the mitochondria in the secondary spermatocytes also, as they are uniformly scattered all over the cytoplasm.



TEXT-FIG. 6. *Ascaris suilla*; *a*, early primary spermatocyte; *b*, secondary spermatocyte.

(*c*) *Spermateleosis*

Three outstanding events take place early in spermateleosis. Firstly, the refringent granules progressively fuse together to form the acrosome of the ripe sperm. Thus are formed big refringent masses, which stain slightly and appear spongy in Champy iron-haematoxylin (Text-fig. 7, *a–c*); but in Kolatchev the refringent masses show no differentiation in their internal structure, appearing as yellowish smooth bodies (Text-fig. 7, *d*).

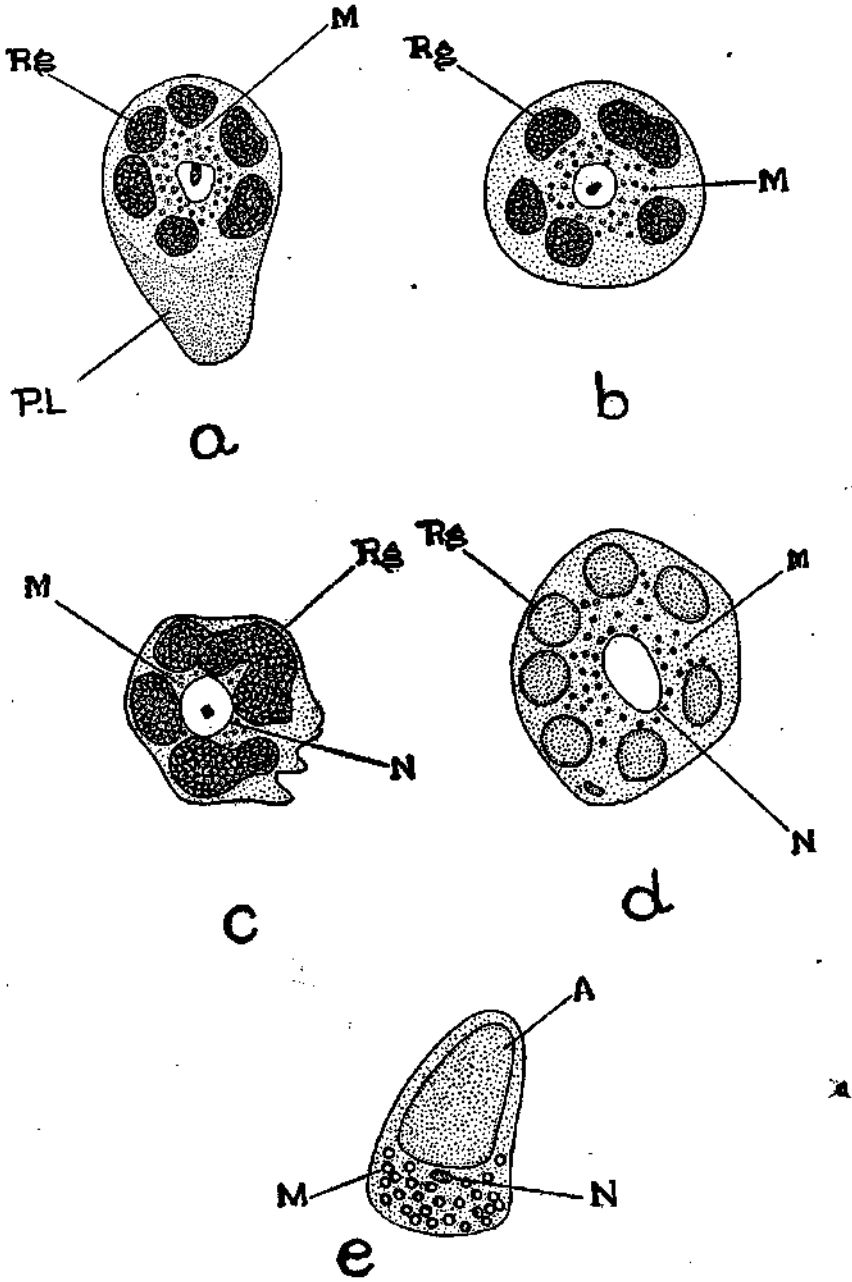
Secondly, the mitochondria now become very prominent and are circum-nuclear in arrangement. They stain feebly in Champy iron-haematoxylin (Text-fig. 7, *a–c*); but in Kolatchev (Text-fig. 7, *d*), they appear yellowish.

Thirdly, there is put out a cytoplasmic lobe devoid of any cytoplasmic inclusions (Text-fig. 7, *a*). This lobe is subsequently cut off.

The ripe sperm from the uterus is a cone-like cell with a broad base and a narrow apex. Towards the base lies a small nucleus surrounded by now very much enlarged mitochondria. In Kolatchev preparations (Text-fig. 7, *e*) the mitochondria appear as vesicles with a slightly blackened cortex and a yellowish medulla. The acrosome is a large cone-like body, appearing brownish in such preparations. In Bouin preparations the mitochondria are not destroyed.

Plate I, figs. 4 and 5 are photomicrographs of living sperms of *Ascaris suilla* from the uterus of the female, studied with phase-contrast microscopy. In Plate I, fig. 4, the large acrosome lies towards the narrower part of the cone-like sperm. Just below the base of the acrosome lies the nucleus containing a tiny 'nucleolus'. Below and round the nucleus are the mitochondria. In Plate I, fig. 5, the acrosome and the mitochondria are very clear, but the nucleus is out of focus.

Lastly, we have not seen any Golgi bodies in the ripe sperm of *A. suilla* either in fixed preparations or in the living material under the phase-contrast microscope.



TEXT-FIG. 7. *Ascaris suilla*; *a–d*, late spermatids; *e*, ripe sperm.

III. DISCUSSION

(*a*) *Morphology of the Golgi Bodies*

Unfortunately Sturdivant (1934) employed Kolatchev and Weigl techniques for the study of Golgi bodies in the spermatogenesis of *Ascaris*

megalocephala. These and also the silver nitrate techniques introduce serious artifacts in the cell as pointed out by Nath (1944, 1956) and Baker (1944, 1949, etc.). Hence the observations of Sturdivant with regard to the morphology of the Golgi bodies and his account of the secretion of the refringent granules by the Golgi bodies are open to serious objection.

According to Sturdivant (1934) in the primary spermatocytes 'the Golgi bodies often present a variable appearance; some appear as closed rings or variously bent rods, but often they appear as typical crescent-shaped Golgi bodies'. Again 'slightly older cells show definite ring-shaped Golgi bodies scattered through the cytoplasm (fig. 54)'. In addition to these ring-shaped bodies, 'there are numerous smaller irregular granules scattered through the cytoplasm'. Sturdivant adds that in the later growth history of the cells, several more of the ring-shaped Golgi bodies can be seen in the cell. 'These bodies at this stage have a granular appearance, which would suggest that they are probably made up of several of the granule-like Golgi bodies (fig. 57)'.

On the contrary, we have shown with Champy technique (a much less drastic procedure) that the Golgi bodies in the young spermatocytes of *Porrocaecum angusticolle* and *Ascaris suilla* are *invariably* in the form of well-defined *granules*, and that in later growth stages several of these Golgi granules grow into Golgi spheroids, each spheroid consisting of a chromophobic sphere completely ensheathed by a chromophilic cortex. This is in strict accord with the views of Hirsch (1939), as quoted by Bourne (1951), and of Nath and Gupta (1956) in *Anadenus* and *Euaustenia*.

This has been fully confirmed by the junior author (Sant Singh), who has examined very recently under the phase-contrast the living sperm-forming cells from the testis as well as from the uterus of the female in a nematode (*Polydelphis* sp.) from the python. Sant Singh's account will be published later on; but it is necessary to state here that his observations with phase-contrast microscopy fully confirm our observations based on material fixed with Champy and stained with 0·5% iron-haematoxylin. In the spermatogonia and the young primary spermatocytes alike the Golgi elements appear invariably in the form of dark, well-defined granules which under the positive phase-contrast give a very high phase-change. In older primary spermatocytes several of the Golgi granules have directly grown into Golgi spheroids having a duplex structure. The cortex of each spheroid is complete and gives a high phase-change, whereas the internal sphere gives a very low phase-change. It is hardly necessary to add that in optical sections the Golgi spheroids appear as rings under the phase-contrast.

(b) Origin of Refringent Granules

By the use of 20 per cent acid fuchsin in anilin water on the Weigl or Kolatchev preparations for two minutes (a procedure which causes considerable shrinkage of the cell and its components) Sturdivant (1934) has claimed that the refringent granules appear for the first time in the chromophobic spheres of the Golgi bodies. Throughout the growth period of the spermatocytes, these granules have a very strong affinity for acid

fuchsin. After their secretion by the Golgi bodies, the refringent granules are released into the cytoplasm, while the whole of the Golgi material is cast off with the cytophore and the plasma lobe.

On the contrary, we have shown in *Porrocaecum angusticolle* and *Ascaris suilla* that the Golgi spheroids undergo radical changes in their chemistry and are transformed *directly* into the refringent granules, which are homogeneous bodies when first formed, and are totally devoid of a duplex structure.

This has been fully confirmed by Sant Singh in his studies under the phase-contrast on the nematode from the python. In late primary spermatocytes this worker has seen Golgi granules, Golgi spheroids and fully matured refringent bodies lying side by side in the whole cytoplasm of the living cell, leaving no doubt about the origin of the refringent bodies directly from the Golgi spheroids. This is further supported by the observations that the secondary spermatocytes have no traces of the Golgi elements, both granules and spheroids, whereas the cytoplasm is now choked with refringent bodies.

(*c*) *Origin of the Acrosome*

As quoted by Sturdivant (1934), all the workers on the nematode sperm without exception (van Beneden and Julin, 1884; Scheben, 1905; Tretjakoff, 1905; Mayer, 1908; Romieu, 1911; Hirschler, 1913; Wildman, 1913) have described these remarkable refringent granules. All agree without exception that these granules fuse to form one large refringent body (the acrosome) of the ripe sperm.

According to Sturdivant (1934) the refringent granules, when first secreted, are homogeneous in structure, but during both the meiotic divisions each granule shows a sharply staining rod in its interior. Early in spermateleosis the rods disappear completely; and the refringent granules by a progressive process of coalescence form one large refringent body, the acrosome of the ripe sperm.

Sturdivant interprets the refringent spheres, when first secreted, as the acrosomal vesicles, the rods as the acrosomal granules and the final matured refringent granules as the proacrosomal granules. We entirely agree so far as *Porrocaecum* is concerned, except that the refringent spheres are formed directly from the Golgi spheroids and are not secretory products of the latter. But we have been unable to observe the acrosomal granules in *Ascaris suilla*, as our preparations were lacking in meiotic stages.

(*d*) *Cytophore and Plasma Lobe*

Sturdivant (1934) has described a cytophore as well as a plasma lobe in the spermatogenesis of *Ascaris megalocephala*.

The cytophore, according to Sturdivant, is a cytoplasmic projection formed at the close of meiosis II towards that part of the earliest spermatid, which is in contact with its sister cell. 'It seems probable that the formation of this structure is an extrusion of cytoplasmic material in the region of separation, because some of the Golgi bodies, which are at this time

collected in this region, and a few mitochondria are carried out into it during its formation' (ref. figs. 63 and 64).

We must confess that we have not seen the cytophore as described by Sturdivant in *Ascaris megalocephala* either in *A. suilla* or in *Porrocaecum angusticolle*, much less the casting off of the Golgi bodies and mitochondria. Sturdivant's figures 63 and 64 are not at all convincing; and we get the impression that the author is unduly influenced by the sloughing off of some of the Golgi elements and the mitochondria, which is almost universal in normal spermatogenesis of a flagellate sperm.

The plasma lobe, according to Sturdivant, is a large cytoplasmic projection formed toward the posterior portion of later spermatids. It consists of 'a great part of the cytoplasm of the cell'. In the meantime the remaining Golgi elements, according to Sturdivant, have fused into one large mass, the Golgi remnant, which is pushed into the plasma lobe.

According to our observations, there is a plasma lobe in *Porrocaecum angusticolle* and in *Ascaris suilla*; and sometimes we have seen more than one lobe in the former species changing their form like the pseudopodia of an *Amoeba*. But in both the species, the plasma lobes are absolutely devoid of Golgi bodies and mitochondria.

(e) Ripe sperm

Sturdivant (1934) describes the ripe sperm of *Ascaris megalocephala* as a cone-like cell with a broad base and a narrow apex. The nucleus with most of the mitochondrial vesicles lies towards the broad end, and the acrosome (refringent body) towards the narrow end. There is no Golgi material in the ripe sperm.

We also did not find any Golgi elements in the ripe sperm of *A. suilla*, either in the fixed cell or in the living cell examined under the phase-contrast. But Collier (1936) includes a few Golgi elements in the ripe sperm of *A. suilla*.

Nevertheless we have seen a few Golgi elements in the living ripe sperm of *Porrocaecum angusticolle* examined under the phase-contrast (Pl. I, fig. 3).

(f) Mitochondria

It has been shown that in *Porrocaecum angusticolle* the mitochondria remain granular and stain feebly throughout spermatogenesis. Hence they cannot be demonstrated unless they are circum-nuclear in arrangement (Text-figs. 1, *a*; 2, *a–f*; 3, *a–c*; and Pl. I, fig. 3). The same is true of *A. suilla* except that in the late spermateleosis the mitochondria grow into prominent vesicles (Text-fig. 7, *a–e*).

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V. Explanation of Plates

All the photomicrographs were taken with a Leitz 'Dialux' phase-contrast microscope fitted with a Heine condenser. The photomicrographs were taken either under Pv Apo. 90/1·15 oil immersion or Pv Apo. 70/0·90 objectives and 10× ocular employing a Leica camera and Leitz photomicrographic attachment. The photomicrographs were further enlarged four times and are untouched.

Plate I

Lettering same as for Text-figures

Fig. 1. Two living spermatids of *Porrocaecum* from the male, showing clear pseudopodia-like plasma lobes.

,, 2. Same as in fig. 1. Here a refringent body containing an acrosomal granule has accidentally wandered into the plasma lobe.

,, 3. Ripe living sperm of *Porrocaecum* from the uterus of the female.

Figs. 4 and 5. Ripe living sperms of *Ascaris suilla* from the uterus of the female.

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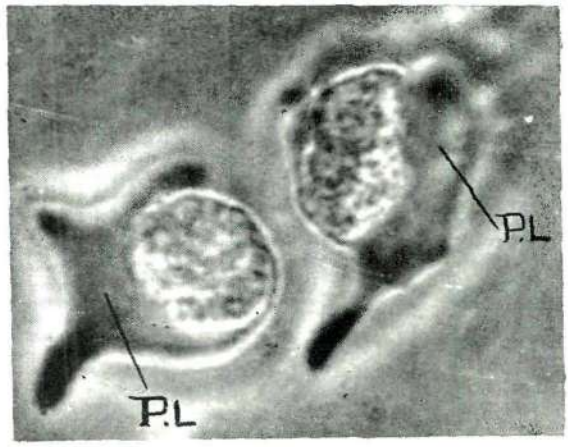

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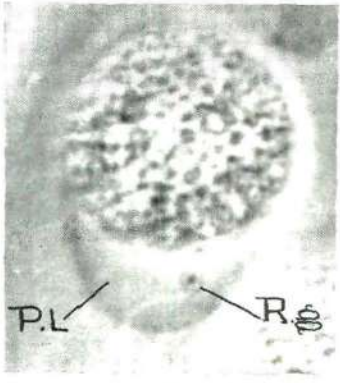

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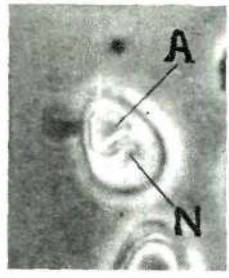

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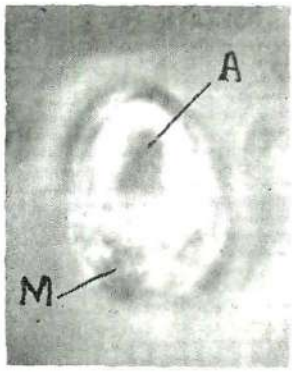

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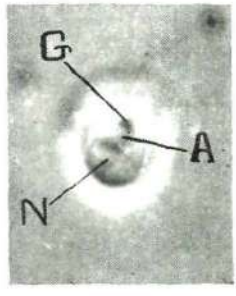

3

RESEARCH BULLETIN
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No. 92 CHEMISTRY Pp. 135–138

August, 1956

EFFECT OF TEMPERATURE OF INCUBATION ON THE CHEMICAL COMPOSITION OF *ASPERGILLUS NIDULANS* FAT

by

J. SINGH and T. K. WALKER

(College of Technology, University of Manchester)

Edited for the Panjab University by Vishwa Nath,
Department of Zoology, Panjab University,
HOSHIARPUR

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was allowed at 17°, 25°, and 30° respectively. These flasks were sterilized by autoclaving for 15 minutes at 10 lbs. steam pressure. The felts were removed and thoroughly washed with water. The washings were made to a known volume with distilled water and the amount of the residual sucrose was determined. The felts were dried in air and then at 60° till the weight became constant. The mycelial mass was then ground and thoroughly extracted with petroleum (b.p. 40–60°). The solvent was removed from the extracted fat by distillation and the last traces of petroleum were removed by heating *in vacuo* for 15 minutes at 100°. Chemical characteristics of the fat were determined according to British Standard Methods of Analysis of Oils and Fats (1950). Spectrophotometric examination of the mixed fatty acids was conducted according to the method of Hilditch *et al.* (1951).

III. Results

The effect of temperature on the weight of felt and fat produced is recorded in Table I. The calculations are based on the average contents of one Glaxo flask and the standard deviation is given beneath the mean felt weight.

Table I

Effect of temperature during incubation period on the formation of felt and fat by Aspergillus nidulans *in 175 ml. of medium*

Temperature (°C.)	Incubation period (days)	Weight of felt (g.)	Weight of fat (g.)	Weight of sugar consumed (g.)	Fat as % of felt	Fat coefficient	Economic coefficient
17 ..	15	7·523 (±0·546)	1·166	26·74	15·50	4·3	28·1
25 ..	9	14·116 (±0·514)	6·310	43·45	44·7	14·5	32·5
30 ..	7	13·253 (±0·331)	4·834	43·10	36·5	11·2	30·7

The chemical characteristics of the fat synthesized at different temperatures are given in Table II.

Table II

Chemical characteristics of the fat of Aspergillus nidulans *at different temperatures*

Temperature (°C.)	Acid value	Saponification value	Saponification equivalent	Iodine value	% Unsaponifiable matter
17 ..	2·5	196·5	285·5	95·8	4·45
25 ..	0·84	193·3	290·3	73·7	1·3
30 ..	0·63	195·1	287·6	78·6	2·0

EFFECT OF TEMPERATURE OF INCUBATION ON THE CHEMICAL COMPOSITION OF *ASPERGILLUS NIDULANS* FAT

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Abstract

The changes in the amounts of component fatty acids of the fat of *Aspergillus nidulans* produced at 17°, 25° and 30° have been studied. The fat synthesized at 17° was more unsaturated than at 25°, but more unsaturation was also observed in the fat produced at 30° than at 25°.

I. Introduction

Ivanow (1926) was the first to demonstrate that the oil produced from linseed grown in a colder climate was more unsaturated in character than that grown in a warmer locality. Pearson and Raper (1927) studied the effect of temperature on the degree of unsaturation of the fatty acids produced by moulds and they also observed more unsaturation in the fatty acids at lower temperature. On the other hand Prill *et al.* (1935) could not confirm these findings with *A. fischeri*. Unfortunately in all these investigations iodine absorption has been used to assess the degree of unsaturation, and the proportions of the different fatty acids were not determined. Recently Barker and Hilditch (1950) have carried out detailed study of the influence of environmental conditions on the composition of sunflower seed oils. During our investigations (1955) of the component fatty acids of *Aspergillus nidulans* fat, it was observed that this micro-organism gave a high yield of fat when grown on a simple medium containing sugar and inorganic salts. It was thought desirable to study the effect of temperature on the composition of *Aspergillus nidulans* fat, as the substrate and cultural conditions can be properly controlled.

II. Experimental

Aspergillus nidulans Eidam was grown under static conditions on the following medium (g./100 ml.): sucrose, 34; NH_4NO_3, 0·6; K_2SO_4, 0·044; $MgSO_4.7H_2O$, 0·5; $ZnSO_4.7H_2O$, 0·005; $FeCl_3.6H_2O$, 0·016; $NaH_2PO_4.2H_2O$, 0·73, made up to volume with distilled water and adjusted to *p*H 3·8. During these investigations a set of Glaxo flasks, each containing 175 ml. of the medium was employed. The number of flasks employed at a particular temperature depended upon the mycelial growth. It was necessary to take 20 flasks at 17°, 5 at 25° and 10 at 30° in order to get sufficient fat for detailed analysis. Incubation period of 15, 9 and 7 days

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The component fatty acids of the fat samples procured from the felts, harvested at different temperatures, are shown in Table III.

TABLE III

The component fatty acids of the fat produced by Aspergillus nidulans *at different temperatures*

Temperature (°C.)	Mixed fatty acids		Composition of acids (% wt.)			
	Iodine value	$E^{1\%}_{1\,cm.}$ @ 234 mμ.	Saturated	Oleic	Linoleic	Linolenic
17 ..	93·9	213	22·1	53·7	22·4	1·8*
25 ..	73·7	155	35·5	47·4	17·1	..
30 ..	74·8	156	34·4	48·4	17·2	..

* $E^{1\%}_{1\,cm.}$ @ 268 mμ was 9, and values less than 5 in other cases were neglected.

The actual weights of the individual fatty acids of the fat, synthesized *per Glaxo flask at different temperatures, are recorded in Table IV.*

TABLE IV

Actual weights of individual fatty acids of Aspergillus nidulans *fat synthesized in 175 ml. of medium at different temperatures*

Temperature (°C.)	Felt weight (g.)	Fat (g.)	Saturated acids (g.)	Oleic acid (g.)	Linoleic acid (g.)
17 ..	7·5	1·2	0·2	0·6	0·3
25 ..	14·1	6·3	2·1	2·8	1·0
30 ..	13·3	4·8	1·5	2·2	0·8

It will be observed from Table I that the optimum temperature for fat formation in the case of *Aspergillus nidulans* is 25°. The amount of fat in the mycelium is 44·7% at 25°, 36·5% at 30° and 15·5% at 17°. From the chemical characteristics recorded in Table II it is also clear that the percentage amount of unsaponifiable matter is minimum when the formation of fat is maximum. Under adverse conditions the percentage amount of unsaponifiable matter increases. The iodine value is maximum at 17°. Larger proportions of unsaturated acids are synthesized at the lowest temperature, whereas the amounts at 30° show a slight increase over the amounts at 25° as given in Table III. From the actual weights of individual fatty acids recorded in Table IV, it is quite clear that the amounts of all the component acids, i.e. linoleic, oleic, and saturated acids increase as the temperature rises from 17° to 25°, and the amounts of all the acids decrease, when the temperature rises from 25° to 30°.

IV. Discussion

Barker and Hilditch (1950) noted that in the case of sunflower seeds the amount of saturated acids remained constant and relative proportions of oleic and linoleic acids varied according to the environmental conditions. Recently Stansbury and Hoffpauir (1952) carried out detailed investigations on the influence of environments upon the composition of cotton-seed oil. 48 samples of cotton-seed from eight varieties and different localities were examined. They observed that with the increase in the iodine value, the linoleic acid content increased while oleic acid and saturated acids decreased. In the present studies it has been found that the relative proportions of all the acids vary with different temperatures. The actual weight of each individual fatty acid synthesized in the case of *Aspergillus nidulans* is maximum at the optimum temperature (25°) and at other temperatures each component acid registers a fall.

The authors take this opportunity to thank the Committee on Chemical Micro-biology of the British Medical Research Council for financial assistance.

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September, 1956

SPERMATOGENESIS OF THE DOMESTIC FOWL, *GALLUS DOMESTICUS*

by

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Abstract

During a careful study of the process of spermatogenesis in the domestic fowl, it has been observed that the mitochondria which appear granular in the fixed preparations are definitely fibrillar in the spermatocytes. They rôle themselves during spermateleosis to form big granules some of which arrange themselves along the axial filament between the two centrosomes to form the middle-piece. All the Golgi elements which are definitely granular in form are consumed in the formation of the vacuole-like proacrosome. This is then directly transformed into the triangular acrosome of the ripe sperm.

Introduction

Zlotnik (1947), who has reviewed the earlier literature on the subject, seems to be the only worker to employ modern cytological techniques to work out the spermatogenesis of the domestic fowl. According to him, the Golgi material 'is in the form of a localized body, composed of rods and granules which lie on the surface of the archoplasm'. He further states that the acrosome is formed by the proacrosome which 'originates within the archoplasmic vacuole inside the Golgi material of the spermatid'. After the acrosome has been formed the Golgi remnant is sloughed off with the residual cytoplasm.

Since 1947, three more papers dealing with avian spermatogenesis in a modern way have come out. Two of these are by Mehrotra (1951*a* and *b*) and the third is by Gupta (1955). Mehrotra has worked out the process of spermatogenesis in the domestic pigeon and the mynah. According to him, the Golgi elements, though 'localized in their distribution being limited to the archoplasm (idioplasm)', are spherical in form. Similarly Gupta, who has carefully studied the spermatogenesis in the domestic duck from the fixed as well as the living material under the phase-contrast microscope, states that the Golgi elements are in the form of distinct granules or spheroids but these are never localized in the earlier stages. In the spermatid, however, they reveal a distinct tendency to come together till ultimately by their fusion a large round or oval chromophilic body—the proacrosome—is formed. Gradually the proacrosome is differentiated into a clear vacuole which is directly transformed into the acrosome of the ripe sperm. As no Golgi remnant could be detected by him in either the fixed or the fresh material, it seems that all the Golgi elements are consumed in the formation of the acrosome.

In view of this recent work which has been carried out in this very laboratory the present investigation was undertaken to throw some more

light on the process of spermatogenesis in the domestic fowl, *Gallus domesticus*.

Material and technique

Most of the material for the present study was obtained from a small-scale poultry farm but sometimes a local restaurant also came to our rescue with a donation of the required material.

The material was fixed from the end of March to the beginning of August. The study of the fresh material under the phase-contrast microscope was also undertaken in the middle of September. It was thought advisable to have the testicular material of the younger specimens only.

The animal was killed by violent twisting and pulling off of the neck or by pithing the head. The testes were quickly dissected out and removed to the Ringer's solution immediately. By the use of a sharp safety razor blade the material was then cut into very small pieces which were instantaneously transferred to a variety of fixing fluids. Soon the material along with the fixative was poured into a deep watch glass and it was teased with the help of fine needles into still smaller pieces.

The fixatives used for the present investigation were Champy, Champy-Kull, F.W.A. (*full strength*), F.W.A. (*diluted*), Kolatchev, Aoyama and Bouin. The material was fixed in these fluids for varying periods but Champy (24 hours) and diluted F.W.A. (48 hours) gave the best results.

After proper dehydration and clearing, the material was embedded in paraffin wax and the sections which were cut 4–6 microns thick were stained with 0·5% Iron-haematoxylin. The stained sections were mounted in Canada balsam. The sections of the material fixed in Aoyama and Kolatchev were, however, mounted unstained.

Smears of the testicular material were also prepared in F.W.A. diluted with an equal quantity of the distilled water. In this case too 0·5% Iron-haematoxylin was employed for staining.

Many a time the material was found to be tinged with the colour of the fixing reagents. To remove this defect bleaching of the sections with 1% $KMnO_4$ followed by 5% oxalic acid was resorted to.

Observations

Sertoli cells.—The sertoli cells in the testicular material of the domestic fowl vary in shape and structure. To begin with, they are somewhat spherical (Pl. I, Fig. 1), the circular outline later flattening at angles (Pl. I, Figs. 2 to 4). Each of them possesses a large nucleus which is usually stained lightly and reveals inside it a prominent nucleolus besides some fine chromatin granules. In the cytoplasm the Golgi material first appears at one pole of the nucleus in the form of a big osmiophilic granule. This particular pole is directed towards the lumen of the tubule. The mitochondria at this stage are too faint to be clearly made out (Pl. I, Fig. 1).

Soon the single large Golgi granule divides into a variable number of clumps of smaller granules (Pl. I, Figs. 2 to 5). The mitochondria also

now put in their appearance in the form of lightly staining and fine, dust-like particles (Pl. I, Fig. 4).

During the subsequent stages of development the sertoli cell begins to elongate at that pole which contains the Golgi elements and the mitochondria (Pl. I, Fig. 5). With the elongation of the cell both these inclusions also spread uniformly throughout the elongating part of the cytoplasm. Finally the groups of very late spermatids become embedded in the grown out cytoplasm of the sertoli cell and the previously scattered Golgi elements can very often be seen approaching the sperm heads (Pl. I, Fig. 6).

Spermatogonia.—The spermatogonia are generally situated at the periphery of the seminiferous tubule. In their earliest stage (Pl. I, Fig. 7), they are elliptical in shape, each with a comparatively large nucleus which hardly reveals any structure save for the presence of a deeply staining prominent nucleolus and a few very small chromatin granules. The nucleus is not exactly central in position, the thin film of cytoplasm surrounding it being a bit wider on one side of it, where a few fine granules are found scattered. The rest of the cytoplasm is clear and does not reveal any granulation whatsoever. At this stage there is no visible differentiation of granules into different categories. Most probably they are all mitochondria.

In a comparatively later stage (Pl. I, Fig. 8), however, it is possible to differentiate one or two bigger and more chromophilic granules embedded amongst the remaining smaller ones. These bigger, darkly stained granules are assigned to the category of the Golgi elements, while the rest of them are the mitochondria. In the nucleus, the nucleolus and a few chromatin granules have become more prominent.

The fully grown spermatogonia are more or less rounded cells (Pl. I, Fig. 9). The thin film of cytoplasm surrounding the nucleus is much wider at one pole, thus placing the latter in an excentric position. This wider cytoplasmic area reveals an aggregation of a number of faintly stained fine granules, especially around a darkly stained mass. A few of these granules are also present on the sides of the nucleus, thus forming a sort of horse-shoe. These granules are the mitochondria. In the centre of this horse-shoe of the mitochondria there is the localized mass of the chromophilic Golgi material which is composed of a number of closely packed granules. In some cases the Golgi granules can be very clearly made out, there being, of course, no other structure amongst them. The background of the Golgi mass which is comparatively darker than the rest of the cytoplasm seems to contain fine mitochondrial granules embedded amongst the bigger and dark Golgi granules (Pl. I, Fig. 9).

These observations on the composition of the Golgi mass of the spermatogonium are substantially supported by the study of the living spermatogonia under the phase-contrast microscope where the Golgi granules can be easily demonstrated.

Primary spermatocyte.—The primary spermatocyte which is the largest cell of the testis is also met with near the wall of the tubule just next to the spermatogonia (Pl. I, Fig. 10). It generally possesses a large vesicular nucleus often containing a prominent nucleolus. The layer of cytoplasm

surrounding the nucleus is wider at one pole at which, in the resting stages, can be seen the Golgi material in the form of a localized body formed, of course, by a number of darkly staining granules. This Golgi material shows a dense cortical region composed of granules placed very close to each other. The comparatively lightly staining central part owes its appearance to the slightly loose arrangement of the granules. The mitochondria which are in the form of faintly staining granules are present mostly around the Golgi material but some of them can be seen scattered down the sides of the nucleus also (Pl. I, Fig. 10).

Besides these cell components, there is often visible in the cytoplasm a small, darkly staining, spherical body. This, in the absence of a more suitable name at the present moment, may be called the 'accessory body' as Zlotnik (1947) terms it (Pl. I, Figs. 10 and 11).

In preparation for the forthcoming first meiotic division, the whole cell, particularly the nucleus, undergoes a series of systematic changes. In the early prophase stages the Golgi elements show signs of dispersal. In some cases it has been observed that the Golgi material spreads out assuming the form of a cap. Inside this cap is visible a deeply staining, small and spherical granule which is most probably the 'accessory body' (Pl. I, Fig. 12). In another case, in addition to the 'accessory body', a similar granule can also be seen on the margin of the Golgi cap (Pl. I, Fig. 13).

The Golgi material subsequently breaks up into a number of clumps which, in turn, further break up into granules (Pl. I, Figs. 14 to 17 and Pl. II, Fig. 18). These granules, with the beginning of the metaphase, become more or less evenly distributed in the cytoplasm.

Some similar observations regarding the Golgi elements have also been made in the living spermatocytes under the phase-contrast microscope (Pl. IV, Figs. 57 and 58), but the mitochondria in them, unlike those in the fixed preparations, are fibrillar instead of granular.

Only a few division stages have been noticed and figured by us. In the metaphase I (Pl. II, Fig. 19 and Pl. IV, Fig. 59) the chromosomes are arranged at the equator. In this position they appear as a darkly stained compact mass in the centre of the cell. Two centrosomes are also visible, one at each pole of the spindle which, however, is not very well defined.

With the arrangement of the chromosomes at the equator, further dispersal of the individual Golgi elements takes place. But, during the division stages, these are visible in a comparatively much smaller number than seen originally in the resting primary spermatocyte.

In the telophase I (Pl. II, Fig. 20) the two chromosomal masses, destined to form the corresponding nuclei of the daughter cells, are seen occupying the respective poles. As the Golgi elements and the mitochondria remain in a state of dispersal, apparently outside the spindle area, they seem to be distributed almost equally to the two daughter cells. The 'accessory bodies' could not be differentiated during the division stages.

The division of the cell, thus, culminates in the formation of two daughter cells—the secondary spermatocytes, each with its approximately equal share of the cytoplasmic contents derived from the parent cell. Soon

the originally dispersed Golgi elements reassemble to form the localized Golgi material of the secondary spermatocyte.

In several cases, a departure from the normal phenomenon of division has been noticed. It results in the formation of a cell with two nuclei, each with its separate mitochondria and the Golgi elements (Pl. II, Figs. 21 and 22). Most probably it is caused by a failure of the cytoplasm to divide after the nuclear division of the cell. But for their size, such cells, of course, are not abnormal as assumed by Zlotnik (1947). Their cytoplasm will divide at a little later stage resulting ultimately in the formation of normal spermatozoa in due course of time.

Secondary spermatocyte.—The secondary spermatocyte is a much smaller cell than the primary but it is slightly larger than the spermatogonium. The Golgi material in it is present in the form of a cap at one pole of the nucleus (Pl. II, Fig. 23). Usually a darkly stained granule is also visible in the cytoplasm, lying in the vicinity of the Golgi material. This is the 'accessory body' (Pl. II, Figs. 23 and 24). The mitochondria are present in a scattered state.

Just before and during meiosis II, the Golgi elements and the mitochondria behave in a similar manner as seen at the time of the division of the primary spermatocyte (Pl. II, Figs. 25 to 27).

Besides the normal cells, formed as a result of the mitotic and meiotic divisions, several large cells with two or more nuclei have been observed (Pl. II, Figs. 28 to 31). The cell with four nuclei results from two consecutive nuclear divisions of a particular cell without any corresponding division of the cytoplasm. In one such case two chromosomal masses have been observed to undergo division, having their own separate spindles and the centrosomes (Pl. II, Fig. 32). It will not be superfluous to add here that each chromosomal mass has its usual complement of the mitochondria and the Golgi elements.

During the study of the fresh material under the phase-contrast microscope also many such large cells containing more than one nucleus are encountered.

Spermatid and its metamorphosis.—The earliest spermatid, which is formed as a result of the second maturation division, is a comparatively small cell with a spherical and slightly excentric nucleus. In the beginning, the nucleus reveals inside it four or five darkly stained chromatin granules scattered through the nucleoplasm. The complement of the Golgi elements received by the newly formed cell is originally in a dispersed state (Pl. II, Fig. 33). Soon, however, they show a marked tendency to come closer together, the granules clumping into smaller groups and the latter, in their turn, jointly form a mass at one pole of the nucleus (Pl. II, Figs. 33 to 36). The dense cortical layer of the Golgi mass is composed of small granules packed closely together, whereas the central part is comparatively lightly staining owing to the slightly loose arrangement of its constituents. This localized mass of the Golgi material formed by the progressive regrouping of the granules undergoes hereafter a series of very important changes.

The faintly staining mitochondria of the spermatid maintain their granular form and are seen scattered throughout the cytoplasm but mostly around the Golgi material.

Sooner or later the loosely arranged granules of the central area of the Golgi mass move to its periphery giving it the appearance of a horse-shoe, surrounding a small clear space in the centre (Pl. II, Fig. 37). This space gradually grows to take up the form of a hyaline vacuole to be known hereafter as the proacrosome (Pl. III, Figs. 38 to 40 and Pl. IV, Fig. 64). Since the entire horse-shoe disappears in the meanwhile and as no Golgi remnants could be detected in any of the later stages, it appears that all the Golgi elements of the spermatid are consumed in the formation of this vacuole-like proacrosome. A deeply staining granule is, however, seen at a much later stage, on the posterior side of the late spermatid nucleus (Pl. III, Figs. 51 and 52). Keeping in view its staining reaction which is similar to that of the Golgi material, this granule should be considered as a part of it. It is undoubtedly homologous to the granules which in the preceding account have been described under the term 'accessory bodies'. But as regards its function and position in the ripe spermatozoon Zlotnik's description cannot be supported as it is no longer visible at that stage. There is every likelihood of its disappearance in the final stages of spermateleosis.

In the meanwhile a deeply staining granule is differentiated in the proacrosomal vacuole towards its top (Pl. III, Fig. 41). This is the acrosomal granule. During the subsequent stages it slowly travels through the hyaline vacuole (Pl. III, Fig. 42), till ultimately it comes to lie in the centre of the nuclear depression (Pl. III, Fig. 43). This depression in the nuclear membrane is caused in one of the preceding stages (Pl. III, Fig. 39) due to the pressure exerted by the proacrosome on the anterior part of the nucleus.

Now that part of the nucleus where the acrosomal granule had taken up its position begins to grow inside the proacrosome (Pl. III, Fig. 44). In the meanwhile the proacrosome itself is condensed to form a deeply staining acrosome which is triangular in shape in the ripe sperm (Pl. III, Figs. 45 to 54 and Pl. IV, Figs. 60 to 63, 65 and 66). For a long time the acrosomal granule can be clearly made out at the anterior tip of the elongating nucleus just at the base of the condensing proacrosome (Pl. III, Fig. 46 and Pl. IV, Fig. 61). But, as there is no trace of it in the ripe sperm, it seems to spread a little to form the narrow base of the acrosomal triangle.

The continued elongation of the nucleus without any corresponding response from the cytoplasm results in the coiling up of the former (Pl. III, Figs. 47 and 48) to give rise ultimately to S- and Z-like figures. On account of the subsequent elongation of the cytoplasm is seen a similar phenomenon of uncoiling which leads to the straightening up of the nucleus inside the cell (Pl. III, Fig. 49). A downward attenuation and shrinkage of the cytoplasm helps the nucleus to become free of the former, but its posterior end still retains some of it in the form of the residual cytoplasm (Pl. III, Figs. 50 to 52). A study of both the fresh and sectioned material clearly reveals that the maturing spermatozoon becomes free of its cytoplasm by the shrinkage and sloughing off of the latter on the one hand and by the

attempts of the former to cast it off on the other. While studying the living material under the phase-contrast microscope it is very interesting indeed to observe the spermatozoon struggling hard to get rid of the residual cytoplasm.

In the spermatid when the scattered Golgi elements are collecting together two centrioles can also be clearly made out in its posterior part (Pl. II, Fig. 35). It seems that these are formed as a result of the division of the originally single centrosome. Soon one of these moves towards the nucleus and comes to lie on that pole of it which is opposite the proacrosome (Pl. III, Figs. 38 and 39). This is the proximal centriole. The other, now known as the distal, also lies nearby and the two are connected by means of an intra-cellular filament which is continued outside the cell in the form of a thin axial filament. As the proximal centriole becomes one with the posterior end of the nucleus it cannot always be clearly differentiated from it. The distal centriole gradually travels down the elongating spermatid till ultimately it comes to lie at a place which is the posterior limit of the future middle-piece (Pl. III, Figs. 40 to 45, 47, 49, 50, 52 and 53). Since the thin axial filament arising from the proximal centriole seems to pass through the distal centriole it appears that the latter is in the form of a ring which is, however, not so conspicuous in this material as in the mammals.

The lightly staining mitochondria of the early spermatid are restricted to the anterior half of the cell (Pl. II, Figs. 33 and 36). With the formation of the proacrosome, however, they appear to be divided into two groups, each situated on either side of it and the nucleus (Pl. III, Figs. 38 to 40). Subsequently when the acrosomal granule moves from the top to the bottom of the proacrosome, the mitochondria become arranged in four groups—two forming the sides of the proacrosome and two covering the postero-lateral margins of the nucleus (Pl. III, Figs. 42 to 44). Gradually all the mitochondria move backwards (Pl. III, Figs. 45 to 52) and with the simultaneous shrinkage of the cytoplasm to the posterior side of the nucleus they come to lie just at the base of it. At this stage, however, the mitochondria are comparatively deeply stained. Very soon, they arrange themselves along the axial filament, constituting later, the spiral sheath of the short middle-piece of the ripe sperm.

During the last stages of the phenomenon of uncoiling of the nucleus a clear zone is sometimes observed at the posterior end of it (Pl. III, Fig. 51 and Pl. IV, Fig. 63). This is very clear indeed at a later stage when the nucleus is almost free from the residual cytoplasm. Since the mitochondria arrange themselves along the axial filament in this clear zone it appears that the lateral boundaries of the middle-piece are formed by it.

It is always very interesting to note that in several cases the development of the two spermatids proceeds without their complete separation from each other after the second meiotic division (Pl. III, Fig. 55). Similar stages have also been met with during the fresh study under the phase-contrast microscope (Pl. III, Fig. 56).

Ripe sperm.—The fully ripe sperm is a moderately long structure, composed of a deeply staining and slightly curved head, followed by a short

middle-piece and a thin vibratile tail (Pl. III, Figs. 53 and 54 and Pl. IV, Figs. 58 and 66). Normally it is always difficult to differentiate the two structures constituting the head of the sperm, namely the acrosome and the nucleus, but in a slightly less stained sperm a conical acrosome, situated at the anterior end of the cylindrical nucleus, can be clearly made out. Immediately behind the head is the short middle-piece, the posterior boundary of which is formed by the distal centriole (Pl. IV, Fig. 65). The proximal centriole, forming the anterior boundary of the middle-piece, is not always clear but a dark point is sometimes present at the posterior extremity of the head of the sperm. Very often a spiral mitochondrial sheath is also visible in the middle-piece region of the sperm. Such an appearance of the sheath is due to the irregular distribution of the mitochondria along the axial filament. The tail is extremely thin and comparatively very long. Consequently in the sectioned material it is difficult to trace out the whole of it. But under the phase-contrast microscope it can be clearly demonstrated in the form of a long thread (Pl. III, Fig. 54 and Pl. IV, Fig. 66).

A study of the fresh material under the phase-contrast microscope reveals all these structures with great clarity, each sharply demarcated from the others. It is very fascinating indeed to see the living sperm *making worm-like but slightly jerky movements.*

Discussion

Golgi material.—Zlotnik (1947), while working on the cytoplasmic components of the germ cells during spermatogenesis in the domestic fowl, has described the Golgi material 'in the form of a localized body, composed of rods and granules which lie on the surface of the archoplasm'. So far as the localized condition of the Golgi material is concerned we are in complete accord with Zlotnik, but we have never come across his rods, the Golgi elements being always in the form of distinct granules. As has been shown earlier by Sharma *et al.* (1953), the rod-like form of the Golgi elements seems to us to be an artifact, produced by the close alignment of the Golgi granules and excessive deposition of silver or osmium not only on the surface of the individual Golgi elements but also in the narrow spaces between them.

Our observations on the form of the Golgi elements are strongly supported by the recent work in this laboratory on the domestic duck by Gupta (1955), according to whom the Golgi elements are in the form of distinct granules and spheroids but these are never localized in the earlier stages. Similarly Mehrotra (1951*a* and *b*) describes the Golgi elements in the domestic pigeon and the mynah as distinct spheres which, according to him, 'are localized in their distribution being limited to the archoplasm (idioplasm) in the spermatogonia and the spermatocytes'. His figures, however, especially those for mynah, do not clearly show the archoplasm. In our own preparations also we have not been able to make out a distinct archoplasm as is generally met with in the mammals. The background of the Golgi mass is, however, comparatively darker than the rest of the

cytoplasm and it seems to contain fine mitochondrial granules embedded amongst which are the bigger and darker Golgi elements.

Acrosome.—In the earliest spermatid of the domestic fowl the Golgi elements are, at first, dispersed but soon they collect together on one side of the nucleus and finally arrange themselves in the form of a horse-shoe, enclosing a clear space in between two of its arms. Gradually this space grows into a hyaline vacuole—the proacrosome, in the formation of which are consumed all the Golgi elements of the spermatid. Consequently there are no Golgi remnants. Soon afterwards a deeply staining granule is differentiated in the proacrosomal vacuole. This is the acrosomal granule. The conical acrosome of the ripe sperm is directly formed by the proacrosome and the acrosomal granule, the latter forming the narrow base of it.

Our observations on the domestic fowl are strongly supported by those of Mehrotra (1951*a* and *b*) on the domestic pigeon and the mynah. According to him, the 'complex of the vesicle (our proacrosome) and the granule becomes applied to the wall of the nucleus and gives rise to the acrosome'. His figures for mynah, showing the direct formation of the acrosome from the vesicle and the granule, are very convincing indeed. Similarly Gupta (1955) has also clearly shown that the Golgi elements in the spermatid of the domestic duck reveal a distinct tendency to come together till ultimately by their fusion a large, round or oval, chromophilic body—the proacrosome—is formed. Gradually the proacrosome is differentiated into a clear vacuole which is directly transformed into the acrosome of the ripe sperm.

On the other hand, Zlotnik (1947), working on the testicular material of the domestic fowl, states that 'the acrosome is formed from the proacrosome' (our acrosomal granule) which 'originates within the archoplasmic vacuole inside the Golgi material of the spermatid' probably as a result of secretion because after the formation of the acrosome the Golgi material moves posteriorly and is sloughed off with the residual cytoplasm as the Golgi remnant. Since, however, the Golgi remnants are conspicuous by their absence in our own material as well as in that of Gupta (1955) and Mehrotra (1951*a* and *b*) we find it difficult to believe the above account of Zlotnik. It appears to us that he has been led to this erroneous conclusion on account of his earlier work on mammals (Gresson and Zlotnik, 1945).

Accessory bodies.—It will be recalled that we described a deeply staining spherical body in the cytoplasm of the spermatocytes—both primary and the secondary. As it has been seen to come out of the localized Golgi material, it appears to us to be just a part of it. Similar are the views of Zlotnik (1947).

Sharma *et al.* (1953) had also described the passing out of a big Golgi granule from the idiosome when most of the Golgi granules in it had fused to form the forerunner of the acrosome and they compared it to the accessory body described by Gresson and Zlotnik (1945 and 1948). They further felt that some of those Golgi granules also which did not come together to be in the localized condition and remained dispersed throughout the cytoplasm of the spermatocytes and the spermatids might have been

labelled as the chromatoid bodies by the earlier workers. Gupta (1955), on the other hand, has described during the spermatogenesis of the domestic duck an enigmatic chromatoid body which, according to him, is quite distinct from the Golgi elements.

Mitochondria.—The behaviour of the mitochondria in the present material is more or less the same as has been described earlier by Zlotnik (1947), Mehrotra (1951*a* and *b*) and Gupta (1955). In our fixed preparations the mitochondria have been observed to be in the form of very fine, dust-like particles from the earliest spermatogonium to the spermatid. During spermateleosis, however, they are appreciably bigger in size and are much more deeply staining. On the other hand, a careful study of the fresh material under the phase-contrast microscope has clearly shown that the mitochondria in the earlier stages of spermatogenesis are distinctly filamentous in form but as they roll over during the later stages they form quite big spheres giving a dark contrast.

During the last stages of the phenomenon of uncoiling of the nucleus a clear zone is observed at the posterior end of it. This zone becomes much more distinct at a little later stage when the nucleus is almost free from the residual cytoplasm. Since the bigger and deeply staining mitochondria of the late elongating spermatids arrange themselves along the axial filament in this clear zone it seems that the lateral boundaries of the middle-piece are formed by it. Zlotnik (1947), therefore, rightly compares it with the manchette of the mammalian spermatids.

Centrosomes.—During the present study, the centrosomes in the earlier stages were observed only at the time of the division in the form of distinct granules, each situated at the respective pole of the spindle which was, however, never very conspicuous. In the spermatid when the scattered Golgi elements are collecting together two centrioles can also be clearly made out in its posterior part. They seem to be formed as a result of the division of the originally single centrosome. Soon one of these moves towards the nucleus and comes to lie on that pole of it which is opposite the proacrosome. This is the proximal centriole. The other, now known as the distal, also lies nearby and the two are connected by means of an intracellular filament which is continued outside the cell in the form of an axial filament. As the proximal centriole becomes one with the posterior end of the nucleus, it cannot always be clearly differentiated from it. The distal centriole gradually travels down the elongating spermatids till ultimately it comes to lie at a place which marks the posterior boundary of the middle-piece. Since the thin axial filament, arising from the proximal centriole, seems to pass through the distal centriole, it appears that the latter is in the form of a ring which is, however, not so conspicuous in this material as in the mammals. Zlotnik (1947), on the other hand, has described a clear, ring-like distal centriole in the same material.

Summary

1. In this paper, the spermatogenesis of the domestic fowl, *Gallus domesticus*, has been worked out with special reference to the cytoplasmic inclusions.

2. The mitochondria, in the fixed preparations, are in the form of lightly staining granules with a distinct tendency to gather on one side of the cell, especially around the Golgi material, excepting, of course, during the division stages when they are dispersed throughout the cytoplasm, apparently outside the spindle. In the fresh material, on the other hand, the mitochondria are distinctly filamentous in form during the earlier stages of spermatogenesis but, as the filaments roll over during the later stages, they form quite big spheres. These move to the posterior side of the elongating nucleus and most of them soon become arranged along the axial filament to form the middle-piece of the ripe sperm. The remaining mitochondria are sloughed off along with the residual cytoplasm.

3. The Golgi material is present in the form of a localized mass composed of closely packed and darkly staining granules. During the maturation divisions the localized Golgi material breaks up gradually into a number of clumps and finally into granules which become dispersed in the beginning of the metaphase and are subsequently sorted out, almost equally, to the two daughter cells. After the completion of each division the scattered Golgi elements reassemble to form the localized mass of the daughter cells. As the process of spermateleosis begins the Golgi granules of the localized mass of the spermatid become arranged in the form of a horse-shoe which encloses a small clear space between two of its arms. This space gradually grows to take up the form of a hyaline vacuole—the proacrosome, in the formation of which are consumed all the Golgi elements of the spermatid. The acrosome of the ripe sperm is directly formed from the proacrosome and the acrosomal granule which is differentiated in the former at an earlier stage.

4. The so-called accessory body which is, in fact, a part of the Golgi material is present in the cytoplasm of the primary spermatocyte and some later stages.

5. Two small centrioles are also present in the early spermatids. As the proximal of these becomes one with the posterior end of the elongating nucleus it cannot be always clearly differentiated. The distal centriole gradually moves down and marks the posterior limit of the short middle-piece. Since the thin axial filament, arising from the proximal centriole, seems to pass through the distal centriole, the latter appears to be ring-like.

6. The ripe sperm consists of a deeply staining head with a conical acrosome situated at the anterior tip of the cylindrical nucleus, followed by a short middle-piece and a thin vibratile tail.

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Explanation of Lettering in Plates

A—Acrosome; *A.b*—Accessory body; *A.f*—Axial filament; *A.g*—Acrosomal granule; *C*—Centrosome; C_1—Proximal centriole; C_2—Distal centriole; *Ch*—Chromosomes; *Chr*—Chromatin; *G*—Golgi elements; *H*—Head; *M*—Mitochondria; *M.p*—Middle-piece; *N*—Nucleus; *n*—Nucleolus; *P*—Proacrosome; *S.f*—Spindle fibres; *T*—Tail.

Explanation of Plates

All the figures on Plates I to III except Figs. 45, 46, 54 and 56 on Plate III have been selected from the sectioned testicular material of the domestic fowl, fixed in Champy's fixative, followed by 0·5% iron-haematoxylin and have been drawn with Beck's camera lucida at the table level, using Beck's 10× eye-piece and a 2 mm. oil immersion objective, giving a total magnification of approximately 1,350 times.

Figs. 45, 46, 54 and 56 on Plate III are free hand drawings from the living material, studied under the phase-contrast microscope.

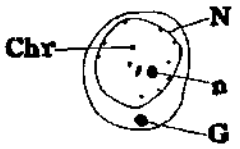
All the figures on Plate IV are the photomicrographs of the living cells, taken with a Contax 35 mm. camera and Carl Zeiss photomicrographic camera attachment, fitted on a Carl Zeiss stand 'W' phase-contrast microscope. The photomicrographs were taken under 1·25/100 oil immersion objective and K 8× ocular and they were further enlarged four times, giving a total magnification of 1,600 times.

Plate I

Figs. 1 to 5. Sertoli cells.
Fig. 6. A part of a sertoli cell showing the sperm heads embedded in its cytoplasm.
Figs. 7 to 9. Spermatogonia.
„ 10 to 13. Primary spermatocytes showing the localized mass of the Golgi elements and the accessory body.
„ 14 to 17. Primary spermatocytes showing the dispersal of the localized Golgi material in the form of granules.

Plate II

Fig. 18. Late primary spermatocyte.
„ 19. Primary spermatocyte—metaphase.
„ 20. Primary spermatocyte—telophase.
Figs. 21 and 22. Primary spermatocytes—syncytia.
„ 23 to 26. Secondary spermatocytes showing the dispersal of the localized Golgi material in the form of granules.
Fig. 27. Secondary spermatocyte—metaphase.
Figs. 28 and 29. Spermatids—syncytia.
„ 30 and 31. Secondary spermatocytes—syncytia.
Fig. 32. Metaphase II—syncytium.
Figs. 33 to 36. Early spermatids showing the progressive regrouping of the Golgi elements into a localized mass.
Fig. 37. Spermatid showing the arrangement of the Golgi elements in the form of a horse-shoe enclosing a clear space.



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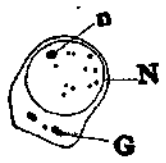

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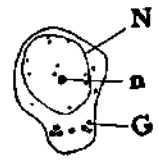

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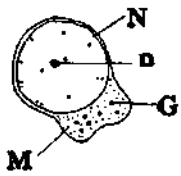

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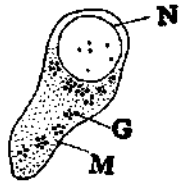

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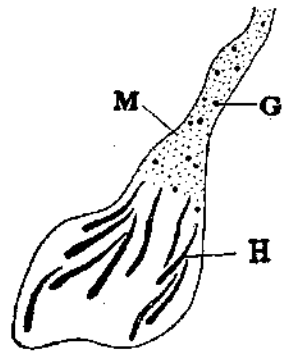

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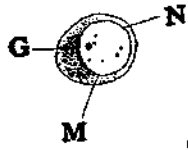

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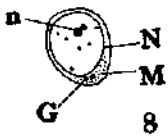

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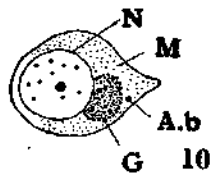

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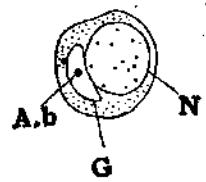

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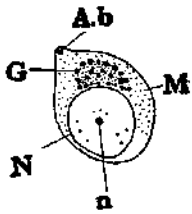

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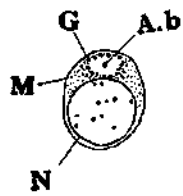

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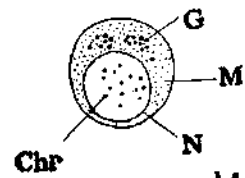

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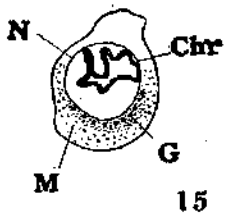

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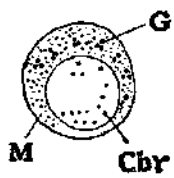

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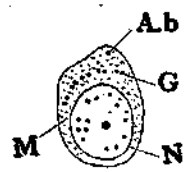

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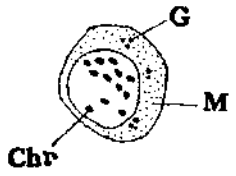
PLATE II.



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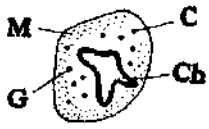

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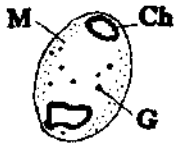

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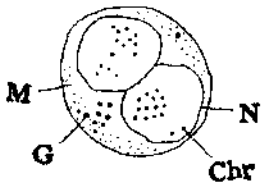

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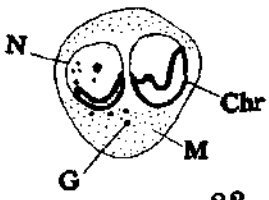

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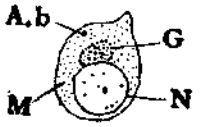

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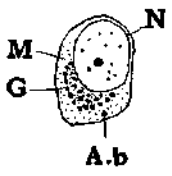

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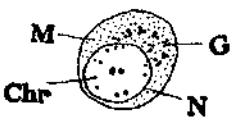

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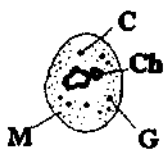

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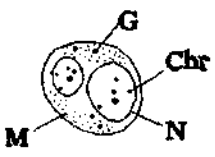

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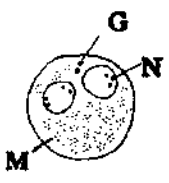

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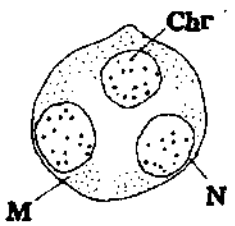

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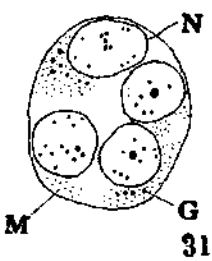

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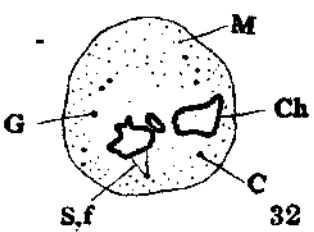

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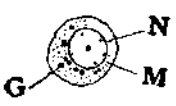

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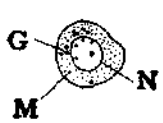

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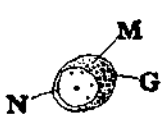

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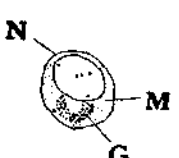

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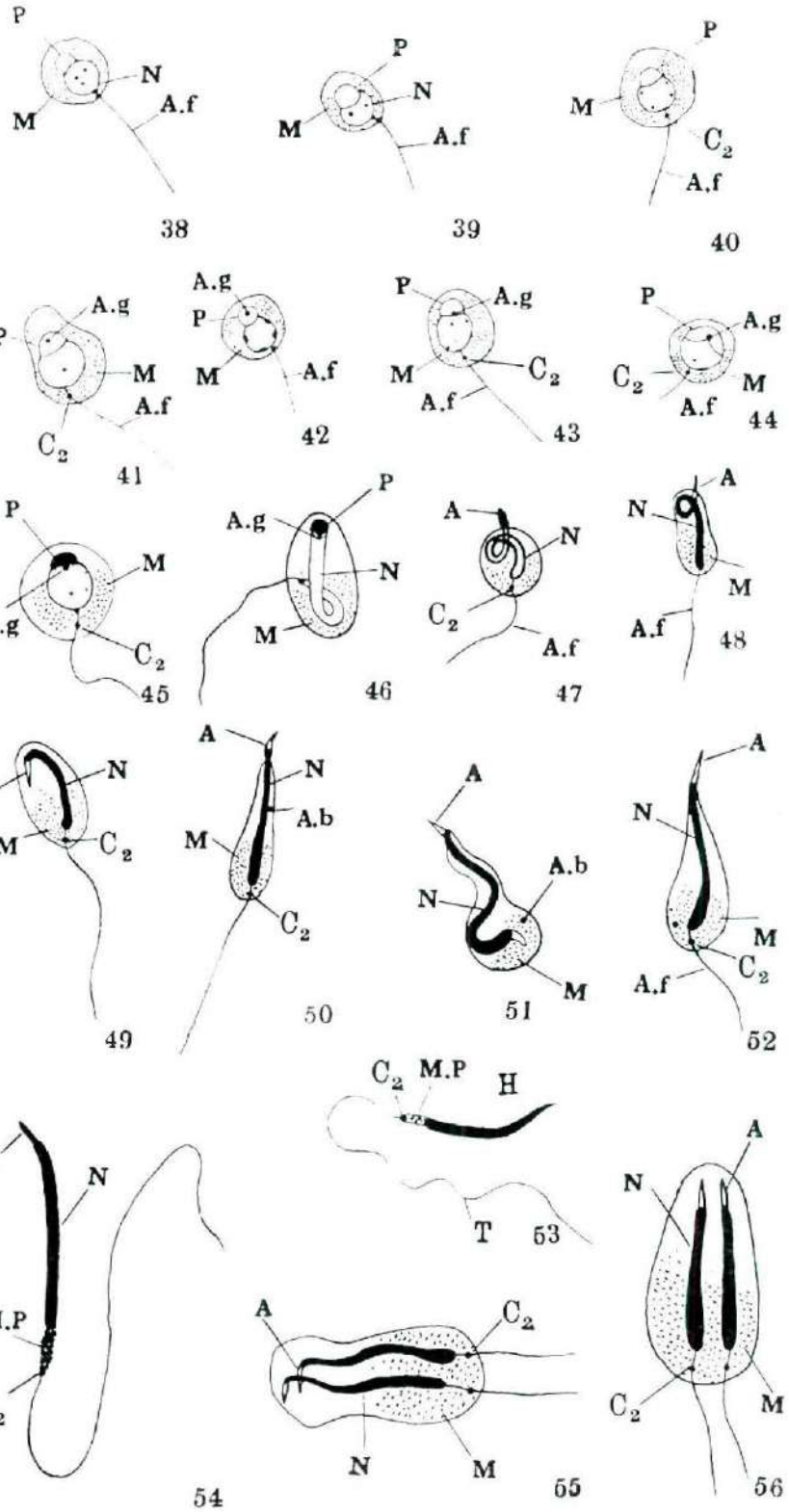
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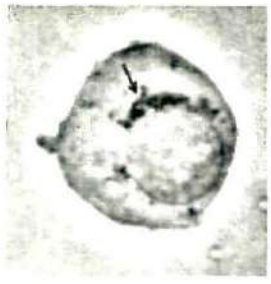
PLATE IV.



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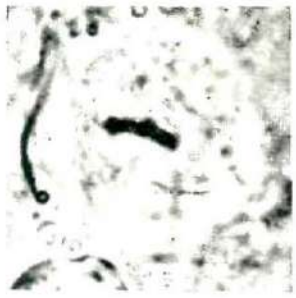

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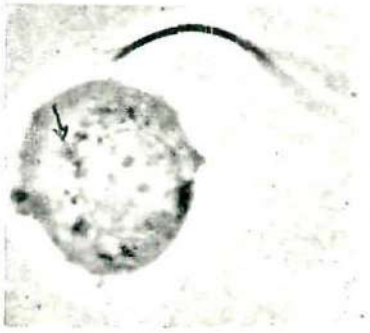

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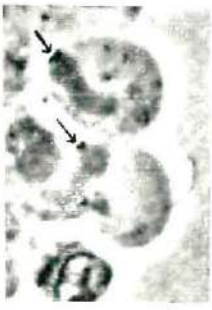

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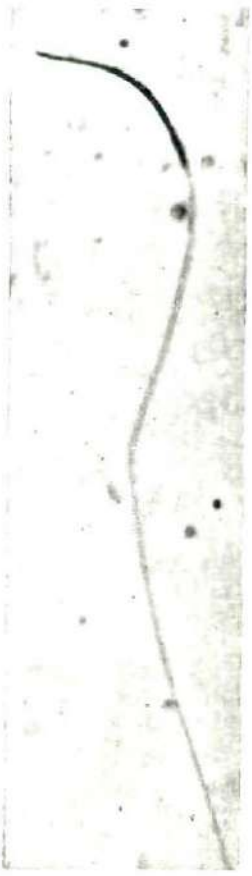

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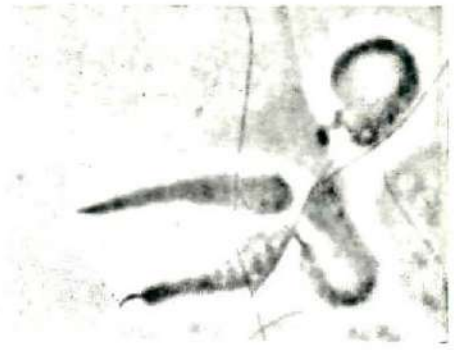

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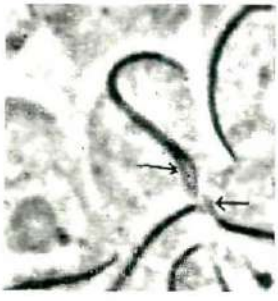

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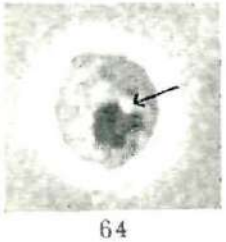

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Plate III

Figs. 38 to 40. Spermatids showing the vacuole-like proacrosome.

„ 41 to 43. Spermatids showing the acrosomal granule inside the proacrosome.

Fig. 44. Spermatid showing the protrusion of the nuclear membrane in the form of a process with the acrosomal granule situated at its tip.

„ 45. Spermatid showing the condensation of the proacrosome with the acrosomal granule lying at its base.

Figs. 46 to 48. Late spermatids showing the elongation and coiling of the nucleus and the differentiation of the acrosome.

„ 49 to 52. Very late spermatids showing the uncoiling of the elongated nucleus and further differentiation of the acrosome.

„ 53 and 54. Ripe sperms.

„ 55 and 56. Very late spermatids—syncytia.

Plate IV

Fig. 57. Primary spermatocyte.

„ 58. Primary spermatocyte and a ripe sperm.

„ 59. Metaphase I (side view).

Figs. 60 and 61. Spermatids showing the elongation of the nucleus and the condensation of the proacrosome.

Fig. 62. Late spermatids showing the differentiation of the acrosome.

„ 63. Late spermatids showing the uncoiling of the nucleus and a clear space at its posterior end. The acrosome is also clearly made out in one of them.

„ 64. Early spermatid showing the vacuole-like proacrosome.

„ 65. Ripe sperm under the negative phase-contrast showing the distal centrosome.

„ 66. Ripe sperm.

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PARTITIONS IN TERMS OF COMBINATORY FUNCTIONS

by

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PARTITIONS IN TERMS OF COMBINATORY FUNCTIONS

By Hansraj Gupta

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1. In connection with our Introduction to the Partition Tables[1], Dr. Miller and the writer obtained three sets of formulae expressing for values of $m \leqslant 12$, the number $p(n, m)$ of partitions of n into at most m non-zero summands, in terms of combinatory functions. It was finally decided to include Dr. Miller's set in the Introduction, as it had some advantages over the others. The other two sets are not, however, without an interest of their own and are given here for record.

2. **Notation.** The circulator

$$A_r(n) = (a_0, a_1, a_2, a_3, \ldots\ldots\ldots, a_{r-1})$$

is defined for integral values of n (positive, negative or zero) in the following manner.

Let $n \equiv k \pmod r$, $0 \leqslant k \leqslant r-1$;

then $A_r(n) = a_k$.

It would thus be seen that $A_r(n)$ is a periodic function of n. In fact

$$A_r(n+r) = A_r(n)$$

for all integral values of n.

3. **The first set.** Writing C_r for $\binom{n+r}{r}$,

we have

$$p(n, 1) = 1$$

$$p(n, 2) = \frac{1}{2} C_1 + \frac{1}{2}(1, 0)$$

$$p(n, 3) = \frac{1}{6} C_2 + \frac{1}{4} C_1 + \frac{1}{4}(1, 0) + \frac{1}{3}(1, 0, 0)$$

$$p(n, 4) = \frac{1}{24} C_3 + \frac{1}{8} C_2 + \left\{\frac{1}{9} + \frac{1}{16}(2, 1)\right\} C_1 + \frac{1}{8}(1, 0) + \frac{1}{9}(2, 1, 0) + \frac{1}{4}(1, 0, 0, 0)$$

$$p(n, 5) = \frac{1}{120} C_4 + \frac{1}{24} C_3 + \frac{31}{288} C_2 + \frac{1}{64}(13, 11)\, C_1 + \frac{13}{64}(1, 0) + \frac{1}{9}(1, 0, 0) + \frac{1}{8}(1, 1, 0, 0) + \frac{1}{5}(1, 0, 0, 0, 0)$$

$$p(n, 6) = \frac{1}{720} C_5 + \frac{1}{96} C_4 + \frac{17}{432} C_3 + \frac{1}{3456}(349, 331)\, C_2$$

---

[1] Tables of Partitions (in press with the Royal Society).

$$+\left\{\frac{1}{25}+\frac{1}{256}(37,25)+\frac{1}{162}(5,2,2)\right\}C_1+\frac{5}{256}(1,0)$$
$$+\frac{1}{81}(8,1,0)+\frac{1}{16}(3,1,2,0)+\frac{1}{25}(4,3,2,1,0)$$
$$+\frac{1}{6}(1,0,0,0,0,0)$$

$$p(n,7)=\frac{1}{5040}C_6+\frac{1}{480}C_5+\frac{47}{4320}C_4+\frac{161}{4320}C_3$$
$$+\left\{\frac{46}{675}+\frac{1}{384}(10,9)\right\}C_2+\left\{\frac{1}{768}(34,9)+\frac{1}{162}(25,24,23)\right\}C_1$$
$$+\frac{1}{4}(1,0)+\frac{1}{324}(52,21,0)+\frac{1}{128}(3,0,-5,0)$$
$$+\frac{1}{25}(2,1,2,0,0)+\frac{1}{12}(0,1,0,0,-1,0)$$
$$+\frac{1}{7}(1,0,0,0,0,0,0)$$

$$p(n,8)=\frac{1}{40320}C_7+\frac{1}{2880}C_6+\frac{83}{34560}C_5+\frac{25}{2304}C_4$$
$$+\left\{\frac{127}{675}-\frac{1}{3072}(468,469)\right\}C_3+\left\{\frac{1}{25}+\frac{1}{192}(10,9)\right\}C_2$$
$$+\left\{\frac{74}{1225}+\frac{1}{4608}(23,4)+\frac{1}{162}(2,1,1)\right.$$
$$\left.+\frac{1}{128}(16,11,15,11)\right\}C_1+\frac{1}{4}(1,0)+\frac{1}{9}(1,0,0)$$
$$+\frac{1}{512}(-1,8,-37,0)+\frac{1}{25}(2,1,1,1,0)$$
$$+\frac{1}{162}(-5,10,0,4,-8,0)+\frac{1}{49}(6,5,4,3,2,1,0)$$
$$+\frac{1}{8}(1,0,0,0,0,0,0,0)$$

$$p(n,9)=\frac{1}{362880}C_8+\frac{1}{20160}C_7+\frac{319}{725760}C_6+\frac{1843}{725760}C_5$$
$$+\frac{929377}{87091200}C_4+\left\{\frac{1}{12288}(1977,1975)-\frac{215}{1701}\right\}C_3$$
$$-\left\{\frac{3}{49}+\frac{1}{24576}(207,289)-\frac{1}{1458}(232,231,231)\right\}C_2$$
$$+\left\{\frac{5}{32}+\frac{235}{16384}(1,-1)+\frac{1}{2916}(84,43,41)\right.$$
$$\left.+\frac{1}{2304}(8,1,-1,-8)\right\}C_1+\frac{11}{16384}(1,0)$$
$$+\frac{1}{2916}(28,43,0)+\frac{1}{512}(53,38,5,0)+\frac{1}{25}(1,0,0,0,0)$$

$$+\frac{1}{72}(15,2,9,8,7,0)+\frac{1}{49}(3,2,4,2,3,0,0)$$

$$+\frac{1}{16}(2,1,2,1,1,0,1,0)+\frac{1}{9}(1,0,0,0,0,0,0,0,0)$$

$$p(n,10)=\frac{1}{3628800}C_9+\frac{1}{161280}C_8+\frac{199}{2903040}C_7+\frac{2867}{5806080}C_6$$

$$+\frac{161111}{62208000}C_5+\left\{\frac{599}{30375}-\frac{1}{122880}(1137,1139)\right\}C_4$$

$$+\left\{\frac{1031299}{13395375}-\frac{1}{12288}(532,537)\right\}C_3-\left\{\frac{174}{6125}\right.$$

$$\left.-\frac{1}{294912}(2103,685)-\frac{1}{4374}(481,480,479)\right\}C_2$$

$$+\left\{\frac{21}{65536}+\frac{1}{73728}(9,14)+\frac{1}{26244}(1060,883,748)\right.$$

$$\left.+\frac{1}{512}(81,63,80,62)+\frac{1}{1250}(7,2,2,2,2)\right\}C_1$$

$$+\frac{29}{65536}(1,0)+\frac{7}{13122}(-20,11,0)+\frac{1}{512}(29,24,0,0)$$

$$+\frac{1}{625}(39,8,7,1,0)+\frac{1}{72}(9,4,9,0,11,0)$$

$$+\frac{1}{49}(2,0,2,2,1,0,0)+\frac{1}{32}(2,1,3,2,2,1,1,0)$$

$$+\frac{1}{27}(6,2,2,5,1,1,4,0,0)+\frac{1}{10}(1,0,0,0,0,0,0,0,0,0,0)$$

$$p(n,11)=\frac{1}{39916800}C_{10}+\frac{1}{1451520}C_9+\frac{1}{107520}C_8+\frac{95}{1161216}C_7$$

$$+\frac{114221}{217728000}C_6+\frac{2262473}{870912000}C_5-\left\{\frac{10961}{107163}\right.$$

$$\left.-\frac{1}{6144000}(691471,691421)\right\}C_4-\left\{\frac{5104}{107163}\right.$$

$$\left.-\frac{1}{12288000}(989971,986921)\right\}C_3+\left\{\frac{1641}{30625}\right.$$

$$\left.+\frac{1}{2949120}(53711,43255)+\frac{1}{13122}(203,200,200)\right\}C_2$$

$$+\left\{\frac{1}{655360}(787,-897)+\frac{1}{26244}(441,256,248)\right.$$

$$\left.-\frac{1}{512}(45,60,46,60)+\frac{1}{1250}(339,338,337,336,335)\right\}C_1$$

$$+\frac{55}{131072}(1,0)+\frac{16}{6561}(53,4,0)+\frac{1}{512}(28,3,-4,0)$$

$$+\frac{1}{1250}(128, 25, 56, -39, 0)+\frac{1}{36}(1, 0, 0, 0, 0, 0)$$
$$+\frac{1}{49}(2, 1, 1, 1, 2, 0, 0)+\frac{1}{32}(7, 1, 7, 2, 7, 1, 7, 0)$$
$$+\frac{1}{27}(0, 1, 2, 0, 2, 1, 0, 0, 0)+\frac{1}{10}(0, 1, 0, 1, 0, 0, 0, 0, 0, 0)$$
$$+\frac{1}{11}(1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)$$

$$p(n, 12)=\frac{1}{479001600}C_{11}+\frac{1}{14515200}C_{10}+\frac{583}{522547200}C_9$$
$$+\frac{3077}{261273600}C_8+\frac{355619}{3919104000}C_7+\frac{121219}{223948800}C_6$$
$$+\left\{\frac{17701}{40186125}+\frac{(6327, 6325)}{2949120}\right\}C_5+\left\{\frac{217349}{4465125}\right.$$
$$\left.-\frac{(3161, 3163)}{81920}\right\}C_4-\left\{\frac{1482}{30625}-\frac{(1203187, 1188419)}{35389440}\right.$$
$$\left.-\frac{(7390, 7387, 7387)}{157464}\right\}C_3$$
$$+\left\{\frac{58529}{8028160000}+\frac{(4, 33)}{1105920}-\frac{(4526, 4635, 4639)}{157464}\right.$$
$$\left.+\frac{(716, 688, 715, 688)}{6144}\right\}C_2+\left\{\frac{170}{5929}+\frac{(2394, 2411)}{39321600}\right.$$
$$+\frac{(127, -155, -326)}{944784}+\frac{(312, 167, 287, 165)}{4096}$$
$$\left.+\frac{(132, 131, 132, 130, 130)}{1250}-\frac{(3, 33, 37, 7, 35, 35)}{2592}\right\}C_1$$
$$+\frac{7}{262144}(1, 0)+\frac{(-73, 508, 0)}{236196}+\frac{(3403, 216, -1979, 0)}{73728}$$
$$+\frac{(-14, 3, -4, 5, 0)}{1250}+\frac{(207, 16, 126, 58, 128, 0)}{648}$$
$$+\frac{1}{49}(2, 1, 1, 1, 1, 1, 0)+\frac{1}{32}(1, 1, 1, 1, 1, 1, 1, 0)$$
$$+\frac{1}{81}(2, 1, 3, 2, 4, 3, 2, 1, 0)+\frac{1}{50}(2, 3, 2, 3, 0, 6, 0, 4, -3, 0)$$
$$+\frac{1}{121}(10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0)$$
$$+\frac{1}{12}(1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0)$$

Writing Glaisher's[1] formula for $p(n, m)$ in terms of combinatory functions we would get

[1] J. W. L. Glaisher (1909). Formulae for Partitions into Given Elements, derived from Sylvester's Theorem. *Quarterly Journal of Pure and Applied Mathematics*, **40**, 275–348.

$$
\begin{aligned}
p(n,12) = {} & \frac{1}{479001600}C_{11} + \frac{1}{14515200}C_{10} + \frac{583}{522547200}C_9 \\
& + \frac{3077}{261273600}C_8 + \frac{355619}{3919104000}C_7 + \frac{121219}{223948800}C_6 \\
& + \left\{\frac{3404664443}{1316818944000} + \frac{(1,-1)}{2949120}\right\}C_5 + \left\{\frac{368650483}{36578304000}\right. \\
& \left. + \frac{(1,-1)}{81920}\right\}C_4 + \left\{\frac{5106621148711}{158018273280000} + \frac{923}{4423680}(1,-1)\right. \\
& \left. + \frac{(2,-1,-1)}{157464}\right\}C_3 + \left\{\frac{13435995840307}{158018273280000} + \frac{4921}{2211840}(1,-1)\right. \\
& \left. + \frac{(74,-35,-39)}{157464} + \frac{1}{12288}(1,0,-1,0)\right\}C_2 \\
& + \left\{\frac{41434452767831663}{229442532802560000} + \frac{1281583}{78643200}(1,-1)\right. \\
& + \frac{(15070,-6635,-8435)}{1889568} + \frac{(25,2,-25,-2)}{8192} \\
& \left. + \frac{(1,0,1,-1,-1)}{1250} + \frac{(2,1,-1,-2,-1,1)}{2592}\right\}C_1 \\
& + \frac{29903093}{176947200}(1,0) \\
& + \frac{1}{472392}(60361, 7577, 0) + \frac{1}{73728}(4399, 1368, -3031, 0) \\
& + \frac{1}{2500}(172, 81, 142, 10, 0) + \frac{1}{648}(13, 7, -12, -25, -19, 0) \\
& + \frac{1}{49}(2,1,1,1,1,1,0) + \frac{1}{64}(1,1,1,2,1,1,1,0) \\
& + \frac{1}{81}(2,1,3,2,4,3,2,1,0) + \frac{1}{100}(2,3,4,6,6,4,3,2,0,0) \\
& + \frac{1}{121}(10,9,8,7,6,5,4,3,2,1,0) \\
& + \frac{1}{72}(2,0,1,0,-1,0,-2,0,-1,0,1,0)
\end{aligned}
$$

To obtain the simpler forms recorded above, I had to play a lot with the circulators.

4. **The second set.** With

$$N = 2n + \frac{m(m+1)}{2},$$

$$^mD_k \equiv D_k = \binom{(N+k-1)/2}{k}$$

we have the following formulae for $p(n, m)$ for values of $m = 1, 2, 3 \ldots, 12$.

$0!\,1!\; p(n,1) = 1$

$1!\,2!\; p(n,2) = D_1 + \frac{1}{2}(1,-1)$

$2!\,3!\; p(n,3) = 2D_2 - \frac{3}{4} - 3(0,1) + 4(1,0,0)$

$$3!\,4!\,p(n,4) = 6D_3 - (2,11)\,D_1 + 16\,(1,0,-1) + 18\,(1,0,-1,0)$$

$$4!\,5!\,p(n,5) = 24D_4 - 50D_2 + 45(1,-1)D_1 + \frac{45}{2} + 320\,(1,0,0)$$
$$-360\,(0,0,1,1) + 576\,(1,0,0,0,0)$$

$$5!\,6!\,p(n,6) = 120D_5 - 425D_3 + 225\,(1,-1)\,D_2 + \left\{\frac{16763}{48} + 1600(1,0,0)\right\}D_1$$
$$-\frac{4725}{4}\,(1,-1) + \frac{1600}{3}\,(0,1,-1) + 5400\,(0,1,-1,0)$$
$$+3456(2,1,0,-1,-2) + 7200(1,0,0,-1,0,0)$$

$$6!\,7!\,p(n,7) = 720D_6 - 3990D_4 + \left\{\frac{62643}{8} + 9450(1,0)\right\}D_2$$
$$+22400(1,0,-1)D_1 + \frac{1235381}{64} - \frac{127575}{4}\,(1,0)$$
$$+246400\,(0,1,0) + 226800(1,0,0,0) - 145152(0,1,0,2,2)$$
$$-604800(0,0,0,0,1,0) + 518400(1,0,0,0,0,0,0)$$

$$7!\,8!\,p(n,8) = 5040D_7 - 41160D_5 + \{153762 + 66150(1,0)\}D_3$$
$$-\{537200 + 1808100(1,0) - 1254400(1,0,0)$$
$$-1587600(1,0,0,0)\}D_1 - 5017600(0,1,-1)$$
$$+1587600(0,1,0,-1) + 8128512(1,0,0,0,-1)$$
$$+11289600(0,1,0,0,0,-1)$$
$$+4147200(3,2,1,0,-1,-2,-3)$$
$$+12700800(1,0,0,0,-1,0,0,0)$$

$$8!\,9!\,p(n,9) = 40320D_8 - 463680D_6 + 2919336D_4 + 1190700(1,-1)D_3$$
$$-\{16735200 - 10035200(1,0,0)\}\,D_2 - \left\{\frac{3472875}{2}\,(1,-1)\right.$$
$$\left.-5017600(0,1,-1) - 57153600(0,1,-1,0)\right\}D_1$$
$$-\frac{2171284325}{2} - 863027200(1,0,0) + 142884000(1,0,0,1)$$
$$+585252864(1,0,0,0,0) + 203212800(0,1,1,0,-1,-1)$$
$$+298598400(3,2,4,2,3,0,0) + 914457600(1,1,1,1,0,0,0,0)$$
$$+1625702400(1,0,0,0,0,0,0,0,0)$$

$$9!\,10!\,p(n,10) = 362880D_9 - 5670000D_7 + 47960073D_5$$
$$+10716300(1,-1)D_4 - \frac{2340015459}{8}\,D_3 - \{317023875(1,-1)$$
$$-301056000(1,0,-1)\}D_2 - \{542798954\tfrac{3}{1250}$$
$$+1053696000(0,1,0) - 2571912000(1,1,0,0)$$
$$-5267275776(1,0,0,0,0)\}D_1 + 1456747031\tfrac{1}{4}(1,-1)$$
$$-15153152000(1,0,-1) + 34720812000(0,0,1,-1)$$
$$+2106910310\tfrac{2}{5}(0,4,3,-3,-4)$$
$$+18289152000(0,1,0,0,-1,0)$$
$$+26873856000(1,-1,1,1,0,-1,-1)$$
$$+82301184000(0,0,0,1,0,0,-1,0)$$
$$+48771072000(1,1,1,0,0,0,-1,-1,-1)$$
$$+65840947200(1,0,0,0,0,-1,0,0,0,0)$$

$$
\begin{aligned}
10!\,11!\,p(n,11) = {} & 3628800D_{10} - 74844000D_8 + 828419130D_6 \\
& - \{5968325673\tfrac{3}{4} + 1178793000(0,1)\}D_4 \\
& + \{57051441358\tfrac{29}{128} - 52701870375(1,0) \\
& + 33116160000(1,0,0)\}D_2 \\
& + \{22077440000(0,1,-1) + 141455160000(1,0,-1,0) \\
& + 115880067072(2,1,0,-1,-2)\}D_1 \\
& + 7344543313\tfrac{795}{1024} - 913794398704\tfrac{11}{16}(0,1) \\
& - 2289154560000(1,0,0) + 3677834160000(0,0,0,1) \\
& + 57940033536(10,-7,-6,-7,10) \\
& + 670602240000(2,1,-1,-2,-1,1) \\
& + 2956124160000(1,0,0,0,1,-1,-1) \\
& - 9053130240000(0,0,0,0,0,0,0,1) \\
& + 5364817920000(0,0,1,0,1,0,0,-1,-1) \\
& + 1448500838400(2,3,2,3,2,-2,-3,-2,-3,-2) \\
& + 13168189440000(1,0,0,0,0,0,0,0,0,0,0)
\end{aligned}
$$

$$
\begin{aligned}
11!\,12!\,p(n,12) = {} & 39916800D_{11} - 1061121600D_9 + 15033775680D_7 \\
& - \{144842873340 + 12966723000(0,1)\}D_5 \\
& + \{767171091071 + 656980632000(0,1) \\
& + 364277760000(1,0,0)\}D_3 \\
& + \{242851840000(0,1,-1) \\
& + 1556006760000(1,0,-1,0)\}D_2 \\
& + \{26530513723093\tfrac{1}{8} + 917377642912\tfrac{1}{2}(0,1) \\
& - 5221314560000(1,0,0) - 9336040560000(0,0,0,1) \\
& + 15296168853504(1,0,1,-1,-1) \\
& - 44259747840000(0,0,0,1,0,0)\}D_1 \\
& - 154747216213333\tfrac{1}{3}(0,1,-1) \\
& - 425373348015000(1,0,-1,0) \\
& + 382404221337 60(3,0,-3,1,-1) \\
& + 206545489920000(0,0,1,0,-1,0) \\
& + 390208389120000(1,0,0,0,0,0,-1) \\
& + 298753297920000(0,0,0,1,0,0,0,-1) \\
& + 708155965440000(0,0,0,0,1,0,0,0,-1) \\
& + 191202110668800(-1,0,1,3,3,1,0,-1,-3,-3) \\
& + 158018273280000(5,4,3,2,1,0,-1,-2,-3,-4,-5) \\
& + 796675461120000(1,0,0,0,0,0,0,-1,0,0,0,0,0).
\end{aligned}
$$

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