Panjab University Research Journal (Science)

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In the year 1954 the journal was named “Research Bulletin of Panjab University” and continued with this name till the year 2002. From the year 1954 till 1958 (volumes 4-9) it published Bulletin 42 to 156. From the year 1959 (volume 10) it started publication in new format and Professor Vishwanath D.Sc. (Cantab), F.N.I. *(Editor-in-Chief)* played a pivotal role in the development of the journal. It is being published regularly as annual volume.

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Published by the Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 1. ZOOLOGY Pp. 1—12

1950

PARALLELISM BETWEEN VARIATIONS OF TAXONOMIC VALUE AND CYTOLOGICAL RESEMBLANCES IN ALLIED SPECIES:

Spermatogenesis of Scorpions

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PARALLELISM BETWEEN VARIATIONS OF TAXONOMIC VALUE AND CYTOLOGICAL RESEMBLANCES IN ALLIED SPECIES: SPERMATOGENESIS OF SCORPIONS

By Vishwa Nath *and* Gurdev Kaur Gill

(Department of Zoology, East Panjab University, Government College, Hoshiarpur)

Introduction

Specimens of the scorpion, *Buthus acute-carinatus*, are easily obtainable in Hoshiarpur throughout the year, but more easily in the months of June to September. In view of its availability and the importance of this animal in general, we hoped that investigation of the spermatogenesis of this classical animal might be profitable and might yield important results. This hope has been fully realized.

Gurdev Kaur started this work in April, 1948, with the object of submitting a thesis for the M.Sc. degree of the East Panjab University under the supervision of the senior author.

It was soon discovered that the mitochondria in the spermatogenesis of this species behave exactly as they do in the classical species of the Arizona scorpion, *Centrurus*, worked out by Wilson (1916) inasmuch as in both species the mitochondria of the primary spermatocyte form a ring which, in both meiotic divisions, divides equally, each spermatid receiving two mitochondrial masses of more or less equal size. It may be noted that in all other genera of scorpions whose spermatogenesis has been worked out (*Opisthacanthus*, *Vejovis*, *Hadrurus*, and *Palamnaeus*) the mitochondrial balls do not fuse to form a ring, but remain separate and are more or less equally sorted out to the four spermatids resulting from one primary spermatocyte.

It may be stated that both *Centrurus* and *Buthus*, whose mitochondria behave alike, belong to the family *Buthidae*.

Another important conclusion that we have arrived at is that the acrosome is formed directly from the Golgi body and is not a secretory product thereof.

Regarding technique live scorpions were dissected in normal saline and the testis was fixed in Flemming-without-acetic from 24 to 72 hours. The material fixed in F.W.A. for 48 hours gave very good results. Champy's fluid was also used, but it gave identical results. Bouin's fixative was used for control. Staining was done in 0·5% iron haematoxylin. The material was studied in smears also.

Previous Literature

Wilson's classical work on the spermatogenesis of scorpions (*Opisthacanthus*, *Vejovis*, *Hadrurus*, and *Centrurus*) was published in 1916. Later,

Gatenby and Bhattacharya (1925) published a paper on the spermatogenesis of *Palamnaeus bengalensis* and in the same year Nath published a paper on *Palamnaeus Madraspatensis*.

In all the scorpions the mitochondria are found to be very small and numerous in the spermatogonia. But in the spermatocytes they coalesce into a number of mitochondrial balls which remain separate in all genera except *Centrurus*. These balls do not divide, but are merely sorted out more or less equally in both the meiotic divisions. In *Palamnaeus bengalensis*, according to Gatenby and Bhattacharya, the number of mitochondria in the spermatid varies from four to eleven. The number of mitochondria in the spermatids of *Palamnaeus Madraspatensis* also varies (Nath, 1925). The same is true of *Opisthacanthus*, *Vejovis*, and *Hadrurus*, except that in the first-named the number of mitochondria in the spermatids is six in about 75% out of the two hundred cases counted, whereas in the remaining 25% the number varied from 5 to 7.

But in *Centrurus* (Wilson, 1916) all the mitochondrial balls of the primary spermatocyte coalesce to form a single ring-shaped body, which places itself tangentially to the spindle of the first meiotic division. It is then cut across transversely into two half-rings. At the close of the first meiotic division each half-ring is cut into two parallel rods. In the second meiotic division these rods are cut across transversely into two shorter rods. The spermatocyte ring is thus divided into eight short rods of equal size, each spermatid receiving two such rods. These rods quickly turn into ball-shaped masses. Later, they become attenuated along the axial filament and twist round each other to form a close double spiral, which ultimately becomes so fine as to become invisible as such.

Observations

Primary Spermatogonia

The primary spermatogonia are fairly large cells. As usual, each has a vesicular nucleus. There are one or two deeply staining nucleoli (Text-fig. I, 1). The mitochondria in the primary spermatogonia are granular, and they occupy the usual juxta-nuclear position. They stain a deep blue colour.

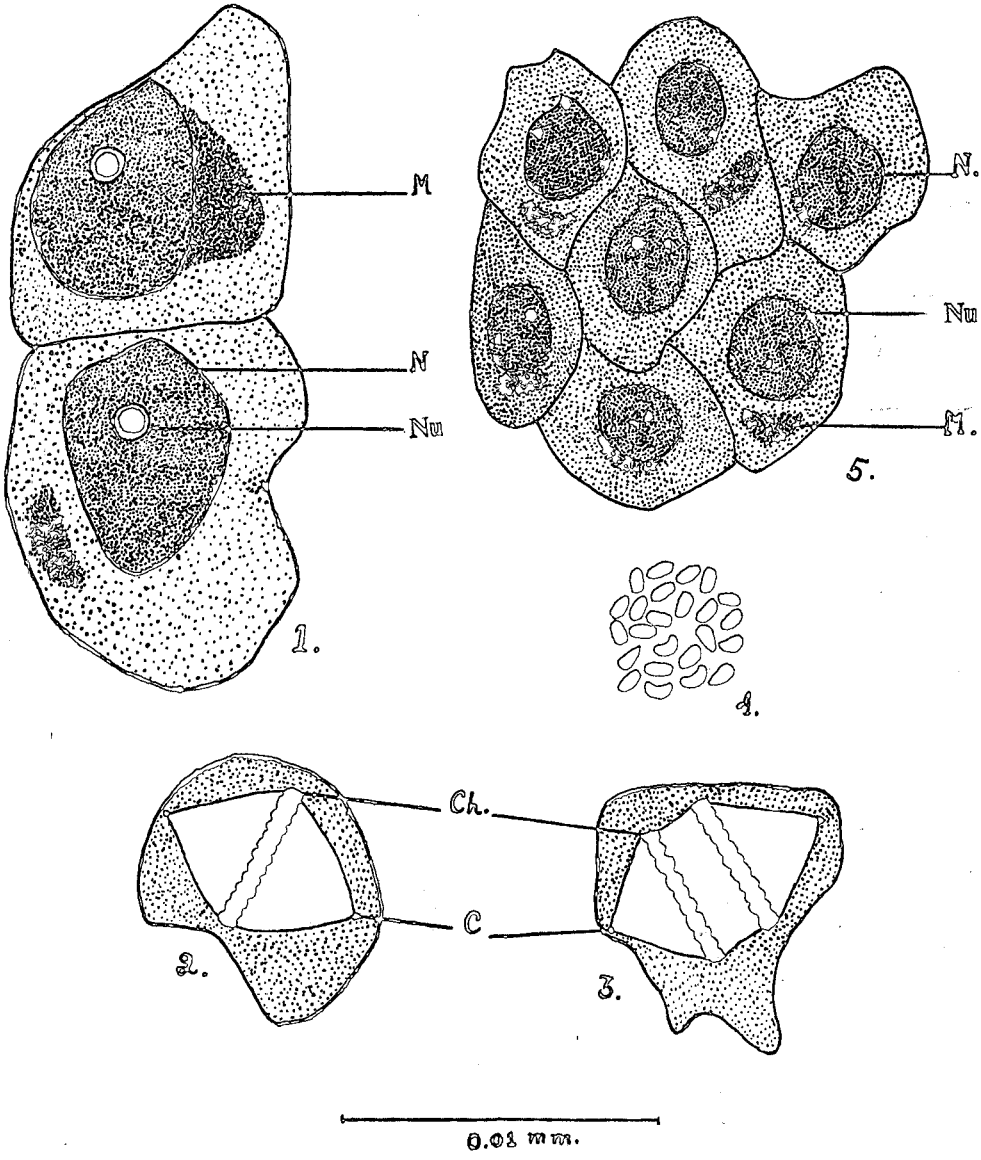
In spite of the most diligent search it has not been possible to observe either the Golgi body or the centrosome, although both these cell elements become conspicuous in subsequent stages of spermatogenesis.

The primary spermatogonia divide to give rise to the secondary spermatogonia (Text-fig. I, 2 and 3). A centrosome is conspicuous at either pole of the spindle. It is worthy of note that the spindle appears as a hyaline fusiform area showing no fibres whatsoever. This picture is similar to the picture of the spindle in the living cells. It is clear therefore that osmic acid causes the least amount of coagulation and that the fibres in the spindle area are artifacts.

The diploid number of chromosomes, as determined by the study of spermatogonial metaphase is 24 in *Buthus acute-carinatus* (Text-fig. I, 4).

Secondary Spermatogonia

The secondary spermatogonia are exactly similar in structure to the primary spermatogonia, except that they are smaller in size (Text-fig. I, 5).



TEXT-FIG. I. 1. Primary Spermatogonia; 2 and 3. Spermatogonial metaphase; 4. Spermatogonial metaphase, polar view (24 chromosomes); 5. Secondary spermatogonia.

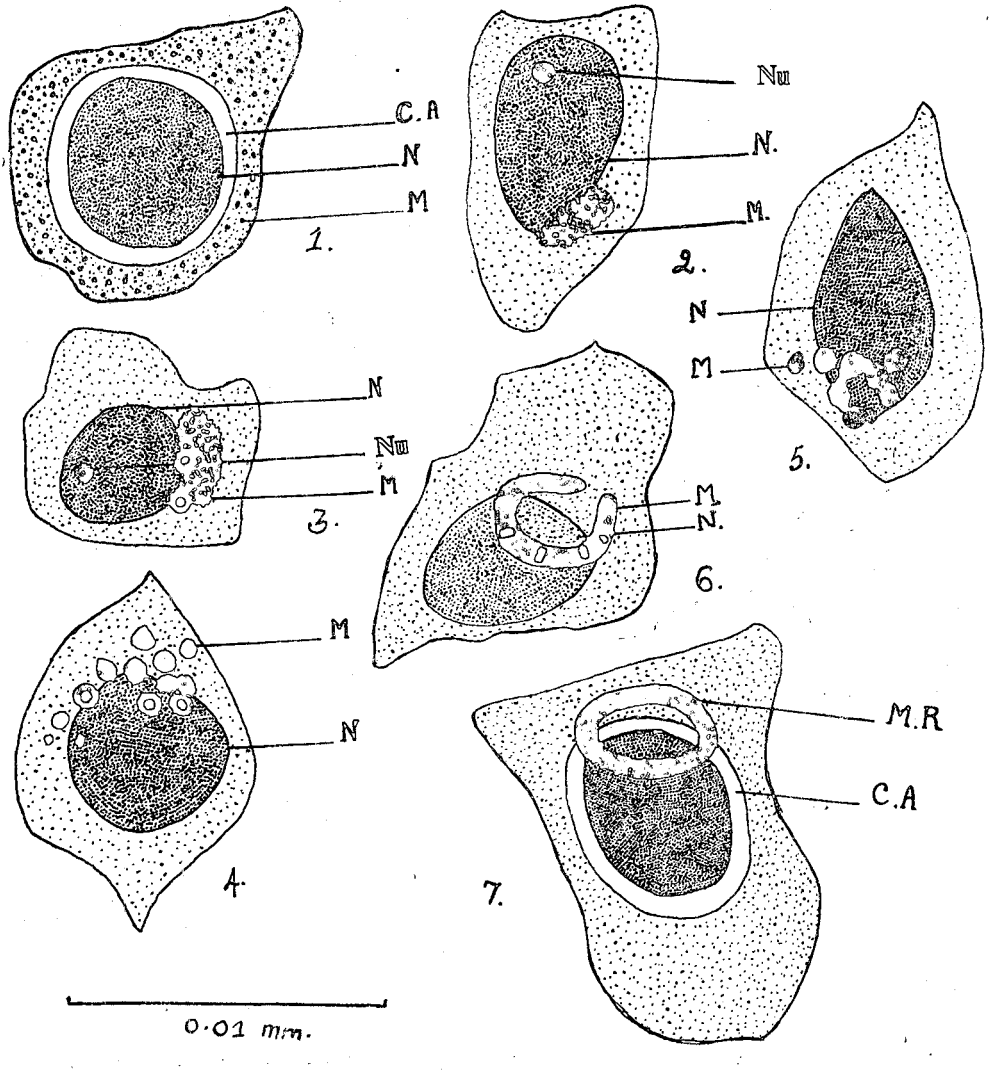
1, 2, 3, and 5 from material fixed in F.W.A. and stained with 0·5% iron haematoxylin, and 4 from material fixed in Bouin's fluid and stained as above.

C., centrosome; *Ch.*, chromosomes; *M.*, mitochondria; *N.*, nucleus; *Nu.*, nucleolus; *T.*, tail.

Spermatocytes

There is no appreciable difference between the primary spermatocytes and the primary spermatogonia, except that the mitochondria in the former do not occupy juxta-nuclear position, but, having grown in size, are distributed evenly throughout the cytoplasm (Text-fig. II, 1).

A remarkable process now takes place which has its parallel only in *Centrurus* as described by Wilson (1916). The mitochondrial granules of the primary spermatocytes, which were hitherto distributed evenly, now take up a juxta-nuclear position and form a dense deeply-staining mass.

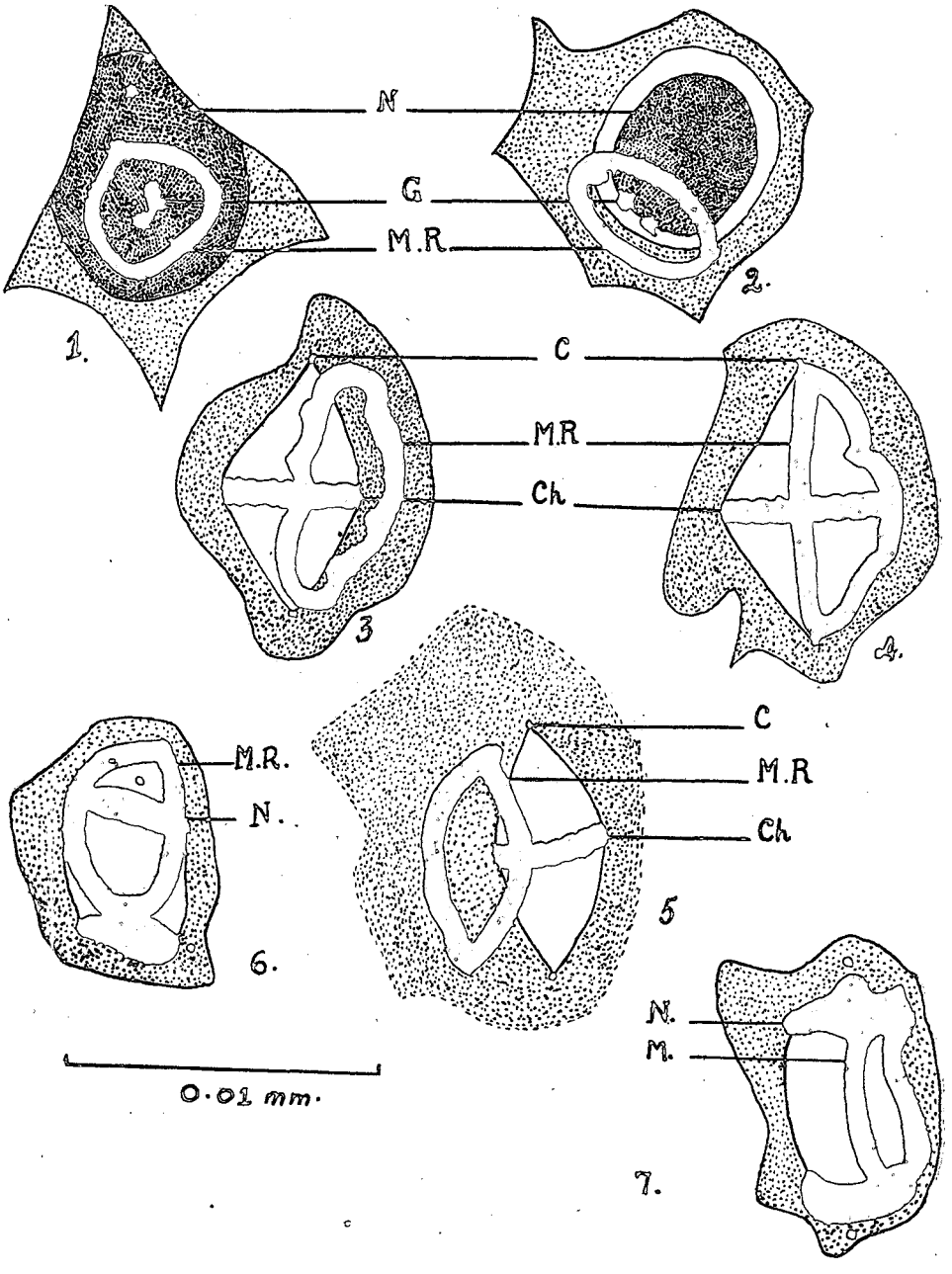


TEXT-FIG. II. 1. Primary spermatocyte with scattered mitochondria; 2. Mitochondria closely aggregated at one side of the nucleus; 3 and 4. Mitochondria assuming a vesicular form; 5 and 6. Mitochondria arranging themselves into a ring; 7. Mitochondrial ring.

All from material fixed in F.W.A.

C.A., clear area; *M.R.*, mitochondrial ring.

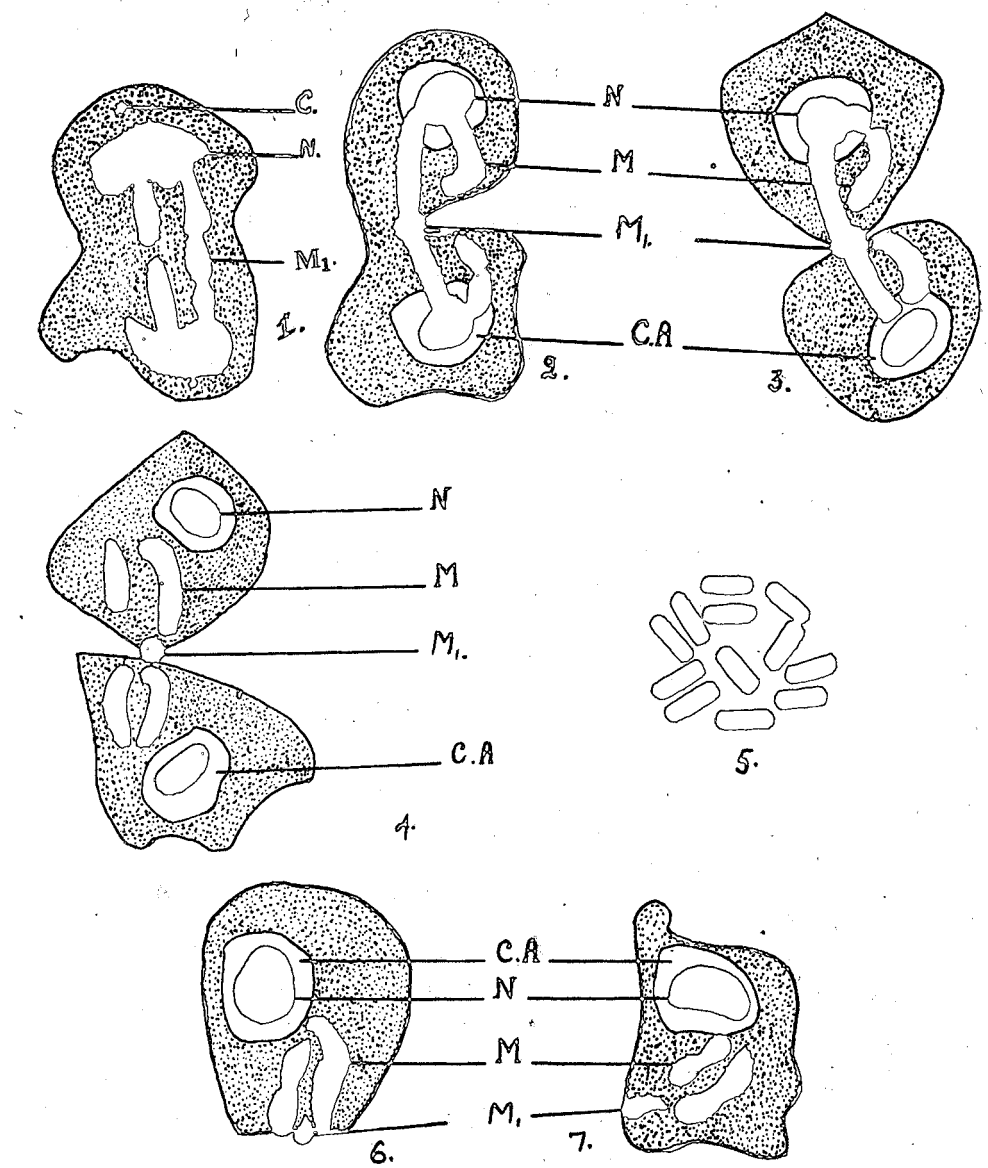
Gradually, by a progressive process of coalescence, the mitochondrial granules form what may be appropriately described as mitochondrial balls or vesicles. With suitable staining it is possible to demonstrate that each



TEXT-FIG. III. Primary spermatocytes before and during their divisions. 1. Top view showing the ring along with the Golgi elements lying over the nucleus; 2. Ring with two Golgi bodies; 3–5. Ring lying at a tangent to the spindle; 6 and 7. Anaphase I showing a complete ring and the two daughter nuclei.

From material fixed in F.W.A.

G., Golgi bodies.



TEXT-FIG. IV. Stages leading to the formation of the secondary spermatocyte. 1. Late anaphase I showing a break on one side of the mitochondrial ring and an indication of a break on the other side; 2. Telophase I, mitochondrial ring dividing into two half-rings and an extra piece; 3 and 4. Division of the two half-rings into two rods each and an extra ball-like mass; 5. Metaphase I, polar view showing twelve chromosomes; 6 and 7. Secondary spermatocytes with two rod-like pieces and an extra piece of mitochondrial material.

1 from material fixed in F.W.A.; 2–4, 6 and 7 from material fixed in Champy's fixative; 5 from material fixed in Bouin's fluid.

M_1, extra mitochondrial piece.

mitochondrial vesicle shows a chromophilic cortex and a chromophobic medulla (Text-fig. II, 2–5).

The next step in the process consists of a gradual and a progressive fusion of the mitochondrial vesicles to form a complete ring, in which the chromophobic material can be still seen in the form of vacuoles, at least in some cases (Text-fig. II, 6-7 and Text-fig. III, 1 and 2).

During metaphase I the mitochondrial ring places itself tangentially to the mitotic spindle (Text-fig. III, 3–5) and continues to occupy this position even during the early telophase I (Text-fig. III, 6 and 7).

During the late telophase stages of the first meiotic divisions the mitochondrial ring is cut into two half-rings, each secondary spermatocyte receiving one half-ring (Text-fig. IV, 1–4), which quickly breaks up into two parallel rods.

There is, however, one important point of difference between *Buthus acute-carinatus* and *Centrurus*, viz., that in the former species a somewhat round mitochondrial piece is differentiated at an early stage in the equatorial region, and this gets pinched off from each of the two half-rings, and it passes to one of the two secondary spermatocytes (see M_1 in Text-fig. IV, 1–4 and also in Text-fig. IV, 6 and 7). This mitochondrial piece, however, soon fuses with one of the two parallel rods in the secondary spermatocyte (Text-fig. V, 1–3).

During metaphase II the two mitochondrial rods in secondary spermatocyte again place themselves tangentially to the mitotic spindle (Text-fig. V, 4 and 5). In the telophases each mitochondrial rod is cut across in the middle, so that each of the two resulting spermatids will receive two short rods.

In Text-fig. IV, 5 is shown a polar view of the early telophase I, showing the haploid number of chromosomes which is twelve. In Text-fig. V, 9 is shown likewise a polar view of the early telophase II, also showing the reduced haploid number of chromosomes.

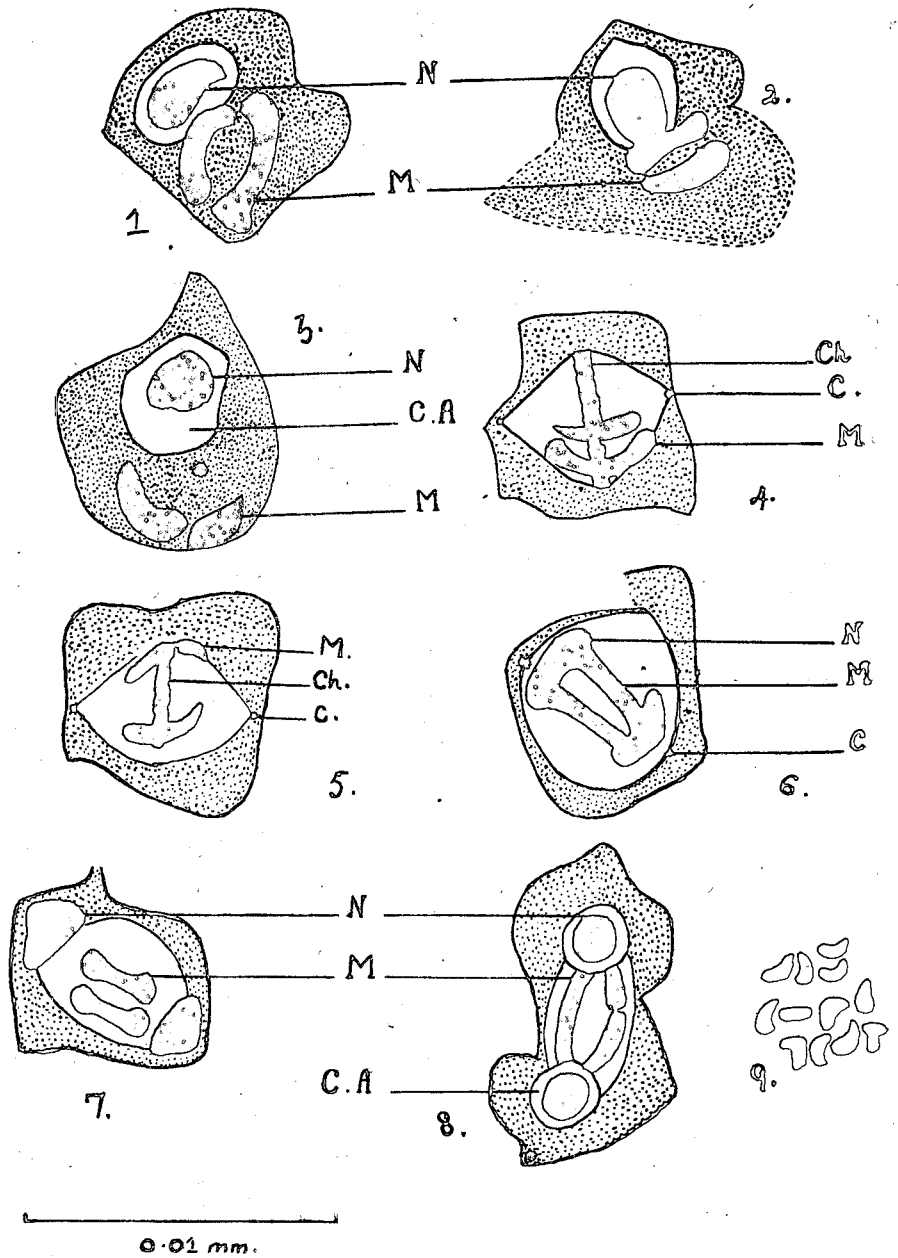
As a rule, it is easy to demonstrate the Golgi body during spermateleosis, but in this species it has been possible to demonstrate it sometimes during earlier stages also (Text-fig. III, 1 and 2). In these two figures the Golgi body clearly consists of two pieces.

Spermateleosis

In the earlier spermatids the two short mitochondrial rods quickly round themselves off and the Golgi apparatus, which hitherto consisted of two pieces, becomes a single compact body by the fusion of these pieces (Text-fig. VI, 1–5). The spermatid nucleus now begins to elongate, and the Golgi body places itself at its anterior end and the two mitochondrial pieces at the posterior end (Text-fig. VI, 6–9).

Soon an axial filament springs up from the base of the nucleus (Text-fig. VI, 10). It soon emerges out of the cell at the posterior end. Due to mechanical disturbance it may not be possible to demonstrate the origin of the axial filament from its natural position, that is, the base of the nucleus (Text-fig. VII, 1–5).

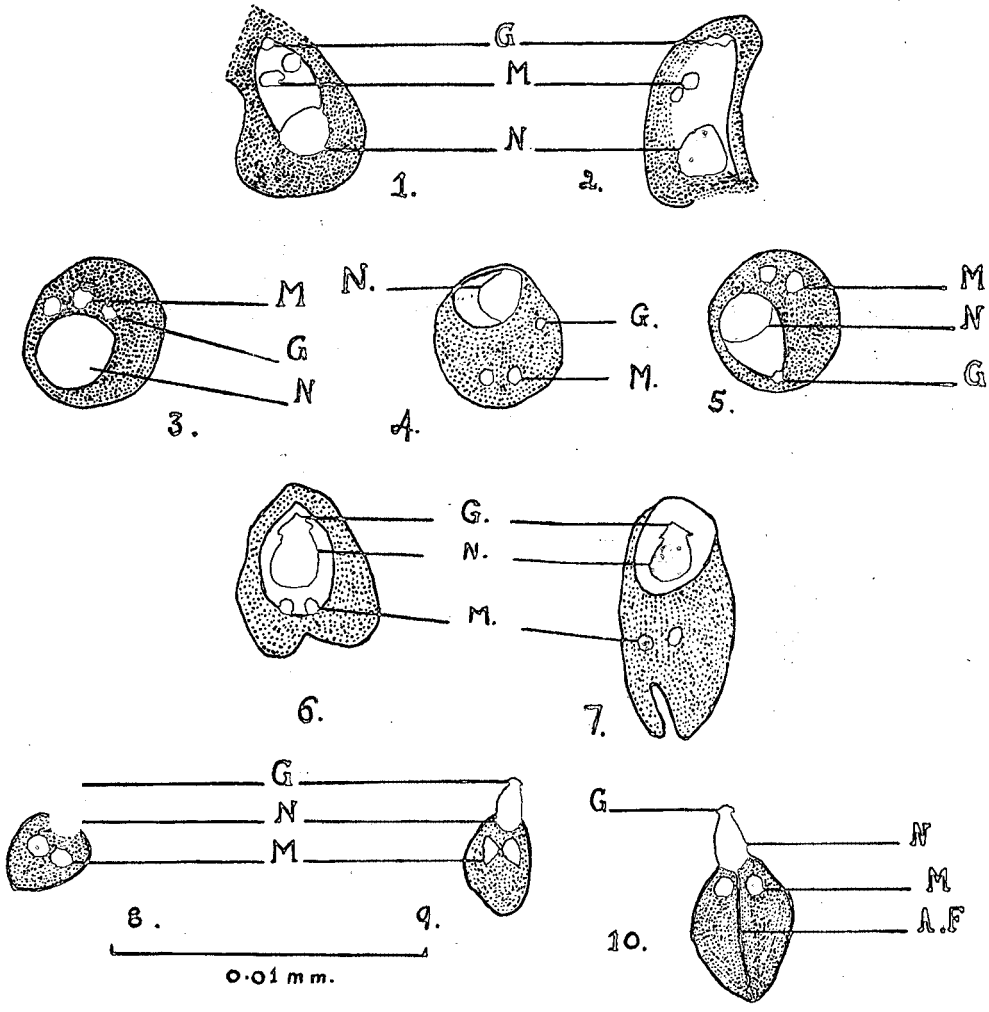
With the elongation of the nucleus of the spermatid the Golgi body begins to grow directly into the acrosome (Text-fig. VII, 6 and 7). At the same time the two mitochondrial masses in each spermatid begin to elongate and assume the form of two thick threads closely twisted round each other



TEXT-FIG. V. Secondary spermatocytes and their divisions. 1–3. Two rod-shaped mitochondria; 4–8. Gradual stages in the division of these two rod-like mitochondria into two equal halves; 9. Metaphase II, polar view.

1–3 from material fixed in Champy's fixative; 4–8 from material fixed in F.W.A.; 9 from material fixed in Bouin's fluid.

(Text-fig. VIII, 1–5). Ultimately the mitochondrial spirals get so attenuated that no trace is left of them in the tail of the ripe sperm, although the sheath of the axial filament is formed definitely from the mitochondrial spirals.



Text-fig. VI. Spermatids. 1. With two mitochondrial pieces and the Golgi body lying in front of them; 2–5. Movement of the Golgi body to the anterior side of the nucleus; 6. Fusion of the Golgi body with the nucleus; 7–9. Early stages in the development of the acrosome; 10. Showing the axial filament arising from the base of the nucleus.

All figs. from material fixed in F.W.A.

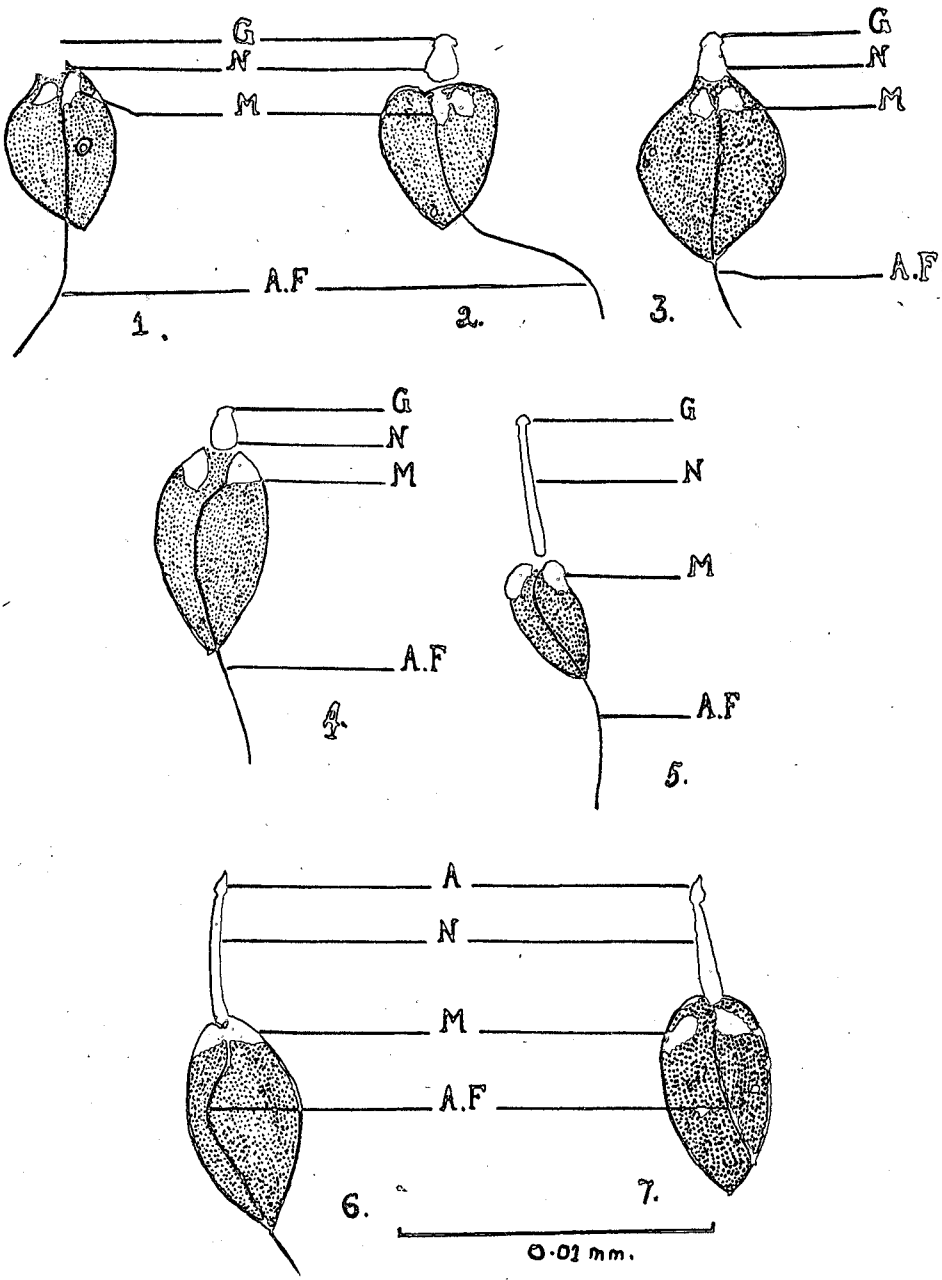
A.F., axial filament.

Discussion

We wish to emphasize only three points in this section: firstly, it has to be noted that the distribution of the mitochondrial material to the four spermatids resulting from one primary spermatocyte is equal only in the quantitative sense and not in the qualitative sense. The division of the mitochondrial material is not on a par with the division of a chromosome, which divides equally not only in the quantitative sense, but also in the

qualitative sense. It has therefore been rightly held that chromatin is the *physical basis of heredity par excellence.*

Secondly, it is to be noted that the acrosome is formed directly from the Golgi apparatus and is not a secretory product thereof (*vide* Nath, 1944).



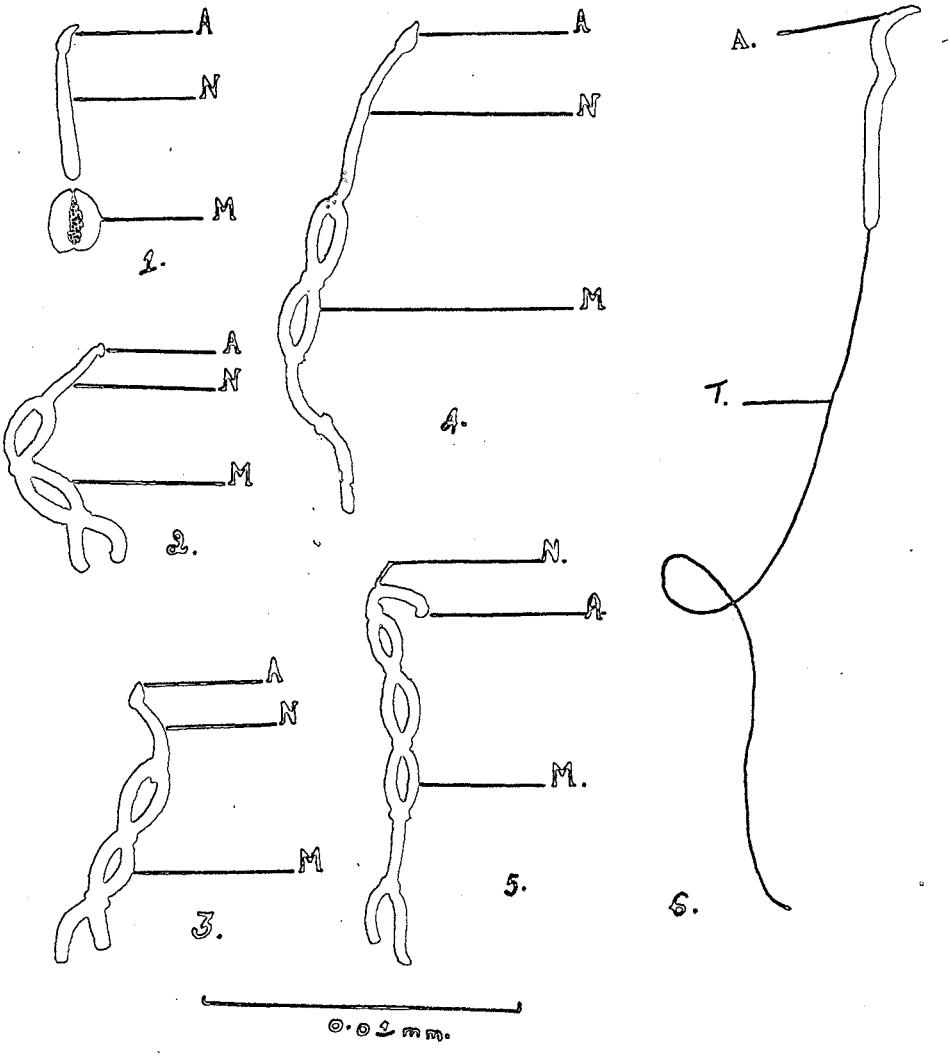
TEXT-FIG. VII. Spermatids showing the formation of the acrosome, elongation of the nucleus, and the origin of the axial filament.

All figs. from material fixed in F.W.A.

A., acrosome.

Lastly, we claim to have produced cytological evidence in support of the taxonomic value of the seemingly unimportant external variations which the taxonomist uses in his work of species building. As has been pointed out earlier by us, *Centrurus* and *Buthus acute-carinatus* have been placed in the scheme of classification close to each other in the family *Buthidae* on the strength of external resemblances which are parallel, as we have proved, to the identical behaviour of the mitochondrial material in these two closely allied species.

A similar parallelism was brought out by Nath in the Decapod sperm (Nath, 1942). In the *Macrura*, as illustrated by *Penaeus indicus*, *Palaemon lamarrei*, and *Panulirus polyphagus*, there is a single large centrosome.



TEXT-FIG. VIII. 1–5. Elongation and twisting of the two mitochondrial pieces to form the tail-sheath of the sperm tail; 6. Ripe sperm with acrosome, head, and the tail.

1–5 from material fixed in F.W.A. and 6 from a smear prepared in F.W.A.

From some Macrura-like ancestors (probably forms somewhat like present-day Penaeidea), with only single centrosome in their sperms, were evolved such Brachyuran forms as have two centrosomes in their sperms (*Paratelphusa* and its allies), and from some of these bicentrosomal forms were, in turn, evolved *Leptodius* and its allies, which have three centrosomes in their sperms. The Anomura seem to have arisen from the Penaeidea-like ancestors by the enormous enlargement of the centrosomal apparatus and its differentiation into three well-defined parts. It was shown by Nath that this process of evolution based on cytological difference runs parallel to the process evolved by the taxonomists inasmuch as the taxonomists are generally agreed that the *Penaeidea* are the most primitive of the *Decapoda*.

Summary

1. The remarkably equal distribution of the mitochondrial material in the spermatogenesis of *Buthus acute-carinatus* has been fully described.

2. The acrosome is formed directly from the Golgi body and is not a secretory product thereof.

3. Cytological evidence in support of the taxonomic value of seemingly unimportant external variations has been produced.

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Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

Nos. 2, 3. MATHEMATICS Pp. 13-63

February, 1950

TABLES OF DISTRIBUTIONS

A TABLE OF VALUES OF LIOUVILLE'S FUNCTION $L(t)$

by

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HOSHIARPUR

Price Rs. 5.

TABLES OF DISTRIBUTIONS[1]

By HANSRAJ GUPTA, *Government College, Hoshiarpur*

1. Let $u(m, a)$ denote the number of distributions of m unlike things into a ($< m$) groups; i.e. the number of ways in which m men can be accommodated in exactly a similar apartments, no apartment being left vacant. Thus $u(4, 3) = 6$, because four men A, B, C and D can be placed in three apartments in the following six ways:—

(1) (A), (B), (C, D); (2) (A), (D), (B, C); (3) (A), (C), (B, D);
(4) (B), (D), (A, C); (5) (B), (C), (A, D); (6) (C), (D), (A, B).

Now suppose, we have to place $(m+1)$ men in a apartments. Corresponding to each of the $u(m, a)$ distributions of the first m men into a apartments there are a ways of accommodating the $(m+1)$th person, because he can be placed in any one of the a apartments. Again, corresponding to each one of the $u(m, a-1)$ distributions of the first m persons into $(a-1)$ apartments, there is one way of accommodating the $(m+1)$th person and that is in the a-th apartment. Hence, we have the fundamental relation:—

$$u(m+1, a) = a.\, u(m, a)+u(m, a-1), \quad .. \quad .. \quad (1)$$

which holds when $m \geqslant a > 1$. Moreover,

$$u(m, 1) = 1 = u(m, m), \quad m > 1; \quad .. \quad .. \quad .. \quad (2)$$

and we take

$$u(0, 0) = 1, \quad .. \quad .. \quad .. \quad .. \quad (3)$$

$$u(m, a) = 0, \text{ when } 0 < m < a, \text{ or } a < 0. \quad .. \quad .. \quad (4)$$

The tables that follow, give the values of $u(n, a)$ for $1 \leqslant a \leqslant n \leqslant 50$; and have been computed mainly with the help of relation (1). We give also the values of

$$U(n) = \sum_{m=1}^{n} u(m, a), \quad .. \quad .. \quad .. \quad (5)$$

for values of n up to 50. These are the unrestricted distributions of n dissimilar things into groups. Thus $U(6) = 203$.

2. Let[2] $S(a) = \sum_{m=a}^{\infty} u(m, a)\,.\,x^{-m}$,

then $$\frac{a}{x}\,.\,S(a) = \sum_{m=a+1}^{\infty} a\,.\,u(m-1, a)\,.\,x^{-m}.$$

Subtracting, we get

$$\frac{x-a}{x}\,S(a) = \sum_{m=a}^{\infty} u(m-1, a-1)\,.\,x^{-m} = \frac{S(a-1)}{x}.$$

Hence $$S(a) = \frac{1}{x-a} . S(a-1) = \frac{1}{(x-a)} \cdot \frac{1}{(x-a+1)} . S(a-2) = \ldots .$$

$$= \{(x-1)(x-2)(x-3) \ldots (x-a)\}^{-1};$$

because $$S(1) = \frac{1}{x} + \frac{1}{x^2} + \frac{1}{x^3} + \ldots = \frac{1}{x-1} .$$

Thus $u(m, a)$ is the coefficient of x^{-m} in the expansion of

$$\{(x-1)(x-2)(x-3) \ldots (x-a)\}^{-1}. \quad .. \quad .. \quad (6)$$

Breaking (6) into partial fractions, we find that

$$(a-1)!\, u(m, a) = a^{m-1} - \binom{a-1}{1}(a-1)^{m-1} + \binom{a-1}{2}(a-2)^{m-1} - \ldots .$$

$$\ldots . + (-1)^{a-1}\binom{a-1}{a-1} . 1^{m-1} . \ldots \quad (7)$$

In particular,

$$u(m, 1) = 1,$$

$$1!\, u(m, 2) = 2^{m-1} - 1,$$

$$2!\, u(m, 3) = 3^{m-1} - 2 . 2^{m-1} + 1.$$

Again[3] $(e^x - 1)^a = e^{ax} - \binom{a}{1} e^{(a-1)x} + \binom{a}{2} e^{(a-2)x} - \ldots . + (-1)^a \binom{a}{a}$.

Coefficient of x^m in the expansion of $(e^x - 1)^a$

$$= \frac{a^m}{m!} - \binom{a}{1}\frac{(a-1)^m}{m!} + \binom{a}{2}\frac{(a-2)^m}{m!} - \ldots + (-1)^{a-1}\binom{a}{a-1}\frac{1^m}{m!}, \; m \geqslant 1;$$

$$= \frac{a!}{m!}\, u(m, a).$$

Hence $U(m)$ is the coefficient of $\frac{x^m}{m!}$ in

$$\sum_{a=1}^{m} \frac{(e^x-1)^a}{a!}, \text{ i.e. in } \sum_{a=0}^{\infty} \frac{(e^x-1)^a}{a!} \text{ or } \frac{e^{e^x}}{e} .$$

In other words,

$$e^{e^x - 1} = 1 + \frac{U(1)}{1!}x + \frac{U(2)}{2!}x^2 + \frac{U(3)}{3!}x^3 + \ldots . + \frac{U(m)}{m!}x^m + \ldots . \quad .. \quad (8)$$

Differentiating, we get

$$e^x . e^{e^x - 1} = \sum_{m=0}^{\infty} \frac{U(m+1)}{m!} x^m .$$

Also $$e^x . e^{e^x - 1} = \sum_{t=0}^{\infty} \frac{x^t}{t!} \cdot \sum_{s=0}^{\infty} \frac{U(s)}{s!} x^s .$$

Equating the coefficients of x^m in these two expressions, we have

$$U(m+1) = U(0) + \binom{m}{1}U(1) + \binom{m}{2}U(2) + \ldots . + \binom{m}{m}U(m). \quad .. \quad (9)$$

where $U(0) = 1$.

The value of $U(50)$ has been checked [4] with the help of (9).

3. The following congruences were very useful to me in checking the results as the work of computation proceeded.

Throughout this section p denotes a prime > 2.

As a direct consequence of Fermat's Theorem, we have

$$u(p, a) \equiv 0 \pmod{p}, 1<a<p; \quad \ldots \quad \ldots \quad \ldots \quad (10)$$

and
$$\equiv 1 \pmod{p}, a = 1 \text{ or } p. \quad \ldots \quad \ldots \quad (11)$$

Moreover

$$u(p+m, a) = u(m+1, a)+u(m, a-p) \pmod{p}. \quad \ldots \quad (12)$$

For supposing (12) to be true for every positive $m \leqslant n-1$, we have

$$\begin{aligned} u(p+n, a) &= a \,.\, u(p+n-1, a)+u(p+n-1, a-1), \\ &= a\{u(n, a)+u(n-1, a-p)\} \\ &\qquad +\{u(n, a-1)+u(n-1, a-p-1)\} \pmod{p}, \\ &\equiv \{a \,.\, u(n, a)+u(n, a-1)\} \\ &\qquad +\{a \,.\, u(n-1, a-p)+u(n-1, a-p-1)\} \pmod{p}, \\ &\equiv u(n+1, a)+u(n, a-p) \pmod{p}. \end{aligned}$$

The result follows readily by induction.

From (12) we obtain without difficulty

$$U(p+m) \equiv U(m+1)+U(m) \pmod{p}; \quad \ldots \quad \ldots \quad (13)$$

where $U(0) = 1$ as already stated.

Using (13), k times in succession, we get

$$\begin{aligned} U(kp+m) \equiv U(m+k)+\binom{k}{1} U(m+k-1)+\binom{k}{2} U(m+k-2)+\ldots. \\ \ldots+\binom{k}{k} U(m) \pmod{p}. \quad \ldots \quad (14) \end{aligned}$$

As a particular case of (14), we have

$$\begin{aligned} U(kp) &\equiv U(k)+\binom{k}{1} U(k-1)+\binom{k}{2} U(k-2)+\ldots.+\binom{k}{k} U(0) \pmod{p}, \\ &\equiv U(k+1) \pmod{p}. \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (15) \end{aligned}$$

4. *An Inequality.* For every $m > 0$,

$$U(m) \leqslant \frac{(m+2)!}{3 \,.\, 2^m}. \quad \ldots \quad \ldots \quad \ldots \quad (16)$$

Supposing this to hold for every $m \leqslant n-1$, we have

$$\begin{aligned} U(n) &= U(n-1)+\binom{n-1}{1} U(n-2)+\binom{n-1}{2} U(n-3)+\ldots. \\ &\qquad +\binom{n-1}{n-1} U(0), \\ &\leqslant \frac{(n+1)!}{3 \,.\, 2^{n-1}}+(n-1).\frac{n!}{3 \,.\, 2^{n-2}}+\frac{(n-1)(n-2)}{2!}.\frac{(n-1)!}{3 \,.\, 2^{n-3}}+\ldots. \\ &\qquad +(n-1).\frac{3!}{3 \,.\, 2}+1, \end{aligned}$$

$$< \frac{(n+1)!}{3 \cdot 2^{n-1}} \left\{ 1 + \frac{2}{1!} + \frac{2^2}{2!} + \frac{2^3}{3!} + \ldots \right\},$$

$$< \frac{(n+1)!}{3 \cdot 2^{n-1}} \cdot e^2,$$

$$< \frac{(n+2)!}{3 \cdot 2^n}, \text{ provided } \frac{n+2}{2} > e^2.$$

The result being true for every $m < 2(e^2-1)$, is true for every $m > 0$.

5. 1. *Some Asymptotic Results.*

Let $(m-1)/a = y$, where y is not necessarily an integer.

Then for large values of m and a,

$$U(m, a) = \frac{1}{(a-1)!} \left\{ a^{m-1} - \binom{a-1}{1}(a-1)^{m-1} + \binom{a-1}{2}(a-2)^{m-1} - \ldots \right\},$$

$$= \frac{a^{m-1}}{(a-1)!} \left\{ 1 - \binom{a-1}{1}\left(1-\frac{1}{a}\right)^{ay} + \binom{a-1}{2}\left(1-\frac{2}{a}\right)^{ay} - \ldots \right\},$$

$$\sim \frac{a^{m-1}}{(a-1)!}(1-e^{-y})^{a-1}.$$

Similarly, $u(m, a-1) \sim \frac{a^{m-1}}{(a-2)!} e^{-y}(1-e^{-y})^{a-2}$,

and $u(m, a+1) \sim \frac{a^{m-1}}{a!} e^{y}(1-e^{-y})^{a}$.

Hence $u(m, a-1) \leqslant u(m, a) \geqslant u(m, a+1)$ for large m, if

$a \leqslant e^y \leqslant a+1$, i.e. $\log a \leqslant y \leqslant \log (a+1)$.

Thus $u(m, a)$ is maximal for a given large m, if

$$\log a < \frac{m-1}{a} < \log (a+1). \quad \ldots \quad \ldots \quad \ldots \quad (17)$$

For $m = 50$, this gives $a = 17$, while $a = 16$ from the table.

5. 2. Let r denote the value of a for which $u(m, a)$ is maximal.

Then $U(m) = \sum_{a=1}^{r} u(m, a) + \sum_{a=r+1}^{\infty} u(m, a)$,

$$\sim r^{m-1}\left\{ \frac{(1-e^{-y})^{r-1}}{(r-1)!} + \frac{e^{-y}(1-e^{-y})^{r-2}}{(r-2)!} + \ldots \right\}$$

$$+ r^{m-1}\left\{ \frac{e^{y}(1-e^{-y})^{r}}{r!} + \frac{e^{2y}(1-e^{-y})^{r+1}}{(r+1)!} + \ldots \right\}, y = \frac{m-1}{r};$$

$$\sim \frac{r^{m-1}}{e^{y(r-1)}} \sum_{t=0}^{m} \frac{(e^y-1)^t}{t!},$$

$$\sim \frac{r^{m-1}}{e^{y(r-1)}} \exp (e^y-1).$$

Since $y = \log r$ nearly,

$$U(m) \sim r^{m-r}\, e^{r-1}. \qquad \ldots \quad \ldots \quad \ldots \quad (18)$$

For $m = 50$, taking $r = 16$, this gives

$$\log_{10} U(50) = 34 \log_{10} 16 + 15 \log_{10} e,$$
$$= 34 \times 1{\cdot}20412 + 15 \times 0{\cdot}43429,$$
$$= 47{\cdot}45443;$$

while

$$\log_{10} U(50) = 47{\cdot}26879.$$

Even if we were to take $r = 17$, as given by (17), we would get

$$\log_{10} U(50) = 47{\cdot}55349.$$

Within the limits of the table, the value of $\log_{10} U(m)$ computed from (18) exceeds the true value by $< 0{\cdot}3$.

For forming a rough estimate of the value of $U(m)$ for large m, (18) is thus very useful indeed.

References

1. This work was done at the suggestion of Dr. D. S. Kothari of the University of Delhi in 1945.
2. See H. Gupta, 'Symmetric Functions in the Theory of Integral Numbers', Lucknow *University Studies, xiv*, 1940, 37–54.
3. See W. A. Whitworth, 'Choice and Chance', Cambridge, 1901, 87–89.
4. See the table at the end.

My pupils Om Prakash, Ranbir Singh and Uma helped me in checking this result.

The Table.

$n \rightarrow$ a	1	2	3	4	5	6	7	8	9	10
1	1	1	1	1	1	1	1	1	1	1
2		1	3	7	15	31	63	127	255	511
3			1	6	25	90	301	966	3025	9330
4				1	10	65	350	1701	7770	34105
5					1	15	140	1050	6951	42525
6						1	21	266	2646	22827
7							1	28	462	5880
8								1	36	750
9									1	45
10										1
	1	2	5	15	52	203	877	4140	21147	1 15975

$n \rightarrow$ a	11	12	13	14
1	1	1	1	1
2	1023	2047	4095	8191
3	28501	86526	2 61625	7 88970
4	1 45760	6 11501	25 32530	103 91745
5	2 46730	13 79400	75 08501	400 75035
6	1 79487	13 23652	93 21312	634 36373
7	63987	6 27396	57 15424	493 29280
8	11880	1 59027	18 99612	209 12320
9	1155	22275	3 59502	51 35130
10	55	1705	39325	7 52752
11	1	66	2431	66066
12		1	78	3367
13			1	91
14				1
	6 78570	42 13597	276 44437	1908 99322

$n \rightarrow$ a	15	16	17	18
1	1	1	1	1
2	16383	32767	65535	1 31071
3	23 75101	71 41686	214 57825	644 39010
4	423 55950	1717 98901	6943 37290	27988 06985
5	2107 66920	10961 90550	56527 51651	2 89580 95545
6	4206 93273	27349 26558	1 75057 49898	11 06872 51039
7	4087 41333	32818 82604	2 57081 04786	19 74624 83400
8	2166 27840	21417 64053	2 04159 95028	18 90360 65010
9	671 28490	8207 84250	95288 22303	10 61753 95755
10	126 62650	1937 54990	27583 34150	3 71121 63803
11	14 79478	289 36908	5120 60978	83910 04908
12	1 06470	27 57118	620 22324	12563 28866
13	4550	1 65620	49 10178	1258 54638
14	105	6020	2 49900	84 08778
15	1	120	7820	3 67200
16		1	136	9996
17			1	153
18				1
	13829 58545	1 04801 42147	8 28648 69804	68 20768 06159

$n \rightarrow$ a	19	20
1	1	1
2	2 62143	5 24287
3	1934 48101	5806 06446
4	1 12596 66950	4 52321 15901
5	14 75892 84710	74 92060 90500
6	69 30816 01779	430 60788 95384
7	149 29246 34839	1114 35540 45652
8	170 97510 03480	1517 09326 62679
9	114 46146 26805	1201 12826 44725
10	47 72970 33785	591 75849 64655
11	12 94132 17791	190 08424 29486
12	2 34669 51300	41 10166 33391
13	28924 39160	6 10686 60380
14	2435 77530	63025 24580
15	139 16778	4523 29200
16	5 27136	223 50954
17	12597	7 41285
18	171	15675
19	1	190
20		1
	583 27422 05057	5172 41582 35372

$n \rightarrow$ a	21	22
1	1	1
2	10 48575	20 97151
3	17423 43625	52280 79450
4	18 15090 70050	72 77786 23825
5	379 12625 68401	1913 78219 12055
6	2658 56794 62804	16330 53393 45225
7	8231 09572 14948	60276 23799 67440
8	13251 10153 47084	1 14239 90799 91620
9	12327 24764 65204	1 24196 33035 33920
10	7118 71322 91275	83514 37993 77954
11	2682 68516 89001	36628 25008 70286
12	683 30420 30178	10882 33560 51137
13	120 49092 18331	2249 68618 68481
14	14 93040 04500	329 51652 81331
15	1 30874 62580	34 56159 43200
16	8099 44464	2 60465 74004
17	349 52799	14041 42047
18	10 23435	533 74629
19	19285	13 89850
20	210	23485
21	1	231
22		1
	47486 98161 56751	4 50671 57384 47323

$n \rightarrow$ a $\downarrow$	23	24
1	1	1
2	41 94303	83 88607
3	1 56863 35501	4 70632 00806
4	291 63425 74750	1168 10566 34501
5	9641 68881 84100	48500 07834 95250
6	99896 98579 83405	6 09023 60360 84530
7	4 38264 19991 17305	31 67746 38518 04540
8	9 74195 50199 00400	82 31828 21583 20505
9	12 32006 88117 96900	120 62257 43260 72500
10	9 59340 12973 13460	108 25408 17849 31500
11	4 86425 13089 51100	63 10016 56957 75560
12	1 67216 27734 83930	24 93020 45907 58260
13	40128 25603 41390	6 88883 60579 22000
14	6862 91758 07115	1 36209 10216 41000
15	847 94044 29331	19582 02422 47080
16	76 23611 27264	2067 71824 65555
17	4 99169 88803	161 09499 36915
18	23648 85369	9 24849 25445
19	797 81779	38807 39170
20	18 59550	1169 72779
21	28336	24 54606
22	253	33902
23	1	276
24		1
	44 15200 58550 84346	445 95886 92948 05289

a	$n = 25$	$n = 26$
1	1	1
2	167 77215	335 54431
3	14 11979 91025	42 36107 50290
4	4677 12897 38810	18722 63569 46265
5	2 43668 49741 10751	12 23019 61602 92565
6	37 02641 70000 02430	224 59518 69741 25331
7	227 83248 29987 16310	1631 85379 79910 16600
8	690 22372 11183 68580	5749 62225 19456 64950
9	1167 92145 10929 73005	11201 51678 09551 25625
10	1203 16339 21753 87500	13199 55537 28468 48005
11	802 35590 44384 62660	10029 07834 09984 76760
12	362 26262 07848 74680	5149 50735 38569 58820
13	114 48507 33437 44260	1850 56857 42535 50060
14	25 95811 03608 96000	477 89861 83962 88260
15	4 29939 46553 47200	90 44903 01911 04000
16	52665 51616 95960	12 72587 72424 82560
17	4806 33313 93110	1 34373 17953 78830
18	327 56785 94925	10702 55461 01760
19	16 62189 69675	643 38390 18750
20	62201 94750	29 06228 64675
21	1685 19505	97591 04355
22	32 00450	2389 29405
23	40250	41 26200
24	300	47450
25	1	325
26		1
	4638 59033 22299 99353	49631 24652 36187 56274

a	$n = 27$	$n = 28$
1	1	1
2	671 08863	1342 17727
3	127 08658 05301	381 26645 24766
4	74932 90385 35350	2 99858 70199 46701
5	61 33820 71584 09090	307 44036 48305 80800
6	1359 80131 80050 44551	8220 14611 91886 76396
7	11647 57177 29112 41531	82892 80372 83837 35268
8	47628 83181 35563 36200	3 92678 22628 13619 31131
9	1 06563 27328 05417 95575	10 06698 29133 84324 96375
10	1 43197 07050 94236 05675	15 38533 97837 47778 52325
11	1 23519 41712 38300 92365	15 01910 65887 15546 21690
12	71823 16658 72819 82600	9 85397 41617 12138 83565
13	29206 89881 91531 09600	4 51512 85123 62724 07400
14	8541 14923 18015 85700	1 48782 98806 43753 09400
15	1834 63407 12628 48260	36060 66030 07443 09600
16	294 06306 60708 24960	6539 64312 83960 47620
17	35 56931 77639 22670	898 74146 80575 10350
18	3 27019 16252 10510	94 43276 70177 11850
19	22926 84874 58010	7 62629 28869 12700
20	1224 62963 12250	47419 44137 03010
21	49 55640 56130	2265 31414 90980
22	1 50155 51265	82 59061 83960
23	3338 32005	2 26936 87380
24	52 65000	4601 92005
25	55575	66 54375
26	351	64701
27	1	378
28		1
	5 45717 04793 60599 89389	61 60539 40459 993465 2455

a	$n = 29$
1	1
2	2684 35455
3	1143 81277 92025
4	11 99816 07443 11570
5	1540 20041 11728 50701
6	49628 31705 59626 39176
7	5 88469 77221 38748 23272
8	32 24318 61397 92791 84316
9	94 52962 84832 72543 98506
10	163 92038 07508 62110 19625
11	180 59551 22596 18786 90915
12	133 26679 65292 61212 24470
13	68 55064 48224 27551 79765
14	25 34474 68413 75267 39000
15	6 89692 89257 55399 53400
16	1 40694 95035 50810 71520
17	21818 24808 53737 23570
18	2598 53127 43763 23650
19	239 33233 18690 53150
20	17 11018 11609 72900
21	94991 03850 13590
22	4082 30775 38100
23	134 78609 93700
24	3 37382 95500
25	6265 51380
26	83 36601
27	74907
28	406
29	1
	713 39801 93886 02751 91172

a	$n = 30$
1	1
2	5368 70911
3	3431 46518 11530
4	48 00408 11050 38305
5	7713 00021 66085 65075
6	2 99310 10274 69486 85757
7	41 68916 72255 30864 02080
8	263 83018 68404 81082 97800
9	883 00984 24892 45687 70870
10	1733 73343 59918 93645 94756
11	2150 47101 56066 68766 19690
12	1779 79707 06107 53333 84555
13	1024 42517 92208 19385 61415
14	423 37710 06016 81295 25765
15	128 79868 07277 06260 40000
16	29 40812 09825 68370 97720
17	5 11605 16780 64343 72210
18	68591 81102 41475 49270
19	7145 84557 98883 33500
20	581 53595 50885 11150
21	37 05829 92462 58290
22	1 84801 80908 51790
23	7182 38803 93200
24	215 75800 85700
25	4 94020 80000
26	8433 03006
27	103 59090
28	86275
29	435
30	1
	8467 49014 51180 93324 50147

a	$n = 31$
1	1
2	10737 41823
3	10294 44923 05501
4	192 05063 90719 64750
5	38613 00516 41478 63680
6	18 03573 61669 83006 79617
7	294 81727 16061 85535 00317
8	2152 33066 19493 79527 84480
9	8210 91876 92436 92272 35630
10	18220 34420 24081 82147 18430
11	25388 91460 76652 50074 11346
12	23508 03586 29357 08772 34350
13	15097 32440 04814 05346 82950
14	6951 70458 76443 57519 22125
15	2355 35731 15172 75201 25765
16	599 32861 64488 00196 03520
17	116 38099 95096 62214 25290
18	17 46257 76624 10902 59070
19	2 04362 87704 20258 85770
20	18776 56468 16585 56500
21	1359 76023 92599 35240
22	77 71469 72449 97670
23	3 49996 73398 95390
24	12360 58024 50000
25	339 26320 85700
26	7 13279 58156
27	11229 98436
28	127 74790
29	98890
30	465
31	1
	1 02933 58946 22637 64850 95653

a	$n = 32$
1	1
2	21474 83647
3	30883 45506 58326
4	768 30550 07801 64501
5	1 93257 07645 98112 83150
6	108 60054 70535 39519 41382
7	2081 75663 74102 81751 81836
8	17513 46256 72012 21757 76157
9	76050 59958 51426 09979 05150
10	1 90414 36079 33255 13744 19930
11	2 97498 40488 67259 32962 43236
12	3 07485 34496 28937 55342 23546
13	2 19773 25306 91939 78281 12700
14	1 12421 18862 75024 10615 92700
15	42282 06426 04034 85538 08600
16	11944 61517 46980 78337 82085
17	2577 80560 81130 57838 33450
18	430 70739 74330 58460 88550
19	56 29152 43003 95820 88700
20	5 79894 17067 51970 15770
21	47331 52970 61171 96540
22	3069 48357 86498 83980
23	158 21394 60625 91640
24	6 46650 65986 95390
25	20842 16045 92500
26	524 71589 97756
27	10 16489 15928
28	14806 92556
29	156 42600
30	1 12840
31	496
32	1
	12 80646 70049 90871 38189 25644

a	$n = 33$
1	1
2	42949 67295
3	92650 57994 58625
4	3073 53083 76713 16330
5	9 67053 68779 98365 80251
6	653 53585 30858 35229 31442
7	14680 89700 89255 11782 14234
8	1 42189 45717 50200 55813 91092
9	7 01968 85883 34847 11569 22507
10	19 80194 20751 83977 47421 04450
11	34 62896 81454 73107 76330 95526
12	39 87322 54444 14509 97069 25788
13	31 64537 63486 24154 72996 88646
14	17 93669 89385 42277 26904 10500
15	7 46652 15253 35546 93687 21700
16	2 33395 90705 55727 38943 21960
17	55767 31051 26200 61589 50735
18	10330 53876 19081 10134 27350
19	1500 24635 91405 79057 73850
20	172 27035 84354 35224 04100
21	15 73856 29450 36581 43110
22	1 14860 16843 64146 44100
23	6708 40433 80894 91700
24	313 41010 44312 81000
25	11 67704 67135 07890
26	34484 77385 34156
27	799 16797 27812
28	14 31083 07496
29	19343 27956
30	190 27800
31	1 28216
32	528
33	1
	162 95958 92846 00760 67647 28147

a	$n = 34$
1	1
2	85899 34591
3	2 77952 16933 43170
4	12295 04985 64847 23945
5	48 38341 96983 68542 17585
6	3930 88565 53930 09741 68903
7	1 03419 81491 55644 17704 31080
8	11 52196 55440 90859 58293 42970
9	64 59909 18667 63824 59936 93655
10	205 03910 93401 74621 85779 67007
11	400 72059 16753 88162 87061 55236
12	513 10767 34784 47227 41162 04982
13	451 26311 79765 28521 46028 78186
14	282 75916 14882 16036 49654 35646
15	129 93452 18185 75481 32212 36000
16	44 80986 66542 27185 16778 73060
17	11 81440 18577 01137 85964 84455
18	2 41717 00822 69660 44006 43035
19	38835 21958 55791 12231 30500
20	4945 65352 78492 83538 55850
21	502 78018 02812 03434 09410
22	41 00780 00010 47803 13310
23	2 69153 46821 24729 53200
24	14230 24684 44402 35700
25	605 33627 22689 78250
26	20 64308 79153 95946
27	56062 30911 85080
28	1199 87123 37700
29	19 92038 18220
30	25051 61956
31	230 02496
32	1 45112
33	561
34	1
	2119 50393 88640 36046 23886 56799

a	$n = 35$
1	1
2	1 71798 69183
3	8 33857 36699 64101
4	49182 97894 76322 38950
5	242 04004 89904 07558 11870
6	23633 69735 20564 26992 31003
7	7 27869 59006 43439 33671 86463
8	93 20992 25018 82520 84051 74840
9	592 91379 23449 65280 97725 85865
10	2114 99018 52685 10043 17733 63725
11	4612 96561 77694 44413 43456 74603
12	6558 01267 34167 54891 81006 15020
13	6379 52820 71733 18006 39536 21400
14	4409 89137 88115 53032 41189 77230
15	2231 77698 87668 48256 32839 75646
16	846 89238 82862 10444 00672 04960
17	245 65469 82351 46528 78181 08795
18	55 32346 33385 55025 78080 59085
19	9 79586 18035 29691 76401 22535
20	1 37748 29014 25647 83002 47500
21	15504 03731 37545 55654 53460
22	1404 95178 03042 55103 02230
23	102 91309 76899 16582 36910
24	6 10679 39247 90386 10000
25	29363 65365 11646 91950
26	1142 05655 80692 72846
27	35 77991 13773 93106
28	89658 70366 40680
29	1777 56230 66080
30	27 43586 76900
31	32182 39332
32	276 46080
33	1 63625
34	595
35	1
	28160 02030 19560 26656 33404 26570

a	$n = 36$
1	1
2	3 43597 38367
3	25 01573 81897 61486
4	1 96740 25436 41989 19901
5	1210 69207 47415 14112 98300
6	1 42044 22416 13289 69511 97888
7	51 18720 82780 24639 62695 36244
8	752 95807 59157 03606 06085 85183
9	5429 43405 36065 70049 63584 47625
10	21742 81564 50300 65712 75062 23115
11	52857 61198 07323 98590 95757 84358
12	83309 11769 87705 03115 15530 54843
13	89491 87936 66898 88974 94976 93220
14	68118 00751 05350 60460 16193 02620
15	37886 54621 03142 76877 33786 11920
16	15782 05520 13462 15360 43592 55006
17	5023 02225 82837 01433 29750 54475
18	1241 47703 83291 36992 83631 72325
19	241 44483 76056 19169 29703 87250
20	37 34551 98320 42648 36450 72535
21	4 63333 07373 14104 51747 70160
22	46412 97648 04481 67921 02520
23	3771 95302 71723 36497 51160
24	249 47615 18848 85848 76910
25	13 44770 73375 81559 08750
26	59057 12416 09657 85946
27	2108 11416 52588 86708
28	60 88434 84033 32146
29	1 41208 01055 57000
30	2600 63833 73080
31	37 41240 96192
32	41029 13892
33	330 45705
34	1 83855
35	630
36	1
	3 81971 47298 94818 33997 55256 81317

a	$n = 37$
1	1
2	6 87194 76735
3	75 04724 89290 22825
4	7 86986 03319 49854 41090
5	6055 42777 62512 12554 11401
6	8 53476 03704 27153 31184 85628
7	359 73090 01877 85767 08379 51596
8	6074 85181 56036 53488 11382 17708
9	49617 86455 83748 34052 78346 13808
10	2 22857 59050 39072 27177 14206 78775
11	6 03176 54743 30864 50213 28398 51053
12	10 52567 02436 59784 35972 82124 42474
13	12 46703 54946 54790 59789 50230 66703
14	10 43143 98451 41607 35417 21679 29900
15	6 36416 20066 52492 13620 22984 81420
16	2 90399 42943 18537 22644 31266 92016
17	1 01173 43359 21691 39726 49351 81081
18	27369 60894 82081 67304 35121 56325
19	5828 92895 28359 01209 48005 30075
20	988 35523 42464 72136 58718 37950
21	134 64546 53156 38843 23152 45895
22	14 84418 55630 12701 46010 25600
23	1 33167 89610 54119 07363 79200
24	9759 38067 24095 96867 97000
25	585 66883 53244 24825 95660
26	28 80255 96194 32663 43346
27	1 15976 20662 29557 27062
28	3812 87592 05521 86796
29	101 83467 14644 85146
30	2 19227 16067 49400
31	3760 42303 55032
32	50 54173 40736
33	51934 22157
34	392 96775
35	2 05905
36	666
37	1
	52 86836 62085 50447 90194 55756 24941

a	$n = 38$
1	1
2	13 74389 53471
3	225 14181 55065 45210
4	31 48019 18002 88707 87185
5	30285 00874 15880 12624 98095
6	51 26911 65003 25431 99663 25169
7	2526 65106 16849 27522 89841 46800
8	48958 54542 50170 13671 99436 93260
9	4 52635 63284 09771 59963 16497 41980
10	22 78193 76959 74471 05824 20414 01558
11	68 57799 61226 78581 79523 26590 40358
12	132 33980 83982 48276 81887 13891 60741
13	172 59713 16741 72062 13236 35123 09613
14	158 50719 33266 37293 55630 53740 85303
15	105 89386 99449 28989 39720 66451 51200
16	52 82807 07157 49087 75929 23255 53676
17	20 10347 80049 87290 97994 70247 70393
18	5 93826 39465 99161 51204 81539 94931
19	1 38119 25905 20902 90284 47222 27750
20	25596 03363 77653 43941 22372 89075
21	3815 91000 58748 87844 44920 01745
22	461 21754 77019 18275 35378 09095
23	45 47280 16672 57440 15377 47200
24	3 67393 03224 32422 32195 07200
25	24401 10155 55202 17516 88500
26	1334 53538 54296 74075 22656
27	60 11613 54076 30709 74020
28	2 22736 73239 84169 57350
29	6766 08139 30222 56030
30	167 60281 96669 67146
31	3 35800 27477 55392
32	5377 75852 58584
33	67 68002 71917
34	65295 12507
35	465 03450
36	2 29881
37	703
38	1
	746 28989 20956 25330 52309 95406 39146

a	$n = 39$
1	1
2	27 48779 06943
3	675 42558 39585 89101
4	125 92301 86193 09896 93950
5	1 51456 52389 97403 51832 77660
6	307 91754 90893 68472 10604 49109
7	17737 82654 82948 18092 28553 52769
8	3 94195 01446 18210 36898 85336 92880
9	41 22679 24099 38114 53340 47913 71080
10	232 34573 32881 54482 18205 20637 57560
11	777 13989 50454 38870 80580 12908 45496
12	1656 65569 69016 57903 62168 93289 69250
13	2376 10252 01624 85084 53959 70491 85710
14	2391 69783 82470 94171 92063 87495 03855
15	1746 91524 25005 72134 51440 50513 53303
16	951 14300 13969 14393 54588 38540 10016
17	394 58719 68005 33034 41839 17466 50357
18	126 99222 90437 72198 19681 37966 79151
19	32 18092 31664 96316 66609 78763 22181
20	6 50039 93180 73971 69108 94680 09250
21	1 05730 14376 11379 88674 65693 25720
22	13962 69605 53170 89902 23238 01835
23	1507 09198 60488 39398 89059 94695
24	133 64712 94056 35575 88059 20000
25	9 77420 57113 12476 70117 19700
26	59099 02157 66917 43472 77556
27	2957 67104 14357 03238 21196
28	122 48242 04791 87457 79820
29	4 18953 09279 60623 82220
30	11794 16598 30312 70410
31	271 70090 48473 84298
32	5 07888 54760 30080
33	7611 19942 31845
34	89 88036 97165
35	81571 33257
36	647 79166
37	2 55892
38	741
39	1
	10738 82333 07746 92832 76885 79864 25209

a	$n = 40$
1	1
2	54 97558 13887
3	2026 27702 67536 74246
4	503 69882 87330 79173 64901
5	7 57408 54251 73210 69060 82250
6	1849 01985 97752 08236 15459 72314
7	1 24472 70338 71530 95118 10479 18492
8	31 71297 94224 28631 13283 11248 95809
9	374 98308 18340 61241 16963 16560 32600
10	2364 68412 52914 82936 35392 54289 46680
11	8780 88457 87879 82061 04586 62630 58016
12	20657 00825 78653 33714 26607 32384 76496
13	32545 98845 90139 64002 63645 09683 83480
14	35859 87225 56218 03491 42853 95422 39680
15	28595 42647 57556 76189 63671 45198 03400
16	16965 20326 48512 02431 24854 67155 13559
17	7659 12534 70059 75978 65854 35470 66085
18	2680 44731 95884 32601 96104 00868 75075
19	738 42976 92072 02214 85267 34468 00590
20	162 18890 95279 75750 48788 72365 07181
21	28 70372 95079 12949 31276 74238 49370
22	4 12909 45697 81139 66523 76929 66090
23	48625 81173 44403 96076 71616 79820
24	4714 62309 17840 93220 02480 74695
25	378 00227 21884 47493 40989 12500
26	25 13995 13212 52330 00409 36156
27	1 38956 13969 54557 30904 49848
28	6387 17881 48529 52056 56156
29	243 97881 73900 45548 64200
30	7 72778 07228 70004 94520
31	20216 89403 33001 83648
32	434 22524 00803 46858
33	7 59058 12856 80965
34	10667 13199 35115
35	118 43033 61150
36	1 01291 83233
37	642 47170
38	2 84050
39	780
40	1
	1 57450 58839 12049 31289 32434 47025 31067

a	$n = 41$
1	1
2	109 95116 27775
3	6078 83163 00168 36625
4	2014 81557 77025 84231 33850
5	37 87546 41141 53384 24477 76151
6	11101 69324 40764 22627 61819 16134
7	8 73157 94356 98468 74062 88814 01758
8	254 94856 24133 00580 01383 00470 84964
9	3406 56071 59289 79801 65951 60291 89209
10	24021 82433 47488 90804 70888 59454 99400
11	98954 41449 19592 85607 85845 43225 84856
12	2 56664 98367 31719 86632 23874 51247 75968
13	4 43754 85822 50468 65748 53993 58274 61736
14	5 34584 20003 77192 12882 63600 45597 39000
15	4 64791 26939 19569 46335 97925 73392 90680
16	3 00038 67871 33749 15089 61346 19680 20344
17	1 47170 33416 39527 94068 44378 70156 37004
18	55907 17709 95977 62813 96726 51108 17435
19	16710 61293 45252 74684 16183 55760 86285
20	3982 20795 97667 17224 61041 81769 44210
21	764 96722 91941 47686 05600 31373 43951
22	119 54381 00430 98021 94799 66691 03350
23	15 31303 12687 02430 76288 24116 01950
24	1 61776 76593 72588 33357 31154 72500
25	14164 67989 64952 80555 27208 87195
26	1031 64100 65410 08073 51632 52556
27	62 65810 90390 25377 34830 82052
28	3 17797 14651 13383 88488 22216
29	13462 56451 91642 72967 17956
30	475 81223 90761 45696 99800
31	13 99501 78731 93061 87608
32	34112 10171 58712 83104
33	684 71442 25078 18703
34	11 21740 61634 74875
35	14812 19375 75365
36	154 89539 57538
37	1 25063 28523
38	750 41070
39	3 14470
40	820
41	1
	23 51152 50774 06176 28200 69407 72437 88988

a	$n = 42$
1	1
2	219 90232 55551
3	18236 49598 95621 37650
4	8059 32309 91266 37093 72025
5	189 39746 87265 43947 06620 14605
6	66648 03492 85726 89149 95392 72955
7	61 23207 29823 30045 41067 83517 28440
8	2048 32007 87421 03108 85126 92580 81470
9	30913 99500 57741 18794 94947 43097 87845
10	2 43624 80406 34178 85848 74837 54841 83209
11	11 12520 38374 63010 32291 15188 34939 32816
12	31 78934 21857 00231 25194 72339 58198 96472
13	60 25478 14059 87812 41363 25791 08817 78536
14	79 27933 65875 31158 46105 44399 96638 07736
15	75 06453 24091 70734 07922 32486 46490 99200
16	52 65410 12880 59555 87769 79464 88276 16184
17	28 01934 35950 05724 14253 15784 12338 49412
18	11 53499 52195 67125 24719 67455 90103 50834
19	3 73408 82285 55779 81813 03214 10564 56850
20	96354 77212 98596 19176 37019 91149 70485
21	20046 51977 28438 18631 78648 40611 67181
22	3394 93105 01423 04168 91192 98576 17651
23	471 74352 92232 53929 49429 21359 48200
24	54 13945 50936 44502 76863 71829 41950
25	5 15893 76334 96406 47239 11376 52375
26	40987 34606 65614 90466 69654 53651
27	2723 40995 05946 93261 92064 67960
28	151 64131 00622 00126 12501 04100
29	7 08211 51756 71023 04536 42940
30	27736 93169 14486 43877 11956
31	909 65779 31451 30615 15648
32	24 91089 04222 71872 46936
33	56707 67765 86293 00303
34	1066 10623 20659 64453
35	16 40167 39786 12650
36	20388 42800 46732
37	201 16881 12889
38	1 53578 89183
39	873 05400
40	3 47270
41	861
42	1
	357 42549 19887 26172 91353 50865 66266 42567

a	$n = 43$
1	1
2	439 80465 11103
3	54709 49016 77096 68501
4	32237 47476 14664 43996 25750
5	947 06793 68637 11001 70194 45050
6	4 00077 60704 01626 78846 78976 52335
7	429 29099 12255 96044 76624 80013 72035
8	16447 79270 29191 54916 22083 24163 80200
9	2 80274 27513 07091 72263 39653 80461 72075
10	24 67162 03563 99529 77282 43322 91516 19935
11	124 81349 02527 27292 41051 41909 39174 44185
12	392 59731 00658 65785 34627 83263 33326 90480
13	815 10150 04635 41792 62917 07623 72830 17440
14	1170 16549 36314 24030 86839 47390 61750 86840
15	1205 24732 27250 92169 64940 31696 94002 95736
16	917 53015 30181 23628 12239 03924 58909 58144
17	528 98294 24031 56866 30073 47794 98030 56188
18	235 64925 75472 13978 59207 29990 34201 64424
19	82 48267 15621 26941 79167 28523 90830 30984
20	23 00504 26545 27703 65340 43612 33558 66550
21	5 17331 68735 95798 10443 88636 43994 81286
22	94735 00287 59745 10347 84894 09287 55503
23	14245 03222 22771 44547 28064 89844 26251
24	1771 09045 14707 21995 94158 45265 55000
25	183 11289 59310 54664 57841 56242 51325
26	15 81564 76108 02393 99373 22394 47301
27	1 14519 41473 26182 08538 55400 88571
28	6969 36663 23362 96793 42093 82760
29	357 02285 01566 59794 44057 49360
30	15 40319 46831 05616 20850 01620
31	55936 32327 89476 92946 97044
32	1706 80628 66578 30534 17600
33	43 62442 40496 19541 56935
34	92955 28954 88720 91705
35	1640 16482 13174 07203
36	23 74150 80602 95038
37	27831 67402 23626
38	259 52879 01843
39	1 87627 99783
40	1011 96200
41	3 82571
42	903
43	1
	5529 50118 79716 54843 21714 69328 07377 67385

a	$n = 44$
1	1
2	879 60930 22207
3	1 64128 47490 11755 16606
4	1 28950 44614 07674 53081 71501
5	4735 66205 90661 69672 94968 51000
6	24 01412 71017 78397 84082 44053 59060
7	3009 03771 46495 73940 15220 39072 56580
8	1 32011 63261 45788 35374 53290 73324 13635
9	25 38916 26887 93017 05286 78967 48319 28875
10	249 51894 63153 02389 45087 72882 95623 71425
11	1397 62001 31363 99746 28848 04326 22435 05970
12	4835 98121 10431 16716 56585 41069 39097 29945
13	10988 91681 60919 09089 52549 82371 80119 17200
14	17197 41841 13034 78224 78669 71092 37342 33200
15	19248 87533 45078 06575 60944 22844 71795 22880
16	15885 72977 10150 70219 60764 94490 36556 26040
17	9910 24017 38717 90355 23488 16439 25429 13340
18	4770 66957 82530 08480 95804 87621 13660 15820
19	1802 82001 72276 25872 63385 71944 59977 53120
20	542 58352 46526 81014 85976 00770 62003 61984
21	131 64469 70000 39463 84662 04977 57449 73556
22	26 01501 75063 10190 38096 56306 48321 02352
23	4 22370 74398 83488 34935 30386 75705 59276
24	56751 20305 75744 72449 87867 76217 46251
25	6348 91284 97470 88610 40197 51328 38125
26	594 31973 38119 16908 41545 38498 81151
27	46 73588 95886 09310 29914 18218 38718
28	3 09661 68043 80345 18754 34028 05851
29	17323 02348 68794 30832 19761 14200
30	819 11849 06498 28280 69557 97960
31	32 74345 48995 79401 02206 09984
32	1 10554 12445 19982 70040 60244
33	3146 41228 02952 75405 96455
34	75 22922 24962 36052 74905
35	1 50361 05829 49813 43810
36	2494 85911 14880 28571
37	34 03922 74485 69200
38	37693 76804 93660
39	332 70370 93380
40	2 28106 47783
41	1168 81611
42	4 20497
43	946
44	1
	87019 63427 38705 50890 23600 53185 57971 48876

a	$n = 45$
1	1
2	1759 21860 44415
3	4 92385 43349 96195 72025
4	5 15803 42584 78188 24082 02610
5	23679 59979 97922 56039 27924 26501
6	144 13211 92312 61048 74167 59290 05360
7	21087 27812 96487 95978 90625 17561 55120
8	10 59102 09863 12802 56936 41546 25665 65060
9	229 82258 05252 82941 82955 63998 08197 73510
10	2520 57862 58418 16911 56164 07797 04556 43125
11	15623 33909 08156 99598 62416 20471 42409 37095
12	59429 39454 56538 00345 07872 97158 91602 65310
13	1 47691 89982 02379 34880 39733 11902 80646 53545
14	2 51752 77457 43406 04236 53925 77665 02911 82000
15	3 05930 54842 89205 76858 92833 13763 14270 76400
16	2 73420 55167 07489 30089 33183 34690 56695 39520
17	1 84359 81272 68355 06258 60063 73957, 68851 52820
18	95782 29258 24259 43012 47975 93619 71311 98100
19	39024 24990 55779 00061 00133 54568 53233 25100
20	12654 49051 02812 46169 82905 87357 00049 92800
21	3307 12216 16535 09755 63879 05299 68448 06660
22	703 97508 21388 63852 22786 43720 20512 25300
23	123 16028 86236 30422 41608 55201 89549 65700
24	17 84399 61737 01361 73732 39213 04924 69300
25	2 15474 02430 12516 87709 92805 59426 99376
26	21801 22592 88589 28229 20377 52297 48051
27	1856 18875 27043 68286 49228 30395 26537
28	133 44116 01112 58975 55035 71004 02546
29	8 12029 36155 75380 12888 07101 17651
30	41896 57820 63742 79253 06500 53000
31	1834 16559 25367 89712 37947 07464
32	68 12077 47242 18847 43505 37792
33	2 14385 72970 17423 58437 43259
34	5704 20584 51673 01199 43225
35	127 85559 28994 79523 08255
36	2 40175 98630 85503 72366
37	3754 31052 70850 88971
38	48 36285 93073 28280
39	50669 21271 35480
40	423 94630 04700
41	2 76027 03834
42	1345 42485
43	4 61175
44	990
45	1
	13 92585 05266 26366 96023 47053 99365 40796 93415

a	$n = 46$
1	1
2	3518 43720 88831
3	14 77156 31809 10447 60490
4	20 63218 62724 56102 92523 82465
5	1 18403 15703 32197 58384 63703 36115
6	885 02051 13855 64215 01044 83664 58661
7	1 47755 07902 67728 32901 08543 82220 91200
8	84 93904 06717 98908 51470 22995 22886 80400
9	2078 99424 57138 59279 03537 17528 99445 27250
10	25435 60883 89434 52057 44596 41968 53762 04760
11	1 74377 30862 48145 12496 42742 32982 71059 51170
12	7 28776 07363 86613 03739 56891 86378 41641 20815
13	19 79424 09220 87469 53790 24403 51895 40007 61395
14	36 72230 74386 10063 94191 94693 99213 21412 01545
15	48 40711 00100 81492 57120 46422 84112 16973 28000
16	46 80659 37516 09034 58288 23766 68812 21397 08720
17	34 07537 36802 69525 36485 54266 91971 27171 37460
18	19 08441 07921 05024 80483 23630 59112 52467 18620
19	8 37243 04078 84060 44171 50513 30421 82743 75000
20	2 92114 06011 12028 23457 58251 01708 54231 81100
21	82104 05590 50049 51038 24365 98850 37459 32660
22	18794 57396 87085 10104 65180 67144 19717 63260
23	3536 66172 04823 63367 79783 13363 80154 36400
24	551 41619 67924 63104 11185 96315 07742 28900
25	71 71250 22490 14283 66480 59352 90599 53700
26	7 82305 89845 15318 21669 22621 19161 48702
27	71918 32225 18748 71964 49541 72969 64550
28	5592 54123 58196 19601 90228 18507 97825
29	368 92967 49629 44999 28789 76938 14425
30	20 68926 70774 87663 90480 02117 07651
31	98755 71157 50147 60336 82859 84384
32	4014 03038 37117 92830 30119 16808
33	138 86806 55257 93825 71940 65339
34	4 08328 72843 74305 99218 12909
35	10179 15159 66490 84507 32150
36	214 31894 79705 57657 13431
37	3 79085 47581 06986 64293
38	5592 09918 07635 63611
39	68 12385 22656 12000
40	67627 06473 23480
41	537 11775 51894
42	3 32535 78204
43	1543 73010
44	5 04735
45	1035
46	1
	226 54182 19334 49400 29284 84444 70539 22761 58355

a	$n = 47$
1	1
2	7036 87441 77663
3	44 31468 98945 75063 70301
4	82 52889 28054 56220 80542 90350
5	5 92036 41735 23712 48026 11040 58040
6	5191 36109 98837 17487 64653 65690 87081
7	10 35150 58269 87953 94522 60851 59210 97061
8	680 98987 61646 58996 44662 92505 65315 34400
9	18795 88725 20965 32419 83304 80756 17894 25650
10	2 56435 08263 51483 79853 49501 37214 37065 74850
11	19 43586 00371 19030 89518 14762 04778 35416 67630
12	89 19690 19228 87501 57371 25444 69523 70754 00950
13	264 61289 27235 23717 03012 74137 61018 61740 18950
14	533 90654 50626 28364 72477 50119 40880 39775 83025
15	762 82895 75898 32452 50998 91036 60895 76011 21545
16	797 31261 00358 26045 89732 26689 85107 59326 67520
17	626 08794 63161 90965 78542 46304 32323 83310 45540
18	377 59476 79381 59971 85183 79617 55996 71580 72620
19	178 16058 85419 02173 19741 83383 37127 24598 43620
20	66 79524 24301 24625 13323 15533 64592 67379 97000
21	20 16299 23411 63067 95260 69936 73366 40877 66960
22	4 95584 68321 65921 73340 58340 75822 71247 24380
23	1 00137 79353 98028 67564 00192 74511 63268 00460
24	16770 65044 35014 77866 48246 24925 65969 30000
25	2344 22875 30178 20195 73200 80137 72730 71400
26	275 11203 58464 12557 29880 47503 88798 19952
27	27 24100 59925 21533 64710 60247 89341 91552
28	2 28509 47685 48242 20817 75930 91193 03650
29	16291 50180 97450 24581 25131 49714 16150
30	989 60768 72875 74916 43190 40450 43955
31	51 30353 76657 42239 60921 70772 23555
32	2 27204 68385 37921 30906 46673 22240
33	8596 67654 60629 89079 04160 72995
34	277 69983 31945 20229 45357 04245
35	7 64599 03432 01485 56974 38159
36	17894 63372 35891 60164 15666
37	354 58057 40205 16162 92272
38	5 91585 24467 97140 81511
39	8248 92941 91224 31611
40	95 17467 81585 51200
41	89648 89269 51134
42	676 78278 36462
43	3 98916 17634
44	1765 81350
45	5 51310
46	1081
47	1
	3745 00595 02461 51119 65053 42096 43151 01201 74682

a	$n = 48$
1	1
2	14073 74883 55327
3	132 94407 03874 12632 88566
4	330 11601 43687 23828 97235 31701
5	29 60264 61565 46616 96351 35745 80550
6	31154 08696 34758 28638 35948 05185 80526
7	72 51245 43999 14514 79145 90614 80167 66508
8	5458 27051 51442 59925 51826 00896 81733 72261
9	1 69843 97514 50334 50774 94406 19311 26363 65250
10	25 83146 71360 35803 30954 78318 52899 88551 74150
11	216 35881 12346 60823 64553 11883 89776 26649 18780
12	1089 79868 31117 69049 77973 20098 39062 84464 79030
13	3529 16450 73286 95822 96536 89233 62765 73376 47300
14	7739 30452 36003 20823 17697 75809 33344 18601 81300
15	11976 34090 89101 15152 37461 15668 54316 79944 06200
16	13519 83071 81630 49186 86715 18074 22617 25238 01865
17	11440 80769 74110 72464 24954 13863 34612 75604 41700
18	7422 79376 92030 70459 11850 79420 40264 71763 52700
19	3762 64595 02343 01262 60278 63901 61414 38951 01400
20	1514 06543 71443 94675 86204 94056 28980 72197 83620
21	490 21808 15945 49052 13797 84205 05287 25811 03160
22	129 19162 26488 13346 08753 53433 41466 08317 03320
23	27 98753 93463 20581 27312 62773 89590 26411 34960
24	5 02633 40418 38383 36359 58102 72727 46531 20460
25	75376 36926 89469 82759 78266 28368 84237 15000
26	9497 14168 50245 46685 50093 15238 81483 90152
27	1010 61919 76444 93965 77066 74197 01029 91856
28	91 22365 95118 72315 47607 86313 42746 93752
29	7 00963 02933 74299 33674 04744 32903 72000
30	45979 73242 83722 72074 20843 63227 34800
31	2580 01735 49255 84344 31763 34389 74160
32	124 00903 64989 55721 49928 64315 35235
33	5 10895 00987 38707 70514 83977 31075
34	18038 47087 46766 76880 46300 17325
35	545 30949 52065 72224 39480 39810
36	14 08805 84836 93583 22884 02135
37	31014 11496 23482 58192 29730
38	579 38296 69988 07513 89690
39	9 13293 49202 54889 14340
40	12055 91654 54644 79611
41	131 93072 41635 47694
42	1 18073 76960 82538
43	848 31673 94724
44	4 76611 97034
45	2013 90300
46	6 01036
47	1128
48	1
	62891 97963 03118 41542 02104 54071 84953 77460 15761

a	$n = 49$
1	1
2	28147 49767 10655
3	398 83221 25696 12782 21025
4	1320 46538 69155 99190 01574 15370
5	148 01653 19428 76772 05585 75964 34451
6	1 86954 12442 70115 18447 12039 66860 63706
7	507 89872 16690 36301 82659 70251 66359 46082
8	43738 67657 55539 93918 93753 97789 34037 44596
9	15 34054 04682 04453 16900 01481 74698 19006 59511
10	260 01311 11118 08367 60322 77591 48310 11881 06750
11	2405 77839 07173 04863 41039 09041 40438 81692 80730
12	13293 94300 85758 89421 00231 53064 58530 40226 67140
13	46968 93727 83848 14748 32952 80135 55017 38358 93930
14	1 11879 42783 77331 87347 44305 50564 29584 33801 85500
15	1 87384 41815 72520 48108 79615 10837 48096 17762 74300
16	2 28293 63239 95189 02142 24904 04856 16192 83752 36040
17	2 08013 56157 41512 81079 10935 53751 11034 10513 10765
18	1 45051 09554 30663 40728 38268 43430 59377 67347 90300
19	78913 06682 36547 94448 57144 93551 07138 11832 79300
20	34043 95469 31221 94779 84377 45027 41028 82907 73800
21	11808 64515 06299 24770 75959 62362 40013 14229 49980
22	3332 43377 98684 42666 06375 59740 17541 08785 76200
23	772 90502 76141 86715 36943 97233 02042 15778 07400
24	148 61955 63504 41781 09942 57239 35049 43160 26000
25	23 87042 63590 75129 05354 14759 81948 52459 95460
26	3 22302 05307 95851 96582 80688 24578 02818 58952
27	36783 86002 14258 83761 30895 18558 09291 70264
28	3564 88166 39769 18799 10086 90972 97944 16912
29	294 50293 80197 26996 24155 23898 96954 81752
30	20 80355 00218 85980 95900 30053 29724 16000
31	1 25960 27043 10653 86748 05507 29309 33760
32	6548 30652 28921 67432 29479 92481 01680
33	292 60438 97573 33075 76918 35566 60710
34	11 24203 01961 28777 84450 58183 20125
35	37124 30320 69067 04734 27414 10675
36	1052 47960 06195 41220 63285 16070
37	25 56328 10197 62438 75999 02145
38	53030 66770 83029 43720 37950
39	935 56742 88887 48190 48950
40	13 95530 15384 40680 98780
41	17465 07623 61699 35065
42	181 52170 73990 14290
43	1 54551 38940 55670
44	1058 02600 64220
45	5 67237 60534
46	2290 37956
47	6 54052
48	1176
49	1
	10 72613 71545 73358 40034 22155 18590 00263 39172 47281

a	$n = 50$
1	1
2	56294 99534 21311
3	1196 49664 05235 88113 73730
4	5281 86553 59845 22456 19078 82505
5	740 09586 43682 53016 27118 81395 87625
6	11 21872 76309 40119 87454 77823 77128 16687
7	3557 16059 29275 24647 97065 03801 31376 86280
8	3 50417 31132 61009 87713 32691 52566 38659 02850
9	138 50225 09795 95618 46019 07089 70073 05096 80195
10	2615 47165 15862 88129 20127 77396 57799 37817 27011
11	26723 57540 90021 61865 11752 77046 93137 10501 94780
12	1 61933 09449 36279 77915 43817 45816 42803 64412 86410
13	6 23890 12762 75784 81149 28617 94826 73756 38892 88230
14	16 13280 92700 66494 37612 53229 88035 69198 11584 90930
15	29 22645 70019 65139 08979 38532 13126 51027 00243 00000
16	38 40082 53654 95544 82384 78079 88536 07181 57800 50940
17	37 64524 17916 00906 80487 10808 18625 03772 62475 19045
18	28 18933 28134 93454 14189 99767 35501 79832 22775 36165
19	16 44399 36519 25074 35251 24022 20900 95001 92170 97000
20	7 59792 16068 60986 90045 44693 94099 27714 69987 55300
21	2 82025 50285 63506 14965 79529 54637 81304 81727 23380
22	85122 18830 77356 63424 16222 76646 25917 07516 26380
23	21109 24941 49947 37119 56086 96099 64510 71681 46400
24	4339 77438 00247 89483 35565 70977 43228 51624 31400
25	745 38021 53273 20008 33796 26234 83762 54659 12500
26	107 66896 01597 67280 16507 12654 20977 25743 28212
27	13 15466 27365 80840 58138 14858 25646 53694 56080
28	1 36600 54661 27796 10136 13328 65801 51728 43800
29	12105 46686 65490 01690 10588 84043 09633 87720
30	918 60943 86763 06425 01164 25497 88679 61752
31	59 85123 38555 16250 85090 00779 38313 62560
32	3 35506 07916 36147 44581 48864 88701 87520
33	16204 25138 48841 58932 67785 66179 05110
34	674 83341 64257 11522 48238 13795 44960
35	24 23553 63185 46124 50150 17676 93750
36	75013 56882 92101 88677 05680 10795
37	1998 32099 83507 51454 75248 96035
38	45 71493 47489 17557 37373 44245
39	89517 79743 49641 23149 47000
40	1493 77949 04263 75430 00150
41	21 11598 27952 70354 36445
42	25088 98794 69285 35245
43	247 97880 48434 08100
44	2 01104 53368 81350
45	1313 28292 88250
46	6 72595 06510
47	2597 78400
48	7 10500
49	1225
50	1
	185 72426 87710 78270 43825 77671 81908 91749 92218 52770

r	$U(50) = \sum_{r=0}^{24} \binom{49}{r} \{U(r) + U(49-r)\}$
0	10 72613 71545 73358 40034 22155 18590 00263 39172 47282
1	30 81707 00188 52802 35559 03122 49520 62734 95547 72338
2	44 04126 99748 94737 16709 02823 05403 45590 13254 28384
3	41 73806 52730 18717 50995 43974 09252 14729 59416 24640
4	29 50553 50617 94881 26066 68844 11959 45178 91251 74680
5	16 59363 48282 69537 15637 76794 76587 30989 05364 20352
6	7 73235 27184 37703 45430 57430 75334 27328 62013 65808
7	3 07030 58498 31889 96693 57645 44053 28414 17993 71296
8	1 06031 82108 11913 76761 14560 74813 06043 53695 30448
9	32347 52483 96925 96713 59352 84027 51645 02314 73676
10	8824 97443 77762 85309 96056 00440 78172 65673 22624
11	2174 38398 04767 73511 08383 51553 56978 15957 33024
12	487 78329 21078 24120 11541 39506 72513 77259 09768
13	100 30448 02966 35527 80866 21813 72927 41902 58056
14	19 01502 19594 86677 27515 28147 02329 01845 02112
15	3 33944 95051 24953 29680 08297 78221 61148 48896
16	54560 64663 77891 61044 08438 04590 47501 09354
17	8323 26919 10537 17074 24727 82014 62717 80232
18	1189 32129 94993 53592 36321 77711 20063 36192
19	159 62645 60902 63682 63307 55870 08526 27136
20	20 17313 20167 38003 16289 47418 92406 04544
21	2 40568 58154 25822 75189 16469 88425 99344
22	27122 30481 03041 57460 35324 17950 37312
23	2898 22950 14528 31743 53654 54149 62880
24	321 37047 40136 69130 44635 96668 22392

$U(50) = 185\ 72426\ 87710\ 78270\ 43825\ 77671\ 81908\ 91749\ 92218\ 52770$

A TABLE OF VALUES OF LIOUVILLE'S FUNCTION $L(t)$

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1. If p denotes a prime ≥ 2, then for positive integral values of t, Liouville's Functions $\lambda(t)$ and $L(t)$ are defined by the relations

$$\lambda(0) = 0, \quad \lambda(1) = 1, \quad \lambda(pt) = -\lambda(t)$$

and
$$L(t) = \lambda(1)+\lambda(2)+\lambda(3)+\ldots+\lambda(t).$$

Thus
$$\lambda(475) = -\lambda(95) = \lambda(19) = -\lambda(1) = -1.$$

In 1919, Polya[1] conjectured that for values of $t \geq 2$,

$$L(t) \leq 0.$$

He verified this conjecture for values of t up to 1500.

In 1940, at the suggestion of Dr. Chowla, I computed a table giving the values of $\lambda(t)$ and $L(t)$ for values of t up to 20000, and found Polya's conjecture to be true to that extent. The following is a specimen of this table:

t		$\lambda(t)$	$-L(t)$
15801	3.5267	−1	145
15802	2.7901	1	144
15803	p	−1	145
15804	2.7902	−1	146
15805	5.3161	−1	147
15806	2.7903	−1	148
15807	3.5269	−1	149
15808	2.7904	1	148
15809	p	−1	149
15810	2.7905	−1	150

The above table is here presented in a condensed form. It gives the value of $-L(t)$ when t is a multiple of 5 and of $\lambda(t)$ when t is not a multiple of 5. The value of $-L(t)$ when t is not a multiple of 5 and of $\lambda(t)$ when t is a multiple of 5 are readily found with the help of the two fundamental relations:

(1) $$L(t) = L(t-1)+\lambda(t), \quad \text{and}$$

(2) $$\lambda(5t) = -\lambda(t);$$

or by making use of the subsidiary tables of section 3.

2. To find the value of $-L(5t)$, we write

$$t = 5m+n, \text{ where } 0 \leq n \leq 4.$$

The value of $-L(5t)$ is then found directly from the table.

Thus, when $t = 1183$, we have $m = 236$ and $n = 3$.
Hence, looking up the entry in front of 236 (in column one) and under 3 (in the top row), we obtain

$$-L(5915) = 53.$$

To find the value of $\lambda(5t+k)$, $k = 1, 2, 3, 4$;
we again write t in the form $5m+n$. Looking up the entry in the same row as m and in the same column as Δ_n, we get a number r between 0 and 15. We write r as a four-figure number in the scale of 2. Thus, if $r = 3$, we write it as 0011. Changing each zero into -1 in this representation of r, we get in order the values of

$$\lambda(5t+1), \lambda(5t+2), \lambda(5t+3) \text{ and } \lambda(5t+4).$$

Thus, to find the value of $\lambda(5918)$ say, we look up the entry in front of 236 and in the column Δ_3. This is found to be 5. Now, 5 is 0101 in the scale of 2. This gives the scheme:

$$-1, 1, -1, 1.$$

Hence, $\lambda(5916) = -1, \lambda(5917) = 1, \lambda(5918) = -1, \lambda(5919) = 1.$

The value of $-L(5918)$ is now readily found.

In fact, $-L(5918) = -L(5915) - \lambda(5916) - \lambda(5917) - \lambda(5918)$,

$$= 53 + 1 - 1 + 1 = 54.$$

Lastly, to find $\lambda(t)$ when t is a multiple of 5, we write

$$t = 5^{\alpha} . t_1, \text{ where } (t_1, 5) = 1.$$

Then

$$\lambda(t) = (-1)^{\alpha} . \lambda(t_1),$$

where $\lambda(t_1)$ can be found from the table as stated above.

3. For ready reference, we give below the values of $\lambda(5t+k)$ corresponding to the numbers 0 to 15 in the Δ—columns, as also the additives for the values of $-L(5t+k)$.

	$\lambda(5t+k)$				$-L(5t+k) = -L(5t) + \ldots$			
k	1	2	3	4	1	2	3	4
0	−1	−1	−1	−1	1	2	3	4
1	−1	−1	−1	1	1	2	3	2
2	−1	−1	1	−1	1	2	1	2
3	−1	−1	1	1	1	2	1	0
4	−1	1	−1	−1	1	0	1	2
5	−1	1	−1	1	1	0	1	0
6	−1	1	1	−1	1	0	−1	0
7	−1	1	1	1	1	0	−1	−2
8	1	−1	−1	−1	−1	0	1	2
9	1	−1	−1	1	−1	0	1	0
10	1	−1	1	−1	−1	0	−1	0
11	1	−1	1	1	−1	0	−1	−2
12	1	1	−1	−1	−1	−2	−1	0
13	1	1	−1	1	−1	−2	−1	−2
14	1	1	1	−1	−1	−2	−3	−2
15	1	1	1	1	−1	−2	−3	−4

4. Let $t(h)$ be the least value of t for which

$$-L(t) = h.$$

Then the following table gives the values of $t(h)$ for values of h from 0 to 150.

$h\rightarrow$ $\downarrow$	0	1	2	3	4	5	6	7	8	9
	0	3	8	13	20	31	32	53	76	79
1	80	117	176	181	182	193	200	283	284	285
2	286	293	440	443	468	661	678	683	684	1075
3	1076	1087	1088	1091	1092	1093	1106	1109	1128	1129
4	1130	1131	1132	1637	1638	1753	1756	1759	1760	2699
5	2700	2703	2712	2713	2714	2715	2720	2731	2732	2739
6	2742	2769	2770	2801	2802	2803	2804	4157	4256	4261
7	4364	4373	4526	4527	4528	6317	6318	6381	6390	6391
8	6392	6397	6398	6399	6480	6481	6482	6575	6582	6859
9	6864	6877	6878	6969	6972	6975	6976	6977	6978	6987
10	6988	6997	7026	7027	9686	9689	9690	9695	9696	9697
11	9698	9699	9700	9719	9720	9721	9724	9725	9726	9789
12	9792	9795	9816	9817	9822	9823	9836	9837	9840	15669
13	15670	15671	15672	15675	15676	15679	15680	15745	15750	15753
14	15756	15791	15792	15795	15798	15799	15804	15805	15806	15807
15	15810	>20000								

Within the limits of the table, $\{L(t)\}^2/t$ is the greatest when $t = 9840$, its value being just less than 5/3 then. It thus appears that

$$\left|L(t)\right| = O(t^{\frac{1}{2}}).$$

5. The following table will show the main variations in the value of $L(t)$ as t increases from 2 to 20000.

t	$-L(t)$	t	$-L(t)$
2	0	3281	7
468	24	4528	74
586	0	5645	9
684	28	7027	103
880	6	8512	14
1132	42	9840	128
1411	3	12798	32
1760	48	15810	150
2264	8	19680	10
2804	66		

References.

1. Polya. (1919). *Jahr. deut. Math. Verein.*, **28**, 38–40.
2. H. Gupta (1943). A Formula for $L(n)$. *Jour. Indian Math. Soc.*, **7**, 68–71. (With the help of a result in this paper, stray values of $L(t)$ for values of t up to 60000 can be computed.)

m \ n	0	Δ_0	1	Δ_1	2	Δ_2	3	Δ_3	4	Δ_4
0	0	9	1	9	0	1	1	8	4	13
1	1	8	4	3	3	11	0	0	5	9
2	6	9	5	14	2	5	1	1	4	1
3	7	4	10	13	7	14	4	11	1	8
4	2	1	5	8	8	8	9	3	10	14
5	9	9	10	7	7	8	8	15	3	8
6	4	4	5	11	2	8	5	9	6	0
7	11	6	12	3	11	5	12	1	15	10
8	16	15	11	9	10	3	9	15	4	9
9	3	10	4	5	3	4	4	0	9	7
10	6	3	7	9	6	4	7	4	10	1
11	13	10	14	0	19	5	20	9	19	15
12	16	14	13	11	12	1	13	1	16	11
13	15	15	10	1	11	9	10	13	9	10
14	8	12	7	2	8	13	5	0	10	12
15	9	12	8	13	7	8	8	11	5	2
16	6	2	9	4	12	11	9	12	10	5
17	11	4	14	0	19	4	22	9	21	12
18	22	11	21	3	20	4	23	9	24	14
19	23	10	24	13	21	11	18	6	17	4
20	20	13	17	0	20	11	17	13	16	4
21	17	15	14	6	13	14	10	7	7	8
22	8	11	7	3	6	5	5	10	4	6
23	5	9	4	11	1	9	2	8	5	4
24	6	0	11	2	14	9	15	0	18	15
25	13	9	14	7	11	8	12	1	15	1
26	16	0	19	0	24	5	25	13	24	9
27	25	9	26	8	27	13	24	3	23	7
28	22	2	25	14	24	7	23	6	24	10
29	25	9	26	13	23	14	20	0	23	11
30	22	2	23	10	24	2	25	12	24	9
31	25	11	24	11	21	1	24	15	21	3
32	22	7	21	6	20	6	19	15	14	1
33	15	0	20	8	21	14	18	14	17	9
34	16	12	15	10	14	5	13	11	10	12
35	9	11	6	1	9	9	10	2	11	11
36	8	9	7	0	10	7	9	4	12	14
37	11	10	10	3	11	9	10	2	13	3
38	12	9	11	3	10	8	11	8	14	3
39	13	1	16	4	17	3	18	6	17	11
40	14	2	17	12	18	13	17	10	18	1
41	21	5	22	0	25	12	24	14	23	14
42	22	0	25	5	24	4	27	6	28	10
43	29	7	28	12	29	1	32	1	35	11
44	34	9	35	4	36	13	33	1	36	12
45	37	8	40	3	39	7	38	12	37	7
46	34	1	35	13	34	9	33	5	34	1
47	37	12	36	1	39	11	36	12	35	15
48	32	7	29	14	26	13	23	3	22	1
49	23	12	22	5	23	10	24	10	25	4

m \ n	0	Δ_0	1	Δ_1	2	Δ_2	3	Δ_3	4	Δ_4
50	28	3	27	12	26	14	25	5	26	11
51	23	10	24	5	23	12	22	7	21	9
52	22	5	21	10	22	7	19	14	16	4
53	19	9	18	2	21	13	18	3	17	13
54	14	9	13	12	12	3	11	11	10	8
55	11	9	12	7	9	5	10	14	7	4
56	8	15	3	2	4	8	5	6	4	4
57	5	0	8	12	9	6	8	9	9	3
58	8	1	11	12	10	8	11	9	12	2
59	15	6	16	5	17	10	18	1	21	4
60	22	13	21	7	20	3	21	6	20	13
61	19	7	18	0	21	14	20	9	21	8
62	22	1	23	0	26	11	23	1	26	5
63	27	12	26	0	29	1	30	15	27	0
64	30	6	31	0	34	4	37	6	38	6
65	37	2	40	10	41	1	44	15	41	13
66	40	13	37	10	36	9	35	10	36	10
67	37	3	38	10	37	7	34	9	35	8
68	38	10	39	14	38	11	35	6	36	4
69	37	12	38	9	37	1	40	0	43	10
70	44	9	45	4	48	15	43	9	42	1
71	45	9	44	13	41	2	44	3	43	5
72	44	3	45	13	44	5	43	15	40	12
73	41	5	40	4	41	15	36	10	35	3
74	34	11	33	6	34	5	33	0	36	1
75	39	8	42	15	39	2	40	15	35	4
76	38	3	39	9	40	13	37	5	38	11
77	35	15	32	1	33	15	28	15	23	0
78	28	7	27	13	24	14	23	15	20	13
79	19	4	20	14	17	3	18	9	17	0
80	22	5	21	2	22	0	27	11	24	8
81	25	8	26	11	25	2	26	13	23	13
82	20	12	21	9	20	5	21	10	22	10
83	23	14	22	0	27	2	28	0	31	2
84	32	15	27	10	28	4	29	13	28	6
85	27	14	24	6	25	2	26	1	27	7
86	24	4	25	5	24	0	27	14	24	15
87	19	12	18	15	15	9	14	9	13	1
88	14	9	15	9	14	4	15	15	12	2
89	15	13	14	13	13	8	14	8	15	11
90	12	3	13	14	10	7	9	0	14	12
91	13	5	12	2	13	10	14	12	15	0
92	20	5	19	12	20	0	23	13	20	11
93	17	13	16	12	15	8	16	5	17	8
94	18	2	21	11	20	15	17	9	16	1
95	17	0	22	0	25	10	26	8	27	0
96	30	14	29	4	32	2	33	3	34	0
97	39	13	38	7	35	0	40	7	39	9
98	40	2	41	10	42	14	41	9	40	9
99	41	3	40	11	39	7	36	12	35	15

m \ n	0	Δ_0	1	Δ_1	2	Δ_2	3	Δ_3	4	Δ_4
100	30	13	29	5	30	2	31	15	28	0
101	33	2	34	6	33	12	32	9	31	0
102	36	1	39	3	38	14	37	13	36	10
103	37	14	36	14	35	5	34	1	37	9
104	36	14	33	8	36	9	35	3	34	6
105	35	5	36	8	39	2	42	14	41	11
106	38	11	35	8	38	13	37	3	36	2
107	39	8	42	13	41	0	46	8	47	8
108	50	9	49	9	50	0	55	10	56	15
109	53	4	56	3	55	0	58	3	57	14
110	56	1	59	13	56	12	57	0	62	14
111	59	9	58	8	59	6	60	6	61	10
112	62	0	65	5	66	15	61	2	64	3
113	65	14	64	12	63	3	64	12	63	10
114	64	5	63	11	62	2	65	15	60	12
115	59	2	62	9	61	9	60	7	59	1
116	62	13	61	12	60	10	61	10	62	11
117	61	11	60	11	57	8	58	15	55	7
118	52	12	53	3	52	12	51	8	52	3
119	51	6	50	15	47	12	46	14	43	10
120	44	2	45	12	44	6	43	4	44	5
121	43	5	42	12	41	3	42	2	43	12
122	42	14	41	4	42	7	39	11	38	10
123	37	6	36	1	37	8	38	11	35	15
124	32	15	29	12	30	7	29	14	28	1
125	31	4	34	15	29	9	28	6	29	13
126	26	10	25	11	24	13	23	10	24	7
127	23	8	26	2	27	1	28	7	25	15
128	22	4	23	6	22	5	21	10	22	6
129	21	14	18	6	17	3	16	12	17	7
130	16	5	15	2	16	11	13	3	12	7
131	11	13	8	8	9	4	10	10	9	8
132	10	0	13	9	14	1	15	6	16	1
133	17	12	18	1	21	7	18	9	19	3
134	18	15	15	0	18	1	19	3	20	4
135	21	5	22	11	19	12	18	3	19	14
136	16	11	15	3	14	0	17	3	16	9
137	17	5	18	12	19	7	16	6	17	10
138	18	2	19	11	16	1	19	8	22	7
139	21	8	22	13	21	15	18	3	19	6
140	18	3	17	8	18	6	19	11	16	10
141	15	0	20	1	23	12	24	7	21	9
142	20	9	19	8	22	10	23	9	24	5
143	23	3	22	5	23	5	24	4	25	9
144	24	15	21	0	24	8	27	8	28	4
145	29	13	28	0	31	1	32	8	35	15
146	30	14	29	8	32	14	29	15	26	0
147	31	1	34	3	35	6	36	7	33	1
148	36	1	37	4	38	14	35	6	34	12
149	35	8	38	5	37	14	36	7	35	13

m \ n	0	Δ_0	1	Δ_1	2	Δ_2	3	Δ_3	4	Δ_4
150	32	3	31	10	30	2	33	0	36	7
151	35	6	36	8	37	4	40	12	39	11
152	36	5	35	1	36	9	37	12	36	0
153	41	13	40	12	41	13	38	11	35	1
154	38	0	43	1	44	5	43	13	42	2
155	43	0	48	2	49	0	54	15	51	11
156	48	14	47	1	48	0	53	8	56	8
157	59	6	58	2	59	4	60	8	63	9
158	62	6	63	15	60	10	61	3	62	7
159	59	5	58	11	55	8	58	15	55	14
160	52	4	53	9	54	5	55	2	58	7
161	55	10	54	11	53	5	54	3	53	10
162	54	1	55	11	54	11	53	1	54	5
163	55	6	56	2	59	12	60	0	65	12
164	66	10	65	11	62	1	63	13	62	12
165	63	8	64	9	63	2	64	11	61	2
166	62	8	65	2	66	7	63	11	60	13
167	59	2	62	15	59	15	56	7	53	6
168	54	0	59	4	62	3	63	2	64	14
169	61	14	60	0	63	13	60	4	63	7
170	62	0	67	6	68	6	67	7	64	5
171	65	3	66	13	63	12	64	12	63	1
172	66	6	65	15	62	6	61	13	60	13
173	59	11	58	10	57	2	60	10	61	0
174	66	10	67	11	66	0	69	5	68	1
175	71	13	70	9	69	13	68	7	67	9
176	68	9	67	8	68	11	65	12	66	4
177	67	15	64	0	67	5	66	7	65	3
178	64	2	65	9	64	8	67	13	64	11
179	63	11	62	1	63	15	60	1	63	5
180	64	13	63	1	64	8	65	2	68	4
181	71	1	72	15	67	14	64	8	65	10
182	66	6	65	13	64	5	65	3	66	11
183	63	7	60	0	65	11	62	7	59	11
184	56	12	57	13	56	2	59	15	54	5
185	53	15	50	10	49	8	52	9	51	4
186	54	2	55	9	54	8	57	14	56	9
187	55	14	54	12	53	12	52	1	55	11
188	54	13	51	3	50	11	49	5	48	0
189	51	4	52	1	53	13	52	15	49	13
190	48	5	49	14	46	15	41	11	40	9
191	41	6	40	9	39	10	40	13	39	14
192	38	2	41	2	42	12	41	11	38	13
193	37	7	36	7	33	5	32	15	27	15
194	22	2	23	13	20	6	21	4	24	7
195	23	10	22	14	19	4	20	9	21	12
196	20	5	19	6	20	9	19	2	20	0
197	25	6	24	1	25	9	26	1	29	8
198	30	5	29	1	32	3	33	0	36	12
199	37	5	38	8	39	11	38	0	43	4

m \ n	0	Δ_0	1	Δ_1	2	Δ_2	3	Δ_3	4	Δ_4
200	46	8	47	12	46	4	49	12	48	5
201	47	7	46	10	47	0	50	13	47	10
202	46	7	45	6	46	6	45	3	46	8
203	47	11	46	1	47	9	48	15	43	14
204	40	5	39	8	40	13	37	0	42	2
205	43	7	40	8	43	12	42	11	41	3
206	40	0	43	6	42	10	41	3	40	6
207	41	12	42	7	41	12	40	9	39	9
208	40	8	43	6	44	11	43	11	40	11
209	37	9	38	5	39	0	44	12	43	7
210	40	12	39	13	36	3	35	15	30	5
211	31	6	30	0	35	6	34	3	35	1
212	38	9	37	0	42	11	39	15	34	8
213	35	13	32	13	31	1	34	5	33	3
214	32	3	33	15	28	10	29	12	28	15
215	23	14	20	3	21	11	20	1	23	10
216	22	11	21	8	24	9	23	8	24	5
217	23	11	20	3	19	3	18	0	23	4
218	24	4	25	11	22	13	19	1	22	7
219	19	10	20	5	19	1	22	2	25	7
220	22	5	23	9	22	2	23	8	26	0
221	29	10	28	7	27	11	24	10	23	3
222	24	1	27	8	30	9	29	14	28	4
223	31	7	28	6	27	7	24	1	27	7
224	24	15	21	15	18	14	15	13	12	0
225	15	5	16	15	11	6	10	5	9	0
226	12	0	15	2	16	10	17	14	16	13
227	15	4	16	0	21	10	22	1	25	7
228	22	6	23	12	24	3	23	0	26	15
229	23	3	22	8	25	1	28	0	33	14
230	32	7	29	3	28	9	27	12	28	11
231	27	12	28	4	31	1	32	7	31	9
232	30	2	33	9	32	0	35	10	36	1
233	39	2	40	11	39	6	38	9	39	4
234	40	3	39	0	42	1	43	0	48	3
235	47	10	48	0	53	0	56	14	53	1
236	56	0	59	11	56	13	53	5	54	8
237	55	11	54	3	53	14	52	13	51	4
238	54	12	55	3	56	10	55	1	56	8
239	59	15	56	2	59	3	60	11	59	7
240	56	15	51	3	52	2	55	15	52	14
241	51	6	52	14	51	11	50	12	49	9
242	50	12	51	9	52	0	55	11	54	13
243	53	4	54	13	51	6	52	0	57	10
244	58	2	59	7	56	12	55	15	52	4
245	55	5	56	1	59	1	60	1	61	2
246	64	4	65	7	64	13	61	13	60	9
247	59	11	58	11	55	5	56	10	55	0
248	58	8	61	5	60	0	65	10	64	4
249	65	4	66	2	69	7	66	15	61	9

m \ n	0	Δ_0	1	Δ_1	2	Δ_2	3	Δ_3	4	Δ_4
250	60	4	61	11	58	8	61	4	64	3
251	65	9	66	14	65	3	64	8	65	12
252	66	1	69	2	72	3	73	1	74	8
253	75	12	74	11	73	3	72	12	73	5
254	72	1	75	10	74	7	73	2	76	13
255	75	12	76	6	75	4	78	2	79	0
256	82	14	79	14	78	12	77	6	78	6
257	79	9	80	11	79	7	76	6	75	2
258	78	2	79	7	78	6	79	6	80	5
259	79	0	84	2	85	15	80	6	81	5
260	80	6	79	13	78	13	75	6	76	3
261	77	4	80	7	77	5	78	12	77	0
262	80	0	83	13	82	9	83	10	84	4
263	87	11	86	3	87	7	86	11	83	10
264	84	5	83	11	82	11	79	12	78	15
265	73	9	74	14	71	3	70	13	69	9
266	70	0	73	10	72	7	71	12	70	12
267	69	4	72	3	73	8	74	5	75	5
268	76	0	79	4	80	13	79	14	78	5
269	79	9	80	13	79	1	80	14	79	11
270	78	14	77	12	76	1	77	5	78	2
271	81	14	80	7	79	4	80	0	83	7
272	82	9	81	5	80	5	81	5	82	13
273	81	10	82	5	81	1	84	3	85	12
274	84	4	87	8	88	8	89	14	86	4
275	89	1	92	12	91	7	88	11	87	6
276	86	15	81	3	82	3	83	0	88	5
277	89	12	88	11	87	15	82	14	81	0
278	84	3	85	10	86	0	91	8	92	2
279	95	1	96	5	97	1	98	4	99	3
280	100	7	99	5	100	9	101	14	100	6
281	101	3	100	11	97	4	100	9	99	6
282	100	15	97	10	96	15	91	12	90	10
283	91	10	90	12	91	4	94	11	91	13
284	90	9	89	8	92	11	89	15	84	3
285	85	2	86	11	83	12	82	14	79	13
286	78	7	77	4	80	12	79	13	76	12
287	75	9	74	14	73	10	74	9	73	13
288	72	8	75	0	78	5	77	14	76	3
289	75	14	72	11	69	10	70	12	71	11
290	70	8	71	8	72	15	67	11	66	15
291	61	13	60	1	63	11	60	10	59	0
292	64	2	67	2	68	11	65	15	60	2
293	63	6	64	0	67	1	68	15	65	10
294	64	7	61	12	60	15	57	4	58	14
295	55	7	52	4	55	4	58	13	55	12
296	54	15	49	9	50	4	51	11	50	10
297	49	6	50	12	49	3	50	8	51	14
298	48	3	47	10	46	6	45	8	48	8
299	49	3	48	6	49	0	52	2	53	8

m \ n	0	Δ_0	1	Δ_1	2	Δ_2	3	Δ_3	4	Δ_4
300	56	13	55	1	58	3	57	3	58	5
301	57	2	58	15	55	10	56	6	57	0
302	60	4	61	2	62	3	63	11	62	12
303	61	10	60	8	63	14	62	3	61	4
304	64	12	65	9	66	7	63	13	62	1
305	63	13	60	10	61	14	60	13	59	14
306	56	10	55	9	54	10	53	10	52	8
307	55	9	56	1	59	0	64	5	63	12
308	62	5	63	11	60	13	57	8	60	12
309	59	9	60	0	63	13	60	5	59	6
310	60	13	57	10	56	7	53	3	54	15
311	51	11	48	10	47	4	48	9	47	1
312	50	8	53	6	52	15	49	9	50	13
313	49	14	46	9	45	15	40	1	43	11
314	40	12	39	3	40	15	35	2	38	12
315	37	10	38	1	41	11	38	9	37	13
316	36	6	35	3	34	10	33	4	34	9
317	35	2	36	5	35	1	36	12	37	0
318	42	4	45	12	46	9	47	5	48	9
319	47	15	42	8	43	1	44	8	45	7
320	44	14	41	10	42	3	43	8	44	14
321	41	12	40	15	35	6	34	4	35	5
322	36	9	35	6	36	11	33	4	34	12
323	33	13	30	7	29	0	34	9	33	6
324	32	5	31	9	32	1	35	1	36	9
325	37	1	38	15	33	13	32	14	29	9
326	28	14	27	7	24	5	25	2	26	2
327	27	10	26	5	25	15	20	2	23	14
328	20	11	19	6	20	11	19	4	22	7
329	19	12	20	0	25	8	26	0	31	15
330	26	11	25	15	22	9	21	12	22	5
331	23	3	24	9	23	1	26	5	25	3
332	26	9	27	15	22	5	21	2	24	12
333	25	4	28	11	25	1	28	8	29	13
334	26	13	25	7	22	0	27	4	28	10
335	27	0	30	6	29	0	34	12	35	7
336	32	15	29	15	24	14	23	14	20	5
337	21	0	24	5	25	2	28	8	31	2
338	34	10	35	7	32	7	29	10	30	10
339	29	9	30	6	29	15	24	12	23	5
340	22	7	21	15	16	12	17	3	16	4
341	17	3	18	12	19	0	24	12	23	13
342	20	15	17	5	16	8	19	12	20	8
343	23	11	20	2	23	14	22	7	19	8
344	20	6	19	2	22	8	23	5	22	12
345	23	2	26	10	27	13	24	0	27	9
346	26	11	25	5	24	9	23	2	26	7
347	25	2	26	3	25	2	26	13	25	10
348	24	1	25	3	24	9	23	4	24	5
349	25	14	24	4	25	8	28	7	25	9

m \ n	0	Δ_0	1	Δ_1	2	Δ_2	3	Δ_3	4	Δ_4
350	24	8	27	13	24	1	25	12	26	2
351	27	12	26	12	27	0	30	9	29	12
352	28	9	29	9	30	11	29	14	28	1
353	31	0	36	0	39	10	38	6	39	10
354	40	8	41	5	40	5	39	10	40	14
355	37	9	38	12	37	1	38	13	37	1
356	40	15	37	7	36	3	35	12	36	9
357	37	14	34	0	37	13	36	1	37	4
358	38	3	37	0	40	5	41	12	42	0
359	47	4	48	15	45	9	44	12	45	12
360	44	2	45	8	46	5	47	13	46	3
361	45	14	44	15	41	6	40	4	43	14
362	40	7	37	12	38	2	39	1	42	10
363	43	7	42	3	43	11	42	1	45	7
364	42	12	43	6	44	10	43	9	42	14
365	39	8	40	13	39	0	42	3	43	5
366	44	4	45	0	50	7	47	15	42	1
367	45	5	46	6	47	0	52	3	53	4
368	56	8	59	10	58	8	61	12	60	7
369	59	3	58	2	59	4	62	6	63	13
370	62	2	65	1	66	11	65	6	66	9
371	65	10	64	1	67	13	66	4	67	11
372	64	13	61	6	62	3	61	12	62	1
373	65	15	60	2	61	2	62	1	63	12
374	64	2	65	6	64	2	65	11	64	0
375	67	11	66	7	63	13	60	1	61	0
376	64	0	69	13	68	13	67	0	72	3
377	71	4	72	4	73	8	76	5	75	11
378	74	12	75	10	76	5	77	9	78	8
379	81	8	82	8	85	5	84	2	85	8
380	86	12	85	13	82	2	85	6	86	2
381	87	5	88	9	87	4	88	3	89	8
382	90	6	91	6	92	11	89	9	90	11
383	89	6	88	0	93	9	92	2	95	3
384	94	7	93	7	90	13	89	6	90	0
385	95	14	94	3	95	5	96	8	99	12
386	98	12	97	5	96	6	95	4	98	14
387	97	8	100	2	103	4	106	10	107	0
388	112	15	109	6	110	10	111	8	114	4
389	117	7	114	12	113	10	112	4	113	0
390	116	3	115	7	114	10	115	2	118	14
391	115	14	114	1	117	8	118	2	121	15
392	118	12	119	9	120	6	121	2	122	1
393	123	12	124	5	125	2	128	13	127	15
394	122	6	123	2	126	7	123	13	122	1
395	123	8	124	13	123	8	124	11	121	15
396	118	6	119	9	120	7	119	12	118	13
397	117	0	120	7	117	15	114	2	117	14
398	114	6	115	13	112	11	109	11	108	1
399	111	5	110	7	107	15	102	14	99	15

m \ n	0	Δ_0	1	Δ_1	2	Δ_2	3	Δ_3	4	Δ_4
400	94	11	91	10	92	0	95	11	94	12
401	95	15	90	10	89	10	90	12	89	9
402	90	4	91	4	92	3	91	2	92	15
403	87	14	86	10	85	9	86	1	89	6
404	90	12	91	1	92	6	91	7	88	14
405	87	3	88	5	87	0	90	1	91	14
406	90	1	93	4	94	5	95	12	96	11
407	93	8	94	2	95	5	96	0	99	6
408	100	12	101	12	102	3	101	15	98	8
409	101	9	102	15	99	15	94	13	93	3
410	94	12	95	0	100	9	99	11	96	0
411	99	15	96	1	97	0	100	13	99	5
412	100	5	99	14	98	4	99	3	100	3
413	99	6	100	5	99	0	104	12	103	7
414	100	10	101	10	100	14	99	4	100	8
415	101	11	100	11	99	9	100	3	99	12
416	98	11	95	15	90	14	87	6	86	11
417	83	0	86	9	85	4	88	9	87	5
418	88	9	87	8	88	4	89	8	90	15
419	87	10	86	10	85	11	84	6	83	4
420	86	2	89	10	90	8	93	13	92	15
421	89	4	92	2	93	4	96	14	93	12
422	92	14	89	2	92	13	89	10	88	6
423	89	7	88	15	85	4	86	7	85	9
424	84	3	83	9	84	5	85	14	82	3
425	83	0	88	2	91	4	94	11	91	11
426	90	0	93	9	94	8	97	9	96	15
427	91	13	88	12	87	8	90	5	89	5
428	90	13	87	0	90	2	91	5	92	9
429	93	6	92	8	95	10	96	10	97	5
430	98	8	99	6	100	7	97	0	100	3
431	101	5	100	7	99	8	100	11	99	2
432	102	9	101	4	102	11	99	15	94	11
433	93	13	92	1	95	11	94	14	91	9
434	92	9	93	4	96	7	95	1	98	15
435	95	4	98	5	99	10	98	6	97	15
436	94	12	95	14	94	11	93	1	96	8
437	97	9	98	13	95	8	96	6	97	0
438	102	9	103	2	104	14	101	13	100	13
439	99	12	98	13	95	2	96	12	97	5
440	98	4	101	9	100	11	97	13	96	15
441	91	3	92	9	91	10	90	15	87	10
442	88	11	85	2	88	4	89	4	90	3
443	91	0	96	9	97	3	98	13	97	1
444	98	15	93	13	90	9	91	10	90	1
445	91	13	90	0	95	2	96	14	95	14
446	92	6	93	4	96	4	97	7	96	4
447	97	1	100	15	95	13	92	6	91	1
448	94	10	95	5	94	0	97	5	96	2
449	99	6	100	10	99	12	100	5	101	11

m \ n	0	Δ_0	1	Δ_1	2	Δ_2	3	Δ_3	4	Δ_4
450	100	6	99	8	100	2	103	5	104	12
451	103	6	104	14	103	8	106	15	101	10
452	102	15	97	15	94	7	93	2	96	1
453	97	6	96	2	97	6	96	8	97	13
454	94	4	97	15	94	7	91	14	88	9
455	89	7	86	13	85	13	82	12	83	0
456	88	14	85	3	84	2	87	11	84	13
457	83	1	86	5	85	11	84	8	85	1
458	86	7	85	5	86	11	83	11	80	5
459	79	13	76	5	75	10	74	0	77	3
460	76	4	77	5	78	7	75	0	80	9
461	81	8	84	10	85	15	80	9	79	0
462	82	2	83	11	80	14	77	15	72	15
463	69	8	72	14	71	1	72	9	73	9
464	74	7	73	2	74	11	73	8	76	7
465	75	11	74	9	75	7	72	15	69	12
466	68	15	65	3	66	11	63	5	62	12
467	63	2	66	3	65	8	66	14	63	10
468	64	5	63	4	66	5	65	10	66	15
469	61	9	62	7	59	11	56	13	53	5
470	54	9	53	7	50	13	49	11	46	15
471	41	10	42	0	45	6	46	15	43	12
472	42	5	43	10	44	1	47	5	48	0
473	51	9	52	4	53	8	54	11	53	10
474	54	11	51	4	52	13	49	0	54	2
475	57	3	56	8	57	8	58	6	57	11
476	54	8	55	10	54	15	49	4	50	1
477	53	3	54	5	53	8	56	1	57	14
478	54	0	59	4	60	15	55	6	54	5
479	55	0	58	3	57	1	58	6	57	4
480	60	10	61	1	64	7	63	4	64	15
481	59	3	58	10	59	1	60	0	63	6
482	62	14	59	7	56	10	57	6	56	1
483	59	5	58	10	57	14	56	9	57	8
484	58	2	59	10	58	1	59	8	60	7
485	57	14	56	11	55	5	54	8	57	12
486	56	14	53	11	52	0	57	9	58	12
487	59	3	58	13	55	14	52	11	49	3
488	48	13	45	3	46	6	47	5	48	2
489	49	11	48	2	49	4	50	6	51	14
490	48	4	49	8	50	5	51	12	50	15
491	47	9	48	7	45	12	46	7	43	6
492	42	4	45	14	44	14	43	5	42	8
493	43	8	46	2	47	9	46	1	49	9
494	50	3	51	1	52	9	51	5	52	12
495	51	8	52	9	51	7	50	5	51	15
496	48	3	49	14	46	12	47	13	46	5
497	45	10	44	9	45	3	46	14	45	15
498	42	4	45	11	44	13	41	3	40	12
499	41	4	44	10	45	0	50	1	53	9

m \ n	0	Δ_0	1	Δ_1	2	Δ_2	3	Δ_3	4	Δ_4
500	54	12	55	11	54	1	55	1	58	11
501	55	10	54	14	53	14	50	5	51	0
502	54	1	55	13	52	2	55	2	56	15
503	53	0	58	9	59	14	58	0	63	15
504	58	7	55	13	52	5	51	2	52	7
505	49	5	48	15	43	8	46	3	45	10
506	46	8	47	14	46	9	47	4	48	5
507	49	5	50	2	53	14	50	14	47	9
508	48	13	47	13	44	1	45	2	48	6
509	49	1	50	13	47	6	46	0	49	8
510	50	10	49	11	46	12	45	7	44	6
511	43	15	38	7	35	6	36	7	35	14
512	34	0	39	2	42	10	43	7	40	0
513	43	14	42	14	41	3	40	4	43	7
514	40	9	41	8	42	3	43	5	42	12
515	41	5	42	14	41	2	44	13	41	2
516	42	7	41	2	44	6	45	0	48	4
517	49	3	48	14	47	6	46	4	49	9
518	50	5	49	10	48	13	45	3	46	0
519	49	4	52	12	51	3	50	12	51	12
520	52	4	55	2	58	10	59	13	56	8
521	59	12	60	12	59	2	60	13	57	4
522	58	12	59	14	56	12	55	5	56	14
523	55	8	56	10	55	15	52	5	53	14
524	52	5	51	10	52	10	53	13	50	3
525	49	9	48	3	49	3	48	12	49	11
526	46	3	47	4	48	5	47	1	48	6
527	47	0	50	1	51	0	56	11	53	3
528	52	12	53	13	52	11	51	0	54	11
529	51	4	54	8	55	10	56	0	61	4
530	64	3	65	12	64	10	65	3	66	15
531	63	0	68	2	69	9	68	3	67	9
532	66	15	63	14	62	1	63	3	64	6
533	63	0	66	2	67	10	68	10	69	11
534	68	6	67	10	66	13	65	1	66	11
535	63	11	62	4	63	13	60	14	57	8
536	58	7	57	10	58	4	59	10	60	2
537	61	13	58	8	59	3	58	6	57	9
538	56	3	57	9	56	0	59	8	60	15
539	57	9	58	2	59	2	60	3	59	0
540	62	8	65	7	62	8	63	15	60	13
541	59	9	60	12	59	9	58	15	55	3
542	54	8	55	6	54	14	51	1	52	12
543	51	11	50	13	47	11	46	4	47	0
544	50	3	51	13	50	4	53	12	54	12
545	53	9	52	12	53	13	50	10	49	12
546	48	3	47	6	46	14	45	12	46	5
547	47	8	48	7	45	1	46	0	49	10
548	50	14	47	10	46	1	49	10	50	1
549	53	10	54	2	57	3	58	6	57	15

m \ n	0	Δ_0	1	Δ_1	2	Δ_2	3	Δ_3	4	Δ_4
550	52	7	49	8	50	8	51	10	52	14
551	49	4	52	1	55	4	56	14	55	2
552	58	8	61	1	64	15	59	5	58	7
553	55	0	58	7	55	14	52	6	51	13
554	48	2	51	10	52	11	51	1	52	0
555	57	0	62	0	65	2	66	13	65	14
556	64	5	65	1	66	9	65	7	62	5
557	63	15	58	1	61	11	60	7	57	6
558	56	15	51	12	52	12	53	9	52	15
559	47	9	48	4	49	15	46	15	41	0
560	44	4	45	4	46	4	47	13	44	11
561	43	8	44	0	49	7	46	12	47	7
562	44	5	45	1	48	3	49	5	50	6
563	51	15	46	1	47	9	48	14	45	3
564	44	8	45	1	46	11	45	7	44	10
565	43	1	46	8	49	14	48	9	47	2
566	48	1	51	2	54	10	53	15	48	12
567	49	11	46	3	45	5	46	10	47	9
568	46	11	45	9	46	3	45	11	42	1
569	45	4	48	2	49	5	50	5	49	0
570	52	7	49	7	48	11	45	5	46	2
571	49	15	46	0	49	2	52	8	55	9
572	54	12	53	1	54	12	55	15	50	0
573	53	10	54	2	57	13	56	0	61	11
574	60	11	59	9	60	10	59	6	58	9
575	59	7	56	11	53	8	56	2	57	13
576	54	3	55	10	54	5	53	14	52	12
577	53	13	52	0	55	3	54	13	53	0
578	58	10	57	3	58	1	61	1	64	11
579	61	6	60	2	61	10	60	11	59	0
580	62	9	63	15	60	3	59	10	60	8
581	61	4	64	9	65	0	68	0	71	0
582	76	2	79	8	80	5	81	13	78	1
583	79	1	82	11	79	6	80	5	79	15
584	74	7	73	7	70	15	67	6	68	3
585	67	0	72	8	73	4	76	13	75	6
586	74	12	75	3	74	5	75	14	74	13
587	73	13	72	8	73	5	72	3	71	6
588	72	14	71	1	74	8	77	11	76	0
589	79	4	80	4	83	11	82	2	85	3
590	86	12	87	4	90	14	87	11	84	4
591	85	14	82	0	85	13	84	10	85	11
592	84	2	87	1	90	9	89	8	90	6
593	91	11	90	1	91	4	92	9	91	7
594	90	6	89	7	86	2	89	10	90	15
595	87	0	90	11	89	10	90	2	91	3
596	92	15	89	5	90	1	93	7	92	12
597	91	2	94	3	95	10	94	9	93	11
598	92	7	91	1	94	14	93	2	94	15
599	91	15	88	13	85	7	84	1	85	15

m \ n	0	Δ_0	1	Δ_1	2	Δ_2	3	Δ_3	4	Δ_4
600	80	8	81	13	78	13	77	9	76	7
601	75	4	78	7	77	5	76	4	77	13
602	76	5	75	6	76	2	79	1	80	9
603	81	3	80	4	83	3	82	5	81	14
604	80	6	79	11	78	13	75	7	74	5
605	75	5	74	3	75	0	78	10	79	14
606	78	11	77	6	78	3	77	14	74	0
607	79	7	76	7	73	0	78	12	79	10
608	78	10	77	11	74	1	77	9	76	14
609	75	1	78	8	81	8	82	7	79	13
610	78	10	79	8	82	9	83	2	84	0
611	87	2	88	0	93	8	94	2	95	6
612	96	3	95	3	96	9	97	8	100	9
613	101	6	102	1	103	3	104	3	105	14
614	102	11	101	12	100	7	99	8	100	13
615	99	11	96	4	99	9	100	0	103	15
616	100	6	99	9	98	1	99	5	100	8
617	103	8	106	9	105	14	102	8	103	11
618	102	3	103	9	102	5	103	11	102	0
619	107	13	106	12	107	12	108	12	109	3
620	108	10	109	9	110	3	111	6	112	4
621	113	1	116	5	117	5	116	2	117	4
622	118	3	117	4	120	3	121	6	122	12
623	121	15	118	1	121	8	124	15	119	9
624	118	1	119	11	116	14	113	2	116	2
625	117	4	118	9	119	12	118	0	121	8
626	122	10	123	3	124	1	127	8	130	2
627	133	4	136	13	133	9	132	3	133	5
628	134	10	133	15	130	15	127	5	126	8
629	127	1	130	5	131	2	132	0	137	10
630	138	9	139	7	136	7	135	9	134	1
631	137	1	140	11	137	8	140	2	143	8
632	144	4	147	2	150	15	145	1	148	9
633	147	7	146	6	145	15	142	3	141	9
634	140	7	137	6	138	6	139	13	138	15
635	133	8	136	2	137	10	136	15	131	11
636	128	4	129	11	126	8	129	14	126	9
637	127	13	124	12	123	8	124	11	123	13
638	122	0	125	0	130	9	131	15	128	5
639	129	12	130	3	131	11	130	4	133	5
640	132	2	133	3	134	11	131	3	130	15
641	127	1	128	1	131	11	130	10	129	3
642	130	10	129	10	130	0	133	0	138	4
643	141	3	142	12	141	10	142	6	141	9
644	140	1	141	13	140	4	143	6	142	11
645	141	1	144	4	147	14	146	2	147	14
646	146	10	145	12	146	14	145	0	148	15
647	145	15	140	9	139	8	142	12	143	7
648	142	12	143	12	144	3	143	8	144	13
649	141	12	140	5	141	13	140	11	139	8

m \ n	0	Δ_0	1	Δ_1	2	Δ_2	3	Δ_3	4	Δ_4
650	140	13	137	13	136	2	137	1	140	8
651	143	13	140	10	139	2	142	11	139	12
652	140	0	145	7	142	14	141	0	146	14
653	145	13	142	13	139	2	142	7	141	6
654	142	11	139	7	138	4	141	12	142	10
655	141	1	144	5	145	7	142	8	145	6
656	146	11	145	1	146	6	145	2	146	3
657	147	1	148	12	149	15	144	14	141	1
658	144	0	149	15	144	15	141	11	138	3
659	139	10	140	7	137	11	134	0	137	1
660	140	11	137	5	136	0	139	4	140	9
661	141	8	144	10	143	14	140	6	141	8
662	142	13	139	5	138	3	137	9	138	13
663	137	9	136	12	137	13	136	15	131	4
664	132	9	131	9	130	10	129	0	134	12
665	135	12	136	5	137	7	134	8	135	10
666	134	6	133	11	130	3	129	5	130	13
667	127	13	124	9	125	11	124	0	129	12
668	130	0	135	13	132	14	129	5	130	15
669	125	14	122	6	121	11	120	3	121	2
670	124	15	121	14	120	4	123	3	124	15
671	119	15	114	10	113	11	110	6	109	1
672	112	8	113	0	116	0	119	4	122	0
673	127	2	128	0	131	6	132	12	133	12
674	132	7	129	11	128	14	125	13	122	7
675	121	6	120	1	123	10	122	5	123	6
676	122	1	125	3	124	6	125	5	126	6
677	127	1	130	1	133	3	132	9	131	7
678	130	3	129	13	126	12	127	3	128	10
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685	143	15	138	10	139	10	138	9	139	15
686	134	11	133	4	136	13	133	2	134	2
687	135	7	132	12	133	0	138	1	141	15
688	138	12	137	2	140	15	137	3	136	11
689	133	11	132	6	131	12	132	2	133	15
690	128	15	123	2	124	11	123	7	120	10
691	121	9	122	13	119	15	116	1	119	8
692	122	3	121	5	120	12	119	8	122	3
693	121	8	124	5	123	7	120	6	119	1
694	120	5	119	6	120	15	117	1	120	15
695	115	2	118	0	121	8	122	9	121	3
696	122	7	121	8	124	15	119	0	124	9
697	123	9	124	6	125	11	124	12	125	12
698	124	10	123	6	122	14	121	11	120	3
699	119	10	118	12	119	4	122	1	123	8

m \ n	0	Δ_0	1	Δ_1	2	Δ_2	3	Δ_3	4	Δ_4
700	126	3	125	10	124	2	127	12	128	13
701	127	13	126	10	125	10	124	7	121	6
702	122	2	123	14	122	8	125	14	122	13
703	119	14	118	3	117	13	116	2	119	10
704	120	11	119	12	118	11	117	12	116	9
705	117	0	120	8	121	7	118	7	115	13
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707	99	6	100	4	103	7	100	11	97	3
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709	93	8	96	1	97	3	96	10	97	2
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712	98	10	99	1	100	6	101	5	100	13
713	97	1	100	10	99	10	98	3	99	13
714	96	0	99	7	98	13	95	15	92	10
715	93	9	92	5	91	12	92	6	93	15
716	90	13	87	0	92	5	91	11	90	4
717	91	8	92	2	95	10	94	7	93	14
718	90	14	87	10	88	8	89	0	92	1
719	95	8	98	0	101	10	100	2	103	11
720	102	13	101	6	102	1	105	15	102	14
721	99	8	100	2	101	12	100	5	99	0
722	104	2	107	2	108	0	111	0	114	12
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724	116	14	113	4	116	10	115	10	114	5
725	115	3	116	5	117	12	116	9	117	2
726	118	12	117	5	116	7	113	4	114	11
727	113	1	114	7	111	13	108	4	111	4
728	114	10	115	11	112	4	113	3	112	1
729	113	6	114	1	117	12	118	0	123	7
730	122	1	125	10	126	2	129	12	128	7
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733	115	0	120	8	123	5	124	5	125	13
734	122	4	123	9	122	6	121	3	120	7
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736	112	1	113	15	108	9	109	3	110	3
737	109	11	106	8	109	14	108	12	107	5
738	106	7	103	1	106	7	105	6	106	6
739	107	11	104	4	105	3	104	0	109	13
740	106	5	105	3	104	13	101	9	102	1
741	105	5	104	14	103	2	104	1	105	12
742	106	9	107	3	108	7	107	9	106	0
743	111	12	110	12	111	11	110	9	109	5
744	110	0	115	13	114	14	111	3	110	7
745	107	1	110	10	109	11	106	15	101	13
746	98	10	97	5	98	7	95	7	94	7
747	93	6	94	5	95	13	94	2	95	14
748	92	7	89	7	88	4	91	2	94	13
749	91	6	92	1	95	5	94	13	93	10

m \ n	0	Δ_0	1	Δ_1	2	Δ_2	3	Δ_3	4	Δ_4
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751	97	9	98	13	95	9	96	5	95	11
752	94	5	93	15	88	10	89	12	88	2
753	89	13	86	15	81	10	80	13	77	4
754	80	14	77	14	76	14	75	10	76	9
755	75	15	70	14	69	9	70	3	69	4
756	70	10	71	11	68	1	69	2	70	14
757	69	13	66	11	65	12	64	3	63	11
758	60	11	59	10	60	9	59	15	54	12
759	55	12	56	7	53	7	52	9	53	10
760	54	2	55	14	54	8	55	9	56	13
761	55	7	52	6	51	3	50	7	49	3
762	50	6	51	9	50	3	49	12	50	12
763	49	14	48	7	47	0	50	11	47	11
764	46	14	43	7	40	12	39	7	36	3
765	35	4	38	1	41	12	40	3	41	4
766	42	14	41	3	42	5	41	15	36	11
767	33	12	34	5	35	7	32	15	29	5
768	30	4	33	0	36	4	39	4	42	2
769	45	12	44	4	45	3	44	15	41	14
770	38	2	39	6	38	7	35	0	38	12
771	37	13	34	11	31	10	30	8	33	14
772	32	2	33	14	32	4	33	8	36	14
773	35	2	38	12	39	15	34	8	37	7
774	34	11	31	14	28	13	27	7	24	4
775	27	10	26	3	25	2	26	5	25	11
776	22	0	25	5	24	6	25	3	24	1
777	27	2	28	9	27	15	22	6	21	10
778	20	12	21	0	26	0	31	10	32	3
779	31	2	34	12	33	14	32	7	31	10
780	32	7	31	0	36	12	37	5	36	3
781	35	3	34	5	33	7	30	0	35	7
782	34	0	37	6	36	15	31	12	30	1
783	31	11	30	5	29	7	26	2	27	0
784	30	8	33	14	30	3	29	9	28	12
785	27	7	24	15	21	7	20	15	15	12
786	16	10	15	15	10	4	13	12	12	7
787	11	3	10	0	15	9	14	2	15	0
788	20	12	21	6	20	15	15	3	14	4
789	15	1	18	10	17	8	18	15	15	12
790	16	1	17	14	16	0	21	9	20	9
791	19	10	20	11	19	4	22	8	25	5
792	24	6	25	7	22	9	23	8	24	14
793	21	1	22	8	23	14	22	0	27	13
794	24	5	23	15	20	4	23	0	28	8
795	31	1	32	15	29	2	30	0	35	6
796	36	14	35	7	32	8	35	8	38	9
797	39	1	42	11	39	1	40	15	35	8
798	36	14	35	13	34	4	37	5	38	8
799	39	4	42	2	45	2	48	0	51	1
800	54									

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN

OF THE

EAST PANJAB UNIVERSITY

No. 4 CHEMISTRY Pp. 65, 66

March, 1950

AIR-CONTROL THERMO-REGULATOR

by

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Edited for the East Panjab University by Vishwa Nath,
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HOSHIARPUR

Price Re. 0-8-0

AIR-CONTROL THERMO-REGULATOR.

By M. L. Lakhanpal.

Temperature changes affect the value of most of the physical and chemical constants so that the measurements of this kind have to be necessarily carried out at fixed temperature in a thermostat. Thermostat has therefore become the need of almost every research bench. Various devices of thermo-regulators have so far been made on the following general types.

1. Gas Regulators .. (1)
2. Electric Relay type .. (2)
3. Thyratron Relay type .. (1)

'Air-control thermo-regulator' is a modification of the second type and is so named for the control at different temperatures is done by decreasing or increasing the quantity of air enclosed in the thermo-regulator by means of a glass stopper.

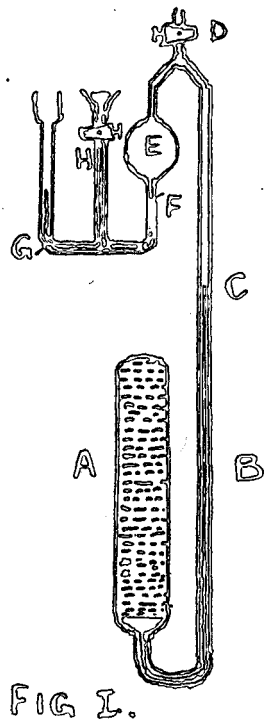

FIG. I.

Construction.

It consists of a toluene tube A of $1\frac{1}{4}''$ diameter bore joined to a glass tube of 5–8 mm. diameter B which is filled with mercury coming to level C. The tube is bent into a U-tube as shown in Fig. 1 and a ground glass stopper is sealed to it at the point D. A bulb of about 1 c.c. in volume is blown in the U-tube at E. Platinum points (3) are sealed at the points F and G. Mercury level in the U-tube is adjusted at F by means of a side tube bearing a glass stopper H.

The platinum joints are joined to the mains or 90 volts battery as shown in the circuit Figure II in series with the electric heater. For better

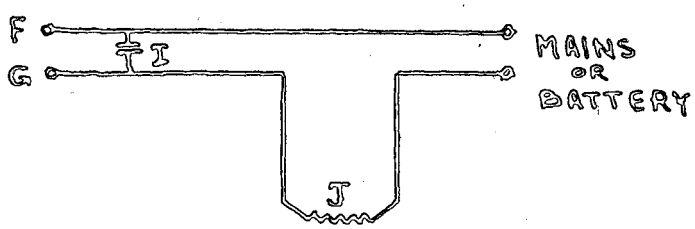

Fig. II.

control of temperature the wattage of the electric heater should depend upon the difference of the required and the atmospheric temperatures,—more the difference, more the wattage. A condenser I of suitable capacity is used as shown to minimize the sparking at F. Two aluminium plates (4) immersed in distilled water may be used as a condenser.

Working of the Apparatus.

For the maintenance of any temperature, open the ground glass stopper at D. Adjust the level of mercury at the platinum point F, by means of ground glass stopper H. Heater circuit being on the required temperature is reached. Close the stopper D. Any further increase in temperature will push down the mercury level at F and thus breaks the heater circuit. Thermostat will cool because of radiation till the circuit is again made. For regulation to exact temperatures adjustment is possible by the stopper H.

This system has the advantage of doing away with relay system which suffers from the possibility of sticking and it consumes much less current. It can also be made available to any laboratory having a glass blower.

Sparking fouls the mercury level if condenser is not used. With the use of a condenser I of suitable capacity and condenser oil over the surface of mercury it is minimized.

It can as well be used with thyratron relay to avoid least amount of fouling of mercury. Thermo-regulators are usually connected in the grid circuit (1) of the tube but in this regulator this connection being impossible, it is connected in the filament circuit as shown in Figure III. In this way with the make and break of filament circuit the current in the heater is switched on and off. Filament voltage and the grid potential will depend upon the characteristic curves of the thyratron tube used.

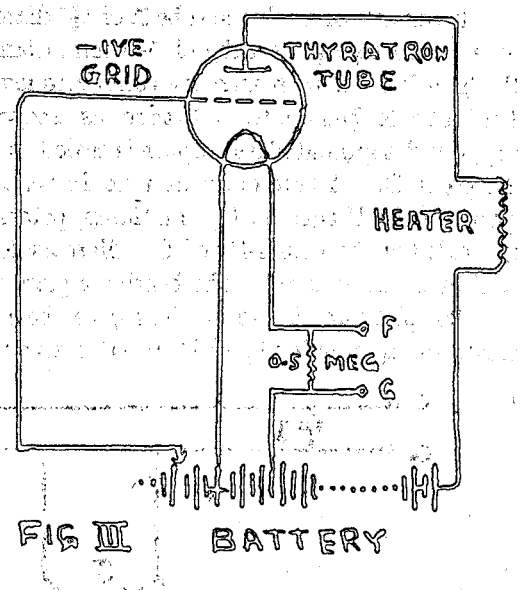

FIG III

It will be clear that filament shall not reach red heat so soon to be able to transfer full current to the heater immediately. Thus the current for the first few seconds will increase with time till it reaches red heat. So an equilibrium will be attained at a certain temperature of filament with the make and break at F, which will transfer just sufficient current to the heater to make up for the radiation of heat.

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Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 5. ZOOLOGY Pp. 67—80

June, 1950

SPERMATOGENESIS IN THE SPIDER, *PLEXIPPUS PAYKULLI*

by

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Price Rs. 2-4-0.

SPERMATOGENESIS IN THE SPIDER, *PLEXIPPUS PAYKULLI*

By GANPATI PARSHAD SHARMA, *M.Sc., Ph.D. (Panjab), Ph.D. (Edinburgh).*

CONTENTS

INTRODUCTION

Warren (1925) in a letter to *Nature* (September 12) reported that the spermatogenesis of some spiders especially *Palystes natalius* Karsch exhibited interesting peculiarities which did not readily accord with the rigid scheme required by the chromosome hypothesis of heredity. According to him the spermatogonial nuclei divide only by amitosis and the spermatozoa arise in the following two different ways:—

(*a*) The nucleus of the spermatocyte buds or fragments in a somewhat irregular manner and gives rise to a small cluster of nuclei, each of which becomes surrounded by differentiated cytoplasm. These small cells are the spermatids and their number seems to be more than four. The nucleus of the spermatid now becomes hollow and the chromatin concentrates at one pole to give rise finally to the spermatozoon which later on spreads over the cytoplasmic vesicle like a fish embryo stretched over its yolk sac.

(*b*) The numerous chromatin granules of the spermatocyte are condensed to form a considerable number of large masses resembling definitive chromosomes. These chromatin masses become more or less rounded and uniform in size. The cytoplasm of the spermatocyte now disappears and each of these free chromatin bodies becomes hollow to produce one spermatozoon which is differentiated peripherally. These spermatozoa are somewhat smaller than those formed by the first method.

At the suggestion of Prof. J. Bronte Gatenby who adversely criticized Warren's work in a letter to *Nature* (October 3, 1925), Dr. S. D. King worked out the spermatogenesis of the spider, *Amaurobius* sp. and published her results in a letter to *Nature* (October 17, 1925). According to her the whole process of spermatogenesis is quite normal without any evidence of amitosis either in the spermatogonial or spermatocyte divisions. Abnormal spermatids, however, are also formed either by an extra division of the ordinary spermatids or by degeneration of normal forms.

In view of these conflicting accounts the present work was undertaken to work out, if possible, the whole process of spermatogenesis in this ancient group of spiders. This material was considered all the more valuable as none of the previous workers had paid any attention whatsoever to such important cell inclusions as the Golgi elements and the mitochondria. Indeed even the names of these important cell components do not figure in the previous accounts.

It gives me great pleasure to acknowledge my deep indebtedness to Dr. Vishwa Nath, for his helpful suggestions and criticism during the course of this investigation which was carried out in the Department of Zoology, Government College, Lahore. For the identification of the material I am thankful to Mr. Sukh Dyal Malik.

Previous Literature

The earliest paper on spider spermatogenesis is that of Carnoy (1885) who has described in detail the process of cell division in male germ cells of a number of spiders. The whole of his Plate V beautifully illustrates the division stages but still his work has no direct bearing on modern spermatogenetic investigations. This is not surprising as the cytologists in those days did not properly follow the sequence of changes during the maturation divisions.

Wagner's (1896*a*) complete paper, published in the Russian language, is not available anywhere. Nor has it been possible to obtain it from Russia. However, from a preliminary report (1896 *b*) and from several reviews I conclude that Wagner's work is quite comprehensive. He has traced the history of the germ cells from the earliest spermatogonium to the ripe sperm in *Agalena*. Previous workers had described the spider spermatozoon as of a disc-like, aberrant form having no resemblance to the normal type. Wagner was the first worker to point out that the peculiar spider sperm is essentially normal inasmuch as it possesses the head, tail and the apical body. He also demonstrated that the disc-like form results from the rolling up of the ripe sperm.

Montgomery (1905) worked on *Lycosa insopita* and described the heterochromosome or the accessory chromosome as arising from two spermatogonial chromosomes. He, however, could not trace the course of this body through the meiotic divisions but on account of the similarity in its formation he suggested that it may behave like the other chromosomes, dividing first reductionally and then equationally.

Bösenberg (1905) in his remarkable paper on the spermatogenesis of Arachnida has described in detail the process of spermateleosis in *Lycosa*. Starting from the telophase of the second meiotic division he has very carefully followed the development of the spermatid nucleus and the idiozome. The latter, according to him, is formed from the disappearing spindle fibres. He has also described the division of the centrosome into a proximal and a distal portion. The distal centrosome moves towards the cell wall and from it is given out a delicate extra-cellular axial filament. The proximal centrosome moves over or through a portion of the nucleus and later becomes quite large. It is then known as the 'connecting piece' or the 'middle piece'. Both the proximal and the distal centrosomes are connected by an intra-cellular filament. The apical body, which, according to Bösenberg, is derived from the idiozome vesicle, reveals in its middle a delicate filament bearing a small granule at its tip. This is derived from the 'Connecting piece'.

Berry (1906) was the first worker to report dimorphism in spider spermatozoa. She worked on *Epeira* and showed that the odd chromosome or the accessory chromosome passed undivided to one pole during the first meiotic division which was reductional for the ordinary chromosomes. During the second meiotic division the odd chromosome also divided equationally like the other ordinary chromosomes. She derived the odd chromosome from one spermatogonial chromosome.

Wallace (1909) published her complete paper on the spermatogenesis of *Agalena nævia*. In this communication, while she pointed out the discrepancies in the conclusions of the previous workers, she also mentioned two chief errors in her own previous investigations (1900, 1905). The first error was that she failed to notice the division of the accessory chromosomes during the second meiotic division and the second was that she could not find the tail of the spermatozoon. Both these points have been fully discussed in her last paper in which she has also attempted to bring the observations of earlier workers in line with her own results.

Painter (1914) worked on thirteen different species of spiders but confined his studies only to the autosomes, accessory chromosomes and Ctetosomes, etc. He even did not describe the process of spermateleosis and the ripe sperm.

Warren (1925 and later) has also made an extensive study of the spermatogenesis in a large number of spiders and has described many different ways with regard to the formation of the ripe spermatozoon. He has consistently stated that the germinal nuclei divide only by amitosis. Spermatogonia can also divide without mitosis to form spermatocytes. In some spiders, according to him, even the primary spermatocytes may further divide amitotically to form secondary spermatocytes and spermatids. Chromatin structures indistinguishable from spermatozoa are also formed in cells which have originated either without mitosis at all or without the full complement of mitotic divisions characteristic of typical spermatogenesis. In many species there may be formed two kinds of spermatozoa originating in different lobules of the testis. The presence of typical spermatogenesis in many spiders, according to Warren, indicates

that the various atypical methods which have been observed are to be regarded as simplification of the typical process rather than as something fundamentally new or as a survival of the primitive methods. Warren has also described the origin of more than one sperm from a single spermatid. In none of his papers he has been able to identify the centrosome, acrosome and chondriosome, etc.

Material and Methods

The spider, *Plexippus paykulli* (Audouin) is commonly met with in houses in Lahore. I collected a large number of them from the vicinity of the Zoological laboratories of the Government College, Lahore. The male can be easily distinguished from the female by the presence of a pair of black longitudinal bands on the white cephalothorax and abdomen. In the female the cephalothorax and the abdomen are dusty brown in colour and these have a pair of deep brown lateral bands.

For preserving the testes the cephalothorax of the spider is first removed by means of a sharp blade of a safety razor. The abdomen is then cut open from the side under normal saline with a fine pair of scissors. The testes which are in the form of a pair of translucent and slightly convoluted tubes lie embedded on the ventral surface of the brown liver. The latter occupies almost the entire abdominal cavity. These testicular tubes are then carefully separated from the liver using a pair of fine needles. In some cases it becomes quite difficult to distinguish the tubular testes from the whitish spinning glands which lie immediately below them.

The testicular tubes thus taken out were fixed in Flemming-without-acetic and were prepared for sectioning in the usual way. Sections were cut 5-6μ thick and these were stained with 0·5% iron-hæmatoxylin. Smears of the testicular material were also made in Flemming-without-acetic diluted with an equal quantity of water and these were also stained with 0·5% iron-hæmatoxylin. Bouin's followed by 0·5% iron-hæmatoxylin was used as control.

Feulgen's reaction was also tried after fixation in strong Flemming solution.

Observations

The earliest spermatogonia of *Plexippus paykulli* (Audouin) are large, more or less, rounded or oval cells with comparatively large nuclei (Plate I, fig. 1). The nucleus which reveals inside it a number of faintly staining chromatin granules arranged on a spireme-like net work, is surrounded by a thin layer of cytoplasm showing no granulation whatsoever. In the late spermatogonia, however, the cytoplasm on one side of the nucleus appears to be denser and very often it is now possible to make out a few greyish granules in this area. These granules are most probably the mitochondria (Plate I, fig. 2).

During the spermatogonial metaphase (Plate I, fig. 3) the rod-like chromosomes are so closely packed together that it is impossible to make an approximately accurate count of them. In Plate I, fig. 4, the chromosomes arrange themselves in such a way that they occupy almost the

whole of the spindle area and consequently it is not possible to observe any spindle fibre. At each pole of the spindle area there is a sharply staining granule, the centrosome (Plate I, figs. 4 and 5). The astral rays are conspicuous by their absence. The cytoplasm is either clear (Plate I, fig. 4) or may reveal fine granules (Plate I, figs. 3 and 5) which are the mitochondria. These are sorted out, more or less evenly, to the two daughter cells. It has not been possible to make out any accessory chromosome during the spermatogonial divisions.

The resting primary spermatocytes are definitely larger cells than the spermatogonia, their nuclei also being larger (Plate I, figs. 6 and 7). During the early prophases faintly staining spireme threads, with chromatin granules arranged on them, appear in the nuclei. In Plate I, fig. 6 the nucleus also reveals two rod-like structures which are most probably the accessory chromosomes. These, very often, fuse together to form a nucleolus-like body (Plate I, fig. 7). In the cytoplasm, as illustrated by figure 6, Plate I, there is a juxta-nuclear mass of fine greyish granules which are the mitochondria. One or more deeply staining granules, appreciably bigger than the mitochondria, can also be made out in this juxta-nuclear mass. These are the Golgi elements. As the spermatocytes grow the Golgi elements as well as the mitochondria become circum-nuclear in arrangement (Plate I, fig. 7). Some of the Golgi elements at this stage reveal a definite duplex structure with a chromophilic cortex and a chromophobic central core (Plate I, fig. 9). The spireme in figure 8, Plate I, appears to break up into chromosomes and simultaneously the cytoplasm reveals two sharply staining granules. These are the centrosomes which will now form the apices of the first meiotic spindle.

During the first meiotic division the chromosomes arrange themselves at the equator of the spindle which is a fibrillar structure with a sharply staining centrosome at each pole (Plate I, figs. 10 and 11). As the chromosomes move towards the respective poles a pair of distinct rod-like bodies can very often be seen passing undivided to one of the two daughter cells only (Plate I, figs. 10, 12 and 13). These are definitely the accessory chromosomes. The mitochondria and the Golgi elements are distributed almost evenly to the two daughter secondary spermatocytes (Plate I, figs. 10–14).

When the process of nuclear reconstruction is going on a number of granules appear at the equator of the dividing cell (Plate I, fig. 13). These granules constitute the so-called mid-body which later on becomes condensed to form a conspicuous deeply staining ring surrounding the remains of the spindle fibres. The latter persist for a long time in the form of a bridge connecting the two fully formed secondary spermatocytes (Plate I, fig. 14). It is important to note that some of the primary spermatocytes do not divide for a long time even after the nuclei of the two daughter secondary spermatocytes have been completely formed in them. One such example is illustrated in fig. 15, Plate I. The spindle and the conspicuous ring-like mid-body can be clearly seen on one side of the cell, the nuclei lying away from the poles of the spindle. This represents the well-known phenomenon of telokinesis.

Each resting secondary spermatocyte is almost similar to the primary, but it is definitely smaller than the latter (Plate I, fig. 16). The nucleus contains inside it a deeply staining nucleolus-like body which appears to be formed by the fusion of the accessory chromosomes. This is also confirmed by the fact that the peculiar nucleolus-like body is met with only in some of the secondary spermatocytes (presumably in one half of the total number). The remaining secondary spermatocytes which do not receive any accessory chromosome during the first meiotic division are conspicuous by the absence of this nucleolus-like body (Plate II, fig. 17).

Just before the second meiotic division the cytoplasm reveals a pair of sharply staining granules (Plate II, fig. 17). These are the centrosomes which are most probably formed by the division of the originally single centrosome. Each of these will now take its position at the respective pole of the second meiotic spindle. During this division even the accessory chromosomes divide equationally like the other ordinary chromosomes (Plate II, fig. 18). Mitochondria and the Golgi elements are again sorted out, more or less evenly, to the two spermatids thus formed (Plate II, figs. 18–23). The formation of the conspicuous ring-like mid-body can also be clearly seen in figs. 19–23, Plate II. It is again quite interesting to note here that the process of spermateleosis may proceed on without the complete separation of the daughter spermatids after the second meiotic division (Plate II, figs. 22–25). Very often, a cytoplasmic neck-like structure (spindle-remains) can be seen connecting the two fully formed spermatids (Plate II, figs. 23 and 24). After the complete separation of the daughter spermatids this neck-like structure degenerates. In the earliest spermatid as shown in fig. 26, Plate II, the chromosomal vesicles are seen fusing together to form the nucleus. The latter during the rest stage reveals a number of fine chromatin granules inside it (Plate II, fig. 27). The mitochondrial granules are distributed uniformly throughout the cytoplasm. A few prominent darkly staining Golgi granules can also be seen here and there. But these now have a distinct tendency to come together to form bigger granules (Plate II, figs. 22, 23, 26, 27 and 28). In figs. 27 and 28 can also be seen an extra-cellular axial filament which has grown out from the granular centrosome situated in the cytoplasm. This axial filament bears at its centre and the tip a bleb-like swelling which stains deeply.

One of the big Golgi granules now takes its position at the anterior pole of the nucleus (Plate II, fig. 29). This will later on grow to form a ring-like acrosome (Plate II, fig. 30). The remaining Golgi elements degenerate in the cytoplasm.

The centrosome in the meanwhile divides into a proximal and a distal portion (Plate II, fig. 29). The former which moves towards or over the nucleus becomes rod-like (Plate II, fig. 31). Both of these, however, remain connected through an intra-cellular axial filament. Ultimately the proximal rod-like centrosome takes its position underneath the nucleus just behind the acrosome (Plate II, fig. 32).

The nucleus which now becomes pear-shaped begins to stain deeply and this makes the task of differentiating the acrosome and the proxima

centrosome a little difficult (Plate II, figs. 32-33 and Plate III, figs. 34-35). The ring-like acrosome becomes triangular and at its apex is differentiated a darkly staining granule (Plate II, fig. 31 and Plate III, fig. 36). The pear-shaped nucleus at this stage becomes sinuous and by its peculiar movements it gradually spreads over the clear space vacated by it (Plate III, figs. 37–40).

As the spermatozoa are undergoing these changes the staining capacity of the nucleus again decreases. It, however, does not reveal any chromatin granule inside it (Plate III, figs. 37–40). Soon after, the sinuous nucleus along with the acrosome and the axial filament wriggles out of the cytoplasm containing the whole of the mitochondrial material and the degenerating Golgi elements (Plate III, figs. 41, 42, 45 and 46). These cytoplasmic masses discarded by the ripe spermatozoa later on degenerate completely.

The ripe sperm, therefore, does not seem to have any trace of the mitochondrial material (Plate III, figs. 43, 44, 47 and 48). The distal centrosome disappears from view in the ripe sperm after it has wriggled out of the cytoplasm (Plate III, figs. 47 and 48). The proximal centrosome also cannot be made out clearly as it completely fuses with the deeply staining nucleus of the ripe sperm. The axial filament increases in length and in the meanwhile its bleb-like swellings completely disappear. The deeply staining granule at the apex of the triangular acrosome appears to be connected with the nucleus by means of a lightly staining fibre (Plate III, figs. 41–44). But in smears all these details are often lost (Plate III, figs. 45–48).

The fully ripe spermatozoa in the lumen of the testis coil themselves in such a way that it becomes increasingly difficult to make out their different parts (Plate III, figs. 49–51).

Discussion

A. *Golgi Material.*

The Golgi elements could not be observed in the spermatogonia of *Plexippus paykulli*. They seem to make their appearance for the first time during the early spermatocyte stage just at the time when the juxtanuclear mass of mitochondrial granules begins to spread out in the cytoplasm. They are in the form of one or more deeply staining granules appreciably bigger than the mitochondria. As the spermatocytes grow the number of the Golgi elements increases and they also become circumnuclear in arrangement. Some of the Golgi elements now reveal a definite duplex structure with a chromophilic cortex and a chromophobic central core.

During the meiotic divisions the Golgi elements are sorted out, more or less evenly, to the daughter cells.

In the earliest spermatid there are a few prominent Golgi elements which have a distinct tendency to come together to form bigger granules. One of these big Golgi granules now takes its position at the anterior pole of the nucleus and thus directly forms the ring-like acrosome. The rest

of the Golgi elements remain in the cytoplasm which is completely discarded by the ripe sperm. As the spermatozoa ripen the ring-like acrosome becomes triangular and at its apex is now differentiated a deeply staining granule which is connected to the nucleus by means of a lightly staining fibre.

None of the previous workers on spider spermatogenesis has described any Golgi element. This is not surprising as most of the previous work is quite old and a few papers which can be considered as recent are concerned mainly with chromosomes.

Wagner (1896) described an apical body in the sperm of *Agalena*, and Bösenberg (1905) derived the apical body of the sperm in *Lycosa* from an idiozome vesicle. The latter, according to him, was formed by the disappearing spindle fibres. Wallace (1909), working on *Agalena nævia*, confirmed Bösenberg's account in every detail.

On the other hand I have clearly shown that the apical body described by these workers is really the acrosome and is formed by the fusion of a few Golgi elements.

My observations are strongly supported by the pioneer work of Montgomery (1911) who, working on the hemipteran *Euschistus*, demonstrated the direct origin of the acrosome from a body called the 'sphere' or the idiozome. This, as we now very well know, is nothing but the Golgi apparatus. Even Gatenby (1917) clearly proved in *Smerinthus* that the acrosome was directly formed by the running together of several acroblasts (Golgi elements).

Later in 1920, the late professor Doncaster and H. G. Cannon showed that the acrosome in the lice (*Pediculus corporis* and *Pediculus capitis*) was directly formed from a body which was considered to be the Golgi apparatus. This account was further confirmed by H. G. Cannon (1922) in the lice, *Hæmatopinus consobrinus*, *Hæmatopinus asini* and *Lignognathus piliferus*.

Bell (1929) also, working out the spermatogenesis of the dog, clearly demonstrated that the pro-acrosomic granules of Gatenby and Woodger (1921) were in reality the Golgi elements and not the secretory products of the Golgi dictyosomes which were merely artifacts. These Golgi granules later on directly fused to form the acrosome in the late spermatid.

Similar observations were made by Gobind Ram (1937) and Nath (1932 and 1942) in *Rhysida* (a scolopendrid) and *Paratelphusa* and its allies respectively.

In ticks also I have clearly shown that the Golgi elements which can be easily seen even in the living cells, directly fuse together in the spermatid to form the acrosome (Sharma, 1944).

B. *Mitochondria.*

In the earliest spermatogonia of *Plexippus paykulli*, the thin layer of cytoplasm does not reveal any granulation whatsoever. In some of the late spermatogonia, however, the cytoplasm on one side of the nucleus appears to be denser and it is now possible to make out a few greyish granules in this area. These are most probably the mitochondria. In

the earliest spermatocyte there is a juxta-nuclear mass of fine mitochondrial granules which begin to spread as the spermatocytes grow.

The sorting out of the mitochondrial granules during the meiotic divisions is almost even.

In the earliest spermatids the mitochondria are uniformly spread throughout the cytoplasm and they do not seem to change in any way during spermateleosis. Ultimately they are completely cast out as the ripe sperm wriggles out of the cytoplasm containing these granules.

This account is quite unique inasmuch as there seems to be no trace of any mitochondrial material in the ripe sperm. In every other animal except the doubtful case of *Peripatus* (Montgomery, 1912) all or most of the spermatid mitochondria form a sheath round either a part or nearly the whole length of the axial filament.

Even in some of the non-flagellate ripe sperms, e.g. the Decapod sperm (Nath, 1942) and the millipede sperm (Sharma, 1942) the mitochondria persist in the form of a clear vesicle which completely fits into the nuclear cup.

None of the previous workers on spider spermatogenesis has described any mitochondrial granule but most of them agree that the ripe sperm, consisting of the nucleus, apical body (acrosome) and the axial filament, wriggles out of the cytoplasm. I have also confirmed the above account and therefore it is quite obvious that the whole of the mitochondrial material which remains in the cytoplasm is also discarded by the ripe sperm.

Thus the absence of mitochondria in the ripe sperm of the spider (*Plexippus paykulli*) and *Peripatus* (Montgomery, 1912) clearly disproves the hereditary function attributed to them.

C. *Spermatogonial and Meiotic Divisions.*

Warren (1925 and later) has consistently described that in spiders the spermatogonial nuclei divide only by amitosis. But a glance at my figures will clearly show that there is typical mitosis during all the spermatogonial divisions. Even in the meiotic divisions no abnormality could be observed. The accessory chromosomes pass undivided only to one cell during the first or the reductional division and divide equationally during the second or the equational division. After each meiotic division, just before the two daughter cells are separated, a number of granules appear at the equator of the dividing cell. These granules constitute the so-called mid-body which later on becomes condensed to form a conspicuous deeply staining ring surrounding the remains of the spindle fibres. These persist for a long time in the form of a bridge connecting the two daughter cells. It is quite remarkable that the neck-like structure connecting the two sister spermatids may persist till the end of spermateleosis.

Similar observations have already been made by Wagner (1896), Bösenberg (1905) and Wallace (1909). Wagner called this neck-like structure as the 'connecting body'.

Carnoy (1885) was the earliest worker who described in detail the division of the male germ cells in a number of spiders, but, as I have

already pointed out, his work has no direct bearing on modern spermatogenetic processes, as he could not properly follow the sequence of changes during maturation.

Wagner (1896), working on *Agalena*, states that the spermatogonia do not divide by the ordinary method of karyokinesis; nor do they divide amitotically. I, therefore, fail to understand the exact method of division observed by him. He has also described a peculiar nucleolus in the growth period and also in the primary spermatocytic division. This, I believe is the fusion product of the accessory chromosomes. He, however, has not been able to trace this body correctly during the meiotic divisions.

Montgomery (1905) has followed in detail the history of the spermatocytes in *Lycosa*. According to him there is no rest stage at any period of the spermatocytic history. But in *Plexippus paykulli* I have clearly shown that well-marked rest stages occur not only in the spermatocytes (both primary and secondary) but also in the spermatids. The mode of division of the bivalent heterochromosome during the meiotic divisions was also not positively determined by Montgomery but it was suggested that it may behave like the other chromosomes, dividing first reductionally and then equationally. On the other hand, I have proved beyond doubt that the accessory chromosomes pass undivided to only one of the two daughter cells during the first meiotic division and divide equationally during the second.

Bösenberg's (1905) observations on *Lycosa* begin with the telophase of the second meiotic division and are mainly concerned with the transformation of the spermatid into the ripe sperm. He, like Wagner, has also mistaken the accessory chromosomes for a nucleolus which, according to him, disappears before the final maturation of the spermatid nucleus.

Berry (1906), working on *Epeira*, described in the telophase of the last spermatogonial division a chromosome which had no mate. This odd chromosome, according to her, passed undivided to only one pole during the first meiotic division. She could not identify this odd chromosome during the second meiotic division but she correctly conjectured that it divided equationally.

Wallace (1909) has very carefully described the behaviour of the accessory chromosomes in the spermatogenesis of *Agalena nævia* but I have not been able to confirm her statement that these accessory chromosomes persist and form an important part of the chromatin plate in one half of the spermatozoa.

Painter's (1914) description of the accessory chromosomes during the meiotic divisions in a number of spiders is also in conformity with my own observations.

D. *Centrosome and Axial filament.*

It will be recalled that I have described a conspicuous sharply staining centrosome at each pole of the spermatogonial and the meiotic spindles. In the earliest spermatid, to begin with, there is a single centrosome from which an extra-celluar axial filament is given out. This axial filament,

which is quite delicate, bears at its centre and the tip a deeply staining bleb-like swelling. Later on the single centrosome divides into a proximal and a distal portion. The former, which moves towards or sometimes over the nucleus, becomes rod-like. Both of them, however, still remain connected through an intra-celluar axial filament. As the spermatozoa ripen the distal centrosome disappears from view and the proximal, which now fuses with the underside of the nucleus, cannot also be made out very clearly. These observations of mine confirm the work of Wagner (1896), Bösenberg (1905) and Wallace (1909) who called the proximal centrosome as 'little tooth', 'middle piece' or the 'connecting piece' and the 'end knob' respectively.

In the ripe sperm the axial filament has considerably increased in length and its bleb-like swellings have also completely disappeared. Similar observations were also made by Bösenberg (1905) and Wallace (1909).

Gatenby (1917), working on the cytoplasmic inclusions of the germ cells in Lepidoptera, has also described large clavate structures at the tip of the outgrowing flagella. The flagellum, according to him, passes through the substance of the clubbed end and bears at its tip a dark spot (terminal body). What exactly is the function of such formations, I have not been able to ascertain but these may be the storehouses for nutritive matter as urged by Gatenby. Their later disappearance also seems to be correlated with the growth of the axial filament.

Summary

1. The spermatogenesis of the spider, *Plexippus paykulli* (Audouin), has been worked out in this paper.

2. Both the mitochondria and the Golgi elements are in the form of granules but the latter are appreciably bigger than the former. Some of the Golgi elements reveal a definite duplex structure.

3. During the division stages both the mitochondria and the Golgi elements are sorted out more or less evenly to the daughter cells.

4. The accessory chromosomes pass undivided to only one pole during the first or the reductional division and divide equationally during the second or the equational division.

5. In the spermatid the Golgi elements have a distinct tendency to come together to form bigger granules. One of these big Golgi granules directly forms the acrosome.

6. The single centrosome of the early spermatid divides into a proximal and a distal portion. The former which moves towards and sometimes over the nucleus becomes rod-like. In the ripe sperm it is difficult to make out any of these.

7. There is an extra-cellular as well as an intra-cellular axial filament. The bleb-like swellings on the former seem to be connected with its growth.

8. This investigation tends to disprove the hereditary function attributed to the mitochondria.

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Explanation of Lettering in Plates

A.—Acrosome.
A.Ch.—Accessory Chromosomes.
A.F.—Axial filament.
C.—Centrosome.
C_1.—Proximal Centrosome.
C_2.—Distal Centrosome.
C.G.—Chromatin granules.
Ch.—Chromosomes.
G.—Golgi elements.
M.—Mitochondria.
N.—Nucleus.
N′.—Nucleolus.

Further explanation of figures will be found in the text.

Explanation of Plates

All figures have been drawn with a camera lucida at the table level with Zeiss 4 eye piece and oil immersion objective giving a magnification of 1300 times.

All figures except figures 45 to 51 have been selected from sectioned material fixed in Flemming-without-acetic followed by 0·5% iron-hæma-

toxylin. Figures 45 to 51 are from smears fixed in Flemming-without-acetic diluted with an equal quantity of water and stained with 0·5% iron-hæmatoxylin.

PLATE I

FIG. 1.—Earliest spermatogonium.
FIG. 2.—Late spermatogonium.
FIG. 3.—Spermatogonial metaphase.
FIG. 4.—Spermatogonial Anaphase.
FIG. 5.—Spermatogonial telophase.
FIGS. 6 and 7.—Resting spermatocytes I.
FIG. 8.—Spermatocyte I. The spireme is seen breaking up into chromosomes. Mitochondria and the Golgi elements are uniformly spread throughout the cytoplasm. Note also the centrosome which has just divided.
FIG. 9.—Spermatocyte I with fully differentiated chromosomes. The Golgi elements reveal a duplex structure.
FIG. 10.—Metaphase I.
FIG. 11.—Anaphase I.
FIG. 12.—Telophase I.
FIG. 13.—Telophase I.
FIG. 14.—Two fully formed secondary spermatocytes which are not yet completely separated. Note the remains of the spindle fibres with the conspicuous ring-like mid-body.
FIG. 15.—Spermatocyte I in which the nuclei of the two daughter secondary spermatocytes have been completely formed and yet no division of the cytoplasm has taken place. Note the spindle fibres and the conspicuous ring-like mid-body on one side of the cell.
FIG. 16.—Resting spermatocyte II with a nucleolus-like body formed by the fusion of the accessory chromosomes.

PLATE II

FIG. 17.—*Resting spermatocyte II without any nucleolus-like body. Note also the* centrosome which has just divided.
FIG. 18.—Metaphase II.
FIG. 19.—Anaphase II.
FIG. 20.—Telophase II.
FIG. 21.—Telophase II
FIG. 22.—Late telophase II with fully formed spermatid nuclei.
FIG. 23.—Two fully formed spermatids which are not yet separated. Note the remains of the spindle fibres and the conspicuous ring-like mid-body.
FIGS. 24 and 25.—Daughter spermatids showing the process of spermateleosis without their complete separation after the second meiotic division.
FIG. 26.—Earliest spermatid.
FIG. 27.—Resting spermatid.
FIG. 28.—Spermatid showing a distinct tendency of the Golgi elements to come together *to form bigger granules. Note also the axial filament growing out from* the centrosome.
FIG. 29.—A later spermatid with one of the big Golgi granules situated at the anterior pole of the nucleus. Note also the division of the centrosome into a proximal and a distal portion.
FIG. 30.—Still later spermatid in which a ring-like acrosome has also been differentiated.
FIG. 31.—A late spermatid. The ring-like acrosome becomes triangular at the apex of which is differentiated a deeply staining granule. Note also the proximal centrosome (which has become rod-like in the meanwhile) lying on the surface of the nucleus.
FIG. 32.—A nearly ripe sperm before it wriggles out of the cytoplasm.
FIG. 33.—Illustrates the beginning of changes in the shape of the spermatid nucleus during spermateleosis. Note also the acrosome and the centrosomes.

Plate III

Figs. 34–36.—As for Fig. 33.

Figs. 37 to 40.—Nearly ripe spermatozoa before they wriggle out of the cytoplasm.

Figs. 41 and 42.—Illustrate the ripe spermatozoa wriggling out of the cytoplasm.

Figs. 43 and 44.—Ripe spermatozoa.

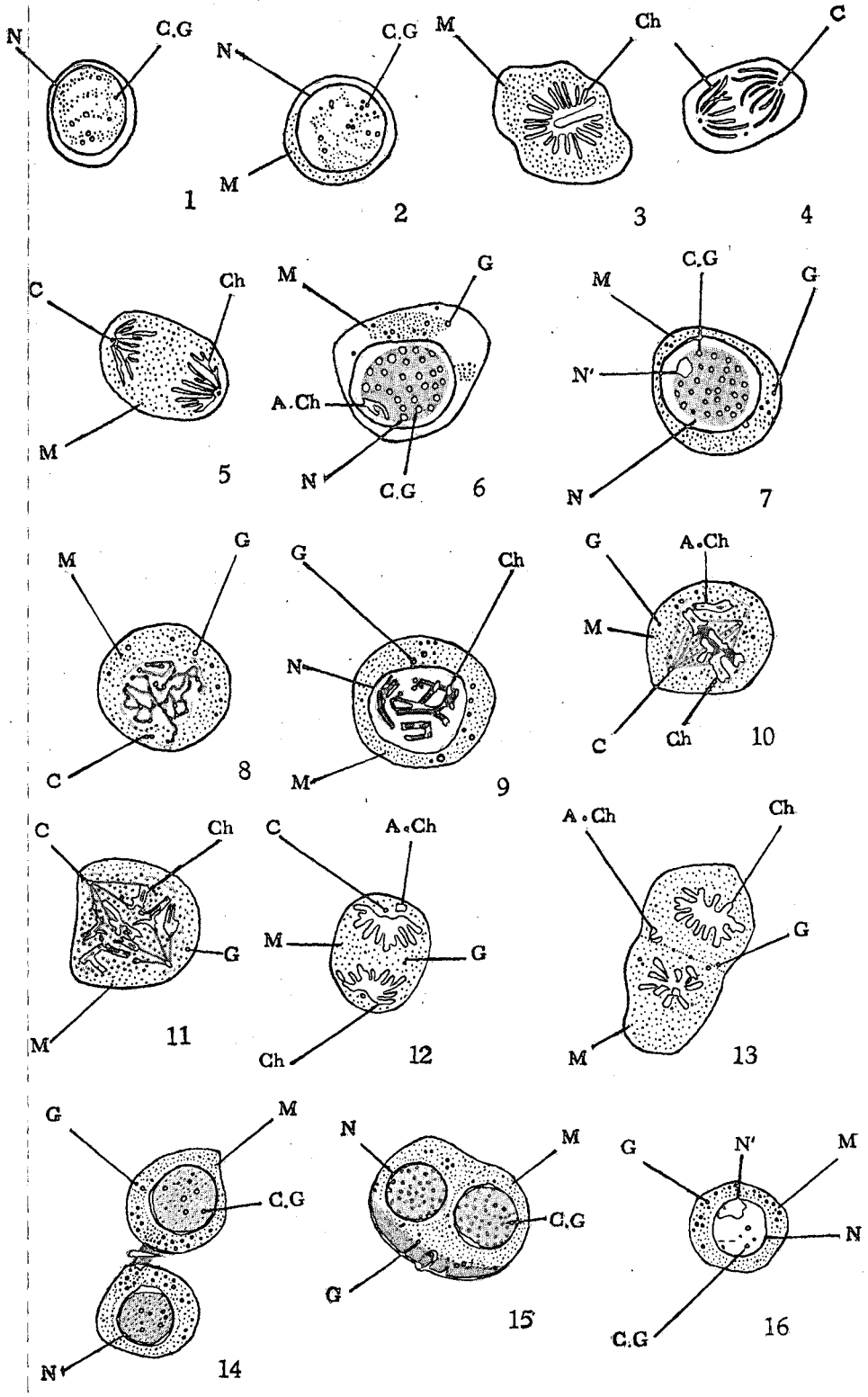
Smears

Figs. 45 and 46.—Illustrate the ripe spermatozoa wriggling out of the cytoplasm.

Figs. 47 and 48.—Ripe spermatozoa.

Figs. 49 to 51.—Coiling up of the ripe spermatozoa.

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.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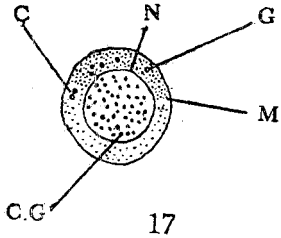

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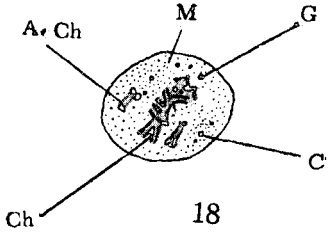

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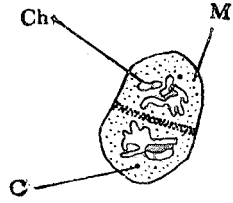

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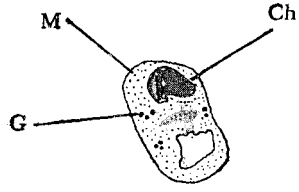

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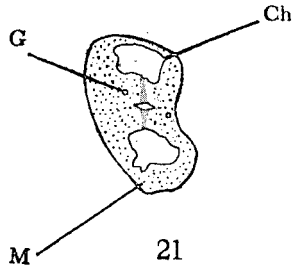

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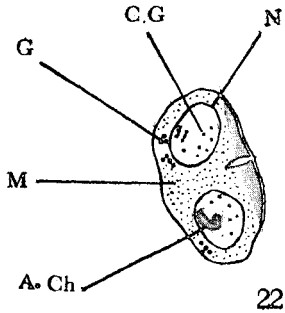

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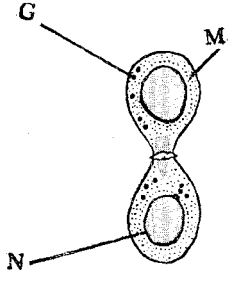

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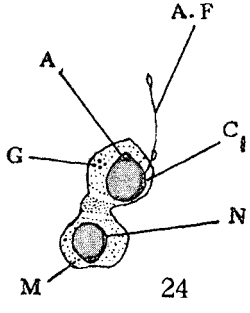

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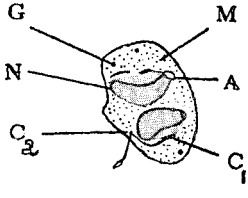

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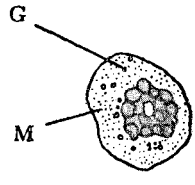

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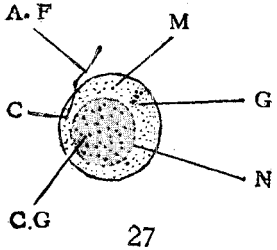

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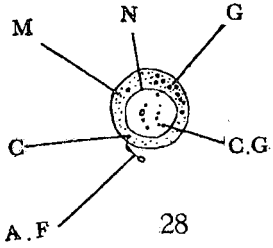

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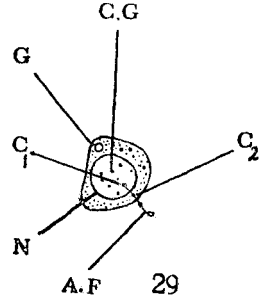

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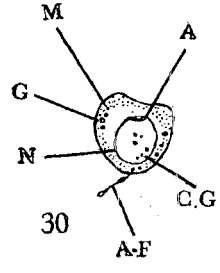

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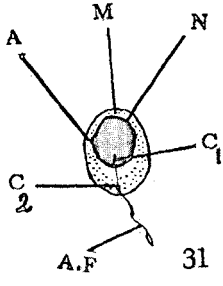

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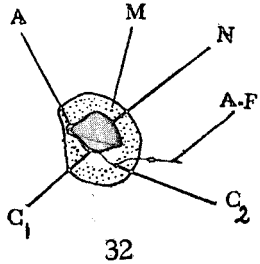

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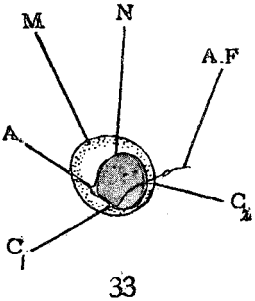

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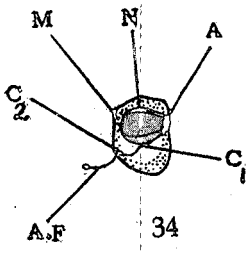

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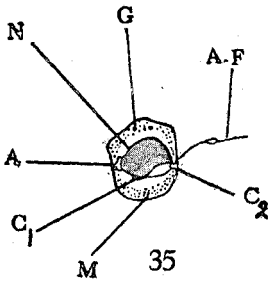

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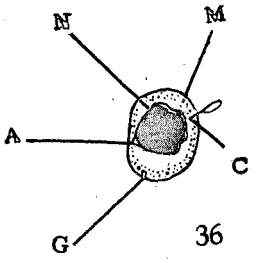

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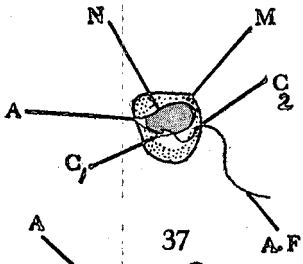

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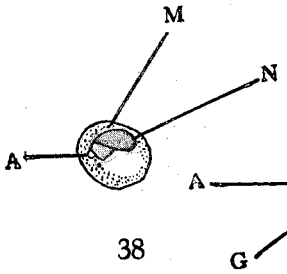

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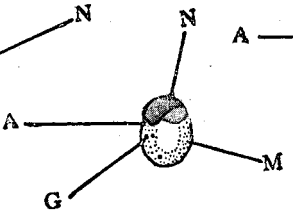

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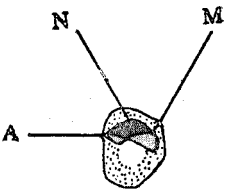

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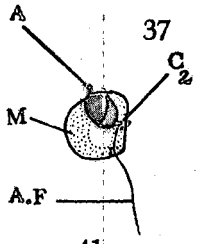

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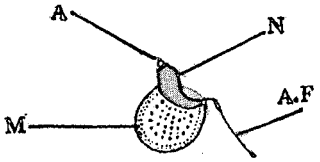

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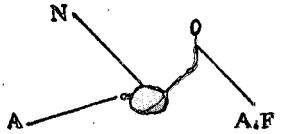

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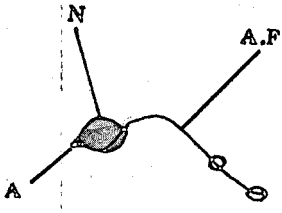

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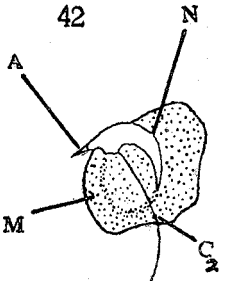

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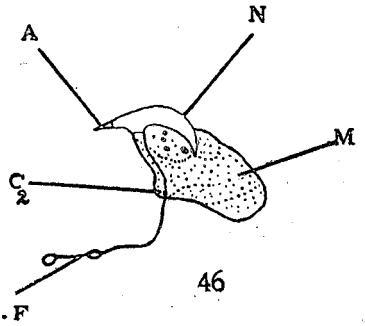

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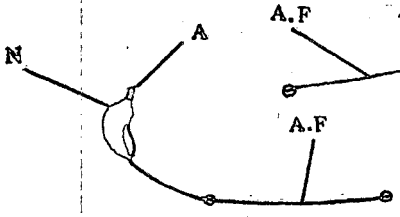

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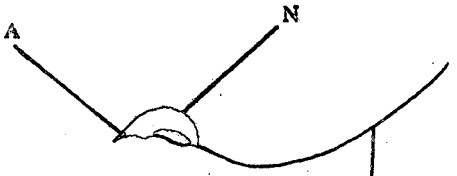

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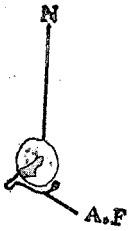

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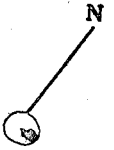

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RESEARCH BULLETIN

OF THE

EAST PANJAB UNIVERSITY

No. 6 MATHEMATICS Pp. 81-82

July, 1950

A SIMPLE PROOF OF THE PRIME NUMBER THEOREM BASED ON SELBERG'S FORMULA

by

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Edited for the East Panjab University by Vishwa Nath,
Department of Zoology, East Panjab University,
HOSHIARPUR

Price Re. 0-8-0

A SIMPLE PROOF OF THE PRIME NUMBER THEOREM BASED ON SELBERG'S FORMULA

By Hansraj Gupta

(*Government College, Hoshiarpur*)

1. *Notations.*

In what follows, p_r denotes the rth prime; p, q run over primes; all small letters other than x denote positive integers unless stated otherwise; and x denotes a positive number. Moreover,

$$\theta(x) = \sum_{p \leqslant x} \log p, \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

and $\pi(x)$ denotes the number of primes not exceeding x.

2. The prime number theorem states that

$$\lim_{x \to \infty} \frac{\pi(x) \log x}{x} = 1;$$

or what is the same thing

$$\lim_{x \to \infty} \frac{\theta(x)}{x} = 1. \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (2)$$

Using elementary methods, Selberg [1] has recently proved that

$$\sum_{p \leqslant x} (\log p)^2 + \sum_{pq \leqslant x} (\log p)(\log q) = 2x \log x + O(x).$$

This has led him to the important result:

$$U + L = 2, \quad \ldots \quad \ldots \quad \ldots \quad \ldots \quad (3)$$

where

$$U = \limsup_{x \to \infty} \frac{\theta(x)}{x}, \text{ and } L = \liminf_{x \to \infty} \frac{\theta(x)}{x}; \quad 0 < L \leqslant U.$$

Erdos and Selberg have obtained proofs of (2). Giving these in a recent paper,[2] Erdos has stated that Selberg has now a more direct proof of (2). This is, however, not available to me. I give here an extremely simple proof of (2) in the hope that it is different from Selberg's.

3. Consider the function $\{\theta(x)/x\}$ as x varies from 2 to ∞.

Let $p_r \leqslant x \leqslant p_{r+1} - \epsilon$,

where ϵ is a preassigned positive quantity however small.

Then since

$$\theta(x) = \theta(p_r),$$

therefore, $\{\theta(x)/x\}$ decreases continuously in this interval from $\{\theta(p_r)/p_r\}$ to $\{\theta(p_r)/(p_{r+1} - \epsilon)\}$.

In fact, $\{\theta(x)/x\}$ is a decreasing function except when x is a prime p. The curve

$$y = \theta(x)/x,$$

consists of portions of several hyperbolas running from one prime value of x to the next.

Now

$$U = \limsup_{x\to\infty} \{\theta(x)/x\} = \lim_{r\to\infty} \{\theta(p_r)/p_r\};$$

and

$$L = \liminf_{x\to\infty} \{\theta(x)/x\} = \lim_{r\to\infty} \{\theta(p_r)/(p_{r+1}-\epsilon)\},$$

$$= \lim_{r\to\infty} \{\theta(p_{r-1})/(p_r-\epsilon)\}.$$

Since

$$\frac{\theta(p_r)}{p_r} = \frac{\theta(p_{r-1})}{p_r} + \frac{\log p_r}{p_r},$$

$$< \frac{\theta(p_{r-1})}{p_r-\epsilon} + \frac{\log p_r}{p_r}.$$

Therefore,

$$U < L + \lim_{r\to\infty} \frac{\log p_r}{p_r}.$$

For every positive quantity δ however small, there is an r_0 such that for every $r \geqslant r_0$,

$$\frac{\log p_r}{p_r} \leqslant \delta.$$

Hence

$$L \leqslant U \leqslant L+\delta.$$

Since

$$L+U = 2,$$

we must have

$$L = U = 1.$$

This proves the theorem.

References.

1. See van der Corput's report entitled 'Demonstration Elementaire du Theoreme sur la Distribution des Nombres Premiers' issued by the Mathematisch Centrum, Amsterdam.
2. P. Erdos, *Proc. National Acad. Sci.*, **35**, 1949, 374–384.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 7. ZOOLOGY Pp. 83—89

August, 1950

SOME OBSERVATIONS ON THE FORMATION OF THE HEN'S EGG WITH SPECIAL REFERENCE TO THE CHALAZIFEROUS LAYER AND THE CHALAZÆ

by

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Price Re. 1-8-0.

SOME OBSERVATIONS ON THE FORMATION OF THE HEN'S EGG WITH SPECIAL REFERENCE TO THE CHALAZIFEROUS LAYER AND THE CHALAZÆ*

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INTRODUCTION

In connection with investigations on the origin of blood and meat spots in the hen's egg opportunities arose to examine the ovaries and the oviducts of a number of Brown Leghorn birds killed from time to time at the Institute of Animal Genetics. Occasionally during such examinations immature eggs in various stages of formation from different levels of the oviduct were obtained. On opening some such eggs obtained from the uterus, no distinct structure resembling the normal twisted chalazæ, so conspicuous in a laid egg, could be found. These observations, in view of the existing confusion in the literature with regard to the formation of the chalaziferous layer and the chalazæ, led to a more extended and detailed study.

PREVIOUS WORK

Pearl and Curtis (1912) stated that the yolk during its passage through the magnum region of the oviduct acquired its chalazæ, chalaziferous layer, the dense albumen and also the inner layer of thin albumen. They were, however, doubtful about the existence of the last named layer. The outer layer of thin albumen, according to them, was added in the isthmus and the uterus by osmosis through the shell membranes already formed.

Surface (1912), in his description of the infundibulum, has described two kinds of epithelial cells lining the folds of the mucous membrane, namely ciliated and non-ciliated, the former being confined to the more superficial parts of the folds. The non-ciliated cells, according to him, line the deeper parts of the grooves between the folds and he regards these as glandular. Consequently the grooves they line are named by Surface as 'glandular grooves' or 'gland pouches'. He has also distinguished certain other non-ciliated cells in the region of mergence of the infundibulum into the magnum and has named these as 'unicellular glands'.

The presence of glands in the infundibulum led Surface to suggest that the chalazæ and the chalaziferous layer are secreted in this part of the oviduct.

Bradley (1928), while confirming Surface's statements, has added that Surface's 'unicellular glands' (his goblet cells) produce a secretion which,

* This work was carried out at the Institute of Animal Genetics, Edinburgh, during the period 1945–47 when the author worked there as Government of India Research Scholar.

'from its staining properties, must be looked upon as either mucin or something akin thereto'.

Richardson (1935) showed that the chalazæ and the chalaziferous layer were secreted in the caudal half of the infundibulum and he called this the 'chalaziferous region' of the oviduct.

Hansen (1933) found that in the magnum region of the oviduct only the very viscid layer of albumen was secreted and as he could not see any chalazæ till the egg had been in the uterus for some time he concluded that they were formed in the uterus. He also doubted the observation made by Pearl and Curtis (1912) with regard to the taking in of the outer layer of thin albumen as such through the shell membranes. According to him, this layer appears as the result of dilution of the dense albumen with the watery solution of salts diffusing through the shell membranes.

Conrad and Phillips (1938) confirmed Hansen's observations. According to them the layer of dense albumen next to yolk becomes more like a fluid before the egg reaches the uterus. There, due to the rotation of the albumen around the yolk, the mucin of this fluid-like gel is segregated to form the chalazæ and the chalaziferous layer, leaving a much more fluid inner thin albumen. They even produced this change experimentally.

Scott and Huang (1941), on the other hand, confirmed Richardson's observation on the formation of the chalaziferous layer. The chalazal formation was first noted by them in the small end of eggs removed from the posterior magnum.

Material and Methods

All eggs removed from the various levels of the oviduct were carefully examined for the nature of the albumen and also for the presence or absence of the chalazæ. The yolks were then carefully separated from the albumen and after being agitated for a few minutes in 0·8% normal saline, were immediately fixed in Formol Saline. The dehydration was done as usual by passing the specimens through up-graded alcohols and cedarwood oil was used as a clearing agent. The yolks were embedded as such but for sectioning small pieces were cut from the two poles and also from the sides.

Sections were cut 5-6μ thick and stained with Delafield's hæmatoxylin and Eosin technique. Thionin blue was also used to differentiate the chalaziferous layer from the vitelline membrane.

Observations

A casual observation of all the eggs removed from the anterior and the mid-magnum region of the oviduct shows the yolk to be surrounded by a very dense envelope of albumen only. No definite chalazæ can be made out at this stage. Figures 1 and 2 are the photo-micrographs of sections of such yolks from the anterior and the mid-magnum region respectively. These clearly reveal two membranes surrounding the yolk. The outer of these is the chalaziferous layer and the inner the vitelline membrane. Both the layers can be easily seen even in preparations stained with Delafield's hæmatoxylin followed by Eosin. Thionin blue, however, gives the best differential results, staining the chalaziferous layer a purplish pink and the

vitelline membrane a pale blue. It is very difficult, however, to preserve this stain during dehydration.

Apparently the eggs from the posterior magnum region of the oviduct are also similar to those described above. However, on closer observation a faint whitish cloud can very often be observed near that pole of the yolk which is towards the isthmus. Figure 3 represents the section of such a yolk from the polar region. Here, in addition to the two membranes covering the yolk one can also see a cluster of fibres outside the chalaziferous layer. These fibres, which constitute the whitish clouds referred to above, stain with all the mucin stains exactly like those of the chalaziferous layer. These are, therefore, nothing but mucin fibres and are the precursors of the chalazæ. Simultaneously with the appearance of the mucin fibres the inner layer of thin albumen also becomes visible. It can be made to flow out by puncturing the envelope of thick albumen. Very often the mucin fibres forming the faint whitish clouds also flow out with it.

Figure 4 is a section of that pole of a yolk which was inside the isthmus. When this egg was removed from the oviduct only approximately one half of it was covered by a thin shell membrane while the other half, not yet within the isthmus, was without it. This section has been particularly selected to show the mucin fibres joining the chalaziferous layer at two points. These represent the bases of the chalazal core. Even in laid eggs the bases of the chalazæ can very often be seen to form cap-like structures at the two poles of the yolk.

In the isthmian eggs faint whitish clouds can be made out at both poles of the yolk. The inner layer of thin albumen is also present in these eggs. The thick albumen is still quite dense and no definite outer layer of thin albumen can be seen.

Figure 5 represents the contents of a shelled egg from the uterus. Here the outer layer of thin albumen is also quite conspicuous. The chalaza towards the narrower end of the egg is larger than that in the other. The normal twisting of the chalazæ is still not very distinct.

Discussion

According to Conrad and Phillips (1938) the layer of dense albumen next to the yolk becomes more like a fluid before the egg reaches the uterus. There, due to the rotation of the albumen around the yolk, the mucin of this fluid-like gel is segregated to form the chalaziferous layer and the chalazæ. On the other hand, it has been clearly shown in the present study that the chalaziferous layer is to be found even in eggs removed from the anterior magnum region.

As early as 1898 Mitrophanow described the envelope of yolk as consisting of two layers—an internal or the vitelline membrane and an external or the albuminous layer. The presence of blood spots in between the two layers led Mitrophanow to suggest that the external albuminous layer (chalaziferous layer) was secreted in the oviduct.

Lécaillon (1910, *a* and *b*) thought that the external layer was completed before the egg reached the oviduct but later on (1910 *c*) he modified his view in favour of that of Mitrophanow (1898).

Surface (1912), Bradley (1928), Richardson (1935) and Scott and Huang (1941) have all presented evidence that the chalaziferous layer is secreted in the posterior portion of the infundibulum to which the name 'chalaziferous region' of the oviduct was given by Richardson (1935). It has not been possible to confirm their observations directly as eggs from the 'chalaziferous region' were not obtained. However, from the present observations and in the light of the previous work it can be concluded that this layer is secreted in the posterior portion of the infundibulum, the true chalaziferous region'.

It would not be out of place to mention here the work of Burmester and Card (1939). They removed sections, 2–7 cm. long, from the 'chalaziferous region' of the oviduct and reported that such resection was conducive to the formation of dwarf or yolkless eggs. It could be suggested that as no chalaziferous layer was secreted, due to the absence of this particular region, the delicate vitelline membrane could not remain intact while passing through the narrow oviduct. The yolk, thus set free, was either pushed back into the body cavity perhaps by antiperistalsis or was included in the dwarf eggs.

As already pointed out by Burmester and Card (1939) that the resection of the 'chalaziferous region' did not have any significant effect in decreasing the weight of the chalazæ, Scott and Huang (1941) also showed that the quantity of mucin secreted in that region could not possibly form the chalazæ. These are, according to them, formed by the mucin secreted in the magnum region. These observations have been confirmed in the present study. Figures 3 and 4 show clearly that the mucin fibres secreted in the magnum region are added on gradually to the chalaziferous layer at the two poles of the yolk and these constitute the material for the chalazæ. The normal twisting of the chalazæ (Almquist, 1936) is seen only after the egg has been in the uterus for some time. This results from the rotation of the thick albumen around the yolk as already proved experimentally by Conrad and Phillips (1938). It may also be pointed out here that in the miniature eggs where, due to the absence of the normal yolk, the thick albumen does not find anything to rotate around, the normal chalazæ are absent.

It is now a well established fact that the inner layer of thin albumen is present long before the egg reaches the uterus. Almquist (1936) suggests that it probably results from syneresis of a part of the dense albumen as dilution of this with water or a watery solution cannot account for the formation of this layer. To support his view he also quotes Almquist and Lorenz (1933) and Romanoff (1929), according to whom, the inner layer of thin albumen has a higher solids content than the dense albumen or the outer layer of thin albumen. The chalaziferous layer, according to them, has even a still higher solids content.

Conrad and Phillips (1938) state that the dense albumen just near the yolk becomes more like a fluid before the egg reaches the uterus. This results not from the chemical destruction of mucin but by the breaking down of the gel structure. They could not, however, decide whether this

change was brought about by some mechanical action of the oviduct or by the presence of chemical agents.

Scott and Huang (1941) noted that the staining reaction of the layer nearest the yolk changed gradually during egg formation. They, therefore, ascribed this change (from the gel to the suspension of mucin fibres) to the presence of some chemical agent.

It is also quite possible that the inner layer of thin albumen may be secreted as such by the anterior magnum region of the oviduct as would appear from the work of Asmundson and Burmester (1936). The resection of 7, 8 or 10 cm. long parts from the anterior portion of the albumen tube resulted in the reduction of liquid albumen while the amount and percentage of firm albumen did not show any significant change. The work of Cole (1937 and 1938) also points towards the same conclusion. He suggests that the number and distribution of the mucin fibres determine the condition of a particular sample of albumen. He further states that the height of the goblet cells which secrete the mucin fibres averages 11·4μ in the anterior portion of the magnum, 15·7μ in the middle region, 30μ in the posterior region and reaches a maximum of 33·9μ at the junction with the isthmus.

There is no doubt that the outer layer of thin albumen appears only after the shell membranes have been formed, but the method of its appearance has been the subject of much discussion.

According to Pearl and Curtis (1912), the egg, during its stay in the isthmus and the uterus, receives its outer layer of thin albumen by a process of osmosis through the shell membranes. McNally (1934), by a study of the amount of the various proteins in mature and immature eggs, found that the former contained more of ovoglobulin than the latter. This, according to him, is taken in through the shell membranes.

Hansen (1933) doubted the observation of Pearl and Curtis (1912) as, according to him, the shell membranes must be impermeable to albumen. This doubt originated from the non-diffusion of albumen when a shell-less egg was placed in water. He, therefore, concludes that only the watery solution of salts is taken in after the shell membranes have been formed. Indirect evidence presented by Asmundson and Jervis (1933) and Asmundson and Burmester (1936 and 1938) is also in support of Hansen's view that no appreciable amount of protein enters the egg after the formation of the shell membranes.

Hughes and Scott (1936) found that after an egg was laid the greatest increase in ovoglobulin was in the inner layer of thin albumen while, according to McNally (1934), it should be in the outer layer of thin albumen. They suggest that the increase in the amount of ovoglobulin is due to a change in the solubility of the egg white proteins.

Similarly Scott, Hughes and Warren (1937) have presented evidence to show that proteins would not pass from the uterine secretion of low protein concentration into the egg white of high protein concentration through the shell membranes.

Beadle, Conrad and Scott (1938) have found that the uterine secretion is not an albuminous solution like that which forms the outer layer of thin albumen in a laid egg. The uterine secretion, according to them, is a

mineral solution made up of sodium, calcium and potassium in the form of chlorides and bicarbonates. From an analysis of uterine and laid eggs they have concluded that the principal additions to the albumen of an egg, while it is in the uterus, are water, potassium and bicarbonate ions with smaller amounts of sodium and chloride ions.

That water is added to the egg, while it passes through the isthmus and the anterior uterus, has also been conclusively proved by Burmester (1940).

It can, therefore, be safely concluded, as has already been suggested by Conrad and Scott (1938), that the outer layer of thin albumen is formed by the inflow of uterine fluid. As the uterine secretion diffuses into the egg the soluble proteins of the dense albumen come out and increase the solids content of the outer liquid layer.

Summary

1. The chalaziferous layer is secreted in the posterior portion of the infundibulum, the so-called 'chalaziferous region' of the oviduct.

2. In eggs removed from the posterior magnum a faint whitish cloud of mucin fibres can often be observed in that end of the egg which is towards the isthmus. These mucin fibres are the precursors of the chalaza.

3. Simultaneously with the appearance of the mucin fibres the inner layer of thin albumen also becomes visible.

4. In the isthmian eggs faint whitish clouds of mucin fibres can be seen at both poles of the yolk.

5. The normal twisting of the chalazæ is observed only after the egg has been in the uterus for some time. This takes place due to the rotation of the thick albumen round the yolk.

6. The outer layer of thin albumen becomes distinct only in the uterus.

7. The mode of formation of the inner and the outer layer of thin albumen is discussed.

Acknowledgments

I must take this opportunity to thank Dr. A. W. Greenwood, D.Sc., F.R.S.E., Director, Poultry Research Centre, Edinburgh, for very kindly going through the manuscript of this paper. Thanks are also due to Mr. G. R. Knight for taking the photo-micrographs.

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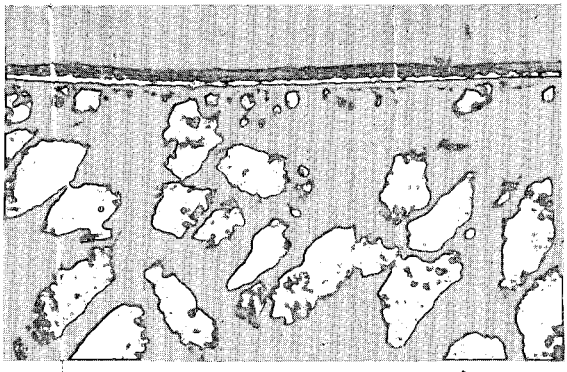
Explanation of Plate

Fig. 1. Section of a yolk from the anterior magnum region of the oviduct. ×500.
Fig. 2. Section of a yolk from the mid-magnum region of the oviduct. ×500.
Fig. 3. Section of that pole of the yolk which is towards the isthmus from an egg obtained from the post-magnum region of the oviduct ×500.
Fig. 4. Section of that pole of the yolk which is inside the isthmus from an egg obtained from the magnum-isthmus junction. ×60.
Fig. 5. Contents of a shelled egg from the uterus.

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.



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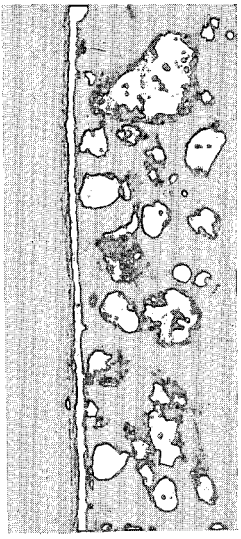

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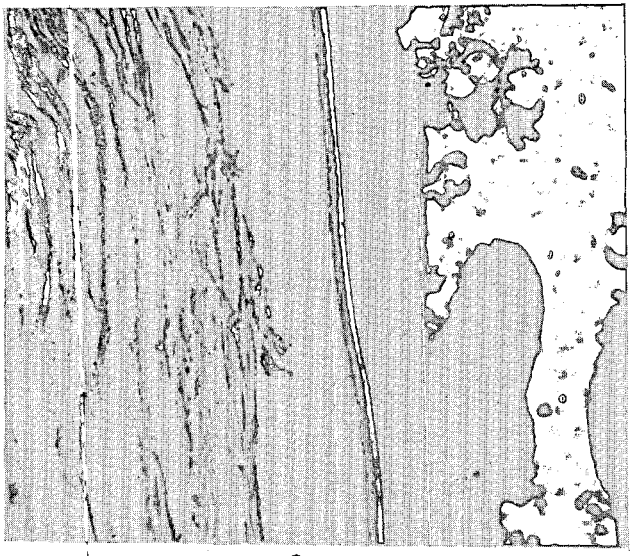

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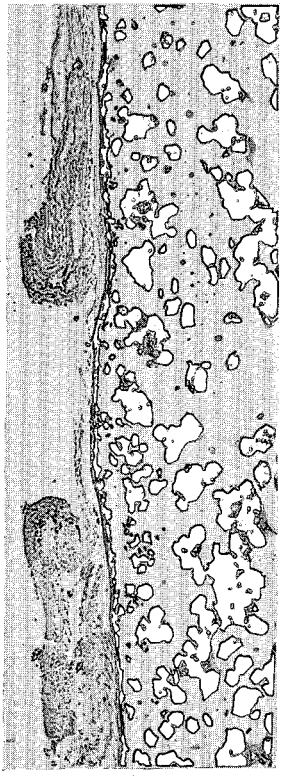

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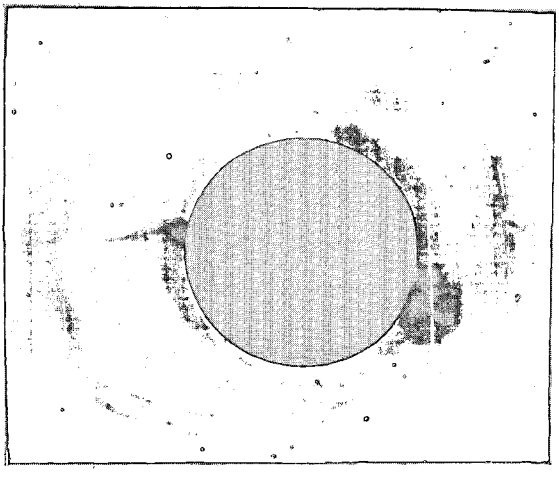

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RESEARCH BULLETIN

OF THE

EAST PANJAB UNIVERSITY

No. 8. ZOOLOGY Pp. 91—101

September, 1950

ANATOMY OF *PARAMPHISTOMUM (CAULIORCHIS) CRASSUM*

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Department of Zoology, East Panjab University
HOSHIARPUR

Price Re. 2-8-0.

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Acknowledgments

I am deeply grateful to Professor Dewan Anand Kumar, M.A. (Cantab.), Vice-Chancellor of the East Panjab University, under whose supervision this work was carried out at Lahore (1940–42), for his valuable suggestions, helpful criticism and sympathetic interest in the completion of this study. I am also indebted to the late Professor Dr. George Matthai, Sc.D. (Cantab.), I.E.S., for his encouragement and provision of facilities in the laboratory to carry on this work. My thanks are also due to Dr. Vishwa Nath, M.Sc., Ph.D. (Cantab.), F.N.I., P.E.S. (I), for editing the paper.

Introduction

In India, where hygienic standards are low, helminths constitute a very real menace to the health of man and his live stock. For a long time, the importance of parasitic worms was not recognized and the serious damage to health caused by them was not properly appreciated. This was perhaps largely due to the fact that the common symptoms of helminthiasis are not always sufficiently spectacular to attract immediate attention. In recent years, however, there has been a gradual realization of the importance of worms as a factor in the health of man and animals.

Prevention is always the best method of control of worm infestations. Helminths do not multiply within the body of their hosts and prevention in helminthology is largely a matter of biology of the parasite. A detailed knowledge of the features of taxonomy, bionomics, anatomy and biology must necessarily be the first pre-requisite in the formulation of effective control measures against them. A considerable amount of work has been done along these lines during the last two decades and useful information on the occurrence of worms in Indian animals is available. However, details of the anatomy and bionomics of some of the common parasites of economic importance still need further investigations. It was at the kind suggestion of Dr. George Matthai that the investigations on the anatomy of *Paramphistomum* (*Cauliorchis*) *crassum* were undertaken.

Material and Technique

Material.—The material for these studies was collected during 1941 from cattle slaughtered in the Municipal abattoir at Lahore. A large collection of Amphistomes was made, out of which five species were selected for detailed study. These are:—

(*a*) *Paramphistomum* (*Cauliorchis*) *crassum* Stiles and Goldberger (1910).

(*b*) *Paramphistomum bathycotyle* Fischoeder (1901).

(*c*) *Paramphistomum dicranocoelium* Fischoeder (1901).

(*d*) *Paramphistomum cervi* Zeder (1790).

(*e*) *Paramphistomum explanatum* Creplin (1847).

The account of only the first species is being published for the present

Technique.—The methods employed in the study of amphistomes are mostly those ordinarily used in Helminthology. For the study of the excretory system special methods of impregnation were used.

Fukui (1929) suggested a method for the study of the excretory system in Amphistomes. It consists in fixing flattened specimens in the acetic sublimate solution for 12 hours and then washing them in running water for 10–20 minutes. After this the specimens are treated with 1–5% aqueous solution of potassium hydroxide, and dehydrated and cleared in xylol or turpentine. The excretory system shows itself prominently in black. The whole mounts of the specimens thus prepared can be studied at leisure. The author has, however, deviated slightly from Fukui's procedure. Only 1% solution of KOH was used instead of the higher strength suggested by Fukui and the specimens kept in the solution for 1–2 hours only. If higher strengths of KOH are used, it materially damages the specimens, possibly due to higher temperatures of our laboratory.

Hypodermic injection of methylene blue in living worms for staining the excretory system was tried with unsatisfactory results.

Amphistomes stain readily and a wide choice of stains is available, though certain stains may give better results with one species than with others. In order to get sharp differentiation it is better to overstain the specimens in dilute solution of the stain and then to differentiate them. Of the several stains borax-carmine, Mayer's haemalum, Delafield's haematoxylin, acetic-acid-alum carmine and Mayer's carm-alum as modified by Gower (1939) were employed by the author, the last one gave by far the best results.

Mayer's carm-alum as modified by Gower is prepared in the following way:—

(*a*) 10 grms. of carmine is added to 100 c.c. of glacial acetic acid and stirred well. The mixture is filtered and the filtrate after drying is called *acidified carmine.*

(*b*) One grm. of acidified carmine and 10 grms. of alum are dissolved in 200 c.c. of distilled water. The stain can be used when required but to prevent any growth of mould a crystal of thymol is added to it. For the differentiation of stained specimens, Mayer's chlorinated alcohol is preferable to 70% acid alcohol. It is prepared by passing chlorine through 70% alcohol. Preserved and flattened specimens are first brought down to water and kept in the stain overnight or even longer depending upon the thickness of the specimen. They are then washed in distilled water, differentiated in chlorinated alcohol and mounted in the usual way.

For section cutting, specimens were embedded by the usual ordinary and double embedding methods. The material after clearing was embedded overnight in 60°C. paraffin wax in an electric bath for ten to twelve hours. Serial sections 8–12 μ thick were cut.

No reliable methods are available for the study of the lymphatic and nervous systems of amphistomes in entire mounts. The excretory and lymphatic systems were studied in living specimens as far as possible. Reconstructions from serial sections were used to confirm the results of the study of these systems in living specimens, and also for the study of the nervous system. Golgi's method failed to make the nervous system clear in whole mounts. Incidentally, a specimen of *Paramphistomum* (*Cauliorchis*) *crassum* which was impregnated to bring out excretory system also revealed the nervous system.

A brief survey of the systems of classification of Amphistomes.

The trematodes with an opening at either end were all referred to as Amphistoma by Rudolphi in 1801. In 1809, he divided 'Amphistoma' into two groups 'Capite discreto' and 'Capite continuo'. Ten years later, Nitsch proposed the genus *Holostomum* for the former group and retained the name *Amphistomum* for the latter. Monticelli (1888) created the family Amphistomidæ, but four years later separated *Gastrodiscus* Leuckart (1877) from the rest and created the sub-family Gastrodiscinae. Fischoeder (1901), finding the name *Amphistoma* preoccupied, proposed the genus *Paramphistomum* and erected the family Paramphistomidae. The family Paramphistomidae has received considerable attention of subsequent workers, several of whom, notably, Fischoeder (1901–1904) Stiles and Goldberger (1910), Maplestone (1923), Stunkard (1925), Fukui (1929), Travassos (1934), Southwell and Kirshner (1937), and Nasmark (1937) have tried to formulate a sound scheme of classification. Nevertheless, opinions differ very widely in regard to the status of the family and its sub-divisions.

Anatomy of

Paramphistomum (*Cauliorchis*) *crassum* Stiles and Goldberger (1910).

Host—*Bos bubalis*.

Habitat—*Rumen*.

Locality—Lahore (Punjab).

External characters.—Live worm is light pink in colour, the extremities being slightly deeper. It has a glassy shine due to mucus. It is conical in shape, tapering postero-anteriorly (Plate I, fig. 1).

The worm may be slightly flexed ventrally due to the contraction. The preserved unpressed specimens measure from 6·1–6·46 mm. in length, 4·18–4·83 mm. in breadth in the middle region, the maximum breadth being 4·27–5·11 mm. The flattened preparations vary from 11·25–13·57 mm. in length, 4·42–5·58 mm. in breadth in the middle region, the maximum breadth being 5·48–6·78 mm.

The outer general surface appears wrinkled owing to transverse grooves formed as a result of contraction. The body is free from hooks and spines

but on its ventral and dorsal surfaces there are small conical papillae which can be best observed in sagittal sections. The mouth is placed at the anterior end of the worm. The genital pore is situated on a slightly raised area in the median line on ventral side of the body (Plate I, fig. 1). In a specimen of average size, it is situated at a distance of 1·395 mm. from the oral aperture and 4·371 mm. from the acetabular. The excretory pore lies in the lower half of the dorsal region. The opening of the Laurer's canal is situated a little posterior to the excretory pore, but both these pores can be seen in sagittal sections only (vide infra). The oral sucker (interpreted by some authors as pharynx) is situated at the anterior end. The acetabulum is a large muscular organ at the posterior end of the body. The acetabular opening, 1·4×1·6 mm. in size, is directed terminally in the natural condition, but in preserved forms it becomes somewhat subventral or ventral in position. It is difficult to measure its length and breadth, as on contraction the opening gets covered by the proximate folds of the body (Plate I, fig. 1).

The Body-wall.—The body-wall (Plate II, fig. 9) consists of (*a*) the cuticle, (*b*) the subcuticle, (*c*) the muscular layer, and (*d*) the layer of subcuticular cells. The cuticle is the external layer measuring 0·024 mm. in thickness, and is beset with small conical papillae both on the dorsal and the ventral sides of the body. The cuticle appears striated in sections. Internally it extends up to oesophagus, ductus ejaculatorius, metraterm, Laurer's canal, excretory canal and the acetabular lumen. The subcuticle is broader than the cuticle, and runs all round the body, and measures about 0·032 mm. in thickness. It is traversed by strands connecting the subcuticular cells and the cuticle and the presence of these makes it striated. This layer does not pick up any stain. The subcuticle is followed by a layer of circular muscle fibres and four to five bands of longitudinal muscles. Oblique muscle fibres are present in groups in between the clusters of subcuticular cells.

The Muscles and Parenchyma.—There are two pairs of inner and outer retractor muscles, which extend from the acetabulum up to the oral sucker (Plate III, fig. 14). The peripheral muscle bands are also in two pairs which run longitudinally on the lateral sides of the worm. There is another pair of longitudinal muscle bands, dorsal to the subcuticular cells. The dorso-ventral muscle fibres running between the two main longitudinal muscle bands are also present.

The body parenchymatous cells are irregular in outline due to the contraction of the body. The cells below the integument are narrower than those surrounding the internal organs. At some places the walls of the adjoining cells become fused.

The Digestive System.—The digestive system (Plate I, fig. 2) consists of the mouth, the oral sucker, the oesophagus and the intestinal caeca. The mouth is a small circular narrow aperture in the centre of the oral sucker and measures 0·36–0·4 mm. in diameter. The oral sucker measures 1·35–1·95 mm.×1·48–1·86 mm.; in sections it is from 1·4–1·65 mm. in length, from 1·05–1·24 mm. in thickness and 1·4 mm. in breadth. It lies in the peri-suctorial cavity and is attached to the body parenchyma at its oral and basal ends, and by delicate mesenterial bands to both the dorsal and the ventral

sides. The lumen of the oral sucker is spindle-shaped having its either side lined by 13 to 17 small conical papillae. It measures from 1·4–1·69 mm. in length and 0·234 mm. in dorso-ventral diameter.

The oesophagus is a thin tube arising from the basal portion of the oral sucker and is continuous with it. It bifurcates into two intestinal caeca which run laterally. On reaching the ventro-lateral aspect of the body, they change their course and each caecum then runs posteriorly in a coil towards the acetabulum along the body-wall. The caeca end blindly, the right caecum is slightly longer than the left and terminates close to the middle of the acetabulum. The blind ends of the caeca are a little dilated (Plate I, fig. 2). The oesophagus is lined with cuticle which is co-extensive with the cuticle of the lumen of the oral sucker. Round the cuticle is a layer of circular muscle fibres and next to this is a thin layer of longitudinal muscle fibres. Subcuticular cells are present all round the oesophagus. The caeca are lined by ciliated columnar epithelium, which is followed by circular and longitudinal muscle fibres (Plate I, fig. 3; Plate IV, fig. 20).

The Suckers.—There are two suckers: the oral sucker and the acetabulum. The oral sucker is a globular muscular organ situated anteriorly (Plate I, fig. 2). It measures from 1·35–1·95×1·48–1·86 mm. in whole mounts and in sections 1·65×1·24 mm. It is composed of cuticle, muscles, parenchymatous cells, subcuticular cells, nerve cells and limiting membrane (Plate II, fig. 7; Plate IV, fig. 17). The cuticle is the extension of the outer cuticle and forms a thin lining to the lumen of the oral sucker.

There are several layers of muscles in the oral sucker. Immediately surrounding the cuticle is a layer of interior circular muscle fibres which are present in bands. It is followed by a layer of interior longitudinal muscle fibres, which are crossed dorsally with some oblique muscle fibres. Next to them, there is some more thick crossing of the same fibres (Plate II, fig. 7). A little further is a layer of poorly developed middle longitudinal and exterior circular muscle fibres. The former layer makes a curve as it extends towards the oral and aboral poles. The limiting membrane forms the outer lining of the oral sucker and underneath it is a layer of exterior longitudinal muscle fibres. Apart from these there are radial muscle fibres, present in bands, extending from the cuticle up to the limiting membrane. Basally there are a few bands of circular muscle fibres, which are feebly developed. In addition to the basal circular muscles, there are 3–4 bands of circular muscle fibres, known as the posterior sphincter muscles (Plate IV, fig. 17).

The parenchymatous cells of the oral sucker are of a smaller size than the body parenchymatous cells and those of the acetabulum. They are more closely packed up in the outer and inner regions.

There are two layers of subcuticular cells, the first consisting of scattered cells is just below the crossing of longitudinal and oblique muscle fibres; while the second layer, slightly apart, has its cells lying in compact groups (Plate II, fig. 7). Besides these there are a few more cells scattered all over its surface. The subcuticular cells are deeply stained with haematoxylin.

In well-differentiated serial sections, scattered nerve cells are clearly visible in the upper region of the oral sucker (Plate II, fig. 7).

The ratio between the length of the oral sucker and the length of the body is 1 : 9·1–1 : 10·5 in flattened specimens and 1 : 4·2–1 : 4·6 in sagittal sections.

The acetabulum is a strong muscular organ placed at posterior extremity of the body. It has a small opening as compared to its large size. The acetabulum measures 2·95–3·48 × 2·9–3·34 mm. and its opening 1·02 1·58 × 1·11–1·76 mm. In transverse sections the dorsal wall measures 0·918–0·99 mm., the lateral wall 0·684 mm. and the ventral wall 0·342 mm. in thickness. The opening of the acetabulum is irregular due to the adjacent folds of the body surface. The acetabulum is attached to the body parenchyma by mesenterial bands. Histologically, it is composed of the cuticle, the muscles, parenchymatous cells, subcuticular cells, the nerve cells and the limiting membrane.

In this there are oblique, circular, longitudinal and radial muscle fibres. Underneath the cuticle there is a meshwork of oblique muscle fibres. On the dorsal side of the oblique muscle fibres are the circular muscle fibres, which are arranged in bands and are better developed on the lateral sides. These lateral circular muscles are called the dorsal exterior circular, dorsal interior circular, ventral exterior circular and ventral interior circular. The dorsal exterior circular muscle fibres are in 25 bands, the dorsal interior circular muscle fibres in 43, the ventral exterior circular muscle fibres in 18 and the ventral interior circular muscle fibres in 55. Underneath the limiting membrane there are bands of longitudinal muscle fibres. The radial muscles are many, and extend from the cuticle to the limiting membrane (Plate III, figs. 12, 16).

Parenchymatous cells are, as compared to those of the body, smaller in size and are present in between the muscle fibres. A little away from the cuticle, there are subcuticular cells present in groups (Plate III, figs. 12, 16). Large-sized nerve cells are found dorsal to the layer of subcuticular cells.

The ratio between the diameter of the acetabulum and the length of the body is 1 : 4·1 in flattened specimens and 1 : 1·9–1 : 2·3 in sagittal and longitudinal sections.

The Excretory System.—There are two main excretory canals each running along either side of the oral sucker and the intestinal caecum. Posteriorly near the acetabulum, the two canals bend inwards and meet to form a prominent excretory vesicle (Plate III, fig. 15). Dorsal to the vas deferens lies a small vesicle, which is connected to the main excretory canals by two connecting canals. From the dorsal side of the excretory vesicle arises a canal, which opens on the dorso-median line of the body. The excretory pore lies just above the opening of the Laurer's canal (Plate IV, figs. 18, 21) in level with the excretory vesicle.

The Lymphatic System.—For a long time the lymphatic system received little attention on account of the difficulty in tracing it, specially in thick forms, and it was therefore considered to be of no taxonomic importance. Van Beneden (1859), Walter (1893), and Looss (1896) considered it as a part of excretory system or excretory system itself. Looss (1902) was the first to recognize the lymphatic system as independent of the excretory system and called it 'Lymphgefass system'. He made it definitely clear

that the lymph system had no connection with the excretory system and the lymph vessels ended blindly. Maccallum (1905) described the lymph vessels as 'endothelial tubes' and thought that they communicated with the excretory channels. Stiles and Goldberger (1910), while describing the species of Paramphistomidæ made no mention of it. In 1912, Looss showed its importance in taxonomy. Stunkard in 1917 described the lymphatic system in *Allassostoma*. Maplestone (1923) also ignored this system and disposed it off as too complicated for practical purposes. Stunkard in 1925 again supported Looss' view on the lymphatic system. Fukui in 1929 described it in his general description of Amphistoma, and Willey (1930) made a detailed study of this system in digenetic trematodes.

The lymphatic system in the species under investigation was studied in living specimens but its details could not be worked out owing to the thickness of the body-wall. Attempts were made to impregnate this system with corrosive sublimate without much success. It has been possible to study the system only from the longitudinal serial sections of the worm. The two main lymph vessels run longitudinally in the body along the intestinal caeca throughout the greater portion of the course (Plate II, fig. 11). They give off a large number of lateral branches, which encircle the various organs without directly communicating with them. Anteriorly they surround the oral sucker and posteriorly the acetabulum. In sections the lymph vessels can be easily differentiated from excretory vessels by the presence of granules in the former. The wall of a lymph vessel is membranous. The close association of the lymph vessels and their branches with the intestinal caeca indicates that they carry the digested food from them to the various organs of the body.

The Nervous System.—A little posterior to the oral sucker there is a mass of nervous tissue, the brain, lying over the oesophagus. Three pairs of nerves arise from its anterior end and three pairs from the posterior end (Plate III, fig. 13).

The Reproductive System.—All amphistomes are hermaphrodite. The male genital system (Plate I, fig. 2) consists of testes, vasa efferentia, vas deferens and ductus ejaculatorius. A pair of testes is situated diagonally in the middle of the body in between the intestinal caeca. They are multilobed giving the appearance of small cauliflowers and show a great affinity for the stains. In flattened preparations the anterior testis is 0·74–1·3 × 1·11 mm. in dimensions; the posterior testis measures 0·93–1·11 × 1·11–1·58 mm. In sagittal sections the anterior testis measures 1·332 mm. transversely and 0·612 mm. longitudinally. The posterior testis measures 0·99 mm. transversely and 0·612 mm. longitudinally (Plate II, fig. 8; Plate IV, fig. 21). In longitudinal sections the anterior testis measures 0·4 mm. transversely, 0·28 mm. longitudinally and the posterior testis measures 0·48 × 0·28 mm. But the testes are placed above the region of the acetabulum and are surrounded by lymph vessels. Arising from the lateral side of each testis is a vas efferens and the two vasa efferentia unite in front of the anterior testis, to form the vas deferens. Its first part is known as the vesicula seminalis, the second as pars-musculosa and the last as pars-prostatica, which is continued into the ductus ejaculatorius. The short and coiled vesicula seminalis

is continued into the pars-musculosa, which is more convoluted. The pars-musculosa is strongly developed in this species. The duct at either end of the pars-musculosa is narrowed. These narrow parts, one between the vesicula seminalis and the pars-musculosa and the other between pars-musculosa and pars-prostatica, are known as pars-intermedia. The pars-musculosa beyond the pars-intermedia is continued as the pars-prostatica. This is a straight tube and is enveloped by the prostate gland cells (Plate I, fig. 5; Plate II, fig. 8). As the pars-prostatica emerges out of the prostate gland cells, it gets considerably reduced in calibre and is called the ductus ejaculatorius. The latter unites with the terminal portion of the uterus and leads into a delicate chamber called the ductus hermaphroditicus (Plate II, fig. 10), which opens out through the genital papilla. The walls of the genital papilla are studded with sphincter papillae (Plate II, fig. 10). The genital atrium is strengthened by the genital-atrial-radial muscles. Besides these muscles there are genital sphincter muscles which are extremely small. The ventral atrium is without such muscles and in itself is not quite distinct. The male genital ducts throughout their course are surrounded by two layers of muscle fibres, an inner circular layer and an outer longitudinal. These are particularly well developed in the region of pars-musculosa (Plate IV, figs. 19, 21).

The female genital system (Plate I, figs. 2, 4) consists of the ovary, the oviduct, the Laurer's canal, the vitellaria and their ducts, Mehlis' glands, the ootype, the uterus and the metraterm. The ovary is situated on the right side of the post-testicular region of the body in front of acetabulum. It is more or less spherical in shape and measures 0·42–0·54 × 0·3–0·35 mm. It is surrounded by lymph vessels. The oviduct emerges from the posterior border of the ovary. After a short free course it enters the Mehlis' gland complex and comes out as the uterus. The Laurer's canal arises from the oviduct at a place where the latter enters Mehlis' gland complex. Then it runs laterally and after crossing the excretory vesicle, opens on the right side of the median line, a little posterior to the excretory pore (Plate I, fig. 4; Plate IV, fig. 18).

The vitellaria are feebly developed. In gravid worms they are prominently seen extending from the sides of the oral sucker along the intestinal caeca up to the middle of the outer sides of the acetabulum. The cells forming the follicles are well differentiated being surrounded by a thin structureless membrane. The nuclei of vitellaria are conspicuous and stain deeply with haematoxylin. The nucleoli pick up a still deeper stain.

The Mehlis' gland complex, like the ovary, is a compact structure and is visible even to the naked eye in flattened specimens. Typically it is more or less in the form of an oval, the long axis being directed transversely. It measures 0·36 × 0·45 mm., and is composed of a large number of cells aggregated together and not enclosed within a membrane. Each cell is club-shaped, radiating out from the ootype, into which it empties itself by a narrow drawn out duct (Plate I, fig. 6; Plate IV, fig. 18). The ootype is a rounded structure, measuring 0·05 × 0·05 mm. It receives a duct of the yolk reservoir, which is formed by the two vitelline ducts, one from each side of the vitellaria.

The uterus winds its way towards the left side of the ovary forming coils, and then proceeds anteriorly dorsal to the testes and opens into the ductus hermaphroditicus through a metraterm, just beneath the ductus ejaculatorius (Plate I, fig. 5). The ductus hermaphroditicus is formed by the union of ductus ejaculatorius and the metraterm, and opens at the tip of the papilla. The genital pore lies in the ventromedian line, just below the bifurcation of the intestinal caeca (Plate I, fig. 2). The eggs, in the specimens sectioned, measure $0{\cdot}104 \times 0{\cdot}06$ mm.

Summary of Conclusions

(*a*) *Paramphistomum* (*Cauliorchis*) *crassum* has been retained as a valid species and is not a synonym of *P. explanatum* Creplin (1847).

The following peculiarities have been observed:—

(*b*) The Body-wall:

Cuticle is thick and papillated.

(*c*) The Muscles:

There are two pairs of retractor muscles which extend from the acetabulum to the oral sucker.

(*d*) The Digestive System:

Intestinal caeca are coiled.

(*e*) The Oral Sucker:

(i) Its lumen is lined with small conical papillæ.

(ii) Interior circular muscles are present in distinct bands.

(iii) Interior longitudinal muscles are interlaced with oblique muscles.

(iv) There is another layer of oblique muscles present next to the interior longitudinal muscles.

(v) Subcuticular cells are in two rows and a few of them are scattered all over its surface.

(vi) Middle longitudinal muscles are present.

(vii) Posterior sphincter muscles are in 3–4 bands.

(viii) Exterior circular muscles are present but feebly developed.

(ix) Basal circular muscles are present in bands at its base.

(x) Anterior sphincter, lateral exterior circular and middle circular muscles are absent.

(*f*) The Acetabulum:

The circular musculature of the acetabulum is not identical in the dorsal and ventral halves.

(*g*) The Excretory System:

The excretory canal is in level with the excretory vesicle.

(*h*) The Lymphatic System:

Two main lymph vessels are longitudinal and their branches surround the internal organs.

(*i*) Ratio between the length of the oral sucker and the length of the body is:—1 : 9·1–1 : 10·5 in flattened specimens and 1 : 4·2–1 : 4·6 in sagittal sections.

(*j*) Ratio between the diameter of the acetabulum and the length of the body is :—1 : 4·1 in flattened specimens and 1 :1·9–1 : 2·3 in sagittal and longitudinal sections.

(*k*) The Genital System:

(i) Cauliflower-like testes are diagonal in position.

(ii) Pars-musculosa and Pars-prostatica are very strongly developed.

(iii) The walls of the genital papilla are studded with genital sphincter papillæ.

(iv) Ventral atrium is without genital atrial-radial layers of muscles.

(v) Genital atrium is with genital atrial-radial layers of muscles.

(vi) Laurer's canal crosses the excretory vesicle.

(vii) The genital pore is situated on an elevated area.

(*l*) Size of the egg 0·104 × 0·06 mm.

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Plate I

Fig. 1. Ventral view of a preserved specimen.
Fig. 2. Shows the internal organs in a balsam mount.
Fig. 3. Sagittal section through the intestinal caecum.
Fig. 4. Sagittal section through the ovary and the Laurer's canal.
Fig. 5. Genital area surrounding the genital pore (enlarged).
Fig. 6. Sagittal section passing through one of the testes, Mehlis' gland complex and excretory vesicle.

Act., acetabulum; *Act. ap.*, acetabular aperture; *B.w.*, body-wall; *D. ej.*, ductus ejaculatorius; *D.h.*, ductus hermaphroditicus; *E.v.*, excretory vesicle; *Fig.*, figure; *G.p.*, genital pore; *Int.c.*, intestinal caecum; *Lym. v.*, Lymph vessel; *L.c.*, Laurer's canal; *M.*, mouth; *M.gl.*, Mehlis' glands; *Mt.*, metraterm; *M.b.*, Mesenterial bands; *Ov.*, ovary; *Oes.*, Oesophagus; *O.s.*, oral sucker; *P.c.*, Perisuctorial cavity; *Pr.g.*, Prostate gland cells; *P.p.*, pars-prostatica; *P.m.*, Pars-musculosa; T_1., anterior testis; T_2., Posterior testis; *Ut.*, Uterus; *Vas. def.*, Vas deferens; *Vit.*, Vitellaria; *Vas.ef.* Vas efferens.

Plate II

Fig. 7. A portion of the oral sucker (highly magnified).
Fig. 8. Median sagittal section through the genital pore and the second testis.
Fig. 9. Body-wall (highly magnified).
Fig. 10. Genital pore (highly magnified).
Fig. 11. Longitudinal section through the main lymph vessels.

C.m., circular muscles; *Cu.*, cuticle; *Cu. pl.*, cuticular papillae; *Ex.C.m.*, exterior circular muscles; *Ex.L.m.*, exterior longitudinal muscles; *G.a.*, genital atrium; *G.a.r.l.*, genital-atrial-radial-layers of muscles; *G.pl.*, genital papilla; *G.s.pl.*, genital sphincter papillae; *G.sph.m.*, Genital sphincter muscles; *I.C.m.*, interior circular muscles; *I.L.m.*, interior longitudinal muscles; *Li.meb.*, limiting membrane; *L.m.*, longitudinal muscles; *Mid.L.m.*, middle longitudinal muscles; *N.c.*, nerve cell; *Ob.m.*, oblique muscles; *R.m.*, radial muscles; *Rt.m.*, retractor muscles; *S.cu.*, subcuticle; *S.cu.c.*, subcuticular cells; *V.a.*, ventral atrium.

Plate III

Fig. 12. A portion of the acetabulum (highly magnified).
Fig. 13. Brain and the nerves in a balsam mount.
Fig. 14. Longitudinal section through the retractor muscles.
Fig. 15. Diagrammatic view of the excretory system.
Fig. 16. Shows the arrangement of lateral circular muscles in the acetabulum.

Br., brain; *C.c.*, connecting canal; *C.v.*, connecting vesicle; *D.e.c.m.*, dorsal exterior circular muscles; *D.i.c.m.*, dorsal interior circular muscles; *E.m.c.*, main excretory canal; *N.*, nerve; *V.e.c.m.*, ventral exterior circular muscles; *V.i.c.m.*, ventral interior circular muscles.

Plate IV

Fig. 17. Diagrammatic view of one half of the oral sucker.
Fig. 18. Diagrammatic view of the relation between the excretory vesicle and the Laurer's canal.
Fig. 19. A portion of the Pars-musculosa (magnified).
Fig. 20. A portion of the intestinal caecum (magnified).
Fig. 21. Sagittal section passing through the excretory pore and the testis.

Ba.C.m., basal circular muscles; *C.e.*, ciliated epithelium; *E.c.*, excretory canal; *E.p.*, excretory pore; *L.c.p.*, Laurer's canal pore; *L.o.s.*, lumen of oral sucker; *P.sph.m.*, Posterior sphincter muscles; *Vit.d.*, vitelline duct.

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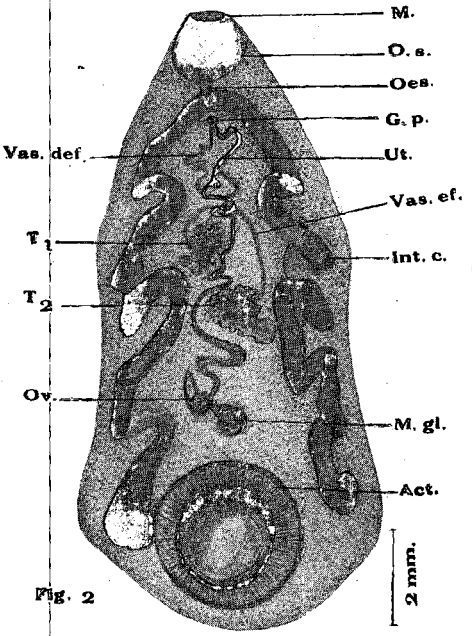

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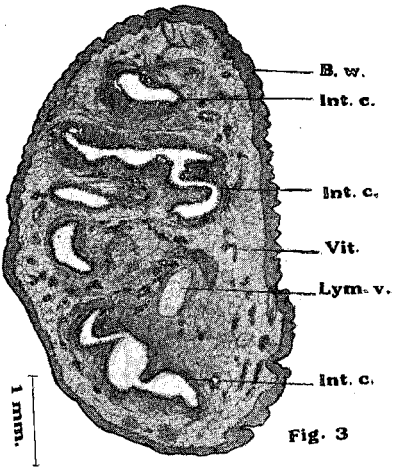

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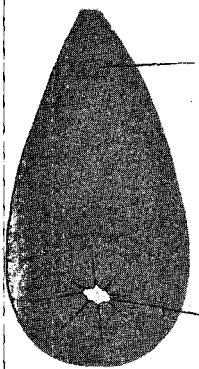

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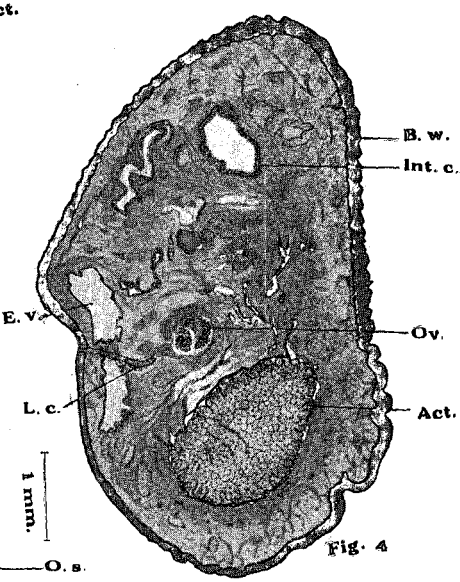

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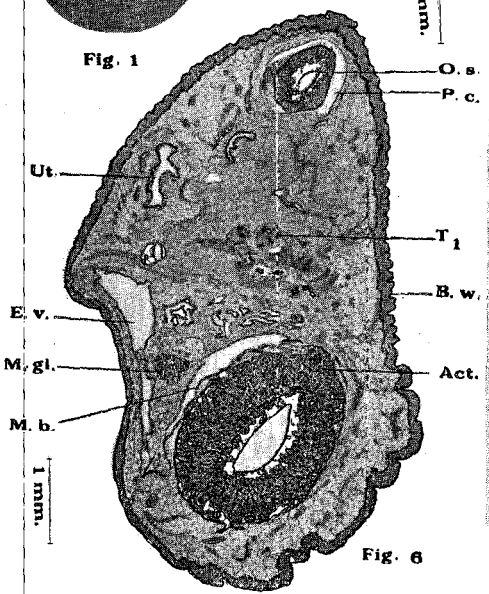

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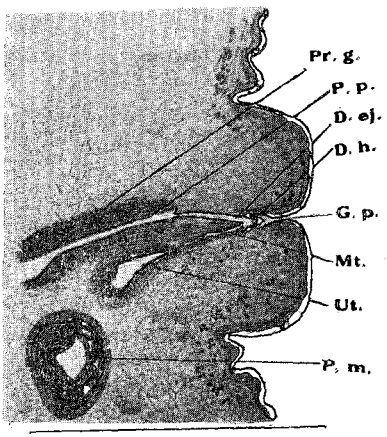

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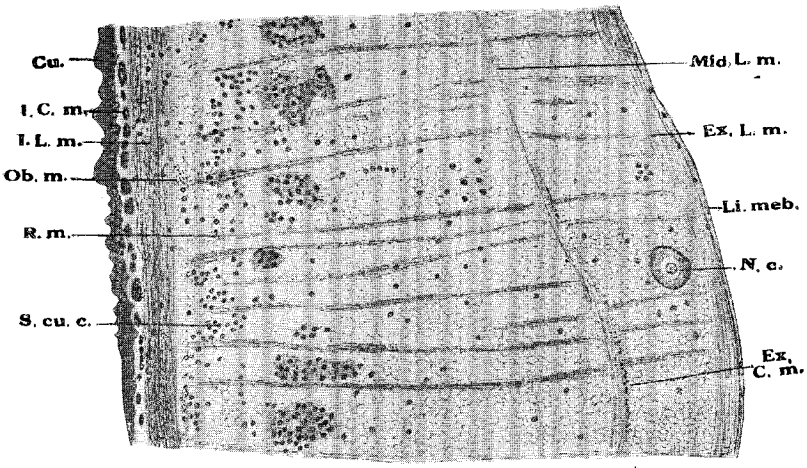

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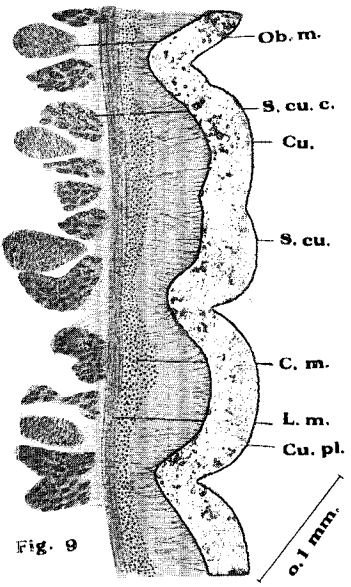

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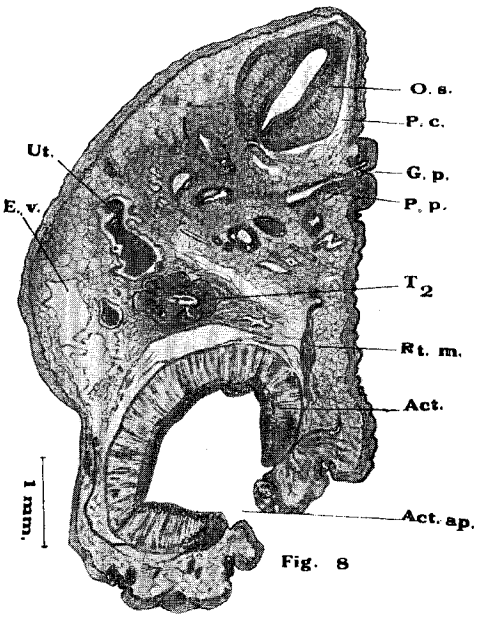

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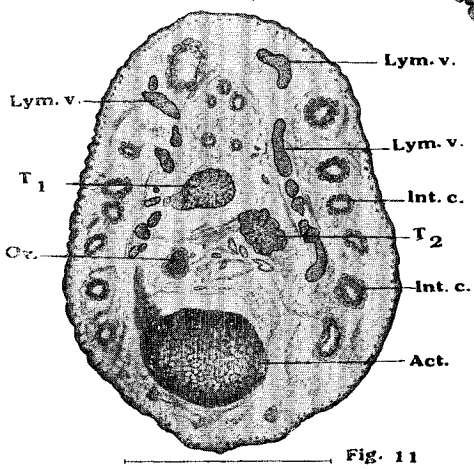

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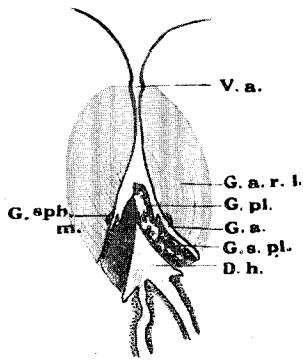

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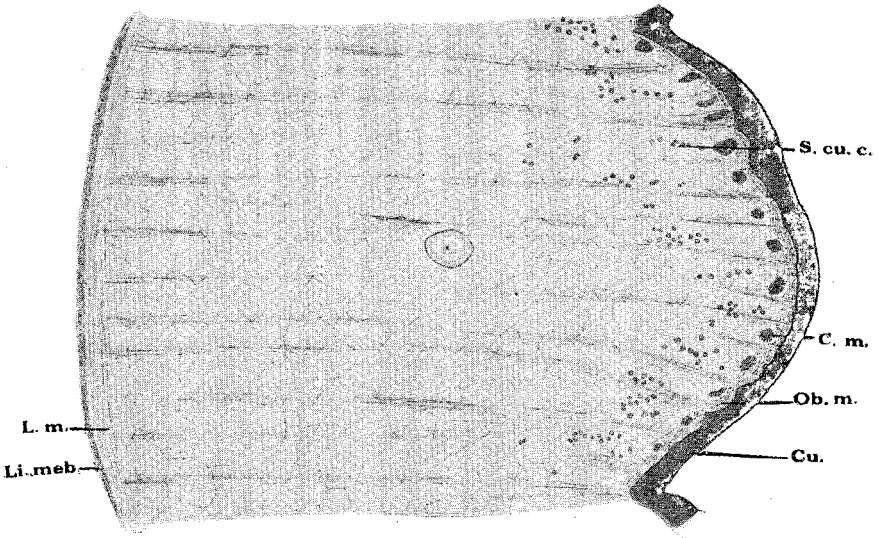

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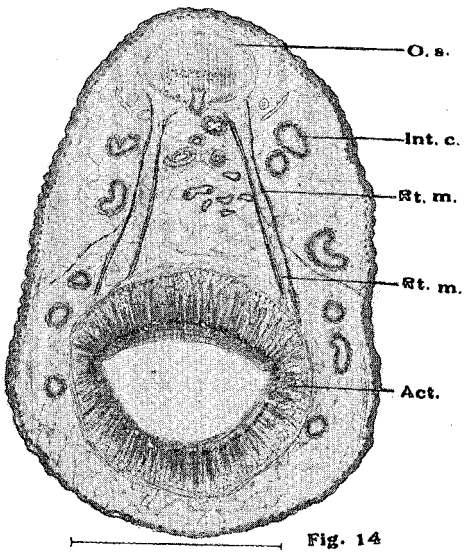

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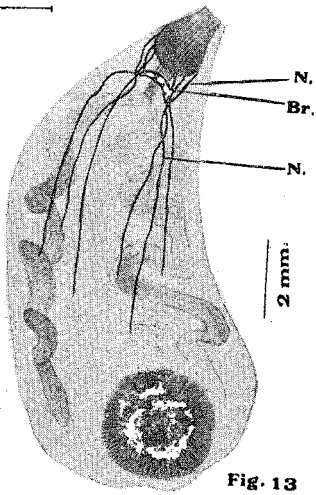
Fig. 14



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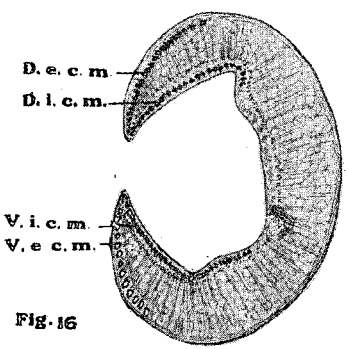

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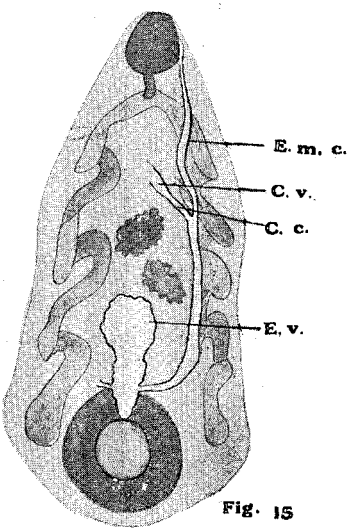


Fig. 15

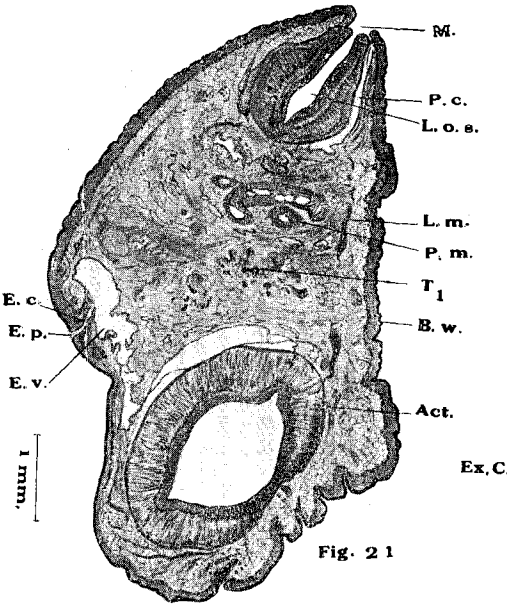


Fig. 21

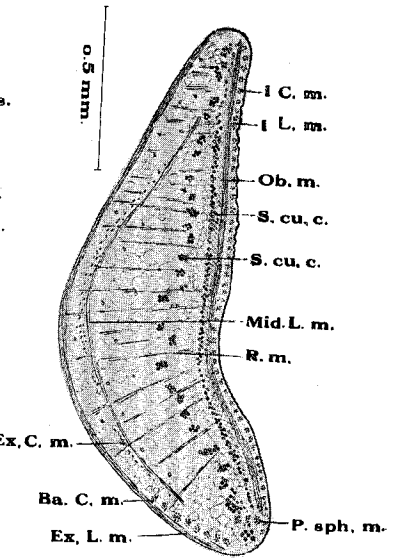


Fig. 17

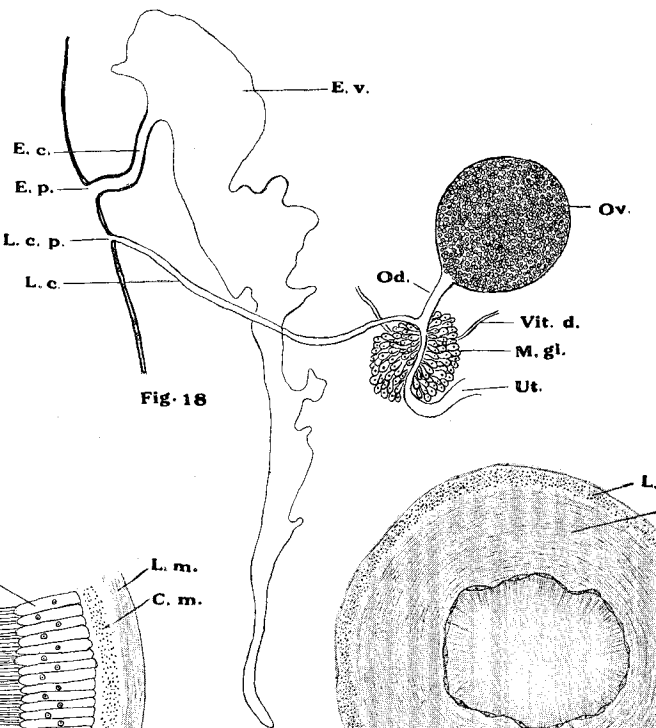
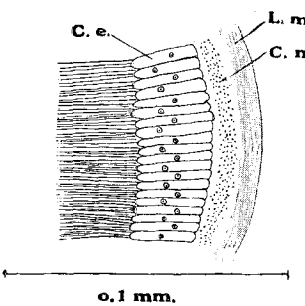


Fig. 18

Fig. 20



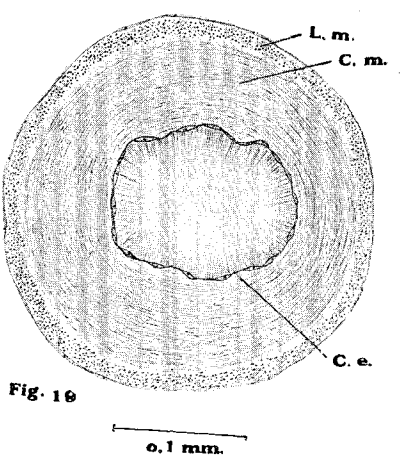


Fig. 19

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 9. ZOOLOGY Pp. 103—110

October, 1950

MORPHOLOGY AND NATURE OF BLOOD AND MEAT SPOTS IN HEN'S EGGS

by

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HOSHIARPUR

Price Rs. 2-4-0.

MORPHOLOGY AND NATURE OF BLOOD AND MEAT SPOTS IN HEN'S EGGS*

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Introduction

Although the eggs with blood and meat spots are quite edible yet the presence of even a slight trace of blood in an opened egg causes such a psychological effect on the mind of the consumers that they cannot help complaining about it to the poultry farmer. The latter, in order to please his customers, takes extra care in candling out these defective eggs and in doing so he sometimes discards even perfectly normal eggs. Since all such eggs are not marketed they constitute a considerable loss to the poultry farmer. The problem of blood and meat spots, therefore, has attracted the attention of a number of workers in recent years.

Previous Literature

Meat spots have frequently been described as pieces of glandular tissue or abnormal growths which have been torn from the wall of the oviduct during the passage of an egg through it. Benjamin and Pierce (1937) believe that these are due to an abnormal condition of the oviduct. Burmester and Card (1938), however, showed that red blood corpuscles or a significant amount of iron was present in all except 3% of the meat spots examined by them.

Evidence was presented by Nalbandov and Card (1941 and 1944) to indicate that the haemorrhages which resulted in the formation of blood clots occurred before ovulation. These blood clots were, according to them, transformed into meat spots by changes in *p*H and high environmental temperatures. The transformation took place either before ovulation, or during egg formation, or even after the egg had been laid. They attempted to prevent intra-follicular haemorrhage experimentally by the administration of vitamins A, C, D, E, K and P but were unsuccessful.

Lucas (1946) prepared smears from blood spots and demonstrated macrophages and fibroblasts in addition to intra-vascular cell types. (It was also suggested that the transfer of viable parent connective tissue cells to the egg might have some significance in the problem of transmission of lymphomatosis and other avian diseases through the egg.)

Denton (1947) observed that in general the eggs from an individual hen contained meat spots which were predominantly of one colour. The

* This work was carried out at the Institute of Animal Genetics, Edinburgh, where the author worked as Government of India Research Scholar during the period 1945–47.

transformation of a blood clot into a meat spot, according to him, was influenced only by the time which elapsed between the haemorrhage and the subsequent removal of the blood clot from the body cavity along with the egg content.

Material and Technique

All the candled out defective eggs laid by the Institute flock of Brown Leghorn birds during the period from November, 1945 to the end of February, 1947 were opened and their contents thoroughly examined. Altogether 297 such eggs formed the basis on which this study was made.

A record was kept of the gross appearance and location of all blood and meat spots encountered.

To determine the actual position of the blood spot the yolk with the blood spot on it was carefully separated from the albumen and after being agitated for a few minutes in 0·8% normal saline was immediately fixed in Formol Saline. Dehydration was done as usual by passing it through up-graded alcohols; cedarwood oil was used as a clearing agent. The yolk was then embedded *in toto* in paraffin but for histological examination only the portion with the blood spot on it was used to prepare sections.

Similarly the coloured and white meat spots were also fixed and prepared for sectioning. During the earlier part of the investigation the fixatives used were mainly Bouin's and Formol Saline, but later on Flemming—without acetic was also employed. Direct study of normal and degenerating yolk immersed in a drop of 2% osmic acid was also made.

Sections were cut 5-6μ thick and, in general, stained with Delafield's hæmatoxylin followed by alcoholic Eosin. The following stains were also used: 0·5% Iron-hæmatoxylin, Thionin blue, Mucicarmine, Mucihæmatin, Giemsa's, Mann's and Wiegert's Fibrin stain. Prussian blue and Feulgen's reactions were employed to detect the presence of iron and nuclear chromatin respectively in white meat spots.

In order to overcome the existing confusion with regard to the classification of foreign substances found in the hen's egg, all the blood formations (dots, streaks, sheets and clots, etc.) which are in close adherence to the vitelline membrane have been included under the term 'blood spot'. The term 'meat spot' has been reserved for all the discrete solid substances lying either free in the egg albumen or entangled in the chalazæ.

Observations

Morphology of Blood Spots

The blood spots vary greatly in size and shape (Plate I, Figs. 1–5). The smallest of them is no larger than a pin-prick and it is extremely difficult to identify such faults by candling. In some eggs, however, the blood spot is so large as to cover almost the whole yolk (Plate I, Fig. 1).

The blood spots are usually in the form of sheets or streaks adherent to the yolk. The sheets may be thin or thick, the latter being darker in colour than the former. Sometimes they are very faint, discontinuous streaks (Plate I, Fig. 3), but they may also be quite thick and distinct (Plate I, Fig. 2). In some cases the blood spot is in the form of a thick and rounded clot intimately attached to the yolk (Plate I, Fig. 4).

Very often it is seen that the blood spot is situated on that pole of the yolk which is towards the broad end of the egg. Occasionally associated with such a blood spot can be seen a blood streak which may be either narrow or broad (Plate I, Fig. 5). This streak may or may not end in a large blood clot but it is usually surrounded by the fibres of the chalaza. Consequently the blood streak also reveals in some cases the typical twisted appearance of a chalaza (Plate I, Fig. 5).

Figure 1 (Plate II) shows the section of a blood-spotted yolk. The spot is in the form of a sheet and can be seen lying in between the two membranes covering the yolk. Figure 2 (Plate II) reveals a portion of the same section under higher magnification. Here can be easily seen a mass of blood cells separated from the yolk by means of the vitelline membrane. The outer membrane covering the blood cells is identified as the chalaziferous layer. Most of the blood cells at this stage are apparently quite normal as their nuclei and cytoplasm take up the usual blood stains (Plate II, Figs. 3 and 4). A few of them, however, degenerate and these are represented only by their nuclei.

Morphology of Meat spots

Meat spots also show great variation in size, shape and colour. They may be found in any part of the egg albumen, lying either free or entangled in the fibres of the chalazæ (Plate I, Figs. 6–8). The smallest of them may be of such an order that it can easily be overlooked by the naked eye in an opened egg, but the largest could readily be distinguished by candling through the shell by even an inexperienced observer. They are usually rounded in shape but may be angular and sometimes elongated. The colour is generally dark red but they may be red, liver-like or of various shades of brown. Some of them are perfectly white or white with some red or brown specks included.

In Figure 5 (Plate II) a section of a red meat spot is shown. It reveals a central mass of blood cells surrounded by a fibrous layer. Figure 1 (Plate III) depicts a portion of the same section under higher magnification. Here the granular substance present in between the central mass of blood cells and the outer fibrous layer is seen infiltrating into the mass of blood cells; the blood cells are still normal except that their cytoplasm does not take up any stain. This, however, is not a general rule but depends rather upon the state of degeneration of the blood cells in particular cases. In Figure 2 (Plate III) a section of another red meat spot is shown in which some of the blood cells are quite normal but the cytoplasm of others does not stain.

With the progress of degeneration the meat spots show extensive vacuolization due to the complete disintegration of blood cells some of which are, at this stage, represented by their nuclei only (Plate III, Fig. 4). These nuclei have a tendency to group together and can be seen in the section of a white meat spot with a red speck in the centre (Plate III, Fig. 5). A piece of the white portion of this meat spot is shown under high magnification (Plate IV, Fig. 1) and reveals it to be made up of coagulated albumen in which are seen masses of degenerating yolk in addition to the blood

cells. A little albumen is invariably coagulated outside the fibrous layer surrounding the coloured meat spots and very often normal and degenerating blood cells can be seen in it (Plate III, Fig. 3). Degenerating yolk can at times be identified in the coagulated albumen and even entangled in the constituent fibres of the layer surrounding the coloured meat spot (Plate IV, Fig. 2).

Generally white meat spots have as their basis such a coagulated mass of albumen but they may show great variation with regard to the amounts of albumen, degenerating blood cells and yolk they contain. In a perfect white meat spot, however, there are few, if any, blood cells and these, if present, are in an extremely degenerate condition. The bulk of a white meat spot consists of coagulated albumen mixed with yolk in various stages of degeneration (Plate IV, Fig. 3).

It is not uncommon to observe a few cells with extreme vacuolation of the cytoplasm in a white meat spot but it becomes increasingly difficult to identify these in view of the general degeneration in progress. Even broken egg membranes are sometimes included in white meat spots. For further details regarding white meat spots please also see Sharma (1949*a*).

Discussion

Origin of Blood and Meat Spots

In general, blood spots are structurally composed of a mass of blood cells lying in between the two membranes enveloping the yolk. The inner covering is the vitelline membrane and the outer is the chalaziferous layer.

As early as 1898, M trophanow also noticed the presence of blood cells in the thickness of the envelope of yolk (enveloppe du jaune) which, according to him, consisted of two layers namely the external or the albuminous layer and the internal or the vitelline membrane. This albuminous coat of the yolk envelope may be identified as the chalaziferous layer.

It has been shown in this study that the coloured meat spots also are encapsuled by a fibrous layer which stains exactly like the chalaziferous layer. It seems highly probable, therefore, that the chalaziferous layer of the ovum and the fibrous covering of the coloured meat spots are similar structures and are secreted in the same region of the oviduct.

During the course of another investigation (Sharma, 1950) evidence has been obtained that the chalaziferous layer is secreted by the posterior half of the infundibulum to which the name 'chalaziferous region' was given by Richardson (1935). It can, therefore, be inferred that blood spots and coloured meat spots are present before the egg reaches this region of the oviduct and in consequence the lower portion of it can safely be excluded as the source of blood and coloured meat spots.

This conclusion is further supported by the fact that in most of the double yolk blood-spotted eggs the blood spot is present on one yolk only.

It was not uncommonly held that blood spots are formed as a result of haemorrhage at the time of ovulation following the rupture of a small blood vessel near the stigma. However, the observations on ovulation in timed laparotomized hens made by Nalbandov and Card (1944) have shown that bleeding may occur at this time but it is so rare and insignificant

a phenomenon that it cannot be held to be an important agency in producing blood spots in eggs. They further state that the bleeding is intra-follicular and that it may occur several days before ovulation. Though they have never observed active intra-follicular bleeding in laparotomized hens they have noted follicles containing small as well as large submembranous haemorrhages in ovaries both excised and in situ. They have also observed the yolks being released with small and large blood clots adhering to them although there was no active bleeding at the time of ovulation.

Experience derived from the present investigation confirms the findings of these workers as follicles were encountered containing submembranous haemorrhages (Plate IV, Fig. 4). How extensive intra-follicular bleeding can be was shown by an abnormal egg laid by a Brown Leghorn hen of the flock at the Institute of Animal Genetics. In this egg, instead of a normal yolk, there was a complete follicle, surrounded by the usual layers of albumen, shell membranes and shell. The follicle appeared to be filled with blood and very little yolk could be detected. (For a detailed description of this egg please see Sharma, 1949*b*.)

As already pointed out by Nalbandov and Card (1944) bleeding may occur anywhere in the follicle. If it is slight the blood remains in between the vitelline membrane and the follicle and thus forms a small blood spot on the former. However, if the bleeding is copious the whole of the vitelline membrane may be smeared with blood and the blood may even accumulate in the pedicel and its funnel-shaped base. This results in the formation of a blood streak with or without a blood clot at its tip and remains attached to the main blood spot on the vitelline membrane. Since many of the formations of this type are seen on that pole of the yolk which is towards the broad end of the egg (Plate I, Fig. 5) and most of the eggs are laid with pointed end first, the hypothesis stated above seems to be quite acceptable. This is further supported by observation on the abnormal egg, referred to above, in which the pedicel contained a blood streak within it.

Frequently during ovulation, this blood streak, with or without a clot at its tip, loses its connection with the main blood spot on the vitelline membrane and may become an inclusion in the albumen of the same egg or of subsequent ones.

When such a blood clot reaches the 'chalaziferous region' of the oviduct it is surrounded by the fibrous layer secreted there and thus forms a meat spot. If this remains lying in the oviduct then, simultaneously with the degeneration of the blood cells, the adherent albumen is also coagulated on its surface giving it a whitish appearance. In these meat spots, however, reddish specks can still be demonstrated in the centre.

According to Nalbandov and Card (1944) all meat spots, including white ones, are merely degenerated blood clots resulting from changes in *p*H of the albumen and that the transformation from red to white meat spot is hastened by high environmental temperatures. It was proved experimentally by using artificial blood clots in albumen and also in buffer solutions of known *p*H exposed to temperatures of 50°F. and 88°F.

A repetition of their experiments carried out at Edinburgh at a temperature of 103°F. did not change an artificial blood clot into a white meat

spot. Blood and meat spots from infertile eggs which had been incubated for more than 7 days were still not white. The colour of the meat spots in such eggs changed from red to dark red, brown or even tan but never to white.

Artificial and natural blood clots kept in 0·8% normal saline changed their colour to pinkish white leaving the medium reddish even at ordinary room temperature after a few days. However, on sectioning these and also the artificial and natural blood clots and coloured meat spots which had been in the incubator for more than a week the same appearance as that of the section of a white meat spot was not obtained. In all sections from the former blood cells in various stages of degeneration could always be seen.

A typical white meat spot, as far as the observations go, is formed in the oviduct by the coagulation of the albumen around free yolk left degenerating in the oviduct by the rupture of the membranes surrounding it. Pieces of the broken membranes are sometimes visible as inclusions in white meat spots. For details please see Sharma (1949*a*).

Frequently blood cells and also some other cellular elements can be detected in white meat spots but, because of the extensive degeneration, it becomes increasingly difficult to identify them. It is quite possible that these cells may be derived, either from the follicular epithelium or from the developing germ of a yolk which has ruptured in the oviduct.

Lucas (1946) has described macrophages and fibroblasts in addition to intra-vascular cell types from smears of blood spots. Though it has not been possible to confirm his findings many of the weird structures figured by him have been noted in sections of white meat spots. It is clear that the problem of white meat spots is one that requires further evidence from an experimental approach towards its solution.

The so-called 'bloody white' appears to be formed by the dissolution of a blood clot as frequent observations have shown that the albumen in the proximity of a large blood spot or a red meat spot is blood coloured. Sometimes it is even possible to detect blood cells in such albumen under the low power of a microscope. This is further substantiated by observations on artificial blood clots which dissolve out, sometimes completely, leaving the medium reddish.

It is also possible that the 'bloody white' may be produced following haemorrhages from the wall of the oviduct. Nalbandov and Card (1944), however, could not succeed in producing 'bloody white' by injecting blood in the magnum region of the oviduct.

The so-called 'cloudy white', according to Nalbandov and Card (1944), results from the bloody white after suitable *p*H changes, provided the temperature is favourable. It appears more probable, however, that the cloudy white is produced by the disintegration of the chalazæ and the dispersion of the free yolk granules.

Summary and Conclusions

1. Blood spots in eggs are due to intra-follicular bleeding. If the haemorrhage is only slight it results in the formation of thin sheets and

streaks of blood in close adherence to the vitelline membrane. Copious bleeding, on the other hand, leads to the formation of large blood clots which, if separated from the yolk, are included in the egg albumen and form coloured meat spots.

2. White meat spots are formed in the oviduct by the coagulation of albumen around a degenerated coloured meat spot, degenerating free yolk, or even broken egg membranes.

3. The so-called 'bloody white' appears to be due to the dissolution of a large blood spot or a coloured meat spot.

4. Cloudy white may be formed by the disintegration of the chalazæ and the dispersion of degenerating free yolk granules.

Acknowledgments

I wish to express my sincere thanks to Dr. A. W. Greenwood for his very kindly going through the manuscript of this paper. Thanks are also due to Mr. G. R. Knight for taking the photo-micrographs.

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Explanation of Plates

Plate I

Figs. 1–5: *Various types of Blood spots.*

Fig. 1. A very large blood spot covering almost the whole yolk.

Fig. 2. Blood spot consists of distinct sheets and streaks adherent to the yolk. The blood streak towards the left (broad end of the egg) is continued into the albumen and ends in a large dark red blood clot.

Fig. 3. Blood spot consists of faint and discontinuous streaks on the yolk.

FIG. 4. Blood spot consists of a dark red thick and rounded blood clot intimately attached to the yolk. Two small blood dots can also be seen near it.

FIG. 5. The blood sheet is situated on that pole of the yolk which is towards the broad end of the egg. It is continued into the albumen in the form of a thick and dark red blood streak which ends into a large blood clot of the same colour. The blood streak presents a typical twisted chalaza-like appearance due to the chalazal fibres surrounding it.

FIGS. 6–8. *Various types of Meat spots.*

FIG. 6. A red meat spot entangled in the free end of the chalaza.

FIG. 7. Meat spots of various colours in the albumen and two small blood streaks on the yolk.

FIG. 8. White meat spots in the albumen and a blood sheet on the yolk.

Plate II

SECTIONS

Unless otherwise mentioned, all sections have been stained with Delafield's hæmatoxylin and eosin.

FIG. 1. Section of a blood-spotted yolk. ×40.

FIG. 2. A portion of the same section. ×350.

FIG. 3. Section of another blood-spotted yolk. ×350.

FIG. 4. Section of a blood-spotted yolk stained with Thionin. Here the chalaziferous layer stains purplish pink while the vitelline membrane is pale blue. ×700.

FIG. 5. Section of a red meat spot stained with Delafield's hæmatoxylin and muci-carmine. ×40.

Plate III

SECTIONS

Unless otherwise mentioned, all sections have been stained with Delafield's hæmatoxylin and eosin.

FIG. 1. A portion of the section shown in Fig. 5, Plate II. ×350.

FIG. 2. Section of another red meat spot. Some of the blood cells are quite normal. ×350.

FIG. 3. Section of a red meat spot where the blood cells can even be seen outside the fibrous layer embedded in the coagulated albumen. ×350.

FIG. 4. Section of a dark red meat spot showing extensive vacuolization due to the disintegration of blood cells. 0·5% Iron-hæmatoxylin and eosin. ×45.

FIG. 5. Section of a white meat spot with a red speck in the centre. ×40.

Plate IV

FIGS. 1–3. *Sections stained with Delafield's hæmatoxylin and eosin.*

FIG. 1. A portion of the section shown in Fig. 5, Plate III. ×350.

FIG. 2. Section of a tan-coloured meat spot from an infertile egg which had been incubated for nearly two weeks. ×300.

FIG. 3. Section of a white meat spot. ×20.

FIG. 4. Excised ovary showing submembranous hæmorrhages in the follicles.

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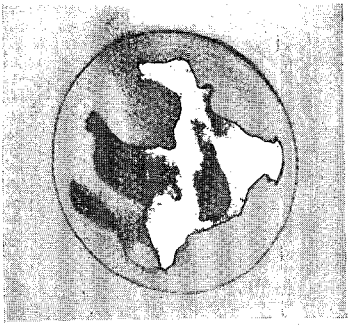

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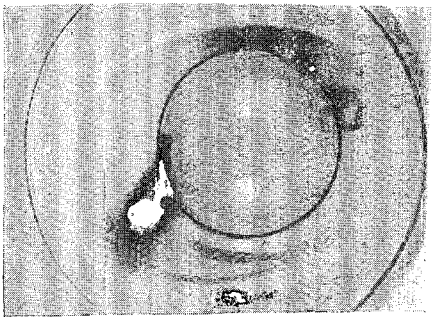

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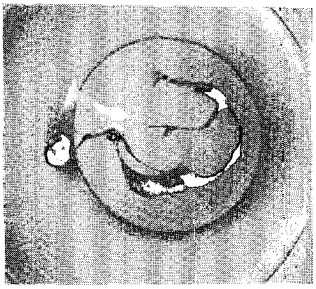

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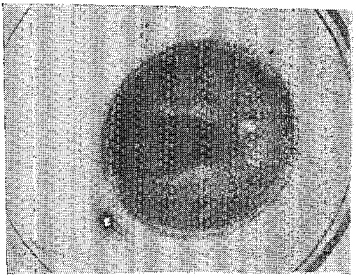

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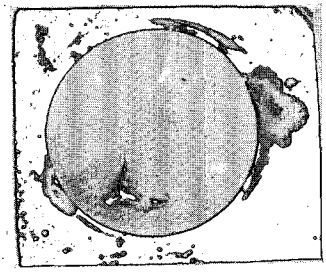

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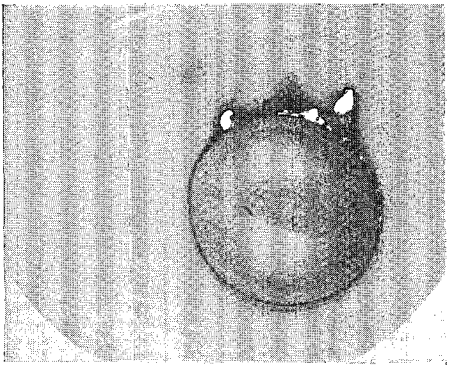

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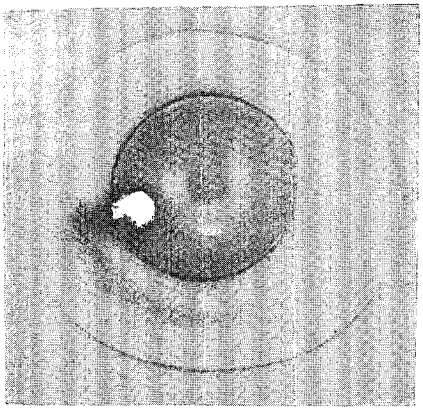

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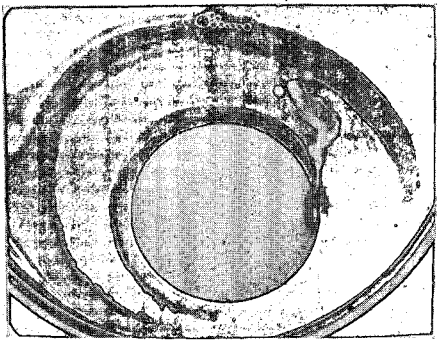

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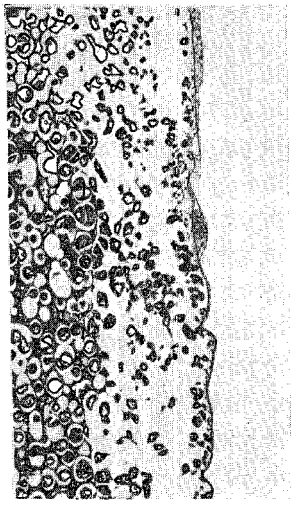

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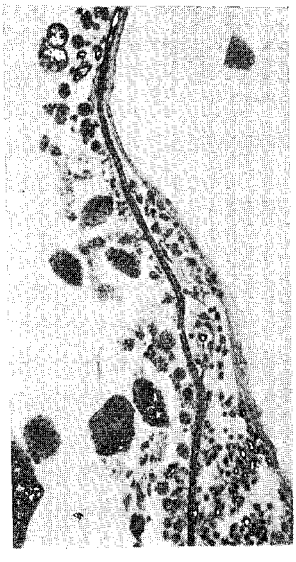

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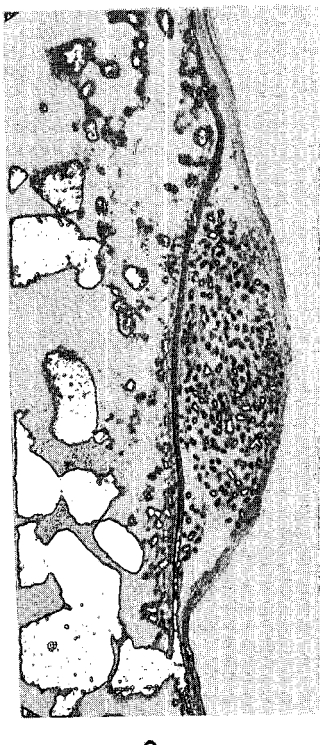

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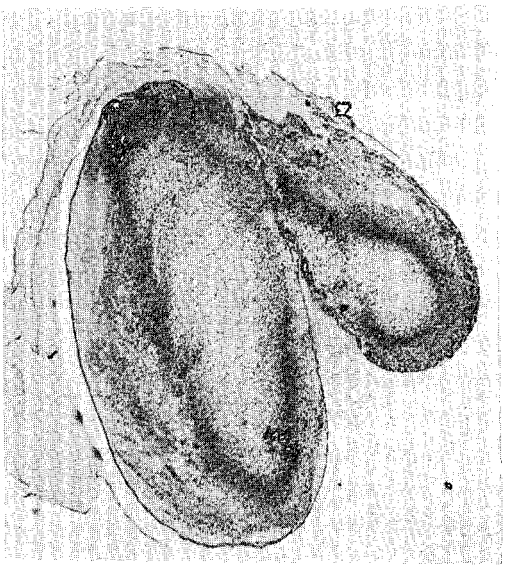

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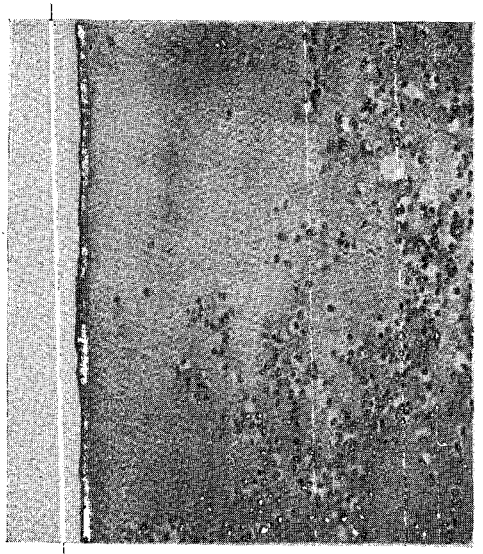

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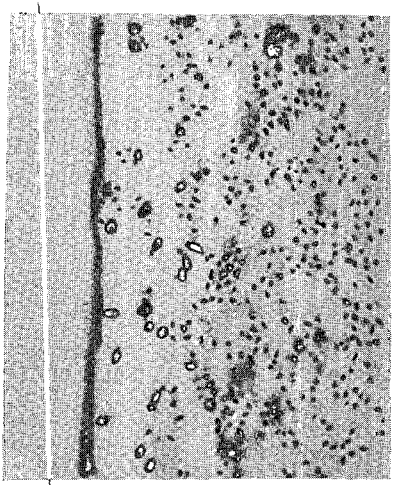

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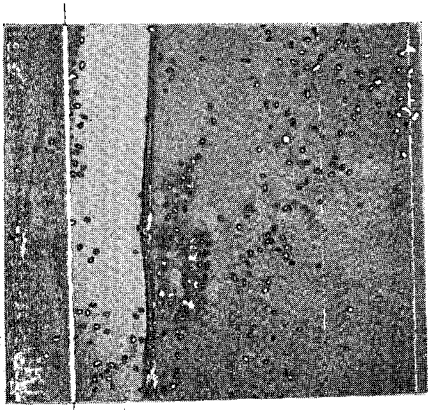

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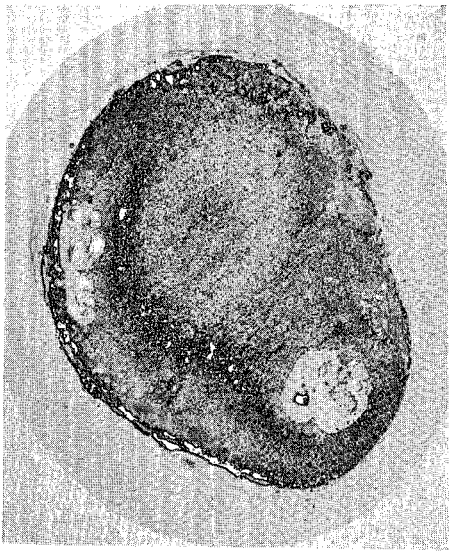

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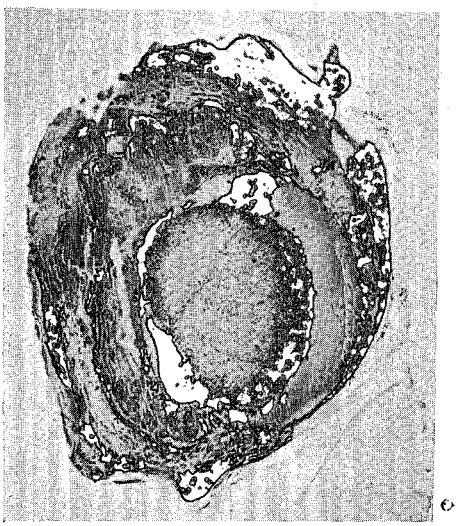

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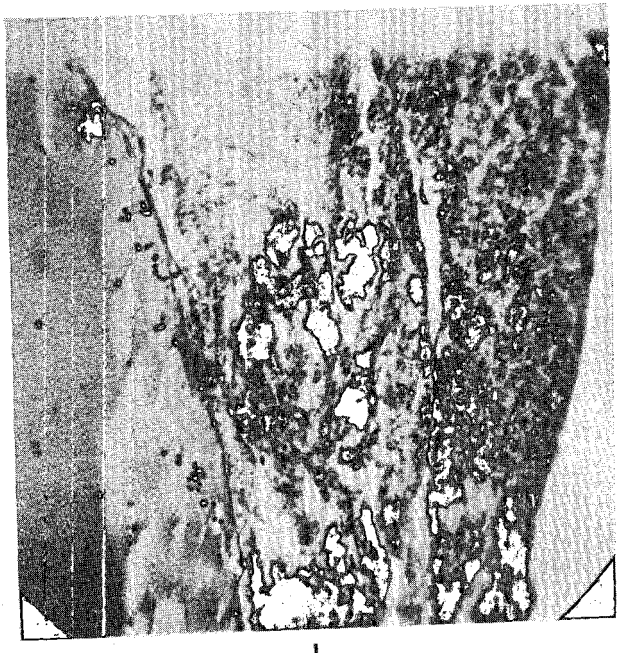

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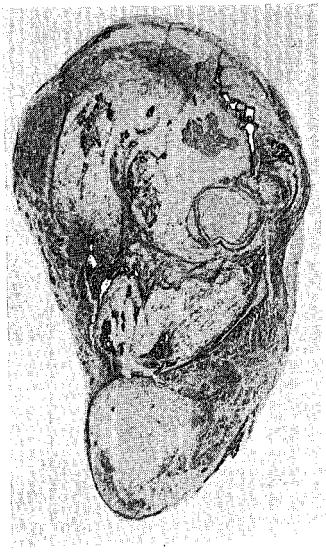

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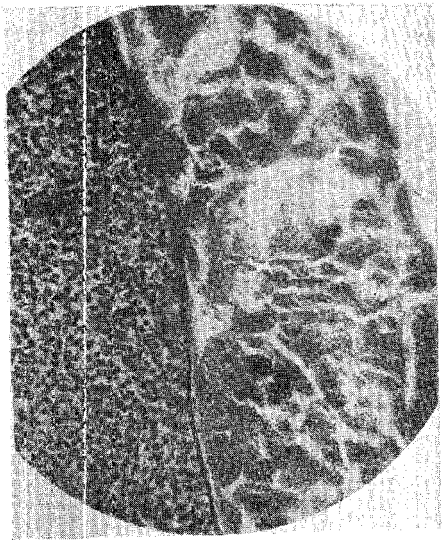

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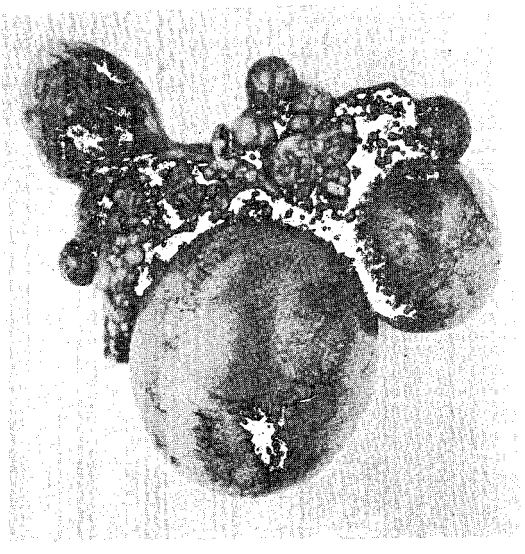

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RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 10. PHYSICS Pp. 111—116

November, 1950

ON THE STORM VARIATIONS OF COSMIC RAY INTENSITY

by

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Edited for the East Panjab University by Vishwa Nath,
Department of Zoology, East Panjab University
HOSHIARPUR

Price Re. 1-4-0.

ON THE STORM VARIATIONS OF COSMIC RAY INTENSITY

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Abstract

A brief discussion of the magnetic storm effects on cosmic radiation is followed by the analysis of observations taken at Lahore (22° N.) during February 12–April 8 (1947), a period of pronounced solar activity. The Cosmic Ray intensity I was measured by a triple coincidence telescope and the magnetic data for the same period obtained from the nearest magnetic observatory at Alibag (Bombay). It is found that over this period of almost two months the correlation coefficient between I and H though showing a slight positive tendency, is not statistically significant. However, during fairly strong storms on February 16, and March 2, the two quantities $\Delta I/I \; \Delta H$ and $H \; \Delta I/I \; \Delta H$ are negative in the first, and positive in the second storm. The variations are discussed in the light of Chapman's and Alfven's hypotheses.

Introduction

It is well known (Hess, 1939) that regular and irregular variations of cosmic ray intensity I with time, after being corrected for temperature and pressure changes, do exist; and that some of these variations are simultaneously observed at widely separated places all over the globe. It is also observed (Forbush, 1937; Hess and Demmelmair, 1937; Duperier, 1942; Hess and Illing, 1935; Sarna and Parkash, 1949), that, generally speaking, during the time of appearance of the sun spots, earth's horizontal magnetic field H shows large fluctuations and simultaneously large changes in I are observed.

For instance, in a storm occurring on February 27–March 6 (1942) Duperier (loc. cit.) observed a 12% decrease in I and in another storm (January 29–February 12) in 1946, Duperier and McCaig observed a 10·5 $\pm$0·4% decrease. A negative correlation existed between I and H during these storms although, according to Clay and others in the case of storms on March 1, 1942, the correlation is found positive if the calculation had been restricted to this day, which from their point of view is undoubtedly the most important of this storm. Forbush (1938) and Demmelmair observed a very pronounced positive correlation (I increasing with H) between the intensity changes of cosmic rays and geo-magnetic horizontal force H during several storms in 1937-38. Forbush also reported the case of a storm occurring on August 21–25 (1937) when a decrease and a following increase of H by 120γ ($\gamma = 10^{-5}$ gauss) produced almost no change in I. There are also cases (Duperier, and McCaig, 1946; Neher

and Roesch, 1948; Dolbear and Elliot, 1947) when no appreciable change in H was observed but C.R.I. showed variations of the same order as observed in strong magnetic storms. The storms are termed 'effective' and 'non-effective' accordingly, though the cause is not clearly understood. Another important observation by Clay and Bruins (1938) at 54° latitude showed that under 110 cm. of Fe shield they did not notice any change in I. This means that harder rays ($E > 8 \times 10^9$ ev) are not affected.

The magnetic storm effects on I are quantitatively expressed as $\Delta I/I/\Delta H/H = H\Delta I/I\Delta H$, i.e. the ratio of the relative change in cosmic ray intensity and of the horizontal magnetic force. It is found (Ref. 1) that this ratio does not remain constant but varies from storm to storm.

Thus, out of this mass of diverse results, it appears that if at all, there is any relation between I and H variations, it will not be so simple. In fact T. H. Johnson (1938) has remarked that in spite of the close relationships observed between I and H the two quantities may not be casually related. However, the fact, that storm variations of cosmic ray intensity are world wide (these variations in I have been observed from 0·6° geomagnetic lat. (Huancayo) to 75° geo. lat. (Godhavan, i.e. equator to the poles)) a study of these effects at different latitudes can be helpful in giving valuable information about the energy distribution in the spectrum of cosmic radiation.

The observations of which the analysis is reported in this paper were taken with a view to find if any correlation existed between I and H at the lat. of observation, i.e. 22° N. (Lahore). These observations extend over two months period (February–April 1947) when there was pronounced solar activity and some strong magnetic storms.

Experimental Procedure and Results

A triple coincidence counter telescope (Parkash and Sarna, 1948) was employed for the present study. The cathodes of the counters were 2·5 cm. in diameter and 35 cm. long and with a distance of 25 cms. between the extreme counters, the zenith resolution was 11·3°. The telescope was set up vertically with its plane in the magnetic meridian and hourly counting rate, observed during the daytime for nearly two months, beginning from 12th February to 8th April, 1947. Hourly values of H for the same period were obtained from the nearest magnetic observatory at Alibag (Bombay). After correcting the counting rate for the temperature (temp. coeff. = −0·18%/°C.) and pressure (pressure coeff. = −6·4%cm. Hg) the daily means were calculated. The mean of the horizontal intensity H was also found for each day for the same period of the day. The results are shown in fig. 1. It is found that the correlation coefficient (r) given by

$$r = \frac{\Sigma (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\Sigma (x_i - \bar{x})^2}\sqrt{\Sigma (y_i - \bar{y})^2}}$$

(where x_i and y_i are the two variables and $\bar{x}$ and $\bar{y}$ their means) between the daily means of I and H over this period is positive. ($r = +0{\cdot}14$), showing a tendency towards parallelism between I and H although its value

is too low to be statistically significant. For the individual storms which occurred on the 16th of February and 2nd March (1947), H changed by 135 and 253 gamma respectively during a period of nearly four hours. Our observations of I in relation to H expressed as

$$\frac{\Delta I}{I \Delta H} \text{ and } \frac{H \Delta I}{I \Delta H}$$

come out to be $+ 0{\cdot}097 \pm 0{\cdot}031\%/\gamma$ and $+ 38{\cdot}3 \pm 12{\cdot}3$ in the storm occurring on 2nd March, and $-0{\cdot}103 + 0{\cdot}060\%/\gamma$ and $-39{\cdot}7 \pm 23{\cdot}0$ in the storm on February 16. (For these particular storms, the calculations were made on the basis of hourly means.) The values seem to correspond with those of other observers, though the errors in our case are somewhat larger.

Discussion

Our results for a period of two months indicate that I values at Lahore and H values at Alibag (Bombay) during magnetically less disturbed days may not be correlated, as the magnetic changes observed at one place may not be effective at another distant place. However, during strong storms, the changes are observed simultaneously all over the earth and in storms of 16th February and 2nd March we found a definite effect on I. The important thing is, that at the same place, the two storms gave opposite correlation, in one negative and in the other positive.

In order to explain these we consider the storms in general. As mentioned earlier, one finds that during a magnetic storm, any one type of change, out of the following might take place :—

(i) An increase in I with H (+ve correlation).
(ii) A decrease in I with H (−ve correlation).
(iii) Large change in I with no apparent change in H.
(iv) No change in I though H might show a large change.

Further that these changes occur and have been observed right from the equator (Huancayo 0·6°) to the pole (Godhavan 75°) and as shown by Clay and Bruins in their observations at 54° Lat., harder rays ($E > 8 \times 10^9$ ev) observed under a shield of 110 cm. Fe remained unaffected.

The above facts may be accounted for in terms of the Störmer and Lemaitre-Vallarta theories. According to these views any change in H should affect the cosmic ray intensity. At a given place the minimum energy, that can reach, is defined by the geomagnetic latitude, hence assuming a certain energy spectrum any change in H will allow or cut off a part of the spectrum thereby producing an increase or a decrease in I. Alfven (loc. cit.), however, has the view that (i) as these changes occur even at 75° Geo = Lat. where changes in H should not produce any effect on I and (ii) that changes in I as much as 20% cannot be accounted for on the above views, the above hypothesis is untenable. Not only that, the above theory demands an antiparallelism between I and H whereas people have reported definite positive correlation. Lemaitre-Vallarta theory completed with a ring-current hypothesis as given by Clay and Bruins gives a satisfactory explanation of the parallelism between I and H.

According to Alfven, the variations observed in I are caused by the electrostatic potential changes of the earth due to the approach of ion clouds from the sun. During a storm a cloud of the ions emitted from the sun, and moving in the sun's magnetic field, is polarized with the advancing side becoming negative and the following one positive and hence a potential difference $\approx 50\times10^6$ volts or even higher (10^9) volts exists between the two ends of the cloud. As the earth quickly attains the potential of the cloud, it becomes negative in the beginning and positive at the end of the storm, giving an increase followed by a decrease in cosmic ray intensity; assuming of course that cosmic rays are predominantly positive. As Alfven has shown, these clouds moving with $v \approx \cdot01$ c. the 'head' of the ion cloud would take about a day in reaching the earth, the storms should start a day after the occurrence of solar prominences and the decrease a day or two still later. This is generally the case.

Alfven's hypothesis is, however, incapable of explaining (i) Clay and Bruins observations which show no change observed in cosmic ray intensity at energies $> 8\times10^9$ volts. The electrostatic potential effect should extend over the whole of the spectrum (though with different magnitudes depending on the shape of the energy spectrum) and a change should be observed even in this region.

(ii) Our case of variations in two storms with apparently no connection between the two.

(iii) The observations of Dolbear and Elliot where an initial increase in I was observed at about 18 hours on the 25th of July and a very intense flare occurring at 17 hour the same day. Neher and Roesch have confirmed the fact that the start of cosmic ray effect and the visual part of the solar flare differ by about an hour, in this particular case.

Stormer's hypothesis of 'Ring Currents' (with which Chapman explains the positive correlation), in a modified form, can explain all the observed effects. According to this ion clouds emitted from the sun reaching the earth, set up ring currents in the equatorial plane round the earth, at a distance of a few times the earth's radius. The ring current increases the field outside it, while inside, it is decreased, and as most of the deflection of charged particles takes place outside the ring, the positive correlation between I and H is explained. This hypothesis, as it is, cannot explain all the observed facts and needs to be modified in the light of fresh results.

According to Clay and Bruins and Forbush all the effects can be explained by assuming (i) that the ring currents always exist and (ii) that their distance and strength may vary from storm to storm (2–16 times the earth's radius and 10^5–10^7 amps).

As the $-$ve ion cloud approaches the earth, the eastwardly $-$ve ion current which we suppose to be always existing is strengthened giving an increase in H inside the ring but a decrease outside. At the beginning of the storm H will, therefore, rise, which is generally so observed in storms, so also the cosmic ray intensity giving a positive correlation. However, if the ring is formed at a greater distance (within the range of course), the deflections may be appreciable inside the ring and we will observe a decrease in I with increase in H (negative correlation). The relative magnitudes of the speci-

fied quantities will depend on the strength of the ring current as well as its distance from the earth. Both these factors may vary from storm to storm giving different magnitudes of the observed effects. Sometimes large changes in I are observed without any appreciable change in H. In a particular case reported by Neher and Roesch 18% increase was observed at a Geo-mag. lat. of 40°, the actual storm started 26·5 hours afterwards. The observed increase coincided with a very prominent solar flare which took place nearly simultaneously. Alfven's reference is probably towards this increase which seems difficult to explain. Neher and Roesch (see also Forbush Gill and Vallarta, 1949) have explained this increase by assuming that under favourable circumstances, the sun might emit very high energy charged particles, and in the case reported by Neher and Roesch at 40° Lat. they must possess energies 6 B ev. Assuming the validity of such a process, the increase observed at 75° may be explained (as at such a latitude, lower energy is needed, and that might be comparatively easy to get). We are inclined to agree with Alfven that normal field fluctuations should produce no effect at so high a latitude. Both solar flares and storms have, therefore, to be considered in discussing the changes observed.

Another case in which H showed large variations though no change in I was observed is explained by Clay and Bruins. According to them, the cosmic rays need not be always affected by storms. A ring current at a distance of 15 times the radius of the earth does not influence the cosmic ray intensity but can give an appreciable variation of H. All other effects can be explained by assuming the existence of the rings between 2–4 × earth's radius.

The observations of Clay and Bruins with 110 cm. Fe shield ($E > 8 \times 10^9$ ev) are also explained in terms of ring current and Stormer Vallarta theories. According to this theory, lower end of the energy spectrum should be affected by changes in H and if this portion is cut off by shield of 110 cm. thickness, the rest of the spectrum should remain unaffected. No change should be observed under such conditions, as shown by Clay and Bruins. This, however, cannot be explained on Alfven's hypothesis, which would demand a variation throughout the energy spectrum, *though the actual magnitudes might differ in different energy regions.*

The changes observed can also be easily explained on the above ideas. However, in order to have a clear and better understanding of the subject, a co-operated programme of observing any such changes with apparatus of similar geometry at different latitudes can be of real help.

Acknowledgments

In the end the authors are grateful to Dr. Alexandar Wolsky, Chief Scientific Officer, UNESCO, for obtaining meteorological observations from Pakistan and to the Director of Colaba and Alibag Observatories, for supplying us with detailed magnetic data necessary for the calculations. They are also thankful to Prof. Clay, University of Amsterdam, for going through the paper and making suggestions.

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Published by the East Panjab University, Hoshiarpur, and Printed by Norman Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

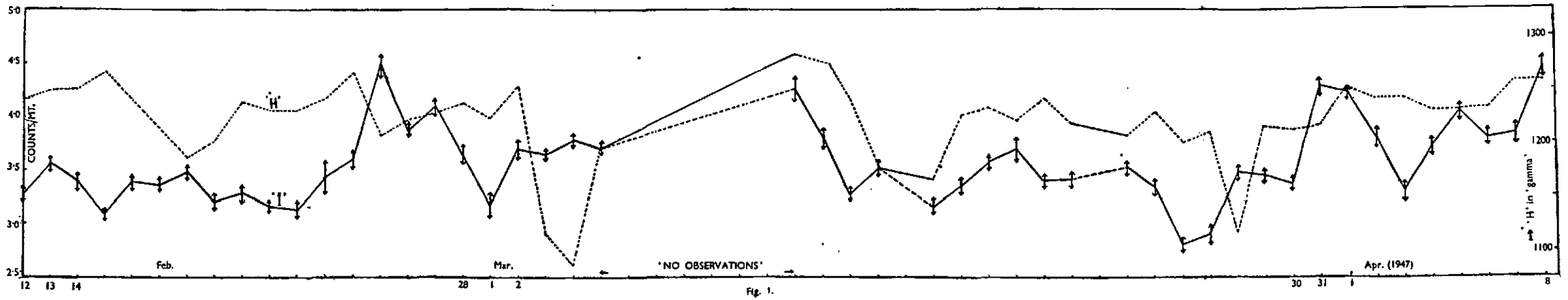
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Fig. 1.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 11. CHEMISTRY Pp. 1—16

January, 1951

THE TERNARY SYSTEM ANTIMONY-CADMIUM-BISMUTH

by

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Edited for the East Panjab University by Vishwa Nath,
Department of Zoology, East Panjab University
HOSHIARPUR

Price Rs.3-0-0

THE TERNARY SYSTEM ANTIMONY-CADMIUM-BISMUTH

By RAM CHAND, M.Sc., Ph.D., *and* VED PARKASH, M.Sc.

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The three binary systems involved in the problem are Antimony-Cadmium, Cadmium-Bismuth and Bismuth-Antimony. These systems are represented in one diagram (Fig. 1) along with the ternary system. The necessary thermal data are taken from 'Landolt—Borenstein Physikalisch—Chemische Tabellen' which contains a summary of previous work, and 'Metal Industry handbook' (1945).

SYSTEM ANTIMONY-CADMIUM

The full curve in the diagram (Fig. IA) corresponds with the condition of stable equilibrium and has been obtained only after inoculation with crystals of the compound Cd Sb. The dotted curve representing a metastable condition is realized in alloys cooled without stirring or inoculation. The stable curve has a maximum at 50 at. % antimony, with a eutectic point Cd Sb—Sb at 58·4 at. % antimony. In the absence of inoculation, however, the curve corresponding with the separation of free antimony is prolonged downwards, and when the separation of a second solid phase does take place it is not the stable compound Cd Sb that crystallizes but the metastable compound Cd_3Sb_2 which has its own solubility curve with a maximum at 40 at. % Sb and 423°C. as maximum temperature, i.e. a temperature 32°C. lower than that of the stable compound. This is in accordance with the general rule that metastable modifications melt or freeze at lower temperatures than the stable ones. To make clear the whole system let us consider an alloy containing 56 at. % antimony. If slowly cooled and inoculated it deposits crystals of Cd Sb (the stable compound) and shows a eutectic point 445°C. If cooled without inoculation, crystallization does not set in until later when antimony separates as the first phase and there is a eutectic arrest at 402°C. corresponding with the simultaneous separation of Sb and Cd_3Sb_2. On further cooling the reaction

$$Cd_3Sb_2 + Sb \rightarrow 3CdSb$$

occurs with considerable evolution of heat, and the solid alloy passes more or less completely, into the stable condition. To the left of the vertical line at 50 at. % antimony the compound Cd_3Sb_2 appears to be stable at low temperatures and occurs along with CdSb. But below 40 at. % antimony, only Cd_3Sb_2 is found along with Cd—Cd_3Sb_2 eutectic at 290°C.

SYSTEM BISMUTH-ANTIMONY

The two metals are completely miscible in both liquid as well as solid state, i.e. they form a series of solid solutions showing neither a maximum nor a minimum temperature.

System Bismuth-Cadmium

The freezing point curve has been determined by Kapp and Stoffel. The system is of a simple type in which there is a eutectic lying at 44·5 at. % or 59·8% Bismuth and 146°C. According to the investigations of Hycock and Neville the two metals do not form solid solutions and are entirely non-miscible in the solid state. But there is a difference of 20°C. in the readings of Kapp. and Stoffel. Barlow has given a summary of the previous work and has given 146°C. as eutectic temperature. G. I. Petrenko and A. S. Fedorov give 140°C. as the eutectic temperature. In view of the existing differences regarding the details of the system, it was repeated. It was found that eutectic temperature is 140°C. and not 146°C. as given by certain authors. The thermal data and diagram for this system are given in Table I and Fig. 2.

Table I.

No.	Percentage Cd	Percentage Bi	Freezing Points.	
			I	II
1	80	20	258	140
2	65	35	220	140
3	55	45	187	140
4	45	55	164	141
5	25	75	178	140
6	20	80	192	140
7	10	90	230	..
8	38	62	..	140

Thermal Investigation.—Antimony, Bismuth and Cadmium metals employed throughout this work were pure, almost free from foreign matter, and their freezing points agreed with the standards. The freezing points and other thermal arrests of the alloys were taken by means of a platinum-nichrome thermocouple. The free ends of the couple were attached to a sensitive mirror-galvanometer. By tracing a spot of light reflected from the galvanometer mirror on to a moving screen, the thermal arrests were obtained on direct time-temperature cooling curves. The couple wires at their brazed end were covered with a very thin layer of fireclay cement and inserted in the molten alloy. This formed a protection for the couple against the attack of metals and at the same time did not impair its sensitiveness. On account of its fragile nature the coating had to be repaired from time to time but this was not a long and tedious process. The thermocouple was standardized by means of the following fixed points: B.P. water = 99·5°C., M.P.Tin 232°C., M.P. Bismuth 268°C., M.P. Cadmium 321°C., M.P. Zinc 418°C., M.P. Antimony 630°C. The standardization was checked after analyzing six samples.

A 150 gram sample of each alloy was prepared by weighing out the requisite quantities of the metals on a sensitive balance. Powdered charcoal was used as protection against oxidation during heating and cooling of alloys. All proportions of the metals in the alloys were reckoned in weight per cent. The rate of cooling and the speed of the screen were regulated

to be the same for all alloys. It took about two hours for an alloy to cool from 630°C. to about 100°C. The results of the thermal analysis are given in Table II.

TABLE II.

Thermal Arrests of Alloys in the System Bi-Sb-Cd.

Alloy No.	Composition in % by weight.			Thermal arrests. °C.			Number of layers in the solidified alloys.
	Cd	Sb	Bi	I	II	III	
1	5	90	5	620	..	..	Only one
2	10	85	5	592	384	..	
3	5	85	10	618	..	..	
4	15	80	5	568	389	..	
5	10	80	10	580	376	..	
6	5	80	15	585	..	..	
7	20	75	5	549	395	..	
8	10	75	15	572	368	..	
9	25	70	5	516	392	..	
10	15	70	15	554	372	..	
11	5	70	25	567	..	..	
12	30	65	5	489	393	..	
13	15	65	20	548	367	..	
14	30	60	10	483	395	..	
15	20	60	20	514	423	..	
16	15	60	25	529	393	..	
17	5	60	35	548	324	..	
18	40	55	5	407	391	..	
19	25	55	20	485	391	..	
20	15	55	30	498	365	..	
21	45	50	5	407	..	..	
22	35	50	15	398	..	..	
23	25	50	25	455	370	..	
24	10	50	40	506	368	..	
25	50	45	5	407	..	..	
26	45	45	10	394	..	..	
27	35	45	20	390	383	..	
28	20	45	35	446	370	..	
29	5	45	50	494	336	..	
30	55	40	5	411	..	..	
31	45	40	15	394	..	..	
32	40	40	20	390	..	..	
33	30	40	30	380	..	..	
34	20	40	40	438	368	..	
35	10	40	50	475	326	..	
36	60	35	5	407	..	..	
37	55	35	10	401	..	154	
38	40	35	25	388	..	..	
39	35	35	30	388	..	..	
40	30	35	35	376	..	..	
41	25	35	40	377	..	..	
42	15	35	50	421	355	..	
43	5	35	60	469	306	..	
44	65	30	5	392	282	144	
45	50	30	20	381	257	148	
46	40	30	30	376	..	158	
47	35	30	35	369	320	175	
48	30	30	40	364	..	..	
49	25	30	45	373	..	..	
50	10	30	60	..	292	..	
51	70	25	5	360	281	144	
52	60	25	15	377	242	150	
53	50	25	25	368	..	158	

TABLE II—(Contd.)

Alloy No.	Composition in % by weight.			Thermal arrests. °C			Number of layers in the solidified alloys.
	Cd	Sb	Bi	I	II	III	
54	40	25	35	..	..	..	
55	30	25	45	..	..	..	
56	25	25	50	325	293	..	
57	15	25	60	..	..	..	
58	5	25	70	419	251	..	
59	75	20	5	323	259	144	
60	60	20	20	327	262	144	
61	50	20	30	346	225	159	
62	40	20	40	322	275	..	
63	30	20	50	331	283	158	
64	20	20	60	307	264	140	
65	10	20	70	..	..	..	
66	80	15	5	302	257	..	
67	70	15	15	317	235	151	
68	60	15	25	317	237	158	
69	50	15	35	330	..	157	
70	40	15	45	336	276	158	
71	35	15	50	334	249	158	
72	30	15	55	337	210	149	
73	25	15	60	289	243 217	144	
74	15	15	70	308	..	150	
75	85	10	5	292	..	..	
76	80	10	10	285	..	158	
77	70	10	20	283	246	155	
78	60	10	30	305	219	155	
79	50	10	40	289	197	163	
80	45	10	45	297	..	176	
81	40	10	50	297	..	166	
82	35	10	55	315	..	141	
83	30	10	60	301	..	142	
84	25	10	65	284	228 208	158	
85	20	10	70	287	..	155	
86	10	10	80	287	..	156	
87	5	10	85	320	..	155	
88	90	5	5	297	..	..	
89	85	5	10	276	..	161	
90	75	5	20	269	..	169	
91	65	5	30	254	..	176	
92	55	5	40	242	201	151	
93	50	5	45	241	181	148	
94	45	5	50	253	164	150	
95	40	5	55	258	..	144	
96	35	5	60	269	..	148	
97	25	5	70	238	..	148	
98	15	5	80	247	..	150	
99	10	5	85	276	..	148	
100	5	5	90	287	..	..	

Only one

The numbering of the alloys.—In Fig. 3 the scheme of numbering of the alloys is given. The percentage of a metal in an alloy is represented on the well-known system of distances from the sides of an equilateral triangle. Starting from the apex or antimony corner the alloys are numbered from left to right on lines parallel to the Cadmium-Bismuth base. By this system it is easy to locate alloys on Fig. 4.

The liquidus surface.—In Fig. 4 the isothermals of the freezing points of the alloys are given. The solid solutions formed by Sb with Bi extend fairly deep into the whole system and this solid solution area is represented by area Sb A B C Bi. Microphotographs I, II and III are from this area. Photograph I is of a pure binary solid solution of Sb and Bi while II and III represent alloys Nos. 43 and 74 which show two phases only. The gradient of the isothermals is regular from Sb corner to Bi corner and isothermals are slightly inclined to the base of the triangle or Bi-Cd line.

Area A B C Cd is very much complicated due to the presence of two binary compounds CdSb and Cd_3Sb_2 and due to the meta-stable part of the curve representing the system Sb-Cd in which Cd_3Sb_2, which sometimes separates earlier than Cd Sb, changes into Cd Sb with evolution of much heat thus masking all the latter arrests. This part is represented on the diagram roughly by area A D E F. Microphotograph No. IV of the alloy No. 40 which lies in this area depicts very clearly the change of one phase into the other represented by the equation $Cd_3Sb_2 + Sb \rightarrow 3$ CdSb.

There is another small area G Cd.C Q in which Cd separates out. The general slope of the isothermals in this small area is from Cd corner to C. Area D E F B Q G has got a small hump near B which may be due to a ternary compound. But from the present study nothing can be said definitely as to its presence or absence. There are chances for the formation of two ternary compounds one of CdSb with Bi and the other of Cd_3Sb_2 with Bi. All over, this area has three arrests which are supported by three phases shown in microphotographs of alloys Nos. 91, 95, 96 on line 5% Sb and micro-photographs of alloys Nos. 59, 63 and 77. These three phases indicate the presence either of a ternary eutectic or a ternary compound or some other complexity. It has not been possible to locate the position of any of these two major possibilities with great definiteness and are therefore, not shown on the diagram.

Isothermals of Binary Arrests.—From the alloys lying in area Sb Bi C B F A a solid solution of Sb-Bi separates out on freezing as first solid phase. The change in composition of the liquid portion of the alloy therefore, always follows a straight line drawn from the Sb-Bi base joining the binary valley A F B C. This is made clear by graphs Nos. 5 and 6 representing section through 5% Bi and median through antimony. The section through 5% Cd Fig. 7 shows very clearly this very fact.

About the rest of the area nothing is very clear as yet. Median through Cadmium and section through 5% Antimony Figs. 9 and 8 fix clearly the position of the two binary valleys.

Ternary arrest which is obtained can most probably be due to a ternary eutectic, but examination of micro-structure of a large number of alloys in this area has so far failed to show the position of the ternary eutectic. The third arrest which is obtained in this area is 140 which coincides exactly with the freezing point of binary eutectic of Cd-Bi and can be due to this very phase, and the ternary eutectic may be entirely absent or the binary and the ternary eutectics may be very close to each other.

The two compounds Cd_3Sb_2 and CdSb are stable at temperatures up to their melting points. To clear up the whole problem it now appears

essential that systems Cd Sb-Bi and Cd_3Sb_2-Bi be studied. Also the large triangle be subdivided into three small ones and three ternary systems Cd-Bi-Cd_3Sb_2, Cd_3Sb_2-Bi-CdSb and CdSb-Bi-Sb, be studied.

Summary

Thermal analysis and microscopic study of a large number of alloys of the ternary system Antimony—Cadmium—Bismuth have been carried out. Cd forms ternary solid solutions with Sb and Bi up to a limited composition. The rest of the area of the ternary diagram is very much complicated due to the presence of two stable compounds of Sb and Cd and also due to the meta-stable part of the curve of the binary system Sb-Cd.

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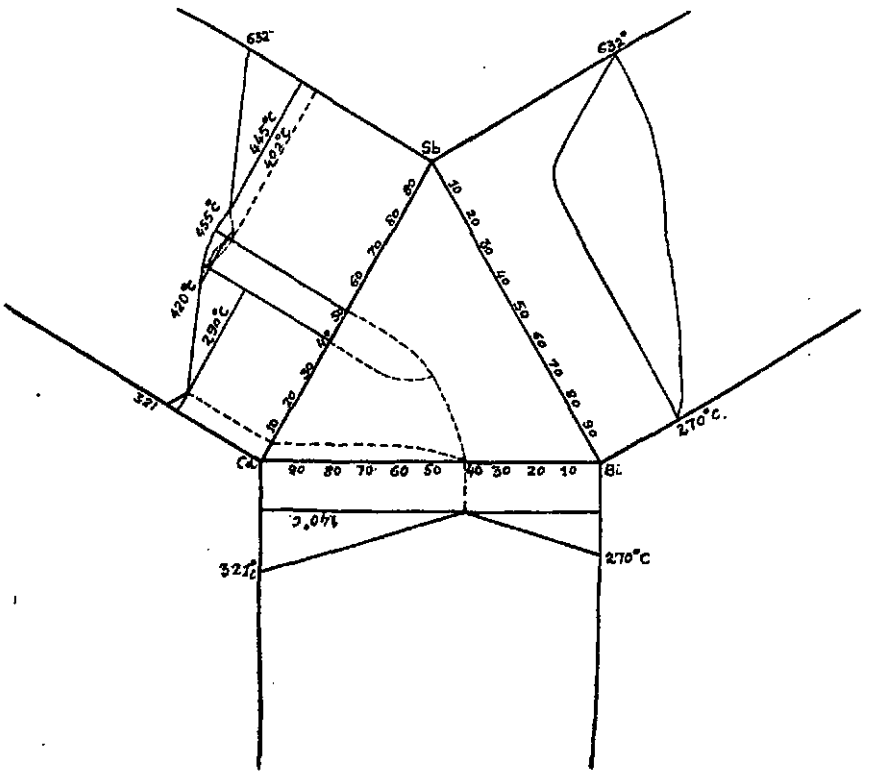
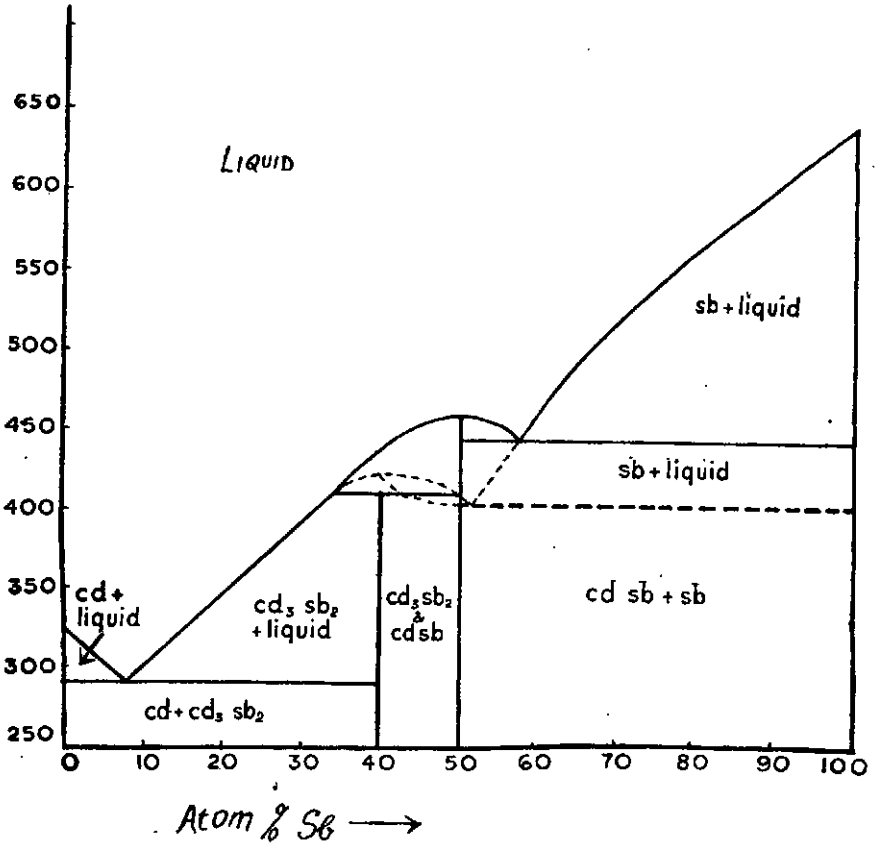
DIAGRAM REPRESENTING THREE BINARY SYSTEMS ENCLOSING THE TERNARY SYSTEM Bi–Sb–Cd

Fig. 1



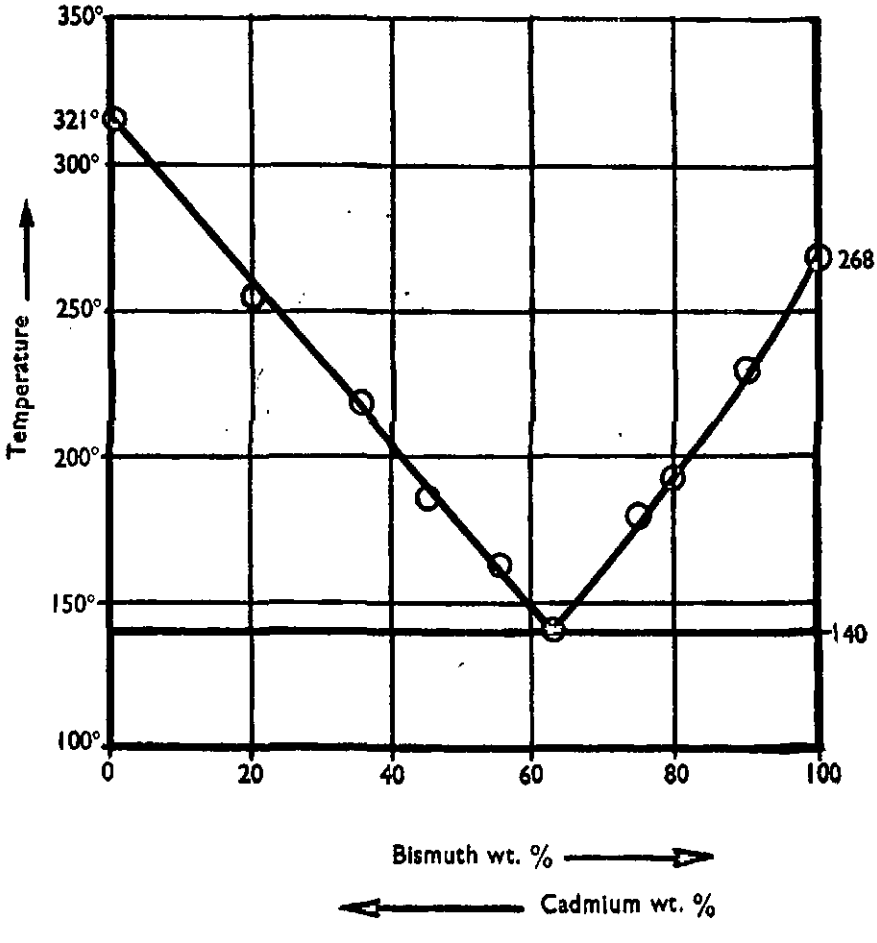
CADMIUM AND ANTIMONY

Fig. 1A



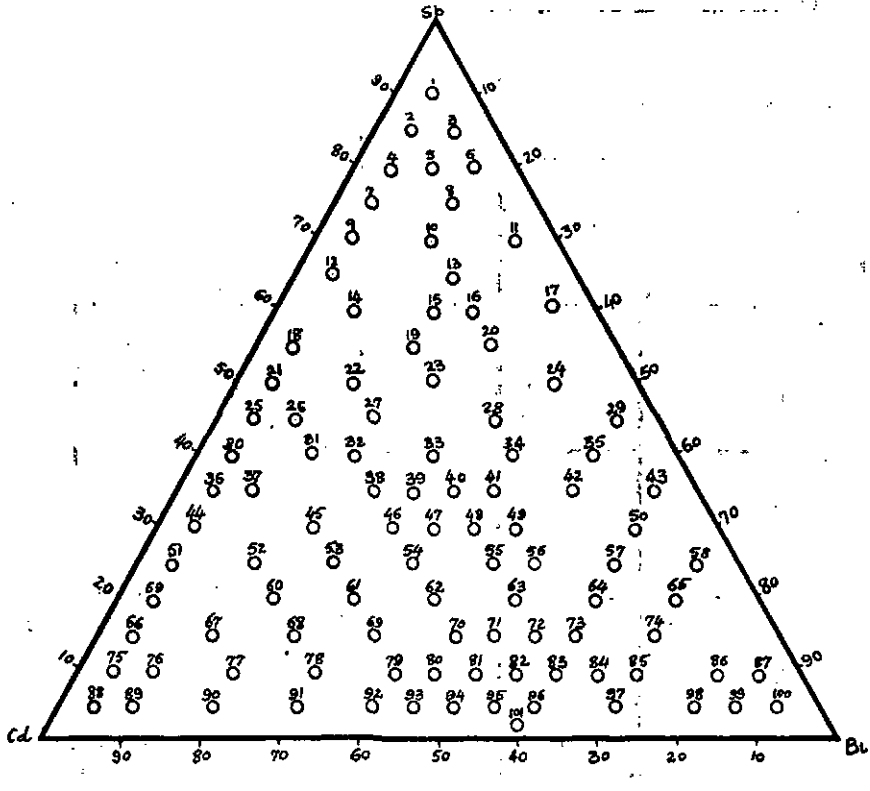
SYSTEM CADMIUM–BISMUTH

Fig. 2



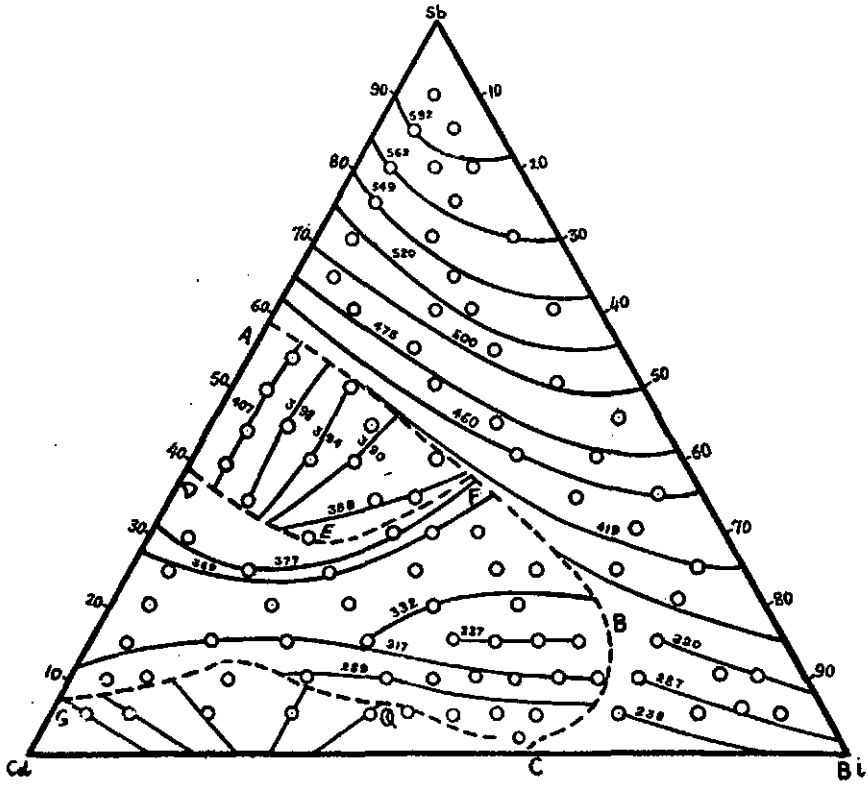
NUMBERING OF ALLOYS

Fig. 3



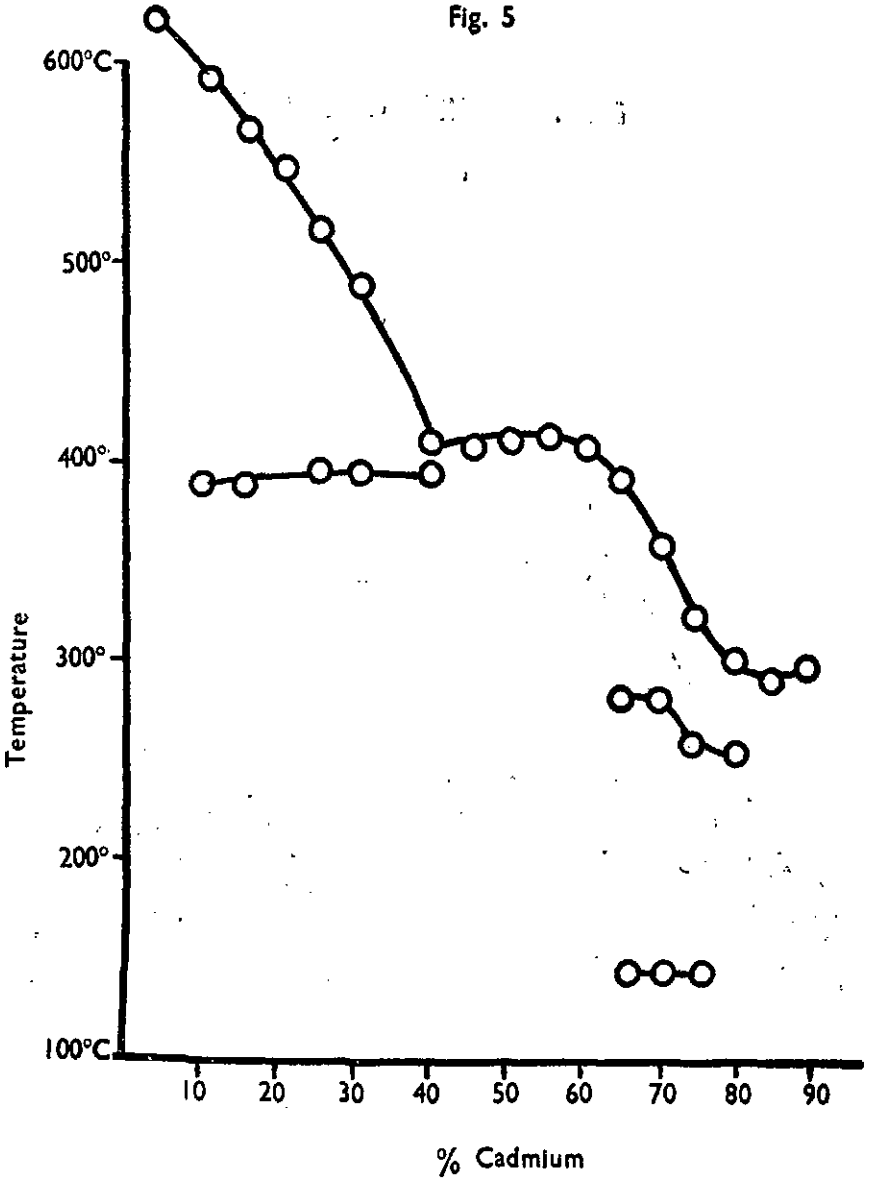
FREEZING POINT OF ALLOYS

Fig. 4



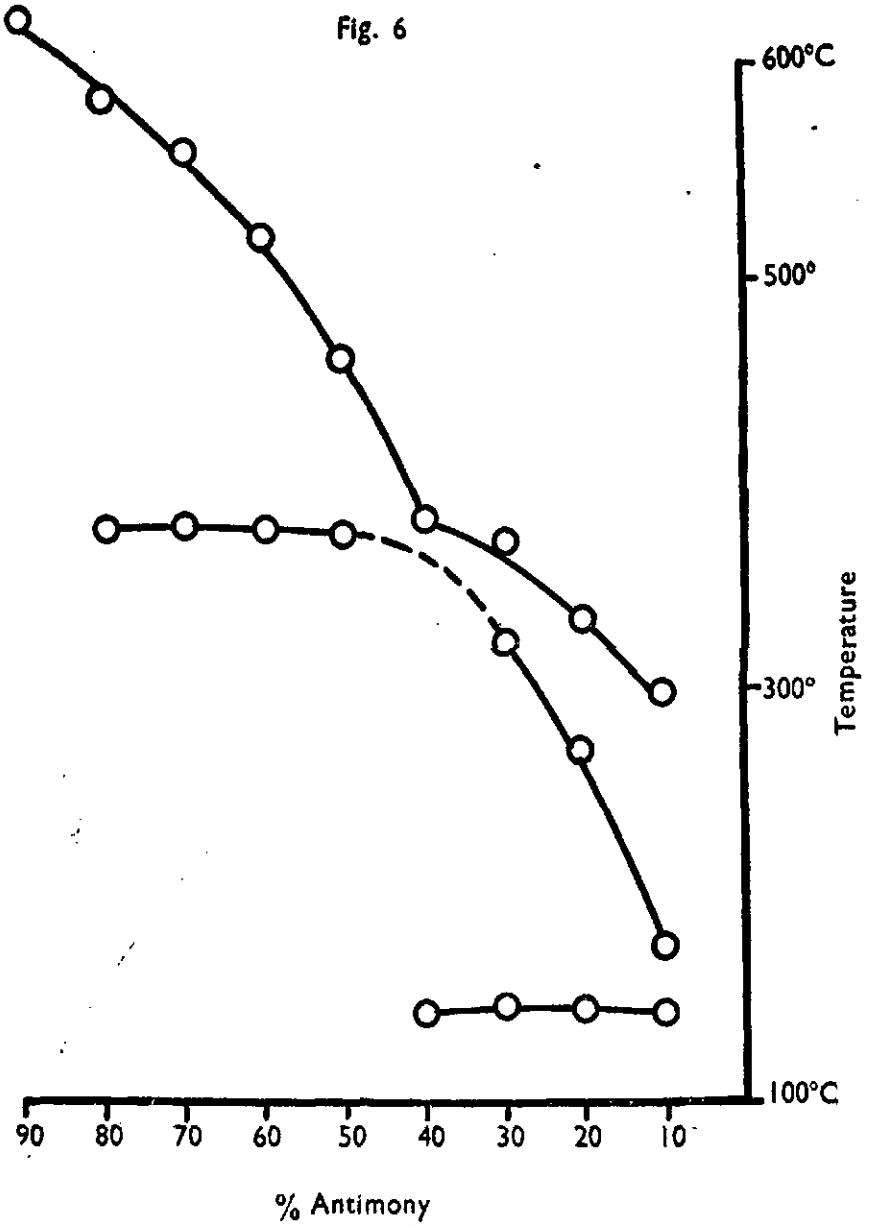
SECTION THROUGH 5% BISMUTH

Fig. 5



MEDIAN THROUGH ANTIMONY

Fig. 6



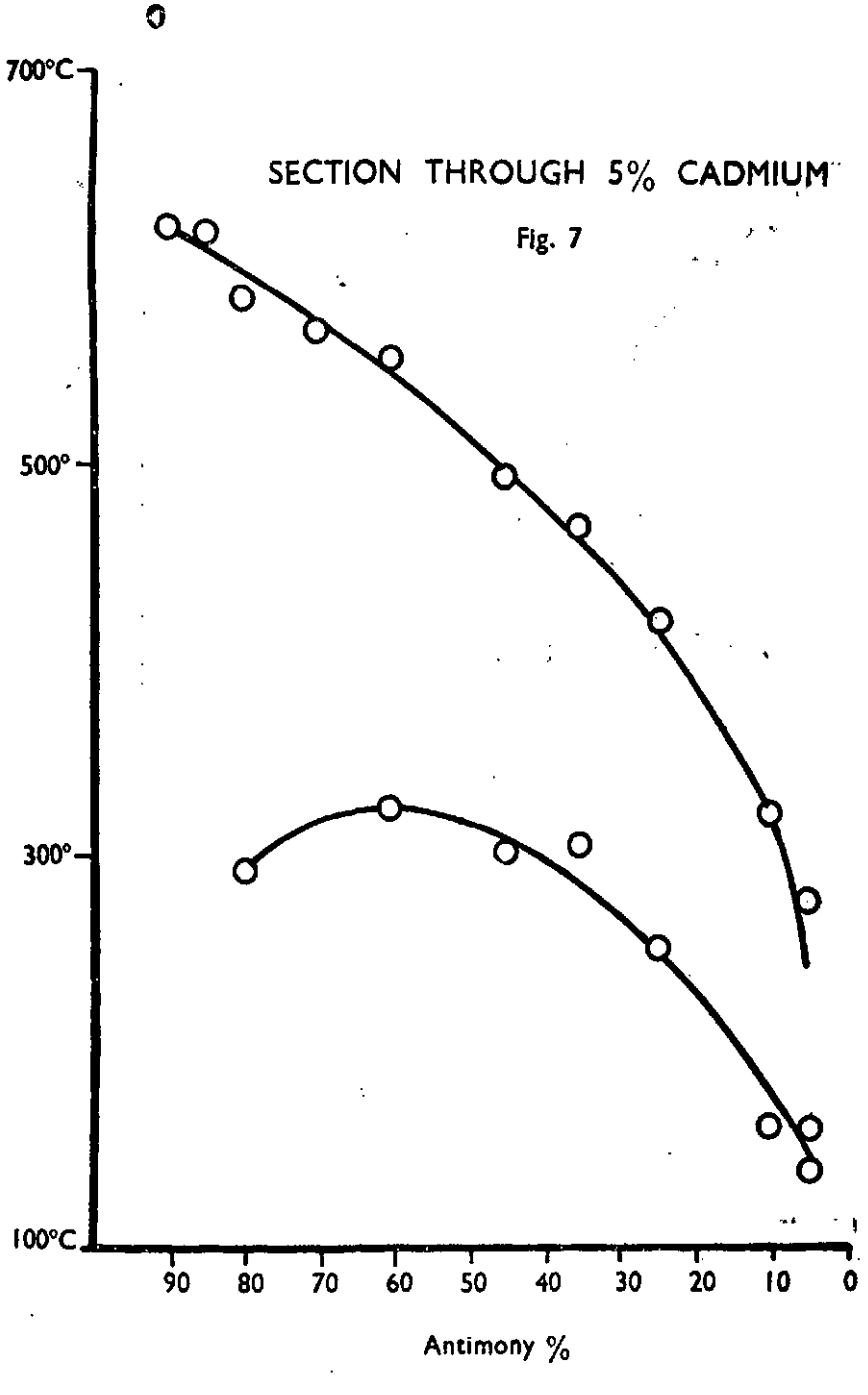
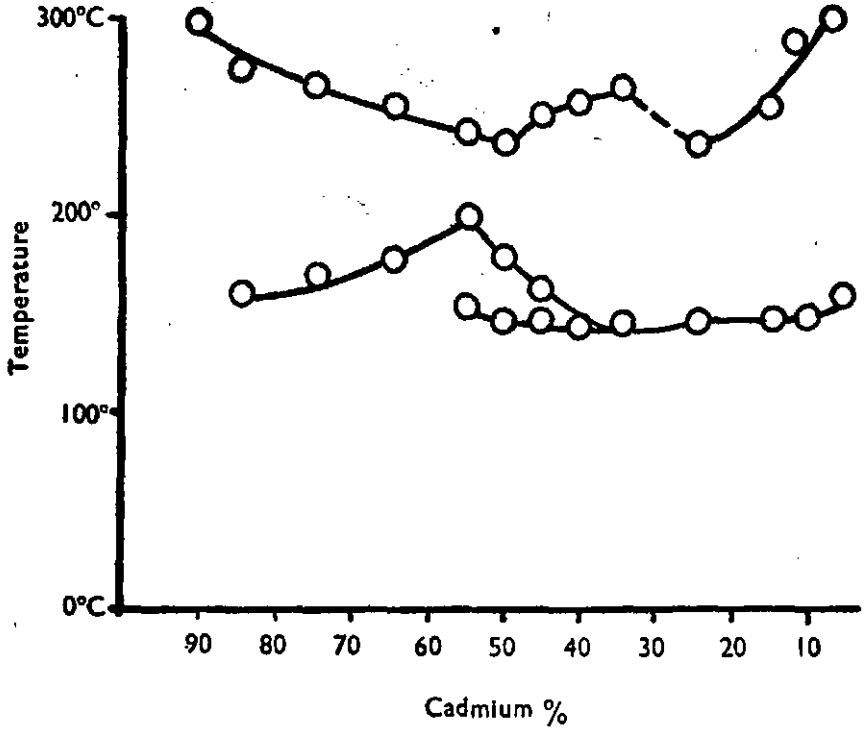

700°C
500°
300°
100°C
SECTION THROUGH 5% CADMIUM
90
80
70
60
50
40
30
20
10
0
Antimony %

Fig. 7

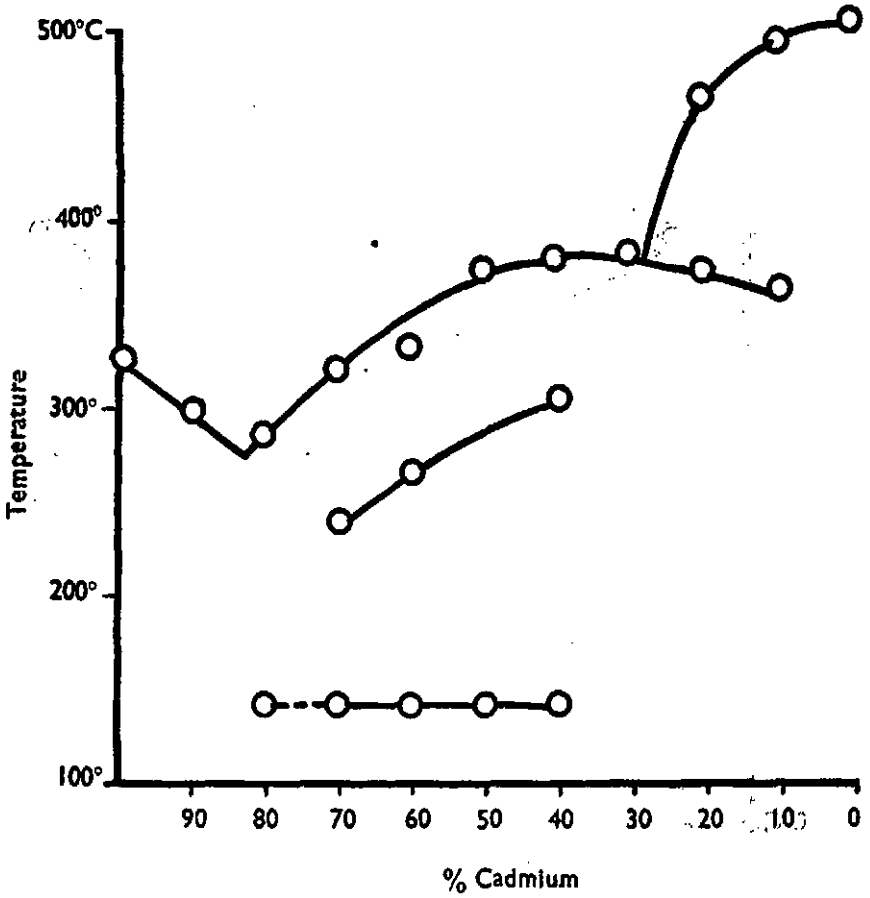
SECTION THROUGH 5% ANTIMONY

Fig. 8



MEDIAN THROUGH CADMIUM

Fig. 9



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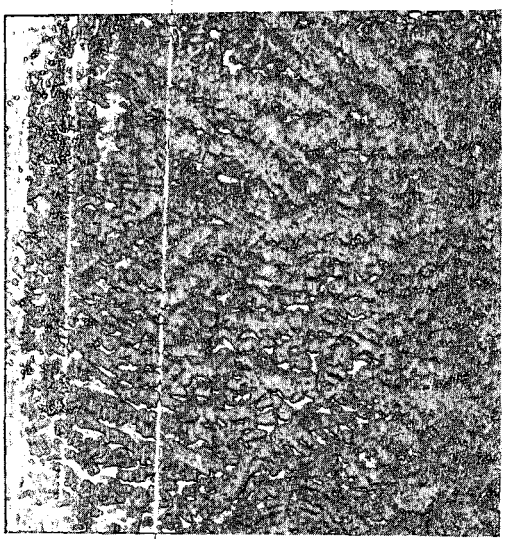
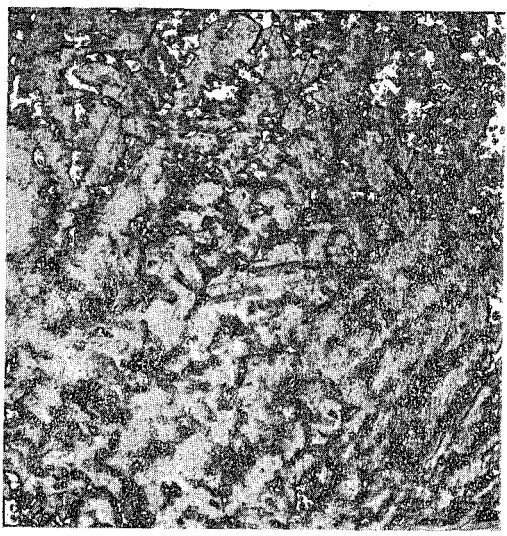
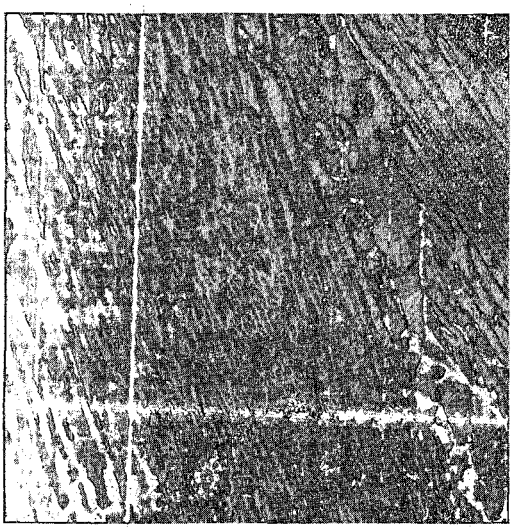
Microphotograph I. Pure Solid Solution
Bi 60%
Sb 40% } ×100



Microphotograph II. Alloy No. 43 ×100
Solid Solution

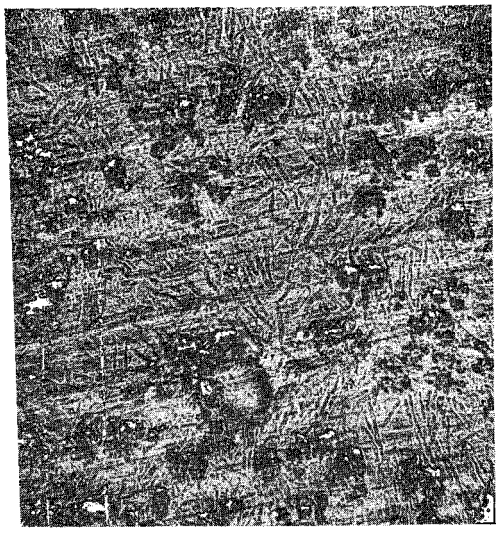


Microphotograph III.
Alloy No. 74 ×100. Two phases

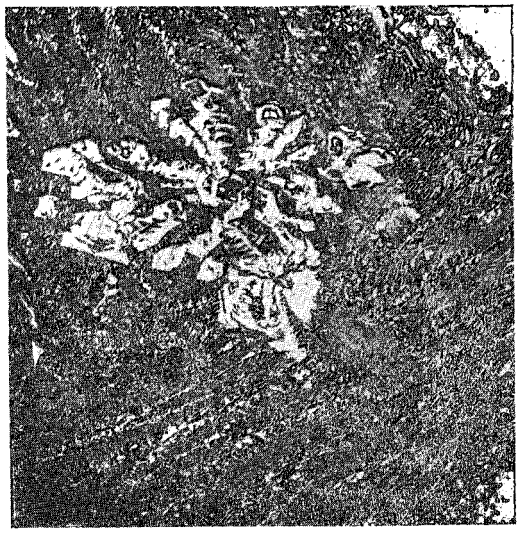


Microphotograph IV.
Alloy No. 40 ×100

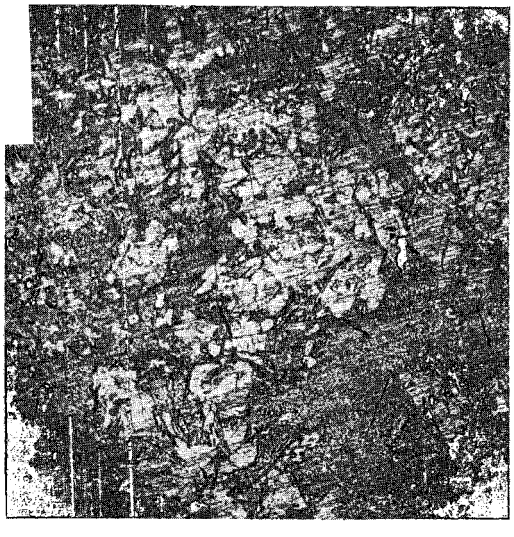
Microphotograph V. Alloy No. 91 ×100



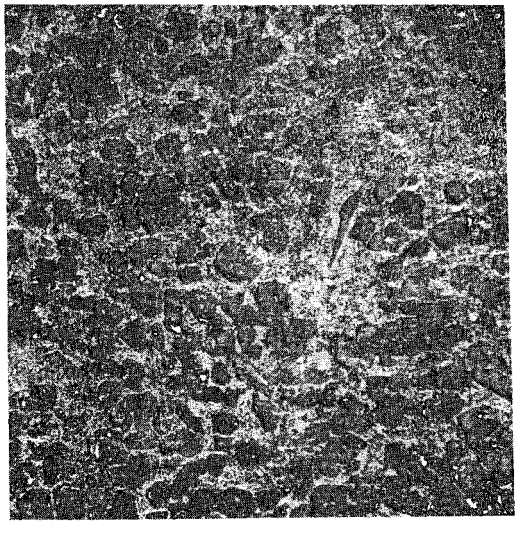
Microphotograph VI. Alloy No. 95 ×100



Microphotograph VII. Alloy No. 96 ×100

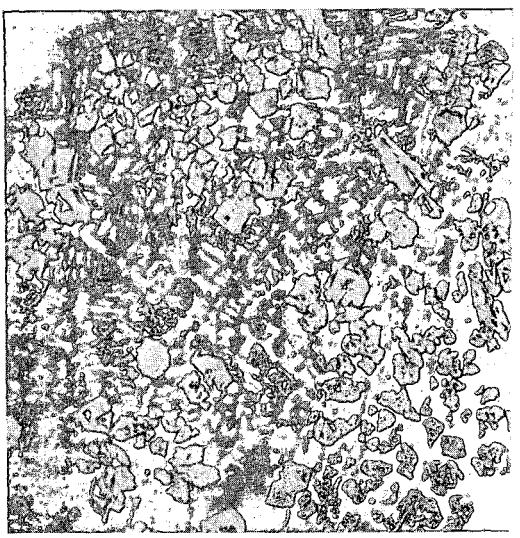


Microphotograph VIII. Alloy No. 59 ×100



Microphotograph IX. Alloy No. 63 ×100

Microphotograph X. Alloy No. 77 ×100



Until and unless the nature of the third phase is exactly defined no explanation can be given for the phases present in these photomicrographs.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 12. PHYSICS Pp. 17—22

ABSORPTION SPECTRUM OF LEAD MOLECULE (Pb_2) IN THE VACUUM ULTRAVIOLET REGION (600–900) A.

by

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Edited for the East Panjab University by Vishwa Nath,
Department of Zoology, East Panjab University,
HOSHIARPUR

Price Re.1-0-0

ABSORPTION SPECTRUM OF LEAD MOLECULE (Pb_2) IN THE VACUUM ULTRAVIOLET REGION (600–900) A.

By B. M. Anand

University Physics Department, Hoshiarpur

Abstract

The absorption spectrum of lead vapour has been studied at 900, 1000 and 1100°C. in the spectral region (600–900) A. with a one metre normal incidence vacuum grating spectrograph using Helium continuum. An intense absorption band extending between 672–676 A. occurs when the lead is heated to 900°C. and broadens as the temperature is raised. This absorption band is attributed to diatomic lead molecule and is being reported for the first time. It shows a sharp edge towards the longer wave-length side.

It is postulated that the upper state involved in this absorption band is a repulsive state of $(Pb_2)^+$ molecule dissociating into a normal neutral atom of Pb in $^3P_0$ ground state and an excited atom of Pb^+ in $^2D_{\frac{5}{2}}$ state of the electronic configuration $6s^2$ 7d.

Introduction

Lead is the last member of the spectroscopically homologous series of elements, C (6), Si (14), Ge (32), Sn (50), Pb (82), having (P^2) electrons in their valence shells, in each case resulting in the ground state ^3P of the neutral atom. The Spectrum of C_2 is well known, but very little is known about the spectra of the elementary molecules of the remaining elements of the series.

Vapour of lead is known to form diatomic molecules (Pb_2) and its band spectrum has been studied by Shawhan (1935), both in absorption and emission. A large number of band-heads lying between 4600–5200 A. were measured and the vibrational analysis was made by him. In the extreme ultraviolet region, however, no data is available for the molecular spectrum of diatomic lead.

For some time past we have been attempting to get the inner transitions of lead, extending Beutler's (1933) investigations of the so called I^b spectra of elements. In a number of our plates a continuous band in absorption appeared, extending between 671–676 A. The band is degraded towards the shorter wave-length side, and shows a sharp edge on the longer wave-length side. This band has been studied at different temperatures and we have no doubt that it is due to diatomic lead molecule in the vaporous state.

The source of continuous radiation was a pyrex glass discharge tube 100 cm. long and 2·4 cm. in diameter, with aluminium cylindrical electrodes, containing pure helium at about 5 mm. of Hg pressure. A 15000 volts luminous tube transformer, with a small condenser of about 5000 cm. capacity in parallel, was connected to the electrodes of the discharge tube through a spark gap of about 2 mm. The weak condensed discharge in Helium produced an intense continuum between 600–900 A., first discovered by Hopfield (1930).

The main absorption chamber was a transparent silica tube 70 cm. long and 1·2 cm. internal diameter. Merck's pure lead, containing less than 0·001% Ag, was contained in a side tube attached to the main absorption tube about 20 cm. from one end. Starting from the side tube 30 cm. of the main quartz tube was wound with S.W.G. 30 nichrome wire using 100 turns. In order to ensure uniform temperature the turns were wound closer near the ends of the 30 cm. length than in its remaining portion. The whole of the 30 cm. length of the tube was lagged closely with successive layers of asbestos paper about 5 mm. thick. Above this asbestos were wound another 100 turns of the same nichrome wire and was similarly lagged with asbestos paper to reduce the loss of heat by conduction. A current 1·7 Amperes through both the heaters simultaneously raised the temperature of the absorption tube to 1100°C. This, of course, is the limiting temperature up to which the nichrome heaters can be used. Proper care is taken to lower the temperature of the heated quartz tube gradually, otherwise it divitrifies when heated above 1000°C. The temperatures above 800°C. were measured by a Cambridge disappearing filament type optical pyrometer. The lower temperatures were measured by an iron-constantan thermo-couple. The side tube containing the lead was similarly wound by a double heater and lagged with asbestos. The side tube was usually kept at a temperature lower than that of the main tube and the temperature of this side tube determined the concentration of the vapour in the absorbing column. On both sides of the furnace, adjacent to the heated portion, about 10 cm. of the silica tube was kept cool by circulating water through flexible metallic tubes wrapped round the quartz tube. This absorption tube was adjusted in between the slit of a normal incidence vacuum grating spectrograph and the helium discharge tube producing the continuum. Two four stage mercury diffusion pumps were suitably arranged to maintain the circulatory differential system. Inside the spectrograph the pressure was maintained less than 0·02 mm., while on the discharge tube side it was anything between 7 to 4 mm.

A one meter speculum metal grating with a ruled area (3×5) cm.^{2} and giving a dispersion of about 17·5 A. per mm. was used.

A technique for preparing our own plates of the Schumann type has been developed. Nelson's gelatine No. I or Kodak photographic gelatine is invariably used. A fresh batch of Schumann plates is prepared a few weeks before the contemplated exposure. The plates are stored in damp-proof tins kept in a refrigerator at 10°C. In general, the plates show good

sensitivity for about a year. The sensitivity of the plates passes through a maximum after which the deterioration is more rapid. The full size of a plate is (16×3·8) cm. and covers range of spectrum up to 2500 A. For trial purposes half plates (8×3·8) cm. are exposed. For good focus extra thin glass plates, which can be curved to 1 meter curvature, are used. On one plate up to four exposures can be given. The exposure time ranged between 40 to 60 minutes.

The condition for the development of the helium continuum are fairly critical. It appears almost equally strong at 4 to 6 mm. pressure of helium, provided the mercury spectrum is subdued to the disappearing stage and the helium is pure and dry. Three pyrex glass traps, cooled by liquid air, are employed to eliminate mercury vapour from the discharge space and the spectrograph. Two activated charcoal traps, cooled to liquid air temperature are put in the circulating system to remove traces of oxygen and nitrogen. An electrically heated silica tube containing copper oxide was introduced in the system to remove hydrogen.

The plates are measured with both a Carl Leiss and a Hilger comparator, reading up to 1/100 of a mm. The following lines, which are always present are taken as reference standards.

584·33 A	..	..	.. He I
736·89 A	..	..	.. Ne I
743·70 A	..	..	.. Ne I

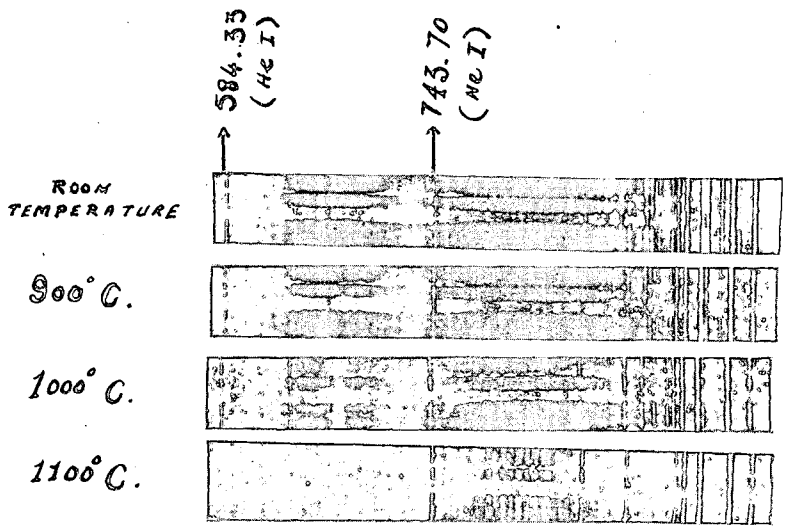

FIG. 1 gives the enlargement of the spectrogram

Results

Table 1 gives the ranges of the absorption band at the various temperatures.

Temperature	Range of Absorption Band		
	λ and $\Delta\lambda$ A.	ν and $\Delta\nu$ in cm.$^{-1}$	E and ΔE in e.v.
900° C.	675·5–671·3 (4·2) A.	148040–148960 (920) cm.$^{-1}$	18·28–18·39 0·11 e.v.
1000° C.	677·7–668·1 (9·6) A.	147560–149680 2120 cm.$^{-1}$	18·22–18·47 0·25 e.v.
1100° C.	706·0–656·8 (49·2) A.	141640–152250 10610 cm.$^{-1}$	17·48–18·80 1·32 e.v.

The band shows no structure, has a sharp head towards the longer wave length side and is degraded towards the short wave length side.

A number of attempts to extend the absorption spectrum of molecular lead in the hydrogen continuum between 1600–2000 A. have not met with success.

Discussion

The correct assignment of a single band to its emitter is usually a matter of some difficulty, as often an unexpected impurity may be responsible for the observed band. In this case, however, the band has appeared a number of times with fairly good intensity, under different conditions of absorption by lead vapour, and the identity of the absorbing molecule is not much in doubt.

It is postulated that this continuous band absorption may be due to the transitions from the ground state X of neutral lead molecule to an upper state B of the ionised molecule.

The lowest state X of the normal lead molecule may be supposed to be dissociated into two normal $^3P_0$, Pb atom and an excited atom of the Pb^+ ion with the electronic energy of 81222 cm.$^{-1}$ above the ground state of Pb^+ ion $^2P_{1/2}$ ($6s^2\,6p$ configuration). Such an excited state of Pb^+ known from the work of Earls and Sawyer (1935) on First Spark Spectrum of lead as the transition $6s^2\,6p$, $^2P_{\frac{1}{2}}$—$6s^2 7d$, $^2D_{2\frac{1}{2}}$ the total energy of this excited state with respect to ground state of neutral lead atom becomes $81222+59821=141043$ cm.$^{-1}$ In this the value 59821 cm.$^{-1}$ corresponds to the ionisation potential of lead, as given in Atomic energy states, Bacher and Goudsmit (1932). The long wave-length limit of the continuous absorption observed at the highest temperature (1100°C.) comes out to be 141640 cm.$^{-1}$ This almost exact correspondence is a very favourable support to the above postulate of the upper state to be the repulsive state B of Pb^+.

Weak ionisation continua occurring below 1000 A. and at the end of Rydberg series of bands, have been observed in the case of O_2 and N_2 by

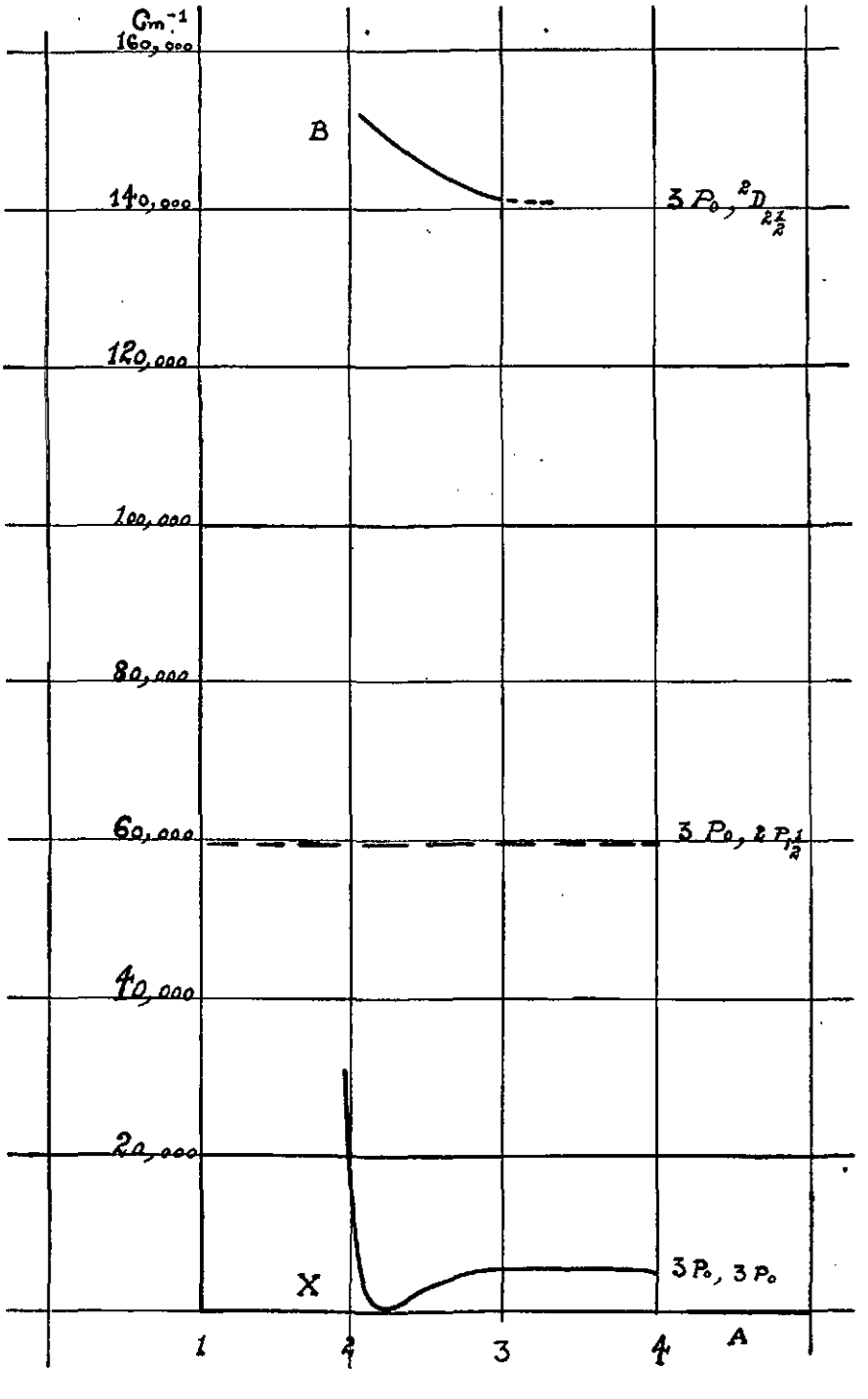

FIG. 2. Potential energy internuclear distance diagram for molecular states of Pb_2. X is the lowermost state and B is the upper repulsive state. The transitions from X to B give the continuous absorption band. The atomic states of the products of dissociation are given on the right. On the left, energy in wave-numbers is given.

Price and Collins (1935), and Worley and Jenkins (1938) respectively. From the convergence limits of the Rydberg series corresponding to the long wave-length limits of the continua, the ionisation potentials of the molecules have been calculated.

The continuous absorption of lead vapour reported in this paper is not accompanied by any discrete bands due to lead molecule. Moreover, the absorption is intense and at the highest temperature fairly extensive. These features lend support to the view that it is the case of a dissociation continuum. The long wave-length limit of the above continuum observed at the lowest temperature should give one of the ionisation potentials of the lead molecule equal to 148040/8066=18·28 volts.

If we subtract the total atomic energy of the excited ion in the product of dissociation from the above molecular energy we shall get the heat of dissociation of the molecule in the ground state.

$$E_{\text{molecule}} - E_{\text{atom}} = D$$
$$18{\cdot}28 - 17{\cdot}49 = {\cdot}79 \text{ volt.}$$

This value is also of the right order as compared to the value found by Shawhan.

Acknowledgments

The author is greatly indebted to Professor P. K. Kichlu and Professor J. B. Seth for their continued interest in this work.

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Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 13. CHEMISTRY Pp. 23—28

March, 1951

POTENTIOMETRIC STUDIES IN OXIDATION-REDUCTION REACTIONS

Part XII. Oxidation with Potassium Meta-Periodate

by

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Price Re.0-15-0

POTENTIOMETRIC STUDIES IN OXIDATION-REDUCTION REACTIONS

Part XII. Oxidation with Potassium Meta-Periodate

By Balwant Singh, Appar Singh *and* Madan Gopal

Potassium meta-periodate reacts with potassium iodide in presence of sulphuric acid

$$KIO_4+7KI+4H_2SO_4 = 4K_2SO_4+4H_2O+4I_2$$

In acid medium potassium chromate, potassium permanganate, potassium dichromate, sodium iodate and potassium bromate react with potassium iodide according to the following equations:—

$$2K_2CrO_4+8H_2SO_4+6KI = 5K_2SO_4+Cr_2(SO_4)_3+8H_2O+3I_2$$
$$2KMnO_4+8H_2SO_4+10KI = 6K_2SO_4+2MnSO_4+8H_2O+5I_2$$
$$K_2Cr_2O_7+7H_2SO_4+6KI = 4K_2SO_4+Cr_2(SO_4)_3+7H_2O+3I_2$$
$$2NaIO_3+6H_2SO_4+10KI = Na_2SO_4+5K_2SO_4+6H_2O+6I_2$$
$$KBrO_3+3H_2SO_4+6KI = KBr+3K_2SO_4+3H_2O+3I_2$$

The reactions have been utilised in the quantitative determination of these substances by the potentiometric method.

Experimental

Potassium meta-periodate was prepared by the method of Bahl and Singh (*J. Ind. Chem. Soc.*, 1940, **17**, 167) by passing a brisk current of chlorine gas through a boiling solution of iodine in potassium hydroxide.

The oxidation-reduction electrode which consisted of a bright platinum foil immersed in a solution to be titrated was coupled with a saturated calomel electrode through an agar-agar potassium chloride bridge. The cell was placed in a water bath, the temperature of which was maintained at 25°C. E.M.F. of the cell was read on a potentiometer.

Potassium iodide, acidified with 7*N* sulphuric acid, was directly titrated against *M*/80 potassium meta-periodate by the potentiometric method. A known weight of each of the other substance was mixed with an excess of potassium iodide and the mixture acidified with 7*N* sulphuric acid. The excess potassium iodide was titrated potentiometrically against standard potassium meta-periodate. The titrations were conducted in an atmosphere of carbon dioxide and the mixture was kept stirred by a mechanical stirrer.

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 0·4357 gm. of potassium iodide mixed with 60 c.c. of 7 N sulphuric acid, against potassium meta-periodate (M/80).

Potassium meta-periodate (c.c.)	E.M.F. (volts)	*E/C* (m. volt/c.c.)	Potassium meta-periodate (c.c.)	E.M.F. (volts)	*E/C* (m. volt/c.c.)
					260
0·00	0·2200		29·90	0·4975	
		29			290
1·00	0·2485		29·95	0·5120	
		11			296
3·00	0·2705		30·00	0·5268	
		7			564
7·00	0·2985		30·05	0·5550	(Maximum)
		6			250
12·00	0·3290		30·10	0·5675	
		4			95
18·00	0·3505		30·20	0·5770	
		4			42
24·00	0·3765		30·50	0·5895	
		7			41
27·00	0·3985		31·00	0·6100	
		12			40
28·00	0·4105		32·00	0·6500	
		18			21
29·00	0·4286		35·00	0·7115	
		37			6
29·50	0·4471		38·00	0·7280	
		65			2
29·70	0·4600		45·00	0·7387	
		115			
29·80	0·4715				

TABLE II

Titration of 0·3103 gm. of potassium chromate mixed with 1·0624 gm. of potassium iodide and 50 c.c. of 7 N sulphuric acid, against potassium meta-periodate (M/80).

Potassium meta-periodate (c.c.)	E.M.F. (volts)	*E/C* (m. volt/c.c.)	Potassium meta-periodate (c.c.)	E.M.F. (volts)	*E/C* (m. volt/c.c.)
					135
0·00	0·3470		17·80	0·4770	
		5			190
1·00	0·3517		18·00	0·5040	
		3			640
3·00	0·3575		18·10	0·5230	
		3			390
6·00	0·3670		18·15	0·5550	(Maximum)
		4			297
9·00	0·3780		18·20	0·5745	
		5			175
12·00	0·3915		18·35	0·6190	
		6			84
14·00	0·4037		18·50	0·6432	
		9			36
15·00	0·4126		18·70	0·6620	
		11			20
16·00	0·4232		19·20	0·6800	
		15			21
16·50	0·4307		20·00	0·6960	
		19			4
17·00	0·4402		21·00	0·7170	
		34			1
17·50	0·4572		23·00	0·7257	
		66			
			25·00	0·7282	

TABLE III

Titration of 0·0948 gm. of potassium permanganate mixed with 0·6640 gm. of potassium iodide and 60 c.c. of 7 N sulphuric acid, against potassium meta-periodate (M/80).

Potassium meta-periodate (c.c.)	E.M.F. (volts)	*E/C* (m. volt/c.c.)	Potassium meta-periodate (c.c.)	E.M.F. (volts)	*E/C* (m. volt/c.c.)
0·00	0·4020	4	11·45	0·5260	160
3·00	0·4130	4	11·50	0·5510	500 (Maximum
5·00	0·4210	5	11·55	0·5660	300
7·00	0·4312	8	11·60	0·5810	300
9·00	0·4480	13	11·80	0·6200	195
10·00	0·4612	22	12·00	0·6370	85
10·50	0·4722	28	12·50	0·6510	28
10·80	0·4805	38	13·00	0·6627	23
11·00	0·4880	45	14·00	0·6725	10
11·20	0·4970	82	16·00	0·6840	6
11·30	0·5052	115	18·00	0·6950	6
11·35	0·5107	146	21·00	0·7090	5
11·40	0·5180				

TABLE IV

Titration of 0·0490 gm. of potassium dichromate mixed with 0·3320 gm. of potassium iodide and 30 c.c. of 7 N sulphuric acid, against potassium meta-periodate (M/80).

Potassium meta-periodate (c.c.)	E.M.F. (volts)	*E/C* (m. volt/c.c.)	Potassium meta-periodate (c.c.)	E.M.F. (volts)	*E/C* (m. volt/c.c.)
0·00	0·3645	2	11·20	0·4710	70
1·00	0·3660	5	11·40	0·4850	300
3·00	0·3757	5	11·50	0·5150	790
5·00	0·3850	6	11·55	0·5545	(Maximum) 350
7·00	0·3970	8	11·60	0·5720	210
8·00	0·4050	10	11·70	0·5930	107
9·00	0·4154	11	12·00	0·6250	28
9·50	0·4210	16	15·00	0·7100	4
10·00	0·4290	20	18·00	0·7312	3
10·50	0·4390	38	20·00	0·7365	1
10·80	0·4505	51	25·00	0·7425	

TABLE V

Titration of 0·2460 gm. of sodium iodate mixed with 1·2460 gm. of potassium iodide and 40 c.c. of 7 N sulphuric acid, against potassium meta-periodate (M/80).

Potassium meta-periodate (c.c.)	E.M.F. (volts)	*E/C* (m. volt/c.c.)	Potassium meta-periodate (c.c.)	E.M.F. (volts)	*E/C* (m. volt/c.c.)
0·00	0·3785		14·95	0·5175	
		3			830
3·00	0·3885		15·00	0·5590	(Maximum)
		4			320
5·00	0·3955		15·05	0·5750	
		4			228
8·00	0·4065		15·10	0·5864	
		6			106
11·00	0·4230		15·20	0·5970	
		8			61
13·00	0·4382		15·50	0·6152	
		20			62
14·00	0·4580		16·00	0·6460	
		40			31
14·30	0·4700		17·00	0·6770	
		40			22
14·60	0·4820		18·00	0·6985	
		60			10
14·80	0·4940		20·00	0·7175	
		90			5
14·90	0·5030		22·00	0·7275	
		290			4
			25·00	0·7386	

TABLE VI

Titration of 0·1670 gm. of potassium bromate mixed with 1·1066 gm. of potassium iodide and 40 c.c. of 7 N sulphuric acid, against potassium meta-periodate (M/80).

Potassium meta-periodate (c.c.)	E.M.F. (volts)	*E/C* (m. volt/c.c.)	Potassium meta-periodate (c.c.)	E.M.F. (volts)	*E/C* (m. volt/c.c.)
					240
0·00	0·3720		8·00	0·5930	
		6			156
2·00	0·3840		8·10	0·6086	
		6			104
4·00	0·3960		8·20	0·6190	
		10			81
5·00	0·4057		8·40	0·6352	
		12			41
6·00	0·4175		8·70	0·6475	
		18			32
6·60	0·4282		9·00	0·6572	
		24			28
7·00	0·4376		9·50	0·6710	
		48			17
7·50	0·4615		10·00	0·6795	
		93			16
7·70	0·4800		11·00	0·6950	
		190			13
7·80	0·4990		12·00	0·7075	
		300			11
7·85	0·5140		14·00	0·7301	
		972			8
7·90	0·5626	(Maximum)	17·00	0·7538	
		368			
7·95	0·5810				

Discussion

In these titrations it is evident that with the addition of the titrant the E.M.F. rose steadily till the equivalence point. At the equivalence point there was a sharp jump in potential followed by a steady rise in each case.

From the volume of potassium meta-periodate used, corresponding to the equivalence point in each titration, the amount of each substance was calculated. In the following tables the values obtained are compared with the amounts of the substance taken.

Table VII.

Potassium Iodide

KI taken (gm.)	KI found (gm.)
0·0726	0·0723
0·1452	0·1448
0·2178	0·2180
0·2905	0·2909
0·3631	0·3634
0·4357	0·4361

Table VIII

Potassium Chromate

K_2CrO_4 taken (gm.)	KI added (gm.)	Excess KI corresponding to KIO_4 (gm.)	KI used for K_2CrO_4 (gm.)	K_2CrO_4 found (gm.)
0·0517	0·3984	0·2656	0·1328	0·0517
0·1293	0·5644	0·2320	0·3324	0·1295
0·2199	0·8632	0·2989	0·5643	0·2199
0·3103	1·0624	0·2641	0·7983	0·3111
0·4138	1·2632	0·2017	1·0615	0·4135

Table IX

Potassium Permanganate

$KMnO_4$ taken (gm.)	KI added (gm.)	Excess KI corresponding to KIO_4 (gm.)	KI used for $KMnO_4$ (gm.)	$KMnO_4$ found (gm.)
0·0316	0·3320	0·1654	0·1666	0·0317
0·0474	0·4150	0·1682	0·2468	0·0469
0·0790	0·5810	0·1661	0·4149	0·0789
0·0948	0·6640	0·1668	0·4972	0·0946
0·1517	1·0790	0·2853	0·7937	0·1511
0·2250	1·4100	0·2337	1·1763	0·2239

TABLE X

Potassium Dichromate

$K_2Cr_2O_7$ taken (gm.)	KI added (gm.)	Excess KI corresponding to KIO_4 (gm.)	KI used for $K_2Cr_2O_7$ (gm.)	$K_2Cr_2O_7$ found (gm.)
0·0490	0·3320	0·1679	0·1641	0·0484
0·0735	0·4482	0·2000	0·2482	0·0732
0·0980	0·4980	0·1659	0·3321	0·0980
0·1225	0·5810	0·1662	0·4148	0·1225
0·1470	0·7470	0·2472	0·4998	0·1476

TABLE XI

Sodium Iodate

$NaIO_3$ taken (gm.)	KI added (gm.)	Excess KI corresponding KIO_4 (gm.)	KI used for $NaIO_3$ (gm.)	$NaIO_3$ found (gm.)
0·0660	0·5533	0·2781	0·2752	0·0654
0·1112	0·6640	0·1995	0·4645	0·1109
0·1510	0·8024	0·1704	0·6320	0·1508
0·2460	1·2460	0·2178	1·0282	0·2452
0·3445	1·6260	0·1820	1·4440	0·3436

TABLE XII

Potassium Bromate

$KBrO_3$ taken (gm.)	KI added (gm.)	Excess KI corresponding to KIO_4 (gm.)	KI used for $KBrO_3$ (gm.)	$KBrO_3$ found (gm.)
0·0556	0·4703	0·1966	0·2737	0·0551
0·0946	0·6363	0·1664	0·4699	0·0945
0·1670	1·1066	0·2805	0·8261	0·1663
0·2337	1·5216	0·3594	1·1622	0·2337
0·3329	2·0050	0·3526	1·6524	0·3324
0·4200	2·7400	0·2308	2·5092	0·4206

The results show that potassium iodide can be determined directly and potassium chromate, potassium permanganate, potassium dichromate sodium iodate and potassium bromate determined indirectly by the potentiometric method, using potassium meta-periodate as an oxidising agent.

The authors are indebted to Dr. Vishwa Nath, Principal, Government College, Hoshiarpur, for providing facilities for the research work.

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 14 MATHEMATICS Pp. 29—32

May, 1951

ON CIRCLES OF ANTI-SIMILITUDE

A THEOREM ON CONFOCALS

COMMUTATIVE LAW IN FOUR DIMENSIONS SPACE S_4

by

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Price Re.0-12-0

ON CIRCLES OF ANTI-SIMILITUDE

(Abstract published in the Proceedings of Indian Science Congress, 1950)

By Sahib Ram Mandan, M.A.

Certain properties of twelve circles of anti-similitude of four circles two at a time are obtained from some simple properties of a triangle by Inversion which may be enumerated as below:

(*i*) *Four sets of 6 circles in each set consisting of 3 externals and 3 internals have a common orthogonal circle.*

(*ii*) *Four sets of 6 circles in each set consisting of three internals and three externals concur.*

(*iii*) *Four sets of 6 circles in each set consisting of all externals or two externals and four internals concur.*

The centres of the circles touching the sides of a triangle $A'B'C'$ form an orthocentric tetrad of points, say $II_1I_2I_3$, $A'B'C'$ being its pedal triangle and the circle $A'B'C'$ its nine-point circle which bisects the six joins of the tetrad; the six circles on them as diameters pass through two vertices of triangle $A'B'C'$ leaving one through which the join under consideration passes; hence they are the six circles of anti-similitude for the circle $A'B'C'$ taken with one of the sides of triangle $A'B'C'$ at a time.

Consider two of them passing through $A'I$; now a circle touching the circle $A'B'C'$ and $A'B'$ and $A'C'$ must be orthogonal to them, hence A', I must be inverse points for that circle; similarly A', I_1 for another and so on for B', I_2, ... i.e., inverse of A' w.r.t. a circle touching the circle $A'B'C'$, $A'B'$ and $A'C'$ is the same as that of B' w.r.t. a circle touching circle $A'B'C'$, $B'C'$ and $B'A'$.

We may note that

(i) The circle orthogonal to any three of the six circles enumerated above has its centre at one of points of the tetrad.

(ii) Through each vertex of triangle $A'B'C'$ there pass two bisectors and four of the above circles.

(iii) Through each point of the tetrad there pass three of them.

Now let us invert the results got above w.r.t. a point O and remembering that the internal bisector of a pair of lines inverts into the external circle of anti-similitude for the inverse circles through O of the pair of given lines and the external bisector into internal circle of anti-similitude; or orthogonal circles invert into orthogonal circles; inverse points for a circle into inverse points for the inverse circle; a circle touching another circle into one touching the inverse of the second circle.

Given four circles OBC, OCA, OAB and ABC, and let O_i ($i = 1, 2, 3, 4$) be the inverses of O w.r.t. four circles touching the 3 circles through O,

then the inverses of A, B, C w.r.t. circles touching the 3 circles through each of them remain same as O_i and hence we have:

(i) Four sets of the twelve circles of anti-similitude of the four given circles two at a time have a common orthogonal circle, each set consisting of six circles, three externals and three internals.

(ii) Four sets of 6 circles in each set consisting of three internals and three externals pass through O, A, B, C: in reality these are determined by the three circles through them.

(iii) Four sets of 6 circles in each set consisting of all externals or two externals and four internals pass through O_i $(i = 1, 2, 3, 4)$.

A THEOREM ON CONFOCALS*

(Abstract published in the Proceedings of Indian Science Congress Association, 1951)

By Sahib Ram Mandan, M.A.

If the normal n *to a quadric* S *at* P *meets it again in* P′ *and another quadric* S′, *confocal with* S, *in* Q_1, Q_2, *the normals* n_1, n_2 *to* S′ *at* Q_1, Q_2, *intersect at a point* R *in the tangent plane* p′ *to* S *at* P′ *and the locus of* R, *as* S′ *varies, is a conic* († B_3, Ex. 8, p. 102) *through* P′.

Since n contains the pole of the tangent plane p to S at P w.r.t. S' the common line a of the tangent planes q_1, q_2 to S' at Q_1, Q_2 lies in p and is at right angles to n and also to n_1, n_2. Hence n_1, n_2 lie in the plane q through n perpendicular to a and intersect, say, at R. As S' varies, to each line a corresponds a point R uniquely. But the lines a envelope a conic († B_3, Ex. 2, p. 100), hence R traces a conic if we prove that it lies in p'.

Let the sections of S and S' by the plane q be the conics s and s'; let q meet the planes p, p', q_1, q_2 in lines l_p, $l_{p'}$, l_{q_1}, l_{q_2} respectively; l_{q_1}, l_{q_2} touch s' at Q_1, Q_2 and meet in R' on the tangent line l_p to s at p where q meets the line a; n_1, n_2 are normals to s' at Q_1, Q_2 and contain the poles of l_{q_1}, l_{q_2} respectively w.r.t. s, which are the poles of q_1, q_2 w.r.t. S; i.e., n_1, l_{q_1} and n_2, l_{q_2} are pairs of conjugate lines for s.

Let the pencils of lines at R and R' be related in the manner that to line l through R correspond the line l', through R', conjugate to l w.r.t. s. We notice that RP, n_1, n_2 meet their corresponding lines l_p, l_{q_1}, l_{q2} respectively in the points P, Q_1, Q_2 which lie on the line n, normal to the conic s at P and it, therefore, contains all the meets of the corresponding rays of the pencils. Now P' is a point of n, hence to $R'P'$ corresponds the line, through P', conjugate to $R'P'$ w.r.t. s, this is no other than the tangent line $l_{p'}$, at P' to s; $l_{p'}$, therefore, must pass through R, i.e. R lies on $l_{p'}$ which again lies in p'.

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* Q. 1810, Math. Student, Vol. X, No. 2, June 1942.

† B_i stands for H. F. Baker, Principles of Geometry, Vol. i.

It is interesting to notice that RR' touches s, as it is the self-corresponding ray of the pencils.

Cor. If the normal n to a conic s at P meets it again in P' and another conic s', confocal with s, in Q_1, Q_2, the locus of the point of intersection of the normals at Q_1, Q_2 to s', as s varies, is the tangent line at P' to s. (*B_2, p. 94.)

COMMUTATIVE LAW IN FOUR DIMENSIONS SPACE S_4

(Abstract published in the Proceedings of the Conference of the Indian Math. Soc. held at Delhi in Dec. 1945)

By Sahib Ram Mandan, M.A.

1

A Set of Five Associated Lines.

Let a, b, c, d, e be a set of five associated lines (*B_4, Chap. 5) and P, B, C' be points on a, Q, A', C on b, R, A, B' on c, P', Q', R' on d, L, M, N on e such that

$A+B+C+A'+B'+C' = 0$ (syzygy determining the space);
$P+B+C' = 0 = Q+C+A' = R+A+B';$
$P'+B'+C = 0 = Q'+C'+A = R'+A'+B;$
$L+A+A' = 0 = M+B+B' = N+C+C';$ and
$A+A' = P+P'; B+B' = Q+Q'; C+C' = R+R'.$

Let $yB+zC'$, $zC+xA'$, $xA+kB'$ be points on the lines a, b, c respectively, if the plane determined by these points is to meet the line e, we must have then $l(yB+zC')+m(zC+xA')+n(xA+kB') \equiv l'L+m'M+n'N$ which is $\equiv l_1(A+A')+m_1(B+B')+n_1(C+C')$, i.e. $mx = nx = l_1$; $nk = ly = m_1$; $lz = mz = n_1$; which proves that $l = m = n$, and hence, $k = y$; i.e. the plane determined by the points $yB+zC'$, $zC+xA'$, $xA+yB'$ meets the lines a, b, c, e. Now $x(yB+zC')+y(zC+xA')+z(xA+yB')$ is a point of this plane, which may be written as $(xyB+yxA')+(yzC+zyB')+(zxA+xzC')$. If $xy = yx$, . . . i.e. if the commutative law holds, this point takes the form $xyR'+yzP'+zxQ'$ save for sign, that is a point on the line d. Hence, *the plane intersecting four lines of an associated set intersects the fifth if and only if the commutative law holds.*

2

Mutually † *Self-polar Pentads in* S_4.

Let $ABCDE$ be a pentad P_0 and A_1 a point, such that $A_1+A+B+C+D+E = 0$. The polar prime of A_1 w.r.t. P_0 contains all the ten points that have the symbols $A-B$, $B-C$, $C-D$, $D-E$, $E-A$, $B-D$, $B-E$, $C-E$, $C-A$, $D-A$. Any point B_1 in this prime may be taken as $A-B$

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* B_i stands for H. F. Baker, Principles of Geometry, Vol. i.

† *Bull. Cal. Math. Soc.*, Vol. 33, No. 4, 1941, p. 148.

$+k(B-C)+l(C-D)+m(D-E)$, i.e. $A+(k-1)B+(l-k)C+(m-l)D-mE$, whose polar prime contains $A+(1-k)B$, $(1-k)B-(k-l)C$, $(k-l)C-(l-m)D$, $(l-m)D-mE$, $A+mE$, ... Now the condition that A_1 may lie in this prime is obtained by making a linear relation of the above points identical with A_1, i.e. $A+B+C+D+E$. Thus we should have:

(i) $A+mE+k[(l-m)D-mE]+l[(k-l)C-(l-m)D]+m[(1-k)B-(k-l)C] \equiv A+B+C+D+E$, i.e. $1=(1-k)m=(k-l)(l-m)=(l-m)(k-l)=m(1-k)$, or $(1-k)m=m(1-k)$, $(k-l)(l-m)=(l-m)(k-l)$, showing that *the commutative law must hold* and $1=(1-k)m=(k-l)(l-m)$.

(ii) $mE+A+(m-1)[A+(1-k)B]+(1-l)[(1-k)B-(k-l)C]+(1-k)[(k-l)C-(l-m)D] \equiv m[A+B+C+D+E]$, i.e. $-m=(1-k)(l-m)=(k-l)^2$ assuming the commutative law to hold here.

Now if we put $m=-r$, we have $1-k=-\frac{1}{r}$ from (i) and. then $l-m=-r^2$ from (ii) and finally $k-l=-\frac{1}{r^2}$ from (i). Putting this value of $k-l$ in (ii) we find $r=-m=(k-l)^2=\frac{1}{r^4}$, i.e. $r^5=1$ showing that r is a 5th root of unity (not unity itself) and there being four such roots we can say that there are four positions of B, only such that its polar prime passes through A_1, say B_i $(i=1, 2, 3, 4)$. Evidently polar prime of each passes through the other three completing a self-polar pentad $B_1B_2B_3B_4A_1$ w.r.t. P_0.

It is easy to note that P_0 is self-polar w.r.t. $B_1B_2B_3B_4A_1$ and they are mutually self-polar.

Hence *we establish the existence of mutually self-polar pentads if and only if the commutative law holds.*

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

50

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 15 ZOOLOGY Pp. 33—38

June, 1951

'ON THE MORPHOLOGY OF *PARAMPHISTOMUM BATHYCOTYLE* FISCHOEDER (1901) A COMMON AMPHISTOME IN THE BILE DUCTS OF INDIAN BOVINES'

by

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Price Re.1-8-0

'ON THE MORPHOLOGY OF *PARAMPHISTOMUM BATHYCOTYLE* FISCHOEDER (1901) A COMMON AMPHISTOME IN THE BILE DUCTS OF INDIAN BOVINES.'

By N. K. Gupta, M.Sc.

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Introduction

Paramphistomum bathycotyle was another worm which I collected from Indian bovines in 1941 at Lahore. This worm is very common in the bile ducts of these animals. Due to their heavy infestation, they generally block the bile duct and the flow of bile is hindered.

This worm is generally confused with *P. explanatum* Creplin (1847) because the latter has got the same habitat. It is also mistaken for *P. cervi* Zeder (1790) for having antero-posteriorly placed testes.

The preliminary description of the worm was given by Fischoeder in 1901 who also recorded it for the first time. In 1923, Maplestone, on the basis of the position of testes synonymised it with *P. cervi*. Later workers, prominent amongst whom were Fukui (1929), Stunkard (1929), Sprehn (1932), Travassos (1934) and Ben Dawes (1936), concurred with Maplestone's view.

Nasmark (1937) revised the family Paramphistomidae and split up the genus Paramphistomum into many genera. He proposed the genus Gigantocotyle for *P. bathycotyle* Fischoeder (1901), *P. explanatum* Creplin (1847), *P. gigantocotyle* Brandes (1896), *Gigantocotyle symmeri* Nasmark (1937), *P. formosanum* Fukui (1929), *G. duplicitestorum* Nasmark (1937), *P. anisocotylea* Faust (1920), *P. birmiense* Railliet (1924), *P. siamense* Stiles and Goldberger (1910) and *P. fraternum* Stiles and Goldberger (1910).

Considering *P. bathycotyle*, he points out that its identity with *P. cervi* is absolutely erroneous because of differences in habitat and in the anatomical structures of the Pharynx (Oral sucker), the acetabulum and the genital atrium.

Ben Dawes (1946), in his book 'The Trematoda', has retained the genus paramphistomum and treated *P. bathycotyle* as synonym of *P. cervi*. From his book it appears that he does not recognise the scheme of classification put forth by Nasmark, 1937.

In view of the diversity of opinion about the validity of this species, its morphology is described here. My observations are based on the freshly collected material of which the whole mounts were prepared and serial sections were cut.

I have great pleasure in expressing my indebtedness to Prof. Dewan Anand Kumar, M.A. (Cantab.), Vice-Chancellor of the Panjab (I) University under whose guidance the work was carried out. I am also grateful to Dr. H. D. Srivastva, D.Sc., Helminthologist, Indian Veterinary Research

Institute, for his valuable suggestions in the preparation of this paper and to Dr. Vishwa Nath, Ph.D. (Cantab.), P.E.S. (I) for accepting it for publication in this bulletin.

Diagnosis

Shape and size: elongated, like a cone. 6–14·48 mm. in length and 4·48–4·84 mm. in maximum breadth. Cuticle: thin. Oral sucker: globular; ratio between its length and that of the body of the worm 1: 12·6–1: 12·7 in fully relaxed and 1: 9·3–1 : 10·3 in unrelaxed specimens. Acetabulum: ratio between its diameter and the length of the body 1 : 3·2–1 : 3·6 in fully relaxed and 1 : 1·8–1 : 2·1 in unrelaxed specimens. Gut: oesophagus 0·68–0·80 mm. in length. Intestinal caeca almost straight (not spiral). Reproductive systems: testes in tandem, one behind the other. Ovary somewhat spherical situated behind the posterior testis. Vitelleria on the lateral sides extending from the level of the oral sucker to the acetabulum. Laurer's canal crosses the excretory vesicle, opening dorsally behind the excretory pore. Genital pore close to the oesophagus in front of intestinal bifurcation. Eggs: 0·12×0·08 mm.

The Morphology

Paramphistomum bathycotyle is found in the bile duct of Indian bovines. Live worm is of light brown colour, reddish at the extremities. It is elongated, conical in shape, and tapering postero-anteriorly to a blunt oral extremity. Fixed specimens may be straight (Plate I, fig. 1) or slightly bent dorso-ventrally. They measure 6–14·48 mm. in length and 4·48–4·84 mm. in maximum breadth. The dorso-ventral side in unflattened specimens measures 2·64–2·8 mm. across the testicular region and 3·6 mm. across the acetabular region. The body surface is generally smooth without any wrinkle or groove. The oral aperture is situated terminally at the anterior end and the genital aperture in the anterior one third on the ventral side of the body (Plate I, fig. 1). The oral sucker is globular in shape and measures 0·64–1·12×0·6–1 mm. The ratio between its length and that of the body is 1 : 12·6–1 : 12·7 in fully relaxed and 1 : 9·3–1 : 10·3 in unrelaxed specimens. The latter specimens were cut sagittally and transversely. The acetabulum is placed at the posterior end of the body and measures 4·0–4·6 mm. in diameter in relaxed and 3·32 mm. in dorso-ventral direction in unrelaxed specimens. The acetabular aperture is either subterminal or ventral in position. It measures 0·83–1·86×2·40–2·88 mm. The ratio between the diameter of the acetabulum and the length of the body is 1 : 3·2–1 : 3·6 in flattened specimens (Plate I, fig. 2) and ratio between the dorso-ventral diameter of the acetabulum and the length of the body in unflattened specimens is 1 : 1·8–1 : 2·1 (Plate I, fig. 4). Nasmark gives 1 : 2·22 the mean ratio for the genus, and this ratio is not applicable to the fully relaxed specimens. Even in unrelaxed specimens Nasmark himself shows that this character varies from 1 : 1·50–1 : 2·60. Such a variable character should not be retained for generic diagnosis.

The cuticle of the integument is very smooth and thin (Plate II, fig. 10). It is without any striation. The subcuticular region is, however, striated.

In the oral sucker, next to the cuticle of its lumen there are bands of interior circular muscles fibres and then a wide layer of interior longitudinal muscle fibres. Oblique muscles interlacing the interior longitudinal muscles as in *P. (Cauliorchis) crassum*, are absent in this species. At about an equal distance from the cuticle and the limiting membrane, there is a row of bands of middle circular muscle fibres, the distal bands of which gradually approach the oral and aboral poles of the oral sucker. On the lateral sides, there are a few bands of anterior lateral circular and basal circular muscle fibres (Plate II, fig. 9). Just below the limiting membrane there are a few bands of exterior longitudinal muscle fibres. The radial muscles connecting the cuticle with the limiting membrane are feebly developed. The subcuticular cells are placed dorsal to the bands of middle circular muscles.

In the acetabulum (Plate II, fig. 12), underneath the cuticle there are prominent bands of circular muscles and on their dorsal side a few bands of longitudinal muscles. The bands of circular muscle fibres on the lateral sides have been counted. They are fifty-four on the dorsal exterior, forty-one on the dorsal interior, twenty-three on the ventral exterior and fifty-four on the ventral interior sides of the acetabulum. These bands were not counted by Nasmark, 1937, probably due to the badly fixed material he had at his disposal. The radial muscle fibres are present in bands alternating with the bands of the circular muscles. There are also present exterior longitudinal muscles underneath the limiting membrane and bands of oblique muscles over the lateral circular muscles.

The mouth is in the centre of the oral sucker (Plate I, figs. 2 and 3). When the specimen is left unstretched or unrelaxed the oral sucker is retracted inside with the result the body surface forms a short canal in front of it. This canal in front of the mouth also forms two papillae like projections which are due to the inward growth of the body surface (Plate I, fig. 4). The oesophagus is a tubular structure measuring 0·68–0·80 mm. in length and 0·2 mm. in thickness. Behind the genital pore, the oesophagus bifurcates into two intestinal caeca, each of which then runs along the lateral wall of the body ending blindly in front of the acetabulum. Both the intestinal caeca may be straight or might show slight curvatures during their course (Plate I, fig. 2). They are not in a spiral as in *P. (C.) crassum*. The cilia of their epithelial cells are grouped in such a way that they form villi like structures projecting into the lumen (Plate I, fig. 7).

The excretory vesicle is situated in the posterior region of the body dorsal to the testes extending backward up to the acetabular region. It is a bag like structure dilated posteriorly and tapering anteriorly to form the excretory canal which opens in the mid-dorsal line in the testicular region (Plate I, fig. 4).

In genital system, the paired testes are placed antero-posteriorly in the middle region in between the two intestinal caeca. They are slightly lobulated (Plates I, fig. 2). The anterior testis measures 2·48–3·2 × 2·40–2·80 mm. and posterior testis 2·0–2·56 × 2·40–3·08 mm. in size. A vas efferens arises from the anterior side of each testis. The vesicula seminalis is very prominent as compared to pars musculosa and pars prostatica which are shorter in size (Plate I, fig. 4). The ductus ejaculatorius unites with

the metraterm and opens into the ductus hermaphroditicus (Plate II, fig. 8).

The ovary is single and somewhat spherical lying behind the posterior testis. It measures 0·48–0·56×0·48–0·52 mm. The Laurer's canal runs on the left lateral side of the excretory vesicle and opens dorsally posterior to the excretory pore (Plate II, fig. 11). The Mehlis' gland-complex is globular in shape and measures 0·46×0·46 mm. (Plate I, fig. 7). The vitelline glands, on both sides, extend from the level of the base of the oral sucker (Plate I, figs. 5 and 6) or from the oesophagus to the acetabulum. They consist of distinct follicles overlapping one another and also the intestinal caeca at some places (Plate I, fig. 2). The two main vitelline ducts, one from each vitellaria open in the yolk reservoir (Plate II, fig. 11) which in its turn opens by a short duct into the ootype. The uterus emerges from the lateral side of the Mehlis' gland-complex and proceeds anteriorly along the left border of the posterior testis then changes its course and becomes dorsal to the anterior testis. Further it runs beneath the vas deferens and finally opens through a metraterm into the small and delicate chamber, the ductus hermaphroditicus (Plate I, fig. 4 and Plate II, fig. 8). It has a wide lumen containing a large number of eggs. The ductus hermaphroditicus opens out on the genital papilla which is strengthened by small sphincter muscle fibres. The eggs measure 0·12×0·08 mm. in size.

Name of the organ.	Measurements as given by Nasmark, 1937.	Measurements according to writer's observations.
Length of the worm ..	10·8 mm. (11–15 mm.)	6–14·48 mm.
Breadth	More than 2/5 of the length of the body.	4·48–4·84 mm.
Dorso-ventral dimension.	4·32 mm.	2·64–2·8 mm. across the posterior testicular region and 3·6 mm. across the acetabular region.
Ratio between the length of the oral sucker and the length of the body.	1 : 10·0 (1 : 12·2–1 : 15·0).	1 : 12·6–1 : 12·7 in fully relaxed and 1 : 9·3–1 : 10·3 in unrelaxed specimens.
Diameter of the acetabulum.	4·64 mm. (4·4–6 mm.).	4·0–4·6 mm. in relaxed and 3·32 mm. in dorso-ventral direction in unrelaxed specimens.
Ratio between the diameter of the acetabulum and the length of the body.	1 : 2·32 (1 : 2·5).	1 : 3·2–1 : 3·6 in flattened specimens 1 : 1·8–1 : 2·1 in unflattened specimens.
Oesophagus ..	1·0 mm. (1·0–1·05 mm.) in length.	0·68–0·80 mm. in length 0·2 mm. in thickness.
Testes	2·0 mm. (1·0–1·3 mm.) in length. 1·5 mm. (1·5–1·8 mm.) D.V. Direction.	Anterior testis 2·48–3·2×2·40–2·80 mm. Posterior testis 2·0–2·56×2·40×3·08 mm.
Egg	0·14×0·078 mm.	0·12×0·08 mm.

Remarks

From the morphology, it is quite evident that *P. bathycotyle* is not a synonym of *P. cervi* Zeder (1790) but a valid species.

With regard to the acceptance of the new genus as proposed by Nasmark, 1937, there are certain objections. His system of classification,

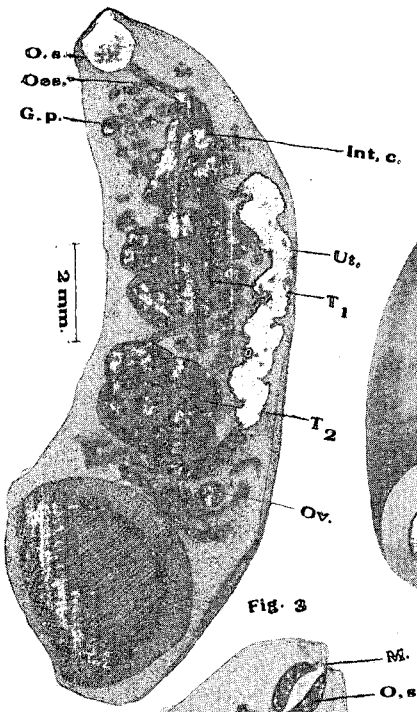

Fig. 3

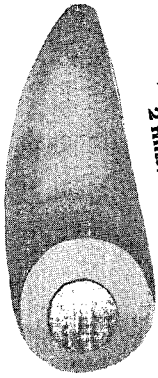

Fig. 1

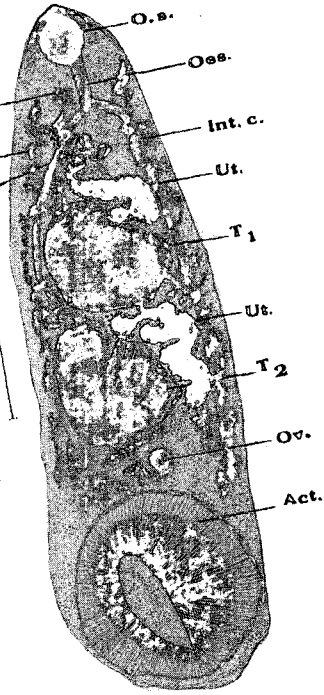

Fig. 2

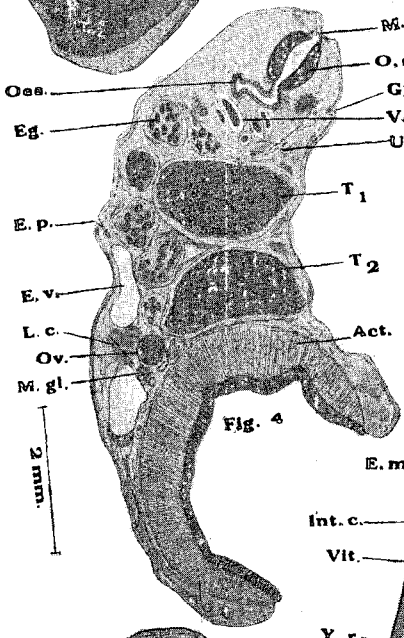

Fig. 4

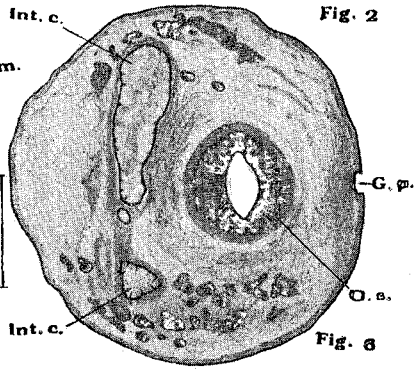

Fig. 6

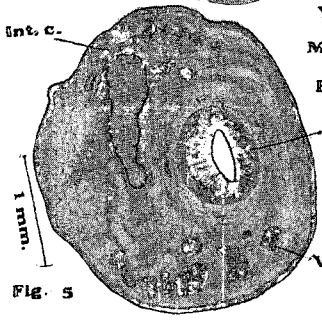

Fig. 5

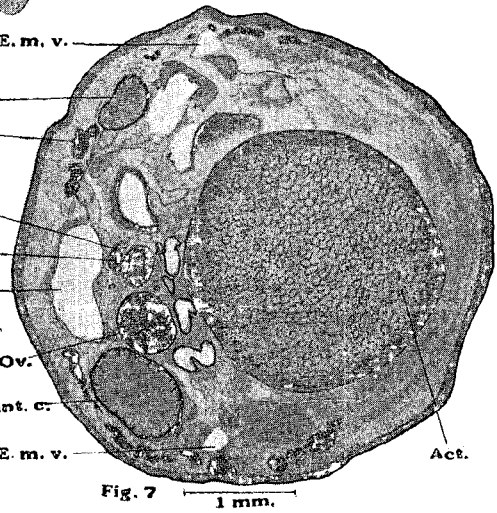

Fig. 7

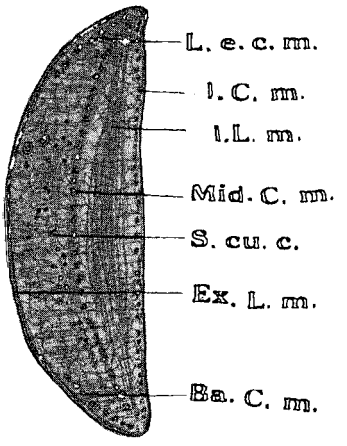

Fig. 9

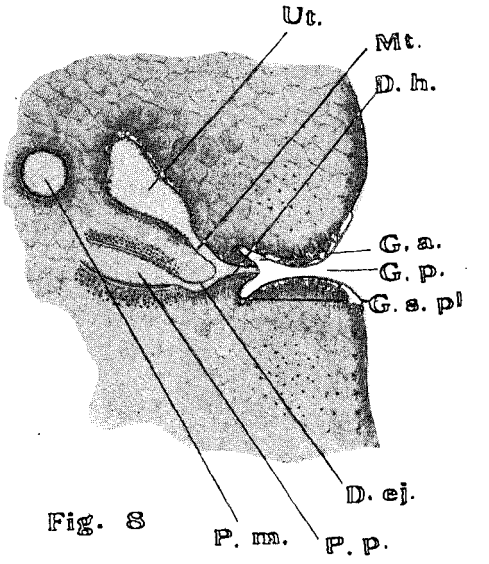

Fig. 8

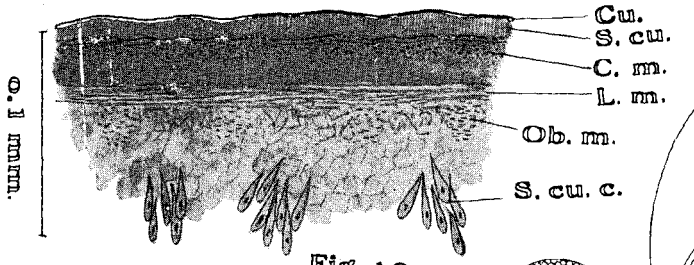

Fig. 10

Fig. 11

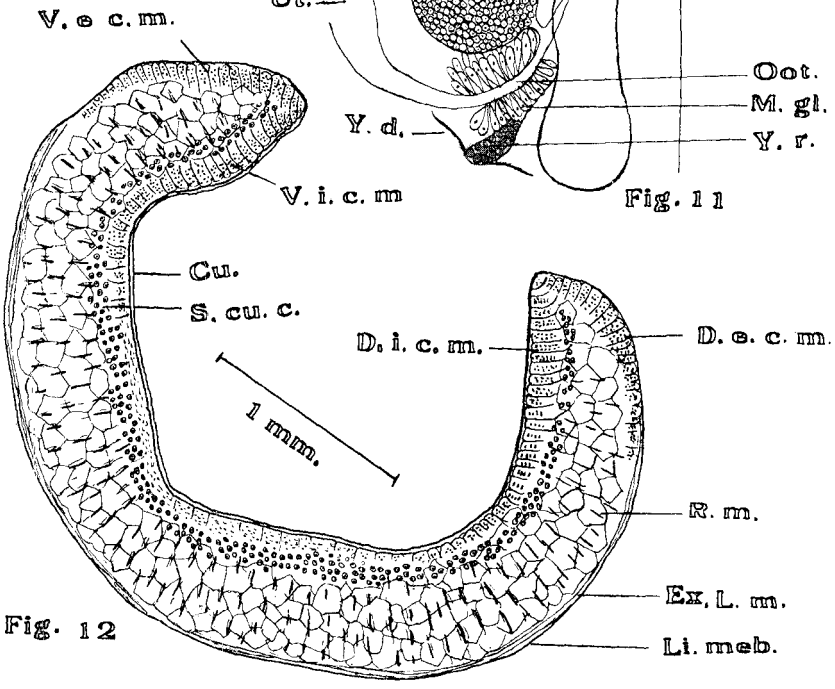

Fig. 12

if adopted, will not prove practicable. In an immature specimen the units of muscles on the basis of which the various types of acetabula have been made, may not be fully developed. Moreover, the shape of acetabulum may vary with fixation.

Similarly the ratio between the diameter of the acetabulum and the length of the body depends upon the state in which the worm has been fixed. In a contracted specimen the ratio will decrease, while in a relaxed specimen, it will increase.

In my flattened specimens, the ratio between the dia. of act. and the length of body is 1 : 3·2–1 : 3·6; whereas according to Nasmark, the mean ratio for the genus is 1 : 2·22. This means that this ratio is not applicable to the whole mounts. I, therefore, prefer to retain this species under the genus Paramphistomum.

PLATE I

FIG. 1. Ventral view of a preserved specimen.
FIG. 2. Ventral view of a flattened specimen showing the internal anatomy.
FIG. 3. Side view of a laterally flattened specimen.
FIG. 4. Median sagittal sectioned specimen showing the excretory vesicle, ovary, a portion of Mehlis' gland-complex, Laurer's canal and testes.
FIG. 5. Transverse section through the oral sucker and lateral intestinal caeca.
FIG. 6. Transverse section through the genital pore.
FIG. 7. Transverse section through the ovary, Mehlis' gland-complex, yolk reservoir and excretory vesicle.

Act., acetabulum; Eg., egg; E.v., excretory vesicle; E.m.v., Main excretory vessel; E.p., Excretory pore; G.p., genital pore; Int.c., intestinal caecum; L.c., Laurer's canal; M., Mouth; M.gl., Mehlis' gland-complex; Oes., Oesophagus; O.s., Oral sucker; Ov., Ovary; T_1., anterior testis; T_2., posterior testis; Ut., uterus; Vas. def., vas deferens; V. sem., vesicula seminalis.

PLATE II

FIG. 8. An enlarged portion of the genital area.
FIG. 9. One half of the oral sucker showing the plan of its component parts.
FIG. 10. A portion of the integument.
FIG. 11. Showing the relation between the Laurer's canal and the excretory vesicle.
FIG. 12. The acetabulum showing its structure.

Ba.C.m., basal circular muscles; C.m., circular muscles; Cu., cuticle; D.h., ductus hermaphroditicus; D.ej., ductus ejaculatorius; Ex.L.m., exterior longitudinal muscles; G.a., genital atrium; G.s.pl., genital sphincter papillae; I.C.m., interior circular muscles; I.L.m., interior longitudinal muscles; L.e.c.m., lateral exterior circular muscles; L.m., Longitudinal muscles; L.c.p., Laurer's canal pore; Mt., metraterm; Mid.C.m., middle circular muscles; Oot., ootype; Ob.m., oblique muscles; P.m., Pars musculosa; P.p., Pars prostatica; S.cu.c., subcuticular cells; S.cu., subcuticle; Y.d., yolk duct.

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Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 16 ZOOLOGY Pp. 39–50

August, 1951

SPERM FORMATION IN CERTAIN COLEOPTERA WITH PARTICULAR REFERENCE TO CHROMOSOME NUMBERS, ACROSOME AND MITOCHONDRIAL NEBENKERN

by

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Price Rs.3-0-0

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CONTENTS

1. INTRODUCTION

A glance at the next section of this communication, 'Previous Literature on Insect Spermatogenesis', will show that most of the previous work on insect spermatogenesis—at any rate, work carried out with the modern technique of studying the Golgi apparatus and the mitochondria—has been carried out on the Lepidoptera and Hemiptera. Bowen (1924), in his remarkable paper on 'the Formation of Sperm in Coleoptera and Aptera', states that the testicular material of *Chelymorpha* is unsuitable both for the study of the condensation of the mitochondrial nebenkern and for the development of the acrosome from the Golgi apparatus.

In view of the above, work in this laboratory on the testicular material of *Plocaederus obesus*, *Coccinella septumpunctata* and *Aulocophora foveicollis* —all coleoptera—was started by Bawa, Bhardwaj and Gupta respectively at the suggestion and under the supervision of the Head of the Department in October, 1949. The investigations were completed early this year and it has been considered best to record the findings of all the three authors in one communication, which has been prepared for the press by the Head of the Department.

Amongst the three beetles studied the testicular material of *Aulocophora foveicollis* turned out to be the most suitable inasmuch as the sperm-forming cells of this beetle are large, and the behaviour of the mitochondria and

Golgi elements can be followed easily throughout the process of spermatogenesis.

2. Previous Literature on Insect Spermatogenesis

To the best of our knowledge Gatenby (1917) was the first worker to study insect spermatogenesis with modern technique, which brings out the Golgi elements and the mitochondria clearly. Prior to this publication all the investigators on insect spermatogenesis used fixatives containing fat solvents and were thus unable to study the mitochondria and the Golgi elements correctly. Indeed, their investigations were confined mostly to the study of the nucleus.

Gatenby (1917) working on *Smerinthus* (Lepidoptera) shows that the acrosome is formed by the Golgi elements, and he also gives details of the formation and ultimate fate of the mitochondrial nebenkern. Although some of the conclusions of Gatenby were challenged subsequently by Bowen, Gatenby's publication must be considered as a pioneer one in this field inasmuch as it stimulated interest in the study of not only insect spermatogenesis but also spermatogenesis in general.

Subsequently Doncaster and Cannon (1920) published a paper on the Sperm of two species of Louse, viz., *Pediculus corporis* and *Pediculus capitis*. This work was continued by Cannon who confirmed the earlier findings in a subsequent paper on the Sperm of Louse published in 1922.

Bowen (1920, 1922*a*, 1922*b* and 1924) published four important papers on insect spermatogenesis covering the *Hemiptera*, *Lepidoptera*, *Coleoptera* and *Aptera*. In his paper on Lepidoptera Bowen (1922*b*) gave an altogether different interpretation of the detailed structure of the mitochondrial nebenkern from the earliest description of Gatenby (1917). Briefly speaking, whereas Gatenby had described a 'spireme' structure in the mitochondrial nebenkern, Bowen advanced the conception of a 'plate-work' structure. Similarly Bowen differed from Gatenby in the interpretation of the detailed formation of the acrosome.

Nath (1925), on an invitation from Prof. Gatenby, was permitted to examine the slides of Lepidopterous testes, which the latter had recently prepared, and confirmed Bowen's conclusions about the 'plate-work' structure of the mitochondrial nebenkern.

Chang-Chun Wu (1946) studied the spermatogenesis of an Orthopterous form *Diestrammena*; and confirmed the earlier conclusions of Bowen.

3. Observations

In this section will be given a comparative account of all the three coleopteran species, so as to avoid unnecessary repetition.

Spermatogonia

Of all the three coleopteran species studied *Aulocophora foveicollis* forms the most favourable material for cytological studies inasmuch as the sperm-forming cells from the spermatogonia to the ripe sperm are comparatively much larger than in either of the other two species, *Plocaederus obesus* and *Coccinella septumpunctata*. In Pl. I, Figs. 1 and 2, are shown

two spermatogonia of *A. foveicollis*. In figure 1 there is a juxtanuclear mass consisting of fine granules, which stain darkly with haematoxylin. Although most of the granules of this juxtanuclear mass are mitochondrial in nature, it is likely that a few granules are Golgi in nature, but the latter cannot be distinguished as such from the mitochondria on account of overcrowding. But this juxtanuclear mass has become almost circumnuclear in figure 2, when the Golgi material appears as a prominent juxtanuclear body of irregular form, staining more intensely with haematoxylin than the mitochondria. This Golgi mass is undoubtedly formed by the running together of closely situated Golgi granules, which could not be distinguished as such in the stage represented by Fig. 1. The nucleus of the spermatogonium reveals a prominent darkly staining nucleolus, round in form.

In Pl. III, Fig. 62, is shown the spermatogonium of *Coccinella septumpunctata*. Here the Golgi elements are not aggregated together in a juxtanuclear mass, but are spread out amongst the mitochondrial granules. One or two of the Golgi elements appear in the form of crescents.

The spermatogonia of *Plocaederus obesus* are the smallest of the three species, and reveal at best mitochondrial granules only. It has not been found possible to observe at this stage any granules, which could be identified as Golgi elements in this species (Pl. IV, Figs. 105 and 106).

Primary Spermatocytes

In the primary spermatocytes of *Aulocophora foveicollis* (Pl. I, Figs. 3 and 4) both the mitochondria and the Golgi elements are in the form of granules, the latter staining more deeply, and both are distributed uniformly throughout the cytoplasm. The Golgi elements remain granular throughout meiosis I, but they grow in size towards the later stages of this phase. On the other hand, radical changes of a chemical nature seem to have taken place in the mitochondrial material. Soon after the stage shown in Fig. 4, Pl. I, the mitochondrial granules run together and arrange themselves in the form of a horseshoe-shaped nebulous mass, which hardly reveals any definite structure within itself (Pl. I, Figs. 5 and 6). In Fig. 8, Pl. I, the mitochondria have appeared in the form of delicate bubbles, which generally put in an appearance as late as the early stages of insect spermateleosis. In other words, the running together of mitochondrial bodies to form a nebenkern in sperm formation is, in this species, *A. foveicollis*, anticipated as early as the spermatocyte stage. During meiosis I, the mitochondrial mass is roughly divided into two; and the Golgi granules which have grown in size by this time, are also sorted out roughly in two halves (Pl. I, Fig. 12).

Although the number of diploid chromosomes in *A. foveicollis* seems to be 30, the chromosomes are large and easy to count. In Fig. 10, Pl. I, thirty diploid chromosomes can easily be counted. In Fig. 9, Pl. I, which gives a polar view of metaphase I, 15 pairs of chromosomes can also be easily counted.

In the resting primary spermatocytes of *Plocaederus obesus* the Golgi elements appear in the form of crescents and granules, and the mitochondria in the form of small granules or vesicles (Pl. IV, Figs. 107–110). The

diploid number of chromosomes in this species seems to be 10 (Pl. V, Fig. 111); and the Golgi elements and the mitochondria are sorted out into two sub-equal halves as illustrated by Figs. 111 and 112, Pl. V.

In the primary spermatocytes of *Coccinella septumpunctata* the Golgi elements and the mitochondria appear in the form of crescents and granules respectively (Pl. III, Fig. 63). The diploid number of chromosomes is here also 10, as is shown by Figs. 65–71, Pl. III. It will be noted that the chromosomes of this species are much larger than the chromosomes of *Plocaederus obesus*, although the diploid number of chromosomes in both is 10.

Secondary Spermatocytes

We now turn to a description of the secondary spermatocytes and meiosis II. In all the three coleopteran species described here there is a distinct pause between the two meiotic phases. In Fig. 13, Pl. I, is shown a resting secondary spermatocyte of *A. foveicollis*; in Fig. 14, Pl. I, metaphase II; and in Figs. 15 and 16, Pl. I, telophase II.

In Figs. 113–116, Pl. V, are shown resting secondary spermatocytes of *Plocaederus obesus*; in Fig. 117 metaphase II; and in Figs. 118 and 119 telophase II. The Golgi elements in the secondary spermatocytes and in meioses II are crescent-like; and the mitochondria, which have now grown in size, are in the form of vesicles.

In Fig. 72, Pl. III, is shown the resting secondary spermatocyte of *Coccinella septumpunctata*; and in Figs. 73 and 74, Pl. III, telophase II.

Spermateleosis

As the details of the process of spermateleosis, occurring in the three species under discussion here, vary, particularly the details of the formation of the acrosome, it will be best to describe this process separately for each of the three species.

Undoubtedly the most favourable of the three species for the study of spermateleosis is *Aulocophora foveicollis*, as, apart from the fact that the cells are large in this species, the details of acrosome formation are easier to follow than in either of the two other species. A study of Figs. 19 and 20, Pl. I, and Figs. 21 and 22, Pl. II, will show that in the early spermatids the mitochondrial nebenkern is an amorphous mass, showing no structure inside its interior, the Golgi elements in these early stages existing in the form of granules varying in size. Most probably, however, the mitochondria exist at this stage in the form of extremely fine and delicate bubbles—more delicate than those figured in the resting secondary spermatocyte (Pl. I, Fig. 13). But the technique used has failed to bring out the real structure of the mitochondria, which is alveolar.

From now onward a partial breakdown and confluence of the mitochondrial bubbles take place, as is usual in insect spermatogenesis. This process results in the formation of a body, which in reality has a 'plate-work' structure (Bowen, 1922*b*), but was erroneously described as a 'spireme' (Gatenby, 1917). For the study of the beginning of this process reference

may be made to Pl. I, Figs. 17 and 18. Soon, however, the mitochondrial nebenkern assumes a spherical form with lightly staining alveoli on the periphery and more heavily staining alveoli in the centre (Pl. II, Fig. 25). The process of coalescence of mitochondrial bubbles continues, till we get a compact sphere having a darkly staining central part, from which radiate outwards a number of septa through a chromophobic peripheral substance (Pl. II, Figs. 26 and 27). Gradually the septa piercing the chromophobic part break down till they all disappear. The mitochondrial nebenkern now consists of a perfectly round central chromophilic sphere and a peripheral chromophobic part. At the same time the axial filament has put in its appearance, and piercing the mitochondrial nebenkern, it divides it into right and left halves (Pl. II, Fig. 28). During the later stages of sperm-formation the mitochondrial nebenkern undergoes a progressive process of lengthening. Side by side with this attenuation of the mitochondrial nebenkern the peripheral chromophobic material is correspondingly reduced, till it completely disappears (Pl. II, Figs. 29–32). With the lengthening of the tail of the sperm the mitochondrial nebenkern also begins to lengthen out (Pl. II, Figs. 35 and 37, and 38–47). It may be noted here that the central substance of Bowen (1922*b*) or 'Sheath substance' of Nath (1925) is conspicuous by its absence in *Aulocophora foveicollis*. But the bleb-like swellings on the tail of the sperm as described by Bowen (1922) in insect spermatogenesis are commonly met with in this species (Pl. III, Figs. 52-53).

While it is often easy to observe the minute centriole lodged in the neck region of the ripe or nearly ripe sperm immediately behind the nucleus, it is not possible to demonstrate axial filament during last stages of spermateleosis of *A. foveicollis*.

As has already been noted in the beginning of this section, it is easier to follow the details of the acrosome formation in *A. foveicollis* than in either of the other two species. The Golgi elements in the earlier spermatids of *A. foveicollis* can be easily made out as deeply staining granules of varying sizes (Pl. I, Figs. 19 and 20 ; Pl. II, Fig. 21). Soon, however, a few Golgi granules seem to come together as illustrated in Fig. 22, Pl. II. It seems likely that these Golgi granules ultimately unite to form a single large vesicle with a chromophilic rim and a chromophobic interior, which, in conformity with the terminology used by Bowen and Gatenby, may be called the acroblast. Soon, however, the acroblast loses the staining capacity of its chromophilic periphery, the whole sphere appearing as a pale structure in the finished preparations, never taking up any stain. This is clearly illustrated by Figs. 23, 24, 26 and 27, Pl. II. It must be clearly noted that only a few Golgi elements fuse together to form the acroblast, whereas the rest of them, along with the acroblast after it has secreted the acrosomal granule, are sloughed off.

The pale sphere, which we have named as the acroblast, seems to go round the nucleus till it has completed a movement through an angle of 180°. This will be made clear from a reference to Figs. 32–35, Pl. II. The meaning of this journey on the part of the acroblast, before it begins to secrete the acrosomal granule, is not clear. A similar journey on the part of acroblast has been described by Bowen in Coleoptera (1924).

Suddenly a deeply staining granule, the acrosomal granule, appears on the border of the pale acroblast (Pl. II, Figs. 38–41). It is difficult to escape the impression that the acrosomal granule is secreted by the acroblast. Soon, however, the acroblast and the acrosomal granule part company, the former shifting backward into the tail to be sloughed off subsequently, and the acrosomal granule beginning to move in front of the nucleus, where it forms the acrosome (Pl. II, Figs. 42–44).

The acrosomal granule, having seated itself in front of the sperm nucleus, applies itself to the anterior aspect of the nucleus and undergoes a process of progressive lengthening out, till it assumes the form of a needle broad at its base and narrow towards the anterior end of the sperm (Pl. III, Figs. 49–51 and 54–61).

As we have already pointed out only a few Golgi elements fuse together to form the acroblast. The remaining Golgi elements are sloughed off along with the 'acroblast' (Pl. II, Figs. 37, 41 and 45–47).

The centrosomal granule, which can be seen often in both meiotic stages, is lost to view in the early stages of sperm formation. It reappears as a definite granule in the last stages of spermateleosis at the base of the nucleus of the sperm (Pl. III, Figs. 54–61). But sometimes in favourable preparations the centriole can be seen as a distinct granule, from which the axial filament springs up (Pl. II, Fig. 31).

In the early stages of spermateleosis in *A. foveicollis* the nucleus reveals a structure which is characteristic of this phase of sperm formation in general. That is, it has a lightly staining central area and a more darkly staining periphery (Pl. I, Figs. 19 and 20; Pl. II, Figs. 21–28 and 32–35). Again, as is usually the case, there takes place a reversal of these two areas of varying staining capacity. That is, the peripheral part of the sperm nucleus now becomes lightly staining, the central part taking a deep stain. In Pl. II, Fig. 36, a beginning of this process of reversal has started and in Pl. II, Fig. 37, the process of reversal has been completed. Soon the central more deeply staining area extends right up to the nuclear membrane. The rest of the process of the maturation of the nucleus consists, as usual, of a process of condensation, till a long, compact and homogeneously staining nucleus has been formed.

In *Coccinella septumpunctata* the details of the formation of mitochondrial nebenkern closely follow those of *A. foveicollis*. But in *Plocaederus obesus* the central chromophilic part of the nebenkern progressively disappears, and the tail sheath is formed from a new substance, the 'central substance' of Bowen (1922*b*) and 'Sheath substance' of Nath (1925). The central substance puts in its appearance in the form of lightly staining granules in the chromophobic area before the final disappearance of the central chromophilic part of the nebenkern (Pl. V, Figs. 131–140).

In *Coccinella septumpunctata* a very prominent acroblast broad at one end and narrow towards the other is formed in the early stages of sperm formation (Pl. III, Fig. 79 and Pl. IV, Figs. 80 and 84). The acroblast, more often than not, stains deeply with haematoxylin. It will be recalled that the acroblast of *Aulocophora foveicollis* is perfectly spherical, whereas, in *Coccinella septumpunctata*, it is an elongated structure broad at one end

and narrow at the other. Sooner or later there appears a vesicle in the immediate vicinity of the acroblast (Pl. IV, Figs. 82, 83, 85, 87–91). This may be called the acrosomal vesicle. Soon a sharply staining granule, the acrosomal granule, puts in its appearance in the interior of the acrosomal vesicle (Pl. IV, Figs. 92–94). The acrosomal vesicle seems to condense and at the same time the acrosomal granule begins to grow in size, the result being that the acrosomal granule completely fills up the space within the acrosomal vesicle. In other words, the acrosomal vesicle as such completely disappears and the acrosomal granule, now grown in size, becomes the definitive acrosome (Pl. IV, Figs. 95–97). The acroblast and the acrosome now part company, and the acroblast begins to travel backwards as is clear from a reference to Figs. 96 and 97, Pl. IV. As it is travelling backwards into the tail the acroblast undergoes shrinkage in size and ultimately degenerates (Pl. IV, Figs. 98 and 99). On the other hand, the acrosome grows in size and begins to travel forwards in front of the nucleus (Pl. IV, Figs. 101 and 102). Seated on the anterior aspect of the nucleus, the acrosome develops into a triangular structure with a broad posterior and a fine needle-like anterior end (Pl. IV, Figs. 103 and 104).

Of all the three coleopteran species under discussion *Plocaederus obesus* seems to have the smallest sperm-forming cells. Whereas the details of the formation of mitochondrial nebenkern and its ultimate fate can be more easily followed in *Plocaederus obesus*, the details of acrosome formation are most difficult to follow in this species, as can be seen from a study of Figs. 122–129, Pl. V. The Golgi elements in the early stages of spermateleosis exist in the form of crescents, each enclosing a chromophobic area. But suddenly the crescent-shaped Golgi elements disappear from view and the only representative of the Golgi material in the cell at this stage is a small acrosomal vesicle, enclosing an acrosomal granule within it (Pl. V, Figs. 130 and 131). The acrosomal granule seems to grow in size and fill up completely the space within the acrosomal vesicle, thus forming the acrosome (Pl. V, Fig. 132). The acrosomal granule now shifts to the anterior aspect of the nucleus and there it grows into a pointed acrosome (Pl. V, Figs. 134, 138–143).

Finally with regard to the changes in the nucleus we note a sharp contrast between *Aulocophora foveicollis* on the one hand and *Plocaederus obesus* and *Coccinella septumpunctata* on the other. Whereas a conspicuous process of reversal of the chromophobic and chromophilic area of the nucleus of the maturing sperm takes place in *A. foveicollis*, in the other two species this process of reversal is conspicuous by its absence. On the contrary in both *C. septumpunctata* and *P. obesus* a sharply staining granule puts in its appearance in the nucleus at an early stage in spermateleosis, to disappear only during the late stages. The significance of this granule is not known.

4. Discussion

A careful study of Bowen's paper on the formation of sperm in Coleoptera and Aptera (1924) leaves the impression that the coleopteran sperm is most unsuitable for the study of the development of the acrosome. Indeed, Bowen says that in the Coleoptera, 'the development of the

acrosome proper is very difficult to follow, owing to its extremely small size'. We are in a position to support this statement so far as the sperm of *Plocaederus obesus* is concerned. In this species there are prominent Golgi elements in the spermatids before the spermatid has begun to elongate. But just before the process of elongation begins there appears a small vesicle with a tiny granule within it, which we have called an acrosomal vesicle and the acrosomal granule respectively. We have no evidence to show that the acrosomal vesicle with its contained granule has been actually derived from the Golgi elements, except on the basis of analogy. The acrosomal granule seems to grow in size and fill up the whole space within the acrosomal vesicle, thus forming the acrosome, which gets itself seated on the anterior aspect of the nucleus. The material, however, is much more favourable for the study of the details of the acrosome formation in *Aulocophora foveicollis* and *Coccinella septumpunctata*, in spite of the fact that in both these species of beetles we have been unable to demonstrate the origin of the acroblast from the Golgi elements; nevertheless the acroblast is a comparatively large structure in both these species, being larger in *Coccinella septumpunctata* than in *Aulocophora foveicollis*.

In *A. foveicollis* the form of the acroblast is perfectly spherical, and the acrosomal granule always puts in its appearance in its close neighbourhood, perhaps pointing towards the conclusion that the acrosomal granule is a secretory product of the acroblast. The acrosome is derived directly from this granule, after it has shifted to the anterior aspect of the sperm nucleus, whereas the acroblast shifts backwards into the tail and disappears.

In *Coccinella septumpunctata*, the acroblast is a much bigger structure: it is elongated in form with one end broad and the other narrower. It stains homogeneously with haematoxylin and never reveals any internal structure. This form of the acroblast in *C. septumpunctata* is in sharp contrast with the perfectly vesicular form in *A. foveicollis*. Suddenly a vacuole, which we have called the acrosomal vesicle, appears in the substance of the acroblast towards one end. Strange as it would appear this end is invariably the broad end of the acroblast, but for a long time the acrosomal vesicle remains closely attached to the acroblast. The staining capacity of the acrosomal vesicle increases and a deeply staining granule soon appears within its interior. This is the acrosomal granule, which grows in size. At the same time the acrosomal vesicle seems to shrink in size, the acrosomal granule thus filling up the whole space in the vesicle. The enlarged acrosomal granule migrates to the anterior aspect of the nucleus, where it forms the acrosome. The elongated acroblast shifts backward into the tail, where it begins to degenerate and ultimately is sloughed off. In *C. septumpunctata* also, as in *A. foveicollis*, it is difficult to escape the conclusion that the acrosome is secreted by the acroblast.

Bowen (1924) has described 'spermatid remnant' in the spermatid of Hemiptera, Coleoptera and Aptera. He has also described a chromatoid body in Coleoptera and Aptera, and a 'juxta-nuclear body' in the beetle *Chelymorpha*. Bowen seems to be doubtful about the homologies of these three bodies. We have not observed any of these bodies in the three species of beetles under discussion in this paper. We venture to suggest

that these bodies all belong to the category of Golgi apparatus. Nath (1944) made out a case in favour of the view that the essential structure of a Golgi element is granular or vesicular—a view which has been fully confirmed by Baker (1949). Therefore, it seems to us that the 'spermatid-remnant', 'chromatoid body' and the 'juxta-nuclear body' of Bowen are all essentially of the nature of Golgi granules or vesicles.

Bowen, who has described the details of the formation of the mitochondrial nebenkern in several species of insects, has directed attention to the development of the 'central substance' in the chromophobic part of the nebenkern in many species, particularly in the Lepidoptera (1922*b*). This 'central substance' usually forms the sheath of the axial filament. For that reason Nath (1925) calls this substance the 'sheath substance'.

It will be recalled that we have described a central substance in *Plocaederus obesus*, whereas in *Aulocophora foveicollis* and *Coccinella septumpunctata* it is completely absent.

Lastly, here we might make reference to some details of the condensation process of the sperm nucleus. It will be recalled that in *A. foveicollis* the nucleus of the early spermatids has a chromophilic periphery and a more lightly staining interior. These deeply staining and lightly staining areas are subsequently reversed, the densely staining area becoming internal. In *C. septumpunctata* and *P. obesus*, however, this process is absent. On the contrary, in both the species, the spermatid nucleus contains a deeply staining round granule, which Bowen (1924) refers to as the 'intra-nuclear body' in the beetle '*Chelymorpha*'. As in *Chelymorpha*, the intra-nuclear body ultimately disappears in both the species mentioned above.

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6. Explanation of Lettering in the Plates

A.—Acrosome.
A'.—Acroblast.
A.F.—Axial filament.
A.v.—Acrosomal vesicle.
A.G.—Acrosomal granule.
C.—Centriole.
Ch.—Chromosomes.
G.—Golgi elements.
M.—Mitochondria.
M.N.—Mitochondrial nebenkern.
N.—Nucleus.
N'.—Nucleolus.
C.S.—Central substance.
S.f.—Spindle fibres.

7. Explanation of the Plates

Figures 1 to 61 have been drawn at the stage level with Spencer 6× eyepiece using an oil immersion objective. They are subsequently enlarged two times, thus giving a total magnification of 1,200. All the figures are selected from sectioned material fixed in Flemming without acetic acid and stained with 0·5% iron haematoxylin.

Figures 62 to 104 have been drawn at the stage level with Spencer 10× eyepiece using an oil immersion objective. They are subsequently enlarged giving a total magnification of 1,900 times. All figures except 66, 69 to 71 are from material fixed in Flemming without acetic acid. Figures 66, 69 to 71 are from material fixed in Bouin's fluid.

Figures 105 to 143 have been drawn at the stage level with Leitz 8× eyepiece using an oil immersion objective. They are subsequently enlarged giving a total magnification of 400 times. All figures have been selected from sectioned material fixed in Flemming without acetic acid followed by 0·5% iron haematoxylin.

Plate I (*Aulocophora foveicollis*)

Fig. 1. Earliest spermatogonium showing large nucleus and cloud of mitochondrial granules lying on one side of the nucleus.

Fig. 2. Late spermatogonium.

Figs. 3 & 4. Early Primary spermatocytes.

Figs. 5 & 6. Early Primary spermatocytes showing loop formed by the mitochondria.

Fig. 7. Nucleus of a Primary spermatocyte showing diplotene threads.

Fig. 8. Late Primary spermatocyte. Mitochondria are vesicular.

Fig. 9. Primary spermatocyte showing bivalent chromosomes.

Fig. 10. Polar view Metaphase I, showing the diploid number of 30 chromosomes.

Fig. 11. Metaphase I. The Golgi and the mitochondrial granules are at the poles of the spindle.

Fig. 12. Telophase I.

Fig. 13. Secondary spermatocyte. The Golgi and the mitochondrial granules lie on one side of the nucleus.

Fig. 14. Metaphase II.

Figs. 15 & 16. Telophase II. The mitochondria form palisade round the spindle.

Figs. 17 & 18. Earliest spermatids showing optical sections of the mitochondrial nebenkern.

Figs. 19 & 20. Early spermatids. The Golgi elements lie scattered.

Plate II (*Aulocophora foveicollis*)

Figs. 21 & 22. Early spermatids.

Figs. 23 & 24. Late spermatids. The Golgi bodies have fused to form the acroblast.

Figs. 25–27. Late spermatids showing the formation of the nebenkern.

Figs. 28–31. Late spermatids showing the chromophilic and chromophobic portions of the nebenkern.

Figs. 32–35. Late spermatids showing the condensation of the nuclear material and the migration of the acroblast around the nucleus.

Figs. 36–39. Late spermatids. Progressive stages in the formation of the acrosome. The acrosomal granule is stained intensely black in every case. Nuclear vesicle is also formed.

Figs. 40 & 41. Late spermatids showing the acroblast with deeply staining acrosomal granule.

Figs. 42–44. Late spermatids. Acroblast separates from the acrosomal granule and it moves backward.

Figs. 45–48. Late spermatids showing the movement of the acrosome to the anterior tip of the nucleus.

Plate III

FIGS. 49 TO 61—*Aulocophora foveicollis*; FIGS. 62 TO 79—*Coccinella septumpunctata*.

FIG. 49. Late spermatid showing a homogeneously staining nucleus with a button-like acrosome in front.

FIGS. 50 & 51. Early sperms showing spindle-shaped nucleus with a button-like acrosome.

FIGS. 52 & 53. Late sperms showing tail vesicles.

FIGS. 54–59. Late sperms. Progressive lengthening out of the nucleus of the sperm.

FIGS. 60 & 61. Mature sperms showing needle-like head with a delicate apical acrosome.

FIG. 62. Spermatogonium. It shows large vesicular nucleus, granular mitochondria and four Golgi bodies.

FIG. 63. Primary spermatocyte.

FIG. 64. Primary spermatocyte in pre-metaphase stage.

FIG. 65. Polar view of chromosomes in metaphase I.

FIG. 66. Polar view of chromosomes in metaphase I (Bouin's preparation).

FIG. 67. Side view of equatorial plate of five pairs of homologous chromosomes in metaphase I (smear F.W.A.).

FIG. 68. Side view of equatorial plate of five pairs of homologous chromosomes in metaphase I (sectioned material F.W.A.).

FIG. 69. Early anaphase I (Bouin's preparation).

FIG. 70. Early anaphase I (Bouin's preparation).

FIG. 71. Late anaphase I. One pair of chromosomes remain linked together (Bouin's preparation).

FIG. 72. Secondary spermatocyte showing vesicular mitochondria and 5 crescent-shaped Golgi bodies.

FIG. 73. Telophase II.

FIG. 74. Telophase II (smear preparation F.W.A.).

FIG. 75. Earliest spermatid showing two Golgi bodies and mitochondria heaped to one side of the cell.

FIGS. 76–79. Spermatids showing progressive coalescing of mitochondria.

Plate IV

FIGS. 80 TO 104—*Coccinella septumpunctata*; FIGS. 105 TO 110—*Plocaederus obesus*.

FIG. 80. Spermatid showing further condensation of the mitochondrial nebenkern.

FIG. 81. Spermatid showing differentiation of acrosomal vesicle.

FIGS. 82 & 83. Spermatids showing the acrosomal vesicle in association with acroblast and the withdrawal of the chromophilic septa traversing the chromophobic portion of the nebenkern.

FIG. 84. Spermatid showing the complete withdrawal of chromophilic septa.

FIG. 85. Spermatid showing the gradual condensation of the nebenkern. The central chromophilic area is very much reduced in size.

FIGS. 86 & 87. Spermatids showing their various components arranging themselves in final order.

FIG. 88. Spermatid showing further elongation of the nebenkern. Acroblast with the vesicle travels back for a short distance.

FIG. 89. Late spermatid showing centriole on the periphery of the nucleus from which the axial filament runs along the nebenkern.

FIGS. 90 & 91. Late spermatids showing progressive lengthening of the nebenkern.

FIGS. 92 & 93. Spermatids showing the appearance of acrosomal granule in the acrosomal vesicle.

FIG. 94. Late spermatid.

FIGS. 95 & 96. Late spermatids. Acrosomal granule separates from the acroblast and becomes attached to nuclear wall.

FIGS. 97 & 98. Spermatids showing the forward migration of the acrosome and the acroblast remnant moving backwards along the tail.

FIG. 99. Late spermatid with the acrosome back at its original position. Acroblast remnant degenerating far behind along the axial filament.

FIG. 100. Spermatid showing the complete elimination of Golgi remnants.

FIGS. 101 & 102. Spermatids showing the ultimate position of the acrosome lying anterior to the nucleus.

FIG. 103. Very late spermatid showing pointed acrosome at the anterior end of elongated nucleus.

FIG. 104. Mature sperm showing the rod-shaped head taking a homogeneous dark stain. Acrosome affixed to its anterior pole.

FIG. 105. Earliest spermatogonium showing the nucleus and the mitochondrial granules.

FIG. 106. Late spermatogonium showing chromatin network in the nucleus.

FIGS. 107 & 108. Resting Primary spermatocytes showing the presence of Golgi granules and crescents interspersed in the mitochondrial granules.

FIG. 109. Primary spermatocyte showing growth of some of the mitochondrial granules into vesicles.

FIG. 110. Primary spermatocyte showing the mitochondrial vesicles encircling the nucleus.

Plate V (*Plocaederus obesus*)

FIG. 111. Metaphase I. The mitochondria and the Golgi elements are being sorted out almost equally to the daughter cells.

FIG. 112. Telophase I.

FIGS. 113 & 116. Secondary spermatocytes in growth period. The mitochondria and Golgi elements exhibit duplex structure. The Golgi bodies show the tendency of becoming juxta-nuclear.

FIG. 117. Metaphase II.

FIGS. 118 & 119. Telophase II.

FIG. 120. Earliest spermatid.

FIG. 121. Spermatid showing optical section of the nebenkern. The nucleus is much decreased in size.

FIGS. 122 & 123. Spermatids showing mitochondrial nebenkern distinguishable into chromophobic and chromophilic portions.

FIGS. 124 & 125. Spermatids showing distinct Golgi bodies with intensely staining chromophilic rim. Also note chromophilic partitions running in the chromophobic portion of the nebenkern.

FIGS. 126 & 127. Spermatids showing disappearance of chromophilic partitions of the mitochondrial nebenkern.

FIG. 128. Late spermatid. Nebenkern distinguishable into central chromophilic mass surrounded by chromophobic cavity.

FIG. 129. Late spermatid.

FIG. 130. Late spermatid showing acrosomal vesicle with the acrosomal granule.

FIG. 131. Spermatid showing the appearance of central substance in the elongated chromophobic portion of the nebenkern. Also note acrosomal vesicle with the acrosomal granule in the centre.

FIGS. 132–134, 138 & 139. Spermatids showing the thinning out of the chromophilic portion of the nebenkern. The acrosome is attached to the nuclear wall.

FIGS. 135 & 137. Cross-sections of the mitochondrial nebenkern showing progressive dwindling of the chromophilic portion.

FIGS. 140 & 141. Late spermatids showing homogeneously staining nucleus and button-like acrosome at its apex.

FIGS. 142 & 143. Sperm heads showing elongated nucleus and needle-like acrosome.

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

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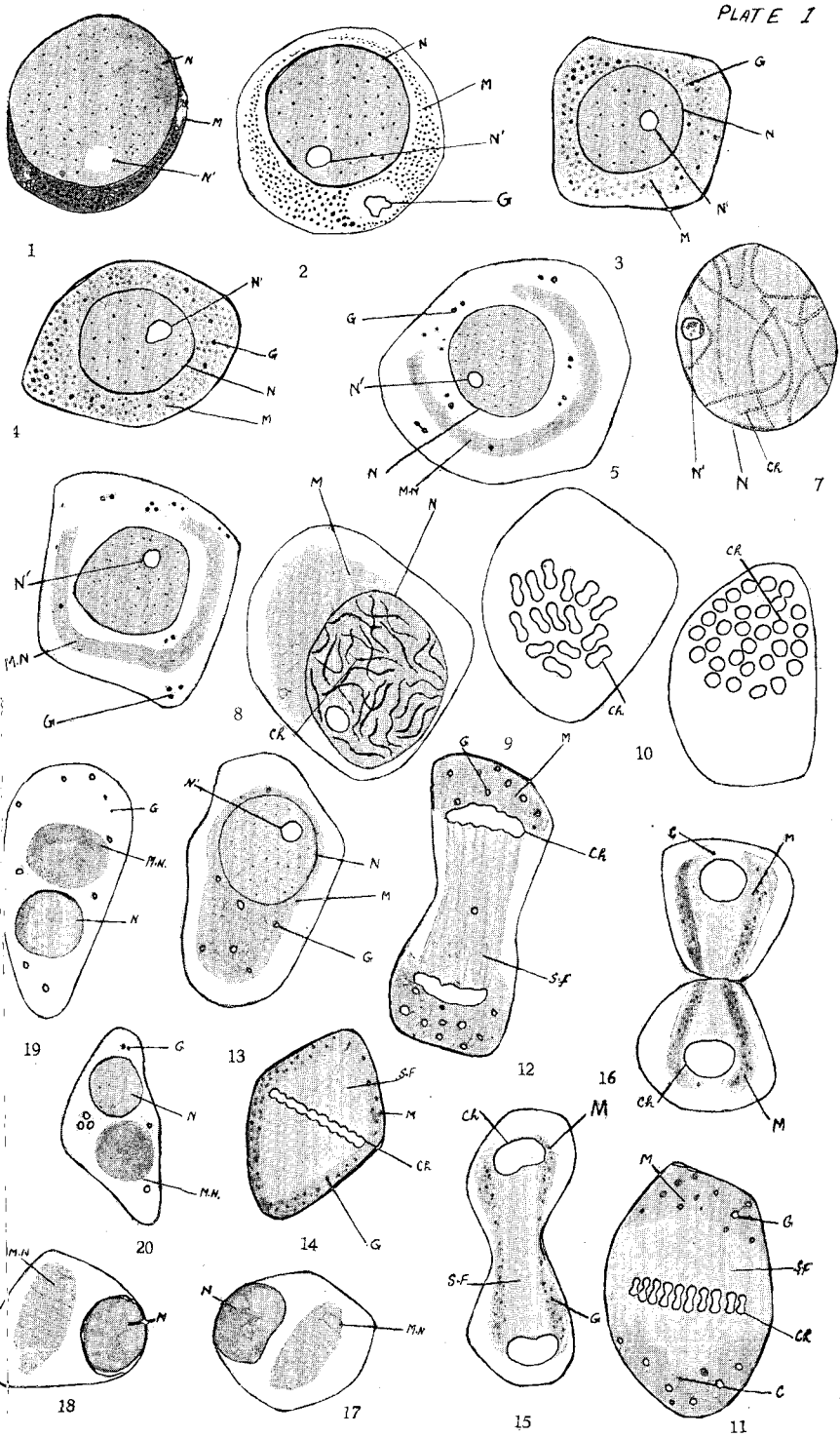

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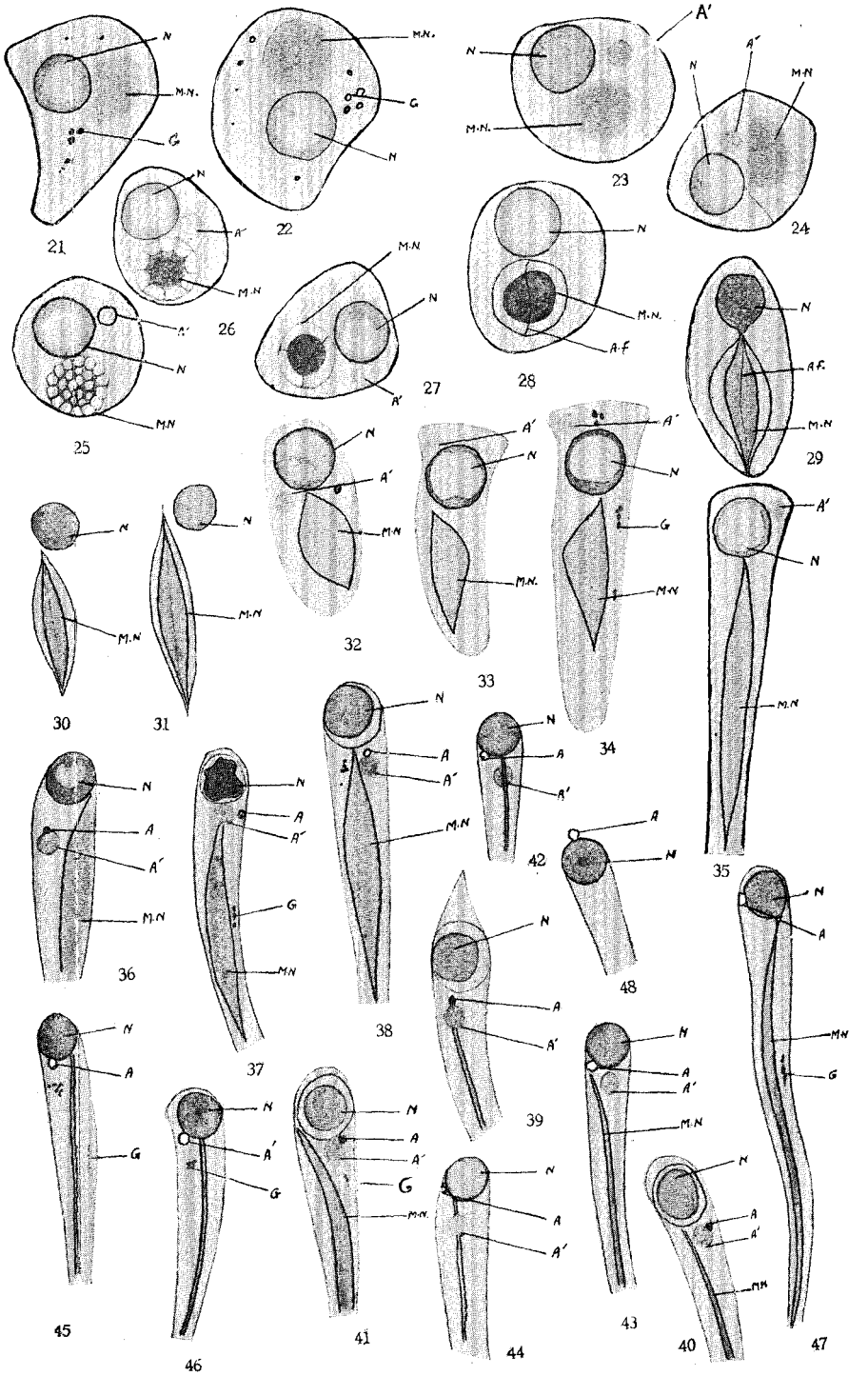
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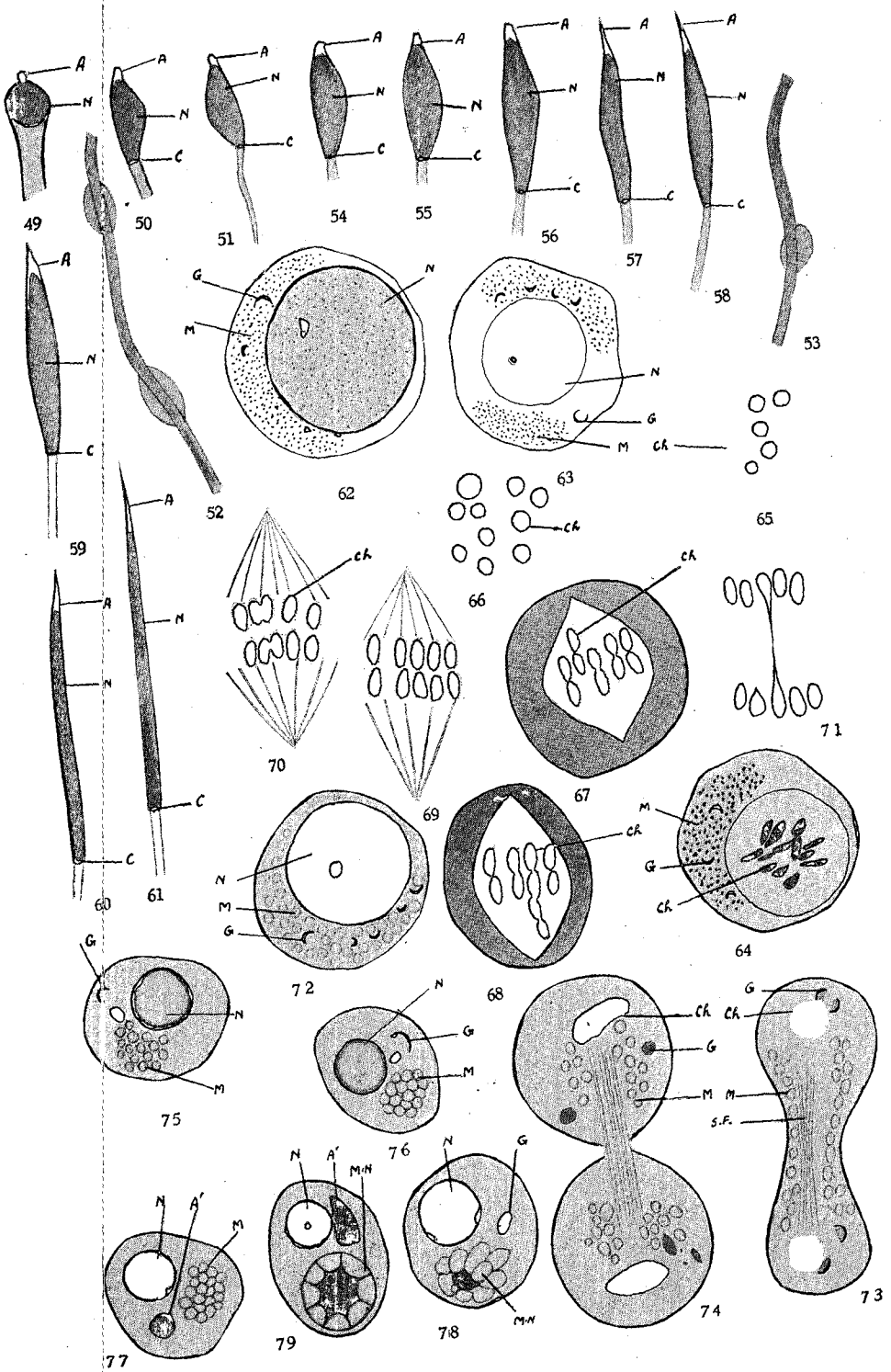
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PLATE IV

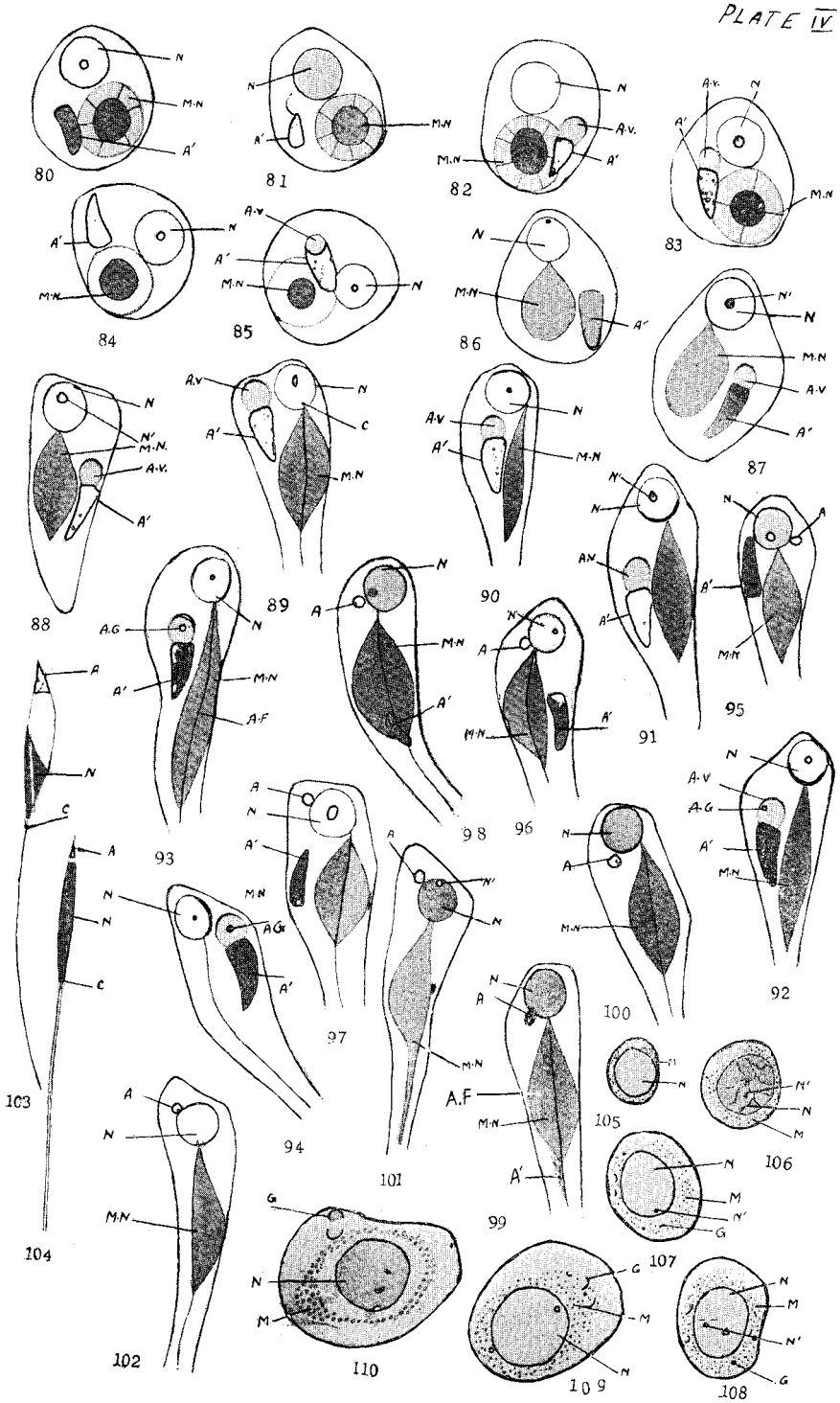
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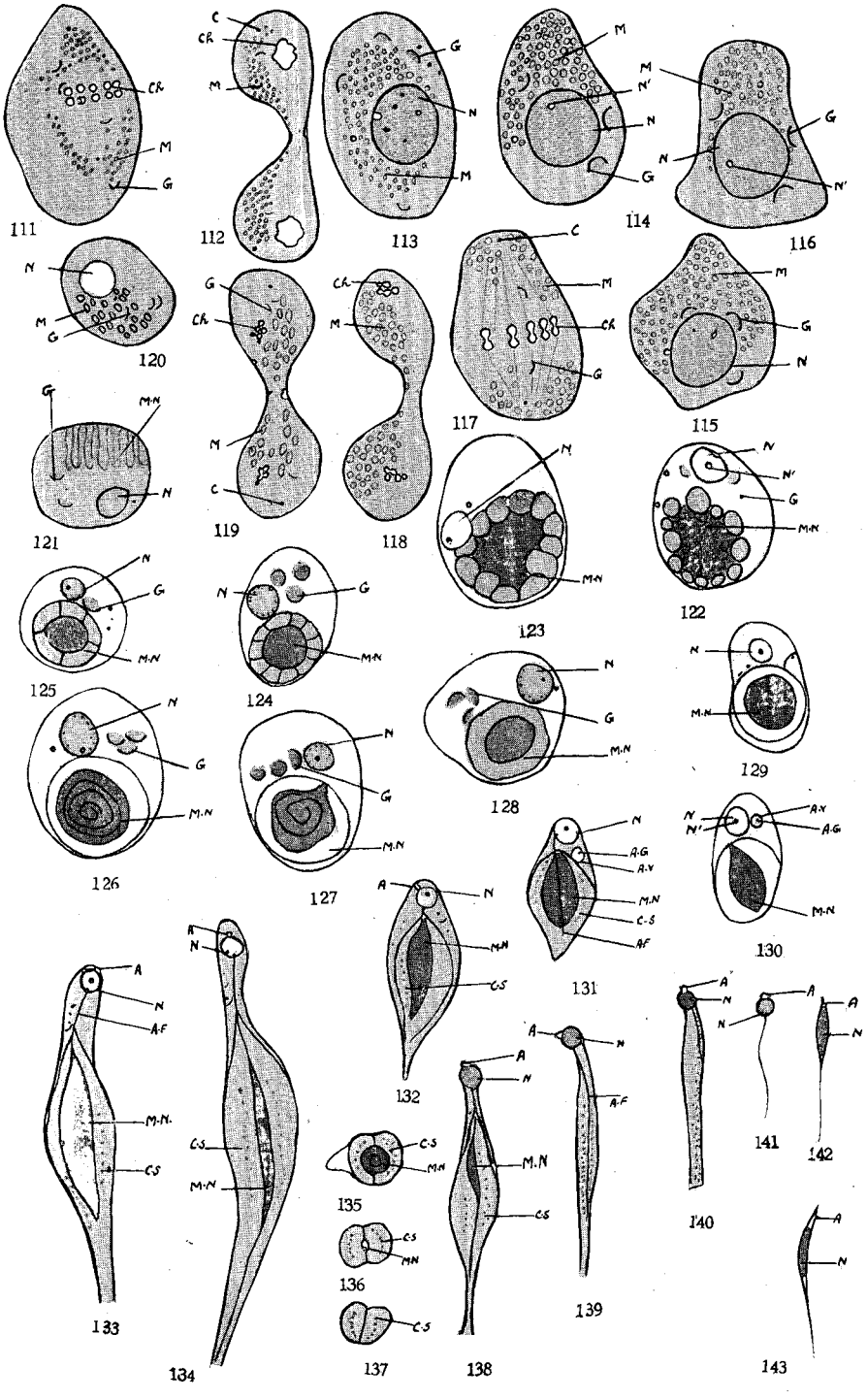
PLATE V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RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 17. CHEMISTRY Pp. 51—56

October, 1951

POTASSIUM META-PERIODATE AS VOLUMETRIC REAGENT

PART II—Indirect Determinations

by

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Edited for the East Panjab University by Vishwa Nath,
Department of Zoology, East Panjab University,
HOSHIARPUR

Price Rs.0-13-0

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PART II—INDIRECT DETERMINATIONS

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In the present investigation, the authors have made an attempt to determine indirectly copper sulphate, hydrogen peroxide, dioxides of lead and manganese, formates, sodium sulphide and potassium iodide in iodine solution by a volumetric method, using potassium meta-periodate as an oxidizing agent.

Potassium meta-periodate was prepared by the method of Bahl and Singh (*J. Ind. Chem. Soc.*, 1940, 17: 167) by passing a brisk current of chlorine gas through a boiling solution of iodine in potassium hydroxide. It was filtered, washed and recrystallized from hot water.

In all titrations with potassium meta-periodate, 5 c.c. of chloroform and enough of hydrochloric acid, to keep its normality between $4N$ to $7N$ were added to titrant in a conical flask. Potassium meta-periodate was added from a burette until the titrant, which at first was strongly coloured with iodine, became pale brown. The conical flask was then stoppered and vigorously shaken, and the chloroform layer acquired purple colour due to iodine. Continued to add small volumes of the meta-periodate solution, shaking vigorously after each addition, until the chloroform layer was faintly violet. Potassium meta-periodate was then added drop-wise, with shaking after the addition of each drop, until the chloroform layer changed its colour from violet to pale yellow, due to formation of iodine monochloride. The end point was very sharp.

(1) DETERMINATION OF COPPER SULPHATE

Dissolved a known weight of copper sulphate in water and saturated the solution with sulphur dioxide. Boiled the solution and added excess of ten per cent ammonium thiocyanate slowly with constant stirring to precipitate cuprous thiocyanate. Allowed the precipitate to settle, filtered and washed with one per cent ammonium sulphate solution till the filtrate was free from ammonium thiocyanate. Put the precipitate along with the filter paper into a conical flask, added to it 30 c.c. of concentrated hydrochloric acid, 20 c.c. of water, 5 c.c. of chloroform and titrated it against standard potassium meta-periodate.

Cuprous thiocyanate reacts with potassium meta-periodate in presence of hydrochloric acid as follows:

$$7\ KIO_4 + 3\ Cu_2(CNS)_2 + 14\ HCl = 7\ KCl + 7\ ICl + 6\ CuSO_4 + 6\ HCN + 4\ H_2O.$$

Hence $$7\ KIO_4 \equiv 3\ Cu_2(CNS)_2 \equiv 6\ CuSO_4 5\ H_2O.$$

A number of titrations were performed and from the volume of standard potassium meta-periodate solution used, corresponding to the end point in each titration, the amount of copper sulphate was calculated. The results are given in Table I.

TABLE I

Copper Sulphate

$CuSO_4 5\ H_2O$ taken. (gm.)	Volume of M/80 KIO_4 used. (c.c.)	$CuSO_4 5\ H_2O$ found. (gm.)
0·0535	20·20	0·0540
0·0722	27·00	0·0722
0·0802	29·90	0·0799
0·0936	35·00	0·0936
0·1069	39·90	0·1067
0·1257	47·10	0·1259

1·00 c.c. M/80 $KIO_4 \equiv$ 0·002674 gm. $CuSO_4 5\ H_2O$.

(2) DETERMINATION OF HYDROGEN PEROXIDE

Hydrogen peroxide reacts with arsenious oxide in alkaline medium (in presence of sodium hydroxide solution) according to the equation:

$$As_2O_3 + 2H_2O_2 = As_2O_5 + 2H_2O.$$

In the determination of hydrogen peroxide, a known excess of arsenious oxide with about 10 c.c. of ten per cent sodium hydroxide was taken in a conical flask and a known volume of approximately $N/10$ hydrogen peroxide run in from burette. The mixture was allowed to stand for a couple of minutes, 50 c.c. concentrated hydrochloric acid, 20 c.c. water, and 5 c.c. of chloroform were added to it. The conical flask was stoppered and the mixture was vigorously shaken. The excess of arsenious oxide, left unreacted in the mixture, was determined by titrating it against standard potassium meta-periodate solution. The reaction is represented as follows:

$$3\ As_2O_3 + 2\ KIO_4 + 4\ HCl = 3\ As_2O_5 + 2\ KCl + 2\ ICl + 2\ H_2O.$$

Table II presents the results of the titrations.

TABLE II

Hydrogen Peroxide

Amount of H_2O_2 taken. (gm.)	Volume of M/60 As_2O_3 added. (c.c.)	Volume of M/60 KIO_4 used. (c.c.)	Volume of M/60 As_2O_3 used for H_2O_2. (c.c.)	Amount of H_2O_2 found. (gm.)
0·0155	20·00	10·90	9·10	0·0155
0·0264	30·00	14·50	15·50	0·0263
0·0387	40·00	17·30	22·70	0·0386
0·0465	45·00	27·60	27·40	0·0466
0·0535	45·00	13·55	31·45	0·0535
0·0697	50·00	9·10	40·90	0·0695

1·00 c.c. of M/60 $As_2O_3 \equiv$ 0·00170 gm. H_2O_2.

(3) Determination of Dioxides of Lead and Manganese

Potassium iodide reacts with the dioxides in presence of concentrated hydrochloric acid:

$$2\ KI + 2\ PbO_2 + 8\ HCl = 2\ PbCl_2 + 2\ KCl + 2\ ICl + 4\ H_2O,$$
$$2\ KI + 2\ MnO_2 + 8\ HCl = 2\ MnCl_2 + 2\ KCl + 2\ ICl + 4\ H_2O.$$

In concentrated hydrochloric acid, potassium iodide reacts with potassium meta-periodate:

$$3\ KI + KIO_4 + 8\ HCl = 4\ KCl + 4\ ICl + 4\ H_2O.$$

A known weight of the dioxide was taken in a conical flask and a known excess of potassium iodide solution was added to it. The mixture was acidified with 50 c.c. of concentrated hydrochloric acid to keep its normality between 4*N* and 7*N*. The excess of potassium iodide was titrated back with standard potassium meta-periodate. About 5 c.c. of chloroform was added to the mixture before each titration to determine the end point. The results are summarised in Tables III and IV.

Table III

Lead Dioxide

Amount of PbO_2 taken.	Volume of M/80 KI added.	Volume of M/80 KIO_4 used.	Volume of M/80 KI used for PbO_2	Amount of PbO_2 found.
(gm.)	(c.c.)	(c.c.)	(c.c.)	(gm.)
0·1352	25·50	10·40	15·10	0·1354
0·1800	31·00	10·95	20·05	0·1799
0·2000	34·00	11·70	22·30	0·2000
0·2770	46·00	15·10	30·90	0·2772

1·00 c.c. M/80 KI ≡ 0·008971 gms. PbO_2.

Table IV

Manganese Dioxide

Amount of MnO_2 taken.	Volume of M/80 KI added.	Volume of M/80 KIO_4 used.	Volume of M/80 KI used for MnO_2	Amount of MnO_2 found.
(gm.)	(c.c.)	(c.c.)	(c.c.)	(gm.)
0·0210	25·00	18·40	6·60	0·0215
0·0350	30·00	19·30	10·70	0·0349
0·0440	30·00	16·50	13·50	0·0440
0·0561	35·00	17·80	17·20	0·0561
0·0685	40·00	19·00	21·00	0·0685

1·00 c.c. M/80 KI ≡ 0·003262 gm. MnO_2.

(4) Determination of Formates

A formate reduces mercuric chloride to mercurous chloride:

$$HCOONa + 2\ HgCl_2 = Hg_2Cl_2 + NaCl + HCl + CO_2.$$

In presence of concentrated hydrochloric acid, potassium meta-periodate oxidizes mercurous chloride to mercuric chloride:

$$KIO_4 + 8\ HCl + 3\ Hg_2Cl_2 = 6\ HgCl_2 + KCl + ICl + 4\ H_2O.$$

A known weight of sodium formate was taken in a conical flask. Some sodium acetate solution, an excess of saturated mercuric chloride solution and enough of water to make the volume to about 75 c.c. were added to it. The mixture was heated on a steam bath for about two hours until the whole of carbon dioxide was evolved. The flask was cooled and the precipitated mercurous chloride was filtered. The precipitate along with the filter paper was transferred to a conical flask, about 50 c.c. of concentrated hydrochloric acid and 5 c.c. of chloroform were added and the mixture titrated against standard potassium meta-periodate. The results are given in Table V.

Table V

Sodium Formate

Amount of HCOONa taken. (gm.)	Volume of M/60 KIO_4 used. (c.c.)	Amount of HCOONa found. (gm.)
0·0400	11·70	0·0398
0·0680	20·30	0·0690
0·0800	23·80	0·0809
0·1000	30·00	0·1020
0·1200	36·00	0·1224

$KIO_4 \equiv 3\ Hg_2Cl_2 \equiv 3\ HCOONa$

1·00 c.c. M/60 $KIO_4 \equiv 0{\cdot}003396$ gm. HCOONa.

(5) Determination of Sodium Sulphide

Sodium sulphide reacts with arsenious oxide in presence of dilute hydrochloric acid according to the equation:

$$3\ Na_2S + As_2O_3 + 6\ HCl = As_2S_3 + 6\ NaCl + 3\ H_2O.$$

Arsenious oxide is oxidized to arsenic oxide by potassium meta-periodate in presence of concentrated hydrochloric acid:

$$3\ As_2O_3 + 2\ KIO_4 + 4\ HCl = 3\ As_2O_5 + 2\ KCl + 2\ ICl + 2\ H_2O.$$

1·50 gm. of sodium sulphide were dissolved in 100 c.c. of water and a known excess of arsenious oxide (2·5070 gm.) dissolved in sodium hydroxide were added to it. The mixture was thoroughly stirred and acidified with hydrochloric acid, when yellow precipitate of arsenious sulphide was formed. The precipitate was filtered, washed with water and the filtrate made up to 250 c.c. To determine the excess of arsenious oxide present in the filtrate, 20 c.c. of the filtrate was mixed with about 30 c.c. concentrated hydro-

chloric acid, 5 c.c. chloroform and titrated against M/60 potassium meta-periodate. The results are tabulated in Table VI.

TABLE VI

Sodium Sulphide

Amount of Na_2S taken.	Volume of the filtrate taken for titration.	Volume of M/60 KIO_4 used.	Amount of As_2O_3 in 250 c.c. filtrate.	Amount of As_2O_3 reacted with Na_2S.	Amount of Na_2S found.
(gm.)	(c.c.)	(c.c.)	(gm.)	(gm.)	(gm.)
1·5000	20·00	20·00	1·2375	1·2695	1·5000
	20·00	20·05			
	20·00	20·00			
	20·00	20·00			

1·00 c.c. M/60 $KIO_4 \equiv$ 0·00495 gms. $As_2O_3 \equiv$ 0·00585 gm. Na_2S.

(6) DETERMINATION OF POTASSIUM IODIDE IN IODINE SOLUTION.

A known volume of iodine solution was titrated against standard sodium thiosulphate, using starch as an indicator. From these titrations, normality of the solution with respect to iodine was calculated.

In presence of concentrated hydrochloric acid, iodine and potassium iodide react with potassium meta-periodate according to the following equations:

$$3\ I_2 + KIO_4 + 8\ HCl = 7\ ICl + KCl + 4\ H_2O.$$

$$3\ KI + KIO_4 + 8\ HCl = 4\ ICl + 4\ KCl + 4\ H_2O.$$

To determine total normality of the iodine solution with respect to iodine and potassium iodide, its known volume was titrated against M/60 KIO_4 in presence of hydrochloric acid and chloroform.

From these two sets of titrations, normality of potassium iodide and its amount in the iodine solution were calculated. The results are given in Table VII.

TABLE VII

Potassium Iodide in Iodine Solution

Amount of KI present in litre of Iodine solution.	Volume of M/60 KIO_4 used.	Normality of I_2 from Thio-sulphate titrations.	Normality of I_2 and KI from KIO_4 titrations.	Normality of KI calculated	Amount of KI found in litre of Iodine solution.
(gm.)	(c.c.)	(N)	(N)	(N)	(gm.)
8·210	13·00	0·0310	0·1300	0·0990	8·217
9·760	19·30	0·0310	0·1485	0·1175	9·752
12·210	17·80	0·0310	0·1780	0·1470	12·200
16·350	22·80	0·0310	0·2280	0·1970	16·350
18·350	25·20	0·0310	0·2520	0·2210	18·350

From the above results given in Tables from I to VII, it is evident that potassium meta-periodate can be used as a volumetric reagent to estimate indirectly copper sulphate, hydrogen peroxide, dioxides of lead and manganese, sodium formate, sodium sulphide and potassium iodide in iodine solution.

The authors are indebted to Dr. Vishwa Nath, Principal, Government College, Hoshiarpur, for providing facilities for the research work.

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 18 ZOOLOGY Pp. 57–68

November, 1951

STUDIES ON THE HARIANA BREED OF CATTLE

by

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Department of Zoology, East Panjab University,
HOSHIARPUR

Price Rs.1-8-0

STUDIES ON THE HARIANA BREED OF CATTLE

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Introduction

Cattle play an important rôle in the agricultural production of our country. They supply much of the power on farms for ploughing and for the transport of agricultural produce to the market. They also transform the rough feed into manure which is so essential for the maintenance of soil fertility. Furthermore they supply the much needed animal protein to a large section of the population in the form of milk and milk products. However, in spite of the fact that we have 38 million heads of milch cattle their milk production is so low that we can provide a *per capita* consumption of 5 ozs. only in most of the States of the Indian Union as against the desirable minimum nutritional quantum of 1 lb. per head. To provide this and for rearing healthy calves it is essential that our total milk production should be increased more than three times. There is, therefore, an increasing demand for the 'dual purpose' animal the progeny of which should be suitable both for 'draught and milk' and not for 'beef and milk' as the term 'dual purpose' is understood in the Western countries. This demand we hope to meet by putting more milk in some of our best draught breeds by careful selective breeding.

With the same object in view the Indian Council of Agricultural Research started some years ago two research schemes—one at the Government Livestock Farm, Hissar, and the other at the Pattagar's Farm at Palayakottai. The breed of cattle maintained at Hissar is Hariana and that at Palayakottai is Kangayam. At both these places an effort is being made to increase the milk yield of these breeds without deteriorating the draught quality which has already been established in them. During the course of our investigations at Hissar we have collected a good deal of valuable data which we propose to record in the following few pages.

Material and Method

As has been stated already the experimental animals at Hissar are of the Hariana breed. The home of this breed is what is popularly known as the 'Hariana tract'. It comprises the districts of Rohtak, Hissar, Gurgaon, part of Karnal and the Delhi State. Pure Hariana animals are found in large numbers in Jhajjar and Rohtak tehsils of the Rohtak district. The cattle of Hissar district are provided with loose thick skin, long sheath, large dewlap, long and thick horns and big drooping ears. They can be

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easily differentiated from the pure Hariana animals which have a long and narrow face with flat forehead and a well marked bony prominence at the centre of the poll. They have short horns, small ears, fine thin skin closely adhering to the body, a small dewlap and a small sheath. The bullocks of the Hariana breed are excellent for draught purposes particularly for fast ploughing and road transport. The cows are moderate milkers. Pedigree herds of this breed are maintained at this farm.

Our experiment was originally started in 1944-45 with a female stock of 230 animals (150 heifers of nearly the same age and 80 dairy cows varying in age). All these animals were so selected that they had a certain standard of body conformation of the Hariana breed. No care was taken to select heifers or cows with good udders or with other body points which are generally looked for while judging a milch cow. Ten young bulls conforming to the Hariana breed were also selected in a similar way. In order to have sufficient variability in our experimental herd five of these bulls were purchased from the Hariana tract while the rest were taken from the farm-bred stock. This whole lot of animals was divided at random into ten groups or families and thus each family had 23 females and one male for breeding.

As far as practicable standard conditions of housing, feeding and management are being provided to the experimental herd.

Results

Sex Ratio.—As a result of breeding in the various families all the females (heifers as well as cows) in the original experimental herd have calved at least once since the beginning of the experiment in 1944-45. In all 691 births of calves have been recorded up to the close of March, 1951. Out of these 352 are male and 339 female calves. From these figures the overall sex ratio (i.e. the number of males for every 100 females) is calculated to be 103·83.

Gestation Period.—There were 517 normal births for each of which the exact date of service was known. It was, therefore, quite easy to calculate the gestation period in each case. The frequency distribution of these gestation periods is presented in Table 1.

Table 1.

Frequency distribution of the gestation periods (in days) for all births.

Class Interval	Class value	Frequency
265–269	267	4
270–274	272	8
275–279	277	17
280–284	282	64
285–289	287	156
290–294	292	156
295–299	297	69
300–304	302	19
305–309	307	10
310–314	312	14
	Total ..	517

On further analysis of the data the mean (with error) of all the gestation periods studied worked out to be 290·15±0·22 days irrespective of the sex of calf born. The corresponding figures for some other Indian and foreign breeds of cattle are:—

Kangayam	.. 285 days (Littlewood, 1937).
Ongole	.. 289·1 days (Littlewood, 1937).
Red Sindhi	.. 283 days (Dave and Singh, 1934).
Jersey	279 days (Wing, 1899). 278·51 days (Copeland, 1930). 278·88±0·176 days (Knoop and Hayden, 1934).
Holstein-Friesian	280 days (Wing, 1899). 278·90 days (Knott, 1932). 278·15±0·154 days (Knoop and Hayden, 1934).
Durham or Improved Short-horned breed	283 days (Spencer, 1840).

For the various other breeds of cattle, reference may also be made to the 'Gestation Periods' compiled by Kenneth (1947).

As early as 1840 the Right Hon. Earl Spencer published some tables from which it appears that the male calf is carried by the dam one to three days longer than the female. However, some of the later workers like Wing (1899) are of the opinion that the average period of gestation is the same regardless of the sex of the offspring. In view of these conflicting accounts in the literature we prepared Tables 2 and 3 showing the frequency distribution of the gestation periods for male and female births separately.

TABLE 2.

Frequency distribution of the gestation periods (in days) for male births.

Class Interval	Class Value	Frequency
265–269	267	2
270–274	272	6
275–279	277	3
280–284	282	24
285–289	287	65
290–294	292	81
295–299	297	49
300–304	302	11
305–309	307	4
310–314	312	7
	TOTAL ..	252

TABLE 3.

Frequency distribution of the gestation periods (in days) for female births.

Class Interval	Class Value	Frequency
265–269	267	2
270–274	272	2
275–279	277	14
280–284	282	40
285–289	287	91
290–294	292	75
295–299	297	20
300–304	302	8
305–309	307	6
310–314	312	7
	TOTAL ..	265

From these two tables the average gestation period for male births works out to be 291·11±0·47 days and for female births 289·19±0·46 days. It will be of interest to note that the difference of about two days between the mean gestation periods for male and female births is statistically significant at 5% level of significance. This clearly indicates that the sex of calf has a significant influence on the gestation period in cattle.

Age at first calving.—For this study the data were available only for 182 cows. These data are presented in Table 4 given below.

TABLE 4.

Frequency distribution of the age at first calving (in days).

Class Interval	Class Value	Frequency
1250–1299	1274·5	3
1300–1349	1324·5	2
1350–1399	1374·5	8
1400–1449	1424·5	2
1450–1499	1474·5	15
1500–1549	1524·5	21
1550–1599	1574·5	34
1600–1649	1624·5	40
1650–1699	1674·5	13
1700–1749	1724·5	15
1750–1799	1774·5	7
1800–1849	1824·5	8
1850–1899	1874·5	7
1900–1949	1924·5	3
1950–1999	1974·5	2
2000–2049	2024·5	2
	TOTAL ..	182

On analysis of the above data the average age (with error) at first calving is found to be 1618·18±10·53 days, i.e. about 4 years and 5 months.

Calving interval.—There were 443 cases in which the interval between two successive calvings could be studied. The data for this study are tabulated below in Table 5.

TABLE 5.

Frequency distribution of the interval (in days) between two successive calvings.

Class Interval	Class Value	Frequency
300–324	312	4
325–349	337	12
350–374	362	19
375–399	387	23
400–424	412	32
425–449	437	31
450–474	462	33
475–499	487	26
500–524	512	40
525–549	537	33
550–574	562	30
575–599	587	37
600–624	612	32
625–649	637	28
650–674	662	21
675–699	687	11
700–724	712	11
725–749	737	3
750–774	762	5
775–799	787	6
800–824	812	1
825–849	837	1
850–874	862	1
875–899	887	1
900–924	912	2
	TOTAL ..	443

The statistical analysis of the above data shows that the average interval (with error) between two successive calvings is 530·85±5·41 days, i.e. one year and 5½ months.

Birth weight of calves.—During the period 1946–51 there were 507 births where the weight of newly born calf had been recorded. Table 6 given below shows the frequency distribution of the birth weight of calves (in lbs.) irrespective of the sex of calf.

TABLE 6.

Frequency distribution of the birth weight (in lbs.) of Hariana calves irrespective of the sex of calf.

Class Interval	Class Value	Frequency
30–32	31	2
33–35	34	4
36–38	37	3
39–41	40	22
42–44	43	36
45–47	46	25
48–50	49	114
51–53	52	61
54–56	55	87
57–59	58	47
60–62	61	62
63–65	64	21
66–68	67	14
69–71	70	4
72–74	73	3
75–77	76	1
78–80	79	1
	TOTAL ..	507

The average weight at birth of calves, irrespective of the sex of calf, works out, on analysis of the above data, to be $52{\cdot}90 \pm 0{\cdot}34$ lbs.

To find out whether the sex of calf has anything to do with the birth weight the following two Tables Nos. 7 and 8 were prepared. They show the frequency distribution of the birth weight of male and female calves separately.

TABLE 7.

Frequency distribution of the birth weight (in lbs.) of male calves.

Class Interval	Class Value	Frequency
30–32	31	1
33–35	34	2
36–38	37	1
39–41	40	8
42–44	43	21
45–47	46	10
48–50	49	49
51–53	52	27
54–56	55	36
57–59	58	29
60–62	61	32
63–65	64	15
66–68	67	11
69–71	70	3
72–74	73	2
75–77	76	1
78–80	79	1
	TOTAL ..	249

TABLE 8.

Frequency distribution of the birth weight (in lbs.) of female calves.

Class Interval	Class Value	Frequency
30–32	31	1
33–35	34	2
36–38	37	2
39–41	40	14
42–44	43	15
45–47	46	15
48–50	49	65
51–53	52	34
54–56	55	51
57–59	58	18
60–62	61	30
63–65	64	6
66–68	67	3
69–71	70	1
72–74	73	1
	TOTAL ..	258

The statistical analysis of the data presented in the above two tables shows that the average birth weight (with error) for male calves is 53·98 ±0·47 lbs. and for female calves is 52·04±0·42 lbs. This difference of about 2 lbs. between the mean birth weights of male and female calves is statistically significant at 5% level of significance. This indicates that the sex of calf has a significant influence on the birth weight of calves, the male calves being heavier than the female calves.

Effect of Season on Calving

Hammond (1927), in his study of the breeding season, has reported the percentage of cows which calve in different months of the year. From this study which is based on a bulk of statistics collected from various authorities on a fairly large number of cows it is obvious that in the foreign breeds maximum number of cows calve in spring and minimum in autumn months. After further investigations Hammond concludes that the seasonal effect on the distribution of calvings is marked mostly due to the intensity of the reproductive force which is maximum in summer months and minimum in winter months.

Joseph and Dave (1931) have published a table based on 1,553 calvings showing the effect of season on the frequency of calvings in Montgomery cows. According to them the distribution of calvings at Pusa Cattle Farm is, more or less, fairly uniform. Thus their finding runs contrary to that of Hammond.

Sikka (1931), as a result of his observations on 1,274 cows of the Military Dairy Farm, Ferozepore, has published a table for studying the effect of season on the frequency of calvings. He concludes 'that percentage of calvings for different months goes on increasing quite regularly from October, in which month only 5·26% of the total calved, to March, calving 16·01% of the total. From this there is a steady decline till September-calving 3·30%, the lowest for the year. This means that in these herds largest number of cows calve in spring and smallest in autumn months.' This result is in close agreement with that of Hammond.

In view of these conflicting accounts in the literature the data presented in Table 9 showing the monthly distribution of calvings in the experimental herd for the period 1946–51, were collected.

Table 9.

Monthly distribution of calvings.

Months	Feb.	March	April	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Total
Season	Spring			Summer			Autumn			Winter			
No. of calvings	78	104	73	51	52	52	35	48	30	42	62	64	691
% of calvings	11·3	15·1	10·6	7·4	7·5	7·5	5·1	6·9	4·3	6·1	8·9	9·3	

From the above table it is clear that the percentage of calvings goes on increasing steadily from November (6·1%) to March (having a maximum of 15·1% of the total calved). After this there is a decline till October (calving 4·3%, the lowest of the year). These observations indicate that the largest number of Hariana cows calve in spring and smallest in autumn months. In order to confirm that the greater or smaller incidence of calvings during these seasons is not due to chance causes, a statistical study was also made. For this purpose comparisons between the means of spring months and other months and between the means of autumn months and other months were made separately. The results are as follows:—

(*a*) Mean of Spring months = 85·00
Mean of other months = 48·45

$$t_{(10)} = \frac{85{\cdot}00 - 48{\cdot}45}{\sqrt{69{\cdot}95}} = 4{\cdot}38$$

Probability for getting such a value of t is less than ·01.

(*b*) Mean of Autumn months = 37·67
Mean of other months = 64·23

$$t_{(10)} = \frac{64{\cdot}23 - 37{\cdot}67}{\sqrt{133{\cdot}54}} = 2{\cdot}30$$

Probability for getting such a value of t is less than ·05.

The above results clearly show that the average number of calvings during spring and autumn seasons is significantly greater and smaller respectively, thereby confirming the views expressed already by Hammond (1927) and Sikka (1931).

We are, therefore, of the opinion that even though the agricultural conditions are poor during summer season at the Govt. Livestock Farm, Hissar, yet the reproductive force is at its maximum partly due to high atmospheric temperature and partly due probably to greater light on account of longer days during this season. The conception rate is, therefore, maximum during the summer season and it results in a larger number of calvings in the succeeding spring season. On the other hand the winter season gives a reverse picture resulting in fewer calvings in the succeeding autumn season. What other factors are responsible for the varying frequency of calvings during various seasons and whether we could regard the temperature as the prime factor in determining the rate of conception, are all points for further research and investigation.

Milk Performance

The lactation-wise milk performance of the experimental herd for the period 1-4-46 to 31-3-51 is tabulated in Table 10.

TABLE 10.

Milk performance of the experimental herd during the period 1-4-46 *to* 31-3-51.

Lactation No.	No. of cows	Average No. of days in milk	Average total yield in lbs.	Average maximum yield in lbs.	Average daily yield in lbs. during lactation
1st ..	133	251·9	1284·9	7·9	4·8
2nd ..	139	274·9	1782·7	10·1	6·1
3rd ..	107	306·1	1780·6	10·4	5·9
4th ..	71	282·3	1675·5	10·1	5·8
5th ..	39	290·2	1827·0	10·0	5·8
6th ..	17	307·9	2077·1	11·9	6·6
7th ..	9	360·9	1972·8	9·9	5·5
8th ..	2	280·0	1553·0	13·5	5·8
TOTAL ..	517				

The average milk yield, irrespective of the order of lactation, works out to be 1749·50±40·25 lbs.

SERVICE PERIOD

To find out the length of the service period (i.e. the interval between the date of calving and the next date of fertile service) the data shown in Table 11 were collected.

TABLE 11.

Frequency distribution of the service period (in days).

Class Interval	Class Value	Frequency
Up to 49	24·5	7
50– 99	74·5	35
100–149	124·5	40
150–199	174·5	55
200–249	224·5	56
250–299	274·5	45
300–349	324·5	51
350–399	374·5	35
400–449	424·5	11
450–499	474·5	7
	TOTAL ..	342

The analysis of the above data shows that the average service period (with error) is 233·71±5·74 days.

Dry period.—Data were also collected to find out the average dry period for the Hariana breed of cattle. These are tabulated below in Table 12.

TABLE 12.

Frequency distribution of the dry period (in days).

Class Interval	Class Value	Frequency
50– 99	74·5	5
100–149	124·5	42
150–199	174·5	66
200–249	224·5	63
250–299	274·5	74
300–349	324·5	48
350–399	374·5	32
400–449	424·5	16
450–499	474·5	5
500–549	524·5	4
550–599	574·5	4
	TOTAL ..	359

On analysis of the above data we find that the average dry period (with error) in this breed is 258·21 ± 5·38 days.

REACTION TIME

Studies for the 'Reaction time' (i.e. the time interval between the bringing of a bull to the cow on heat and the actual time of ejectment) were also made for two years on the ten breeding bulls for the services they performed. The average 'Reaction time' ranges from 5 minutes and 3 seconds to 41 minutes and 11 seconds with an overall average of 23 minutes and 55 seconds ± 3 minutes and 9 seconds. The analysis of variance of the 'Reaction time' of these ten bulls is tabulated below in Table 13.

TABLE 13.

Analysis of Variance.

Source	S.S.	D.F.	M.S.	F. Ratio
Between Bulls ..	29,564,613·12	9	3,284,957·01	2·116*
Within Bulls ..	240,596,694·13	155	1,552,236·7	
TOTAL ..	270,161,307·25	164		

* Significant at 5% point.

The above table shows that the difference between the mean 'Reaction times' of these ten breeding bulls is statistically significant.

As has been stated already two types of bulls have been used for breeding in this experiment. One type is bred at the farm itself and the other is purchased from the Hariana tract. It has been observed that on an average the purchased bulls take only 1064·75 seconds in serving a cow while the farm-bred ones take as many as 1529·04 seconds. This difference of 464·29 seconds in the average 'Reaction times' which may be due to the very

limited exercise for the farm-bred bulls, as a result of stall feeding, has been found to be statistically significant.

Discussion

It will be seen from the foregoing account that for the Hariana breed we have, like the other Indian breeds, very long (i) age at first calving, (ii) calving interval, (iii) service period, (iv) dry period and (v) 'Reaction time'. All these factors contribute to the poor performance of our cattle. It is mainly because of them that we have not so far been able to make dam-daughter comparison at Hissar. Out of a total grown up female progeny of 131 only 75 heifers have so far been mature enough to be served. From these 31 have calved and 5 have completed their respective lactations. Thus in spite of the fact that more than six years have passed we are still not in a position to say anything about the transmitting ability of our animals in the original experimental herd. On the other hand most of the foreign breeds of cattle mature at a much earlier age and thus we can know about their transmitting abilities when they are in the prime of their life. To illustrate our point we give below a comparative account of the age of proving a bull in England and in India.

	England		India	
	Year.	Month.	Year.	Month.
Age of bull when he starts first service	1	6	3	6
Calf is born at	0	10	0	10
This calf becomes mature and receives bull at	1	6	3	6
Gestation period	0	10	0	10
End of first lactation	0	10	0	10
,, ,, second ,, ..	..	..	1	6
Total ..	5	6	11	0

In England a vast majority of heifers prove their quality in the first lactation but, as is apparent from our Table 10, the heifers in India do not show their milk quality in the first lactation. A second lactation has therefore to be taken. Consequently a bull in India has passed the greater part of his effective service life (which is about 13 years) by the time we are able to estimate his transmitting ability. It is mainly because of these factors that we have few, if any, proven sires in India.

To bring about a change in this deplorable state of affairs every effort should be made to lower the age at maturity of our cattle. This should be possible by selective breeding accompanied by better feeding and management as has been achieved in the Sahiwal herd of cattle at the Indian Agricultural Research Institute. Maximum advantage should also be taken of the latest biological technique of artificial insemination for the improvement of our cattle.

Summary

In this paper some interesting details about the Hariana breed of cattle are given. These are:—

1. The Sex Ratio is 103·83.

2. The average gestation period is 290·15±0·22 days for all births. The sex of calf has a significant influence on the gestation period and therefore for male births the gestation period is 291·11±0·47 days and for female births it is 289·19±0·46 days.

3. The average age at first calving is 1,618·18±10·53 days.

4. The average calving interval is 530·85±5·41 days.

5. The average weight at birth of calves, irrespective of the sex of calf, is 52·90±0·34 lbs. The sex of calf has a significant influence on the birth weight of calves, the male calves being heavier than the female calves. The average birth weight for male calves is 53·98±0·47 lbs. and for female calves is 52·04±0·42 lbs.

6. More calves are born during the months of February, March and April and less during the months of August, September and October. It is, therefore, concluded that season has some influence on the frequency of calving.

7. The average milk yield, irrespective of the order of lactation, is 1,749·50±40·25 lbs. The lactation-wise milk performance of the herd is also given in this paper.

8. The average service period is 233·71±5·74 days.

9. The average dry period is 258·21±5·38 days.

10. The average 'Reaction time' ranges from 5 minutes 3 seconds to 41 minutes and 11 seconds with an overall average of 23 minutes and 55 seconds ± 3 minutes and 9 seconds. On an average the bulls purchased from the Hariana tract take only 1,064·75 seconds in serving a cow while the farm-bred ones take 1,529·04 seconds.

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Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 19 | CHEMISTRY | Pp. 1–11

January, 1952

PREPARATION OF ACTIVE CARBON FROM A FEW INDIGENOUS SOURCES AND COMPARATIVE EFFICIENCY OF DIFFERENT ACTIVATION TREATMENTS

by

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Edited for the East Panjab University by Vishwa Nath,
Department of Zoology, East Panjab University,
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Price Rs.1-8-0

PREPARATION OF ACTIVE CARBON FROM A FEW INDIGENOUS SOURCES AND COMPARATIVE EFFICIENCY OF DIFFERENT ACTIVATION TREATMENTS

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Diverse methods of preparing active carbon have been developed in comparatively recent times. These may be divided, broadly speaking, into two categories. In the first category are included what are known as 'chemical processes' in which the carbonaceous materials or sometimes their chars are impregnated with one or more of a variety of chemicals, such as zinc chloride, alkali carbonates, sulphates, sulphuric acid, phosphoric acid, etc., and then carbonized or heated to a high temperature, as the case may be. In the second category of treatments known as 'gas activation processes', charcoal is heated to some temperature between 350° and 1000°C with steam, CO_2, O_2, Cl_2, etc. (1, 8, 12, 16). Steam, which is more commonly used for this purpose, is known to produce maximum activation at temperatures around 1000°C (19) and to be ineffective below 800°C (8) and above 1100°C (10). For steam treatment to be successful the temperature of carbonization should not exceed 600°–650°C (17).

The mechanism of activation is not generally agreed upon. At least three theories have been advanced to explain activation of charcoal by chemical reagents. One of these (2) explains activation as due to catalytic decomposition of residual hydrocarbon complexes, second one as due to enclosure of carbon particles by salt which is subsequently extracted to provide a greater surface area (9) while a third one is based on the dissolution of cellulose by zinc chloride, etc. (6). As regards gas activation Knight, Garner, and McKee (13) consider it to be caused by appreciable increase in surface area of charcoal while Chaney (7) is of the opinion that the adsorbed hydrocarbons are burnt away in this process releasing thereby the active carbon base. Allmand and co-workers (3) suggest that the process simply consists in cleaning up the charcoal surface of the tarry materials.

It appears from perusal of literature that while different methods of activation have been known and several theories have been advanced to explain the mechanism of activation, no systematic investigation has been undertaken to compare the relative merits of the various activation processes. In the present paper the study of the relative effects of three representative and well-known methods of activation on the adsorptive and decolorizing properties of five specimens of charcoal prepared from some common raw-materials has been made with a view to assess the

relative efficiencies of these treatments. The results obtained also give some insight into the mechanism of activation.

Experimental

Raw Materials.—Three out of the five raw materials selected for this investigation were different varieties of wood, namely acacia wood, pine wood and cotton stalk. Coconut shell and sugar were the two other starting materials. Sugar was carbonized by sulphuric acid and then washed free of sulphate ions. The rest of the materials were carbonized by heating them in small pieces in closed vessels between 550°–600°C. The chars in each case were ground and screened through 100 mesh sieve.

Activation.—The following methods of activation were tried :—

1. *Activation by Phosphoric Acid.*—Charcoal was made into a paste with phosphoric acid and allowed to stand for about 30 minutes. The mass was then broken into small lumps, dried in an electric oven and then placed in a silica tube, 1″ wide and 30″ long, and heated in an electric furnace to about 950°C in an atmosphere of nitrogen (4) for 9 hours. The mass was subsequently cooled, washed once with hydrochloric acid, then with water to remove chloride ions, and finally dried at 120°C in an electric oven.
2. *Activation by Zinc Chloride.*—Charcoal was agglomerated with 50% solution of zinc chloride and heated to 650° for 5 hours in a closed vessel provided with a few small holes near the top to serve as exit for the products of volatilization (20). The mass was cooled, washed first with dilute HCl, then with water and finally dried at 120°C.
3. *Activation by Steam.*—A silica pipe, 30″ long and 1″ wide, was filled three-fourths with charcoal and heated to about 950°C in an electric furnace, steam being passed continuously over the charge. As complete information regarding optimum time for steam activation was not available from the literature, a few preliminary experiments were performed to see the influence of the time of activation on moisture as well as methyl alcohol vapour adsorption. It was found that the intensity of adsorption increased with increase in the time of activation up to 10 hours after which it began to decrease gradually. Steam treatment was, therefore, restricted to 10 hours in all the charcoals.

Properties Examined.—The following properties of the activated as well as unactivated materials were examined:—

(i) Adsorption of water and methyl alcohol vapours.
(ii) Adsorption of iodine and potassium permanganate from solutions.
(iii) True density, bulk density and pore space.
(iv) Wettability by water, methyl alcohol, acetone and benzene.

Results

(i) *Adsorption of Water and Methyl Alcohol Vapours.*—0·5 gram portions of the various adsorbents, weighed in small watch glasses, were kept in desiccators containing sulphuric acid-water or methyl alcohol-glycerine mixtures corresponding to different relative vapour pressures of water or methyl alcohol, as the case may be. The increase in weight was determined when it became constant which required about 4 or 5 days time. The results are given in Tables I and II.

(ii) *Adsorption of Iodine and Potassium Permanganate from Aqueous Solutions.*—These tests are reported to be indicative of the ability of a charcoal to remove odours and flavours as well as colloid and colour bodies from solutions (14). The procedure used in these determinations was similar to the one described by Mantell (14). In brief, iodine adsorption was measured by mixing 0·5 gram of charcoal with 30 ml. of 5% HCl solution and 150 ml. of 0·05 *N* iodine solution and allowing it to stand for 24 hours with occasional shaking by hand. Fifty ml. of the supernatant liquid was then titrated against a standard sodium thiosulphate solution, using starch as indicator. Permanganate test was performed by mixing 0·5 gram of charcoal with 100 ml. of 0·5 *N* $KMnO_4$ solution and allowing the mixture to stand for 24 hours with occasional shaking and then titrating an aliquot portion of the supernatant liquid against a standard oxalic acid solution.

The results of these determinations are given in Tables III and IV.

3. *Density, Bulk Density and Pore Space.*—Density was determined by the pyknometer method using methyl alcohol as the immersion liquid. Bulk density was determined by dividing the weight of the dry material that fills a graduated cylinder to the top graduation, on gentle tapping, by the volume of the cylinder. Pore space for 100 gram charcoal, V_p, was calculated by the formula,

$$V_p = 100\left(\frac{1}{D_b} - \frac{1}{D_t}\right),$$

where D_b and D_t are bulk and true densities respectively. The results are given in Table V.

4. *Wettability of Charcoal Surface by Different Liquids.*—Among the properties of porous surfaces that are of scientific and industrial importance the wetting characteristics are particularly significant. These involve measurements of the angle of contact which a porous surface subtends with the wetting liquids.

The height to which a liquid rises in a capillary tube of a solid is given by the well-known relationship,

$$h = \frac{4\gamma}{gd\rho}\cdot\cos\theta,$$

where γ is surface tension and ρ is density of the liquid, d is diameter of the capillary tube and θ is the angle of contact. Cos θ obviously is a measure of the wetting power of the liquid for the capillary surface in question. If, therefore, for a particular porous surface the values of h in the case of

any two liquids of known surface tension and density are h_1 and h_2, then their relative wetting powers for the surface will be given by

$$\frac{\cos\theta_1}{\cos\theta_2} = \frac{h_1\gamma_2\rho_1}{h_2\gamma_1\rho_2}.$$

By means of this equation relative wetting powers of a number of liquids for a particular surface may be expressed with reference to a certain standard liquid whose wetting power ($\cos\theta$) may be taken as unity.

This principle was made use of in determining the relative wetting powers of water, acetone and benzene for the various charcoals with respect to that of methyl alcohol taken as unity. The value of h, the capillary pull, was determined by a simple apparatus described by Mills (15). The results are given in Table VI.

Discussion

Considering the effect of various activation treatments on the moisture adsorption capacity of the charcoals (Table I) it is seen that phosphoric acid treatment produces easily the best products, hygroscopicity increasing appreciably throughout the entire range of vapour pressure. The other two methods of activation, namely treatment with zinc chloride and steam, are far less effective. Comparing these two, however, with one another it is seen that while the former treatment is more effective in the lower vapour pressure range, the latter is more so in the higher vapour pressure range. Since smaller capillary spaces are involved in vapour adsorption at lower vapour pressures and larger ones in adsorption at higher vapour pressures it follows that activation with steam does not result in increasing the number of smaller capillaries and that its cleansing and oxidation action is also confined to the hydrocarbon complexes held up in comparatively larger microspaces only. It is obvious that steam treatment is to be preferred if the object is to remove vapours from atmospheres of higher vapour pressures and zinc chloride treatment if the aim is to remove moisture from atmospheres of lower relative vapour pressures.

The efficiency of the various activation treatments as far as adsorption of methyl alcohol vapours is concerned is shown in Table II. The phosphoric acid treatment is seen again to yield the best materials. The relative superiority of the other two methods is seen to vary with the nature of the charcoal; while zinc chloride treatment is seen to yield better adsorbents in the case of acacia wood and cotton stalk chars, steam treatment is seen to be more successful in the case of coconut shell and pine wood chars. Both the methods, however, are of little consequence in the case of sugar charcoal which gets activated by phosphoric acid treatment only.

Decolorizing properties of the charcoals, as adjudged from iodine and permanganate values (Tables III and IV), are seen to be enhanced appreciably on activation by phosphoric acid and zinc chloride treatments, the former again producing the maximum effect in all the charcoals. Steam, on the other hand, seems to be a poor activator as it causes only a slight improvement in iodine adsorption and actual deterioration in

permanganate adsorption values. It is interesting to note in this connection that while zinc chloride and steam treatments yield somewhat comparable values as far as vapour adsorption properties are concerned their effects on iodine and permanganate adsorption values are so markedly different from each other. Behrman and Gustafson (5) have reported that decolorizing properties of charcoal are favoured by low and hindered by high *p*H values. The cause of low decolorizing properties of steam activated materials appears to lie in their high *p*H values which were found to vary from 6·5 to 7·04 while those for phosphoric acid and zinc chloride activated materials were quite low, varying from 2·12 to 3·47 in the case of different samples. The views of King (11) and Smith, Thornhill and Bray (18) that iodine adsorption depends only upon the total extent of charcoal surface and is independent of its nature does not appear to be quite justifiable. The calculations of surface areas from the results of iodine adsorption (18) therefore should not be considered reliable.

Considering the various adsorption values on the whole (Tables I to IV) it is obvious that phosphoric acid treatment is the best process of activation and that of the various raw materials examined cotton stalks provide the best material for the preparation of active carbon. It is also evident that chars obtained from the three cheap wood sources yield better products on activation, than those obtained from coconut shell and cane sugar and therefore the common belief (14) that chars from nut-shells or fruit pits or sugars provide better materials for activation than the common varieties of wood does not appear to be justifiable. The iodine and permanganate tests were also performed with a commercial sample of 'Decolorizing Charcoal', manufactured by a well-known American firm. The values obtained were 0·579 and 3·02 gms. respectively. The superiority of the charcoals prepared by us is quite obvious (Tables III and IV).

True density is seen to increase and bulk density to decrease (both leading to increased pore space) on activation in all cases (Table V). These changes seem to be closely connected with the increased 'activity' produced in a charcoal. For instance, phosphoric acid treatment which produces the maximum activation also produces the maximum relative increase in true density and maximum relative decrease in bulk density of the various products and just as cotton stalk, acacia wood and pine wood chars get activated to greater extents, so do they suffer greater relative changes in true and bulk density values than sugar and coconut shell chars. It is also significant that cotton stalk char which shows the maximum adsorption undergoes the maximum relative changes in the density values on activation. It appears, therefore, that the effectiveness of activation treatment in a particular case can be broadly assessed from the measurement of the relative changes in true and bulk densities undergone by the material.

Two major factors appear to be responsible for bringing about increased adsorption on activation. They are: firstly cleansing of the charcoal surface of the adsorbed hydrocarbons—and other complexes—which is shown clearly by the increased wettability towards water and decreased wettability towards organic liquids in most of the cases on

activation (Table VI)—and secondly, increase in pore space and internal surface of charcoal (Table V). The cleansing of charcoal surface seems to be brought about by a sort of differential oxidation in which the hydrocarbon and other complexes formed during carbonization and strongly adsorbed by elementary carbon are burnt away, in part or as a whole, thereby releasing the corresponding adsorptive forces. The increase in density which takes place at the same time appears to produce an internal shrinkage resulting in the formation of numerous smaller spaces within the charcoal granules increasing thereby the internal surface area of charcoal.

Summary

Active carbon was prepared from five different carbonaceous materials, including three common varieties of wood, by employing three well-known activation processes. It was found that the method involving the use of phosphoric acid yields the best adsorbents irrespective of the nature of the starting material. The success of an activation process can be assessed from density and bulk density measurements.

Active carbons obtained from cheap wood sources were shown to be better adsorbents than those obtained from coconut shell and cane sugar. The common belief that the latter provide better materials is, therefore, not justified.

Cotton stalks when suitably treated yield excellent adsorbents for vapours as well as for decolorizing properties.

Most of the activated materials showed better decolorizing effects than a commercial 'decolorizing charcoal'.

The changes in the wetting characteristics of charcoal on activation were also measured. There was a tendency for charcoal to become more hydrophillic and less organophillic on activation.

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TABLE I

Moisture Absorption—Vapour Pressure Relationships of the various Charcoals before and after Activation.

	Description.	Percentage moisture absorption at different relative humidities.						
		10%	35%	44·8%	74·3%	88·3%	95·9%	99·5%
ACACIA WOOD CHARCOAL.	Unactivated	4·00	5·00	5·54	7·04	7·92	8·46	10·15
	$ZnCl_2$ treated	12·25	16·80	17·86	20·00	21·40	22·70	23·50
	Steam treated	3·46	9·16	10·92	14·48	20·92	27·00	31·54
	H_3PO_4 treated.	18·50	48·68	53·72	57·60	57·76	61·06	61·52
PINE WOOD CHARCOAL.	Unactivated	3·97	6·80	8·10	9·90	10·00	10·20	10·61
	$ZnCl_2$ treated	10·00	12·52	13·06	14·05	16·02	16·50	16·75
	Steam treated	2·68	9·63	12·08	14·50	18·04	20·21	23·25
	H_3PO_4 treated.	11·50	46·32	58·04	64·00	65·02	65·52	66·00
COTTON STALK CHARCOAL.	Unactivated	2·50	3·55	4·40	8·04	11·32	16·10	17·48
	$ZnCl_2$ treated	12·25	16·06	16·56	19·52	20·92	22·61	23·51
	Steam treated	3·25	12·24	13·02	17·06	19·20	22·00	24·10
	H_3PO_4 treated.	18·50	55·76	62·81	68·68	71·50	72·51	74·12
COCONUT SHELL CHARCOAL.	Unactivated	4·20	7·60	8·32	10·35	12·30	14·02	15·40
	$ZnCl_2$ treated	9·00	11·05	11·55	12·86	14·28	14·75	15·12
	Steam treated	2·52	15·28	17·32	19·80	21·48	28·56	30·54
	H_3PO_4 treated.	18·56	37·04	38·92	41·28	42·36	42·60	42·88
CANE SUGAR CHARCOAL.	Unactivated	8·00	12·40	12·52	14·43	16·56	18·35	19·90
	$ZnCl_2$ treated	9·25	11·50	12·50	14·06	16·05	18·00	19·50
	Steam treated	2·80	8·65	12·25	17·00	20·05	25·05	29·09
	H_3PO_4 treated.	10·50	36·76	43·64	48·84	51·56	52·80	53·00

TABLE II

Adsorption of Methyl Alcohol Vapours by the Various Charcoals before and after Activation Treatments.

	Description.	Percentage of methyl alcohol vapours adsorbed at different relative vapour pressures.				
		56·80%	68·50%	81·30%	95·30%	99·7%
ACACIA WOOD CHARCOAL.	Unactivated ..	6·10	7·80	9·20	10·40	12·08
	$ZnCl_2$ treated	13·30	18·64	21·25	24·00	25·80
	Steam treated	8·78	10·48	13·84	20·36	23·00
	H_3PO_4 treated	31·00	43·48	45·24	46·20	52·08
PINE WOOD CHARCOAL.	Unactivated ..	5·00	9·50	13·00	15·40	15·66
	$ZnCl_2$ treated	11·50	13·30	14·70	16·52	17·50
	Steam treated	10·75	13·00	15·50	19·06	20·06
	H_3PO_4 treated	32·00	44·80	45·00	48·04	56·52
COTTON STALK CHARCOAL.	Unactivated ..	3·70	7·40	9·52	13·64	14·05
	$ZnCl_2$ treated	11·32	16·40	21·48	24·84	25·52
	Steam treated	8·75	11·10	12·56	16·42	18·81
	H_3PO_4 treated	33·72	50·80	54·22	57·65	62·84
COCONUT SHELL CHARCOAL.	Unactivated ..	5·60	9·52	11·24	12·31	15·14
	$ZnCl_2$ treated	10·40	12·22	15·54	16·96	17·20
	Steam treated	15·02	15·80	16·92	20·05	21·16
	H_3PO_4 treated	28·46	33·12	33·24	34·40	36·50
CANE SUGAR CHARCOAL.	Unactivated ..	9·60	12·00	15·42	17·62	19·35
	$ZnCl_2$ treated	9·64	13·72	17·20	19·80	20·40
	Steam treated	9·32	10·28	12·84	17·28	20·06
	H_3PO_4 treated	27·40	34·40	35·48	38·72	44·02

TABLE III

Adsorption of Iodine by the Various Charcoals before and after Activation.

Charcoal.	Grams of iodine adsorbed/gm. charcoal.			
	Unactivated.	Zinc chloride activated.	Steam activated.	Phosphoric acid activated.
1. Acacia wood ..	0·312	1·414	0·317	1·341
2. Pine wood ..	0·219	1·196	0·242	1·392
3. Cotton stalk ..	0·289	1·312	0·295	1·702
4. Coconut shell..	0·255	1·144	0·344	1·274
5. Cane Sugar ..	0·192	1·065	0·370	1·850

TABLE IV

Adsorption of Potassium Permanganate by the Various Charcoals before and after Activation.

Charcoal.	Gms. of $KMnO_4$ adsorbed/gm. charcoal.			
	Unactivated.	Zinc chloride activated.	Steam activated.	Phosphoric acid activated.
1. Acacia wood ..	0·495	3·014	0·206	3·122
2. Pine wood ..	0·602	1·326	0·270	2·401
3. Cotton stalk ..	1·716	3·132	0·570	3·165
4. Coconut shell..	0·726	0·774	0·506	3·154
5. Cane sugar ..	1·406	1·615	0·806	2·706

TABLE V

Density, Bulk Density and Pore Space of Charcoals before and after Activation Treatments.

		Acacia wood.	Pine wood.	Cotton stalk.	Coconut shell.	Cane sugar.
TRUE DENSITY	Unactivated ..	1·60	1·58	1·56	1·60	1·78
	$ZnCl_2$ treated	1·71	1·60	1·62	1·64	1·74
	Steam treated	1·89	1·71	1·82	1·63	1·64
	H_3PO_4 treated	1·92	1·90	1·95	1·90	1·90
BULK DENSITY	Unactivated ..	0·56	0·60	0·46	0·81	0·84
	$ZnCl_2$ treated	0·47	0·59	0·44	0·81	0·91
	Steam treated	0·46	0·53	0·44	0·79	0·66
	H_3PO_4 treated	0·42	0·43	0·28	0·63	0·62
PERCENTAGE PORE SPACE.	Unactivated ..	116·10	103·10	153·3	60·95	60·10
	$ZnCl_2$ treated	154·30	105·90	165·5	62·40	52·40
	Steam treated	164·50	130·20	172·9	65·20	90·10
	H_3PO_4 treated	186·00	179·90	305·6	106·10	108·60

TABLE VI

Relative Wetting Powers of Different Liquids towards the various Charcoals before and after Activation.

Liquid.	Treatment.	Ratio: cos θ liquid/cos θ methyl alcohol.				
		Acacia wood.	Pine wood.	Cotton stalk.	Coconut shell.	Cane sugar.
WATER	Unactivated ..	0·848	0·780	0·922	0·892	0·809
	$ZnCl_2$ activated	0·912	0·839	0·935	0·921	0·839
	Steam activated	0·928	0·828	0·989	1·000	0·928
	H_3PO_4 activated.	0·951	0·972	0·991	0·956	1·058
ACETONE	Unactivated ..	1·059	1·137	1·109	1·020	1·089
	$ZnCl_2$ activated	1·000	1·040	1·000	0·999	1·046
	Steam activated	0·975	1·012	1·020	1·040	0·998
	H_3PO_4 activated.	1·000	0·968	1·015	0·998	0·986
BENZENE	Unactivated ..	0·902	0·924	0·901	0·967	0·787
	$ZnCl_2$ activated	0·817	0·900	0·894	0·969	0·853
	Steam activated	0·815	0·980	..	0·957	..
	H_3PO_4 activated.	0·800	0·883	0·885	0·954	0·781

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 20 | MATHEMATICS | Pp. 13–93

March, 1952

A TABLE OF VALUES OF $N_2(t)$

by

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Price Rs.10-12-0

A TABLE OF VALUES OF $N_2(t)$

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In 1940, I listed numbers [1] less than 20,000 which cannot be represented by Ramanujan's ternary quadratic form:

$$x^2+y^2+10z^2.$$

The table presented here was computed then. It gives the non-negative solutions of the equation:

$$x^2+y^2 = t, \tag{1}$$

with $x \leqslant y$ and $t \leqslant 20{,}000$. If $n_2(t)$ be the number of the non-negative solutions of (1), the solutions (a, b) and (b, a) being considered different when a and b are unequal, then the table gives also the values of $N_2(t)$ defined by the relation:

$$N_2(t) = \sum_{j=0}^{t} n_2(j). \tag{2}$$

If t be the greatest integer, not exceeding k, which is listed in the table, then

$$N_2(k) = N_2(t). \tag{3}$$

The table was of use to me also in computing [2] a 'Table of Values of $N_3(t)$' giving the number of non-negative solutions of

$$x^2+y^2+z^2 \leqslant t, \quad t \leqslant 10{,}000. \tag{4}$$

Evidently

$$N_2(t) = \sum_{j=0}^{[\sqrt{t}]} [\sqrt{t-j^2}] \tag{5}$$

where $[x]$ denotes as usual the integral part of x.

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t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
0	0	0	1	104	2	10	94
1	0	1	3	106	5	9	96
2	1	1	4	109	3	10	98
4	0	2	6	113	7	8	100
5	1	2	8	116	4	10	102
8	2	2	9	117	6	9	104
9	0	3	11	121	0	11	106
10	1	3	13	122	1	11	108
13	2	3	15	125	2	11	
16	0	4	17		5	10	112
17	1	4	19	128	8	8	113
18	3	3	20	130	3	11	
20	2	4	22		7	9	117
25	0	5		136	6	10	119
	3	4	26	137	4	11	121
26	1	5	28	144	0	12	123
29	2	5	30	145	1	12	
32	4	4	31		8	9	127
34	3	5	33	146	5	11	129
36	0	6	35	148	2	12	131
37	1	6	37	149	7	10	133
40	2	6	39	153	3	12	135
41	4	5	41	157	6	11	137
45	3	6	43	160	4	12	139
49	0	7	45	162	9	9	140
50	1	7		164	8	10	142
	5	5	48	169	0	13	
52	4	6	50		5	12	146
53	2	7	52	170	1	13	
58	3	7	54		7	11	150
61	5	6	56	173	2	13	152
64	0	8	58	178	3	13	154
65	1	8		180	6	12	156
	4	7	62	181	9	10	158
68	2	8	64	185	4	13	
72	6	6	65		8	11	162
73	3	8	67	193	7	12	164
74	5	7	69	194	5	13	166
80	4	8	71	196	0	14	168
81	0	9	73	197	1	14	170
82	1	9	75	200	2	14	
85	2	9			10	10	173
	6	7	79	202	9	11	175
89	5	8	81	205	3	14	
90	3	9	83		6	13	179
97	4	9	85	208	8	12	181
98	7	7	86	212	4	14	183
100	0	10		218	7	13	185
	6	8	90	221	5	14	
101	1	10	92		10	11	189

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
225	0	15		340	4	18	
	9	12	193		12	14	290
226	1	15	195	346	11	15	292
229	2	15	197	349	5	18	294
232	6	14	199	353	8	17	296
233	8	13	201	356	10	16	298
234	3	15	203	360	6	18	300
241	4	15	205	361	0	19	302
242	11	11	206	362	1	19	304
244	10	12	208	365	2	19	
245	7	14	210		13	14	308
250	5	15		369	12	15	310
	9	13	214	370	3	19	
256	0	16	216		9	17	314
257	1	16	218	373	7	18	316
260	2	16		377	4	19	
	8	14	222		11	16	320
261	6	15	224	386	5	19	322
265	3	16		388	8	18	324
	11	12	228	389	10	17	326
269	10	13	230	392	14	14	327
272	4	16	232	394	13	15	329
274	7	15	234	397	6	19	331
277	9	14	236	400	0	20	
281	5	16	238		12	16	335
288	12	12	239	401	1	20	337
289	0	17		404	2	20	339
	8	15	243	405	9	18	341
290	1	17		409	3	20	343
	11	13	247	410	7	19	
292	6	16	249		11	17	347
293	2	17	251	416	4	20	349
296	10	14	253	421	14	15	351
298	3	17	255	424	10	18	353
305	4	17		425	5	20	
	7	16	259		8	19	
306	9	15	261		13	16	359
313	12	13	263	433	12	17	361
314	5	17	265	436	6	20	363
317	11	14	267	441	0	21	365
320	8	16	269	442	1	21	
324	0	18	271		9	19	369
325	1	18		445	2	21	
	6	17			11	18	373
	10	15	277	449	7	20	375
328	2	18	279	450	3	21	
333	3	18	281		15	15	378
337	9	16	283	452	14	16	380
338	7	17		457	4	21	382
	13	13	286	458	13	17	384

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
461	10	19	386	584	10	22	484
464	8	20	388	585	3	24	
466	5	21	390		12	21	488
468	12	18	392	586	15	19	490
477	6	21	394	592	4	24	492
481	9	20		593	8	23	494
	15	16	398	596	14	20	496
482	11	19	400	601	5	24	498
484	0	22	402	605	11	22	500
485	1	22		610	9	23	
	14	17	406		13	21	504
488	2	22	408	612	6	24	506
490	7	21	410	613	17	18	508
493	3	22		617	16	19	510
	13	18	414	625	0	25	
500	4	22			7	24	
	10	20	418		15	20	516
505	8	21		626	1	25	518
	12	19	422	628	12	22	520
509	5	22	424	629	2	25	
512	16	16	425		10	23	524
514	15	17	427	634	3	25	526
520	6	22		637	14	21	528
	14	18	431	640	8	24	530
521	11	20	433	641	4	25	532
522	9	21	435	648	18	18	533
529	0	23	437	650	5	25	
530	1	23			11	23	
	13	19	441		17	19	539
533	2	23		653	13	22	541
	7	22	445	656	16	20	543
538	3	23	447	657	9	24	545
541	10	21	449	661	6	25	547
544	12	20	451	666	15	21	549
545	4	23		673	12	23	551
	16	17	455	674	7	25	553
548	8	22	457	676	0	26	
549	15	18	459		10	24	557
554	5	23	461	677	1	26	559
557	14	19	463	680	2	26	
562	11	21	465		14	22	563
565	6	23		685	3	26	
	9	22	469		18	19	567
569	13	20	471	689	8	25	
576	0	24	473		17	20	571
577	1	24	475	692	4	26	573
578	7	23		697	11	24	
	17	17	478		16	21	577
580	2	24		698	13	23	579
	16	18	482	701	5	26	581

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
706	9	25	583	832	16	24	681
709	15	22	585	833	7	28	683
712	6	26	587	841	0	29	
720	12	24	589		20	21	687
722	19	19	590	842	1	29	689
724	18	20	592	845	2	29	
725	7	26			13	26	
	10	25			19	22	695
	14	23	598	848	8	28	697
729	0	27	600	850	3	29	
730	1	27			11	27	
	17	21	604		15	25	703
733	2	27	606	853	18	23	705
738	3	27	608	857	4	29	707
740	8	26		865	9	28	
	16	22	612		17	24	711
745	4	27		866	5	29	713
	13	24	616	872	14	26	715
746	11	25	618	873	12	27	717
754	5	27		877	6	29	719
	15	23	622	881	16	25	721
757	9	26	624	882	21	21	722
761	19	20	626	884	10	28	
765	6	27			20	22	726
	18	21	630	890	7	29	
769	12	25	632		19	23	730
772	14	24	634	898	13	27	732
773	17	22	636	900	0	30	
776	10	26	638		18	24	736
778	7	27	640	901	1	30	
784	0	28	642		15	26	740
785	1	28		904	2	30	742
	16	23	646	905	8	29	
788	2	28	648		11	28	746
793	3	28		909	3	30	748
	8	27	652	914	17	25	750
794	13	25	654	916	4	30	752
797	11	26	656	922	9	29	754
800	4	28		925	5	30	
	20	20	659		14	27	
801	15	24	661		21	22	760
802	19	21	663	928	12	28	762
808	18	22	665	929	20	23	764
809	5	28	667	932	16	26	766
810	9	27	669	936	6	30	768
818	17	23	671	937	19	24	770
820	6	28		941	10	29	772
	12	26	675	949	7	30	
821	14	25	677		18	25	776
829	10	27	679	953	13	28	778

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
954	15	27	780		17	28	878
961	0	31	782	1076	20	26	880
962	1	31		1082	11	31	882
	11	29	786	1088	8	32	884
964	8	30	788	1089	0	33	886
965	2	31		1090	1	33	
	17	26	792		19	27	890
968	22	22	793	1093	2	33	892
970	3	31		1096	14	30	894
	21	23	797	1097	16	29	896
976	20	24	799	1098	3	33	898
977	4	31	801	1105	4	33	
980	14	28	803		9	32	
981	9	30	805		12	31	
985	12	29			23	24	906
	16	27	809	1108	18	28	908
986	5	31		1109	22	25	910
	19	25	813	1114	5	33	912
997	6	31	815	1117	21	26	914
1000	10	30		1124	10	32	916
	18	26	819	1125	6	33	
1009	15	28	821		15	30	920
1010	7	31		1129	20	27	922
	13	29	825	1130	13	31	
1013	22	23	827		17	29	926
1017	21	24	829	1138	7	33	928
1018	17	27	831	1145	11	32	
1021	11	30	833		19	28	932
1024	0	32	835	1152	24	24	933
1025	1	32		1153	8	33	935
	8	31		1154	23	25	937
	20	25	841	1156	0	34	
1028	2	32	843		16	30	941
1033	3	32	845	1157	1	34	
1037	14	29			14	31	945
	19	26	849	1160	2	34	
1040	4	32			22	26	949
	16	28	853	1165	3	34	
1042	9	31	855		18	29	953
1044	12	30	857	1168	12	32	955
1049	5	32	859	1170	9	33	
1053	18	27	861		21	27	959
1058	23	23	862	1172	4	34	961
1060	6	32		1181	5	34	963
	22	24	866	1184	20	28	965
1061	10	31	868	1186	15	31	967
1066	15	29		1189	10	33	
	21	25	872		17	30	971
1069	13	30	874	1192	6	34	973
1073	7	32		1193	13	32	975

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
1201	24	25	977	1321	5	36	1076
1202	19	29	979	1322	19	31	1078
1205	7	34		1325	10	35	
	23	26	983		13	34	
1210	11	33	985		22	29	1084
1213	22	27	987	1332	6	36	1086
1217	16	31	989	1341	21	30	1088
1220	8	34		1345	7	36	
	14	32	993		16	33	1092
1224	18	30	995	1346	11	35	1094
1225	0	35		1348	18	32	1096
	21	28	999	1352	14	34	
1226	1	35	1001		26	26	1099
1229	2	35	1003	1354	25	27	1101
1233	12	33	1005	1360	8	36	
1234	3	35	1007		24	28	1105
1237	9	34	1009	1361	20	31	1107
1241	4	35		1369	0	37	
	20	29	1013		12	35	1111
1249	15	32	1015	1370	1	37	
1250	5	35			23	29	1115
	17	31		1373	2	37	1117
	25	25	1020	1377	9	36	1119
1252	24	26	1022	1378	3	37	
1256	10	34	1024		17	33	1123
1258	13	33		1381	15	34	1125
	23	27	1028	1384	22	30	1127
1261	6	35		1385	4	37	
	19	30	1032		19	32	1131
1268	22	28	1034	1394	5	37	
1274	7	35	1036		13	35	1135
1277	11	34	1038	1396	10	36	1137
1280	16	32	1040	1402	21	31	1139
1282	21	29	1042	1405	6	37	
1285	14	33			26	27	1143
	18	31	1046	1409	25	28	1145
1289	8	35	1048	1412	16	34	1147
1296	0	36	1050	1413	18	33	1149
1297	1	36	1052	1417	11	36	
1300	2	36			24	29	1153
	12	34		1418	7	37	1155
	20	30	1058	1421	14	35	1157
1301	25	26	1060	1424	20	32	1159
1305	3	36		1429	23	30	1161
	24	27	1064	1433	8	37	1163
1306	9	35	1066	1440	12	36	1165
1312	4	36	1068	1444	0	38	1167
1313	17	32		1445	1	38	
	23	28	1072		17	34	
1314	15	33	1074		22	31	1173

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
1448	2	38	1175	1570	7	39	
1450	9	37			27	29	1275
	15	35		1573	22	33	1277
	19	33	1181	1576	26	30	1279
1453	3	38	1183	1585	8	39	
1458	27	27	1184		17	36	1283
1460	4	38		1586	19	35	
	26	28	1188		25	31	1287
1465	13	36		1588	12	38	1289
	21	32	1192	1594	15	37	1291
1466	25	29	1194	1597	21	34	1293
1469	5	38		1600	0	40	
	10	37	1198		24	32	1297
1476	24	30	1200	1601	1	40	1299
1480	6	38		1602	9	39	1301
	18	34	1204	1604	2	40	1303
1481	16	35	1206	1609	3	40	1305
1489	20	33	1208	1613	13	38	1307
1490	11	37		1616	4	40	1309
	23	31	1212	1618	23	33	1311
1492	14	36	1214	1620	18	36	1313
1493	7	38	1216	1621	10	39	1315
1508	8	38		1625	5	40	
	22	32	1220		16	37	
1513	12	37			20	35	
	27	28	1224		28	29	1323
1514	17	35	1226	1629	27	30	1325
1517	19	34		1636	6	40	1327
	26	29	1230	1637	26	31	1329
1521	0	39		1640	14	38	
	15	36	1234		22	34	1333
1522	1	39	1236	1642	11	39	1335
1525	2	39		1649	7	40	
	9	38			25	32	1339
	25	30	1242	1657	19	36	1341
1530	3	39		1658	17	37	1343
	21	33	1246	1664	8	40	1345
1537	4	39		1665	12	39	
	24	31	1250		24	33	1349
1538	13	37	1252	1666	21	35	1351
1544	10	38	1254	1669	15	38	1353
1546	5	39	1256	1681	0	41	
1549	18	35	1258		9	40	1357
1552	16	36	1260	1682	1	41	
1553	23	32	1262		29	29	1360
1556	20	34	1264	1684	28	30	1362
1557	6	39	1266	1685	2	41	
1565	11	38			23	34	1366
	14	37	1270	1690	3	41	
1568	28	28	1271		13	39	

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
	27	31	1372	1813	7	42	1471
1693	18	37	1374	1818	27	33	1473
1696	20	36	1376	1825	12	41	
1697	4	41	1378		15	40	
1700	10	40			23	36	1479
	16	38		1828	8	42	1481
	26	32	1384	1832	26	34	1483
1706	5	41	1386	1844	20	38	1485
1709	22	35	1388	1845	9	42	
1714	25	33	1390		18	39	1489
1717	6	41		1849	0	43	1491
	14	39	1394	1850	1	43	
1721	11	40	1396		13	41	
1730	7	41			25	35	1497
	19	37	1400	1853	2	43	
1732	24	34	1402		22	37	1501
1733	17	38	1404	1856	16	40	1503
1737	21	36	1406	1858	3	43	1505
1741	29	30	1408	1861	30	31	1507
1744	12	40	1410	1864	10	42	1509
1745	8	41		1865	4	43	
	28	31	1414		29	32	1513
1746	15	39	1416	1872	24	36	1515
1753	27	32	1418	1873	28	33	1517
1754	23	35	1420	1874	5	43	1519
1762	9	41	1422	1877	14	41	1521
1764	0	42	1424	1882	19	39	1523
1765	1	42		1885	6	43	
	26	33	1428		11	42	
1768	2	42			21	38	
	18	38	1432		27	34	1531
1769	13	40		1889	17	40	1533
	20	37	1436	1898	7	43	
1773	3	42	1438		23	37	1537
1777	16	39	1440	1901	26	35	1539
1780	4	42		1906	15	41	1541
	22	36	1444	1908	12	42	1543
1781	10	41		1913	8	43	1545
	25	34	1448	1921	20	39	
1789	5	42	1450		25	36	1549
1796	14	40	1452	1922	31	31	1550
1800	6	42		1924	18	40	
	30	30	1455		30	32	1554
1801	24	35	1457	1928	22	38	1556
1802	11	41		1930	9	43	
	29	31	1461		29	33	1560
1805	19	38	1463	1933	13	42	1562
1808	28	32	1465	1936	0	44	1564
1810	17	39		1937	1	44	
	21	37	1469		16	41	1568

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
1940	2	44		2061	6	45	1669
	28	34	1572	2066	29	35	1671
1945	3	44		2069	25	38	1673
	24	37	1576	2074	7	45	
1949	10	43	1578		15	43	1677
1952	4	44	1580	2080	12	44	
1954	27	35	1582		28	36	1681
1960	14	42	1584	2081	20	41	1683
1961	5	44		2084	22	40	1685
	19	40	1588	2088	18	42	1687
1962	21	39	1590	2089	8	45	1689
1970	11	43		2097	24	39	1691
	17	41	1594	2098	27	37	1693
1972	6	44		2105	13	44	
	26	36	1598		16	43	1697
1973	23	38	1600	2106	9	45	1699
1985	7	44		2113	32	33	1701
	31	32	1604	2116	0	46	1703
1989	15	42		2117	1	46	
	30	33	1608		31	34	1707
1993	12	43	1610	2120	2	46	
1994	25	37	1612		26	38	1711
1997	29	34	1614	2122	21	41	1713
2000	8	44		2125	3	46	
	20	40	1618		10	45	
2005	18	41			19	42	
	22	39	1622		30	35	1721
2009	28	35	1624	2129	23	40	1723
2017	9	44	1626	2132	4	46	
2018	13	43	1628		14	44	1727
2020	16	42		2137	29	36	1729
	24	38	1632	2138	17	43	1731
2025	0	45		2141	5	46	1733
	27	36	1636	2146	11	45	
2026	1	45	1638		25	39	1737
2029	2	45	1640	2152	6	46	1739
2034	3	45	1642	2153	28	37	1741
2036	10	44	1644	2161	15	44	1743
2041	4	45		2164	20	42	1745
	21	40	1648	2165	7	46	
2042	19	41	1650		22	41	1749
2045	14	43		2169	12	45	1751
	26	37	1654	2173	18	43	
2048	32	32	1655		27	38	1755
2050	5	45		2176	24	40	1757
	23	39		2178	33	33	1758
	31	33	1661	2180	8	46	
2053	17	42	1663		32	34	1762
2056	30	34	1665	2186	31	35	1764
2057	11	44	1667	2192	16	44	1766

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
2194	13	45	1768	2313	3	48	1867
2196	30	36	1770	2314	17	45	
2197	9	46			33	35	1871
	26	39	1774	2320	4	48	
2205	21	42	1776		32	36	1875
2209	0	47	1778	2329	5	48	
2210	1	47			27	40	1879
	19	43		2330	11	47	
	23	41			31	37	1883
	29	37	1786	2333	22	43	1885
2213	2	47	1788	2336	20	44	1887
2216	10	46	1790	2340	6	48	
2218	3	47	1792		24	42	1891
2221	14	45	1794	2341	15	46	1893
2225	4	47		2344	30	38	1895
	17	44		2349	18	45	1897
	25	40	1800	2353	7	48	
2228	28	38	1802		12	47	1901
2234	5	47	1804	2357	26	41	1903
2237	11	46	1806	2362	29	39	1905
2245	6	47		2368	8	48	1907
	33	34	1810	2372	16	46	1909
2248	22	42	1812	2377	21	44	1911
2249	20	43		2378	13	47	
	32	35	1816		23	43	1915
2250	15	45		2381	34	35	1917
	27	39	1820	2384	28	40	1919
2257	24	41		2385	9	48	
	31	36	1824		33	36	1923
2258	7	47	1826	2386	19	45	1925
2260	12	46		2389	25	42	1927
	18	44	1830	2393	32	37	1929
2269	30	37	1832	2401	0	49	1931
2273	8	47	1834	2402	1	49	1933
2276	26	40	1836	2404	10	48	1935
2281	16	45	1838	2405	2	49	
2285	13	46			14	47	
	29	38	1842		17	46	
2290	9	47			31	38	1943
	21	43	1846	2410	3	49	
2293	23	42	1848		27	41	1947
2297	19	44	1850	2417	4	49	1949
2304	0	48	1852	2420	22	44	1951
2305	1	48		2421	30	39	1953
	28	39	1856	2425	11	48	
2306	25	41	1858		20	45	
2308	2	48	1860		24	43	1959
2309	10	47	1862	2426	5	49	1961
2312	14	46		2434	15	47	1963
	34	34	1865	2437	6	49	1965

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
2440	18	46			31	40	2066
	26	42	1969	2564	8	50	2068
2441	29	40	1971	2570	13	49	
2448	12	48	1973		19	47	2072
2450	7	49		2578	27	43	2074
	35	35	1976	2581	9	50	
2452	34	36	1978		30	41	2078
2458	33	37	1980	2592	36	36	2079
2465	8	49		2593	17	48	2081
	16	47		2594	35	37	2083
	23	44		2597	14	49	2085
	28	41	1988	2600	10	50	
2466	21	45	1990		22	46	
2468	32	38	1992		34	38	2091
2473	13	48	1994	2601	0	51	
2474	25	43	1996		24	45	2095
2477	19	46	1998	2602	1	51	2097
2482	9	49		2605	2	51	
	31	39	2002		29	42	2101
2493	27	42	2004	2609	20	47	2103
2498	17	47	2006	2610	3	51	
2500	0	50			33	39	2107
	14	48		2612	26	44	2109
	30	40	2012	2617	4	51	2111
2501	1	50		2621	11	50	2113
	10	49	2016	2624	32	40	2115
2504	2	50	2018	2626	5	51	
2509	3	50			15	49	2119
	22	45	2022	2628	18	48	2121
2512	24	44	2024	2633	28	43	2123
2516	4	50		2637	6	51	2125
	20	46	2028	2642	31	41	2127
2521	35	36	2030	2644	12	50	2129
2522	11	49		2645	23	46	2131
	29	41	2034	2650	7	51	
2525	5	50			21	47	
	26	43			25	45	2137
	34	37	2040	2657	16	49	2139
2529	15	48	2042	2664	30	42	2141
2533	18	47		2665	8	51	
	33	38	2046		19	48	
2536	6	50	2048		27	44	
2545	12	49			36	37	2149
	32	39	2052	2669	13	50	
2548	28	42	2054		35	38	2153
2549	7	50		2677	34	39	2155
2554	23	45	2058	2682	9	51	2157
2557	21	46	2060	2689	33	40	2159
2560	16	48	2062	2690	17	49	
2561	25	44			29	43	2163

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
2692	24	46	2165		37	38	2264
2693	22	47	2167	2817	36	39	2266
2696	14	50	2169	2818	3	53	2268
2701	10	51		2824	18	50	2270
	26	45	2173	2825	4	53	
2704	0	52			11	52	
	20	48	2177		35	40	2276
2705	1	52		2826	15	51	2278
	32	41	2181	2833	23	48	2280
2708	2	52	2183	2834	5	53	
2713	3	52	2185		25	47	2284
2720	4	52		2836	30	44	2286
	28	44	2189	2837	34	41	2288
2722	11	51	2191	2842	21	49	2290
2725	15	50		2845	6	53	
	18	49			27	46	2294
	31	42	2197	2848	12	52	2296
2729	5	52	2199	2853	33	42	2298
2738	23	47		2857	16	51	2300
	37	37	2202	2858	7	53	2302
2740	6	52		2861	19	50	2304
	36	38	2206	2866	29	45	2306
2741	25	46	2208	2873	8	53	
2745	12	51			13	52	
	21	48	2212		32	43	2312
2746	35	39	2214	2880	24	48	2314
2749	30	43	2216	2885	22	49	
2753	7	52	2218		26	47	2318
2754	27	45	2220	2888	38	38	2319
2756	16	50		2890	9	53	
	34	40	2224		17	51	
2762	19	49	2226		37	39	2325
2768	8	52	2228	2896	36	40	2327
2770	13	51		2897	31	44	2329
	33	41	2232	2900	14	52	
2777	29	44	2234		20	50	
2785	9	52			28	46	2335
	24	47	2238	2906	35	41	2337
2788	22	48		2909	10	53	2339
	32	42	2242	2916	0	54	2341
2789	17	50	2244	2917	1	54	2343
2792	26	46	2246	2920	2	54	
2797	14	51	2248		34	42	2347
2801	20	49	2250	2925	3	54	
2804	10	52	2252		18	51	
2809	0	53			30	45	2353
	28	45	2256	2929	15	52	
2810	1	53			25	48	2357
	31	43	2260	2930	11	53	
2813	2	53			23	49	2361

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
2932	4	54	2363		36	42	2462
2938	27	47		3061	6	55	2464
	33	43	2367	3065	16	53	
2941	5	54			19	52	2468
	21	50	2371	3074	7	55	
2952	6	54	2373		35	43	2472
2953	12	53	2375	3076	24	50	2474
2957	29	46	2377	3077	26	49	
2960	16	52			31	46	2478
	32	44	2381	3085	13	54	
2962	19	51	2383		22	51	2482
2965	7	54		3088	28	48	2484
	38	39	2387	3089	8	55	2486
2969	37	40	2389	3092	34	44	2488
2977	24	49		3098	17	53	2490
	36	41	2393	3104	20	52	2492
2978	13	53	2395	3106	9	55	2494
2980	8	54		3109	30	47	2496
	26	48	2399	3112	14	54	2498
2984	22	50	2401	3114	33	45	2500
2986	31	45	2403	3121	39	40	2502
2989	35	42	2405	3125	10	55	
2993	17	52			25	50	
	28	47	2409		38	41	2508
2997	9	54	2411	3130	23	51	
3001	20	51	2413		27	49	2512
3005	14	53		3133	18	53	
	34	43	2417		37	42	2516
3016	10	54		3136	0	56	2518
	30	46	2421	3137	1	56	2520
3025	0	55		3140	2	56	
	33	44	2425		32	46	2524
3026	1	55		3141	15	54	2526
	25	49	2429	3145	3	56	
3028	18	52	2431		21	52	
3029	2	55			29	48	
	23	50	2435		36	43	2534
3033	27	48	2437	3146	11	55	2536
3034	3	55		3152	4	56	2538
	15	53	2441	3161	5	56	
3037	11	54	2443		35	44	2542
3041	4	55	2445	3169	12	55	2544
3042	21	51		3170	19	53	
	39	39	2448		31	47	2548
3044	38	40	2450	3172	6	56	
3049	32	45	2452		16	54	2552
3050	5	55		3176	26	50	2554
	29	47		3177	24	51	2556
	37	41	2458	3181	34	45	2558
3060	12	54		3185	7	56	

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
	28	49	2562	3313	8	57	2661
3188	22	52	2564	3314	17	55	2663
3194	13	55	2566	3316	20	54	2665
3200	8	56		3321	36	45	2667
	40	40	2569	3328	32	48	2669
3202	39	41	2571	3329	25	52	2671
3204	30	48	2573	3330	9	57	
3205	17	54			27	51	2675
	33	46	2577	3332	14	56	2677
3208	38	42	2579	3338	23	53	2679
3209	20	53	2581	3341	29	50	
3217	9	56	2583		35	46	2683
3218	37	43	2585	3349	10	57	
3221	14	55	2587		18	55	2687
3226	25	51	2589	3357	21	54	2689
3229	27	50	2591	3361	15	56	2691
3232	36	44	2593	3362	31	49	
3233	23	52			41	41	2694
	32	47	2597	3364	0	58	
3236	10	56	2599		40	42	2698
3240	18	54	2601	3365	1	58	
3245	29	49	2603		34	47	2702
3249	0	57	2605	3368	2	58	2704
3250	1	57		3370	11	57	
	15	56			39	43	2708
	21	53		3373	3	58	2710
	35	46	2613	3380	4	58	
3253	2	57	2615		26	52	
3257	11	56	2617		38	44	2716
3258	3	57	2619	3385	24	53	
3265	4	57			28	51	2720
	31	48	2623	3386	19	55	2722
3272	34	46	2625	3389	5	58	2724
3274	5	57	2627	3392	16	56	2726
3277	19	54		3393	12	57	
	26	51	2631		33	48	2730
3280	12	56		3394	37	45	2732
	24	52	2635	3400	6	58	
3281	16	55			22	54	
	40	41	2639		30	50	2738
3234	28	50	2641	3412	36	46	2740
3235	6	57		3413	7	58	2742
	39	42	2645	3418	13	57	2744
3293	22	53		3425	17	56	
	38	43	2649		20	55	
3298	7	57			32	49	2750
	33	47	2653	3428	8	58	2752
3301	30	49	2655	3433	27	52	2754
3305	13	56		3434	25	53	
	37	44	2659		35	47	2758

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
3442	29	51	2760		38	46	2859
3445	9	58		3562	9	59	
	14	57			31	51	2863
	23	54		3573	18	57	2865
	41	42	2768	3577	21	56	2867
3449	40	43	2770	3578	37	47	2869
3457	39	44	2772	3581	10	59	2871
3460	18	56		3589	16	58	
	34	48	2776		33	50	2875
3461	31	50	2778	3592	26	54	2877
3464	10	58	2780	3593	28	53	2879
3466	21	55	2782	3600	0	60	
3469	38	45	2784		36	48	2883
3474	15	57	2786	3601	1	60	
3481	0	59	2788		24	55	2887
3482	1	59	2790	3602	11	59	2889
3485	2	59		3604	2	60	
	11	58			30	52	2893
	26	53		3609	3	60	2895
	37	46	2798	3610	19	57	2897
3488	28	52	2800	3613	42	43	2899
3490	3	59		3616	4	60	2901
	33	49	2804	3617	41	44	2903
3492	24	54	2806	3620	16	58	
3497	4	59			22	56	2907
	19	56	2810	3625	5	60	
3501	30	51	2812		12	59	
3505	16	57			32	51	
	36	47	2816		40	45	2915
3506	5	59	2818	3626	35	49	2917
3508	12	58	2820	3636	6	60	2919
3509	22	55	2822	3637	39	46	2921
3517	6	59	2824	3645	27	54	2923
3524	32	50	2826	3649	7	60	
3528	42	42	2827		20	57	2927
3529	35	48	2829	3650	13	59	
3530	7	59			25	55	
	41	43	2833		29	53	2933
3533	13	58	2835	3653	17	58	
3536	20	56			38	47	2937
	40	44	2839	3656	34	50	2939
3538	17	57		3664	8	60	2941
	27	53	2843	3665	23	56	
3541	25	54	2845		31	52	2945
3545	8	59		3673	37	48	2947
	29	52	2849	3677	14	59	2949
3546	39	45	2851	3681	9	60	2951
3554	23	55	2853	3688	18	58	2953
3557	34	49	2855	3690	21	57	
3560	14	58			33	51	2957

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
3697	36	49	2959	3812	26	56	3058
3698	43	43	2960	3816	30	54	3060
3700	10	60		3821	10	61	3062
	28	54		3825	15	60	
	42	44	2966		24	57	
3701	26	55	2968		39	48	3068
3706	15	59		3826	35	51	3070
	41	45	2972	3833	32	53	3072
3709	30	53	2974	3842	11	61	
3712	24	56	2976		19	59	3076
3716	40	46	2978	3844	0	62	3078
3721	0	61		3845	1	62	
	11	60	2982		38	49	3082
3722	1	61	2984	3848	2	62	
3725	2	61			22	58	3086
	19	58		3853	3	62	3088
	35	50	2990	3856	16	60	3090
3728	32	52	2992	3860	4	62	
3730	3	61			34	52	3094
	39	47	2996	3865	12	61	
3733	22	57	2998		27	56	3098
3737	4	61		3866	29	55	3100
	16	59	3002	3869	5	62	
3744	12	60	3004		37	50	3104
3746	5	61	3006	3872	44	44	3105
3748	38	48	3008	3874	25	57	
3754	27	55	3010		43	45	3109
3757	6	61		3877	31	54	3111
	29	54		3880	6	62	
	34	51	3016		42	46	3115
3761	25	56	3018	3881	20	59	3117
3764	20	58	3020	3889	17	60	3119
3769	13	60	3022	3890	13	61	
3770	7	61			41	47	3123
	17	59		3893	7	62	
	31	53			23	58	3127
	37	49	3030	3897	36	51	3129
3778	23	57	3032	3898	33	53	3131
3785	8	61		3904	40	48	3133
	43	44	3036	3908	8	62	3135
3789	42	45	3038	3917	14	61	3137
3793	33	52	3040	3920	28	56	3139
3796	14	60		3922	21	59	
	36	50	3044		39	49	3143
3797	41	46	3046	3924	18	60	3145
3802	9	61	3048	3925	9	62	
3805	18	59			26	57	
	21	58	3052		30	55	3151
3809	28	55		3929	35	52	3153
	40	47	3056	3940	24	58	

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
	32	54	3157	4068	42	48	3256
3944	10	62		4069	10	63	
	38	50	3161		15	62	3260
3946	15	61	3163	4072	34	54	3262
3961	19	60		4073	37	52	3264
	44	45	3167	4082	19	61	
3965	11	62			41	49	3268
	22	59		4084	22	60	3270
	34	53		4090	11	63	
	43	46	3175		29	57	3274
3969	0	63	3177	4093	27	58	3276
3970	1	63		4096	0	64	3278
	37	51	3181	4097	1	64	
3973	2	63			31	56	3282
	42	47	3185	4100	2	64	
3977	16	61			16	62	
	29	56	3189		40	50	3288
3978	3	63		4105	3	64	
	27	57	3193		36	53	3292
3985	4	63		4106	25	59	3294
	41	48	3197	4112	4	64	3296
3986	31	55	3199	4113	12	63	3298
3988	12	62	3201	4114	33	55	3300
3989	25	58	3203	4121	5	64	
3994	5	63	3205		20	61	3304
4000	20	60		4122	39	51	3306
	36	52	3209	4129	23	60	3308
4001	40	49	3211	4132	6	64	3310
4005	6	63		4133	17	62	3312
	33	54	3215	4138	13	63	3314
4010	17	61		4141	35	54	
	23	59	3219		45	46	3318
4013	13	62	3221	4145	7	64	
4018	7	63	3223		44	47	3322
4021	39	50	3225	4148	28	58	
4033	8	63			38	52	3326
	28	57	3229	4149	30	57	3328
4034	35	53	3231	4153	43	48	3330
4036	30	56	3233	4157	26	59	3332
4040	14	62		4160	8	64	
	26	58	3237		32	56	3336
4041	21	60	3239	4162	21	61	3338
4045	18	61		4165	14	63	
	38	51	3243		42	49	3342
4049	32	55	3245	4168	18	62	3344
4050	9	63		4176	24	60	3346
	45	45	3248	4177	9	64	3348
4052	44	46	3250	4178	37	53	3350
4057	24	59	3252	4181	34	55	
4058	43	47	3254		41	50	3354

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
4194	15	63	3356	4322	29	59	3455
4196	10	64	3358	4325	10	65	
4201	40	51	3360		31	58	
4205	19	62			46	47	3461
	22	61		4328	22	62	3463
	29	58	3366	4329	27	60	
4210	27	59			45	48	3467
	31	57	3370	4330	19	63	
4212	36	54	3372		39	53	3471
4217	11	64	3374	4337	44	49	3473
4225	0	65		4338	33	57	3475
	16	63		4346	11	65	
	25	60			25	61	3479
	33	56		4349	43	50	3481
	39	52	3384	4352	16	64	3483
4226	1	65	3386	4356	0	66	3485
4229	2	65	3388	4357	1	66	3487
4232	46	46	3389	4360	2	66	
4234	3	65			38	54	3491
	45	47	3393	4361	35	56	3493
4240	12	64		4365	3	66	
	44	48	3397		42	51	3497
4241	4	65	3399	4369	12	65	
4244	20	62	3401		20	63	3501
4250	5	65		4372	4	66	3503
	23	61		4373	23	62	3505
	35	55		4381	5	66	
	43	49	3409		30	59	3509
4253	38	53	3411	4384	28	60	3511
4258	17	63	3413	4385	17	64	
4261	6	65	3415		41	52	3515
4264	30	58		4388	32	58	3517
	42	50	3419	4392	6	66	3519
4265	13	64		4394	13	65	
	28	59	3423		37	55	3523
4273	32	57	3425	4397	26	61	3525
4274	7	65	3427	4405	7	66	
4276	26	60	3429		34	57	3529
4282	41	51	3431	4409	40	53	3531
4285	21	62		4410	21	63	3533
	37	54	3435	4418	47	47	3534
4289	8	65	3437	4420	8	66	
4292	14	64			18	64	
	34	56	3441		24	62	
4293	18	63	3443		46	48	3542
4297	24	61	3445	4421	14	65	3544
4304	40	52	3447	4426	45	49	3546
4306	9	65	3449	4432	36	56	3548
4321	15	64		4436	44	50	3550
	36	55	3453	4437	9	66	

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
	39	54	3554	4562	29	61	3654
4441	29	60	3556	4570	9	67	
4442	31	59	3558		33	59	3658
4450	15	65		4573	27	62	
	27	61			42	53	3662
	43	51	3564	4580	22	64	
4453	22	63			38	56	3666
	33	58	3568	4581	15	66	3668
4456	10	66	3570	4586	19	65	3670
4457	19	64	3572	4589	10	67	
4468	42	52	3574		35	58	3674
4469	25	62		4594	25	63	3676
	38	55	3578	4597	41	54	3678
4474	35	57	3580	4608	48	48	3679
4477	11	66	3582	4610	11	67	
4481	16	65	3584		47	49	3683
4489	0	67	3586	4612	16	66	3685
4490	1	67		4616	46	50	3687
	41	53	3590	4618	37	57	3689
4493	2	67	3592	4621	30	61	3691
4496	20	64	3594	4624	0	68	
4498	3	67			32	60	3695
	23	63	3598	4625	1	68	
4500	12	66			20	65	
	30	60	3602		23	64	
4505	4	67			40	55	3703
	28	61		4626	45	51	3705
	32	59		4628	2	68	
	37	56	3610		28	62	3709
4513	47	48	3612	4633	3	68	
4514	5	67			12	67	3713
	17	65	3616	4637	34	59	3715
4516	40	54	3618	4640	4	68	
4517	46	49	3620		44	52	3719
4520	26	62		4645	17	66	
	34	58	3624		26	63	3723
4525	6	67		4649	5	68	3725
	13	66		4657	39	56	3727
	45	50	3630	4658	13	67	
4537	21	64			43	53	3731
	44	51	3634	4660	6	68	
4538	7	67	3636		36	58	3735
4545	24	63		4666	21	65	3737
	36	57	3640	4672	24	64	3739
4546	39	55	3642	4673	7	68	3741
4549	18	65	3644	4680	18	66	
4552	14	66	3646		42	54	3745
4553	8	67		4682	31	61	3747
	43	52	3650	4685	14	67	
4561	31	60	3652		29	62	3751

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
4688	8	68	3753		33	61	
4689	33	60	3755		47	51	3854
4693	38	57	3757	4813	18	67	3856
4698	27	63	3759	4817	41	56	3858
4705	9	68		4820	14	68	
	48	49	3763		46	52	3862
4706	35	59		4825	8	69	
	41	55	3767		27	64	
4709	22	65			35	60	3868
	47	50	3771	4834	45	53	3870
4714	15	67	3773	4840	22	66	3872
4717	19	66		4842	9	69	3874
	46	51	3777	4849	15	68	
4721	25	64	3779		40	57	3878
4724	10	68	3781	4850	19	67	
4729	45	52	3783		25	65	
4733	37	58	3785		37	59	3884
4736	40	56	3787	4852	44	54	3886
4744	30	62	3789	4861	10	69	3888
4745	11	68		4868	32	62	3890
	16	67		4869	30	63	3892
	32	61		4874	43	55	3894
	44	53	3797	4877	34	61	3896
4753	28	63	3799	4880	16	68	
4754	23	65	3801		28	64	3900
4756	20	66		4882	11	69	3902
	34	60	3805	4885	23	66	
4761	0	69	3807		39	58	3906
4762	1	69	3809	4889	20	67	3908
4765	2	69		4896	36	60	3910
	43	54	3813	4900	0	70	
4768	12	68	3815		42	56	3914
4770	3	69		4901	1	70	
	39	57	3819		26	65	
4772	26	64	3821		49	50	3920
4777	4	69		4904	2	70	3922
	36	59	3825	4905	12	69	
4778	17	67	3827		48	51	3926
4786	5	69	3829	4909	3	70	3928
4789	42	55	3831	4913	17	68	
4793	13	68	3833		47	52	3932
4797	6	69		4916	4	70	3934
	21	66	3837	4925	5	70	
4801	24	65	3839		38	59	
4802	49	49	3840		46	53	3940
4804	48	50	3842	4930	13	69	
4805	31	62	3844		21	67	
4808	38	58	3846		31	63	
4810	7	69			41	57	3948
	29	63		4932	24	66	3950

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
4933	33	62	3952		24	67	4051
4936	6	70	3954	5066	5	71	
4937	29	64	3956		29	65	4055
4941	45	54	3958	5069	13	70	
4946	35	61	3960		35	62	4059
4948	18	68	3962	5072	44	56	4061
4949	7	70	3964	5077	6	71	4063
4954	27	65	3966	5081	40	59	4065
4957	14	69	3968	5085	18	69	
4961	44	55	3970		27	66	4069
4964	8	70		5090	7	71	
	40	58	3974		37	61	4073
4969	37	60	3976	5096	14	70	4075
4973	22	67	3978	5098	43	57	4077
4981	9	70		5101	50	51	4079
	25	66	3982	5105	8	71	
4985	19	68			49	52	4083
	43	56	3986	5108	22	68	4085
4986	15	69	3988	5113	48	53	4087
4993	32	63	3990	5114	25	67	4089
4996	30	64	3992	5120	32	64	4091
5000	10	70		5121	39	60	4093
	34	62		5122	9	71	
	50	50	3997		19	69	4097
5002	39	59		5125	15	70	
	49	51	4001		30	65	
5008	48	52	4003		34	63	
5009	28	65	4005		47	54	4105
5013	42	57	4007	5128	42	58	4107
5017	16	69		5140	28	66	
	36	61	4011		36	62	4111
5018	23	67		5141	10	71	
	47	53	4015		46	55	4115
5021	11	70	4017	5153	23	68	4117
5024	20	68	4019	5156	16	70	4119
5032	26	66		5161	20	69	
	46	54	4023		45	56	4123
5041	0	71	4025	5162	11	71	
5042	1	71	4027		41	59	4127
5044	12	70		5165	26	67	
	38	60	4031		38	61	4131
5045	2	71		5184	0	72	4133
	41	58	4035	5185	1	72	
5050	3	71			12	71	
	17	69			33	64	
	45	55	4041		44	57	4141
5057	4	71		5186	31	65	4143
	31	64	4045	5188	2	72	4145
5058	33	63	4047	5189	17	70	4147
5065	21	68		5193	3	72	4149

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
5194	35	63	4151		40	61	4250
5197	29	66	4153	5328	12	72	4252
5200	4	72		5329	0	73	
	24	68			48	55	4256
	40	60	4159	5330	1	73	
5202	21	69			17	71	
	51	51	4162		29	67	
5204	50	52	4164		43	59	4264
5209	5	72	4166	5333	2	73	4266
5210	13	71		5337	24	69	4268
	49	53	4170	5338	3	73	
5213	37	62			37	63	4272
	43	58	4174	5341	21	70	4274
5218	27	67	4176	5345	4	73	
5220	6	72			47	56	4278
	48	54	4180	5353	13	72	
5224	18	70	4182		27	68	4282
5233	7	72	4184	5354	5	73	4284
5234	47	55	4186	5364	42	60	4286
5237	14	71	4188	5365	6	73	
5242	39	61	4190		18	71	
5245	22	69			39	62	
	42	59	4194		46	57	4294
5248	8	72	4196	5378	7	73	4296
5249	25	68		5380	14	72	
	32	65	4200		32	66	4300
5252	34	64		5381	34	65	4302
	46	56	4204	5384	22	70	4304
5256	30	66	4206	5386	25	69	4306
5261	19	70	4208	5389	30	67	
5265	9	72			45	58	4310
	36	63	4212	5392	36	64	4312
5266	15	71	4214	5393	8	73	4314
5273	28	67	4216	5402	19	71	
5274	45	57	4218		41	61	4318
5281	41	60	4220	5408	28	68	
5284	10	72	4222		52	52	4321
5288	38	62	4224	5409	15	72	4323
5290	23	69	4226	5410	9	73	
5297	16	71	4228		51	53	4327
5300	20	70		5413	38	63	4329
	26	68		5416	50	54	4331
	44	58	4234	5417	44	59	4333
5305	11	72		5426	49	55	4335
	51	52	4238	5429	10	73	
5309	50	53	4240		23	70	4339
5314	33	65	4242	5437	26	69	4341
5317	31	66		5440	16	72	
	49	54	4246		48	56	4345
5321	35	64		5441	20	71	4347

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
5444	40	62	4349		43	61	4449
5445	33	66	4351	5573	47	58	4451
5449	43	60	4353	5576	10	74	
5450	11	73			26	70	4455
	31	67		5578	33	67	4457
	35	65	4359	5581	35	66	4459
5458	47	57	4361	5584	20	72	4461
5465	29	68		5585	16	73	
	37	64	4365		31	68	4465
5473	12	73		5594	37	65	4467
	17	72	4369	5597	11	74	
5476	0	74			46	59	4471
	24	70	4373	5602	29	69	4473
5477	1	74	4375	5608	42	62	4475
5480	2	74		5617	24	71	
	46	58	4379		39	64	4479
5482	21	71	4381	5618	17	73	
5485	3	74			53	53	4482
	42	61	4385	5620	12	74	
5490	27	69			52	54	4486
	39	63	4389	5625	0	75	
5492	4	74	4391		21	72	
5498	13	73	4393		45	60	4492
5501	5	74	4395	5626	1	75	
5506	45	59	4397		51	55	4496
5508	18	72	4399	5629	2	75	
5512	6	74			27	70	4500
	34	66	4403	5634	3	75	4502
5513	32	67		5636	50	56	4504
	52	53	4407	5641	4	75	4506
5517	51	54	4409	5645	13	74	
5521	36	65	4411		34	67	4510
5524	30	68	4413	5648	32	68	4512
5525	7	74		5650	5	75	
	14	73			41	63	
	22	71			49	57	4518
	25	70		5652	36	66	4520
	41	62		5653	18	73	4522
	50	55	4425	5657	44	61	4524
5536	44	60	4427	5661	6	75	
5537	49	56	4429		30	69	4528
5540	8	74		5666	25	71	4530
	38	64	4433	5668	22	72	
5545	19	72			48	58	4534
	28	69	4437	5669	38	65	4536
5553	48	57	4439	5672	14	74	4538
5554	15	73	4441	5674	7	75	4540
5557	9	74	4443	5684	28	70	4542
5569	40	63	4445	5689	8	75	4544
5570	23	71		5690	19	73	

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
	47	59	4548	5818	43	63	4648
5693	43	62	4550	5821	14	75	4650
5696	40	64	4552	5825	7	76	
5701	15	74	4554		28	71	
5706	9	75	4556		40	65	4656
5713	23	72		5832	54	64	4657
	33	68	4560	5834	53	55	4659
5714	35	67	4562	5837	19	74	
5716	46	60	4564		46	61	4663
5717	26	71	4566	5840	8	76	
5722	31	69	4568		52	56	4667
5725	10	75		5849	35	68	4669
	37	66		5850	15	75	
	53	54	4574		33	69	
5729	20	73			51	57	4675
	52	55	4578	5857	9	76	4677
5732	16	74	4580	5858	23	73	
5733	42	63	4582		37	67	4681
5737	51	56	4584	5860	26	72	
5741	29	70	4586		42	64	4685
5746	11	75		5861	31	70	4687
	39	65		5864	50	58	4689
	45	61	4592	5869	45	62	4691
5749	50	57	4594	5876	10	76	
5760	24	72	4596		20	74	4695
5765	17	74		5877	39	66	4697
	49	58	4600	5881	16	75	4699
5769	12	75	4602	5882	29	71	
5770	21	73			49	59	4703
	27	71	4606	5897	11	76	4705
5776	0	76	4608	5904	48	60	4707
5777	1	76		5905	24	73	
	41	64	4612		44	63	4711
5780	2	76		5906	41	65	4713
	34	68		5913	27	72	4715
	44	62	4618	5914	17	75	4717
5785	3	76		5917	21	74	
	32	69			34	69	4721
	36	67		5920	12	76	
	48	59	4626		36	68	4725
5792	4	76	4628	5924	32	70	4727
5794	13	75	4630	5929	0	77	4729
5800	18	74		5930	1	77	
	30	70			47	61	4733
	38	66	4636	5933	2	77	
5801	5	76	4638		38	67	4737
5809	25	72		5938	3	77	4739
	47	60	4642	5941	30	71	
5812	6	76	4644		54	55	4743
5813	22	73	4646	5945	4	77	

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
	13	76			52	58	4846
	43	64		6073	12	77	4848
	53	56	4751	6074	43	65	4850
5949	18	75	4753	6082	51	59	4852
5953	52	57	4755	6084	0	78	
5954	5	77			30	72	4856
	25	73	4759	6085	1	78	
5956	40	66	4761		46	63	4860
5960	22	74		6088	2	78	4862
	46	62	4765	6089	40	67	4864
5965	6	77		6093	3	78	4866
	51	58	4769	6098	13	77	4868
5968	28	72	4771	6100	4	78	
5972	14	76	4773		18	76	
5978	7	77	4775		50	60	4874
5981	50	59	4777	6101	25	74	4876
5986	19	75		6109	5	78	
	35	69	4781		22	75	4880
5989	33	70		6113	28	73	4882
	42	65	4785	6120	6	78	
5993	8	77			42	66	4886
	37	68	4789	6121	45	64	4888
5994	45	63	4791	6122	49	61	4890
6001	15	76		6125	14	77	
	49	60	4795		35	70	4894
6002	31	71	4797	6130	33	71	
6005	23	74			37	69	4898
	26	73	4801	6133	7	78	4900
6010	9	77		6137	19	76	4902
	39	67	4805	6145	31	72	
6025	20	75			39	68	4906
	29	72		6148	8	78	
	48	61	4811		48	62	4910
6029	10	77	4813	6152	26	74	4912
6032	16	76		6154	15	77	
	44	64	4817		23	75	4916
6037	41	66	4819	6161	44	65	
6050	11	77			55	56	4920
	55	55	4822	6165	9	78	
6052	24	74			54	57	4924
	54	56	4826	6170	29	73	
6053	47	62	4828		41	67	4928
6056	34	70	4830	6173	53	58	4930
6057	36	69	4832	6176	20	76	4932
6058	27	73		6178	47	63	4934
	53	57	4836	6184	10	78	4936
6065	17	76		6185	16	77	
	32	71	4840		52	59	4940
6066	21	75	4842	6196	36	70	4942
6068	38	68		6197	34	71	4944

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
6201	24	75		6317	29	74	5045
	51	60	4948	6322	9	79	
6205	11	78			51	61	5049
	27	74		6329	20	77	5051
	38	69		6337	36	71	5053
	43	66	4956	6338	43	67	5055
6208	32	72	4958	6340	16	78	
6212	46	64	4960		34	72	5059
6217	21	76	4962	6341	10	79	
6218	17	77	4964		46	65	5063
6221	50	61	4966	6344	38	70	
6224	40	68	4968		50	62	5067
6228	12	78	4970	6352	24	76	5069
6229	30	73	4972	6353	32	73	5071
6241	0	79	4974	6354	27	75	5073
6242	1	79	4976	6361	40	69	5075
6245	2	79		6362	11	79	5077
	49	62	4980	6370	21	77	
6250	3	79			49	63	5081
	25	75		6373	17	78	5083
	45	65	4986	6376	30	74	5085
6253	13	78		6381	45	66	5087
	18	77		6385	12	79	
	42	67	4992		56	57	5091
6257	4	79	4994	6388	42	68	5093
6260	22	76		6389	55	58	5095
	28	74	4998	6397	54	59	5097
6266	5	79		6400	0	80	
	35	71	5002		48	64	5101
6269	37	70	5004	6401	1	80	
6272	56	56	5005		25	76	5105
6273	33	72		6404	2	80	5107
	48	63	5009	6408	18	78	5109
6274	55	57	5011	6409	3	80	
6277	6	79	5013		28	75	
6280	14	78			35	72	
	54	58	5017		53	60	5117
6282	39	69	5019	6410	13	79	
6290	7	79			37	71	5121
	19	77		6413	22	77	5123
	31	73		6416	4	80	5125
	53	59	5027	6418	33	73	5127
6292	44	66	5029	6421	39	70	5129
6301	26	75	5031	6425	5	80	
6304	52	60	5033		44	67	
6305	8	79			52	61	5135
	23	76		6434	47	65	5137
	41	68		6436	6	80	5139
	47	64	5041	6437	14	79	
6309	15	78	5043		31	74	5143

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
6442	41	69	5145	6568	22	78	5244
6445	19	78		6569	13	80	5246
	51	62	5149	6570	3	81	
6449	7	80	5151		51	63	5250
6452	26	76	5153	6577	4	81	5252
6458	23	77	5155	6581	41	70	5254
6464	8	80	5157	6586	5	81	
6466	15	79			31	75	5258
	29	75	5161	6596	14	80	
6469	50	63	5163		50	64	5262
6472	46	66	5165	6597	6	81	5264
6473	43	68	5167	6602	19	79	5266
6480	36	72	5169	6605	26	77	
6481	9	80	5171		46	67	5270
6484	20	78	5173	6610	7	81	
6485	34	73			43	69	5274
	38	71	5177	6613	23	78	
6497	16	79			57	58	5278
	49	64	5181	6617	29	76	
6498	57	57	5182		56	59	5282
6500	10	80		6625	8	81	
	32	74			15	80	
	40	70			36	73	
	56	58	5190		55	60	5290
6505	24	77		6626	49	65	5292
	27	76	5194	6628	38	72	5294
6506	55	59	5196	6632	34	74	5296
6514	45	67	5198	6637	54	61	5298
6516	54	60	5200	6641	20	79	
6521	11	80	5202		40	71	5302
6525	21	78		6642	9	81	5304
	30	75		6649	32	75	
	42	69	5208		45	68	5308
6529	48	65	5210	6653	53	62	5310
6530	17	79		6656	16	80	5312
	53	61	5214	6658	27	77	5314
6544	12	80	5216	6660	24	78	
6548	52	62	5218		48	66	5318
6553	37	72	5220	6661	10	81	5320
6554	25	77		6664	42	70	5322
	35	73	5224	6673	52	63	5324
6560	28	76		6676	30	76	5326
	44	68	5228	6682	11	81	
6561	0	81	5230		21	79	5330
6562	1	81		6689	17	80	5332
	39	71	5234	6697	44	69	
6565	2	81			51	64	5336
	18	79		6698	37	73	
	33	74			47	67	5340
	47	66	5242	6701	35	74	5342

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
6705	12	81		6826	51	65	5443
	39	72	5346	6829	30	77	5445
6709	25	78	5348	6833	47	68	5447
6713	28	77	5350	6836	44	70	5449
6714	33	75	5352	6841	21	80	5451
6722	41	71	5354	6845	11	82	
6724	0	82			37	74	
	18	80	5358		58	59	5457
6725	1	82		6849	57	60	5459
	22	79		6850	17	81	
	50	65	5364		35	75	
6728	2	82			39	73	5465
	58	58	5367	6856	50	66	5467
6730	13	81		6857	56	61	5469
	57	59	5371	6865	33	76	
6733	3	82	5373		41	72	5473
6736	56	60	5375	6866	25	79	5475
6737	31	76	5377	6868	12	82	
6740	4	82			28	78	5479
	46	68	5381	6869	55	62	5481
6746	55	61	5383	6877	46	69	5483
6749	5	82		6884	22	80	5485
	43	70	5387	6885	18	81	
6757	14	81			54	63	5489
	49	66	5391	6889	0	83	5491
6760	6	82		6890	1	83	
	26	78			31	77	
	54	62	5397		43	71	
6761	19	80	5399		49	67	5499
6770	23	79		6893	2	83	
	29	77	5403		13	82	5503
6772	36	74	5405	6898	3	83	5505
6773	7	82		6905	4	83	
	38	73	5409		53	64	5509
6778	53	63	5411	6914	5	83	5511
6781	34	75	5413	6917	26	79	5513
6784	40	72	5415	6920	14	82	
6786	15	81			38	74	5517
	45	69	5419	6921	36	75	5519
6788	8	82	5421	6922	19	81	5521
6793	48	67	5423	6925	6	83	
6800	20	80			29	78	
	32	76			45	70	5527
	52	64	5429	6928	48	68	5529
6805	9	82		6929	23	80	
	42	71	5433		40	73	
6813	27	78	5435		52	65	5535
6817	16	81		6932	34	76	5537
	24	79	5439	6938	7	83	5539
6824	10	82	5441	6948	42	72	5541

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
6949	15	82	5543		40	74	5642
6953	8	83		7081	5	84	
	32	77	5547		59	60	5646
6957	51	66	5549	7082	29	79	5648
6961	20	81	5551	7085	14	83	
6962	59	59	5552		19	82	
6964	58	60	5554		34	77	
6970	9	83			58	61	5656
	27	79		7090	23	81	
	47	69			51	67	5660
	57	61	5562	7092	6	84	5662
6976	24	80	5564	7093	42	73	
6977	44	71	5566		57	62	5666
6980	16	82		7105	7	84	
	56	62	5570		56	63	5670
6984	30	78	5572	7108	32	78	5672
6989	10	83		7109	47	70	5674
	50	67	5576	7114	15	83	5676
6994	37	75		7120	8	84	
	55	63	5580		44	72	5680
6997	39	74	5582	7121	55	64	5682
7001	35	76	5584	7124	20	82	
7002	21	81	5586		50	68	5686
7010	11	83		7129	27	80	5688
	41	73	5590	7137	9	84	
7012	54	64	5592		24	81	5692
7013	17	82	5594	7141	30	79	
7016	46	70	5596		54	65	5696
7018	33	77	5598	7145	16	83	
7025	25	80			37	76	5700
	28	79		7146	39	75	5702
	49	68	5604	7154	35	77	5704
7033	12	83		7156	10	84	5706
	43	72	5608	7157	41	74	
7034	53	65	5610		46	71	5710
7045	22	81		7162	49	69	5712
	31	78	5614	7165	21	82	
7048	18	82	5616		53	66	5716
7056	0	84	5618	7173	33	78	5718
7057	1	84	5620	7177	11	84	5720
7058	13	83		7178	17	83	
7060	2	84			43	73	5724
	52	66	5626	7184	28	80	5726
7065	3	84		7186	25	81	5728
	48	69	5630	7193	52	67	5730
7066	45	71	5632	7200	12	84	
7069	38	75	5634		60	60	5733
7072	4	84		7202	31	79	
	36	76	5638		59	61	5737
7076	26	80		7204	48	70	5739

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
					59	62	5841
7208	22	82			52	68	5843
	58	62	5743	7328	21	83	
7209	45	72	5745	7330	33	79	5847
7213	18	83	5747		58	63	5849
7218	57	63	5749	7333			
					17	84	
7220	38	76	5751	7345	28	81	
7225	0	85			48	71	
	13	84			57	64	5857
	36	77			11	85	5859
	40	75		7346			
					25	82	5861
	51	68	5761	7349	45	73	5863
7226	1	85	5763	7354	31	80	
7229	2	85	5765	7361	56	65	5867
7232	56	64	5767		51	69	5869
7234	3	85	5769	7362			
					12	85	5871
7237	26	81	5771	7369	22	83	
7240	34	78		7373	38	77	5875
	42	74	5775		40	76	5877
7241	4	85		7376	18	84	
	29	80	5779	7380			
					36	78	5881
7250	5	85			55	66	5883
	19	83		7381	42	75	5885
	47	71		7389	47	72	5887
	55	65	5787	7393	13	85	5889
7252	14	84	5789	7394			
					0	86	5891
7253	23	82	5791	7396	1	86	
7261	6	85		7397	34	79	5895
	50	69	5795		2	86	
7265	32	79		7400	26	82	
	44	73	5799				
					50	70	5901
7272	54	66	5801		29	81	5903
7274	7	85	5803	7402	3	86	
7281	15	84	5805	7405	54	67	5907
7289	8	85			4	86	
	20	83	5809	7412			
					44	74	5911
7290	27	81	5811		19	84	5913
7297	39	76	5813	7417	23	83	5915
7298	37	77		7418	5	86	
	53	67	5817	7421	14	85	5919
7300	24	82					
					32	80	5921
	30	80		7424	6	86	5923
	46	72	5823	7432	53	68	5925
7301	49	70	5825	7433	49	71	
7306	9	85		7442	61	61	5928
	41	75	5829				
					60	62	5930
7309	35	78	5831	7444	7	86	
7312	16	84	5833	7445	46	73	5934
7321	60	61	5835		15	85	
7325	10	85		7450	39	77	
	43	74					

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
	59	63	5940	7578	3	87	6040
7453	27	82		7585	4	87	
	37	78	5944		23	84	
7456	20	84	5946		32	81	
7457	41	76	5948		49	72	6048
7460	8	86		7586	19	85	6050
	58	64	5952	7589	58	65	6052
7461	30	81	5954	7592	14	86	
7465	24	83			46	74	6056
	52	69	5958	7594	5	87	6058
7466	35	79	5960	7604	52	70	6060
7474	43	75		7605	6	87	
	57	65	5964		39	78	
7477	9	86	5966		57	66	6066
7481	16	85	5968	7610	37	79	
7488	48	72	5970		41	77	6070
7489	33	80	5972	7618	7	87	
7492	56	66	5974		27	83	6074
7496	10	86	5976	7621	15	86	6076
7497	21	84	5978	7624	30	82	6078
7501	45	74		7625	20	85	
	51	70	5982		35	80	
7508	28	82	5984		43	76	
7514	17	85			56	67	6086
	25	83		7632	24	84	6088
	55	67	5990	7633	8	87	
7517	11	86	5992		48	73	6092
7522	31	81	5994	7642	51	71	6094
7528	38	78	5996	7649	55	68	6096
7529	40	77	5998	7650	9	87	
7537	36	79	6000		33	81	
7538	47	73	6002		45	75	6102
7540	12	86		7652	16	86	6104
	22	84		7666	21	85	6106
	42	76		7669	10	87	6108
	54	68	6010	7673	28	83	6110
7541	50	71	6012	7677	54	69	6112
7549	18	85	6014	7681	25	84	6114
7556	34	80	6016	7684	40	78	
7561	44	75	6018		50	72	6118
7565	13	86		7685	17	86	
	26	83			31	82	
	29	82			38	79	
	61	62	6026		47	74	6126
7569	0	87		7688	62	62	6127
	60	63	6030	7690	11	87	
7570	1	87			61	63	6131
	53	69	6034	7693	42	77	6133
7573	2	87	6036	7696	36	80	
7577	59	64	6038		60	64	6137

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
7706	59	65	6139	7829	50	73	6239
7709	22	85		7834	47	75	6241
	53	70	6143	7837	21	86	
7712	44	76	6145		59	66	6245
7713	12	87	6147	7840	28	84	6247
7717	34	81	6149	7841	40	79	6249
7720	18	86		7844	10	88	
	58	66	6153		38	80	6253
7730	29	83		7848	42	78	6255
	49	73	6157	7850	25	85	
7732	26	84	6159		31	83	
7738	13	87			53	71	6261
	57	67	6163	7853	58	67	6263
7741	46	75	6165	7857	36	81	6265
7744	0	88	6167	7858	17	87	6267
7745	1	88		7865	11	88	
	52	71	6171		44	77	6271
7748	2	88		7873	57	68	6273
	32	82	6175	7877	49	74	6275
7753	3	88	6177	7880	22	86	
7754	23	85	6179		34	82	6279
7757	19	86	6181	7888	12	88	
7760	4	88			52	72	6283
	56	68	6185	7892	46	76	6285
7762	39	79	6187	7893	18	87	6287
7765	14	87		7897	29	84	
	41	78	6191		56	69	6291
7769	5	88		7901	26	86	6293
	37	80	6195	7913	13	88	
7778	43	77	6197		32	83	6297
7780	6	88		7921	0	89	
	48	74	6201		39	80	6301
7785	27	84		7922	1	89	
	51	72	6205		41	79	6305
7786	35	81		7925	2	89	
	55	69	6209		23	86	
7789	30	83	6211		55	70	6311
7793	7	88	6213	7929	48	75	6313
7794	15	87	6215	7930	3	89	
7796	20	86	6217		19	87	
7801	24	85			37	81	
	45	76	6221		51	73	6321
7808	8	88	6223	7933	43	78	6323
7813	33	82		7937	4	89	6325
	62	63	6227	7938	63	63	6326
7816	54	70	6229	7940	14	88	
7817	61	64	6231		62	64	6330
7825	9	88		7946	5	89	
	16	87			61	65	6334
	60	65	6237	7949	35	82	6336

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
7954	27	85		8082	39	81	6438
	45	77	6340	8089	60	67	6440
7956	30	84		8090	13	89	
	60	66	6344		43	79	6444
7957	6	89		8093	37	82	6446
	54	71	6348	8098	23	87	6448
7969	15	88		8100	0	90	
	20	87	6352		54	72	6452
7970	7	89		8101	1	90	6454
	59	67	6356	8104	2	90	6456
7972	24	86	6358	8105	19	88	
7976	50	74	6360		59	68	6460
7978	33	83	6362	8109	3	90	
7985	8	89			45	78	6464
	47	76	6366	8114	35	83	6466
7988	58	68	6368	8116	4	90	6468
7993	53	72	6370	8117	14	89	6470
8000	16	88		8125	5	90	
	40	80	6374		27	86	
8002	9	89	6376		30	85	
8005	38	81			50	75	
	42	79	6380		58	69	6480
8009	28	85	6382	8136	6	90	6482
8010	21	87		8138	47	77	
	57	69	6386		53	73	6486
8017	31	84	6388	8144	20	88	6488
8020	36	82		8145	24	87	
	44	78	6392		33	84	6492
8021	10	89		8146	15	89	6494
	25	86	6396	8149	7	90	
8026	49	75	6398		57	70	6498
8033	17	88		8161	40	81	6500
	52	73	6402	8164	8	90	
8036	56	70	6404		42	80	6504
8042	11	89	6406	8168	38	82	6506
8045	34	83		8177	16	89	
	46	77	6410		44	79	
8053	22	87	6412		49	76	
8065	12	89			56	71	6514
	63	64	6416	8180	28	86	
8066	29	85			52	74	6518
	55	71	6420	8181	9	90	6520
8068	18	88	6422	8185	21	88	
8069	62	65	6424		36	83	6524
8072	26	86	6426	8186	31	85	6526
8077	51	74		8192	64	64	6527
	61	66	6430	8194	25	87	
8080	32	84			63	65	6531
	48	76	6434	8200	10	90	
8081	41	80	6436		46	78	

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
	62	66	6537	8329	52	75	6637
8209	55	72	6539	8330	7	91	
8210	17	89			49	77	6641
	61	67	6543	8333	38	83	
8212	34	84	6545		62	67	6645
8221	11	90	6547	8336	44	80	6647
8224	60	68	6549	8345	8	91	
8226	51	75	6551		61	68	6651
8228	22	88	6553	8352	36	84	6653
8233	48	77	6555	8353	28	87	6655
8237	29	86	6557	8354	55	73	6657
8242	41	81		8356	16	90	6659
	59	69	6561	8357	31	86	
8244	12	90	6563		46	79	6663
8245	18	89		8361	60	69	6665
	26	87		8362	9	91	
	39	82			21	89	6669
	54	73	6571	8369	25	88	6671
8249	32	85		8377	51	76	6673
	43	80	6575	8381	10	91	
8258	37	83	6577		34	85	
8264	58	70	6579		59	70	6679
8266	45	79	6581	8388	48	78	6681
8269	13	90	6583	8389	17	90	6683
8273	23	88	6585	8392	54	74	6685
8276	50	76	6587	8402	11	91	6687
8281	0	91		8405	22	89	
	35	84	6591		41	82	
8282	1	91			58	71	6693
	19	89	6595	8410	29	87	
8285	2	91			39	83	
	53	74	6599		43	81	6699
8290	3	91		8420	26	88	
	57	71	6603		32	86	6703
8293	47	78	6605	8424	18	90	6705
8296	14	90		8425	12	91	
	30	86	6609		37	84	
8297	4	91	6611		45	80	6711
8298	27	87	6613	8429	50	77	6713
8306	5	91	6615	8433	57	72	6715
8314	33	85	6617	8434	53	75	6717
8317	6	91	6619	8450	13	91	
8320	24	88			23	89	
	56	72	6623		35	85	
8321	20	89			47	79	
	64	65	6627		65	65	6726
8324	40	82	6629	8452	64	66	6728
8325	15	90		8458	63	67	6730
	42	81		8461	19	90	6732
	63	66	6635	8464	0	92	6734

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
8465	1	92			29	88	
	56	73	6738		53	76	
8468	2	92			64	67	6840
	62	68	6742	8586	45	81	6842
8469	30	87	6744	8593	32	87	
8473	3	92			63	68	6846
	27	88	6748	8594	37	85	6848
8477	14	91	6750	8597	26	89	6850
8480	4	92		8605	18	91	
	52	76	6754		62	69	6854
8482	61	69	6756	8608	12	92	6856
8485	33	86		8609	47	80	6858
	49	78	6760	8612	56	74	6860
8488	42	82	6762	8621	35	86	
8489	5	92			61	70	6864
	40	83	6766	8629	23	90	6866
8497	24	89		8633	13	92	
	44	81	6770		52	77	6870
8500	6	92		8641	60	71	6872
	20	90		8642	19	91	
	38	84			49	79	6876
	60	70	6778	8644	30	88	6878
8501	55	74	6780	8649	0	93	6880
8506	15	91	6782	8650	1	93	
8513	7	92	6784		27	89	
8516	46	80	6786		55	75	6886
8521	36	85	6788	8653	2	93	
8522	59	71	6790		42	83	6890
8528	8	92		8656	40	84	6892
	28	88	6794	8658	3	93	
8530	31	87			33	87	6896
	51	77	6798	8660	14	92	
8537	16	91	6800		44	82	6900
8541	21	90		8665	4	93	
	54	75	6804		59	72	6904
8545	9	92		8669	38	85	6906
	48	79	6808	8674	5	93	6908
8546	25	89	6810	8676	24	90	6910
8548	58	72	6812	8677	46	81	6912
8552	34	86	6814	8681	20	91	6914
8564	10	92	6816	8685	6	93	
8570	17	91			51	78	6918
	41	83	6820	8689	15	92	6920
8573	43	82	6822	8692	36	86	
8577	39	84	6824		54	76	6924
8578	57	73	6826	8693	58	73	6926
8581	65	66	6828	8698	7	93	6928
8584	22	90		8704	48	80	6930
	50	78	6832	8705	28	89	
8585	11	92			31	88	6934

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
8712	66	66	6935		46	82	
8713	8	93	6937		58	74	7037
8714	65	67	6939	8842	51	79	7039
8720	16	92		8845	3	94	
	64	68	6943		14	93	
8722	21	91	6945		54	77	
8725	25	90			66	67	7047
	34	87		8849	65	68	7049
	57	74	6951	8852	4	94	7051
8730	9	93		8857	24	91	
	63	69	6955		64	69	7055
8737	41	84	6957	8861	5	94	7057
8738	43	83		8864	20	92	7059
	53	77	6961	8865	36	87	
8741	50	79	6963		48	81	7063
8744	62	70	6965	8869	63	70	7065
8746	39	85	6967	8872	6	94	7067
8749	10	93		8874	15	93	
	45	82	6971		57	75	7071
8753	17	92	6973	8882	31	89	7073
8761	56	75	6975	8884	28	90	7075
8762	29	89		8885	7	94	
	61	71	6979		62	71	7079
8765	22	91		8893	53	78	7081
	37	86	6983	8900	8	94	
8768	32	88	6985		34	88	
8770	11	93			50	80	7087
	47	81	6989	8905	16	93	
8776	26	90	6991		21	92	
8784	60	72	6993		43	84	
8788	18	92			61	72	7095
	52	78	6997	8906	25	91	
8793	12	93	6999		41	85	7099
8794	35	87	7001	8912	56	76	7101
8801	49	80		8914	45	83	7103
	55	76	7005	8917	9	94	
8810	23	91			39	86	7107
	59	73	7009	8929	60	73	7109
8818	13	93	7011	8933	47	82	7111
8820	42	84	7013	8936	10	94	7113
8821	30	89	7015	8938	17	93	
8825	19	92			37	87	7117
	40	85		8941	29	90	7119
	44	83	7021	8945	32	89	
8829	27	90	7023		52	79	7123
8833	33	88	7025	8948	22	92	7125
8836	0	94	7027	8954	55	77	7127
8837	1	94	7029	8957	11	94	
8840	2	94			26	91	
	38	86			59	74	7133

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
8962	49	81	7135	9089	8	95	
8969	35	88	7137		25	92	7236
8973	18	93	7139	9090	21	93	
8978	67	67	7140		39	87	7240
8980	12	94		9092	16	94	7242
	66	68	7144	9098	47	83	7244
8986	65	69	7146	9104	52	80	7246
8989	42	85		9106	9	95	
	58	75	7150		59	75	7250
8992	44	84	7152	9109	55	78	7252
8993	23	92	7154	9113	37	88	
8996	40	86			67	68	7256
	64	70	7158	9117	66	69	7258
9000	30	90		9122	29	91	7260
	54	78	7162	9124	32	90	7262
9001	51	80	7164	9125	10	95	
9005	13	94			17	94	
	46	83	7168		49	82	
9010	19	93			65	70	7270
	27	91		9133	22	93	7272
	33	89		9137	64	71	7274
	63	71	7176	9140	26	92	
9013	38	87	7178		58	76	7278
9025	0	95		9146	11	95	
	57	76	7182		35	89	7282
9026	1	95	7184	9153	63	72	7284
9028	48	82		9157	54	79	7286
	62	72	7188	9160	18	94	
9029	2	95	7190		42	86	7290
9032	14	94	7192	9161	44	85	7292
9034	3	95	7194	9162	51	81	7294
9040	24	92		9169	12	95	
	36	88	7198		40	87	7298
9041	4	95	7200	9172	46	84	7300
9049	20	93	7202	9173	62	73	7302
9050	5	95		9178	23	93	
	53	79			57	77	7306
	61	73	7208	9181	30	91	7308
9061	6	95		9188	38	88	7310
	15	94		9189	33	90	7312
	31	90		9193	27	92	
	50	81	7216		48	83	7316
9065	28	91		9194	13	95	7318
	56	77	7220	9197	19	94	
9074	7	95			61	74	7322
	43	85	7224	9209	53	80	7324
9076	60	74	7226	9216	0	96	7326
9077	34	89		9217	1	96	
	41	86	7230		36	89	7330
9081	45	84	7232	9220	2	96	

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
	56	78	7334	9337	11	96	7433
9221	14	95	7336	9341	46	85	7435
9224	50	82	7338	9344	40	88	7437
9225	3	96		9346	61	75	7439
	24	93		9349	18	95	7441
	60	75	7344	9360	12	96	
9232	4	96	7346		48	84	7445
9236	20	94	7348	9364	30	92	7447
9241	5	96	7350	9365	23	94	
9242	31	91	7352		38	89	7451
9245	43	86	7354	9370	33	91	
9248	28	92			53	81	7455
	68	68	7357	9376	60	76	7457
9250	15	95		9377	56	79	7459
	41	87		9378	27	93	7461
	45	85		9385	13	96	
	67	69	7365		68	69	7465
9252	6	96	7367	9386	19	95	7467
9256	34	90		9389	50	83	
	66	70	7371		67	70	7471
9257	59	76	7373	9396	36	90	7473
9265	7	96		9397	66	71	7475
	39	88		9409	0	97	
	47	84			65	72	7479
	52	81	7381	9410	1	97	
9266	55	79			59	77	7483
	65	71	7385	9412	14	96	
9274	25	93	7387		24	94	7487
9277	21	94	7389	9413	2	97	7489
9280	8	96		9418	3	97	
	64	72	7393		43	87	7493
9281	16	95	7395	9421	45	86	7495
9290	37	89		9425	4	97	
	49	83	7399		20	95	
9293	58	77	7401		31	92	
9297	9	96	7403		41	88	
9298	63	73	7405		55	80	
9305	29	92			64	73	7507
	32	91	7409	9428	52	82	7509
9314	17	95	7411	9433	28	93	7511
9316	10	96		9434	5	97	
	54	80	7415		47	85	7515
9320	22	94		9437	34	91	7517
	62	74	7419	9441	15	96	7519
9325	26	93		9442	39	89	7521
	35	90		9445	6	97	
	51	82	7425		63	74	7525
9332	44	86	7427	9448	58	78	7527
9333	42	87		9457	49	84	7529
	57	78	7431	9458	7	97	7531

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
9461	25	94	7533	9601	24	95	7632
9466	21	95	7535	9602	41	89	7634
9469	37	90		9604	0	98	7636
	62	75	7539	9605	1	98	
9472	16	96	7541		14	97	
9473	8	97	7543		47	86	
9477	*54*	*81*	*7545*		58	79	*7644*
9488	32	92	7547	9608	2	98	7646
9490	9	97		9610	31	93	7648
	29	93		9613	3	98	7650
	51	83		9616	20	96	7652
	57	79	7555	9620	4	98	
9497	61	76	7557		28	94	
9505	17	96			34	92	
	44	87	7561		62	76	7660
9506	35	91	7563	9621	39	90	7662
9508	42	88	7565	9626	49	85	7664
9509	10	97		9629	5	98	7666
	22	95	7569	9634	15	97	7668
9512	26	94		9640	6	98	
	46	86	7573		54	82	7672
9521	40	89	7575	9649	57	80	7674
9522	69	69	7576	9650	25	95	
9524	68	70	7578		37	91	
9529	48	85			61	77	7680
	60	77	7582	9653	7	98	7682
9530	11	97		9657	21	96	
	67	71	7586		51	84	7686
9533	53	82	7588	9661	69	70	7688
9536	56	80	7590	9665	16	97	
9540	18	96			68	71	7692
	66	72	7594	9668	8	98	7694
9544	38	90	7596	9673	32	93	
9549	30	93	7598		67	72	7698
9553	12	97		9677	29	94	7700
	33	92	7602	9680	44	88	7702
9554	23	95		9684	60	78	7704
	65	73	7606	9685	9	98	
9556	50	84	7608		42	89	
9565	27	94			46	87	
	59	78	7612		66	73	7712
9572	64	74	7614	9689	35	92	7714
9577	19	96		9697	56	81	7716
	36	91	7618	9698	17	97	
9578	13	97	7620		53	83	7720
9586	55	81	7622	9700	22	96	
9593	43	88			40	90	
	52	83	7626		48	86	7726
9594	45	87		9701	26	95	
	63	75	7630		65	74	7730

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
9704	10	98	7732	9841	25	96	
9721	64	75	7734		60	79	7833
9722	59	79	7736	9850	7	99	
9725	11	98			21	97	
	38	91			65	75	7839
	50	85	7742	9857	44	89	7841
9723	18	97	7744	9860	16	98	
9736	30	94	7746		32	94	
9738	33	93	7748		46	88	
9745	23	96			56	82	7849
	63	76	7752	9864	42	90	7851
9748	12	98	7754	9865	8	99	
9749	55	82	7756		53	84	7855
9754	27	95	7758	9866	29	95	7857
9760	36	92		9872	64	76	7859
	52	84	7762	9873	48	87	7861
9764	58	80	7764	9874	35	93	7863
9769	45	88	7766	9881	40	91	
9770	19	97			59	80	7867
	43	89	7770	9882	9	99	7869
9773	13	98		9892	26	96	7871
	62	77	7774	9893	17	98	
9778	47	87	7776		22	97	7875
9781	41	90	7778	9896	50	86	7877
9792	24	96	7780	9898	63	77	7879
9797	31	94		9901	10	99	7881
	49	86	7784	9908	38	92	7883
9800	14	98		9914	55	83	7885
	70	70	7787	9922	11	99	7887
9801	0	99	7789	9925	30	95	
9802	1	99			33	94	
	39	91			58	81	7893
	69	71	7795	9928	18	98	
9805	2	99			62	78	7897
	34	93		9929	52	85	7899
	54	83		9938	23	97	7901
	61	78	7803	9941	70	71	7903
9808	68	72	7805	9945	12	99	
9809	20	97			27	96	
	28	95	7809		36	93	
9810	3	99			69	72	7911
	57	81	7813	9946	45	89	7913
9817	4	99	7815	9949	43	90	7915
9818	67	73	7817	9953	47	88	
9826	5	99			68	73	7919
	51	85	7821	9962	41	91	
9829	15	98	7823		61	79	7923
9832	66	74	7825	9965	19	98	
9833	37	92	7827		67	74	7927
9837	6	99	7829	9970	13	99	

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
	49	87	7931	10090	17	99	
9972	54	84	7933		69	73	8032
9973	57	82	7935	10093	38	93	8034
9981	66	75	7937	10100	10	100	
9985	24	97			52	86	
	39	92	7941		68	74	8040
9986	31	95	7943	10114	33	95	
9992	34	94	7945		67	75	8044
9997	14	99		10116	30	96	8046
	51	86	7949	10121	11	100	
10000	0	100			61	80	8050
	28	96		10125	18	99	
	60	80	7955		45	90	8054
10001	1	100		10130	43	91	
	65	76	7959		47	89	8058
10004	2	100		10132	36	94	
	20	98	7963		66	76	8062
10009	3	100	7965	10133	23	98	8064
10016	4	100	7967	10138	27	97	
10018	37	93	7969		57	83	8068
10025	5	100		10141	54	85	8070
	56	83		10144	12	100	8072
	64	77	7975	10145	41	92	
10026	15	99	7977		49	88	8076
10034	25	97		10154	65	77	8078
	53	85	7981	10161	60	81	8080
10036	6	100		10162	19	99	8082
	44	90	7985	10169	13	100	8084
10037	46	89	7987	10170	39	93	
10042	59	81	7989		51	87	8088
10045	21	98		10177	31	96	8090
	42	91	7993	10180	24	98	
10048	48	88	7995		64	78	8094
10049	7	100		10181	34	95	8096
	32	95	7999	10192	56	84	8098
10053	63	78	8001	10193	28	97	8100
10057	16	99		10196	14	100	8102
	29	96	8005	10201	0	101	
10061	35	94	8007		20	99	8106
10064	8	100		10202	1	101	8108
	40	92	8011	10205	2	101	
10069	50	87	8013		37	94	
10081	9	100			53	86	
	55	84	8017		59	82	8116
10082	71	71	8018	10210	3	101	
10084	70	72	8020		63	79	8120
10085	26	97		10216	46	90	8122
	62	79	8024	10217	4	101	
10088	22	98			44	91	8126
	58	82	8028	10225	15	100	

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
	48	89		10357	39	94	8230
	71	72	8132	10361	19	100	
10226	5	101	8134		56	85	8234
10228	42	92	8136	10368	72	72	8235
10229	25	98		10369	63	80	8237
	70	73	8140	10370	13	101	
10237	6	101			31	97	
	69	74	8144		59	83	
10240	32	96	8146		71	73	8245
10242	21	99	8148	10372	34	96	8247
10244	50	88		10376	70	74	8249
	62	80	8152	10377	24	99	8251
10249	40	93		10378	53	87	8253
	68	75	8156	10386	69	75	8255
10250	7	101		10388	28	98	8257
	29	97		10394	37	95	8259
	35	95		10397	14	101	
	55	85	8164		46	91	8263
10253	58	83	8166	10400	20	100	
10256	16	100	8168		44	92	
10265	8	101			68	76	8269
	67	76	8172	10404	0	102	
10273	52	87	8174		48	90	8273
10280	26	98		10405	1	102	
	38	94	8178		62	81	8277
10282	9	101		10408	2	102	8279
	61	81	8182	10413	3	102	
10285	22	99			42	93	8283
	66	77	8186	10418	67	77	8285
10289	17	100	8188	10420	4	102	
10301	10	101	8190		58	84	8289
10305	33	96		10421	50	89	
	57	84	8194		55	86	8293
10306	45	91	8196	10426	15	101	
10309	30	97			25	99	8297
	47	90		10429	5	102	8299
	65	78	8202	10433	32	97	8301
10312	54	86	8204	10436	40	94	8303
10313	43	92	8206	10440	6	102	
10321	36	95	8208		66	78	8307
10322	11	101		10441	21	100	
	49	89	8212		35	96	8311
10324	18	100		10445	29	98	
	60	82	8216		61	82	8315
10330	23	99		10448	52	88	8317
	41	93	8220	10453	7	102	8319
10333	27	98	8222	10457	16	101	8321
10337	64	79	8224	10466	65	79	8323
10345	12	101		10468	8	102	8325
	51	88	8228	10469	38	95	8327

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
10474	57	85	8329		50	90	8429
10477	26	99	8331	10601	20	101	8431
10484	22	100	8333	10609	0	103	8433
10485	9	102		10610	1	103	
	54	87	8337		61	83	8437
10489	45	92		10613	2	103	8439
	60	83	8341	10618	3	103	8441
10490	17	101		10625	4	103	
	47	91	8345		25	100	
10496	64	80	8347		40	95	
10498	33	97			52	89	
	43	93	8351		65	80	8451
10501	49	90	8353	10628	32	98	8453
10504	10	102		10629	15	102	8455
	30	98	8357	10634	5	103	
10512	36	96	8359		35	97	8459
10513	72	73	8361	10642	21	101	
10517	41	94			29	99	8463
	71	74	8365	10645	6	103	
10522	51	89	8367		57	86	8467
10525	11	102		10656	60	84	8469
	18	101		10657	64	81	8471
	70	75	8373	10658	7	103	
10529	23	100	8375		73	73	8474
10530	27	99		10660	16	102	
	63	81	8379		38	96	
10532	56	86	8381		54	88	
10537	59	84			72	74	8482
	69	76	8385	10666	71	75	8484
10546	39	95	8387	10673	8	103	
10548	12	102	8389		47	92	8488
10553	53	88		10674	45	93	8490
	68	77	8393	10676	26	100	
10562	19	101	8395		70	76	8494
10565	31	98		10682	49	91	8496
	34	97	8399	10685	22	101	
10568	62	82	8401		43	94	8500
10573	13	102		10690	9	103	
	67	78	8405		69	77	8504
10576	24	100	8407	10693	17	102	
10580	46	92	8409		33	98	
10585	28	99			63	82	8510
	37	96		10701	30	99	
	44	93			51	90	8514
	48	91	8417	10705	36	97	
10589	58	85	8419		56	87	8518
10594	55	87	8421	10706	41	95	
10597	66	79	8423		59	85	8522
10600	14	102		10708	68	78	8524
	42	94		10709	10	103	8526

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
10728	18	102	8528		69	78	8628
10729	27	100	8530	10852	6	104	8630
10730	11	103		10853	38	97	8632
	23	101		10858	47	93	
	53	89			63	83	8636
	67	79	8538	10861	45	94	8638
10733	62	83	8540	10865	7	104	
10737	39	96	8542		16	103	
10753	12	103	8544		49	92	
10756	66	80	8546		68	79	8646
10760	34	98		10874	43	95	8648
	58	86	8550	10877	26	101	
10762	31	99	8552		59	86	8652
10765	19	102		10880	8	104	
	46	93	8556		56	88	8656
10768	48	92	8558	10882	51	91	8658
10769	55	88	8560	10888	22	102	8660
10772	44	94	8562	10889	67	80	8662
10777	24	101		10890	33	99	8664
	61	84	8566	10897	9	104	
10778	13	103			41	96	8668
	37	97	8570	10898	17	103	8670
10781	50	91	8572	10900	30	100	
10784	28	100	8574		36	98	
10786	65	81	8576		62	84	8676
10789	42	95	8578	10909	53	90	8678
10804	20	102		10916	10	104	8680
	52	90	8582	10917	66	81	8682
10805	14	103		10930	27	101	
	73	74	8586		39	97	8686
10809	72	75	8588	10933	18	103	
10816	0	104			23	102	
	40	96	8592		58	87	8692
10817	1	104		10937	11	104	8694
	71	76	8596	10946	55	89	
10818	57	87	8598		61	85	8698
10820	2	104		10949	65	82	8700
	64	82	8602	10952	46	94	
10825	3	104			74	74	8703
	32	99		10953	48	93	8705
	60	85	8608	10954	73	75	8707
10826	25	101	8610	10957	34	99	8709
10829	35	98		10960	12	104	
	70	77	8614		72	76	8713
10832	4	104	8616	10961	31	100	
10834	15	103	8618		44	95	8717
10837	54	89	8620	10964	50	92	8719
10841	5	104		10970	19	103	
	29	100	8624		71	77	8723
10845	21	102		10973	37	98	8725

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
					74	75	8827
10980	24	102			17	104	
	42	96	8729	11105			
10984	70	78	8731		73	76	8831
10985	13	104		11106	9	105	8833
	28	101		11108	58	88	8835
	52	91		11113	72	77	8837
	64	83	8739	11114	65	83	8839
10993	57	88	8741	11117	61	86	8841
10996	60	86	8743	11125	10	105	
11002	69	79	8745		39	98	
					55	90	
11009	20	103			71	78	8849
	40	97	8749		27	102	8851
11012	14	104	8751	11133	23	103	8853
11016	54	90	8753	11138	18	104	
11024	32	100		11140			
	68	80	8757		48	94	8857
11025	0	105		11141	46	95	
	63	84	8761		70	79	8861
11026	1	105		11146	11	105	8863
	35	99	8765	11149	50	93	8865
11029	2	105		11152	44	96	
	25	102	8769		64	84	8869
11034	3	105	8771	11156	34	100	8871
11041	4	105		11161	69	80	8873
	15	104	8775	11162	31	101	8875
11042	29	101	8777	11168	52	92	8877
11045	47	94	8779	11169	12	105	
11048	38	98	8781		60	87	8881
11050	5	105		11170	37	99	
	21	103			57	89	8885
	45	95		11173	42	97	8887
	49	93		11177	19	104	8889
	59	87		11185	24	103	
	67	81	8793		68	81	8893
11057	56	89	8795	11188	28	102	8895
11061	6	105	8797	11194	13	105	
11065	43	96			63	85	8899
	51	92	8801	11197	54	91	8901
11069	62	85	8803	11204	40	98	8903
11072	16	104	8805	11213	67	82	8905
11074	7	105	8807	11216	20	104	8907
11080	26	102		11221	14	105	8909
	66	82	8811	11225	32	101	
11089	8	105			35	100	
	33	100	8815		59	88	8915
11090	41	97		11234	25	103	
	53	91	8819		47	95	8919
11093	22	103	8821	11236	0	106	
11097	36	99	8823		56	90	8923
11101	30	101		11237	1	106	

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	49	94	8927		57	90	9026
11240	2	106		11353	52	93	9028
	62	86	8931	11357	11	106	
11241	45	96	8933		34	101	9032
11245	3	106		11365	31	102	
	29	102			63	86	9036
	38	99		11368	42	98	9038
	66	83	8941	11369	37	100	9040
11250	15	105		11378	67	83	9042
	51	93		11380	12	106	
	75	75	8946		54	92	9046
11252	4	106		11386	19	105	9048
	74	76	8950	11392	24	104	9050
11257	21	104	8952	11393	28	103	9052
11258	43	97		11401	40	99	
	73	77	8956		75	76	9056
11261	5	106	8958	11402	59	89	9058
11268	72	78	8960	11405	13	106	
11272	6	106	8962		74	77	9062
11273	53	92	8964	11412	66	84	9064
11281	16	105		11413	62	87	
	65	84	8968		73	78	9068
11282	71	79	8970	11417	56	91	9070
11285	7	106		11425	20	105	
	26	103			47	96	
	41	98			72	79	9076
	58	89	8978	11426	35	101	
11290	33	101			49	95	9080
	61	87	8982	11428	32	102	9082
11296	36	100	8984	11432	14	106	9084
11300	8	106		11434	45	97	9086
	22	104		11437	51	94	9088
	70	80	8990	11441	25	104	
11304	30	102	8992		71	80	9092
11306	55	91	8994	11444	38	100	9094
11314	17	105	8996	11449	0	107	9096
11317	9	106	8998	11450	1	107	
11321	64	85	9000		29	103	
11322	39	99			65	85	9102
	69	81	9004	11453	2	107	
11329	48	95	9006		43	98	9106
11332	46	96	9008	11458	3	107	
11336	10	106			53	93	9110
	50	94	9012	11461	15	106	
11338	27	103	9014		70	81	9114
11344	60	88	9016	11464	58	90	9116
11345	23	104		11465	4	107	
	44	97	9020		61	88	9120
11348	68	82	9022	11466	21	105	9122
11349	18	105		11474	5	107	9124

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11482	41	99	9126	11602	71	81	9225
11485	6	107		11617	49	96	9227
	69	82	9130	11618	13	107	
11489	55	92	9132		47	97	9231
11492	16	106		11621	65	86	9233
	26	104		11624	70	82	9235
	64	86	9138	11626	51	95	9237
11493	33	102	9140	11629	35	102	
11497	36	101	9142		45	98	9241
11498	7	107	9144	11633	32	103	9243
11509	22	105		11636	20	106	9245
	30	103	9148	11642	61	89	9247
11513	8	107		11645	14	107	
	68	83	9152		38	101	
11520	48	96	9154		53	94	
11521	39	100			58	91	9255
	60	89	9158	11650	25	105	
11525	17	106			43	99	
	46	97			69	83	9261
	50	95	9164	11657	29	104	9263
11530	9	107		11664	0	108	9265
	57	91	9168	11665	1	108	
11538	63	87	9170		64	87	9269
11540	44	98		11668	2	108	9271
	52	94	9174	11673	3	108	9273
11545	27	104		11674	15	107	
	67	84	9178		55	93	9277
11549	10	107	9180	11677	21	106	9279
11552	76	76	9181	11680	4	108	
11554	23	105			68	84	9283
	75	77	9185	11681	41	100	9285
11560	18	106		11689	5	108	9287
	34	102		11698	33	103	9289
	74	78	9191	11700	6	108	
11565	42	99			36	102	
	54	93	9195		60	90	9295
11570	11	107		11701	26	105	9297
	31	103		11705	16	107	
	37	101			76	77	9301
	73	79	9203	11709	75	78	9303
11581	59	90		11713	7	108	
	66	85	9207		48	97	
11584	72	80	9209		57	92	
11588	62	88	9211		63	88	9311
11593	12	107	9213	11714	67	85	9313
11597	19	106	9215	11716	30	104	
11600	28	104			50	96	9317
	40	100		11717	74	79	9319
	56	92	9221	11720	22	106	
11601	24	105	9223		46	98	9323

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
11722	39	101	9325		55	94	9424
11728	8	108	9327	11866	29	105	
11729	52	95			75	79	9428
	73	80	9331	11876	74	80	9430
11737	44	99	9333	11881	0	109	
11738	17	107	9335		60	91	9434
11745	9	108		11882	1	109	
	72	81	9339		41	101	9438
11752	54	94		11885	2	109	
	66	86	9343		67	86	9442
11754	27	105	9345	11889	15	108	9444
11762	59	91	9347	11890	3	109	
11764	10	108			21	107	
	42	100	9351		63	89	
11765	23	106			73	81	9452
	34	103		11897	4	109	
	62	89		11898	57	93	9456
	71	82	9359	11905	33	104	
11773	18	107			36	103	9460
	37	102	9363	11906	5	109	9462
11777	31	104	9365	11908	48	98	
11785	11	108			72	82	9466
	56	93	9369	11909	50	97	9468
11789	70	83	9371	11912	26	106	9470
11794	65	87	9373	11917	6	109	
11801	40	101	9375		46	99	9474
11808	12	108	9377	11920	16	108	
11809	28	105	9379		52	96	9478
11810	19	107		11925	30	105	
	49	97	9383		39	102	
11812	24	106	9385		66	87	9484
11813	47	98	9387	11930	7	109	
11817	51	96			71	83	9488
	69	84	9391	11933	22	107	9490
11821	61	90	9393	11936	44	100	9492
11826	45	99	9395	11941	54	95	9494
11828	58	92	9397	11944	62	90	9496
11833	13	108	9399	11945	8	109	
11834	35	103			59	92	9500
	53	95	9403	11953	17	108	9502
11840	32	104		11956	70	84	9504
	64	88	9407	11962	9	109	9506
11848	38	102	9409	11965	27	106	
11849	20	107			42	101	9510
	43	100		11969	65	88	9512
	68	85	9415	11972	34	104	
11858	77	77	9416		56	94	9516
11860	14	108		11978	23	107	
	76	78	9420		37	103	9520
11861	25	106		11981	10	109	9522

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11986	31	105		12113	52	97	9624
	69	85	9526	12114	33	105	9626
11988	18	108	9528	12116	4	110	
12002	11	109			46	100	9630
	61	91	9532	12125	5	110	
12004	40	102	9534		26	107	
12005	49	98	9536		62	91	
12010	47	99			70	85	9638
	51	97	9540	12130	39	103	
12013	58	93			59	93	9642
	77	78	9544	12132	54	96	9644
12017	64	89		12136	6	110	
	76	79	9548		30	106	9648
12020	28	106		12137	16	109	
	68	86	9552		44	101	9652
12025	12	109		12146	65	89	9654
	19	108		12148	22	108	9656
	24	107		12149	7	110	9658
	45	100		12157	69	86	9660
	53	96		12161	56	95	9662
	75	80	9564	12164	8	110	9664
12037	74	81	9566	12168	42	102	
12041	35	104	9568		78	78	9667
12049	32	105	9570	12170	17	109	
12050	13	109			77	79	9671
	43	101		12176	76	80	9673
	55	95	9576	12178	27	107	9675
12053	38	103		12181	9	110	
	73	82	9580		34	105	9679
12058	67	87	9582	12185	37	104	
12064	20	108			61	92	9683
	60	92	9586	12186	75	81	9685
12069	63	90	9588	12193	23	108	
12073	72	83	9590		68	87	9689
12074	25	107	9592	12196	64	90	9691
12077	14	109		12197	31	106	9693
	29	106	9596	12200	10	110	
12085	41	102			58	94	
	57	94	9600		74	82	9699
12097	71	84	9602	12202	49	99	9701
12100	0	110		12205	18	109	
	66	88	9606		51	98	9705
12101	1	110	9608	12209	40	103	
12104	2	110			47	100	9709
	50	98	9612	12218	53	97	
12105	21	108			73	83	9713
	48	99	9616	12221	11	110	9715
12106	15	109	9618	12226	45	101	9717
12109	3	110	9620	12233	28	107	
12112	36	104	9622		67	88	9721

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12240	24	108		12357	6	111	9823
	72	84	9725	12365	22	109	
12241	55	96	9727		74	83	9827
12242	19	109	9729	12368	68	88	9829
12244	12	110	9731	12370	7	111	
12249	60	93	9733		61	93	9833
12250	35	105		12373	42	103	9835
	63	91	9737	12377	64	91	9837
12253	43	102	9739	12385	8	111	
12260	32	106			73	84	9841
	38	104	9743	12389	17	110	
12266	71	85	9745		58	95	9845
12269	13	110	9747	12392	34	106	9847
12274	57	95	9749	12393	27	108	9849
12277	66	89	9751	12394	37	105	9851
12281	20	109	9753	12401	49	100	9853
12289	25	108	9755	12402	9	111	
12290	29	107			51	99	9857
	41	103	9759	12409	72	85	9859
12296	14	110		12410	23	109	
	70	86	9763		31	107	
12301	50	99	9765		47	101	
12304	48	100	9767		67	89	9867
12308	52	98		12413	53	98	9869
	62	92	9771	12416	40	104	9871
12317	46	101		12421	10	111	9873
	59	94	9775	12424	18	110	9875
12321	0	111		12429	45	102	9877
	36	105	9779	12433	63	92	9879
12322	1	111		12434	55	97	9881
	21	109	9783	12436	60	94	9883
12325	2	111		12437	71	86	9885
	15	110		12442	11	111	9887
	33	106		12448	28	108	9889
	54	97		12456	66	90	9891
	65	90		12457	24	109	9893
	78	79	9795	12458	43	103	9895
12329	77	80	9797	12461	19	110	
12330	3	111			35	106	9899
	69	87	9801	12465	12	111	
12337	4	111			57	96	9903
	39	104		12469	38	105	
	76	81	9807		70	87	9907
12340	26	108		12473	32	107	9909
	44	102	9811	12482	79	79	9910
12346	5	111	9813	12484	78	80	9912
12349	30	107		12490	13	111	
	75	82	9817		77	81	9916
12352	56	96	9819	12493	62	93	9918
12356	16	110	9821	12497	41	104	9920

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12500	20	110			71	87	10022
	50	100		12613	47	102	10024
	76	82	9926	12618	63	93	10026
12505	29	108		12625	9	112	
	48	101			31	108	
	52	99			40	105	
	69	88	9934		60	95	10034
12506	25	109		12629	23	110	
	59	95			55	98	10038
	65	91	9940	12634	45	103	10040
12514	75	83	9942	12637	66	91	10042
12517	14	111	9944	12641	79	80	10044
12520	46	102		12644	10	112	
	54	98	9948		70	88	10048
12532	36	106		12645	18	111	
	74	84	9952		78	81	10052
12538	33	107	9954	12653	77	82	10054
12541	21	110	9956	12658	57	97	10056
12544	0	112	9958	12665	11	112	
12545	1	112			28	109	
	44	103			43	104	
	56	97			76	83	10064
	68	89	9966	12674	35	107	10066
12546	15	111		12676	24	110	10068
	39	105	9970	12680	38	106	
12548	2	112	9972		62	94	10072
12553	3	112	9974	12681	75	84	10074
12554	73	85	9976	12682	19	111	
12557	26	109			69	89	10078
	61	94	9980	12688	12	112	
12560	4	112			32	108	10082
	64	92	9984	12689	65	92	10084
12564	30	108	9986	12697	59	96	10086
12569	5	112	9988	12701	50	101	
12577	16	111	9990		74	85	10090
12580	6	112		12704	52	100	10092
	42	104		12706	41	105	10094
	58	96		12708	48	102	10096
	72	86	9998	12713	13	112	10098
12584	22	110	10000	12717	54	99	10100
12589	67	90	10002	12721	20	111	10102
12593	7	112	10004	12722	29	109	10104
12601	51	100	10006	12724	68	90	10106
12602	49	101	10008	12725	25	110	
12605	34	107			46	103	
	37	106	10012		73	86	10112
12608	8	112	10014	12740	14	112	
12610	17	111			56	98	10116
	27	109		12745	36	107	
	53	99			64	93	10120

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
12746	61	95	10122	12868	18	112	10221
12752	44	104	10124	12869	10	113	
12753	33	108			62	95	10225
	72	87	10128	12872	74	86	10227
12757	39	106	10130	12874	43	105	
12762	21	111	10132		65	93	10231
12769	0	113		12884	28	110	10233
	15	112	10136	12889	35	108	10235
12770	1	113		12890	11	113	
	67	91	10140		59	97	10239
12773	2	113		12893	38	107	10241
	58	97	10144	12897	24	111	10243
12776	26	110	10146	12898	73	87	10245
12778	3	113	10148	12904	50	102	10247
12781	30	109	10150	12905	19	112	
12785	4	113			32	109	
	71	88	10154		52	101	
12789	42	105	10156		68	91	10255
12794	5	113	10158	12913	12	113	
12800	16	112			48	103	10259
	80	80	10161	12916	54	100	10261
12802	51	101		12917	41	106	10263
	79	81	10165	12928	72	88	10265
12805	6	113		12932	46	104	
	22	111			64	94	10269
	49	102		12937	56	99	
	63	94	10173		61	96	10273
12808	78	82	10175	12938	13	113	10275
12809	53	100	10177	12941	29	110	10277
12816	60	96	10179	12944	20	112	10279
12818	7	113		12946	25	111	10281
	37	107		12953	67	92	10283
	47	103		12960	36	108	10285
	77	83	10187	12961	44	105	
12820	34	108			80	81	10289
	66	92	10191	12962	71	89	10291
12821	70	89	10193	12965	14	113	
12826	55	99	10195		79	82	10295
12829	27	110	10197	12968	58	98	10297
12832	76	84	10199	12970	33	109	
12833	8	113			39	107	10301
	17	112	10203	12973	78	83	10303
12836	40	106	10205	12985	21	112	
12841	45	104	10207		77	84	10307
12842	31	109	10209	12994	15	113	
12850	9	113			63	95	10311
	23	111		12996	0	114	10313
	75	85	10215	12997	1	114	
12853	57	98	10217		26	111	10317
12861	69	90	10219	13000	2	114	

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	30	110		13121	64	95	10421
	42	106		13122	81	81	10422
	70	90	10325	13124	32	110	
13001	76	85	10327		80	82	10426
13005	3	114		13130	19	113	
	51	102			41	107	
	66	93	10333		61	97	
13009	60	97	10335		79	83	10434
13010	49	103		13136	56	100	10436
	53	101	10339	13138	67	93	10438
13012	4	114	10341	13140	12	114	
13021	5	114			78	84	10442
	75	86	10345	13141	46	105	
13025	16	113			71	90	10446
	47	104		13154	77	85	10448
	55	100	10351	13162	29	111	10450
13028	22	112	10353	13165	13	114	
13032	6	114	10355		58	99	10454
13033	37	108	10357	13169	20	113	
13037	34	109	10359		25	112	10458
13042	69	91	10361	13172	44	106	
13045	7	114			76	86	10462
	74	87	10365	13177	36	109	10464
13049	40	107	10367	13181	70	91	10466
13050	27	111		13185	39	108	
	45	105			63	96	10470
	57	99	10373	13189	33	110	10472
13058	17	113	10375	13192	14	114	
13060	8	114			66	94	10476
	62	96	10379	13194	75	87	10478
13061	31	110		13204	60	98	10480
	65	94	10383	13210	21	113	
13073	23	112			51	103	10484
	73	88	10387	13213	42	107	
13077	9	114	10389		53	102	10488
13085	43	106		13217	49	104	10490
	59	98	10393	13220	26	112	
13088	68	92	10395		74	88	10494
13093	18	113	10397	13221	15	114	
13096	10	114	10399		30	111	10498
13105	28	111		13225	0	115	
	72	89	10403		69	92	10502
13106	35	109	10405	13226	1	115	
13108	38	108			55	101	10506
	52	102	10409	13229	2	115	10508
13109	50	103	10411	13234	3	115	
13117	11	114			47	105	10512
	54	101	10415	13241	4	115	10514
13120	24	112		13249	57	100	10516
	48	104	10419	13250	5	115	

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	37	109			75	88	10620
	65	95		13378	63	97	10622
	73	89	10524	13381	66	95	10624
13252	16	114	10526	13385	29	112	
13253	22	113			44	107	10628
	62	97	10530	13394	13	115	
13256	34	110	10532		25	113	10632
13261	6	115		13396	20	114	
	45	106	10536		36	110	10636
13264	40	108	10538	13397	74	89	10638
13273	27	112		13401	60	99	10640
	68	93	10542	13402	39	109	10642
13274	7	115	10544	13410	33	111	
13282	31	111			69	93	10646
	59	99	10548	13417	51	104	10648
13284	72	90	10550	13418	53	103	10650
13285	17	114		13421	14	115	10652
	81	82	10554	13426	49	105	10654
13289	8	115		13428	42	108	10656
	80	83	10558	13429	55	102	
13297	79	84	10560		73	90	10660
13298	23	113		13437	21	114	10662
	43	107	10564	13441	65	96	10664
13306	9	115	10566	13444	30	112	10666
13309	78	85	10568	13445	26	113	
13312	64	96	10570		47	106	10670
13313	52	103	10572	13448	62	98	
13316	50	104	10574		82	82	10673
13320	18	114		13450	15	115	
	54	102	10578		57	101	
13322	71	91	10580		81	83	10679
13325	10	115		13456	0	116	
	35	110			80	84	10683
	38	109		13457	1	116	10685
	61	98		13460	2	116	
	67	94			68	94	10689
	77	86	10592	13465	3	116	
13328	28	112	10594		72	91	10693
13329	48	105	10596	13466	79	85	10695
13337	56	101	10598	13469	37	110	10697
13345	24	113		13472	4	116	10699
	32	111		13474	45	107	10701
	41	108		13477	34	111	10703
	76	87	10606	13480	22	114	
13346	11	115	10608		78	86	10707
13352	46	106	10610	13481	5	116	
13357	19	114	10612		16	115	
13364	58	100			40	109	
	70	92	10616		59	100	10715
13369	12	115		13492	6	116	10717

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13498	27	113			80	85	10819
	77	87	10721	13626	51	105	10821
13505	7	116		13633	33	112	10823
	31	112		13634	55	103	
	64	97			65	97	10827
	71	92	10729	13637	49	106	
13513	43	108	10731		79	86	10831
13514	17	115		13645	42	109	
	67	95	10735		62	99	10835
13520	8	116		13648	72	92	10837
	52	104		13649	68	95	10839
	76	88	10741	13652	14	116	10841
13522	61	99	10743	13653	57	102	
13525	23	114			78	87	10845
	50	105		13658	47	107	10847
	54	103	10749	13666	21	115	10849
13537	9	116	10751	13669	30	113	10851
13540	48	106		13672	26	114	10853
	56	102	10755	13673	77	88	10855
13544	38	110	10757	13681	15	116	10857
13546	35	111		13682	59	101	10859
	75	89	10761	13689	0	117	
13549	18	115			45	108	10863
	70	93	10765	13690	1	117	
13553	28	113	10767		37	111	
13556	10	116	10769		71	93	10869
13562	41	109	10771	13693	2	117	10871
13565	46	107		13697	76	89	10873
	58	101	10775	13698	3	117	10875
13568	32	112	10777	13700	34	112	
13572	24	114			40	110	
	66	96	10781		64	98	10881
13573	63	98	10783	13705	4	117	
13576	74	90	10785		67	96	10885
13577	11	116	10787	13709	22	115	10887
13586	19	115	10789	13712	16	116	10889
13597	69	94	10791	13714	5	117	10891
13600	12	116		13721	61	100	10893
	44	108		13725	6	117	
	60	100	10797		27	114	
13610	29	113			75	90	10899
	73	91	10801	13729	52	105	10901
13613	82	83	10803	13730	31	113	
13617	36	111			43	109	10905
	81	84	10807	13732	54	104	10907
13621	25	114		13736	50	106	
	39	110	10811		70	94	10911
13625	13	116		13738	7	117	10913
	20	115		13745	17	116	
	53	104			56	103	10917

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13753	8	117		13877	71	94	11018
	48	107	10921	13885	14	117	
13754	23	115	10923		59	102	11022
13757	74	91	10925	13896	30	114	11024
13765	38	111		13897	21	116	
	66	97	10929		64	99	11028
13768	58	102	10931	13898	67	97	11030
13769	35	112	10933	13901	26	115	11032
13770	9	117		13906	45	109	
	63	99	10937		75	91	11036
13778	83	83	10938	13913	37	112	11038
13780	18	116		13914	15	117	11040
	28	114		13921	40	111	11042
	46	108		13922	61	101	11044
	82	84	10946	13924	0	118	11046
13781	41	110	10948	13925	1	118	
13786	69	95			34	113	
	81	85	10952		70	95	11052
13789	10	117	10954	13928	2	118	11054
13793	32	113		13933	3	118	11056
	73	92	10958	13940	4	118	
13796	80	86	10960		22	116	
13801	24	115			52	106	
	60	101	10964		74	92	11064
13810	11	117		13941	54	105	11066
	79	87	10968	13945	16	117	
13817	19	116			83	84	11070
	44	109	10972	13949	5	118	
13828	78	88	10974		43	110	
13829	65	98	10976		50	107	
13833	12	117			82	85	11078
	72	93	10980	13952	56	104	11080
13834	53	105	10982	13954	27	115	11082
13837	29	114		13957	31	114	
	51	106	10986		81	86	11086
13840	36	112		13960	6	118	
	68	96	10990		66	98	11090
13841	55	104	10992	13968	48	108	11092
13842	39	111	10994	13969	63	100	
13844	62	100	10996		80	87	11096
13850	25	115		13973	7	118	
	49	107			58	103	11100
	77	89	11002	13977	69	96	11102
13856	20	116	11004	13978	17	117	
13858	13	117			73	93	11106
	33	113		13985	23	116	
	57	103	11010		79	88	11110
13864	42	110	11012	13988	8	118	
13873	47	108	11014		38	112	11114
13876	76	90	11016	13994	35	113	11116

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
13997	46	109	11118	14132	26	116	11217
14002	41	111	11120	14138	37	113	11219
14004	60	102	11122	14144	40	112	
14005	9	118			80	88	11223
	78	89	11126	14149	15	118	11225
14009	28	115	11128	14152	34	114	
14013	18	117	11130		54	106	11229
14020	32	114		14153	52	107	11231
	72	94	11134	14157	66	99	11233
14024	10	118	11136	14161	0	119	
14026	65	99	11138		56	105	11237
14029	77	90	11140	14162	1	119	
14032	24	116	11142		79	89	11241
14033	68	97	11144	14164	50	108	11243
14036	44	110	11146	14165	2	119	
14045	11	118			73	94	11247
	53	106		14170	3	119	
	62	101	11152		43	111	
14050	19	117			63	101	
	51	107			69	97	11255
	55	105	11158	14173	22	117	11257
14057	76	91	11160	14177	4	119	11259
14065	36	113		14180	16	118	
	39	112			58	104	11263
	49	108		14184	78	90	11265
	57	104	11168	14185	27	116	
14066	29	115			48	109	11269
	71	95	11172	14186	5	119	
14068	12	118	11174		31	115	11273
14081	25	116	11176	14197	6	119	11275
14085	33	114		14209	60	103	
	42	111	11180		72	95	11279
14089	20	117		14210	7	119	
	75	92	11184		77	91	11283
14090	47	109		14213	17	118	
	59	103	11188		38	113	11287
14093	13	118		14216	46	110	11289
	67	98	11192	14218	23	117	11291
14096	64	100	11194	14221	35	114	11293
14112	84	84	11195	14225	8	119	
14114	83	85	11197		41	112	
14116	70	96	11199		65	100	11299
14120	14	118		14228	68	98	11301
	82	86	11203	14240	28	116	
14125	30	115			76	92	11305
	45	110		14242	9	119	11307
	61	102		14248	18	118	
	74	93	11211		62	102	11311
14130	21	117		14249	32	115	11313
	81	87	11215	14257	44	111	

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	71	96	11317	14386	15	119	11417
14258	53	107	11319	14389	58	105	11419
14261	10	119		14393	43	112	
	55	106	11323		77	92	11423
14265	24	117		14400	0	120	
	51	108	11327		72	96	11427
14274	57	105		14401	1	120	11429
	75	93	11331	14404	2	120	
14281	84	85	11333		48	110	11433
14282	11	119		14408	22	118	11435
	49	109	11337	14409	3	120	11437
14285	19	118		14416	4	120	
	83	86	11341		60	104	11441
14290	39	113		14417	16	119	
	67	99	11345		31	116	11445
14292	36	114	11347	14418	27	117	11447
14293	82	87	11349	14425	5	120	
14297	29	116			68	99	
	59	104			76	93	11453
	64	101	11355	14426	65	101	11455
14305	12	119		14436	6	120	11457
	81	88	11359	14437	46	111	11459
14308	42	112	11361	14440	38	114	11461
14309	47	110		14449	7	120	11463
	70	97	11365	14450	17	119	
14312	74	94	11367		35	115	
14314	25	117			41	113	
	33	115	11371		71	97	
14321	80	89	11373		85	85	11472
14324	20	118	11375	14452	84	86	11474
14330	13	119		14453	23	118	
	61	103	11379		62	103	11478
14341	79	90	11381	14458	83	87	11480
14346	45	111	11383	14461	75	94	11482
14354	73	95	11385	14464	8	120	11484
14356	30	116		14468	82	88	11486
	66	100	11389	14473	28	117	
14357	14	119	11391		53	108	11490
14365	21	118		14474	55	107	11492
	26	117		14480	32	116	
	37	114			44	112	11496
	54	107		14481	9	120	11498
	69	98		14482	51	109	
	78	91	11403		81	89	11502
14368	52	108	11405	14485	18	119	
14369	40	113	11407		57	106	11506
14372	56	106	11409	14489	67	100	11508
14373	63	102	11411	14500	10	120	
14381	34	115			24	118	
	50	109	11415		64	102	

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	80	90	11516	14642	1	121	11616
14501	49	110		14645	2	121	
	74	95	11520		22	119	
14504	70	98	11522		71	98	
14506	59	105	11524		82	89	11624
14517	39	114	11526	14650	3	121	
14521	11	120			31	117	
	36	115	11530		75	95	11630
14522	19	119		14653	27	118	11632
	79	91	11534	14656	16	120	11634
14530	29	117		14657	4	121	11636
	47	111	11538	14660	46	112	
14533	42	113	11540		62	104	11640
14537	61	104	11542	14661	81	90	11642
14544	12	120	11544	14666	5	121	11644
14545	33	116		14669	38	115	11646
	73	96	11548	14677	6	121	
14548	78	92	11550		41	114	11650
14549	25	118	11552	14681	35	116	
14557	66	101	11554		80	91	11654
14561	20	119	11556	14689	17	120	
14562	69	99	11558		55	108	11658
14569	13	120		14690	7	121	
	45	112	11562		23	119	
14578	63	103			53	109	
	77	93	11566		67	101	11666
14580	54	108	11568	14692	74	96	11668
14585	52	109		14698	57	107	11670
	56	107	11572	14701	51	110	
14589	30	117	11574		70	99	11674
14593	72	97	11576	14705	8	121	
14594	37	115	11578		44	113	
14596	14	120			64	103	
	40	114	11582		79	92	11682
14600	26	118		14708	28	118	11684
	50	110		14713	32	117	11686
	58	106	11588	14717	59	106	11688
14602	21	119	11590	14722	9	121	
14612	34	116			49	111	11692
	76	94	11594	14724	18	120	11694
14618	43	113	11596	14733	78	93	11696
14621	85	86	11598	14737	24	119	11698
14624	68	100	11600	14738	73	97	11700
14625	15	120		14741	10	121	11702
	48	111		14746	39	115	
	60	105			61	105	11706
	84	87	11608	14752	36	116	11708
14629	65	102	11610	14753	47	112	11710
14633	83	88	11612	14760	42	114	
14641	0	121	11614		66	102	11714

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	69	100	11718	14893	3	122	
14762	11	121	11720		67	102	11819
14765	29	118		14897	16	121	11821
	77	94	11724	14900	4	122	
14778	33	117	11726		38	116	
14785	12	121			70	100	11827
	83	104	11730	14906	41	115	
14786	25	119	11732		55	109	11831
14788	72	98	11734	14909	5	122	
14792	86	86	11735		53	110	11835
14794	45	113		14912	64	104	11837
	85	87	11739	14913	57	108	11839
14797	54	109	11741	14914	35	117	11841
14800	20	120		14920	6	122	
	56	108			78	94	11845
	84	88	11747	14922	51	111	11847
14801	76	95	11749	14929	23	120	11849
14804	52	110	11751	14930	17	121	
14810	13	121			59	107	11853
	83	89	11755	14932	44	114	11855
14813	58	107	11757	14933	7	122	
14821	50	111	11759		73	98	11859
14824	30	118		14945	28	119	
	82	90	11763		49	112	11863
14825	37	116		14948	8	122	
	40	115			32	118	11867
	68	101	11769	14954	77	95	11869
14834	65	103	11771	14957	61	106	11871
14836	60	106	11773	14962	69	101	11873
14837	14	121		14965	9	122	
	26	119	11777		18	121	
14841	21	120			66	103	
	75	96	11781		86	87	11881
14842	71	99		14969	85	88	11883
	81	91	11785	14976	24	120	11885
14845	34	117		14977	39	116	
	43	114	11789		84	89	11889
14848	48	112	11791	14978	47	113	11891
14864	80	92	11793	14984	10	122	11893
14866	15	121	11795	14985	36	117	
14869	62	105	11797		72	99	11897
14884	0	122		14989	42	115	
	22	120	11801		83	90	11901
14885	1	122		14992	76	96	11903
	31	118		14994	63	105	11905
	46	113		15002	19	121	
	74	97	11809		29	119	11909
14888	2	122	11811	15005	11	122	
14890	27	119			82	91	11913

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15016	54	110	11917		86	88	12016
15017	56	109	11919	15145	4	123	
15021	45	114	11921		51	112	
15025	25	120			59	108	
	52	111			77	96	12024
	81	92	11927	15146	85	89	12026
15028	12	122		15149	35	118	12028
	58	108		15154	5	123	12030
	68	102	11933	15156	84	90	12032
15034	75	97	11935	15161	44	115	12034
15041	20	121		15165	6	123	
	65	104			69	102	12038
	71	100	11941	15170	23	121	
15044	50	112	11943		49	113	
15049	60	107			61	107	
	80	93	11947		83	91	12046
15053	13	122	11949	15172	66	104	12048
15056	40	116	11951	15173	17	122	12050
15058	37	117	11953	15178	7	123	12052
15061	30	119	11955	15184	28	120	
15073	48	113	11957		72	100	12056
15074	43	115	11959	15185	32	119	
15076	26	120	11961		76	97	12060
15077	79	94	11963	15188	82	92	12062
15080	14	122		15193	8	123	12064
	34	118		15205	47	114	
	62	106			63	106	12068
	74	98	11971	15208	18	122	12070
15082	21	121	11973	15210	9	123	
15098	67	103	11975		39	117	
15101	70	101	11977		81	93	12076
15109	15	122		15217	24	121	12078
	78	95	11981	15220	36	118	
15112	46	114	11983		42	116	12082
15121	64	105	11985	15229	10	123	
15122	31	119	11987		75	98	12086
15125	22	121		15233	68	103	12088
	55	110	11991	15236	56	110	
15129	0	123			80	94	12092
	27	120	11995	15237	54	111	12094
15130	1	123		15241	29	120	12096
	53	111		15242	71	101	12098
	57	109		15245	19	122	
	73	99	12003		58	109	12102
15133	2	123		15248	52	112	12104
	38	117	12007	15250	11	123	
15137	41	116	12009		33	119	
15138	3	123			45	115	
	87	87	12012		65	105	12112

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15266	25	121			35	119	12218
	79	95	12118	15386			
15269	50	113	12120	15392	4	124	
15273	12	123	12122		44	116	12222
15277	74	99	12124	15397	49	114	
15284	20	122	12126		81	94	12226
15289	40	117	12128	15401	5	124	12228
15293	37	118		15412	6	124	12230
	62	107	12132	15413	23	122	12232
15298	13	123	12134	15418	17	123	
15300	30	120			63	107	12236
	48	114		15424	32	120	12238
	78	96	12140	15425	7	124	
15304	70	102	12142		28	121	
15305	43	116			80	95	12244
	67	104	12146	15426	75	99	12246
15313	87	88	12148	15434	47	115	12248
15317	26	121		15440	8	124	
	34	119			68	104	12252
	86	89	12154	15445	39	118	
15325	14	123			71	102	12256
	21	122		15453	18	123	
	85	90	12160		42	117	12260
15329	73	100	12162	15457	9	124	
15332	64	106	12164		36	119	
15337	84	91	12166		56	111	
15338	77	97	12168		79	96	12268
15341	46	115	12170	15460	24	122	
15346	55	111	12172		54	112	12272
15349	57	110	12174	15461	65	106	12274
15353	53	112		15464	58	110	12276
	83	92	12178	15473	52	113	12278
15354	15	123	12180	15476	10	124	
15361	31	120	12182		74	100	12282
15362	59	109	12184	15481	45	116	
15368	22	122			60	109	12286
	38	118	12188	15482	29	121	12288
15370	27	121		15488	88	88	12289
	41	117		15489	33	120	12291
	51	113		15490	19	123	
	69	103	12196		87	89	12295
15373	82	93	12198	15493	78	97	12297
15376	0	124	12200	15496	50	114	
15377	1	124	12202		86	90	12301
15380	2	124		15497	11	124	12303
	76	98	12206	15506	85	91	12305
15381	66	105	12208	15508	62	108	12307
15385	3	124		15509	25	122	
	16	123			70	103	12311

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
15514	67	105	12313	15650	5	125	
15520	12	124			71	103	
	84	92	12317		79	97	12417
15524	40	118	12319	15658	23	123	12419
15529	20	123		15661	6	125	12421
	48	115	12323	15665	17	124	
15530	37	119			32	121	
	73	101	12327		47	116	
15533	77	98	12329		88	89	12429
15538	43	117		15668	28	122	12431
	83	93	12333	15669	87	90	12433
15541	30	121	12335	15674	7	125	
15545	13	124			65	107	12437
	64	107	12339	15677	74	101	
15556	34	120	12341		86	91	12441
15560	26	122		15680	56	112	12443
	82	94	12345	15682	39	119	12445
15569	55	112	12347	15685	54	113	
15570	21	123			58	111	12449
	57	111	12351	15688	42	118	
15572	14	124			78	98	12453
	46	116	12355	15689	8	125	
15577	69	104			85	92	12457
	76	99	12359	15696	36	120	12459
15578	53	113	12361	15700	18	124	
15581	59	110	12363		52	114	
15586	81	95	12365		60	110	12465
15588	72	102	12367	15705	24	123	
15592	66	106	12369		84	93	12469
15597	51	114	12371	15706	9	125	12471
15601	15	124	12373	15714	45	117	12473
15602	31	121		15716	70	104	12475
	61	109	12377	15725	10	125	
15605	38	119			29	122	
	41	118	12381		50	115	
15613	22	123			62	109	
	27	122	12385		67	106	
15616	80	96	12387		83	94	12487
15625	0	125		15730	33	121	
	35	120			77	99	12491
	44	117		15733	73	102	12493
	75	100	12395	15737	19	124	12495
15626	1	125		15746	11	125	12497
	49	115	12399	15749	82	95	12499
15629	2	125	12401	15754	25	123	12501
15632	16	124	12403	15760	48	116	
15633	63	108	12405		64	108	12505
15634	3	125	12407	15761	40	119	12507
15641	4	125	12409	15769	12	125	
15649	68	105	12411		37	120	12511

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
15773	43	118	12513	15898	47	117	12612
15776	20	124		15901	5	126	12614
	76	100	12517	15905	23	124	
15777	81	96	12519		56	113	12618
15784	30	122	12521	15908	32	122	
15786	69	105	12523		58	112	12622
15793	57	112		15912	6	126	
	72	103	12527		54	114	12626
15794	13	125		15913	28	123	12628
	55	113	12531	15914	17	125	
15797	34	121	12533		83	95	12632
15802	59	111	12535	15921	39	120	
15805	26	123			60	111	12636
	46	117		15925	7	126	
	53	114			42	119	
	66	107	12543		70	105	12642
15809	80	97	12545	15929	52	115	
15817	21	124	12547		77	100	12646
15821	14	125		15937	36	121	12648
	61	110	12551	15938	67	107	
15826	51	115			73	103	12652
	75	101	12555	15940	8	126	
15842	41	119			82	96	12656
	89	89	12558	15944	62	110	12658
15844	38	120		15949	18	125	
	88	90	12562		45	118	12662
15845	31	122		15952	24	124	12664
	79	98	12566	15956	50	116	12666
15850	15	125		15957	9	126	12668
	63	109		15970	29	123	
	87	91	12572		81	97	12672
15857	49	116		15973	33	122	12674
	71	104	12576	15976	10	126	12676
15858	27	123	12578	15977	64	109	
15860	22	124			76	101	12680
	44	118		15986	19	125	12682
	68	106		15993	48	117	12684
	86	92	12586	15997	11	126	
15866	35	121	12588		69	106	12688
15874	85	93	12590	16000	40	120	
15876	0	126	12592		72	104	12692
15877	1	126	12594	16001	25	124	12694
15880	2	126		16004	80	98	12696
	74	102	12598	16010	37	121	
15881	16	125	12600		43	119	12700
15885	3	126		16018	57	113	12702
	78	99	12604	16020	12	126	
15889	65	108	12606		66	108	12706
15892	4	126		16021	55	114	
	84	94	12610		89	90	12710

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
16025	20	125		16153	32	123	
	59	112			67	108	12814
	88	91	12716	16154	5	127	
16029	30	123			23	125	12818
	75	102	12720	16160	28	124	
16033	87	92	12722		52	116	12822
16034	53	115	12724	16162	39	121	12824
16040	34	122		16164	42	120	12826
	46	118	12728	16165	6	127	
16042	61	111			17	126	
	79	99	12732		62	111	
16045	13	126			81	98	12834
	86	93	12736	16178	7	127	12836
16052	26	124	12738	16180	36	122	
16057	51	116	12740		76	102	12840
16061	85	94	12742	16186	45	119	12842
16066	21	125		16189	50	117	12844
	71	105	12746	16193	8	127	12846
16069	63	110	12748	16196	64	110	12848
16072	14	126	12750	16200	18	126	
16073	68	107	12752		90	90	12851
16081	41	120		16201	24	125	
	84	95	12756		80	99	12855
16084	78	100	12758	16202	89	91	12857
16085	38	121		16208	88	92	12859
	74	103	12762	16209	72	105	12861
16090	31	123		16210	9	127	
	49	117	12766		69	107	12865
16097	44	119	12768	16217	29	124	12867
16101	15	126	12770	16218	33	123	
16105	27	124			87	93	12871
	83	96	12774	16228	48	118	12873
16106	65	109	12776	16229	10	127	12875
16109	22	125		16232	86	94	12877
	35	122	12780	16234	75	103	12879
16129	0	127	12782	16237	19	126	
16130	1	127			66	109	12883
	77	101	12786	16241	40	121	
16132	16	126			79	100	12887
	56	114	12790	16245	57	114	12889
16133	2	127		16249	43	120	12891
	47	118		16250	11	127	
	58	113			25	125	
	82	97	12798		55	115	
16136	70	106	12800		59	113	
16138	3	127	12802		85	95	12901
16141	54	115	12804	16253	37	122	12903
16144	60	112	12806	16265	53	116	
16145	4	127			61	112	12907
	73	104	12810	16272	84	96	12909

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
16273	12	127	12911		80	100	13011
16276	20	126		16405	23	126	
	30	124	12915		39	122	
16277	46	119			42	121	
	71	106	12919		87	94	13019
16285	34	123		16409	5	128	
	78	101	12923		28	125	13023
16288	68	108	12925	16417	64	111	13025
16290	51	117		16418	17	127	13027
	63	111	12929	16420	6	128	
16292	74	104	12931		72	106	13031
16298	13	127		16421	86	95	13033
	83	97	12935	16424	50	118	13035
16301	26	125	12937	16425	36	123	
16317	21	126	12939		45	120	
16322	41	121	12941		69	108	13041
16325	14	127		16433	7	128	13043
	49	118		16441	75	104	
	65	110	12947		85	96	13047
16328	38	122		16442	79	101	13049
	82	98	12951	16448	8	128	13051
16333	77	102	12953	16452	24	126	13053
16336	44	120	12955	16453	18	127	13055
16337	31	124	12957	16456	66	110	13057
16349	70	107	12959	16465	9	128	
16354	15	127			33	124	
	27	125			48	119	
	35	123			84	97	13065
	73	105	12967	16466	29	125	13067
16360	22	126		16474	57	115	13069
	58	114	12971	16477	59	114	13071
16361	56	115	12973	16481	55	116	13073
16362	81	99	12975	16484	10	128	
16369	60	113	12977		40	122	13077
16370	47	119		16488	78	102	13079
	67	109	12981	16490	19	127	
16372	54	116	12983		43	121	
16381	90	91	12985		61	113	
16384	0	128	12987		71	107	13087
16385	1	128		16493	83	98	13089
	16	127		16498	37	123	
	76	103			53	117	13093
	89	92	12995	16501	25	126	
16388	2	128			74	105	13097
	62	112	12999	16505	11	128	
16393	3	128			68	109	13101
	52	117		16513	63	112	13103
	88	93	13005	16516	46	120	13105
16400	4	128		16525	30	125	
	32	124			51	118	

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
	82	99	13111		39	123	
16528	12	128	13113		75	105	13212
16529	20	127	13115	16657	4	129	13214
16532	34	124	13117	16658	23	127	13216
16538	77	103	13119	16660	28	126	
16546	65	111	13121		84	98	13220
16552	26	126	13123	16661	50	119	13222
16553	13	128	13125	16666	5	129	
16561	81	100	13127		45	121	13226
16562	49	119		16672	36	124	13228
	91	91	13130	16673	17	128	13230
16564	70	108		16677	6	129	
	90	92	13134		66	111	13234
16565	41	122		16690	7	129	
	73	106	13138		83	99	13238
16570	21	127		16693	78	103	13240
	89	93	13142	16704	48	120	13242
16573	38	123	13144	16705	8	129	
16577	44	121	13146		24	127	
16580	14	128			57	116	
	88	94	13150		71	108	13250
16586	31	125	13152	16706	59	115	13252
16589	58	115		16708	18	128	13254
	67	110	13156	16712	74	106	13256
16592	56	116		16714	33	125	
	76	104	13160		55	117	13260
16594	87	95	13162	16717	29	126	
16596	60	114	13164		61	114	13264
16601	35	124		16722	9	129	13266
	80	101	13168	16724	68	110	
16605	27	126			82	100	13270
	54	117	13172	16729	40	123	13272
16609	15	128		16733	43	122	
	47	120	13176		53	118	13276
16612	86	96	13178	16738	63	113	13278
16613	22	127		16741	10	129	13280
	62	113	13182	16745	19	128	
16628	52	118	13184		37	124	
16633	72	107	13186		77	104	
16634	85	97	13188		91	92	13288
16640	16	128		16749	90	93	13290
	64	112	13192	16754	25	127	13292
16641	0	129	13194	16757	46	121	
16642	1	129			89	94	13296
	69	109	13198	16762	11	129	
16645	2	129			51	119	
	79	102	13202		81	101	13302
16648	42	122	13204	16769	65	112	
16649	32	125	13206		88	95	13306
16650	3	129		16776	30	126	13308

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
16778	73	107	13310	16913	23	128	
16781	34	125			28	127	13412
	70	109	13314	16916	4	130	13414
16784	20	128	13316	16921	36	125	13416
16785	12	129		16922	71	109	13418
	87	96	13320	16925	5	130	
16801	49	120			74	107	
	76	105	13324		82	101	13424
16804	80	102	13326	16928	92	92	13425
16805	26	127		16930	17	129	
	86	97	13330		91	93	13429
16810	13	129		16936	6	130	
	41	123			90	94	13433
	67	111	13336	16937	59	116	13435
16820	38	124		16938	57	117	13437
	44	122		16945	48	121	
	58	116	13342		68	111	13441
16825	21	128		16946	61	115	
	56	117			89	95	13445
	60	115	13348	16949	7	130	
16829	85	98	13350		55	118	13449
16837	14	129		16954	77	105	13451
	31	126	13354	16960	24	128	
16840	54	118			88	96	13455
	62	114	13358	16964	8	130	13457
16848	72	108	13360	16965	18	129	
16850	35	125			33	126	
	47	121			63	114	
	79	103	13366		81	102	13465
16857	84	99	13368	16970	29	127	
16858	27	127	13370		53	119	13469
16861	69	110		16976	40	124	13471
	75	106	13374	16978	43	123	
16865	52	119			87	97	13475
	64	113	13378	16981	9	130	13477
16866	15	129	13380	16993	73	108	13479
16868	22	128	13382	16994	37	125	
16889	83	100	13384		65	113	13483
16893	42	123	13386	17000	10	130	
16897	16	129			46	122	
	39	124	13390		70	110	
16900	0	130			86	98	13491
	32	126		17001	51	120	13493
	50	120		17002	19	129	13495
	66	112		17009	25	128	
	78	104	13400		80	103	13499
16901	1	130	13402	17012	76	106	13501
16904	2	130	13404	17021	11	130	13503
16909	3	130		17026	85	99	13505
	45	122	13408	17029	30	127	13507

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
17032	34	126	13509		77	106	13609
17033	67	112	13511	17168	28	128	
17041	20	129	13513		68	112	13613
17042	49	121	13515	17170	3	131	
17044	12	130	13517		23	129	
17053	58	117	13519		59	117	
17056	60	116			81	103	13621
	84	100	13523	17172	36	126	13623
17057	41	124		17173	57	118	
	79	104	13527		87	98	13627
17060	26	128		17177	4	131	
	56	118	13531		61	116	13631
17065	44	123		17186	5	131	
	72	109	13535		55	119	13635
17069	13	130		17188	48	122	13637
	38	125		17189	17	130	13639
	62	115	13541	17194	63	115	13641
17074	75	107	13543	17197	6	131	
17077	54	119	13545		86	99	13645
17082	21	129		17209	53	120	13647
	69	111	13549	17210	7	131	
17090	31	127			73	109	13651
	83	101	13553	17216	80	104	13653
17092	64	114	13555	17217	24	129	13655
17093	47	122	13557	17218	33	127	13657
17096	14	130	13559	17221	65	114	
17101	35	126	13561		70	111	13661
17104	52	120	13563	17224	18	130	13663
17109	78	105	13565	17225	8	131	
17113	27	128			29	128	
	92	93	13569		40	125	
17117	91	94	13571		43	124	
17125	15	130			76	107	
	22	129			85	100	13675
	66	113		17242	9	131	
	90	95	13579		51	121	13679
17128	82	102	13581	17245	37	126	
17137	89	96	13583		46	123	13683
17140	42	124		17257	84	101	13685
	74	108	13587	17258	67	113	13687
17141	50	121		17261	10	131	
	71	110	13591		19	130	13691
17146	39	125	13593	17266	25	129	
17153	32	127			79	105	13695
	88	97	13597	17282	11	131	13697
17154	45	123	13599	17284	30	128	
17156	16	130	13601		72	110	13701
17161	0	131	13603	17285	34	127	
17162	1	131	13605		49	122	13705
17165	2	131		17288	58	118	13707

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
17289	60	117		17425	1	132	
	75	108	13711		28	129	
17293	83	102	13713		36	127	
17297	56	119	13715		55	120	
17298	93	93	13716		63	116	
17300	20	130			80	105	13818
	62	116		17426	85	101	13820
	92	94	13722	17428	2	132	13822
17305	12	131		17429	23	130	
	69	112	13726		73	110	13826
17306	41	125		17433	3	132	
	91	95	13730		48	123	13830
17312	44	124	13732	17440	4	132	
17316	54	120			76	108	13834
	90	96	13736	17444	70	112	13836
17317	26	129	13738	17449	5	132	13838
17320	38	126		17450	17	131	
	78	106	13742		53	121	
17321	64	115	13744		65	115	13844
17330	13	131		17460	6	132	
	89	97	13748		84	102	13848
17333	82	103	13750	17473	7	132	
17338	47	123	13752		33	128	13852
17341	21	130	13754	17474	43	125	13854
17345	31	128		17476	24	130	
	52	121	13758		40	126	13858
17348	88	98	13760	17477	79	106	13860
17352	66	114	13762	17482	29	129	13862
17354	35	127	13764	17485	18	131	
17357	14	131			51	122	
	74	109	13768		67	114	
17362	71	111	13770		93	94	13870
17370	27	129		17488	8	132	13872
	87	99	13774	17489	92	95	13874
17377	81	104	13776	17492	46	124	13876
17378	77	107	13778	17497	91	96	13878
17384	22	130		17498	37	127	
	50	122	13782		83	103	13882
17386	15	131	13784	17505	9	132	
17389	42	125	13786		72	111	13886
17393	68	113	13788	17506	75	109	13888
17396	86	100	13790	17509	90	97	13890
17397	39	126	13792	17522	19	131	13892
17401	45	124	13794	17524	10	132	
17405	59	118	13796		60	118	13896
17408	32	128	13798	17525	25	130	
17410	57	119			58	119	
	61	117	13802		89	98	13902
17417	16	131	13804	17530	49	123	
17424	0	132	13806		69	113	13906

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
17533	62	117		17672	94	94	14007
	78	107	13910	17674	93	95	14009
17536	56	120	13912	17680	16	132	
17540	34	128			36	128	
	82	104	13916		48	124	
17541	30	129	13918		92	96	14017
17545	11	132		17681	65	116	14019
	88	99	13922	17684	28	130	14021
17552	64	116	13924	17689	0	133	14023
17557	41	126		17690	1	133	
	54	121	13928		23	131	
17561	20	131			79	107	
	44	125	13932		91	97	14031
17568	12	132	13934	17693	2	133	
17569	87	100	13936		53	122	14035
17573	38	127	13938	17698	3	133	14037
17576	26	130		17704	90	98	14039
	74	110	13942	17705	4	133	
17581	66	115	13944		83	104	14043
17585	47	124		17713	17	132	14045
	71	112	13948	17714	5	133	
17586	81	105	13950		67	115	14049
17588	52	122	13952	17722	89	99	14051
17593	13	132		17725	6	133	
	77	108	13956		43	126	
17597	86	101	13958		75	110	14057
17602	21	131		17728	72	112	14059
	31	129	13962	17729	40	127	14061
17609	35	128	13964	17730	33	129	
17620	14	132			51	123	14065
	68	114	13968	17737	24	131	14067
17629	27	130		17738	7	133	14069
	50	123		17741	29	130	
	85	102	13974		46	125	14073
17636	80	106	13976	17744	88	100	14075
17640	42	126	13978	17748	18	132	
17642	59	119	13980		78	108	14079
17645	22	131		17749	82	105	14081
	61	118	13984	17753	8	133	
17649	15	132			37	128	14085
	57	120	13988	17757	69	114	14087
17650	39	127		17761	60	119	14089
	45	125		17764	58	120	14091
	73	111	13994	17768	62	118	14093
17657	76	109	13996	17770	9	133	
17658	63	117	13998		87	101	14097
17665	32	129		17777	49	124	
	84	103	14002		56	121	14101
17666	55	121	14004	17785	19	132	
17669	70	113	14006		64	117	14105

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
17786	25	131	14107		65	117	
17789	10	133	14109		83	105	14209
17797	34	129		17921	89	100	14211
	74	111		17924	32	130	14213
	81	106	14115	17929	48	125	14215
17800	30	130		17937	36	129	14217
	54	122		17938	53	123	14219
	86	102	14121	17945	16	133	
17810	11	133			28	131	
	41	127			67	116	
	71	113			88	101	14227
	77	109	14129	17946	75	111	14229
17812	44	126		17953	23	132	
	66	116	14133		72	113	14233
17824	20	132	14135	17956	0	134	14235
17828	38	128	14137	17957	1	134	14237
17833	12	133		17960	2	134	
	52	123	14141		82	106	14241
17834	47	125		17965	3	134	
	85	103	14145		78	109	14245
17837	26	131	14147	17972	4	134	14247
17849	68	115		17973	87	102	14249
	80	107	14151	17977	51	124	14251
17858	13	133	14153	17978	17	133	
17861	31	130			43	127	14255
	94	95	14157	17981	5	134	14257
17865	21	132		17984	40	128	14259
	93	96	14161	17986	69	115	14261
17866	35	129	14163	17989	33	130	14263
17872	84	104	14165	17992	6	134	
17873	73	112			46	126	14267
	92	97	14169	18000	24	132	
17876	50	124			60	120	14271
	76	110	14173	18002	29	131	14273
17881	59	120	14175	18005	7	134	
17882	61	119	14177		58	121	
17885	14	133			62	119	
	91	98	14181		86	103	14281
17890	27	131		18010	37	129	
	57	121	14185		81	107	14285
17893	42	127		18013	18	133	14287
	63	118	14189	18020	8	134	
17896	70	114	14191		56	122	
17901	45	126			64	118	
	90	99	14195		74	112	14295
17905	39	128		18026	49	125	14297
	79	108	14199	18029	77	110	14299
17908	22	132	14201	18037	9	134	
17909	55	122	14203		71	114	14303
17914	15	133		18041	85	104	14305

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
18045	54	123			82	107	14406
	66	117	14309	18178	67	117	
18049	25	132	14311		87	103	14410
18050	19	133		18180	48	126	
	95	95	14314		72	114	14414
18052	94	96	14316	18181	15	134	14416
18056	10	134		18184	78	110	14418
	34	130	14320	18185	32	131	
18058	93	97	14322		53	124	14422
18061	30	131	14324	18196	36	130	14424
18064	80	108	14326	18208	28	132	14426
18065	41	128		18212	16	134	
	44	127	14330		86	104	14430
18068	92	98	14332	18217	69	116	14432
18077	11	134	14334	18218	23	133	14434
18080	52	124		18225	0	135	
	68	116	14338		81	108	14438
18081	84	105	14340	18226	1	135	
18082	91	99	14342		51	125	14442
18085	38	129		18229	2	135	14444
	47	126	14346	18233	43	128	14446
18089	20	133	14348	18234	3	135	14448
18097	76	111	14350	18241	4	135	
18098	73	113	14352		40	129	
18100	12	134			60	121	
	26	132			95	96	14456
	90	100	14358	18244	62	120	14458
18121	61	120	14360	18245	17	134	
18122	31	131			46	127	
	59	121			74	113	
	79	109			94	97	14466
	89	101	14368	18248	58	122	14468
18125	13	134		18250	5	135	
	35	130			33	131	
	50	125			77	111	
	70	115			85	105	14476
	83	106	14378	18253	93	98	14478
18130	21	133		18257	64	119	14480
	63	119	14382	18261	6	135	14482
18133	57	122	14384	18265	24	133	
18148	42	128			29	132	
	88	102	14388		56	123	
18149	65	118	14390		92	99	14490
18152	14	134	14392	18266	71	115	14492
18153	27	132	14394	18269	37	130	14494
18154	45	127		18274	7	135	14496
	55	123	14398	18277	49	126	14498
18162	39	129	14400	18280	18	134	
18169	75	112	14402		66	118	14502
18173	22	133		18281	80	109	

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
	91	100	14506		86	105	14606
18289	8	135	14508	18432	96	96	14607
18292	54	124		18433	48	127	14609
	84	106	14512	18434	53	125	
18301	90	101	14514		95	97	14613
18306	9	135	14516	18440	22	134	
18313	68	117	14518		94	98	14617
18314	25	133	14520	18442	81	109	14619
18317	19	134		18448	32	132	14621
	34	131	14524	18450	15	135	
18320	44	128			69	117	
	76	112	14528		93	99	14627
18322	41	129	14530	18457	36	131	14629
18324	30	132	14532	18461	85	106	14631
18325	10	135		18464	92	100	14633
	73	114		18472	74	114	14635
	89	102	14538	18473	28	133	
18329	52	125	14540		77	112	14639
18338	47	127		18477	51	126	14641
	83	107	14544	18481	16	135	14643
18341	79	110	14546	18482	91	101	14645
18344	38	130	14548	18484	60	122	14647
18346	11	135	14550	18485	23	134	
18353	88	103	14552		62	121	14651
18356	20	134		18490	43	129	14653
	70	116	14556	18493	58	123	14655
18362	61	121	14558	18496	0	136	
18365	26	133			64	120	14659
	59	122	14562	18497	1	136	
18369	12	135			71	116	14663
	63	120	14566	18500	2	136	
18376	50	126	14568		40	130	
18378	57	123	14570		46	128	
18385	31	132			80	110	14671
	87	104	14574	18504	90	102	14673
18386	35	131		18505	3	136	
	65	119	14578		84	107	14677
18388	82	108	14580	18512	4	136	
18394	13	135			56	124	14681
	75	113	14584	18513	33	132	14683
18397	21	134	14586	18514	17	135	14685
18401	55	124	14588	18517	66	119	14687
18405	42	129		18521	5	136	14689
	78	111	14592	18530	29	133	
18409	45	128			37	131	
	72	115	14596		49	127	
18413	67	118	14598		89	103	14697
18418	27	133	14600	18532	6	136	
18421	14	135			24	134	14701
	39	130		18541	54	125	14703

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
18545	7	136			45	129	14805
	76	113	14707	18674	85	107	14807
18548	68	118	14709	18682	39	131	14809
18549	18	135	14711	18685	27	134	
18553	83	108	14713		53	126	
18554	73	115	14715		69	118	
18560	8	136			91	102	14817
	88	104	14719	18688	48	128	14819
18562	79	111	14721	18692	14	136	14821
18577	9	136		18698	77	113	14823
	44	129	14725	18701	74	115	14825
18580	34	132		18709	22	135	
	25	126	14729		90	103	14829
18581	25	134		18713	32	133	14831
	41	130	14733	18720	36	132	
18586	19	135	14735		84	108	14835
18589	30	133		18721	15	136	
	70	117	14739		80	111	14839
18593	47	128	14741	18728	62	122	14841
18594	87	105	14743	18729	60	123	14843
18596	10	136	14745	18730	51	127	
18605	38	131			71	117	14847
	61	122		18737	64	121	
	82	109	14751		89	104	14851
18610	59	123		18740	28	134	
	63	121	14755		58	124	14855
18617	11	136	14757	18749	43	130	14857
18621	75	114	14759	18752	16	136	14859
18625	20	135		18754	23	135	14861
	57	124		18756	66	120	14863
	65	120		18757	46	129	14865
	96	97	14767	18761	40	131	
18628	78	112	14769		56	125	14869
18629	50	127		18769	0	137	
	95	98	14773		88	105	14873
18632	26	134		18770	1	137	
	86	106	14777		83	109	14877
18637	94	99	14779	18772	76	114	14879
18640	12	136		18773	2	137	14881
	72	116	14783	18778	3	137	
18649	35	132			33	133	14885
	93	100	14787	18785	4	137	
18650	31	133			17	136	
	55	125			49	128	
	67	119	14793		68	119	
18661	81	110	14795		73	116	
18664	42	130	14797		79	112	14897
18665	13	136		18792	54	126	14899
	92	101	14801	18793	37	132	14901
18666	21	135		18794	5	137	14903

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
					45	130	
18797	29	134	14905		77	114	15006
18801	24	135	14907				
18805	6	137		18932	74	116	15008
	87	106	14911	18937	21	136	
18818	7	137			84	109	15012
	97	97	14914	18938	13	137	
18820	18	136			53	127	15016
	96	98	14918	18944	80	112	15018
18824	70	118		18945	39	132	
	82	110	14922		48	129	15022
18826	95	99	14924	18946	89	105	15024
18833	8	137		18954	27	135	15026
	52	127	14928	18965	14	137	
18836	44	130			71	118	15030
	94	100	14932	18973	62	123	15032
18842	41	131	14934	18976	60	124	15034
18845	34	133		18980	22	136	
	86	107	14938		32	134	
18850	9	137			64	122	
	25	135			88	106	15042
	47	129		18985	36	133	
	61	123			51	128	15046
	75	115		18989	58	125	
	93	101	14950		83	110	15050
18853	63	122		18994	15	137	15052
	78	113	14954	18997	66	121	15054
18856	30	134	14956	19001	76	115	15056
18857	19	136		19009	28	135	15058
	59	124	14960	19010	43	131	
18866	65	121	14962		79	113	15062
18868	38	132		19012	56	126	15064
	92	102	14966	19013	97	98	15066
18869	10	137	14968	19016	46	130	15068
18873	72	117	14970	19017	96	99	15070
18874	57	125	14972	19018	73	117	
18882	81	111	14974		87	107	15074
18884	50	128	14976	19024	40	132	
18889	67	120			68	120	15078
	85	108	14980	19025	16	137	
18890	11	137			23	136	
	91	103	14984		95	100	15084
18896	20	136	14986	19037	94	101	15086
18901	26	135		19042	49	129	15088
	55	126	14990	19044	0	138	15090
18913	12	137	14992	19045	1	138	
18914	35	133	14994		33	134	
18916	90	104	14996		54	127	
18917	31	134	14998		82	111	15098
18922	69	119	15000	19048	2	138	15100
18925	42	131		19053	3	138	

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
	93	102	15104	19186	31	135	
19058	17	137			45	131	15206
	37	133	15108	19188	12	138	
19060	4	138			42	132	15210
	86	108	15112	19193	53	128	
19061	70	119	15114		88	107	15214
19066	29	135	15116	19202	71	119	15216
19069	5	138	15118	19204	48	130	15218
19072	24	136	15120	19208	98	98	15219
19073	92	103	15122	19210	21	137	
19080	6	138			39	133	
	78	114	15126		83	111	
19081	75	116	15128		97	99	15227
19088	52	128	15130	19213	13	138	15229
19093	7	138		19216	96	100	15231
	18	137	15134	19220	62	124	15233
19097	44	131		19225	27	136	
	61	124			60	125	
	91	104	15140		64	123	15239
19098	63	123	15142	19226	95	101	15241
19105	41	132		19232	76	116	15243
	81	112	15146	19233	87	108	15245
19106	59	125		19237	79	114	15247
	85	109	15150	19240	14	138	
19108	8	138			58	126	
	72	118	15154		66	122	
19109	47	130			94	102	15255
	65	122	15158	19242	51	129	15257
19112	34	134	15160	19249	32	135	15259
19121	25	136	15162	19252	36	134	15261
19125	9	138		19253	22	137	
	30	135			73	118	15265
	57	126		19258	93	103	15267
	90	105	15170	19265	56	127	
19130	19	137			68	121	15271
	67	121	15174	19268	82	112	15273
19133	38	133	15176	19269	15	138	15275
19141	50	129	15178	19273	43	132	15277
19144	10	138	15180	19277	46	131	
19154	55	127			86	109	15281
	77	115	15184	19280	28	136	
19156	84	110	15186		92	104	15285
19157	89	106	15188	19289	40	133	15287
19161	69	120	15190	19298	23	137	15289
19165	11	138		19300	16	138	
	74	117	15194		54	128	
19169	20	137			70	120	15295
	80	113	15198	19301	49	130	15297
19172	26	136	15200	19306	91	105	15299
19181	35	134	15202	19309	78	115	15301

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19314	33	135		19433	83	112	15403
	75	117	15305	19441	71	120	15405
19321	0	139	15307	19442	11	139	15407
19322	1	139	15309	19444	20	138	15409
19325	2	139		19445	26	137	
	37	134			94	103	15413
	85	110	15315	19449	45	132	15415
19330	3	139		19450	35	135	
	81	113	15319		53	129	
19333	17	138	15321		87	109	15421
19336	90	106	15323	19453	42	133	15423
19337	4	139		19457	31	136	15425
	29	136	15327	19465	12	139	
19345	24	137			48	131	
	52	129			76	117	
	63	124			93	104	15433
	72	119	15335	19466	79	115	15435
19346	5	139		19469	62	125	15437
	61	125	15339	19472	64	124	15439
19354	65	123	15341	19476	60	126	15441
19357	6	139		19477	39	134	15443
	59	126	15345	19485	21	138	
19360	44	132	15347		66	123	15447
19368	18	138	15349	19489	92	105	15449
19370	7	139		19490	13	139	
	41	133			73	119	15453
	47	131		19493	58	127	
	89	107	15357		82	113	15457
19373	67	122	15359	19496	86	110	15459
19377	84	111	15361	19498	27	137	15461
19378	57	127	15363	19501	51	130	15463
19381	34	135	15365	19508	68	122	15465
19385	8	139		19517	14	139	
	77	116	15369		91	106	15469
19394	25	137	15371	19520	32	136	
19396	30	136			56	128	15473
	80	114	15375	19521	36	135	15475
19400	38	134		19528	22	138	15477
	50	130		19538	43	133	15479
	74	118	15381	19540	46	132	
19402	9	139			78	116	15483
	69	121	15385	19541	70	121	15485
19405	19	138		19546	15	139	
	98	99	15389		85	111	15489
19408	88	108	15391	19549	75	118	
19409	55	128			90	107	15493
	97	100	15395	19553	28	137	15495
19417	96	101	15397	19556	40	134	15497
19421	10	139	15399	19557	54	129	
19429	95	102	15401		81	114	15501

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19562	49	131	15503	19674	93	105	15602
19573	23	138	15505	19681	9	140	15604
19577	16	139	15507	19682	19	139	
19584	72	120	15509		71	121	15608
19585	33	136		19697	79	116	15610
	89	108	15513	19700	10	140	
19594	37	135			76	118	
	63	125	15517		92	106	15616
19597	61	126	15519	19709	53	130	15618
19600	0	140		19714	45	133	15620
	84	112	15523	19717	86	111	15622
19601	1	140		19720	26	138	
	65	124	15527		42	134	
19602	99	99	15528		62	126	
19604	2	140			82	114	15630
	52	130		19721	11	140	
	98	100	15534		20	139	
19609	3	140	15536		35	136	
19610	17	139			64	125	15638
	29	137		19728	48	132	15640
	59	127		19729	60	127	
	97	101	15544		73	120	15644
19616	4	140	15546	19730	31	137	
19618	67	123			91	107	15648
	77	117	15550	19732	66	124	15650
19620	24	138		19744	12	140	15652
	96	102	15554	19746	39	135	15654
19625	5	140		19748	58	128	15656
	44	133		19753	68	123	15658
	80	115		19762	21	139	
	88	109	15562		51	131	15662
19633	47	132		19764	90	108	15664
	57	128	15566	19769	13	140	
19634	95	103	15568		85	112	15668
19636	6	140	15570	19773	27	138	
19637	41	134			78	117	15672
	74	119	15574	19777	56	129	15674
19645	18	139		19784	70	122	15676
	69	122	15578	19786	75	119	
19649	7	140	15580		81	115	15680
19652	34	136		19792	36	136	15682
	94	104	15584	19793	32	137	15684
19658	83	113	15586	19796	14	140	15686
19661	50	131	15588	19801	99	100	15688
19664	8	140	15590	19802	89	109	15690
19666	55	129	15592	19805	22	139	
19669	25	138			43	134	
	30	137			46	133	
	38	135			98	101	15698
	87	110	15600	19813	97	102	15700

t	x	y	$N_2(t)$	t	x	y	$N_2(t)$
19816	54	130	15702	19937	76	119	15802
19825	15	140		19940	38	136	
	40	135			86	112	15806
	49	132		19944	30	138	15808
	72	121		19945	8	141	
	84	113			91	108	15812
	96	103	15714	19946	25	139	15814
19828	28	138	15716	19949	82	115	15816
19841	95	104	15718	19961	19	140	15818
19844	88	110	15720	19962	9	141	15820
19845	63	126	15722	19970	53	131	
19850	23	139			73	121	15824
	61	127		19972	64	126	15826
	65	125	15728	19973	62	127	15828
19853	77	118	15730	19981	10	141	
19856	16	140			45	134	
	80	116	15734		66	125	
19858	33	137	15736		90	109	15836
19861	94	105	15738	19984	60	128	15838
19865	37	136		19989	42	135	15840
	52	131		19993	48	133	15842
	59	128		19994	35	137	
	67	124	15746		85	113	15846
19876	74	120	15748	19997	26	139	15848
19881	0	141	15750	20000	20	140	
19882	1	141	15752		68	124	
19885	2	141			100	100	15853
	29	138					
	83	114					
	93	106	15760				
19889	17	140	15762				
19890	3	141					
	57	129					
	69	123					
	87	111	15770				
19892	44	134	15772				
19897	4	141					
	24	139	15776				
19898	47	133	15778				
19906	5	141					
	41	135	15782				
19913	92	107	15784				
19917	6	141	15786				
19924	18	140					
	50	132	15790				
19925	34	137					
	55	130					
	71	122	15796				
19930	7	141					
	79	117	15800				

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 21 | CHEMISTRY | Pp. 95-98

August, 1952

EXPERIMENTS ON THE CONSTITUTION OF CHAKSINE, THE ALKALOID FROM THE SEEDS OF *CASSIA ABSUS* LINN. PART I

by

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Price Rs.0-11-0

EXPERIMENTS ON THE CONSTITUTION OF CHAKSINE, THE ALKALOID FROM THE SEEDS OF *CASSIA ABSUS* LINN. PART I.*

By S. D. LALA *and* I. SEN GUPTA.

The presence of an alkaloidal principle in the seeds of *Cassia Absus* Linn. was first reported by Dymock[1] and also by Siddiqui[2] *et al.* The latter workers found two water-soluble, isomeric quaternary bases, chaksine and isochaksine and the molecular formula $C_{12}H_{21}O_2N_3$ was advanced for these on the basis of the analysis of chaksine bicarbonate ($C_{12}H_{20}ON_3HCO_3 \cdot \frac{1}{2} H_2O$). Ray[3] *et al.* objected to accept the loss in vacuo at 100° C. to be due to half a molecule of water in the bicarbonate and further due to discrepancy in the percentage of iodine in chaksine iodide. These authors put forward the formula $C_{11}H_{21}O_3N_3$ for chaksine base on the analyses of sulphate, nitrate, chloride and bromide of chaksine which were prepared by them in high state of purity.

This new formula ($C_{11}H_{21}O_3N_3$) was not accepted by Siddiqui[4] *et al.* in a note saying that except for sulphate which melts at 317° C. and is highly incombustible none of the other salts are noted by them to have been dried under vacuo to constant weight before analysis. Neglect of careful dehydration of the salt may have been partly responsible for their low carbon values and further that the halogen value in case of chloride and bromide was not determined.

The present investigation was therefore undertaken to ascertain the molecular formula of chaksine by preparing new compounds of chaksine with various organic acids. A majority of these acids gave well crystalline products by reacting chaksine chloride with the saturated solution of sodium salt of the acid. These products were isolated in a high state of purity and dried thoroughly before analysis. The compounds were analyzed for carbon and hydrogen values only, the estimation of nitrogen value, being useless as it cannot differentiate between C_{11} or C_{12} formula, the molecular weight of both the formula being nearly the same and both having the same number of nitrogen atoms. Our analytical results are in agreement with the molecular formula $C_{11}H_{21}O_3N_3$ for chaksine base, put forward by Ray *et al.* (*loc. cit.*).

In a further communication Siddiqui[5] *et al.* have also reported the preparation of acetate, tartrate, oxalate, succinate and citrate by the action of the acid on bicarbonate or carbonate of chaksine. Out of these acetate and tartrate have been reported to be neutral, whereas oxalate, succinate and citrate are acidic in character due to acid salt formation

* A part of the thesis submitted by one of us (S. D. Lala) for his M.Sc. degree of the Panjab University, Lahore, in December, 1945.

with feebly basic nitrogen. The oxalate, citrate, succinate and other derivatives prepared by us are all neutral in character these being prepared by the action of chaksine chloride with saturated solution of sodium salt of the acid and as such the salt formation with feebly basic nitrogen is not possible. Further, succinic acid being weaker acid than tartaric acid, it is quite surprising that succinic acid should form an acid salt formation as reported by Siddiqui *et al.* (loc. cit.), with feebly basic nitrogen and the reaction with tartaric acid should not give acid salt formation. The analytical results of the compounds support our contention.

Experimental.

Chaksine acetate. ($C_{11}H_{20}O_2N_3CH_3COO$).

Saturated solution of sodium acetate (5 c.c.) was added to 2 gms. of 20% chaksine chloride solution in water. The white precipitate so obtained was filtered after washing several times with water. The product was crystallized from absolute alcohol-ether mixture in colourless needles. m.p. 218–220° C. Found: carbon, 54·55%; hydrogen, 8·3%. $C_{13}H_{23}O_4N_3$ requires: carbon, 54·73%; hydrogen, 8·07%.

Chaksine oxalate. $(C_{11}H_{20}O_2N_3)_2\begin{pmatrix}COO\\|\\COO\end{pmatrix}$.

Saturated sodium oxalate (5 c.c.) was added to 2 gms. of 20% chaksine chloride solution. The white precipitate so obtained was filtered, washed with water and crystallized from water in colourless long needles. m.p. 306–8° d.

Found: carbon, 53·15%; hydrogen, 7·62%. $C_{24}H_{40}O_8N_6$ requires: carbon, 53·33%; hydrogen, 7·40%.

Chaksine malonate. $(C_{11}H_{20}O_2N_3)_2\begin{pmatrix}COO\\\diagdown\\CH_2\\\diagup\\COO\end{pmatrix}$.

Sodium salt of malonic acid was prepared by neutralizing 2 gms. of malonic acid with 10% sodium chloride solution. It was then added to 2 gms. of 20% chaksine chloride solution. The white precipitate after washing with cold water, was crystallized from hot water in beautiful long needles. m.p. 180–82° C. (effer.)

Found: carbon 54·21%; hydrogen, 7·46%. $C_{25}H_{42}O_8N_6$ requires: carbon, 54·15%; hydrogen, 7·58%.

The other derivatives as given in Table I were prepared in similar manner.

From the table it is evident that the percentage of carbon and hydrogen as found by us is quite in agreement with those calculated from Dr. Ray's formula ($C_{11}H_{20}O_2N_3X$) and not with that put forward by Dr. Siddiqui ($C_{12}H_{20}ON_3X$).

The authors take this opportunity to thank Dr. Bashir Ahmad, Ph.D. (London), for his keen interest and encouragement in the work.

TABLE I.

No.	Chaksine Salt.	Solvent for crystallization.	m.p. °C.	Molecular formula.	$C_{11}H_{20}O_2N_3X$				$C_{12}H_{20}ON_3X$ Calculated. (Siddiqui's formula).	
					Found.		Calculated. (Ray's formula).			
					% C	% H	% C	% H	% C	% H
1	Acetate	Absolute alcohol-ether mixture.	218–220 C. ..	$C_{13}H_{23}O_4N_3$	54·55	8·3	54·77	8·07	59·78	8·18
2	Oxalate	Water ..	306–308 d ..	$C_{24}H_{40}O_8N_6$	53·15	7·62	53·33	7·4	58·64	7·51
3	Malonate	Water ..	179–182 effer.	$C_{24}H_{42}O_8N_6$	54·21	7·46	54·15	7·58	59·34	7·69
4	Succinate	Water ..	192-93 effer.	$C_{26}H_{44}O_8N_6$	54·88	7·6	54·92	7·74	60·0	7·85
5	Adipate	Water ..	199-200 ..	$C_{28}H_{48}O_8N_6$	56·62	8·4	56·37	8·05	61·22	8·16
6	Tartrate	Water ..	290 d ..	$C_{26}H_{44}O_{10}N_6$	52·4	7·6	52·0	7·33	56·75	7·43
7	Citrate	Water ..	210 d ..	$C_{30}H_{66}O_{13}N_9$	53·72	7·84	53·91	7·6	58·87	7·71
8	Maleiate	Water ..	232 d ..	$C_{26}H_{42}O_8N_6$	55·54	7·2	55·12	7·42	60·21	7·52
9	Citraconate	Water ..	190 d ..	$C_{27}H_{44}O_6N_6$	55·12	7·94	55·86	7·58	60·84	7·69
10	Aconitate	Water ..	205 d ..	$C_{30}H_{54}O_{12}N_9$	55·3	7·77	55·06	7·42	60·21	7·52
11	Phenyl Acetate ..	Water ..	210 ..	$C_{19}H_{27}O_4N_3$	62·94	7·64	63·15	7·48	67·22	7·56
12	Cinnamate	Water ..	176–78 ..	$C_{20}H_{27}O_4N_3$	64·76	7·64	64·34	7·24	68·29	7·31
13	Phthalate	Water ..	200 ..	$C_{30}H_{40}O_8N_6$	58·04	7·82	58·44	7·14	63·15	7·23
14	Sulphamilate	Water ..	215 ..	$C_{17}H_{26}O_5N_4S$	50·88	6·81	51·25	6·53	56·98	6·59

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Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 22 ZOOLOGY Pp. 99-119

September, 1952

THE MILLIPEDE SPERM

by

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Price Rs.3-6-0

THE MILLIPEDE SPERM

By VISHWA NATH *and* GANPATI PARSHAD SHARMA

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PREVIOUS WORK

To the best of our knowledge the earliest work on the sperm of chilognaths is by Gilson (1886), who gave a very brief account of the ripe sperms of *Glomeris marginata, Polydesmus complanatus*, three species of *Julus*, and *Blaniulus guttulatus*. In the last-named species he described a filiform sperm, but in *Julus* he described a cup-shaped sperm reminding one of the Decapod sperm. Judging from the scanty data available Gilson also described a cup-shaped sperm in *Polydesmus complanatus*, although the cup in this form seems to be very shallow as compared with that of the *Julus* sperm. In *Glomeris marginata* he figures a peculiar spindle-shaped sperm in which the nuclear matter, according to the author, gets dispersed in the cytoplasm on the dissolution of the nuclear membrane.

In 1909 Oettinger published a long paper illustrated by many figures on the maturation and formation of spermatozoa in *Pachyiulus varius*. Although this paper deals mainly with chromosomes, the author, in an attempt to prove that the millipede sperm is a normal sperm, has described an axial filament, a sharp point (acrosome?) and a middle-piece. It is very difficult to follow this author, and, in our opinion, most of Oettinger's homologies are wrong.

In 1914 Sokoloff described in *Polyxenus* sp. a diatom-shaped spermatozoon in the male, which, on transference to the receptaculum seminis of the female, becomes transformed into a long, tightly-wound spiral thread. It is difficult to accept this account.

The last paper on the subject is by Warren (1934). In the five species of millipedes examined (*Odontopyge* sp., *Poratophilus diplodontus, Chersastus ruber, Ulodesmus bispinosus* and *Sphaerotherium punctulatum*), Warren described the ripe or semi-ripe spermatozoa as rounded, oval or sub-triangular in front view, and bi-convex (bilateral) or plano-convex or saucer-shaped (unilateral) in edge view, and, according to Warren, they are presumably non-motile. Warren's main object is to show that normal spermatozoa can arise from sex-cells, which have undergone amitosis, and that nuclei without the production of chromosomes may give rise to more than one spermatozoon. Warren is of the opinion that the extent to which amitosis occurs in growing tissues has been gravely underestimated by cytologists through theoretical considerations, and that the general view that amitosis in the metazoa is to be regarded as a degenerate process is not true. 'In the whole course of the investigation of the five

species of millipedes, karyokinesis has not been observed in the case of the nuclei of the walls of the vasa deferentia or reticular ribbon, although many individuals of various ages have been sectioned, and notwithstanding the fact that these nuclei are capable of producing spermatozoa. Mitosis, however, occurs very abundantly in certain of the lateral lobes, but it is remarkable that just those lobes which exhibit it in an unmistakable manner are the ones which ultimately produce abortive spermatozoa or glandular tissue. It would almost appear as if the physiological functions of glandular tissues require karyokinesis, and that mitosis is not necessary for the sex-cells.' (Warren, p. 356.)

All will agree with Warren that his findings, *if true*, will lead to a 'complete reversal of the prevailing views as to the intimate connection of karyokinesis with the determination of sex and the transmission of hereditary characters'. But a careful examination of Warren's script and figures in the light of our own work has convinced us that his conclusions are wholly unwarranted and that he has grossly misinterpreted appearances which are normal in spermatogenesis and with which a student of spermatogenesis is so familiar. Under the chapter on Discussion we shall have occasion to refer to Warren's figures and to show how and where he has erred in the light of our own observations. But here it is necessary to give a summary of Warren's work.

The ground-plan of the male generative organs of the five species of millipedes examined by Warren is as follows:—

On each side of the body, situated ventral to the gut, and immediately above the nerve-cord, are two vasa deferentia extending backwards from the external apertures and ending bluntly at some little distance from the posterior extremity of the body. The right and left vasa deferentia are connected together by a series of regularly spaced transverse tubes, like the rungs of a ladder. The vasa deferentia bear laterally a series of stalked structures, which Warren calls the lateral lobes. In their origin and general appearance these lobes are similar, but they differ in their ultimate fate; some are spermatic lobes and produce sperm, others are of the nature of accessory genital glands, and these glandular lobes may vary among themselves in histological character. Typically the walls of the vasa deferentia and of the transverse connections are capable of producing spermatozoa, and such spermatozoa differ in some respects from those formed in the spermatic lobes. Some of the lobes produce abortive sperm, and the purely glandular lobes which do not form spermatozoa are not all the same. Warren, therefore, concludes that there is a very considerable complexity in the histology and physiological functions of the different parts of the generative organs. As we shall show later on this is a wholly unwarranted conclusion of Warren, who does not seem to be familiar with even the broad outlines of spermatogenesis in general.

According to Warren the sperm-development of all the five species described by him conforms to the same general plan, but each species exhibits some variation and some points of special interest. Thus in *Odontopyge* the histological character of the lateral lobes is particularly diverse—some giving rise to bilateral sperm and others to unilateral sperm,

and others again producing abortive sperm or purely glandular tissue; in *Poratophilus* the lateral lobes, so far as observed, are mostly glandular, but a few produce obviously defective sperm; in *Chersastus* the spermatozoa are all unilateral, and in certain lateral lobes they are produced singly or in 2's, 3's, 4's and occasionally in 5's or even 6's from the cell elements; in *Ulodesmus* the spermatozoa are extremely minute, with a diameter of about 0·7μ; in *Sphaerotherium* the lateral lobes exhibit a simpler condition —some of them produce spermatozoa from special nuclei, while the remaining cell-elements are gradually absorbed as the central mass of sperm increases in size, and the rest of the lobes form only small-celled glandular tissue or degenerate sperm.

Material and Methods

In view of the confused state of literature available on the subject, particularly in view of the revolutionary claims made by Warren, and further, bearing in mind the experience gained in this Laboratory of the non-flagellate sperms of the Decapod Crustacea (Nath, 1932, 1937 and 1942), it was considered profitable to study the sperm of the large millipede, *Thyroglutus malayus*, available at Solan in Simla Hills in the Punjab. Consequently one of us (G.P.S.) paid a visit to Solan in the Summer of 1940 with the object of fixing the male gonads of the millipede.

A plentiful supply of the millipede cannot be obtained till about the middle of August. The males can be easily distinguished from the females by the presence of a pair of dark copulatory appendages on the ventral surface of the 7th segment. The male gonads of *Thyroglutus malayus*, as is usual in the millipedes, are unpaired, consisting of a bunch of grape-like lobes and lying underneath the alimentary canal. They extend almost to the posterior end of the animal.

The testicular material was fixed in Flemming, without acetic, for at least 24 hours and washed in running water for the same period so as to remove all traces of chromic acid and osmic acid. The material was dehydrated as usual and cleared in cedar-wood oil. It was subsequently embedded in paraffin at Lahore in the month of October 1940, and sections were cut 5 to 6 microns thick. These were stained with 0·5% iron-haematoxylin. Bouin's fluid followed by 0·5% iron-haematoxylin was also used as control.

The millipedes were identified as *Thyroglutus malayus* (Carl.) belonging to the order Spirostreptoidea and family Harapagophoridae by the Zoological Survey of India.

Although our investigations were completed by the end of 1941, it has not been possible to go to the press earlier on account of the non-availability of Oettinger's paper in this country. Nor was it possible to obtain it from abroad on account of the war. It was only in 1946 that one of us (G.P.S.) studied Oettinger's paper at Edinburgh where he was sent as State Scholar by the Government of India. From there he sent photographs of Oettinger's figures and an English translation of his (Oettinger's) script to his co-author (V.N.) who prepared the paper for publication at Lahore. Before it could be sent to the press, however,

partition of the country took place and the manuscript along with figures was, therefore, left at Lahore (Pakistan). A few years later the senior author (V.N.), while going through his papers which were sent to him from Pakistan by the courtesy of Dr. Nazir Ahmed, came across the manuscript of this paper, which he had prepared at Lahore. In 1951, while conducting an examination at Allahabad, he also got hold of a slide showing the serial sections of the testicular material of *Thyroglutus malayus*. This had been presented to Dr. Bhattacharya of Allahabad, when he had gone to Lahore to examine the junior author (G.P.S.) for the award of an M.Sc. degree in 1941. The figures presented in this paper have been recently redrawn by the junior author (G.P.S.) from this slide as well as from fresh preparations, which were made by him last year from the testicular material of another large millipede, which is also available in Hoshiarpur.

Observations

Spermatogonia.—As is usual in spermatogenesis the spermatogonia have large vesicular nuclei (Plate I, figs. 1 to 3). The spermatogonial nucleus hardly reveals any structure save for the presence of a nucleolus, which is generally excentric. As revealed by haematoxylin-staining, the nucleolus appears to be of the nature of an amphinucleolus, showing a ground substance of a lightly staining material in which are embedded a number of small deeply staining granules (Plate I, figs. 2 and 3). The presence of a single prominent nucleolus is one of the diagnostic features of a spermatogonial cell.

The mitochondria appear as pale, fine granules in the cytoplasm arranged in the form of a juxta-nuclear mass generally situated opposite the nucleolus (Plate I, fig. 1). They are delicate granules, and they stain so feebly with iron-haematoxylin that it is not always possible to demonstrate them.

In sharp contrast with mitochondria the Golgi elements stain deeply with haematoxylin. They exist in the form of granules, appreciably bigger than the mitochondria, and are generally situated in the vicinity of the nuclear membrane (Plate I, fig. 1). In the fully grown spermatogonia, both the mitochondria and the Golgi elements tend to become circum-nuclear in arrangement (Plate I, fig. 3).

During spermatogonial mitosis the mitochondria and the Golgi elements are more or less evenly sorted out to the two daughter cells (Plate I, fig. 4). In this process it is always possible to follow the sorting out of the mitochondrial granules as they are so numerous, but the Golgi elements, being few in number, are not always so easily traceable. The spermatogonial spindles are not well defined. Each spindle is a fibrillar and fusiform structure with a sharply staining centrosome at each pole (Plate I, fig. 4). Very often the spindle is completely masked by a mantle of mitochondrial granules. Astral rays are conspicuous by their absence.

A testicular lobe consists of many follicles. The spermatogonia studied and described by us are from follicles which do not contain any other kind of sperm-forming cell. At any rate we have never observed

spermatogonia co-existing with spermatocytes or spermatids or sperms in the same follicle.

Each spermatogonial follicle in a testicular lobe is ensheathed by a thick connective tissue covering in which very small nuclei can be seen here and there. A follicle has its own lumen and the lumina of all the follicles in a lobe unite together to open into the vas deferens.

Nurse-cells.—A follicle containing primary spermatocytes or secondary spermatocytes and spermatids in different stages of spermateleosis has invariably a very distinct layer of what we must define as nurse-cells, lying immediately below the outermost sheath of connective tissue.

A nurse-cell is a fairly large cell (Plate I, fig. 5). Its nucleus has a number of not very deeply staining minute granules distributed uniformly. A small nucleolus is also invariably met with immediately under the nuclear membrane.

It seems likely that in a developing follicle the outlying spermatogonia directly form the nurse cells, while the remaining ones form the primary spermatocytes after the usual process of growth. It is hardly necessary to point out that this conclusion is in conformity with what happens in spermatogenesis and even in oogenesis in general.

Primary spermatocytes.—The primary spermatocytes are definitely larger cells than the spermatogonia, their nuclei also being larger (Plate I, figs. 6 to 8). The complete absence of a nucleolus is one of the diagnostic features of the primary spermatocyte in sharp contrast with the prominent nucleolus of a spermatogonium. During early prophase I faintly staining spireme threads appear in the nucleus and the nucleolus seems to be completely broken up into a large number of deeply staining chromatin granules which are generally arranged on the inner surface of the nuclear membrane (Plate I, figs. 6 to 8).

The mitochondria of the primary spermatocyte are granular as in the spermatogonia but, on account of their uniform distribution in the cytoplasm, it is not always easy to demonstrate them. On the other hand, the Golgi elements have a distinct tendency to come together and form fairly big bodies which can be easily demonstrated (Plate I, figs. 6 to 8).

During the first meiotic divisions, beautiful and well-defined spindles can be seen (Plate I, figs. 9 to 11). Like the spermatogonial spindles, these are fibrillar and fusiform structures with a sharply staining centrosome at each pole, but these are definitely bigger. Both the mitochondria and the Golgi elements are seen to be distributed almost evenly to the two daughter secondary spermatocytes.

Secondary Spermatocytes.—Each resting secondary spermatocyte is similar to the primary but it is definitely smaller than the latter (Plate I, fig. 12). The second meiotic divisions are definitely of two kinds, early and late. In the early divisions all cells of a follicle are found to be dividing —some cells are in metaphase, others in anaphase and still others in early or late telophases. In these early second meiotic divisions the spindles are large and the centrosomes are only slightly larger, if at all, than the centrosomes found at the poles of the spindles of the first meiotic divisions (Plate II, figs. 14 to 16). But follicles are often met with in which most of

the cells have gone far beyond telophase II and have already passed through the early stages of spermateleosis. Amongst such spermatids one may come across cells which are still in metaphase II. Two such cells have been drawn in figs. 17 and 18, Plate II. It may be noted that in these figures the spindles are very small and the centrosomes are much more prominent.

The more or less even sorting out of both the mitochondria and the Golgi elements can be seen again during the second meiotic divisions (Plate II, figs. 13 to 18).

Spermateleosis.—In the earliest spermatid, as shown in fig. 19, Plate II, the chromosomes are seen fusing together to form the nucleus. The latter during the resting stage reveals a number of fine chromatin granules inside it (Plate II, fig. 20). In these early spermatids the mitochondria are distributed uniformly throughout the cytoplasm and a few prominent deeply staining Golgi granules are seen here and there. In fig. 19, Plate II, can also be seen another darkly staining granule which is surrounded by a clear area. This is most probably the centrosome. In fig. 20, Plate II, the centrosome has grown fairly big in size and can be easily distinguished from the Golgi elements.

In the ensuing process of spermateleosis important changes take place both in the nucleus and the cytoplasm by means of which the ripe spermatozoon assumes an atypical and saucer-shaped appearance. This important process is heralded by the movement of the centrosome and the nucleus towards the periphery of the cell (Plate II, figs. 21 to 24). As this movement is going on the centrosome grows still bigger in size and ultimately becomes ring-like. In fig. 22, Plate II, a sharply staining granule can also be seen at the junction of the nucleus and the ring-like centrosome. This is the centriole. The wall as well as the contents of the anterior end of the nucleus at this stage start taking up deeper stain than the posterior end (Plate II, figs. 21 to 24). This is the beginning of the formation of a cup-like nucleus. The centrosome now fuses completely with the nucleus and as this also stains intensely it is difficult to make out its real structure. In some of the top views of the maturing spermatids, however, the centrosome can be clearly seen to be in the form of a deeply staining ring with a sharply staining granule or centriole in the centre (Plate II, fig. 27 and Plate III, figs. 28 to 33). Outside the ring-like centrosome there is a clear area, which does not take up any stain. In side views of the maturing spermatids the centrosome generally appears as a slightly compressed and conical body staining uniformly and deeply with haematoxylin (Plate II, figs. 23 and 24). Sometimes, however, the ring-like character of the centrosome may be revealed even in the side views when it may appear as a crescent with a sharply staining granule or centriole at the tip of the cone (Plate II, figs. 25 and 26). The clear area surrounding the centrosome can also be seen just below the crescent.

As the nucleus is attaining its cup-like form small vacuoles appear in the cytoplasm and both the mitochondria and the Golgi elements seem to merge into these (Plate II, figs. 24 to 27 and Plate III, figs. 28 to 30).

When the wide and shallow nuclear cup is completely formed it appears to be quite empty (Plate III, figs. 33 to 42 and Plate IV, figs. 43, 44 and 46 to 48). The chromatin in it is distributed in what we consider a unique manner. The whole of it, in the form of fine chromatin granules, seems to be aggregated just in the mouth of the nuclear cup.

In all these late spermatids the cytoplasm appears frothy due to the presence of a large number of vacuoles. As they always stand out prominently both in smears and sections they seem to have a firm consistency. The mitochondria are still granular but the Golgi elements at this stage have a distinct tendency to come together to form bigger masses (Plate III, fig. 41). Nevertheless, like the mitochondria, they also merge into these vacuoles, and thus no such structure as the acroblast or the acrosome is formed.

By a two-fold process of growth and coalescence the vacuoles in the cytoplasm form a large vesicle, the cytoplasmic vesicle (Plate II, figs. 24 to 27; Plate III, figs. 28 to 30 and 34 to 42 and Plate IV, figs. 43 to 48). In appearance it is structureless and hyaline. Judging from the fact that it never becomes distorted in form in finished preparations it seems to be quite firm. This cytoplasmic vesicle which has a glue-like consistency goes on growing till the entire cytoplasm is absorbed.

Just before the ripe sperm is formed the cytoplasmic vesicle either bursts or is pushed inside the nuclear cup. The shape of the nucleus of the ripe sperm and the arrangement of the chromatin granules in it, however, strongly indicate that the cytoplasmic vesicle is pushed inside the nuclear cup. It will be recalled that in the late spermatids the chromatin granules are arranged uniformly only in the mouth of the nuclear cup, the rest of it being quite empty. In the ripe sperm, however, the chromatin granules can also be seen arranged just beneath the nuclear wall (Plate IV, figs. 50 to 52). This seems to be the result of the entry of the cytoplasmic vesicle into the nuclear cup. It is further proved by a section of the ripe sperm (Plate IV, fig. 49) in which no chromatin granules are seen beneath the nuclear wall. Instead there is only a shaded area which is most probably the cytoplasmic vesicle. Similarly in the top view of a ripe sperm (Plate IV, fig. 53) one can see a ring-like centrosome with a sharply staining granule or the centriole in the centre. Outside the clear area round the centrosome there is a darkish granular area; and a similar dark granular area is seen just inside the outer deeply staining line, which represents the rim of the nuclear cup. Both these darkish areas represent chromatin concentration at the base and the mouth of the nuclear cup, the intervening area being lighter.

The ripe sperm, therefore, is a simple structure, consisting of three cell-elements only, viz., the nuclear cup, the large ring-like centrosome and the cytoplasmic vesicle. The acrosome, the middle-piece and the axial filament of the typical flagellate sperm are conspicuous by their absence.

The rim of the nuclear cup in the very much condensed ripe sperm of *Thyroglutus malayus* or any other millipede is very misleading indeed. In side views it takes up intense stain and appears to be a new structure

that has put in its appearance at the distal end of the cytoplasmic vesicle to plug the mouth of the nuclear cup (Plate IV, figs. 49 to 51). But a careful examination of all the stages in spermateleosis has clearly revealed that this interpretation is wholly wrong. The deeply staining plug-like thing in side views is only the chromatin-rich rim of the nuclear cup along with a portion of the cytoplasmic vesicle which is showing through it.

Mention may be made here of a very fine process on each side of the mouth of the nuclear cup which may sometimes appear in the side view of a ripe sperm (Plate IV, figs. 50 and 51). Such a process has been figured by Gilson (1886) also in the ripe sperm of *Julus*, but he does not make any mention of it in the text. This process is obviously due to the stretching out of the rim of the nuclear cup consequent on the compression of the sperm from side to side.

Lastly we may also refer to the so-called bilateral spermatozoa described by Warren (1934). In fig. 57, Plate IV, we have shown a cell which, according to Warren, will be a bilateral spermatozoon. In reality, however, these are two ripe spermatozoa which have arisen quite normally from their respective spermatids with the only difference that the furrow separating the two daughter spermatids has not so far appeared in this particular secondary spermatocyte. This will be made amply clear if a careful study is made of our figs. 54 to 56, Plate IV. In all these figures it will be noticed that the process of spermateleosis is going on without the complete separation of the daughter spermatids after the second meiotic division. Such a phenomenon has also been noticed in the spider, *Plexippus paykulli* (Sharma, 1950).

Discussion

To sum up: the spermatozoon of *Thyroglutus malayus* is a simple cell, consisting of three cell-elements only, viz., the nucleus, the centrosome and the cytoplasmic vesicle. The nucleus is in the form of a shallow cup with a circular mouth. At the bottom of the nuclear cup lies the ring-shaped centrosome; and the cavity of the nuclear cup is completely filled up by the cytoplasmic vesicle. The result is that the chromatin of the nucleus is not uniformly spread out all over the cup, but is confined to a narrow area just beneath the walls of the nuclear cup. The chromatin-rich rim of the nuclear cup, therefore, appears as a granular disc perforated by the circular mouth in front view (Plate IV, fig. 53) and as a deeply staining transverse bar in side view (Plate IV, figs. 50 and 51). This deeply staining area round the mouth of the nuclear cup has been figured by Gilson (1886) in the side view of the sperm of *Julus* (figs. 770, 771, 773 and 775) and by Warren (1934) in a similar view of the sperm of *Odontopyge* sp. (Pl. XVIII, figs. 26 to 28); but both these authors have not made any attempt to explain what the structure really is.

The mitochondria and the Golgi elements have been followed from the spermatogonium up to the spermatid in *Thyroglutus malayus*; but both these cell-elements degenerate and merge into the cytoplasmic vesicle during spermateleosis.

In its general make-up the millipede sperm closely resembles the Decapod sperm (Nath, 1932, 1937 and 1942), more particularly the sperm of the Macruran forms, *Penaeus indicus* and *Panulirus polyphagus*, except that the centrosome in the millipede sperm is proximal and not distal. The millipede sperm also resembles more particularly the sperm of 19 Brachyuran genera described by Nath, except that in the millipede sperm there is a single centrosome, whereas in the latter there may be two centrosomes in some species and three in others. Other points of resemblance between the millipede sperm and the Decapod sperm are (1) the cup-like nucleus, (2) the presence of a cytoplasmic vesicle which fills up the cavity of the nuclear cup, (3) the absence of an acrosome on account of the complete disappearance of the Golgi material of the spermatid, (4) the absence of a middle-piece, and (5) the absence of the flagellum-like tail.

The details of the process of formation of the cytoplasmic vesicle in *Thyroglutus malayus* seem to be similar to those found in the spermateleosis of many species of Brachyura described by Nath (1932 and 1942). But in *Thyroglutus* the mitochondria are too small and difficult to stain and for this reason it is impossible to be certain whether the vacuoles which appear in the cytoplasm of the spermatid arise *de novo* or they are the mitochondria which become vacuolar as in the Brachyura.

Warren's figures (1934) are generally correct, but his interpretations being grossly inaccurate, he has arrived at startling conclusions, which cannot be allowed to go unchallenged. Warren states that he has seen ripe spermatozoa in the vasa deferentia or reticular ribbon of *Odontopyge* sp. He further states that he failed to see karyokinesis in the case of the nuclei of the walls of these tubes. From this he jumps at the conclusion that 'these spermatozoa in the reticulum have not simply been received from the lateral lobes, but they have originated *in situ*, for isolated islands of developing sperm can be seen in chambers in the tubes, bounded in front and behind by the walls, which may be so swollen that the lumen of the tube is closed (text-fig. 2, *is*).'—(p. 361).

In Warren's text-fig. 2, the 'isolated islands of developing sperm' are *not* 'bounded in front and behind by the walls'; on the contrary these islands of the so-called developing sperm communicate through the lumen of the tubes with the lumen of the lateral lobes. But even if the lumina of the vasa deferentia were temporarily blocked, it certainly does not follow that the sperms found in these tubes 'have originated *in situ*' and have not come from the lateral lobes. Equally unwarranted is the conclusion of Warren that the spermatozoa found in these tubes originate from the nuclei lining them by a process of amitosis, simply because Warren failed to see karyokinesis in the case of the nuclei of the walls of the vasa deferentia. Moreover, Warren's figures of amitosis in the nuclei of the walls of vasa deferentia are unconvincing (e.g., fig. 8, Pl. XVIII).

While describing the genesis of the spermatozoa found in the reticulum (vasa deferentia), Warren has mixed up these tubes with the lateral lobes in which all stages of spermatogenesis can be found. By itself this error perhaps would not have been very serious, but Warren has grossly.

misinterpreted the stages he has seen in the lateral lobes and given a very inaccurate account of spermatogenesis.

Warren repeats that mitosis has not been observed in the vasa deferentia, while describing the genesis of spermatozoa found in these tubes. 'The nuclei of the wall proliferate, and the inner ones subsequently swell by the absorption of cytoplasm. At the same time the swollen nuclei become surrounded by a sharply defined membrane differentiated out of the cytoplasm immediately in contact with them. The structures are rounded but slightly compressed, and thus the future bilateral symmetry is early indicated (Pl. XVIII, figs. 10 to 13). The chromatin becomes dissolved and loses its basophil character, and there appears a somewhat large, central, globular body which entirely fails to stain with Delafield haematoxylin but stains black with iron-haematoxylin (fig. 20). The nature of this body is unknown; possibly it is chondriosomal, but it may provisionally be called plasmosome. Except for the globular body or plasmosome, the substance of the nucleus becomes homogeneous, and it is also neutrophil (fig. 10, *h.n.*; fig. 20).'—(p. 360).

Fig. 20, Pl. XVIII of Warren illustrating the homogeneous and swollen nucleus, which is alleged to have arisen amitotically and forms an entire sperm, is really the front view of the ripe sperm. The so-called plasmosome is really the large centrosome[1], which has taken its position at the bottom of the nuclear cup. The dark outermost circular line in Warren's fig. 20 is the wall of the nuclear cup and the homogeneous body (Warren's nucleus) is really the hyaline structureless cytoplasmic vesicle, which fills up the cavity of the nuclear cup. Similarly the homogeneous nucleus (*h.n.*) of Warren's fig. 10 is most certainly the developing cytoplasmic vesicle.

That Warren has mixed up the sections of the vasa deferentia and the spermatic lobes is clear from his own description of figures. Figs. 10 to 13, Pl. XVIII should be all sections of the vasa deferentia as he is describing the genesis of the spermatozoa of the reticulum, insisting that they originate *in situ* and have not come from the spermatic lobes. And yet in the legend he describes fig. 10 as representing a section of the spermatic lobe. Again figs. 11, 12 and 13 represent, according to the legend, sections of the reticulum tubes, but in the explanation of fig. 13 he talks of primary spermatocytes transforming into bilateral spermatozoa.

Warren gives a most fantastic and inaccurate account of the origin of the so-called bilateral spermatozoa found in the vasa deferentia from a single homogeneous nucleus, which, as we have already shown, is really the cytoplasmic vesicle. On p. 360 Warren continues: 'The plasmosome, *which is really the centrosome*[2], divides into two, and the halves migrate from a central position to the middle of the two opposite, slightly compressed convex surfaces (figs. 21, 22, *P*). An indication of a change in the character of the homogeneous substance is now given by the assumption of a slight

[1] It may be stated here at once that Warren does not make a mention of the centrosome even once in his paper, although he has described and sketched mitotic figures in the lateral lobes.

[2] Italicized part ours.

basophil tendency at the periphery of the two convex surfaces, each of which carries a plasmosome (fig. 23). The compression of the nucleus now increases, and ultimately the structure becomes biconvex lens-shaped (fig. 25). A small eminence[1], often flat-topped, is formed in the centre of the two opposite convex surfaces immediately over the site of the plasmosome.'

These so-called bilateral spermatozoa of Warren are really formed from two sister spermatids, which do not separate for a long time or from a secondary spermatocyte whose nucleus has divided into 2 spermatid nuclei but in which the appearance of the cytoplasmic furrow has been delayed. Warren has himself admitted 'that the bilateral spermatozoa formed in the reticulum fairly frequently split in two symmetrical halves ....'—(p. 361). From a single 'homogeneous nucleus' Warren derives not one spermatozoon (unilateral) but two spermatozoa (bilateral); and he considers the homogeneous nuclei which produce the bilateral structures in the vasa deferentia as equivalent to those of the secondary spermatocytes. This last statement of Warren would be correct if the homogeneous nuclei were not really the cytoplasmic vesicles of mature sperms.

Equally arbitrary is the division of the lateral lobes or spermatic lobes of the testis in six categories in *Odontopyge* sp. Warren has based this division on the histological appearances of the lobes, although 'externally there is no marked difference in the lobes, except that they diminish in size both at the anterior and posterior ends of the series, and the stalk of attachment to the reticular tubes varies in width and length (text-figs. 1, *a*; 2).'—(p. 362). These six classes of lobes are (1) smaller lobes situated mostly at the anterior end of the series and producing bilateral spermatozoa, (2) lobes bearing unilateral spermatozoa, which arise in pairs, (3) large-celled lobes which apparently have a glandular function, (4) lobes producing solitary, unilateral spermatozoa which are apparently non-functional, (5) small-celled lobes of glandular function, and (6) reticular lobes, mainly glandular in function and confined to the more posterior portion of the series.

Warren is obviously ignorant of the manner in which various stages of spermatogenesis are met with in a testis. He does not seem to know that a testicular follicle or lobe will show at different periods all stages of spermatogenesis. He has seen a spermatic lobe in a particular stage and has placed it in a category of its own, not knowing that the contents will change at a later stage. In spite of the very confused account which Warren has given of the spermatic lobes, it is possible to say what stages in spermatogenesis the contents of these lobes represent.

1. *Lobes Producing Bilateral Sperm.*—A reference to Warren's text-fig. 2 and figs. 15 and 16, Pl. XVIII will at once show that the lobes figured there are not special lobes but ordinary lobes containing ripe spermatozoa. In figs. 15 and 16 inactive spermatogonia showing vesicular nuclei are also present towards the periphery of the lobes. As has already been explained

[1] This eminence is really formed by the centrosome.

the so-called bilateral sperms are two sperms arising from two sister spermatids which have not yet separated.

2. *Lobes Bearing Unilateral Sperms in Pairs.*—According to Warren these lobes (Pl. XIX, figs. 41, 50 and 51) may retain a few vesicular nuclei with scattered chromatin grains, and he believes that they are probably special gland cells. On the contrary we believe that the vesicular nuclei are those of the inactive spermatogonia. Fig. 51 shows a large number of spermatogonial nuclei towards the periphery, whereas towards the interior are to be found a large number of cytoplasmic vesicles (Warren's homogeneous nuclei) to each of which is attached either one nuclear cup or two nuclear cups, each nuclear cup having its own centrosome (Warren's plasmosome). By figs. 52 to 61 Warren attempts to illustrate the exact manner in which these spermatozoa arise from 'homogeneous nuclei', but his account is grossly inaccurate. Figs. 52 and 53 really represent spermatogonial nuclei with scattered chromatin grains (Warren's special gland cell nuclei). These nuclei, according to Warren, give rise to 'homogeneous nuclei' as shown in figs. 54 to 61. Now a glance at these figures will show that figs. 54 and 55 represent two separately growing sperms in front view, and Warren has mistaken the cytoplasmic vesicle for the homogeneous nucleus, the centrosome for the plasmosome, whereas the real nucleus is indicated by the outermost dark circular line. Similarly each of the figures 56 to 61 represents two maturing spermatids in side view, which have not separated. Warren derives the swollen nuclei, which are really spermatogonial nuclei (figs. 52 and 53), directly from the small, oval nuclei of the young lobe, but we must reject the account of amitosis of these vesicular nuclei as given by him (figs. 32 to 34).

3. *Large-celled Glandular Lobe.*—The cells forming these lobes are of considerable size, with large nuclei rich in chromatin. Cell-outlines are conspicuously present. 'Subsequently the chromatin of the large nuclei breaks up into a cluster of rounded chromosomes, and the nuclear membrane disappears (text-fig. 3, *g*). These cells may then undergo mitotic division, but the cells thus formed are still of considerable size and the chromatin passes into an amorphous condition (Pl. XVIII, fig. 29). At the same time, the cytoplasm becomes impoverished, and ultimately it mostly disappears, and there remains a skeletal network of cell membranes with each compartment containing a ragged lump of deeply staining chromatin which represents the degenerated nucleus (text-fig. 3, *J*).'—(p. 365). Warren assumes 'that the lobe has a secretory function, and possibly it produces a hormone. Most certainly it never forms spermatozoa of any kind.'—(p. 365).

We have no doubt that the large cells described by Warren in these lobes are the primary spermatocytes and the mitotic divisions described by him are the first meiotic divisions. Probably he has mixed up the second meiotic divisions with the first, as is strongly indicated by the fact that he is really describing maturing spermatids with vacuoles developing in the cytoplasm when he talks of the cytoplasm becoming impoverished, and ultimately disappearing, there remaining a skeletal network of cell membranes with each compartment containing a ragged lump of deeply staining chromatin, which represents the degenerated nucleus.

4. *Lobe producing solitary abortive spermatozoa.*—Under this heading Warren begins correctly, but unfortunately ends wrongly. He begins with a description of the large-celled lobes (Pl. XIX, figs. 36 to 38). These figures undoubtedly represent primary spermatocytes undergoing mitosis. Warren says that these cells undergo two mitotic divisions (which means that he has seen both the meiotic divisions), and the small cells thus formed are doubtless to be regarded as spermatids. The chromosomes of the spermatids form a small nucleus with nuclear membrane. The nucleus passes to the periphery of the spermatid (Pl. XIX, fig. 43). The cytoplasm of the spermatid practically disappears, and there is left a mere hollow vesicle to which the transformed nucleus is fused (Pl. XIX, figs. 35, 49, *a.sp.*; text-fig. 3, *i*). At last Warren describes correctly the cytoplasmic vesicle of the maturing spermatid as a hollow vesicle, and not erroneously as 'homogeneous nucleus' (vide *supra*). So far Warren's account is correct. But he erroneously assumes that 'active absorption clearly occurs in such lobes, and the nuclei, which are apparently quite incapable of separating from the vesicles as functional spermatozoa, degenerate.'—(p. 366). It is not known why Warren, having given a more or less correct account of the genesis of the sperm, should suddenly arrive at the conclusion that these spermatids, really maturing in a perfectly normal way, degenerate.

5. *Small-Celled Glandular Lobes.*—According to Warren, 'the spermatids produced by mitotic divisions in a large-celled lobe (text-fig. 3, *H*) may be converted into a mass of very small cells; and such a lobe is undoubtedly an accessory genital gland (Pl. XIX, figs. 39, 40, 42, *gl.t*; text-fig. 3, *k*).' A comparison of Warren's figures with ours clearly shows that Warren is describing the earliest spermatids; but his conclusion that such a lobe is an accessory genital gland is wholly unwarranted.

6. *Reticular Lobes.*—'These lobes are situated in the more posterior part of the series. The young club-shaped lobe develops into a lobulated structure with permanent wide canals (text-fig. 3, *d*). The walls of these canals consist of a plasmodium with rounded, granular nuclei (Pl. XVIII, fig. 18, *gl.n.*). Mitotic division can be found taking place among the nuclei (figs. 17, 18).'—(p. 366). According to Warren these rounded granular nuclei are undoubtedly secretory in function. This again is a wholly unwarranted conclusion. A comparison of Warren's fig. 18 with our figs. 1 to 4, Plate I, will clearly show that in fig. 18 Warren is really dealing with a lobe containing resting and dividing spermatogonia whose nuclei show one large nucleolus, which is definitely more prominent than the other granules found in the nuclei. Some of the spermatogonial cells in fig. 18 are dividing, showing metaphases and telophases. Scattered amongst the spermatogonial cells are a few minute, bilateral spermatozoa which Warren erroneously considers as non-functional.

Warren concludes in the case of *Odontopyge* sp.—(1) that abundant sperm is produced in a simple, direct manner in the walls of the reticulum, where no mitosis has been observed, and (2) that although in certain lateral lobes mitosis is prevalent and typical spermatids may be formed, yet these lobes are mostly converted into glandular tissues and produce very few, if any, functional spermatozoa. As shown above these conclusions of

Warren are wholly unwarranted as they are based on grossly inaccurate interpretations of faulty preparations. Warren used alcoholic Bouin as a fixative and in many cases he fixed the entire animal without dissecting out the testes.

In *Poratophilus diplodontus* Warren describes bilateral spermatozoa which, according to him, are produced in the vasa deferentia (figs. 65, 69 and 70, Pl. XIX). The so-called bilateral spermatozoa are really two spermatozoa arising from two sister spermatids, which have not separated; and Warren again erroneously describes them as having originated in the vasa deferentia. He also describes unilateral, abortive or degenerate spermatozoa, arising singly or in pairs from 'the homogeneous swollen nuclei', which, as in *Odontopyge*, are really the cytoplasmic vesicles (figs. 71 and 72, Pl. XIX) of maturing spermatids.

In *Chersastus ruber* Warren describes unilateral spermatozoa in the ladder-system (vasa deferentia and their transverse bridges), which he again erroneously derives from the walls of the tubes. The spermatozoa are shallow saucers in shape (fig. 95), which Warren compares to the unilateral sperm or to half of a bilateral sperm of *Odontopyge* or *Poratophilus*. They arise more usually in a single condition. As in *Odontopyge* and *Poratophilus* they are erroneously described having arisen from the so-called 'homogeneous nuclei', which are indeed the cytoplasmic vesicles of maturing spermatids (figs. 89 to 92). Pairs of unilateral spermatozoa are sometimes produced (figs. 93 to 95).

Warren again quite arbitrarily divides the spermatic lobes in *Chersastus ruber* into four classes:—(1) hollow lobes with thick walls producing sperm mostly in pairs, (2) lobes with moderately thick walls producing sperm mostly in groups of 2 to 6, (3) large-celled glandular lobes with extremely thin walls, and (4) lobes producing abortive sperm. It is easy to show, as we have shown in the case of *Odontopyge* sp., that the lobes are really all alike, but they simply contain sperm-forming cells in different stages of development.

The hollow lobe in the young condition increases in size by the multiplication of the undifferentiated nuclei through fission. These nuclei are situated as a layer surrounding the incipient lumen, and they may become conspicuously elongated before splitting transversely (Pl. XX, fig. 99). The outer nuclei thus formed become transformed into 'rosette-nuclei'. Ultimately all the undifferentiated nuclei in the body of the lobe are converted into rosette-nuclei. The thick wall of the body of the lobe bears mostly rosette-nuclei (Pl. XX, fig. 96). 'Some of the nuclei situated more internally swell and the chromatin dissolves, and there is formed a homogeneous, globular nucleus (*h.n.*). Subsequently, on opposite sides, two saucers of faintly staining chromatin are condensed around plasmosomes (fig. 98). The spermatozoa pass into the wide cavity of the lobe, and they may remain for some time coupled together in pairs (figs. 96, 97). The members of the pairs (*S.bi*), however, are only loosely applied to each other and readily separate (fig. 98). It would appear that a good many of the homogeneous swollen nuclei often fail to form the chromatin plates,

and they remain in the tissue as residual globules of large size.'—(pp. 372 to 374).

It is clear that once more Warren has made a serious error in mistaking cytoplasmic vesicles of sperms seen from front for the so-called homogeneous nuclei. Warren is rightly puzzled to have seen that a good many of the 'homogeneous swollen nuclei' often fail to form the 'chromatin plates', because the last-named structures, which are really the nuclear cups, cannot be seen except in the side view of the sperm.

Warren's figures of amitosis (Pl. XX, fig. 99) are unconvincing and his so-called 'rosette-nuclei' are possibly the nuclei of primary spermatocytes in prophase.

Warren's statement that 'these lobes, for some unknown reason, are not very satisfactory sources of production for functional sperm' is wholly unwarranted.

In the second type of lobe described in *Chersastus* by Warren (that is, lobes with moderately thick walls) he has described a peculiar phenomenon which clearly needs re-investigation. 'As the young lobe grows, the inner rosette-nuclei expand and become homogeneous, and the cavity of the lobe may become almost wholly occupied with the developing groups of spermatozoa (Pl. XX, figs. 101 to 106); the outer nuclei remain unchanged in a fairly thick, presistent wall (text-fig. 4, *c*). The expanded homogeneous nuclei (fig. 103, *h.n.*) give rise to a variable number of spermatozoa; some seem to produce none at all, the remainder form from one to six. The spermatozoa arise peripherally, the chromatin gradually condensing around plasmosome as faintly staining saucer-shaped structures. *The commonest number produced is four* (italics ours), but there are many 3's, also 2's and 1's. Less frequently 5 or even 6 can be counted.'

Strangely enough Warren rejects the only reasonable explanation of groups of four spermatozoa, viz., that a group of 4 spermatozoa has arisen from a primary spermatocyte which has undergone the usual two meiotic divisions without cytoplasmic cleavage. Regarding group of 3 spermatozoa it is possible that these are really groups of 4 spermatozoa but in section three spermatozoa are being seen from the side showing their respective nuclear cups, whereas the fourth sperm is being seen from the front (see Warren's fig. 103). Groups of 2 spermatozoa will obviously arise from 2 spermatids which have failed to separate; and a single sperm offers no difficulty whatsoever. Lastly Warren's account of the genesis of a sperm from the 'homogeneous nucleus' is so fantastic that no reliance can be placed on his statement that there are groups of 5 and 6 spermatozoa. It may be noted that the so-called 'rosette-nuclei' shown in figs. 101 and 103 are probably those of primary spermatocytes in prophase.

The large-celled glandular lobes of *Chersastus* have large nuclei with abundant floccular chromatin and surrounded by well-marked specialized cytoplasm (Pl. XX, figs. 115 and 116). A look at these figures will convince anybody that these nuclei are those of primary spermatocytes in or approaching the synizesis stage, the chromatin having already formed a knot in a few cases. Yet, for some unknown reason, Warren erroneously believes that these nuclei persist without further change and further supposes

that the tissue produces some substance which passes down the hollow stalk into the vas deferens, or is absorbed in the blood. It may be noted that these so-called glandular lobes actually contain spermatozoa in their lumen.

Lastly Warren describes lobes producing abortive sperm in *Chersastus*. He has described similar lobes in *Odontopyge* (vide *supra*). In these lobes he correctly describes the formation of spermatids from the primary spermatocytes by two mitotic divisions (Pl. XX, figs. 114, 117 and 118), but, for some unknown reason, he considers the spermatids maturing in a perfectly normal fashion as abortive.

In *Ulodesmus bispinosus* Warren describes the vasa deferentia as wide tubes extending backwards nearly straight. These tubes are connected by transverse tubes, forming a ladder-system. The lumen of the system contains a dense mass of very minute sperms which, according to Warren, are formed in the walls of the vasa deferentia. The spermatozoa have a diameter of about $0{\cdot}7\mu$ which is only about three times the diameter of the virus particle ($0{\cdot}23\mu$) of vaccinia. In support of this statement Warren puts forth the most astounding argument that because 'there seemed to be more sperm in the ladder-system than could be easily accounted for by the partially emptied lobes, it is quite likely that at an earlier stage the walls themselves had produced sperm.'—(p. 378).

The lateral lobes of *Ulodesmus* include both spermatic and glandular lobes. As the material was sparse and young specimens were not available, Warren says that he has not been able to give the full developmental history of these lobes. He has not observed mitotic figures in any part of the genital system of this species, but he has seen binary fission.

The account of the origin of spermatozoa in this species is perhaps more fantastic than that given for other species; and it is impossible to accept it. 'The nuclei of the concentric layers swell so that they come into mutual contact. The chromatin at first is irregularly dispersed in the form of granules and delicate branching strands (figs. 128, 130, 132 and 136, *p. sp.*), and there is a small rounded karyosome.'—(p. 379). Warren regards these nuclei as those of primary spermatocytes but, since these nuclei show the characteristic karyosome, they are probably those of the spermatogonia.

Warren continues: 'On expansion occurring the chromatin concentrates into a number of rounded or oval bodies which pass to the periphery of the nucleus (figs. 127, 137, *sp.*); the karyosome generally disappears entirely. In this millipede the nuclei do not pass into a homogeneous condition with the temporary disappearance of the chromatin, as in the previous species, but there is the gradual production of the above-mentioned minute bodies by the concentration of chromatin granules which are strongly basophil. These bodies are directly transformed into exceptionally small spermatozoa, and, so far as it is possible to ascertain, each nucleus may produce a dozen or more of such sperms.'—(pp. 380 to 381).

Warren anticipates that this account is bound to be received with scepticism by the genetic cytologist whom, however, he invites to inspect the drawings of the sections, and to endeavour to fit them into the typical scheme of spermatogenesis. We suspect that the nuclei described above

by Warren as having a number of rounded or oval bodies which are said to form the very minute spermatozoa are those of primary spermatocytes in the stage known as diakinesis, common in Prophase I, in which the chromosomes are arranged just under the nuclear membrane.

The so-called glandular lobes of *Ulodesmus* with small nuclei (Warren's figs. 138 and 139) are most certainly lobes which are full of the earliest spermatids and he erroneously considers them as glandular in function as in the case of the small-celled glandular lobes of *Odontopyge* (vide *supra*).

Sphaerotherium punctulatum is the last millipede described by Warren. In this millipede 'there is very little evidence that the walls of the vasa deferentia produce spermatozoa', although Warren has not produced any evidence whatsoever in favour of his claim that the spermatozoa found in the vasa deferentia of *Odontopyge* sp., *Poratophilus diplodontus*, *Chersastus ruber* and *Ulodesmus bispinosus* originate *in situ*.

While Warren derives the sperm from what he calls the 'homogeneous nucleus', which is really the cytoplasmic vesicle of a maturing spermatid, in *Odontopyge*, *Poratophilus* and *Chersastus*, and from chromosomes of the nuclei of primary spermatocytes in diakinesis in *Ulodesmus*, here in *sphaerotherium* he derives the entire sperm from an undifferentiated nucleus which becomes homogeneous.

'The spermatozoa arise in a central cluster continuous with the incipient lumen which is developed (text-fig. 6, *b*; Pl. XXII, fig. 149). The spermatozoa are not formed from the large cells which enclose the cluster, but they originate by a transformation of the undifferentiated small nuclei which must be regarded as potential sex nuclei (Pl. XXII, fig. 151, *u.n.*, *h.n.*, *sp.*).'—(p. 385). Because the undifferentiated nuclei are found immediately around the growing cluster of spermatozoa and because they also occur around the incipient lumen and extend into the embedded stalk of the spermatic lobe, Warren jumps to the conclusion that the sperms must arise from these nuclei.

The fact is that in *Thyroglutus malayus* the nurse-cell nuclei of the walls of the spermatic lobes often project into the interior of the lobes. The same phenomenon has been observed by Nath (1942) in the spermatogenesis of the Decapoda. Indeed this is a very wide-spread phenomenon and Warren has produced no evidence whatsoever in favour of his claim that these 'undifferentiated nuclei' directly form the spermatozoa.

Warren continues: 'As the cluster extends the large cells (Pl. XXII, fig. 150, *gl.n.*) with their nuclei break down, and doubtless thereby yield nutritive substances to the growing mass of sperm.'—(p. 385). This is a wholly unwarranted statement for which no evidence whatsoever has been produced by Warren. As a matter of fact the large nuclei shown in Pl. XXII, figs. 149 to 151 are perfectly healthy nuclei and are certainly those of the primary spermatocytes in or approaching the synizesis stage in Prophase I.

The spermatozoa of *Sphaerotherium* are unilateral in character and arise by the direct transformation of the undifferentiated small nuclei. In general form they are similar to those of other species, but on the concave side of the nuclear cup Warren has described 'a small conical projection

over the site of the plasmosome' (text-fig. 7, *é*). The 'plasmosome' is, of course, the centrosome, which appears to be elongated and running through the cytoplasmic vesicle of the sperm, thus forming 'a small, conical projection over the site of the plasmosome'. Gilson (1886) has figured a similarly situated rod-like structure in the sperm of *Julus sabulosus*, which is probably the centrosome.

In addition to the spermatic lobes Warren has described glandular lobes in *sphaerotherium*. The cells forming these lobes are small and Warren correctly regards these cells as spermatids. He has also figured somewhat smaller spermatozoa in these lobes, but he considers these spermatozoa as abortive. 'The small-celled tissue with the abortive sperm ultimately breaks down, and it is suggested that it produces nutritive or stimulating substances for the growth and maintenance of the functional spermatozoa formed in the spermatic lobes.'—(p. 387). No evidence has been produced by Warren in support of this view.

Oettinger (1909), working on *Pachyiulus varius*, confines himself mainly to chromosomes, but it is impossible to follow him through the process of spermateleosis in spite of the excellent figures he has produced as his homologies are mostly wrong and the fixatives used (Hermann's and Flemming's Osmium mixtures) contain acetic acid. Oettinger has described a very unusual structure in the sperm of *Pachyiulus varius*, viz., a tail filament, which, however, is conspicuous by its absence in the sperms of *Thyroglutus malayus* described by us, of *Glomeris marginata*, *Polydesmus complanatus* and three species of *Julus* (Gilson, 1886), and of *Odontopyge* sp., *Poratophilus diplodontus*, *Chersastus ruber*, *Ulodesmus bispinosus*, and *Sphaerotherium punctulatum* (Warren, 1934).

But it is not easy to dismiss the tail filament of *Pachyiulus* as an artifact, because Oettinger has figured it in the living spermatozoa and he lays particular stress on the extraordinary distinctness of this structure. Oettinger also claims to have demonstrated this structure on the occasion of a lecture held in Marburg in the Society for furthering the science of living spermatozoa. With the progress of the transformation of the spermatid the flagellum grows on to a relatively long fibre, which, in its final form, amounts to approximately three times the size of the whole cell. Oettinger is not certain whether the flagellum is capable of making any movement. We have repeatedly examined Oettinger's figures and script with great care in the hope of offering some constructive criticism in the light of our own observations, as we have done in the case of Warren's account, but we have failed to evolve any order out of the existing chaos. Evidently a comprehensive and intensive survey of the millipede sperm is urgently needed particularly the flagellate sperm of *Pachyiulus* and the filiform sperm of *Blaniulus guttulatus* (Gilson, 1886).

Summary and Conclusions

1. In this paper the spermatogenesis of *Thyroglutus malayus* has been completely worked out.

2. In the earliest spermatogonia, the nucleus hardly reveals any structure save for the presence of an excentric nucleolus, which seems to

be of the nature of an amphinucleolus. The mitochondria exist as pale fine granules in the cytoplasm arranged in the form of a juxta-nuclear mass and the Golgi elements, in the form of darkly staining bigger granules, are scattered amongst them. In the fully grown spermatogonia both the mitochondria and the Golgi elements become circum-nuclear in arrangement.

3. The primary spermatocytes are definitely larger cells than the spermatogonia. The nucleolus seems to break up into a large number of deeply staining chromatin granules. The resting secondary spermatocytes are similar to the primary but they are definitely smaller than the latter.

4. During the spermatogonial and the meiotic divisions the mitochondria and the Golgi elements are seen to be sorted out, more or less equally, to the daughter cells.

5. Beautiful spindles appear during the above stated divisions. These are fibrillar and fusiform structures with a sharply staining centrosome at each pole.

6. The earliest spermatid has, beside a nucleus, a few prominent Golgi elements, a large centrosome and fine granular mitochondria.

7. During spermateleosis the vesicular nucleus of the early spermatid is converted into a wide and shallow cup. Simultaneously vacuoles appear in the cytoplasm and both the mitochondria and the Golgi elements merge into these. By a two-fold process of growth and coalescence these vacuoles form a large cytoplasmic vesicle, which is pushed inside the nuclear cup just before the ripe sperm is formed.

8. The ripe sperm is a simple structure consisting of three cell-elements only, viz., the nuclear cup, the centrosome and the cytoplasmic vesicle. The acrosome, the middle-piece and the axial filament of the typical flagellate sperm are conspicuous by their absence.

9. The structure of the centrosomal apparatus has been studied in detail and its large size is supposed to be in conformity with the belief that it is in some way connected with the opening out of the sperm.

10. Since the ripe sperm is completely devoid of an acrosome, this investigation tends to disprove its alleged boring function.

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Explanation of Lettering in the Plates

C —Centrosome.
C_1 —Centrosomal granule or centriole.
C_2 —Centrosomal ring.
Ch —Chromosomes.
C.G.—Chromatin granules.
C.V.—Cytoplasmic vesicle.
G —Golgi elements.
M —Mitochondria.
N —Nucleus.
N_1 —Nucleolus.
V —Vacuole.

Further explanation of figures will be found in the text.

Explanation of Figures in the Plates

All figures have been drawn with a camera lucida at the table level with Beck 25 MM(×10) eye-piece and oil immersion objective giving a magnification of 1,700 times.

All figures except figs. 50 to 53 on Plate IV have been drawn from sectioned material fixed in Flemming, without acetic, followed by 0·5% iron-haematoxylin. Figures 50 to 53 are from smears fixed in Flemming, without acetic, diluted with an equal quantity of water and stained with 0·5% iron-haematoxylin.

Plate I.

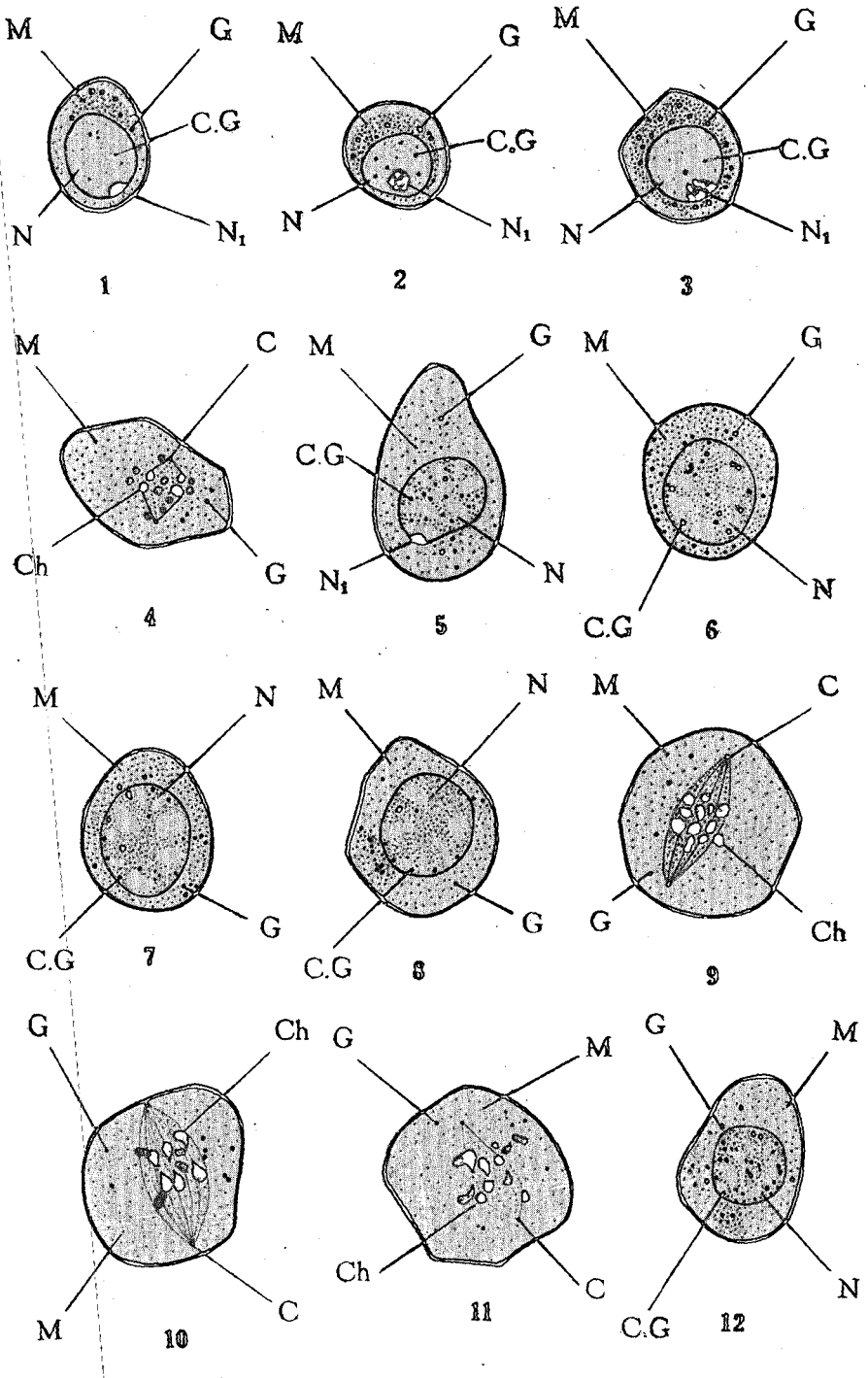
Fig. 1.—Earliest spermatogonium.
Fig. 2.—Spermatogonium at a little later stage.
Fig. 3.—Fully grown spermatogonium.
Fig. 4.—Spermatogonial metaphase.
Fig. 5.—Nurse-cell.
Figs. 6 to 8.—Primary spermatocytes.
Fig. 9.—Metaphase I.
Fig. 10.—Early Anaphase I.
Fig. 11.—Late Anaphase I.
Fig. 12.—Secondary spermatocyte.

Plate II.

Fig. 13.—Metaphase II in polar view.
Fig. 14.—Metaphase II in side view.
Fig. 15.—Late Anaphase II.
Fig. 16.—Telophase II.
Fig. 17.—Metaphase II.
Fig. 18.—Metaphase II.
Fig. 19.—Earliest spermatid.
Fig. 20.—Resting spermatid.
Figs. 21 to 26.—Spermatids showing the movement of the nucleus towards the periphery and its transformation into a wide and shallow cup-like structure. Vacuoles have started appearing in the cytoplasm.
Fig. 27.—Nearly top view of a maturing spermatid.

Plate III.

Figs. 28 to 30.—Nearly top views of maturing spermatids.
Figs. 31 and 32.—Top views of maturing spermatids.
Fig. 33.—Top view of a nuclear cup only.
Figs. 34 to 42.—Late spermatids. The nuclear cup has been completely formed. The centrosome has grown to its maximum size. Cytoplasm is almost full of vacuoles into which both the Golgi elements and the mitochondria are seen merging.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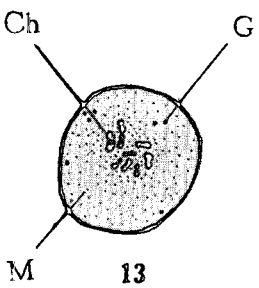

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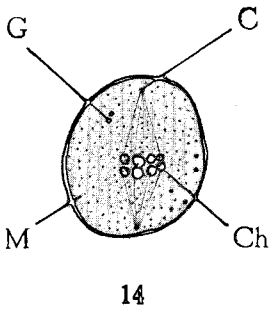

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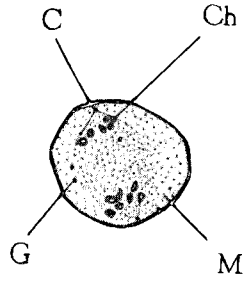

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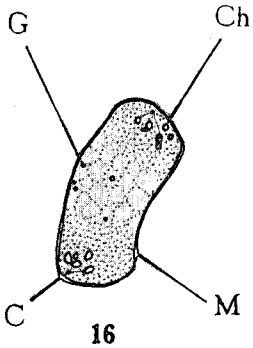

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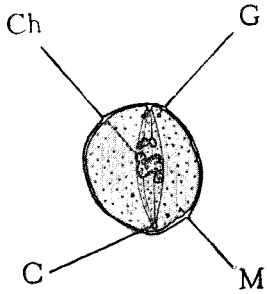

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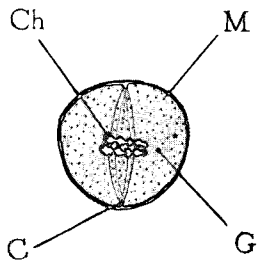

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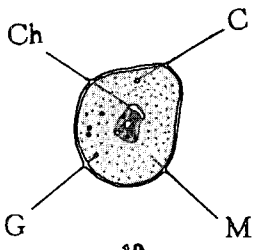

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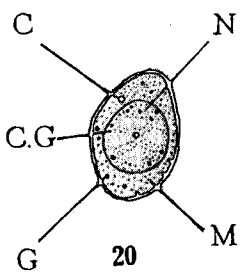

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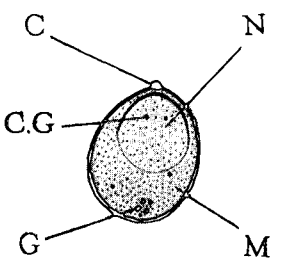

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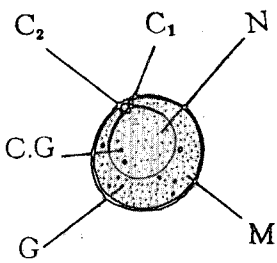

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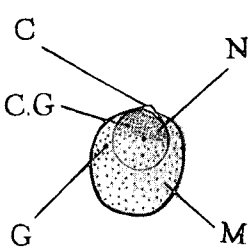

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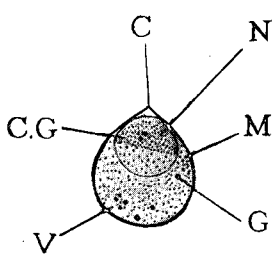

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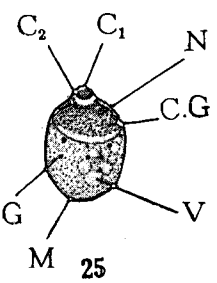

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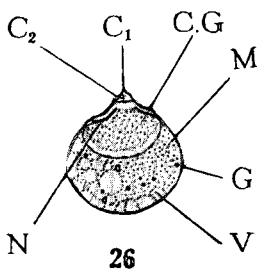

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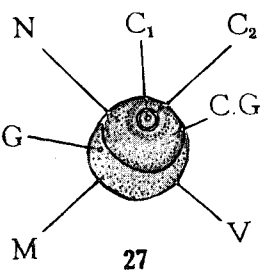

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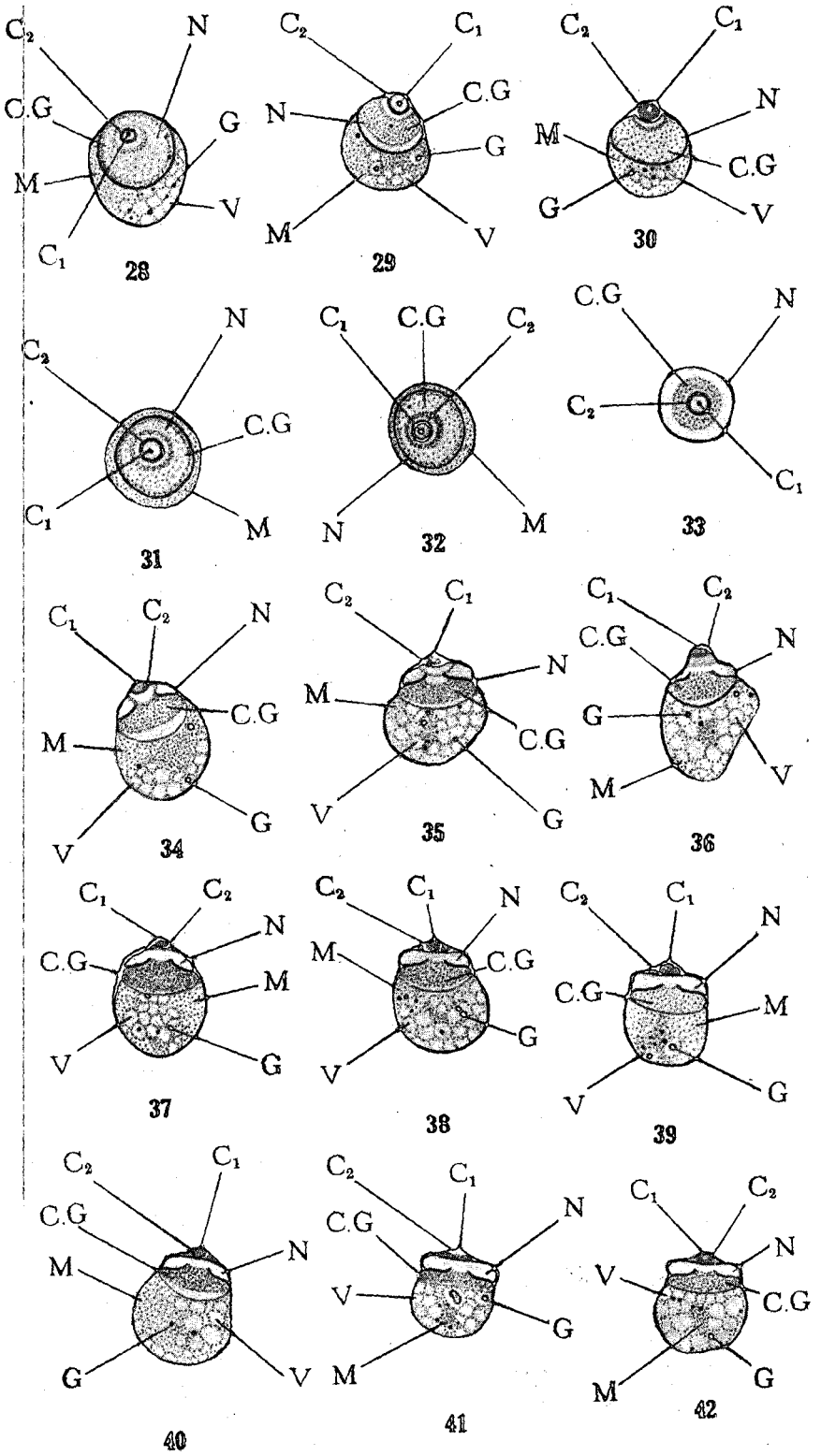
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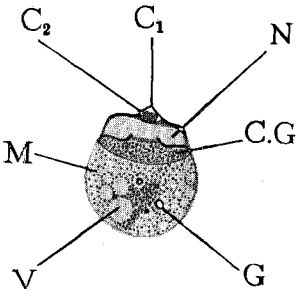

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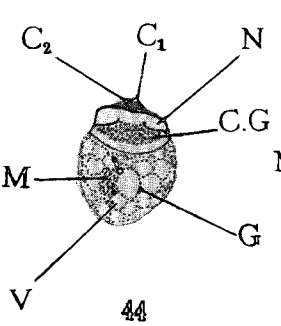

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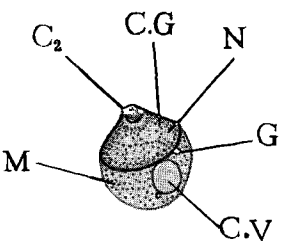

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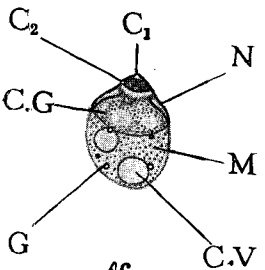

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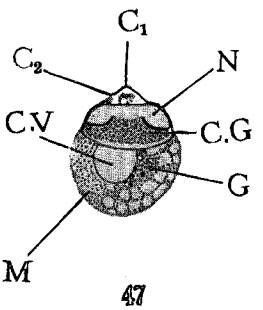

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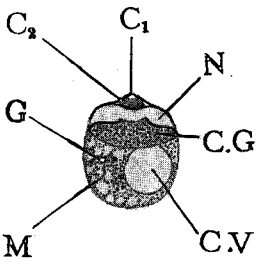

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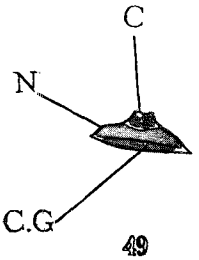

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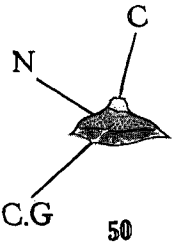

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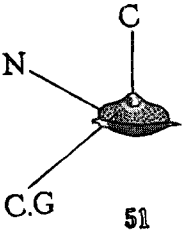

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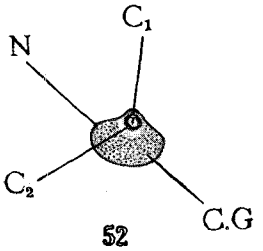

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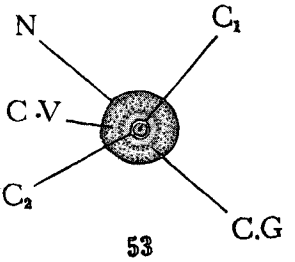

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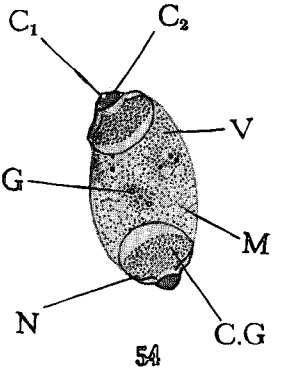

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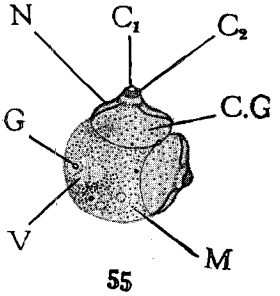

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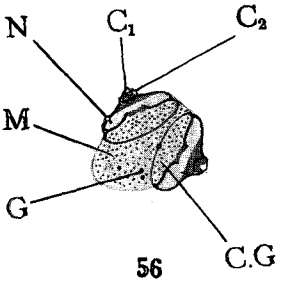

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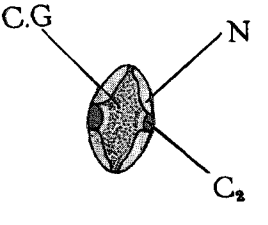

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Plate IV.

FIGS. 43 and 44.—Same as for figs. 34 to 42.

FIG. 45.—Nearly top view of a late spermatid.

FIGS. 46 to 48.—Late spermatids showing the formation of the cytoplasmic vesicle.

FIG. 49.—Ripe sperm (from a section). Side view.

FIGS. 50 and 51.—Ripe sperms (from smears). Side views.

FIG. 52.—Nearly top view of a ripe sperm (from smears).

FIG. 53.—Top view of a ripe sperm (from smears).

FIGS. 54 to 56.—Daughter spermatids showing the process of spermateleosis without their complete separation after the second meiotic division.

FIG. 57.—Two fully ripe spermatozoa formed in a perfectly normal way from their respective spermatids which have not, however, separated so far after the meiotic division of their parent secondary spermatocyte.

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 23 | CHEMISTRY | Pp. 121-122

October, 1952

CONSTITUTION OF PERIODATE OF URANIUM

by

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Price Rs.0-8-0

CONSTITUTION OF PERIODATE OF URANIUM.

By Balwant Singh, Apar Singh *and* Roshan Singh.

C. F. Rammelsberg (*Pogg. Ann.*, 1839, 55, 70) found that when uranium tetra-chloride is treated with potassium periodate, a greyish green precipitate of uranous periodate is formed, which soon passes into yellowish white uranyl periodate.

In the present investigation, an attempt has been made to prepare periodate of uranium and to determine its constitution.

Experimental.

On adding potassium meta-periodate solution to a warm saturated aqueous solution of uranyl nitrate, a yellowish white precipitate was formed. It was filtered, washed with hot water till free from nitrate, dried in an electric air oven at 60° C. and analyzed. Uranium content of the periodate was determined by Oxine method (Vogel, *Quantitative Inorganic Analysis*, 1948, page 557). Iodine and available oxygen in the compound were estimated by Kimmins' method modified by Bahl and Partington (*J. Chem. Soc.*, 1934, page 1087). The analytical results are given in the following tables:

Table I—*Uranium.*

Sample No.	Periodate taken. (gm.)	Oxine Compound of Uranium. (gm.)	Percentage of Uranium in Periodate.
1	0·1901	0·3311	58·96
	0·2352	0·4120	59·30
2	0·1228	0·2150	59·27
	0·2712	0·4720	58·91
3	0·1572	0·2744	59·09
	0·2292	0·3907	58·57
4	0·1416	0·2450	58·57
	0·2693	0·4692	58·98
5	0·1482	0·2570	58·70
	0·2061	0·3610	59·29
			Mean value 58·96

Table II—*Iodine.*

Sample No.	Periodate taken. (gm.)	$N/10$ $Na_2S_2O_3.5H_2O$ used. (c.c.)	Percentage of Iodine in Periodate.
1	0·1150	15·15	20·91
	0·1751	23·05	20·90
2	0·0806	10·37	20·42
	0·1425	18·30	20·39
3	0·1300	16·90	20·64
	0·1589	20·70	20·68
4	0·1200	16·00	21·17
	0·1607	21·35	21·10
5	0·1020	13·50	21·01
	0·1589	21·10	21·08
			Mean value 20·83

TABLE III—*Available Oxygen.*

Sample No.	Periodate taken. (gm.)	Dry Oxygen collected at N.T.P. (c.c.)	Percentage of Oxygen in Periodate.
1	0·1435	10·24	10·20
	0·2080	14·98	10·29
2	0·1260	9·08	10·29
	0·1762	12·71	10·30
3	0·1492	10·83	10·37
	0·1990	14·42	10·35
4	0·1194	8·62	10·31
	0·1750	12·66	10·33
5	0·1500	10·67	10·16
	0·1982	14·17	10·21
			Mean value 10·28

DISCUSSION.

On adding potassium meta-periodate solution to a warm saturated aqueous solution of uranyl nitrate, a yellowish white precipitate of $UO_2(IO_4)_2 . 2\ UO_3$ is formed according to the following reactions:

$$UO_2(NO_3)_2 + 2\ KIO_4 = UO_2(IO_4)_2 + 2\ KNO_3.$$
$$UO_2(NO_3)_2 + 2\ H_2O \rightleftarrows H_2UO_4 + 2\ HNO_3.$$
$$2\ H_2UO_4 = 2\ UO_3 + 2\ H_2O.$$
$$UO_2(IO_4)_2 + 2\ UO_3 = UO_2(IO_4)_2 . 2\ UO_3.$$

On heating the compound decomposes to give

$$UO_2(IO_4)_2 . 2\ UO_3 = UO_2 + 2\ UO_3 + I_2 + 4\ O_2.$$

10·46 per cent of available oxygen. Calculated values of uranium and iodine in this compound are 58·35 per cent and 20·74 per cent respectively.

The analytical and the calculated values of uranium, iodine and available oxygen in the periodate of uranium agree with its formula $UO_2(IO_4)_2 . 2\ UO_3$.

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Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 24 CHEMISTRY Pp. 123–127

November, 1952

ALKALINE PERMANGANATE AS A VOLUMETRIC REAGENT (PART I)

Estimation of Organic Hydroxy Acids

by

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Price Rs.0-14-0

ALKALINE PERMANGANATE AS A VOLUMETRIC REAGENT (PART I).

Estimation of Organic Hydroxy Acids.

By Ram Chand Paul, V. S. Gaind, O. P. Malhotra *and* Rajkumari Nahan (Miss).

Holluta (1922) has shown that the reaction between potassium permanganate and a formate in alkaline solutions proceeds in two distinct stages:—

(i) The reduction of permanganate to manganate

$$MnO_4^- + e^- \longrightarrow MnO_4^{--} \quad .. \quad .. \quad (1)$$

and (ii) The reduction of manganate to manganite

$$MnO_4^{--} \longrightarrow MnO_3^{--} + O \quad .. \quad .. \quad (2)$$

The first of these two reactions proceeds much more rapidly than the second one, which is in conformity with the findings of Sackur and Taegener (1912) who determined the normal potentials of the reactions (1) and (2) to be +0·61 and +0·50 volt (at about 18° C. and 1 *N* KOH solution). Stamm (1934) found that under the conditions in which only the first stage is involved many substances, otherwise difficult to attack, can be quantitatively oxidized at room temperature in a few minutes.

The second stage of the reaction can be written as

$$MnO_4^{--} + 3H_2O + 2\,e^- \rightleftarrows H_2MnO_3 + 4OH^- \quad .. \quad (3)$$

This reaction can be hindered by increasing the hydroxyl ion concentration, because an increase in the (OH)' concentration will shift the equilibrium towards left (1·2 *N* alkaline solution can satisfy the above condition). The effect of this increase of the OH^- ion-concentration upon the reaction (1) is to shift the equilibrium to the right, i.e. to help the reaction:—

$$MnO_4^- + OH^- \longrightarrow MnO_4^{--} + OH \quad .. \quad .. \quad (4)$$

This shows that by keeping the solution strongly alkaline the first stage (1) is facilitated while the second stage (2) is checked, thus creating strong oxidizing conditions (Stamm, *loc. cit.*). The free hydroxyl which appears on the right-hand side of equation (4) is much more mobile than permanganate and manganate ions and may be the real oxidizing agent.

Thus the principle of procedure for titration in strongly alkaline medium should be such as to involve only the first rapid stage, i.e. the reduction of permanganate to manganate while the second stage involving the conversion of manganate to manganite should not be allowed to occur. This is achieved by the addition of some barium salt solution which causes the precipitation of sparingly soluble barium manganate and thus removes

the manganate ions from the reaction mixture, while the permanganate ions are still there because barium permanganate is quite soluble, in conformity with the Weitz and Stamm (1928) rule. The solubility product of barium manganate has been found by Schlesinger and Seims (1924) at 25° C. to be $2{\cdot}46 \times 10^{-10}$, which is less than that of even barium carbonate. This shows that by the addition of an excess of barium chloride solution the manganate ions can be completely removed from any further action of the reducing agent; in fact the removal is so complete that in the absence of permanganate ions or any other coloured material the supernatant solution is colourless. The precipitate of barium manganate is dark green but with practice the colour of supernatant solution can be judged easily. *Alternatively the solution can be made acidic when the oxidation is complete* and $KMnO_4$ left unreacted titrated against oxalic acid.

Keeping the above principles in view attempt here has been made to estimate certain oxidizable compounds. In this communication the estimation of certain organic hydroxy acids has been dealt with. The results about other classes of compounds will be reported shortly.

Procedure.

20 c.c. of 0·5 *N* $KMnO_4$, about 10 c.c. of 30% NaOH and 7–15 c.c. (a known volume) of approximately 0·1 *N* solution of the acid to be estimated were measured together in a 250 c.c. flask and allowed to stand for 10 to 15 minutes. The excess of permanganate is estimated by any one of the following two methods.

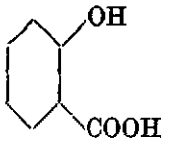
Method A:—About 15 c.c. of 30% barium chloride solution were then added and the contents diluted with water to approximately 100 c.c. The excess permanganate was then back titrated against a standard formate solution in the alkaline medium, end point being the disappearance of the colour of permanganate from the supernatant solution.

Method B:—After allowing the oxidation to complete as above the solution was acidified with 10 c.c. of dilute sulphuric acid (1 : 1) and then 20 c.c. of 0·5 *N* oxalic acid solution were added. The contents were warmed to 60–70° C. and the excess oxalic acid determined by titration against a standard (0·1–0·2 *N*) $KMnO_4$ in acidic medium.

The acids estimated by this procedure were salicylic acid, mandelic acid, lactic acid, citric acid, glycollic acid, tartaric acid and malic acid. The results are tabulated in Tables I and II.

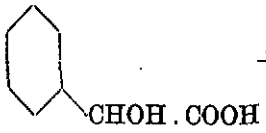
Discussion of Results.

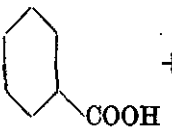
The oxidation products in most of the cases are carbon dioxide and water, excepting in the case of mandelic acid which gives benzoic acid, which is stable in alkaline as well as acidic medium, and tartaric acid in which case in the alkaline medium the reaction ceases at the oxalic acid stage, but when the medium is made acidic for *Method B*, the oxidation proceeds to completion yielding carbon dioxide and water. The reactions may be represented as:—



$+14\,O \longrightarrow 7\,CO_2+3\,H_2O$

(Salicylic Acid)



$+2\,O \longrightarrow$  $+CO_2+H_2O$

(Mandelic Acid)

$CH_3.CHOH.COOH + 6\,O \longrightarrow 3\,CO_2+3\,H_2O$

(Lactic Acid)

$CH_2.COOH$
$C(OH).COOH \quad +9\,O \longrightarrow 6\,CO_2+4\,H_2O$
$CH_2.COOH$

(Citric Acid)

CH_2OH
$+3\,O \longrightarrow 2\,CO_2+2\,H_2O$
$COOH$

(Glycollic Acid)

$CHOH.COOH$
$+4\,O \xrightarrow[\text{Medium}]{\text{Alkaline}} COOH \quad +2\,CO_2+2\,H_2O$
$CHOH.COOH \qquad\qquad COOH$

(Tartaric Acid)

$CHOH.COOH$
$+5\,O \xrightarrow[\text{Medium}]{\text{Acidic}} 4\,CO_2+3H_2O$
$CHOH.COOH$

(Tartaric Acid)

$CH_2.COOH$
$+6\,O \longrightarrow 4\,CO_2+3\,H_2O$
$CHOH.COOH$

(Malic Acid)

In all the cases the best results are obtained when 80–120% excess of $KMnO_4$, over the theoretically required amount is used. Both the methods yield comparable results except in the case of citric acid when Method B gives better results than Method A.

It may be added that Method B is more convenient.

TABLE I.

Results with Method A.

Vol. of $KMnO_4$ taken = 20 c.c. 0·5 *N*. Time allowed for reaction = 10–15 minutes.

Acid soln. taken.	Excess $KMnO_4$ %age.	Salicylic acid. Amt. present is 0·9857gms./litre		Mandelic acid. Amt. present 3·8000 g./l.		Lactic acid. Amt. present 0·7500 g./l.		Citric acid. Amt. present 1·1660 g./l.		Glycollic acid. Amt. present 1·2000 g./l.		Tartaric acid.* Amt. present. 3·5250 g./l.	
		Found g./l.	Diff.	Found g./l.	Diff.	Found g./l.	Diff.	Found g./l.	Diff.	Found g./l.	Diff.	Found g./l.	Diff.
15·0	32·6	0·8800	0·1057	..	..	..	..	..	..	..	..	..	..
14·0	42·8	0·9044	0·0813	3·6482	0·1518	..	..	..	..	..	..	..	..
13·0	53·7	0·9150	0·0707	3·6542	0·1458	0·7398	0·0102	1·1422	0·0138	1·1710	0·0820	3·4750	0·0500
12·0	66·6	0·9660	0·1970	3·6888	0·1112	0·7404	0·0096	1·1360	0·0300	1·2063	0·0063	3·4842	0·0408
11·0	81·8	0·9800	0·0057	3·8423	0·0423	0·7494	0·0006	1·1480	0·0180	1·1192	0·0008	3·5624	0·0374
10·0	100·0	0·9880	0·0023	3·7912	0·0088	0·7510	0·0010	1·1688	0·0028	1·2048	0·0048	3·5090	0·0160
9·0	122·2	1·0040	0·0183	3·7802	0·0098	0·7480	0·0020	1·1548	0·0112	1·2090	0·0090	3·5402	0·0152
8·0	150·0	0·9940	0·0083	3·7420	0·0580	0·7515	0·0015	1·1420	0·0240	1·2298	0·0298	3·5620	0·0370
						Results with Method B.						Amt. present 2·8200 g./l.	
15·0	32·6	0·8745	0·1112	3·6246	0·1754	..	..	..	..	..	..	..	..
14·0	42·8	0·8876	0·0981	3·7764	0·0236	0·7014	0·0486	..	..	..	..	..	..
13·0	53·7	0·9145	0·0712	3·7020	0·0980	0·7206	0·0294	1·1500	0·0160	1·1900	0·0100	2·7482	0·0718
12·0	66·6	0·9044	0·0813	3·8412	0·0412	0·7314	0·0186	1·1482	0·0178	1·1920	0·0080	2·7354	0·0846
11·0	81·8	0·9792	0·0065	3·7900	0·0100	0·7428	0·0072	1·1644	0·0016	1·1978	0·0022	2·8186	0·0014
10·0	100·0	0·9867	0·0010	3·7840	0·0160	0·7524	0·0024	1·1620	0·0040	1·2020	0·0020	2·8302	0·0102
9·0	122·2	0·9942	0·0085	3·8014	0·0014	0·7404	0·0096	1·1502	0·0058	1·2042	0·0042	2·8442	0·0058
8·0	150·0	1·0082	0·0225	3·8226	0·0226	0·7702	0·0202	1·1742	0·0082	1·2196	0·0196	2·7540	0·0660

* Eqivalent weight of tartaric acid is 35·25 for Method A and 28·2 for Method B (vide p. 125).

TABLE II.

Results for Malic acid (Methods A and B).

Vol. of $KMnO_4$ taken = 20·0 c.c. 0·55 *N*.
Time allowed for reaction = 10–15 minutes.
Amount of acid present = 1·1166 gms./litre.

Acid soln. taken c.c.	Excess $KMnO_4$ %age.	Method A.		Method B.	
		Found g./l.	Diff.	Found g./l.	Diff.
15·0	49·1	1·0794	0·0372	1·0154	0·1012
14·0	57·0	1·0845	0·0321	1·0340	0·0826
13·0	69·4	1·0846	0·0320	1·0640	0·0526
12·0	83·5	1·1082	0·0084	1·1132	0·0044
11·0	100·0	1·1096	0·0070	1·1174	0·0008
10·0	121·1	1·1074	0·0092	1·1160	0·0006
9·0	142·0	1·1247	0·0081	1·1341	0·0274
8·0	174·0	1·1324	0·0158	1·1469	0·0294

SUMMARY.

Certain aromatic and aliphatic hydroxy acids have been estimated volumetrically by using potassium permanganate in the alkaline medium using two different methods. In most of the cases acidic permanganate does not yield quantitative results.

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Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN

OF THE

EAST PANJAB UNIVERSITY

No. 25 ZOOLOGY Pp. 1–21

January, 1953

THE EXTERNAL MORPHOLOGY OF *DIPRION PINI* (L)

(Symphyta-Hymenoptera)

by

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Price Rs.2-3-0

THE EXTERNAL MORPHOLOGY OF *DIPRION PINI* (L)*

(Symphyta-Hymenoptera)

By G. L. Arora, M.Sc., D.I.C., Ph.D. (Lond.), F.R.E.S.

The literature on the external morphology of the sawflies has been accumulating for the last half a century. Van Dine (1905) described and illustrated the mouth parts of a few sawflies ; Snodgrass (1910) gave a detailed account of the thorax of *Tremex* and some other Hymenoptera; Crampton (1919) treated the male genitalia in detail; Bird (1926) dealt with the external anatomy of the adult *Hoplocampa halcyon*; Ross (1936) made a thorough study of the wing venation of the Symphyta; Reeks (1937) described the external morphology of *Diprion polytomum ;* Ross (1937) made a comparative morphological survey of the Nearctic sawflies and Snodgrass (1941) gave a detailed comparative account of the male genitalia of the Hymenoptera with a section on the Chalastogastra. In addition to these, several passing references on sawfly morphology appear in text-books and in papers on anatomy and phylogeny.

The present study of *Diprion pini* is an attempt to bring out characters which have some phylogenetic interest, difficult to secure from the writings of earlier morphologists. A fair amount of well preserved material of this sawfly could be obtained from the collections in the Department of Entomology, Imperial College, London. I am indebted to Dr. O. W. Richards of the Imperial College, London, for his invaluable advice and guidance and to Mr. R. G. Davies for his general assistance. My thanks are due to Dr. Vishwa Nath for getting the paper published in the Research Bulletin of the East Panjab University.

1. The Head

(Pl. I, Figs. 1, 2)

The head is hypognathous, globular, broader than long, convex in front and almost flattened behind and bears laterally the oval, slightly bulging compound eyes. The cranium is strongly sclerotized, highly pigmented in the male, and beset with setae and pits all over. Its surface is marked by sutures and ridges which divide it into definite areas. In the middle of the dorsal surface is a raised area, the Vertex, cut off by the temporal sutures laterally and by a horizontal pigmented line ventrally. On the anterior surface and immediately below the vertex lie the three ocelli in a triangle, with a heavily pigmented area between the lateral

* Submitted as part of the thesis for the Degree of Doctor of Philosophy of London University.

ones. The epicranial suture or the Cleavage line of the larva (Snodgrass, 1947) has no counterpart in the adult but from the middle of the horizontal line mentioned above run downward two faint frontal sutures (post-frontal sutures of Ferris, 1942), one on either side of the median ocellus and stopping short there. The fate of the epicranial suture is quite variable in the insect head. In the generalized forms the frontal bifurcation may reach the epistomal suture or even the labro-clypeal suture and the area with the median ocellus in between these is termed the Frons. In the present species, because of the obliteration of the sutures, a broad continuous area, the Fronto-parietal, comes to lie below and on the sides of the vertex, bearing the ocelli, the antennae and the eyes, and extending ventrally up to the clypeus which is separated from it by the horizontal epistomal or clypeo-frontal suture; the latter is identified by the broad anterior tentorial pits at its two ends in front of the antennae which mark the posterior limits of the clypeus. The antennae lie almost in the middle of the fronto-parietal, one on each side, a little above the anterior tentorial pit. Each antenna lies in an oval antennal socket enclosed by the antennal suture and articulates with the socket by a peg-like projection from its ventromesal border. In line with the anterior tentorial pits and immediately above the antennae can be made out a pair of dark patches, the dorsal tentorial patches or 'tentorial maculae', marking the position of the coalescence of the distal ends of the dorsal tentorial arms with the cranium. Two lateral, slightly curved ridges run from the lateral ocelli to the antennae dividing the fronto-parietal into a median area with the median ocellus and two parietal areas bearing the lateral ocelli, the antennae and the eyes. The eyes are marked by the ocular sutures and each is supported by an internal, sclerotized inflected margin or shelf. Anterior to the fronto-parietal lies the transverse, more or less trapezoidal clypeus extending to the mandibular articulations on the sides. It is bounded above by the epistomal suture and separated from the terminal flap-like, semicircular labrum by the labro-clypeal suture. The ventral border of the clypeus is somewhat inflected and covers the base of the labrum, foreshadowing a condition that one finds in other sawflies like *Pamphilius* and *Megalodontes*, in which the labrum is invaginated and only its tip is seen. The labrum (Pl. I, Fig. 3) is semicircular in outline and overhangs the oral fossa anteriorly. Its free anterior border carries long bristles, two or three rows deep. This surface is continuous with the ventral or epipharyngeal surface which is beset with setae. At the junction of the labrum with the clypeus on the epipharyngeal surface and at the outer angles of the former lie a pair of small posteriorly pointed tormae, concealed by the latter. The inner surface of the clypeus is closely attached to a highly sclerotized plate, the Pharyngeal sclerite, which is bifid posteriorly and bears setae and pits.

The genae, below and behind the eyes, are reduced due to the ventro-lateral position of the eyes. Their ventral edges bear the posterior mandibular articulations. The posterior surface of the cranium shows a vertically elongated occipital foramen in the centre with the tentorial bridge running across. A broad V-shaped area, the occiput, lies above

the foramen. It is bounded laterally by two highly pigmented sutures which are continuations of the temporal sutures mentioned before. Directed postero-ventrally below the occipital foramen is the oral fossa containing the movable mouth-parts suspended from the cranium. There are no occipital sutures and as such the limits of the genae and postgenae cannot be determined. Even a ridge (crassa) mentioned by Ross (1937) in *Tenthredo basilaris* is missing. The postgenae are, however, the broad areas on the sides of the occipital foramen, each extending from the dorsal cranial edge above to the side of the oral fossa below. The ventral edge of each postgena is weakly differentiated into an oblique ridge-like hypostoma, bounding the oral fossa laterally. The two hypostomal ridges do not meet mesally to form a hypostomal bridge but each meets the maxillaria of its side. A postoccipital suture surrounds the occipital foramen dorsally and laterally, separating the occiput and the postgenae from the narrow horse-shoe-shaped postocciput which bounds the foramen magnum. The postocciput is a narrow, pigmented sclerotized ridge continuous dorsally with the neck membrane and broadening out laterally a little above the tentorial bridge to form the occipital condyles for the articulation of the lateral cervical sclerites to be mentioned later. In front of the occipital condyles, the postocciput is continued on each side into a narrow curved pigmented sclerite, the maxillaria, affording articular surface for the maxillary cardo of its side. The maxillariae do not meet in the middle and the membranous part of the labium between them is continuous with the neck membrane. The head of *Diprion pini* is therefore of the simplest type called the 'Open Head' (Ross, 1937) characterized by the following:—

(1) The maxillariae are not fused in the middle line. They provide articulation for the cervical sclerites and the maxillary cardines.

(2) The postgenae are not produced mesally to form a postgenal bridge.

(3) The occipital foramen is continuous with the oral fossa.

(4) The anterior margin and the sides of the clypeus are free.

(5) The head capsule does not surround the mandibles at their bases. As such the oral fossa is a single one containing all the mouth-parts.

2. The Tentorium

(Pl. II, Fig. 3)

The tentorium is a well-developed, H-shaped, sclerotized structure indistinguishably fused with the pigmented sclerotized ridge which bounds the occipital foramen. It consists of three pairs of arms, the posterior, the anterior and the dorsal. The posterior arms, which are closely associated with the pigmented ridge, run inward in front of the occipital condyles to meet in the middle forming the tentorial bridge (body of tentorium) across the occipital foramen with the posterior tentorial pits at the two ends of the bridge. The anterior tentorial pits lie at the ends

of the epistomal suture a little in front of the antennae. These invaginations lead to the broad anterior tentorial arms which run upward and backward to meet the anterior margin of the tentorial bridge. The proximal half of each is fused with the maxillaria of its side and the inner margin is produced into a process. The dorsal tentorial arms originate from the outer borders of the anterior arms, each broad proximally and narrowing distally to fuse with the inner wall of the cranium at the dorsal tentorial macula.

3. The Appendages of the Head

The appendages of the head are the antennae and the mouth-parts.

A. *The Antennae*—(Pl. I, Fig. 1; Pl. II, Figs. 1, 2, 4)

The antennae show a sexual dimorphism. They are strongly pectinate in the male, consisting of 28 segments. The scape articulates with the antennal socket, there being, according to Ferris (1942) no such thing as an antennal sclerite. It is broader than long. The Pedicel is small and broad with a sensory girdle (Johnston's organ) running round its distal end. The flagellum is 26-segmented, the segments being broader than long in the proximal half and longer than broad in the distal half. All the segments bear small spines and pubescence. The basal twenty-three segments of the flagellum carry long, narrow paired prolongations, beset with spines all round whereas the last three are without *such prolongations. The paired prolongations on the proximal segments* are long, becoming smaller gradually towards the distal end of the antennae. The antennae are serrate in the female, the flagellar segments being produced into small blunt inner processes. Each antenna has twenty-four segments. The scape is large and is longer than broad. The pedicel is bulb-like with the Johnston's organ as in the male. The flagellar segments are broader than long, becoming narrow distally. They bear spinules and hair. The terminal segment carries in addition, four sense papillae near its tip.

B. *The Mouth Appendages*

The mouth-appendages include the mandibles and the maxillo-labia complex.

(*a*) *The Mandibles*—(Pl. I, Figs. 1, 2, 4)

The mandibles are triangular, highly sclerotized paired jaws, each bound to the head by a membrane round its base and articulating by an anterior ginglymus and a posterior condyle with the ventral genopostgenal border below the eye. The ginglymus is a basal anterior concavity of the mandible into which fits a knob on the side of the clypeus and the condyle is the posterior convexity at its base which fits into a socket on the lower edge of the postgena. The inner border of each mandible has a pronounced incisor area with three well-developed teeth, the distal one being the longest

and the other two progressively smaller. The mandibles are asymmetrical, lying crossed below the labrum, the left one having its distal tooth longer than that of the right and bent inward. No molar area is differentiated. A tuft of bristles is borne on the outer edge of the mandible near its middle.

(*b*) *The Maxillo-Labial Complex*—(Pl. I, Fig. 2)

The two maxillae and the labium unite to form this composite structure contained in the oral fossa and suspended from the maxillariae by the maxillary cardines, the basal membranous part of the labium (postmentum) between them being continuous with the neck membrane.

(i) *The Maxillae*—(Pl. I, Fig. 2; Pl. II, Fig. 5)

The maxillae are the lateral structures in the complex, each consisting of the proximal cardo and the distal stipes, the latter bearing two terminal lobes, the outer galea and the inner lacinia, and a lateral segmented maxillary palp. The maxillae are united to the labium by a narrow strip of membrane on either side of the latter between the inner margins of the cardo and stipes on one hand and the outer margin of the postmentum on the other; the prementum of the labium does not take part in the union. The cardo and the stipes lie postero-lateral to the labium while the galea and the lacinia come to occupy a position a little anterior to the prementum and the paraglossae. The cardo is more or less triangular, sclerotized and produced proximally into a process for articulation with the maxillaria of its side. It is produced mesally into a blunt angle which reaches as far as the median sclerite of the postmentum. Distally the cardo articulates with the stipes. The stipes is elongate and sclerotized, and bears a few bristles. It does not show any division. The galea is a globular lobe bearing setae with a special set of strong setae along its distal margin. The lacinia is a narrow, soft blade-like lobe pressed against the galea and beset with setae and pubescence on its inner surface. The maxillary palp is six-segmented with a small palpifer at its base. It is fairly long with its segments sclerotized and bearing setae and pubescence; the last segment is narrow and the longest.

(ii) *The Labium*—(Pl. I, Fig. 2).

The labium is the median component of the maxillo-labial complex, lying between the maxillae with which it is united by lateral membranous strips. It consists of a basal part, the postmentum and a distal part, the prementum. The postmentum is a membranous rectangular piece with a small sclerotized, pear-shaped setiferous plate in the middle in between the mesally directed inner angles of the cardines. The exact homologies of this sclerite are not certain. Ross (1937), Reeks (1937) and other hymenopterists have labelled it submentum. Snodgrass (1935) compares it also to the lorum (= submentum) of higher Hymenoptera but it is not connected with the cardines. It is better to call it a postmental sclerite, for in certain sawflies such as *Megalodontes klugii* there are three such sclerites, a median and two lateral. The prementum is a sclerotized piece, faintly bifid at its distal end. Its adoral surface is beset with small spines while its oral surface has the hypopharynx

immovably united with it. It carries distally three terminal conical lobes and laterally a pair of labial palps. Of the three lobes, the side ones are the paraglossae and the middle one the alaglossa formed by the fusion of the glossae. The paraglossae and the alaglossa are adorned with spines and bristles at their distal extremities. The labial palps are four-segmented, the third broadened distally and the fourth conical. Small spines are borne on all the segments.

4. The Hypopharynx

(Pl. I, Fig. 5)

The hypopharynx is a raised sclerotized lobe intimately fused with the oral surface of the prementum at the base of the alaglossa and incapable of any free movements. Its surface bears small spines. The salivary duct can be seen opening into it. Its sides are supported by a pair of suspensorial rods, one on either side.

5. The Cervix

(Pl. I, Fig. 2; Pl. III, Figs. 2, 4)

The cervix or neck region is the membranous inter-segmental portion between the head and the prothorax which is strengthened ventrolaterally by a pair of sclerites, the cervical sclerites. The latter are broad proximally and narrow distally, their distal prolongations articulating with the occipital condyles on the sides of the occipital foramen. Each sclerite sends a small process inwards and backwards just before its articulation with the occipital condyle while its proximal broad part is fused with the pro-episternum. The suture between the two has been almost obliterated; only a faint indication of one remains, showing where fusion has taken place. These neck sclerites were regarded by Snodgrass (1910) as episterna but Crampton's (1917) and Weber's (1933) view that they are cervical sclerites which have fused with the propleura, particularly when an indication of such a fusion is noticeable, seems to be more satisfactory. The cervical sclerites bend inward but do not meet each other mesally nor do they meet the prosternum, the area between them being membranous.

6. The Thorax

The thorax is formed by three segments, the prothorax, the mesothorax and the metathorax, each bearing a pair of legs. The meso- and the metathorax are the wing bearing segments and together constitute the pterothorax.

(*a*) *The Prothorax*—(Pl. III, Figs. 1, 2, 4)

The tergum of the prothorax or the pronotum is a narrow sclerotized collar in front of the mesothorax. It expands into a broad triangular plate on either side immediately above the cervical sclerite with which it is connected by a membrane. Each lateral pronotal plate is beset with small setae and its posterior ventral angle is produced into a process which

reaches as far back as the anterior edge of the mesepisternum of its side. The pronotum, thus, is associated more closely with the mesothorax than with the propleural elements. The propleuron on either side has a distinct pleural suture which runs vertically up, is marked by a pleural ridge on the inner side, and is produced backward into an apodeme. In front of the pleural suture lies the episternum faintly marked off from the cervical sclerite and behind it lies the narrow epimeron. The ventral end of the pleural suture articulates with the coxal ridge of the first coxa on the dorsal aspect of the latter, forming the pleural articulation. The venter of the prothorax shows a T-shaped eusternum, not differentiated into the basi and furcasternal elements. At either end of the T, in the membranous part in front of each coxa, lies a small sclerite, the trochantin, bearing small setae. Snodgrass (1910) calls it the accessory precoxal plate in *Arge*. Weber (1933) recognizes a small trochantin in a number of sawflies. A similar trochantin has been observed in a number of panorpoid orders. The eusternum is bifid posteriorly, each knob-like bifurcation establishing a sternal articulation with the anterior inner angle of the coxal ridge. A similar condition exists in some other sawflies also. Weber (1933) describes it as a secondary sternal articulation in *Athalia* and Snodgrass (1910) mentions an articulating plate between prosternum and coxa of *Tremex columba*. The eusternum carries a pair of furcae on the inner surface. Behind the eusternum is the membranous spinasternum with a small spine near its posterior margin.

(*b*) *The Mesothorax*—(Pl. III, Figs. 1, 2, 4)

The mesothorax is the middle segment of the thorax, well developed and much bigger than the metathorax. It carries the fore wings. The mesotergum consists of the notum (also called the alinotum by Snodgrass, 1935) and the postnotum, the former comprising the prescutum, the scutum and the scutellum. The anterior edge of the mesonotum carries an inflected ridge on the inner side, the first phragma. The prescutum is heart-shaped, lies anteriorly in the middle, bounded by two convergent sutures (notaulices) that meet in the mid-line. A median suture divides it into two. The scutum lies behind and on the sides of the prescutum and extends backward laterally on the sides of the scutellum. It thus forms two lateral areas connected by a median bridge between the approaching ends of the prescutum and the scutellum. The lateral margins of the scutum are produced outwards to form the anterior notal wing processes in front and the posterior notal wing processes behind. A scuto-scutellar suture separates the scutum from the V-shaped scutellum. A pair of lateral sutures, the parapsidal furrows, start from the sides of the scutellum and run forward and outward, setting off two latero-posterior areas of the scutum, the parapsides or parascutellar areas of Ross (1937). The scutellum is a large V-shaped sclerite in the middle with the apex of the V directed forward. Its posterior border is somewhat thickened and is termed post-tergite by Ross (1937). It is produced laterally to become continuous with the axillary cords of the wings. The postnotum is a broad sclerotized band, notched in the middle

and lying across behind the scutellum with which it is connected by a small membrane; the latter is almost covered over by the scutellum. Laterally, the postnotum runs outward, downward and forward to reach the posterior edge of the mesepimeron on either side. The posterior border of the postnotum is produced backwards into a large bifid, heavily pigmented second phragma, extending beneath the metatergum and a part of the first abdominal tergum. The mesopleuron on either side shows externally a well-developed pleural suture which runs upward and forward, dividing it into an anterior episternum and a posterior epimeron. Ventrally the pleural suture forms a dorsal pleural articulation with the ridge of the mid-coxa and is continued into an articular wing process at the other end. The episternum is fused with the basisternum, the pleurosternal suture having disappeared. There is a triangular piece cut off from the anterodorsal portion of the episternum which has been variously named parapterum, prepectus, pre-episternum and so on and has been regarded as peculiar to the hymenoptera. This piece can safely be compared to the anepisternum of the neuroptera in which the division of the episternum into an anepisternum and katepisternum is a common feature; the former may be larger, equal to or smaller than the latter. The episternum of the taxonomists corresponds then to the katepisternum which has fused with the basisternum. The mesepimeron is divided into a horizonal, narrow dorsal portion, the anepimeron and a vertical broad ventral portion, the katepimeron. A trochantin, though weakly sclerotized, starts from the lower end of the pleural suture and below the episternum and runs along the posterior margin of the sternum between it and the coxa. It is intimately fused with the sternum, its inner end curving round to articulate with the anterior coxal ridge on its inner side, a little in front and below (above when seen from the ventral side) the sternal coxal articulation. In front of the anepisternum lies the mesothoracic spiracle in a weakly sclerotized area. Behind the epimeron at the junction of the anepimeron and the katepimeron lies the meta-thoracic spiracle. The mesosternum consists of the presternum, the basisternum, the furcasternum and the spinasternum. The presternum is a narrow strip cut off from the anterior margin of the sternum, extending on the sides up to where the lateral pronotal plates meet the episterna. The basisternum is not demarcated from the episterna as it is in some other sawflies. There is, however, a prominent median suture running in the mid-ventral line with a strong ridge on the inner side. The furcasternum is a reduced sclerite behind the basisternum in the middle. Internally it bears two well-developed furcal arms which meet in the middle and run forward as a common median ridge on the inner side of the median line of the basisternum. Posteriorly the furcasternum sends a pair of processes, one on either side of the middle line, each articulating with the coxa of its side on the latter's mesal surface. These sternal coxal articulations lie dorsal and a little posterior to the trochantinal articulations mentioned before. The spinasternum is a small membranous part between the two coxae and behind the furcasternum. It can be better seen when the legs are pulled up and apart.

(c) *The Metathorax*—(Pl. III, Figs. 1, 2, 4)

The metathorax is much smaller than the mesothorax and bears the hind wings. The metanotum lacks the prescutum. The scutum consists of two lateral parts, connected by a narrow bridge in front of the scutellum. A pair of elongated, slightly raised, membranous areas, the cenchri, are differentiated on the scutum, one on either side of the middle, along its anterior border. They are narrow towards the inner side and broaden out gradually, extending to more than half the breadth each way. The lateral margins of the scutum are produced into the anterior and the posterior wing processes as in mesoscutum. There are no parapsidal sutures but faint indications exist. The scutellum is broadly triangular and punctate with a 'post-tergite,' continuous laterally with the axillary cords. The postnotum is a narrow sclerite behind the scutellum. Its hinder edge forms a weak phragma. The metapleuron shows the prominent pleural suture. The episternum is divided into anepisternum and katepisternum, the latter fused with the basisternum. The epimeron is not completely divided into two as the dividing suture bends back and stops short in the middle. The mesopleura and the metapleura are so similar that one can safely say that the so-called prepectus in the mesopleuron is nothing else but the anepisternum. The trochantin is similar to that in the mesothorax but is considerably bigger. The coxae, as in the mesothorax, have three articulations, the pleural, the trochantinal and the sternal. The metasternum has the same parts as the mesosternum except that the spinasternum is missing. The various sternal elements are, however, comparatively small.

7. The Thoracic Appendages

The thoracic appendages include the legs and the wings.

A. *The Legs*—(Pl. III, Fig. 3; Pl. IV, Figs. 3, 5)

There are three pairs of legs, the prothoracic, the mesothoracic and the metathoracic; the hind pair is the longest. The legs lie quite approximated together on the ventral side. They are similar, each consisting of the coxa, the trochanter, the femur, the tibia and the tarsus. All the segments bear small spines.

In the prothoracic leg the coxa is conical, broad proximally and narrow distally, with a thickened marginal ridge, the basicoxite, running round its proximal end. It articulates dorsally with the ventral end of the pleural suture to form the pleural articulation and ventrally with the backwardly directed process of the furcasternum to form the sternal articulation. Distally the coxa articulates with the trochanter. The latter is small, slightly broader distally and presents a two-segmented appearance because it is closely associated with a small proximal conical piece marked off from the femur by a strong suture. The femur is cylindrical and possesses distally a membranous part on its ventral aspect. The tibia is the longest segment of the leg. It is narrow proximally and broad distally and bears at its distal end two similar, finger-shaped bluntly pointed tibial spurs,

which are of classificatory importance. The tarsus is elongate and divided into five tarsomeres. Of these the first is the longest, the second, the third and the fourth progressively smaller, and the fifth longer than the second but smaller than the first. The first four tarsal segments each bear an oval, slightly cupped tarsal pulvillus. The last tarsal segment carries a pair of claws and some other structures, together designated as the pretarsus. The claws or ungues are articulated to the distal bilobed rim, the unguifer, on the dorsal side of the last tarsal segment. Each claw is curved at its extremity and bears a small tooth at its distal one-third on the ventral side. From the notch of the unguifer arises a pear-shaped sclerite, the orbicula. Its surface is marked into small areas and bears two pairs of long setae on the distal half. Lying in the terminal part of the cavity of the last tarsal segment on the ventral side is a sclerotized, sculptured sclerite, the unguitractor plate. It is more or less bell-shaped and grooved in the middle with a conspicuous apodeme at its proximal end, running through the tarsal segments. Arising between the claws and distal to the unguitractor plate is a membranous stalked adhesive organ, the arolium. It is somewhat rounded in outline. Its stalk is supported dorsally by the orbicula and ventrally by another triangular plate, the planta. The latter lies distal to the unguitractor and bears a pair of spines. The body of the arolium is supported by a pigmented ring-like camera. On the sides of the planta and at the bases of the claws can be made out two small sclerites, the basipulvilli, also known as the auxilia.

The mesothoracic and the metathoracic legs are similar to the prothoracic legs except in the number of coxal articulations. In these the coxa has three articulations, a pleural with the pleural suture, a sternal with the sternal process and a trochantinal with the trochantin. The trochantin in the meso and metathoracic legs is a weakly sclerotized element fused with the postero-lateral margin of the sternum and articulates with the coxa, a little below and in front of the sternal articulation. It will be noticed that in close association with these articulations, one finds definite sense-areas similar to the proprioceptor organs described by Pringle (1938) in the leg of *Periplaneta*. These sense-areas may be in the form of 'hair-plates' or areas with campaniform sensillae; both types may occur simultaneously in the same leg. According to Pringle's electrophysiological study these sense-areas may be regarded as 'tension receptors.' Such areas have also been observed in other insects. In *Diprion pini* there are three such sense-areas in connection with the meso and the metathoracic legs and two with the prothoracic. Ferris and Pennibaker (1939) named the trochantinal area with setae in *Agulla* (Raphidiidae) as 'trochantinal signum' which according to them is a landmark to determine whether a trochantin is present. The hind tibia in the male possesses two unequal spurs, while those of the female are equal.

B. *The Wings and their Articulations*

There are two pairs of membranous wings, the fore-wings and the hind-wings, the former slightly longer than the latter. The two wings on

either side are held together by a few hook-like hamuli along the anterior margin of the hind wing which catch on the slightly curved posterior margin of the fore-wing. Fine pubescene is borne on the wing margins.

(*a*) *The Wing Articulations*—(Pl. IV, Fig. 4)

The wings are hinged on to the sides of the mesothorax and the metathorax by their membranous bases which are strengthened by a number of articular sclerites. The anteriormost of these in the fore-wing is a small flat piece, the humeral plate, at the anterior margin of the wing-base and in association with the costa. Behind the humeral and articulating with the anterior notal wing-process is the first axillary sclerite which sends a process to the humeral plate in front and articulates by its outer margin with the inner margin of the second axillary sclerite. The latter is irregular in shape and sends an inner long process to the post-notal wing process and an outer short process to the third axillary. The second axillary is produced anteriorly into another process which reaches the base of the common subcostal+radial vein. The third axillary sclerite is also irregular and forked anteriorly, the inner limb broader than the outer and called accessory sclerite of the third axillary by Snodgrass (1910). Its outer border is closely associated with the bases of the anal veins, whereas its posterior angle reaches the tip of the prolongation of the post-notal wing process. The fourth axillary sclerite of other insects is absent. A large, laterally elongated, weakly sclerotized median plate lies outer to and in front of the second axillary. Its anterior inner angle is confluent with the common base of the $Sc+R+M+Cu$. The posterior margin of the wing is thickened to form the axillary cord, continuous with the lateral prolongation of the scutellum. Lying above the base of the wing, inner and anterior to the humeral plate, is a sclerotized scale like plate, the tegula. It has the shape of a quadrant, beset with small spines on its dorsal surface and its outer angle produced into a point.

(*b*) *The Wings*

Several systems of nomenclature for the wings of hymenoptera have been offered but Ross's system (1936) based on their derivation from the *Sialis*-like panorpoid, has been adopted because it agrees with conclusions drawn from other characters studied.

(i) *The Fore-Wing*—(Pl. IV, Fig. 1)

The fore-wing is 8·5 mm. long and 3·4 mm. broad. There is a thickened dark patch, the pterostigma, on the anterior margin near its middle, covered with small hair. The anterior margin of the wing is straight and the number of veins is relatively small, compared with some of the sawflies. The costa runs along the anterior margin. The subcosta, radius, media and cubitus are proximally fused to form a common basal vein. This divides into two branches, an anterior $Sc+R$ and a posterior $M+Cu_1$. The subcosta separates off before the pterostigma beyond which point M meets the radius. The common $R+M$ divides into R_1 and $RS+M$, the former running along the inner and the posterior margin of the

pterostigma to the border. $RS+M$ are confluent for some distance and then they separate off, each running straight to the margin of the wing. There is one cross-vein between R_1 and RS, the second having been lost, resulting in the formation of a large cell Rl_2+Rl_3. M separates from Cu_1, runs for some distance with RS and leaves it to reach the margin unbranched. There are two cross-veins between RS and M. Cu_1, after M has separated off, runs out for some distance and then divides into Cu_{1a} and Cu_{1b}, the latter receiving the common anal vein before it reaches the border. There are two cross-veins between M and Cu_1. There is no Cu_2. There is one cross-vein between Cu_1 and $1A$ and one cross-vein between $1A$ and combined $2A$ and $3A$. $2A$ and $3A$ meet and the combined vein meets $1A$, the resulting common anal running into Cu_{1b} before the latter reaches the border. There is a small jugal lobe with spinules along its margin.

(ii) *The Hind-Wing*—(Pl. IV, Fig. 2)

The hind-wing is 6·5 mm. long and 3·4 mm. broad. The pterostigma is very much reduced. Some 9–12 hook-shaped hamuli are borne on the anterior margin near its middle and a few fine setae near the base. There is no cross-vein between R_1 and RS, so a large radial cell is formed ($R_{1\ 1+2+3}$). RS and M do not run together as they do in the fore-wing. There are two cross-veins between RS and M (same as fore-wing) but only one cross-vein between M and Cu_1 (reduction). There is one cross-vein between Cu_1 and A. The anals form one anal cell and the petiole of the cell meets $Cu\ a$. An anal lobe is present.

8. The Abdomen

(Pl. V, Figs. 1, 2).

The thorax passes into the abdomen without a constriction. The abdomen consists of ten segments. The first six segments and the last are similar in the two sexes but the 7th, 8th and 9th are modified differently for oviposition and copulation.

The first segment (Pl. III, Fig. 1) has its tergum sclerotized to form two dorso-lateral pieces, narrowing in the mid-dorsal line, with a membranous part between and behind them. The sternum is small, membranous and invaginated. The abdominal spiracles of the first pair are large and are borne on the tergum, one on either side.

Segments 2–7 (Pl. V, Fig. 2) are identical, each with a dorso-lateral band-like tergum, a similar ventrolateral sternum and a membranous strip on either side between the two. Each tergum is dorsally divided into a narrow anterior portion and a broad posterior portion, the latter bearing the paired spiracles on the sides. These spiracles are smaller than those of the first pair.

The seventh sternum in the female covers posteriorly the gonopore in the membranous infolding of the VIII sternite.

The eighth tergum in the male is slightly smaller than the preceding one and bears the last pair of spiracles laterally. The sternum is not a

continuous band ventrally but is notched in the middle to form two ventro-lateral pieces. The eighth tergum in the female is comparatively larger. The eighth sternum lies behind the seventh. It is mostly membranous but there is a small sclerotized piece, partially divided into two, lying between the bases of the first valvulae. This can possibly be the remnant of the sclerotization of the VIII sternum.

A. *The Female External Genital Apparatus*

The eighth sternum and the one following are modified to form the female external genital apparatus or the ovipositor (Pl. V, Figs. 3, 4). There are two triangular sclerites, the first valvifers, one on either side behind the eighth tergum and below the ninth tergum, articulating with the latter dorsally and with the second valvifer ventrally. These sclerites belong to the eighth segment but have shifted backward. Each valvifer (also called coxite of VIII) sends inward and backward a sclerotized pigmented ramus which is continued into a blade-like ventral valve or stylet, the first valvula (also called saw; gonapophysis of 8th) broader proximally and tapering gradually towards its distal end. Each valve has a smooth inner margin and bears on its ventral surface rows of serrations on raised ridges and is produced dorsally into a longitudinal ridge which fits into a corresponding ventral groove on the inner valve, affording a sliding movement forward and backward.

The ninth tergum in the female is narrow dorsally but broadens out laterally. Its antero-lateral margins articulate with the first valvifers whereas its postero-lateral margins partially cover the second valvifers and the third valvulae. A pair of elongate second valvifers (also called coxites of IX), one on either side, lies below the first valvifers with which they articulate. The second valvifers do not articulate with the ninth segment. Laterally each second valvifer is produced backward. From its ventromesal border is given off a ramus (similar to the one given off by the first valvifer) which connects it to the blade-like second or the inner valvula (also called saw-guide, gonapophysis of 9th), while distally it articulates with a broad, sheath-like third valvula (also called style of the 9th; gonostyle). The two inner or second valvulae are connected by membrane along their inner margins throughout their length except at their posterior extremities. They are dorsally convex and ventrally concave with a groove each for the dorsal ridge of the first valvula. The surface of the fused second valvulae is marked by faint annular ridges and the margins are produced into small processes. Distally the two valvulae are unequal in length, the right one longer and slightly broader. The connecting membrane between the second valvifers and valvulae is comparable to the ninth sternum.

The tenth tergum is a small piece forming a proctiger above the anus in both sexes. It bears laterally, near its distal margin, a pair of small more or less club-shaped appendages, the cerci (also called socii), beset with strong setae. Snodgrass (1941) calls these the pygostyles. He observed a pair of cerci on the invaginated anal lobes (XI Seg.) in the male of the ichneumonid *Megarhyssa lunator* in addition to the socii on the 10th

segment. This is a solitary example where the socii and the cerci are said to be met with in the same animal. The tenth sternum is missing.

B. *The Male External Genital Apparatus*—(Pl. V, Figs. 2, 5)

The ninth tergum in the male is reduced, covered over by the eighth, concealed from view dorsally, and appearing as two lateral pieces on the sides. The ninth sternum is the largest and forms a deeply concave subgenital plate or hypandrium, covering the male genitalia ventrally.

The genital apparatus is of the strophandrious type in which the pear-shaped, dorsoventrally flattened phallus lies loosely attached in the cocavity of the hypandrium, or the genital chamber, with its primary dorsal and ventral surfaces inverted to become the lower and the upper surfaces respectively. It consists of a two-segmented phallobase, a pair of parameres (harpes), a pair of volsellae, a pair of parapenes and the aedeagus. The basal part of the phallobase, the basal ring or the gonocardo of Crampton (1919) and the gonobase of Michener (1944), is a sclerotized ring, pointed anteriorly with an oblique anteroventral opening. It articulates with the two distal, laterally placed sclerotized pieces, the parameral plates or the gonostipites of Crampton (1919) and the gonocoxites of Michener (1944), which are connected by membrane dorsally and are closely associated ventrally with a pair of broad parapenial lobes meeting in the median line. Each parameral plate carries a more or less triangular paramere or the harpe of Crampton (1919) and the gonostylus of Michener (1944), bearing setae at its distal end. On the dorsal aspect and attached to the inner sides of the parameral plates are the two broad lobes, the volsellae, each with a bifurcate beak-shaped distal end and provided with a prominent basal apodeme, running anteriorly. The aedeagus consists of a median membranous part, which receives the ejaculatory duct, and a pair of elongated penis valves, one on each side of the medium part and lying ventral to the volsellae. The penis valves are rod-like proximally and enlarge distally, the inner margins of the distal parts being concave. Each penis valve is provided with an aedeagal apodeme, running anteriorly.

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Abbreviations

A—Anal vein; *Ab.*—Abdominal segment; *Ae. ap*—Aedeagal apodeme; *Aem*—Anepimeron; *Aes*—Anepisternum; *An. L.*—Anal lobe; *Ant*—Antenna; *Ar*—Arolium; *Ata*—Ant. Tentorial arm; *Atp*—Ant. Tentorial pit; *Ax*—Axillary sclerite; *Ax. C*—Axillary Cord.

B.P.—Basipulvilli; *B.R.*—Basal ring; *B.S.*—Basisternum; *B.T.*—Body of the tentorium.

C—Costa; *Ca*—Camera; *Cam. Sen.*—Campaniform sensilla; *Cd*—Cardo; *Ce*—Cercus; *Cen*—Cenchri; *Cl*—Claw; *Cly*—Clypeus; *C.Sc.*—Cervical solerite; *Cu*—Cubitus; *Cu a*—Cubito-anal cross-vein; *Cx*—Coxa.

Dta—Dorsal tentorial arm.

E—Eye; *Emp*—Epimeron; *Eps*—Episternum.

F—Femur; *Fl*—Flagellum; *Fr.Pa*—Fronto-parietal; *Fr.S*—Frontal suture; *F.S.*—Furca sternum.

Ga—Galea; *Gl*—Glossa.

Ham—Hamuli; *H.P.*—Humeral plate; *Hy*—Hypandrium; *Hyp*—Hypopharynx; *Hys*—Hypostoma.

I.T.—Incisor tooth.

Kem—Katepimeron; *Kes*—Katepisternum.

Labr—Labrum; *Lac*—Lacinia; *Lp*—Labial palp.

M.—Media; *m cu*$_1$—Medio-cubital cross-vein; *Md*—Mandible; *M.P.*—Median plate; *Mxl*—Maxillaria; *Mxp*—Maxillary palp.

Oc.l—Ocellus lateral; *Occ*—Occiput; *O.F.*—Occipital foramen; *Orb*—Orbicula.

Ped—Pedicel; *Pf*—Palpifer; *P.G.*—Postgena; *Pgl*—Paraglossa; *Phr*—Phragma; *Ph.Sc*—Pharyngeal Sclerite; *Pl*—Planta; *Pl.S.*—Pleural suture; *Pm*—Postmentum; *Pm.p*—Parameral plate; *Pmr*—Paramere; *P.N.*—Post Notum; *P.Occ*—Post occiput; *Pp.l*—Parapenial lobe; *Prm*—Prementum; *Pr.N*—Pronotum; *Pr.S*—Presternum; *Pr.Sc*—Prescutum; *Pr.T*—Pretarsus; *Ps*—Parapsides; *Pt*—Post-tergite; *Ptp*—Posttentorial pit; *Pts*—Pterostigma; *P.V.*—Penis valve.

R—Radius; *Rs*—Radial sector; *rm*—radio-medial cross-vein.

S—Sternum; *S.a*—Sternal articulation; *SC*—Subcosta; *Sc*—Scutum; *Sca*—Scape; *Scl*—Scutellum; *Sl.D*—Salivary Duct; *Sp*—Spiracle; *S.pp*—Sense papillae; *Sp.R*—Suspensorial Rod; *St.*—Stipes.

T—Tergum; *Ta*—Tarsus; *T.Ant.S*—Terminal Antennal Segment; *Teg*—Tegula; *Tem.S*—Temporal Suture; *Ti*—Tibia; *T.m*—Tentorial Macula; *Tor*—Tormae; *Tp*—Tarsal pulvilli; *Tr*—Trochantin; *Tr.a*—Trochantinal articulation; *Tro*—Trochanter; *T.S.*—Tibial Spur.

Uf—Unguifer; *Utp*—Unguitractor plate.

Ve—Vertex; *Vl*—Valvula; *Vlf*—Valvifer; *Vol*—Volsella.

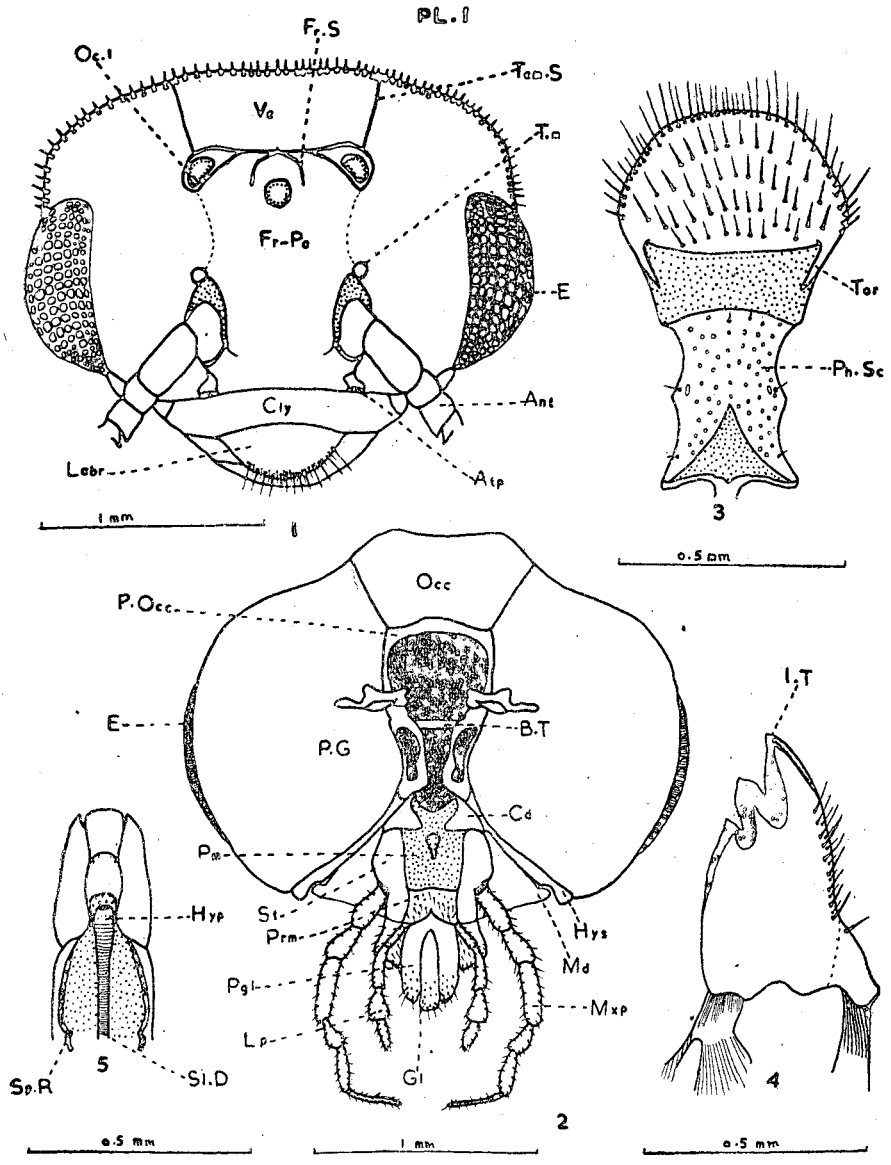

FIG. 1. Head of Diprion pini—dorsal view.
„ 2. Head of Diprion pini—ventral view.
„ 3. Labrum of Diprion pini—ventral view.
„ 4. Right Mandible of Diprion pini.
„ 5. Hypopharynx of Diprion pini—dorsal view.

PL. II

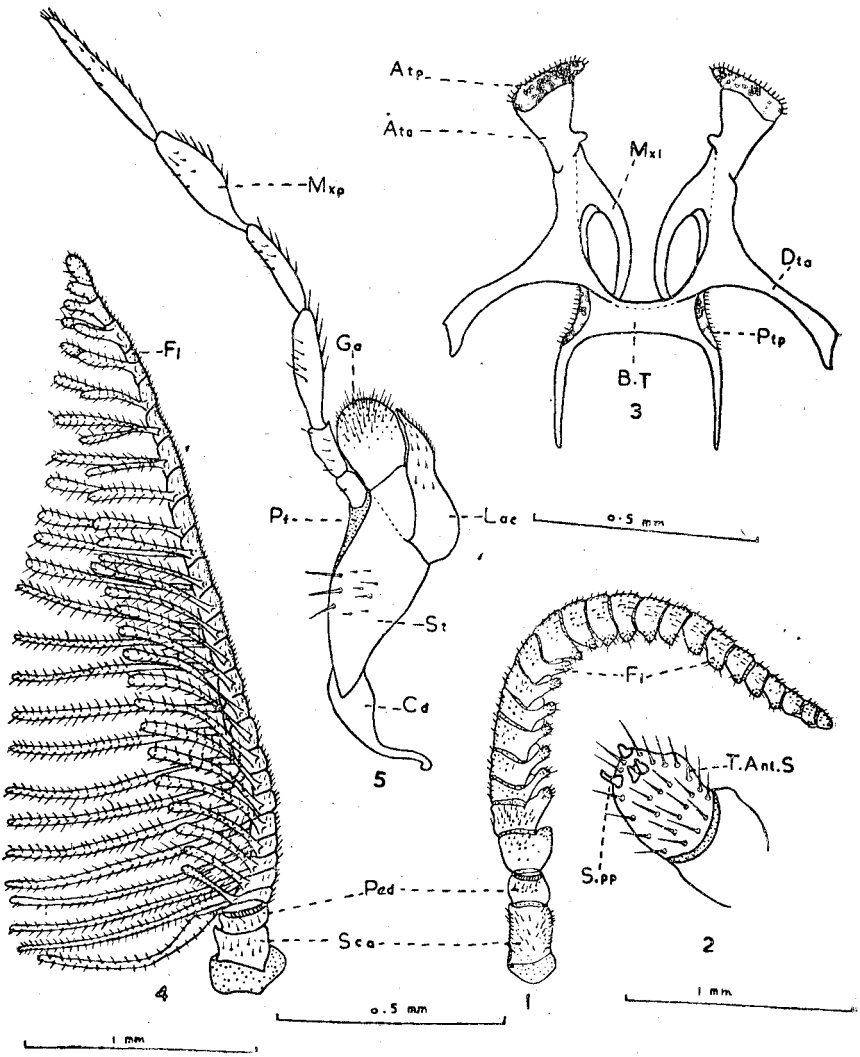

FIG. 1. Antenna of Diprion pini (female).
„ 2. Terminal antennal segment of Diprion pini.
„ 3. Tentorium of Diprion pini—dorsal view.
„ 4. Antenna of Diprion pini (male).
„ 5. Maxilla of Diprion pini.

PL. III

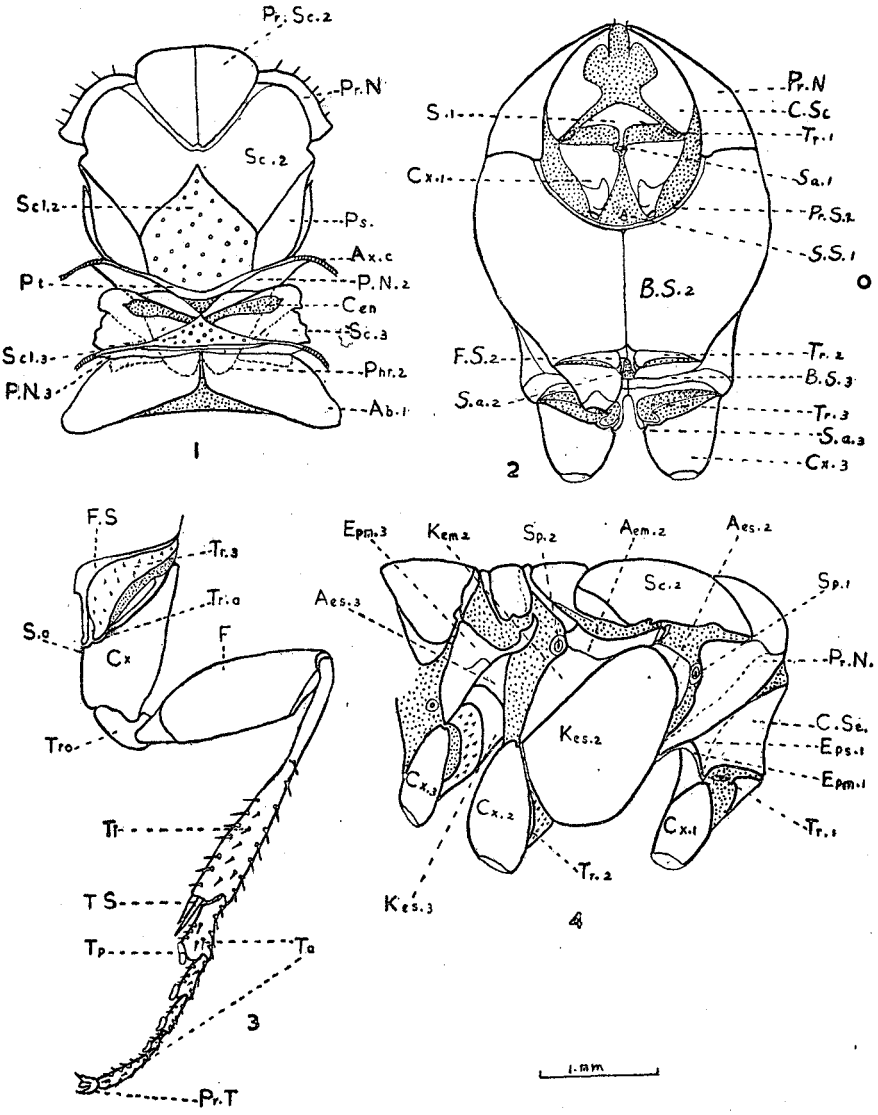

FIG. 1. Ptero-thorax of Diprion pini—dorsal view.
,, 2. Thorax of Diprion pini—ventral view.
,, 3. Hind leg of Diprion pini.
,, 4. Thorax of Diprion pini—lateral view.

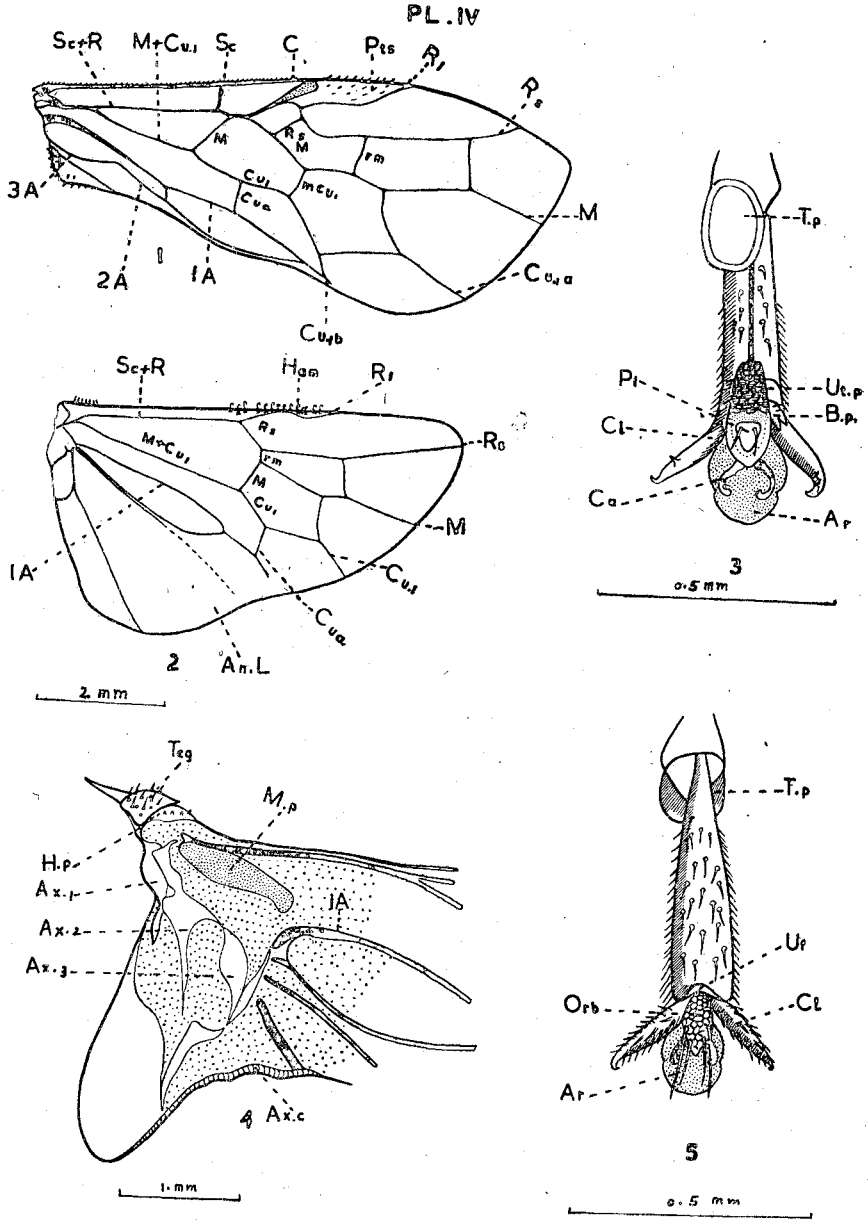

FIG. 1. Fore-wing of Diprion pini.
,, 2. Hind-wing of Diprion pini.
,, 3. Pretarsus of Diprion pini—ventral view.
,, 4. Wing bases of Diprion pini.
,, 5. Pretarsus of Diprion pini—dorsal view.

PL. V

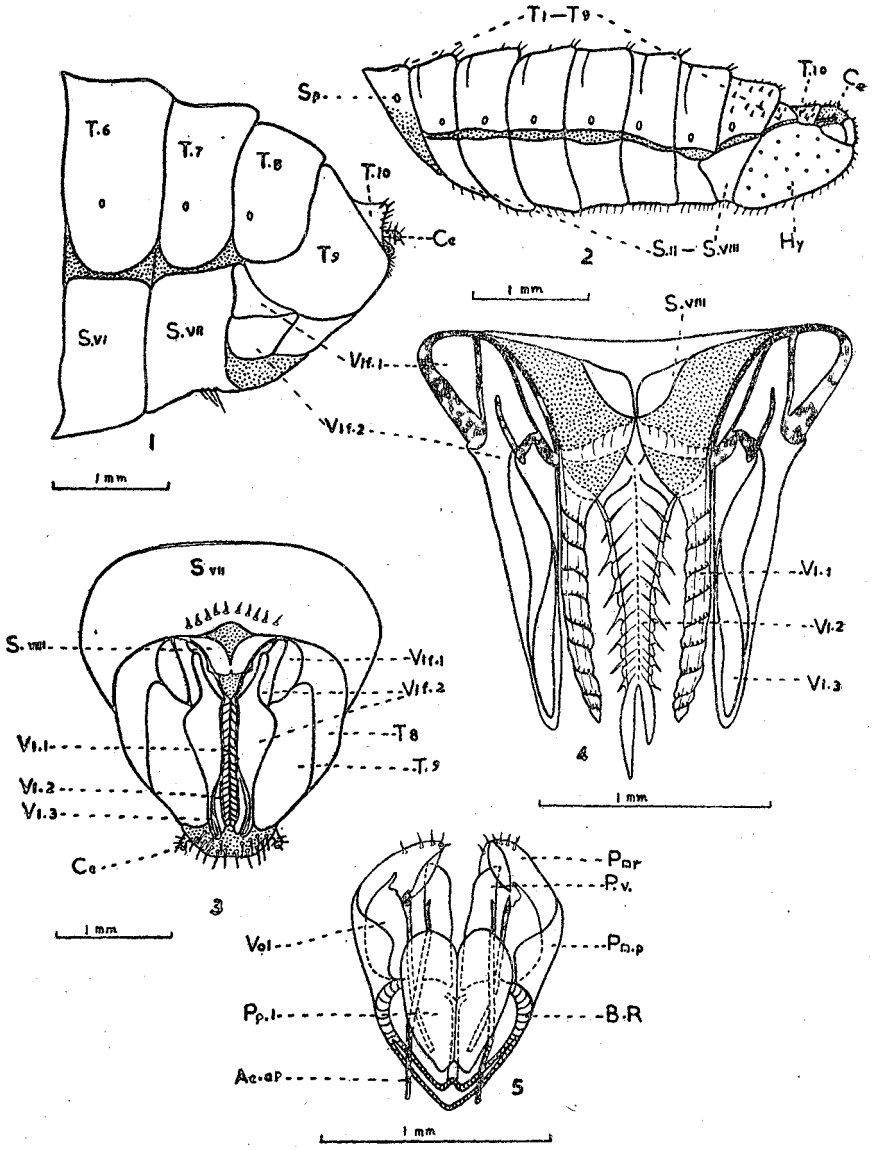

FIG. 1. Terminal abdominal segments of Diprion pini—(female).
„ 2. Abdomen of Diprion pini—(male) lateral view.
„ 3. Terminalia and Ovipositor of Diprion pini—ventral view.
„ 4. Ovipositor of Diprion pini—ventral view.
„ 5. Phallus of Diprion pini—ventral view.

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 26 VETERINARY Pp. 23-32

April, 1953

A PRELIMINARY INVESTIGATION TO STUDY THE EFFECT OF FEEDING CONCENTRATES ON THE FREQUENCY OF OESTRUS IN CATTLE

by

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Edited for the East Panjab University by Vishwa Nath,
Department of Zoology, East Panjab University,
HOSHIARPUR

Price Rs.1-2-0

A PRELIMINARY INVESTIGATION TO STUDY THE EFFECT OF FEEDING CONCENTRATES ON THE FREQUENCY OF OESTRUS IN CATTLE

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Introduction

A common difficulty felt in this country is that sufficient number of females on heat is, as a rule, not available when work on artificial insemination is in hand. To overcome this difficulty, a small-scale experiment on 40 hill-goats was conducted at this farm in 1944-45 by Dr. J. K. Makhijani, the then Animal Geneticist, and he concluded that feeding of *methi* seed (*Trigonella foenumgroecum*) could be successfully employed for increasing the frequency of oestrus in goats.

In 1946, while adopting the Annual Report of the Scheme to Supplement Livestock Investigation, Hissar, for the year 1944-45, the Animal Breeding Committee and the Advisory Board of the Indian Council of Agricultural Research emphasized upon the necessity of further investigational work on feeding *methi* seed for bringing animals into oestrus. In response to these remarks an experiment on 40 young Hariana heifers, each about $2\frac{3}{4}$ years old, was conducted during the year 1946-47. Four groups, each of ten animals, were formed and each group was given a different treatment. The data collected for one year's experimentation revealed that the number of animals put in each group was not sufficient, as some animals under different treatments did not come on heat even once during the entire experimental period. The statistical analysis of the data was, thus, rendered difficult and no useful results of practical importance could be achieved. Fresh experimental work was, therefore, started on the 6th May, 1948.

Previous Work

From a careful study of the Literature, it appears that very little work dealing specifically with the influence of nutrition on the frequency of oestrus in farm animals has been done. Some references to the effect of feeding on early onset of oestrus, and on the length of oestrous cycle are, however, available.

Darlow and Hawkins (1933) found that breeding was more effective with ewes receiving modified rations than with ewes receiving only prairie

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hay. Clark (1934) stated that flushing did not appear to have any significant influence on accelerating onset of heat in ewes and that gain in weight was not in itself indicative of higher ovulation rate. Okulicev (1934) found that flushing had good influence on lamb crops by reducing the percentage of barren ewes and raising the percentage of multiple births. It also reduced the death rate amongst the new-born lambs and the percentage of still-births. It seemed that feeding of barley was the most and that of millet the least satisfactory. Tebbe (1935) stated that a *slimming diet may induce oestrus in cows.* Polovceva *et al.* (1938) concluded that feeding of concentrates to ewes increased the number of multiple ovarian follicles and of lambs born. Addition of phosphates did not appear to affect ovarian function. Kelley (1939), working with sheep, noticed that the incidence of oestrus decreased during the spring months and increased in the summer months, the higher incidence being maintained during late summer, autumn and winter. His data, however, did not support the suggestion that the reproductive rhythm was directly associated with periodicity of the plane of nutrition. Sinicyn (1939) made observations on three groups of ewes which grazed respectively on natural steppe pasture, lucerne and winter barley aftermath, and showed that the rate of lambing was lowest in the group grazing on natural pasture. This result was ascribed to differences in chemical composition, vitamin and water content of the fodder. Ovsjannikov (1940) observed that a ration rich in proteins caused a 25% increase in fertility in pigs. Underwood and Shier (1941) found that flushing produced a highly significant increase in fertility due entirely to an increased proportion of twins. It, however, did not bring the ewes into oestrus any earlier, nor did it increase the percentage of ewes which conceived. Palmer *et al.* (1941) concluded that a combined deficiency of phosphorus and protein in the bovines, analogous to similar deficiencies in animals reared largely on prairie hay in the phosphorus deficient regions, delayed sexual maturity and repressed normal evidences of oestrum, missing thereby periods of oestrum, but did not interfere with the normal regularity of ovulation or the ease of conception. Briggs *et al.* (1942) did not observe any difference in time of first oestrus between flushed and unflushed groups of ewes. The unflushed ewes had, however, significantly longer oestrous cycles.

Material and Methods

Sixty young Hariana heifers, each of about $2\frac{3}{4}$ years in age and possessing normal conformation, were selected from the farm-bred stock at Hissar for this experiment. These animals were divided into 15 blocks, each consisting of 4 animals of as equal an age as possible. This was done to eliminate, as far as possible, the effect of age which seemed to influence the onset of oestrus to a great extent. Since the lay-out of the experiment was on a Fisherian 'Randomized Block Design', the four proposed treatments were allotted at random to the animals within each block. Thus, by separating the block differences, i.e. differences between the various age groups from the main error, a true and proper estimation of the experimental error was obtained.

The plan of feeding in the four treatments was as follows:

Treatment No. 1 .. Grazing only (Control).
Treatment No. 2 .. Grazing plus one seer of gram per head daily.
Treatment No. 3 .. Grazing plus half seer of gram and half seer of *bajra* (Pearl millet) per head daily.
Treatment No. 4 .. Grazing plus half seer of gram and half seer of *methi* seed (Fenugreek) per head daily.

It may be stated here that free grazing was preferred over stall-feeding as it was observed that the animals in the Hariana tract in general and at the Hissar Farm in particular did not thrive well if they were stall-fed. If the rains are normal the grasses in the *bir* (reserved forest) attached to the Hissar Farm grow in such abundance that the animals do not care to look at the other fodder crops. However, when grazing is not adequate roughages are also fed to all the animals. The roughages normally available at the Farm are:

1. Grasses .. Anjan (*Pennisetum chenchorides*); Dub (*Cynodon dactylon*).
2. Straws .. Oat and Wheat *bhusa*.
3. Hays .. *Jowar* and grasses.
4. Greens .. Berseem, *Jowar* and Lucerne.
5. Silage .. *Jowar* and grasses.

Bajra and *methi* seed, used as feeds in treatment No. 3 and 4 respectively, were analysed at the Animal Nutrition Section of the Indian Veterinary Research Institute, Izatnagar, and the results of analyses on dry matter basis were as follows:

	Bajra %	*Methi* %
Ether extract	2·766	7·962
Ash	2·309	4·438
Crude protein	13·369	24·797
Crude fibre	1·862	11·917
Nitrogen-free extract	79·694	50·886

Since the various signs of oestrus in an animal are displayed best when a bull or other cows are present nearby, all the experimental animals were let loose in an enclosure along with two vasectomized bulls. They were always kept under close observation and were tied only at the time of feeding. A careful record was made of the date and time when a particular animal came on and went off heat. The following description of the signs of heat in cows is from Hammond (1927):

'At the onset of heat a cow becomes restless and frequently when tied in the stall is seen standing while the others are lying down. She twitches her tail frequently and often raises it. When out at grass the cow does not usually continue to feed but wanders about the field and frequently goes off by herself or with another cow which she rides or is ridden by. She will also frequently jump the bull and when he attempts to serve her will stand still and not move away as happens when the animal is not on heat. A cow on heat also frequently lowers the hips and small of the back and raises the tail

head; she also, more especially after jumping other cows (and after service by the bull), arches her back and strains, a string of glairy mucus coming away from the vulva at this time.'

'When on heat she will also frequently play with the bull by horning him or will stand and lick him, and when separated from other cattle will low or "blar". The bull will "hang round" a cow that is just coming on or going off, although she is not actually on heat at the time, and will not stand to service.'

During the course of this investigation, which continued for full one year, it was observed that besides one heifer which calved, there were four others which did not show any signs of heat at all. While the inclusion of the already pregnant heifer in the experiment could reasonably be ascribed to the fact that her early pregnancy was not detected at the time of her selection, no satisfactory reason for the absence of oestrus in the other four animals could be given without a careful study of their future behaviour. These animals were, therefore, retained for further study, of course, under normal conditions. Out of these four animals three came on heat later and this was probably the result of their late maturity. The fourth heifer seemed to be an abnormal case inasmuch as her reproductive organs were found, on rectal and vaginal examinations, to be under-developed. A zero was, therefore, put in Table I to represent the number of heats recorded for all those animals which did not show any signs of heat throughout the experimental period but were normal. The data thus collected were as follows (dashes representing the abnormal cases):

TABLE I

Number of heats recorded

Treatments / Blocks	I (Control)	II (Gram)	III (Gram & *Bajra*)	IV (Gram & *Methi* seed)
1	1	4	11	4
2	1	9	7	13
3	2	0	10	6
4	2	3	2	—
5	4	10	11	1
6	2	—	2	4
7	3	7	9	4
8	1	2	2	2
9	8	4	9	3
10	14	1	1	5
11	3	1	3	2
12	0	6	5	6
13	0	7	4	6
14	1	2	7	3
15	1	1	9	9
TOTAL ..	43	57	92	68

STATISTICAL ANALYSIS AND THE RESULTS

Obviously the straightforward analysis of variance of the data could not be made as these were non-orthogonal. Moreover, from a look at the data, the animals in the 'control' group did not appear to behave so

consistently as those in the other groups. This might, of course, arise out of chance effects. The data, however, were examined in various ways by one of us (K. N. Vali) in consultation with the statistical section of the Indian Council of Agricultural Research. The details of the analyses and the results achieved were as follows:

1. *Study on the three treated groups leaving out the 'control'.*—In view of the apparent lack of uniformity in the data for the four groups, as explained above, it was thought desirable to confine the statistical analysis to the three treated groups only leaving out the 'control'. As the data were non-orthogonal, recourse was made to the method of 'Fitting of constants' for their analysis. The following Table II for the analysis of variance was constructed after evaluating the block and treatment effects and finding out, subsequently, the various sums of squares involved:

TABLE II

Analysis of Variance

Variance due to	D.F.	S.S.	M.S.	F. Ratio	5% point of F.
Blocks (ignoring treatments)	14	202·74	14·48	1·58	2·10
Treatments (adjusted for blocks)	2	35·36	17·68	1·93	3·37
Error	26	237·81	9·15	..	..
TOTAL ..	42	475·91			

The above table shows that neither the treatment nor the block mean square was significant as compared to the error. This implies that the treatments Nos. 2, 3 and 4 did not appear to be significantly different in inducing oestrum.

2. *Study on all the four treatments.*—The question whether chance could be the reason for the apparent inconsistency in the data for the 'control' group as compared to the other groups was investigated as follows:

A straightforward analysis of variance as above on all the four groups instead of three was first carried out and the results achieved were as follows:

TABLE III

Analysis of Variance

Variance due to	D.F.	S.S.	M.S.	F. Ratio	5% point of F.
Blocks (ignoring treatments)	14	152·90	10·92	0·89	1·95
Treatments (adjusted for blocks)	3	76·37	25·457	2·09	2·84
Error	40	487·21	12·180		
TOTAL ..	57	716·48			

It would be apparent from the foregoing table that the block variation was not significant. This means that the data for the four groups could be taken as random samples from possibly four different populations. The comparison of the variances of these four populations should reasonably give us a criterion for judging whether the data for the four groups were really inconsistent to justify a joint analysis of variance on all the four groups. If these variances did not differ significantly, there was every reason to believe that the differences were only due to chance effects. For this purpose a test of homogeneity of variances of these four populations was made. The value of χ^2 (chi-square) was found to be as low as 0·507. It was, therefore, concluded that these four variances did not differ significantly and thus a joint analysis of variance on all the four groups for the comparison of the four treatments appeared to be justified.

From Table III it was obvious that the treatments were not significant and that the calculated value of F in this case was slightly less than the value at the 5% level of significance while in the previous case the calculated value of F was very small and had a very high probability. This clearly showed that the difference in treatments, though not significant, tended to approach significance when 'control' was also included in the study. It was, therefore, reasonable to compare the means of the three treated groups with that of the untreated one, i.e. 'control'. This comparison showed that only treatment No. 3 (*bajra* plus gram) was significantly better than 'control', the result which would make it possible to recommend the feeding of a mixture of *bajra* and gram for increasing the frequency of oestrus in cattle.

3. *Estimation of missing values.*—To exploit the data for deriving some more useful inferences which might confirm the results already achieved it was thought desirable to restore the orthogonality of the data by estimating the two missing values. Recourse had, therefore, to be made to the 'Technique of the estimation of missing values' as evolved by Allan and Wishart (*1930*) and later generalized by Yates (*1933*). Employment of this technique was found quite reasonable in view of the fact that the two missing values occurred in the experiment merely due to random causes. On these grounds the problem was taken to be analogous to the one commonly arising in agricultural experiments where the yield of a plot or plots was occasionally lost or distorted by some disturbing factor beyond human control.

The two missing values were estimated both by approximate and exact methods. The values thus calculated were 2·03 and 2·73 respectively and with them the data were completed. The analysis of variance is given in Table IV.

From this study as well, it was clear that the calculated value of F (2·36) was slightly less than the value of F (2·84) at the 5% level of significance and it, therefore, completely corroborated the inference derived already in the previous case.

4. *Splitting the three degrees of freedom for treatments.*—For making a more detailed study of the data the three degrees of freedom assigned to

TABLE IV

Analysis of Variance

	D.F.	S.S.	M.S.	F. Ratio	Sum of squares calculated from
Total	57	716·483	..	..	Original data
Error	40	478·951	11·974	..	Completed data
Differences = Blocks + Treatments ..	17	237·532	..	..	..
Blocks	14	152·903	..	..	Original data
Differences = Treatments	3	84·629	28·209	2·36	..

treatments were further subdivided into three individual orthogonal degrees of freedom comprising the following three comparisons:

(i) Treatments (II+III+IV) ~ 3 I (Treated versus untreated).

(ii) Treatments (III+IV) ~ 2 II (Mixture versus no-mixture).

(iii) Treatment III ~ IV (*Bajra* versus *methi* seed).

After calculating the mean square for each of the above comparisons and comparing it with the error mean square it was found that only the first comparison, i.e. the treated versus the untreated, seemed to be significant at the 5% level of significance. It was, therefore, concluded that the feeding of a mixture of *bajra* and gram, due to its effectiveness, made the three treated groups as a whole to appear superior to the 'control'.

5. *Test of significance of the difference between the largest and the smallest means.*—To confirm the result already achieved the test of significance of the difference between the largest and the smallest means in a given group of sample means, as propounded by Tippett (1941), was employed. The range (the difference between the largest and the smallest means) and the standard error of difference in our case were calculated to be 3·26 and 1·27 respectively. Their ratio being just 2·6 (for a set of four means) was found to be significant at the 5% point according to the table prepared by Tippett for this purpose. This result, therefore, also confirmed the effectiveness of feeding a mixture of *bajra* and gram for the purpose in question.

DISCUSSION

It will be recalled that under the heading 'Previous work' we recorded only two references in which work on oestrus in cattle was mentioned. The first one was that of Tebbe (1935) who stated that a slimming diet may induce oestrus in cows. What actually constituted the so-called slimming diet, could not be ascertained as his original paper was not available anywhere in this country; nor could it be obtained from abroad. The second reference was that of Palmer *et al.* (1941) who concluded that a combined deficiency of phosphorus and protein in the bovines, analogous to similar deficiencies in animals reared largely on prairie hay in the phosphorus

deficient regions, delayed sexual maturity and repressed normal evidences of oestrum so that periods of oestrum appeared to be missed, but did not interfere with the normal regularity of ovulation or the ease of conception. These observations also run contrary to our own, as by chemical analyses the crude protein has been found to be less in *bajra* than in *methi*, while the former mixed with gram has been seen to be more effective for increasing the frequency of oestrus in cattle. It may, however, be pointed out that Makhijani (1944-45), working with goats in this Section, observed that *methi* seed containing more of protein was slightly better than *bajra* for increasing the frequency of oestrus.

The present experiment has further shown clearly that the interval between two successive heats in animals of the same breed and even of the same age varies considerably. The individual differences in the number of heats recorded from treatment to treatment and even within each treatment are so large as to warrant the statement that it is futile to assess the normal condition of oestrus in a breed by merely including a 'control' group for subsequent comparisons. In an experiment of such a nature, every individual animal should, therefore, have her own 'control' as the knowledge of her behaviour under normal conditions can possibly serve as the ideal 'control' which will enable a statistician to derive definite and precise conclusions after employing suitable statistical techniques. With this device the inconsistent 'within block variation' can also be avoided and properly accounted for, which, otherwise, is liable to vitiate the precision of the results.

In such experiments, it is ideal to study the oestrus in every individual animal for some reasonable period under normal conditions before they are given the proposed treatments. This would avoid the chance of losing some precision of the results by estimating certain missing values. This will also help in giving due consideration to the genetical differences between the various animals so far as frequency of oestrum is concerned. Finally, by using the number of heats per animal as a concomitant variate, the data will be easily analysed by the use of the well-known technique of 'Analysis of Covariance' and quite trustworthy results will be achieved.

Summary

1. In this investigation, feeding of a mixture of gram and *bajra* has been found to be quite effective for increasing the frequency of oestrus in cattle.

2. Some of the difficulties which are likely to be encountered in such investigations are presented in this paper.

3. A proper design of an experiment of this nature for tiding over some major difficulties is given.

4. Methods and suggestions for carrying out the statistical analysis of the data collected for such investigations have been put forth in detail.

Acknowledgments

The authors wish to express their sincere thanks to Shri B. N. Handa, B.Sc., M.R.C.V.S., the then Superintendent of the Government Livestock

Farm, Hissar, for his ever ready help and constant encouragement during the course of this investigation. They are thankful to Shri A. R. Roy, M.A., M.Sc., Statistician, Indian Council of Agricultural Research, for his valuable suggestions in carrying out the statistical analysis of the data. Thanks are also due to Dr. N. D. Kehar, Sc.D., Officer-in-Charge, Animal Nutrition Section, Indian Veterinary Research Institute, Izatnagar, for getting the samples of *bajra* and *methi* seed chemically analysed in his laboratories. They are also indebted to Major A. C. Aggarwala, B.Sc. (Hons.), M.R.C.V.S., A.I.R.O., Director, Veterinary Services and Warden of Fisheries, Panjab, for very kindly going through the manuscript of this paper. Lastly the grants from the Indian Council of Agricultural Research and the Panjab Government which made this investigation possible are also gratefully acknowledged.

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Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 27 ZOOLOGY Pp. 33-43

April, 1953

THE SPERM OF *LEPISMA DOMESTICA*

by

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Edited for the East Panjab University by Vishwa Nath,
Department of Zoology, East Panjab University,
HOSHIARPUR

Price Rs.2-0-0

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INTRODUCTION

In the last of the series of his epoch-making publications on insect spermatogenesis, Bowen (1924) gave a remarkable account of the formation of the sperm in Coleoptera (*Chelymorpha, Lixus* and *Cicindela*) and Aptera (*Lepisma domestica*) with a general discussion on flagellate sperms.

According to Bowen the sperm of *Lepisma* is an atypical flagellate sperm inasmuch as (1) the centrosome travels to the extreme anterior tip of the elongated nucleus, giving backwards an axial filament, which lies on the nucleus and then enters into the tail of the sperm, and (2) the acrosome, which is a product of the Golgi apparatus, remains at the base of the nucleus in the neck region, from where it sends backwards into the tail region a filamentary process, which gets lost along with the axial filament in the ripe sperm. In other words, the centrosome, which in typical flagellate sperms lies at the base of the sperm nucleus in the neck region, has, in *Lepisma* sperm, travelled to the extreme anterior tip of the nucleus, whereas the acrosome has departed from its typical position at the extreme tip of the nucleus, remaining in the neck region and sending backwards, instead of forwards, a long filamentary process.

Earlier Charlton (1921) had given an account of *Lepisma* sperm, which was surprisingly correct; but Charlton's homologies were all wrong due to the faulty technique he had used. According to Charlton also the centrosome migrated to the extreme anterior tip of the sperm nucleus, but, for the reason that the centrosome took up a position at the tip of the nucleus, he mistook it for the acrosome. Likewise, not knowing that the acrosome is always a product of the Golgi apparatus and puzzled by the unusual position of the acrosome, he mistook it for the 'middle-piece'. Again, considering that Charlton used fixatives containing fat-solvents, he gave a faithful account of the mitochondrial nebenkern as he saw it in his faulty preparations. It was subsequent to Charlton's publication that Bowen had given a very clear account of the origin, structure and ultimate fate of the mitochondrial nebenkern, particularly in insect spermatogenesis, as also of the origin of the acrosome from the Golgi apparatus in general.

Bowen's description of the sperm of *Lepisma* started a controversy which has not, up till this day, been settled. Gatenby and Mukerji (1929) and Mukerji (1929) questioned the findings of Bowen. According to these authors the spermatogenesis of *Lepisma* could be compared with the same

process as found in some other insects such as a Lepidopteran or a Cicindelid. Mukerji stated that Bowen, instead of trying to bring this *Lepisma* spermatogenesis in line with these insects, had gone so far as to suggest that the sperm of *Lepisma* was of an atypical type, differing in every respect from all other typical flagellate sperms. According to these authors what Bowen calls the acrosome in the neck region of the sperm is not the acrosome, but a new type of body, the post-nuclear body, 'as yet undescribed by all authors except Gatenby (1922 and 1929), who noted its presence in every type of spermatogenesis studied by him' (Mukerji 1929, p. 1). Mukerji goes on to say that their post-nuclear body is the 'middle-piece' of Charlton (1921).

Gatenby and Mukerji and Mukerji describe in both the papers a peculiar funnel-shaped structure immediately behind their post-nuclear body. This funnel-shaped structure has also been described and figured by Bowen (1924), but Bowen gives convincing evidence, which has been supported by our own observations, that the funnel is really a part of the attenuating acrosome, sending backwards in the tail region a filamentary process in later stages of spermateleosis.

Our observations on *Lepisma* spermatogenesis support Bowen's interpretations and homologies in every respect—the centrosome, the acrosome and the mitochondrial nebenkern. We have been fortunate in having a series of brilliant slides prepared by one of us (C. L. B.), which have enabled us to give, what we believe, is a correct account of the process. While supporting the homologies of Bowen we have filled up the gaps necessarily left by him. Likewise we have given reasons why we cannot accept the interpretations of Gatenby and Mukerji.

Work on the spermatogenesis of *Lepisma* was undertaken by one of us (C. L. B.) at the suggestion and under the supervision of the senior author at the Panjab University, Lahore, some years before August, 1947, when the partition of the Panjab took place. The work was completed at Lahore, but it could not be sent to the press before the partition. Some two years after the partition the junior author succeeded in bringing his *Lepisma* slides from Lahore. Subsequently he paid three visits to Hoshiarpur at wide intervals, when both the authors re-examined the slides. All the figures are prepared by C. L. B., but the manuscript has been prepared by the senior author.

The junior author obtained such brilliant preparations with Flemming-without-acetic followed by ·5% iron-haematoxylin that it was not considered necessary to use any other technique.

Observations

Spermatogonia and Spermatocytes :—

Although we have concentrated on the study of spermateleosis and have paid scant attention to earlier stages, it is necessary to examine the mitochondria and the Golgi elements in the spermatogonia and the spermatocytes.

In the spermatogonia (Pl. 1, fig. I) the Golgi elements exist in the form of vesicles, each vesicle showing a chromophilic cortex and a chromophobic medulla. In optical sections the Golgi vesicles appear either in the form of rings or crescents, each crescent generally showing the chromophobic sphere attached to it. It has not been possible to find any granules etc. in the spermatogonium, which can be assigned to the category of mitochondria; and we believe the mitochondria are differentiated for the first time in the primary spermatocytes.

In the primary spermatocytes also (Pl. I, fig. 2) the Golgi elements stain brilliantly and appear under the same forms as in the spermatogonia, but the mitochondria appear as a nebulous, juxta-nuclear mass of ill-defined granules.

Our material did not show any secondary spermatocytes or any spermatogonial and spermatocyte mitoses, but it abounded in different stages of spermateleosis. As we were concerned primarily with the maturing sperm, we did not consider it worth our while to prepare fresh preparations.

Spermateleosis :—

The earliest spermatid that we have been able to get has been drawn in Plate I, figure 3. The Golgi elements still lie scattered in the cytoplasm, but the mitochondria have fused together to form a deeply-staining, oval and curved mass, the mitochondrial nebenkern. A rod-shaped centrosome has appeared, which lies on the surface of the nucleus, giving rise posteriorly to the axial filament.

The mitochondrial nebenkern quickly assumes a spherical form and reveals a structure, which is highly characteristic of insect sperm, as shown by Bowen. It now consists of a deeply-staining medulla and a lightly-staining cortex, consisting of clear vacuoles (Pl. I, figs. 4, 7 and 13). But more often the medullary part of the mitochondrial nebenkern does not stain at all, with the result that the mitochondrial nebenkern is liable to escape notice altogether (Pl. I, figs. 5, 6, 8 to 10 and 12). The mitochondrial nebenkern now loses its duplex structure, and, since it has completely lost its staining capacity, it becomes increasingly difficult to demonstrate it for a long time. Indeed we have not figured it in figs. 15 and 16, Plate I. At a later stage, however, when the nucleus has completed its condensation and has lost its chromaticity, and the centrosome has placed itself at the anterior aspect of the nucleus, there can be noticed in a very small percentage of cells a faintly-staining, fusiform structure, stretching over the nucleus, between the centrosome and the acrosome, the latter lying immediately behind the nucleus (Pl. II, figs. 27 and 28). This is the degenerating mitochondrial nebenkern. But for the fact that the limits of the degenerating mitochondrial nebenkern are well-defined with the deeply-staining centrosome in front and the acrosome behind, and the nucleus provides an excellent background, it would have been almost impossible to demonstrate this structure. There can be no doubt whatsoever that the mitochondrial nebenkern completely pales off and disappears in the

later stages of spermateleosis. In one case only we have seen the mitochondrial nebenkern lying on the side of the pale nucleus, and taking up the stain (Pl. II, fig. 26).

When the mitochondrial nebenkern is passing through early stages of its formation as described above, most of the Golgi elements, *but by no means all*, come together to form the acrosome. Those Golgi elements, which do not co-operate in the formation of the acrosome, are ultimately sloughed off on entering the tail region of the sperm; but the acrosome-forming Golgi elements place themselves on the posterior side of the nucleus and, fusing together, form a prominent vesicular acrosome, which attaches itself closely to the posterior aspect of the nucleus (Pl. I, figs. 4 to 16). In figs. 13, 15 and 16, the large vesicular acrosome, clinging to the posterior side of the nucleus, is well represented; in fig. 12 the formation of the acrosome has been almost completed; in figs. 11 and 14 the vesicular nature of the acrosome-forming Golgi elements is well represented; and lastly in figs. 9, 10, 15 and 16 the Golgi elements, which did not co-operate in the formation of the acrosome, are seen being sloughed off in the tail region (*G'*).

It has to be noted that the vesicular acrosome, when first formed, shows a duplex structure consisting of an outer chromophilic cortex and an inner chromophobic medulla, exactly like the vesicular Golgi elements, which participated in its formation. But soon the acrosome begins to stain homogeneously; nevertheless it retains its spherical form (Pl. I, figs. 17 and 18).

Condensation of the acrosome continues till it becomes a shallow cup with a small process in its middle, sticking firmly to the posterior aspect of the nucleus (Pl. II, figs. 19, 20 and 21). By the elongation of this small process towards the posterior side, the cup-shaped acrosome is converted into a short, broad cone, which continues to stain deeply (Pl. II, figs. 22, 24, 25 and 27). The short, broad cone-like acrosome is gradually converted into a long, narrow cone (Pl. II, figs. 23, 26, 28 to 33, 35 and 39), till ultimately the apex of the cone-like acrosome is drawn out posteriorly into a long filament, which gets lost in the tail of the ripe sperm along with the axial filament, the upper broad part of the cone continuing to stick firmly to the posterior aspect of the nucleus (Pl. II, figs. 38 and 40).

The centrosome is recognizable as such for the first time in the earliest spermatid as a small thick rod, highly stainable, and giving off the axial filament (Pl. I, fig. 3). During the earlier stages of spermateleosis the centrosome keeps hovering in the vicinity of the nucleus generally towards its posterior aspect (Pl. I, figs. 4 to 10). But when the condensation processes within the nucleus have been completed and the nucleus begins to stain uniformly, the rod-like centrosome begins to travel forward, carrying the axial filament with it. A study of figs. 19 to 40 of Plate II will make this anteriorward migration of the centrosome very clear. In nearly ripe or ripe sperms (Pl. II, figs. 36 to 38 and 40) the rod-like centrosome is found to be placed at the extreme tip of the sperm nucleus. The axial filament runs over the surface of the elongated nucleus, and since the latter is sinuous the axial filament also takes a sinuous course. Behind the

nucleus the axial filament enters the sperm tail and gets lost along with the posterior filamentary process of the acrosome.

During the whole of this process of its migration anteriorward, the centrosome grows in size, becoming longer and thicker. On account of its elongated form, the centrosome is liable to break up into two, or even three, parts (Pl. II, figs. 29 and 32). We regard this breaking up of the centrosome as an artifact (*vide infra*).

Discussion

Our investigations confirm the findings of Bowen (1924) on the homologies of the various cell-components of the sperm of *Lepisma*.

There is complete consensus of opinion amongst the earlier investigators on the sperm of *Lepisma* with regard to the migration of the rod-like centrosome towards the anterior tip of the sperm nucleus, where it occupies a position generally assigned to the acrosome. Charlton (1921) clearly described this migration of the centrosome anteriorwards, but he mistook it for the acrosome on account of its unusual position at the tip of the elongated sperm nucleus. Besides Charlton seems to have been ignorant of the paper of Gatenby (1917), who for the first time had given a clear account of the direct origin of the acrosome from the Golgi elements, his *acroblasts*, in the sperm of the Lepidopteran, *Smerinthus*. Bowen (1924), Mukerji (1929) and Gatenby and Mukerji (1929) all confirm this migration of the centrosome anteriorwards. This unusual position of the centrosome at the anterior tip of the sperm nucleus in *Lepisma* is paralleled by the sperm of the tiger beetle *cicindela*, as worked out for the first time by Goldsmith (1919) and later confirmed by Bowen (1924). Our investigations on the *Lepisma* sperm also fully confirm these findings of the earlier investigators.

We agree with Bowen (1924) that 'in the primary spermatocytes there are numerous and unusually large scattered Golgi bodies.....' We also agree with him that 'in well-fixed spermatids the Golgi elements are very clearly demonstrated, each one being composed of a small mass of idiosomic material, to which is applied a darkly stained Golgi rodlet .....'. Bowen goes on to say that these spermatid 'Golgi bodies, at first scattered, may collect gradually into a more or less concentrated mass (fig. 92), they may remain for a time in a looser association (fig. 93), or they may be for quite a period rather widely scattered in smaller aggregates'. We entirely agree. If Bowen's figs. 92 and 93 are compared with our figs. 7 to 11 and fig. 14, it will be discovered that there is an essential agreement between us on this point. Bowen is quite correct in interpreting these clusters as clusters of Golgi bodies, which had been earlier considered by Charlton as derivatives of the mitochondria separated off from the nebenkern mass at an early stage in its condensation.

But we cannot agree with Bowen when he says that 'in addition, there occur in the cytoplasm several darkly stained granules (figs. 93 and 94) perhaps related to the *chromatoid body* (italics ours), and also rather ill-defined masses possibly to be compared with the *spermatid remnant*

(italics ours)'. Bowen goes on to say that these additional components (i.e., his chromatoid body and spermatid remnant) combine to make a very confusing cytoplasmic picture, but he does not consider them as Golgi material. On the contrary, without giving any reasons for building up new categories of cytoplasmic inclusions, he dismisses the 'chromatoid body' and the 'spermatid remnant' as unworthy of further consideration, and interprets in Charlton's figs. 80, 81 and 83 the body marked X as chromatoid granule of some kind, while the masses labelled *m* by Charlton are interpreted by Bowen as poorly preserved Golgi clusters for the most part.

We cannot agree with Bowen that the cytoplasmic picture offered by the maturing spermatid of *Lepisma* is confusing. Once it is understood that the Golgi elements, which are essentially granular and vesicular, may appear under different forms in fixed and sectioned material, there is no difficulty in regarding the 'chromatoid body' and the 'spermatid remnant' as the Golgi material. At any rate Bowen does not supply any information whatsoever with regard to the origin, morphology, fate and functions of his 'chromatoid body' and 'spermatid remnant.' On the contrary, he dismisses these bodies as unworthy of further consideration.

We invite comparison of Bowen's fig. 93 and our figs. 7 to 10 and 12. In Bowen's fig. 93 there is shown a darkly-staining spherical body on the left-hand side. This is presumably the 'chromatoid body' of Bowen; and admittedly this body has no fixed position in the cell. In our figures Bowen's chromatoid body appears in different forms, but it has been labelled by us as *G*, that is, the Golgi element. In fig. 7 it lies on the left of the nucleus and appears in the form of a ring; in fig. 8 it lies in front of the nucleus and consists of two tiny crescents; in fig. 9 it lies in the top corner of the cell on the left and appears in the form of a few granules; in fig. 10 also it appears as a granule immediately above the mitochondrial nebenkern; and in fig. 12 it appears in the form of three granules, one large and the other two small, lying immediately above the mitochondrial nebenkern.

Likewise we have had no difficulty in interpreting the 'spermatid remnant' of Bowen as the Golgi elements, which do not co-operate in the formation of the acrosome and are ultimately sloughed off. Bowen in his fig. 94 doubtfully labels his 'spermatid remnant' as S; whereas we have labelled the same material as *G'* in our figs. 9, 10, 15 and 16, that is, the sloughing-off Golgi material.

Before leaving the subject of 'chromatoid body' it will be profitable to dispose off references to this body made by Gatenby and Mukerji (1929). Even a casual study of the figures produced by these authors leaves no doubt that they are really describing the Golgi element under a different name. In their figs. 2, 5 and 7, Plate I, they have figured the 'chromatoid body' (*CB*), which is in fact, the Golgi element. In fig. 2 the 'chromatoid body' has been figured as a ring with rather a thick chromophilic cortex and a chromophobic medulla; in fig. 5 the cortex of the 'chromatoid body' (our Golgi element) is rather attenuated with a more prominent medulla; and in fig. 7 two small Golgi rings, touching each other, have been interpreted and labelled as the chromatoid body (*CB*).

We now address ourselves directly to that structure in the sperm of *Lepisma*, which has been described by Bowen (1924) as the acrosome. According to this author the acrosome of *Lepisma* sperm, which, like all other sperms, is formed by the Golgi elements, is unique inasmuch as it remains in the neck region. The same structure has been described earlier by Charlton (1921) as the 'middle-piece' and later by Gatenby and Mukerji (1929) and Mukerji (1929) as the 'post-nuclear body'.

We agree with Bowen that the acrosome of the sperm of *Lepisma* remains in the neck region, but we do not agree with him when he says that the acrosome is secreted by the Golgi elements (acroblasts). On the contrary, we have no doubt that the acrosome in the sperm of *Lepisma* is formed directly *by the fusion of vesicular Golgi elements.* We invite a reference to our figs. 11 to 16, Plate I, whose study, we believe, will make our point very clear. In figs. 11 and 14 the vesicular Golgi elements have come together to form the acrosome; in fig. 12 the large vesicular acrosome has been formed but its composite nature is still clear; and in figs. 13, 15 and 16 all traces of the composite nature of the acrosome have completely disappeared.

Indeed Bowen is himself doubtful about the acrosome being a secretory product of the Golgi elements (his acroblasts). In his figs. 93 to 95 he correctly shows the Golgi elements coming together, but 'this multiple construction of the acroblast makes it difficult to observe the actual deposition of the acrosome, which is perhaps deposited a little at a time as in the Lepidoptera'. Bowen admits that in the scanty material at his command, 'the steps in the deposition of the acrosome could not be followed satisfactorily; but once the process is well advanced, the acrosome becomes conspicuous as a more or less flattened, probably somewhat elongate, mass applied to the nuclear membrane at a point corresponding to the former position of the acroblast (figs. 97 and 98).' Bowen considers this mass to be homologous with the acrosomal granule of other forms, 'but I have not been able to make out the acrosomal vesicle, the occurrence of which one would expect.'

From the above it is clear that Bowen has not admittedly produced any evidence in favour of his theory of the acrosome being a secretory product of the acroblasts. Study of our preparations, on the contrary, has convinced us that the acrosomal mass, which Bowen considers 'to be homologous with the acrosomal granule of other forms', is a composite structure to begin with, formed as it is by the direct fusion of Golgi vesicles, and later this mass is converted into an acrosomal vesicle, which Bowen failed to observe in the scanty material at his disposal. A close study of Bowen's figs. 98, 99 and 106 to 108 leads one to suspect that his acrosome mass, which he has homologized with acrosomal granule, has after all a composite structure!

Nevertheless we strongly support Bowen's valuable conclusion that the acrosome, having been formed by the Golgi bodies, remains in the neck region, and corresponds to the 'middle-piece' of Charlton and to the 'post-nuclear body' of Gatenby and Mukerji.

We have also to make due allowance for the limitations of Bowen, considering that his conclusions are based on the study of Charlton's slides, which were admittedly prepared with fixatives containing fat-solvents. As stated by Bowen (1924) 'Charlton's attempts with specific Golgi technique were unsuccessful....'.

We believe that the use of fat-solvents by Charlton is also responsible for some of Bowen's conclusions with regard to the structural details of the mitochondrial nebenkern and its ultimate disposition in the sperm, with which we cannot agree. It will be recalled that in late stages of spermatelcosis we have described the nebenkern as a fusiform and faintly-staining structure stretching over the surface of the nucleus with the centrosome in front and the acrosome behind (Pl. II, figs. 27 and 28). A reference to Charlton's figure 87, Plate 6, will make it clear that this author also has defined the limits of the nebenkern in an identical manner,—that is, the centrosome (his acrosome) at the anterior end and the acrosome (his middle-piece) at the posterior end of the nebenkern. But Bowen figures (fig. 107, Pl. 4) the nebenkern as extending beyond the acrosome into the tail-region of the sperm. We cannot agree with this part of Bowen's conclusion. We believe that the nebenkern does not extend beyond the acrosome behind; and as we have already stated it completely disappears from view, whereas, according to Bowen, it forms the sheath of the axial filament of the tail. We must, however, state, in fairness to Bowen, that neither Charlton nor we have ever been able to get that fine differentiation of stain in the tail region of the sperm behind the acrosome which Bowen obtained in his figure 87, Plate 6, and in all our figures in Plate II, the tail region consists of deeply-staining acrosome in front and the fine axial filament behind.

Nor did we ever get the stage of the nebenkern as figured by Bowen in his figure 95, Plate 4, where the nebenkern has considerably elongated and the 'central substance', which consists of delicate vesicles, has appeared in its interior. In our preparations (see our figs. on Plate I), the nebenkern remains spherical and simply pales off; and it can be seen in a very small percentage of cells at a much later stage as an almost hyaline and fusiform structure stretching over the surface of the nucleus between the centrosome in front and the acrosome behind.

But the 'central substance' of Bowen is conspicuous by its absence in our preparations. While going through Bowen's paper one gets the impression that Bowen, while recording some of his statements, was unconsciously influenced by what he had seen and described earlier in other insect orders, such as the Lepidoptera and the Hemiptera, in which there is a typical mitochondrial nebenkern.

It may be that Bowen has mistaken the vacuolar system described by Mukerji (1929) for the 'central substance'. According to this author there is a system of neutral red-staining vacuoles, consisting of small vesicles aggregated together, as in the germ-cells of *Abraxes, Cavia, Saccocirrus* and *Macrothylacia*. In *Lepisma* the vacuoles are ultimately thrown down the tail.

Our belief that Bowen has mistaken these delicate vacuoles for his 'central substance' is strengthened by the study of our figures 9 to 11, Plate I, in which we have figured delicate vacuoles (*V*) in the tail region independent of the nebenkern.

While our observations support the broad conclusions arrived at by Bowen, we must strongly controvert the conclusions of Gatenby and Mukerji (1929) and Mukerji (1929), viz., (1) the acrosome in the *Lepisma* sperm lies as usual at the anterior tip of the nucleus and (2) the 'acrosome' of Bowen or the 'middle piece' of Charlton is the 'post-nuclear body'.

Talking of the centrosome in *Lepisma*, Bowen (1924) says that this rod-shape is retained by the centriole in all the subsequent stages which have been studied and there is practically no indication of a division into two parts such as commonly occurs in the spermatid. But 'rarely, at about the stage of figure 109, I found an apparent division of the rod into two rather distinct, elongate granules—possibly indicative of a potential duplicity in the outwardly single centriole'. Charlton (1921) also describes the breaking up of the centrosome into two or three parts.

We have also noticed in a few cases a similar breaking-up of the rod-shaped centrosome into two or three parts (Pl. II, figs. 29, 32 and 33), which we consider to be an artifact. We are convinced that Gatenby and Mukerji (1929) have mistaken the anterior part of the centrosome for the acrosome. Indeed these authors also describe the breaking up of the centrosome in two parts (their fig. 6, Pl. I). In their figure 4, Plate I, they seem to have misinterpreted the anterior of these two granules as the acrosome! Again Gatenby and Mukerji have failed to establish the origin of their 'acrosome' from the Golgi bodies, as was done by Bowen, whose observations we have fully supported. Both Bowen and the present writers have shown numerous Golgi bodies in the neck region where they form the acrosome, which continues to remain in this region of the sperm. After all a structure cannot be called an acrosome unless it is proved to have its origin in the Golgi bodies.

In addition to the 'post-nuclear body', which is really the acrosome, Gatenby and Mukerji have described a clear funnel-shaped structure, the 'post-nuclear funnel', which lies immediately behind their 'post-nuclear body'. This funnel has neither been described by Charlton, nor by Bowen, nor again by the present writers. Judging from the very scanty data furnished by Gatenby and Mukerji, one gets the impression that their preparations are not satisfactory. A reference to our figures on Plate II will show that the acrosome, when fully developed, is a funnel-shaped structure, staining deeply with iron-haematoxylin. We are inclined to believe that Gatenby and Mukerji have failed to stain this funnel-shaped acrosome except its anterior part, which they have called as the 'post-nuclear body', and the posterior unstained part as the 'post-nuclear funnel'.

If the 'post-nuclear body' of Gatenby and Mukerji (1929) in the *Lepisma* sperm is the acrosome, that of *Cavia* as described by Gatenby and Wigoder (1929) is nothing but the posterior region of the nucleus. This was reported by Nath (1944) in his Presidential address to the Section

of Zoology and Entomology, 31st Indian Science Congress held at Delhi. It will be profitable to quote what Nath said:

'Reverting to the silver methods, I feel no hesitation in saying that these methods, in spite of the occasionally brilliant results they have yielded, have grievously led us astray with respect to the form of the Golgi apparatus. But what is worse is that these methods (and also the long osmication methods) can blacken mitochondria, nuclear membrane and several other structures in the cell totally unrelated to the Golgi apparatus (see Bowan, 1928*b* for this phenomenon). Indeed it appears that granules of silver and osmium can settle down on many kinds of surfaces. Friend (1935), working on the sperms of British Muridae, discovered that there was an asymmetrical deeply staining area in the posterior part of the nucleus in all the Muridae sperms he had seen, which gave the characteristic stain with Feulgen. He named this area as the "dense posterior region", and he pointed out that in position it agreed with Gatenby's "post-nuclear body". G. W. Vaidya, the King Edward Memorial Scholar from Nagpur working at Lahore on the sperm of the squirrel, has confirmed Friend's conclusions. He employed Feulgen's reaction on smears and sections fixed in about seventeen fixatives. The posterior part of the nucleus in each case took up the characteristic stain, showing that the "post-nuclear body" is only a part of the nucleus. In Da Fano (chilled) preparations no post-nuclear granules were observed in any stage of spermatogenesis, but in Da Fano smears silver was deposited on the posterior part of the nucleus. Gian Chand has arrived at identical results in the sperm of Cavia.'

The degeneration of the fully-formed acrosome in the non-flagellate sperm of *Paratelphusa spinigera* and its allies has been reported by Nath (1932 and 1942), who also reported in several species of the Decapod sperm the complete degeneration of the Golgi elements *before* they have had a chance to form the acrosome.

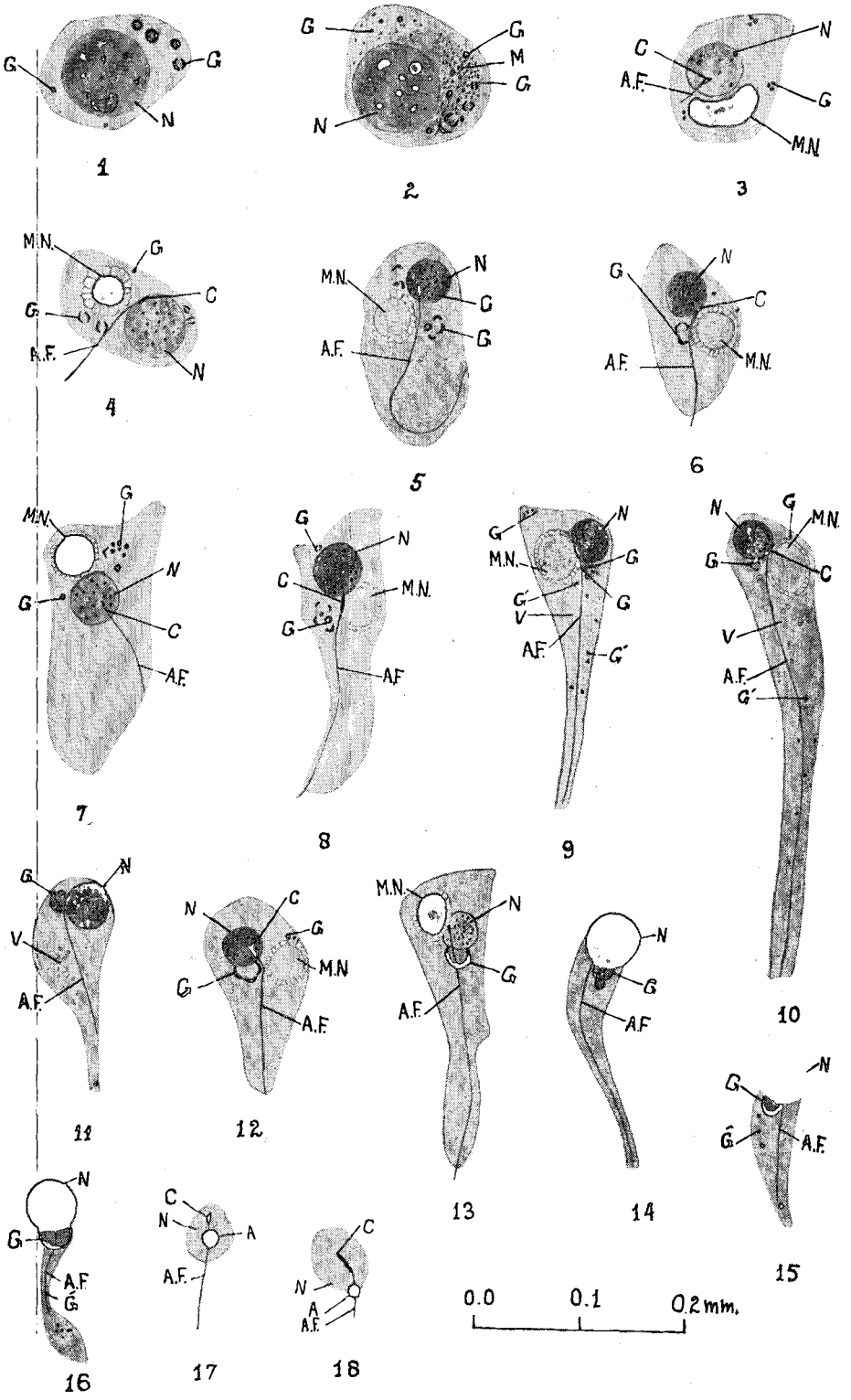
But *Lepisma domestica* seems to be the first case on record of the complete paling off of the fully-formed mitochondrial nebenkern. The climax seems to have been reached in the spermatogenesis of a local dragon-fly, in which Rishi (unpublished) has reported in this laboratory a complete absence of the mitochondrial material in the spermatid—not to talk of the mitochondrial nebenkern!

Summary

1. We are in general agreement with the broad conclusions of Bowen (1924) on the spermateleosis of *Lepisma domestica*, although we have important differences of detail.

2. We have been able to confirm the important discovery of Bowen that the acrosome of the *Lepisma* sperm remains in the neck region, from where it sends backwards into the tail region a filamentary process, which gets lost with the axial filament.

3. But, according to our investigations, on *Lepisma* sperm, the acrosome is formed by the direct fusion of the Golgi vesicles and is not a secretory product thereof as described by Bowen.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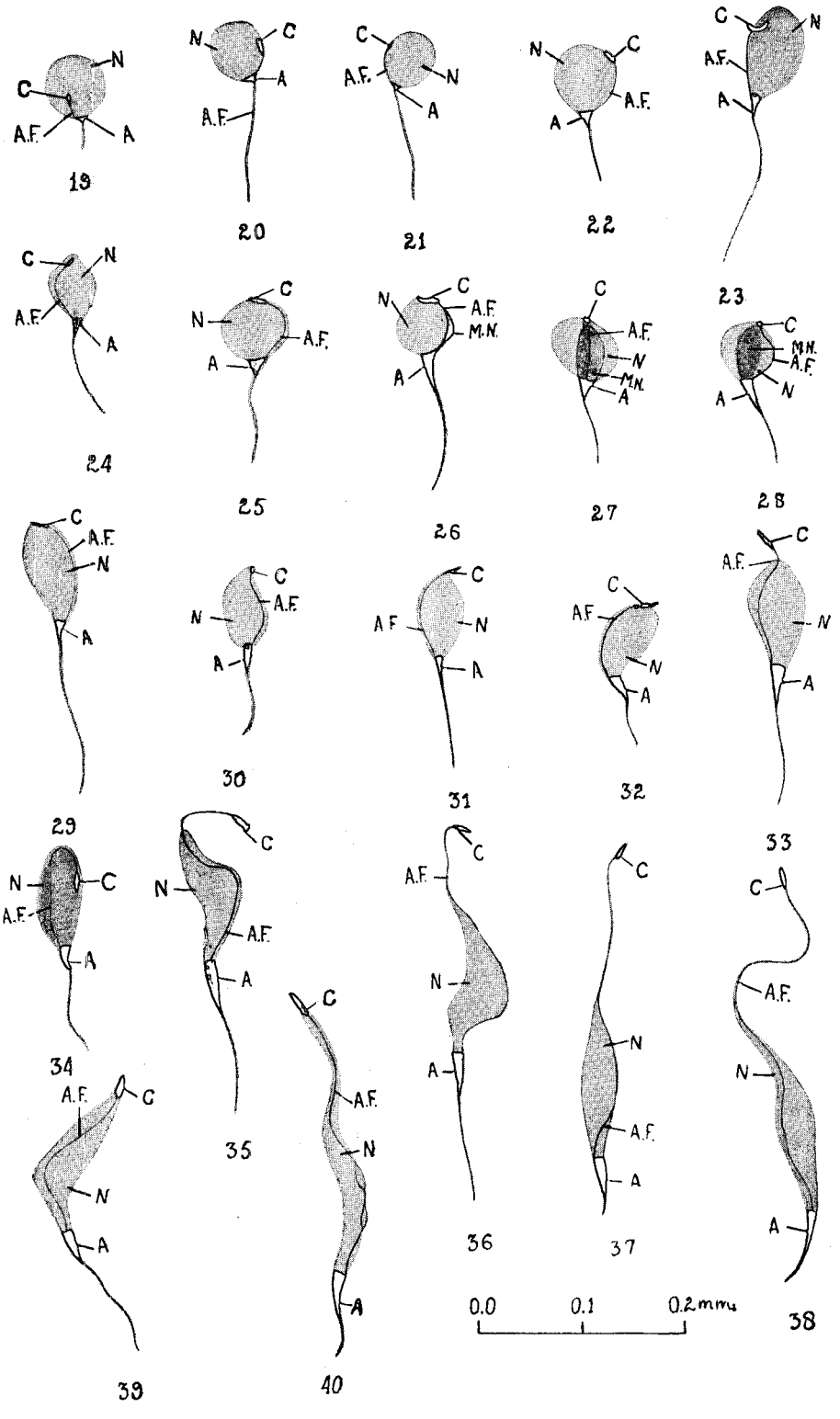
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4. The 'chromatoid body' of Bowen, Gatenby and Mukerji (1929) and Gatenby (1929) is only that part of the Golgi apparatus, which does not merge into the acrosome. The 'spermatid remnant' of Bowen also represents the non-participating Golgi elements, which are ultimately sloughed off down the region of the sperm tail.

5. The structure described as acrosome by Bowen corresponds to the 'middle-piece' of Charlton (1921) and to the 'post-nuclear body' of Gatenby and Mukerji, and Mukerji.

6. The 'post-nuclear funnel' of Gatenby and Mukerji, and Mukerji is again the posterior part of the funnel-shaped acrosome, which they have failed to stain.

7. The rod-shaped centrosome gets itself placed at the extreme anterior tip of the attenuated nucleus, from where it sends back, on the side of the nucleus, an axial filament, which enters the sperm tail behind the nucleus.

8. Bowen, Charlton, and the present authors—and even Gatenby and Mukerji, and Mukerji—report the breaking up of the rod-shaped centrosome into two or three parts. The last-mentioned authors seem to have mistaken one of these parts of the centrosome as the acrosome.

9. A typical insect mitochondrial nebenkern is formed in *Lepisma*, but it seems to pale off and disappear completely without forming the sheath of the axial filament in the typical insect manner. This is contrary to Bowen's conclusions.

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Lettering.

A, acrosome; *AF*, axial filament; *C*, centrosome; *G*, Golgi elements; G', sloughing-off Golgi elements; *M*, mitochondria; *M.N.*, mitochondrial nebenkern; *N*, nucleus; *V*, vacuoles.

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN

OF THE

EAST PANJAB UNIVERSITY

No. 28 | CHEMISTRY | Pp. 45–49

May, 1953

VISCOSITY OF CONDENSED PHOSPHATES IN SOLUTION

by

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Price Re.0-13-0

VISCOSITY OF CONDENSED PHOSPHATES IN SOLUTION

By Ram Chand Paul, Kundan Lal, V. S. Gaind *and* S. S. Bawa

As early as 1833, T. Grahm [1] demonstrated the existence of three different types of sodium metaphosphates: glassy soluble metaphosphates, crystalline soluble metaphosphate, and insoluble metaphosphate. Glassy metaphosphate was also described in some details by Proust.[2] An early paper interesting because of its insight into polymeric aspects of the structure of condensed phosphates and their hydrolytic instability in the presence of acids is that of Flietmann and Hanneberg.[3] That a century of work following these earlier papers only resulted in creating confusion about the chemistry of metaphosphates is apparent from the many contradictory systems of nomenclature adopted by various authors during this period. Most of the trouble can, however, be traced back to the inadequacy of the methods used in establishing the chemical individuality and to assign the degree of polymerization. In recent years a number of helpful reviews have been made.[4] Mention may also be made of the two modern experimental papers [5] which have thoroughly dealt with the phase equilibria of the system Na_2O–P_2O_5 in the range $NaPO_3$–$Na_4P_2O_7$. Recently Monk and Davies [6] and Lamm [7] have shown that the so-called sodium hexa metaphosphate is in fact a polymer with molecular weight of about 13,000, e.g. 130 units of $NaPO_3$. It has also been noticed that some of the condensed phosphates give highly viscous solutions. But a comparative study of the viscosities of different solutions of condensed phosphates has not been made. In the present work attempt has been made to do so and to estimate roughly the molecular size of the phosphate polymers by applying the well-known Staudinger's equation [8] relating intrinsic viscosity with molecular weight. Much work has been done on organic polymers by Staudinger and others (*loc. cit.*) who have concluded that the equation

$$(\eta) = KM^a \qquad .. \qquad .. \qquad (1)$$

gives results with a close degree of approximation as long as the solute-solvent system remains the same in cases of polymers with molecular

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[1] Grahm, T., *Phil. Trans.*, 1833, **123**, 253.

[2] Proust, J. L., *Ann. Chim. Phys.*, 1820, **14**, 281.

[3] Flietmann, T. and Hanneberg, W., *Annalene*, 1848, **65**, 304.

[4] Terry, H., *Ann. Reports*, 1937, **34**, 115; Karbe, K. and Jander, G., *Koll. Beihefte.*, 1942, **54**, 1; Topley, B., *Quart. Revs.*, 1949, **3**, 345.

[5] Partridge, E. P., Hicks, V. and Smith, G. W., *J.A.C.S.*, 1941, **63**, 454; Morey, G. W. and Ingerson, E., *Amer. Journal, Sci.*, 1944, **42**, 1.

[6] Monk, C. B. and Davies, C. W., *J.C.S.*, 1949, 413.

[7] Lamm, O., *Arch. Chem. Min. Jeo.*, 1944, **17**, No. 25.

[8] Staudinger, H., 1932, *die hoschmolecular organschen verbindugen springle, Berline*; Staudinger, H. and Howarth, J., *J. Prakt. Chem.*, 1940, **153**, 24; Staudinger, H. and Fischer, K., *J. Prakt Chem.*, 1941, **19**, 1585.

weight higher than 10,000. In the present work an attempt has been made to keep the solute-solvent system same.

Nomenclature used is the one suggested by E. Partridge.[1]

Experimental.—Preparation of metaphosphates.

Sodium (1 : 1) phosphate glass I.—Two different methods that of Monk and Davies (*loc. cit.*) and Pascal[2] were used for preparing this sample. Both the products gave similar results. Other sodium metaphosphates were prepared according to the method described by Topley.[3]

Sodium metaphosphate II.—Dehydrated NaH_2PO_4 was heated for 10–12 hours at 350–380°C. in an electric oven.

Sodium metaphosphate III.—This is obtained mixed with $NaPO_3$ I and the unchanged sodium phosphate when dehydrated NaH_2PO_4 is heated for 8 hours at 250–280°C. The soluble impurities were removed by repeated washings with water in which $NaPO_3$ III is insoluble.

Sodium metaphosphate IV.—The sodium metaphosphate melt was cooled to 500°C. till crystallization began. It was then heated to 550–600°C. to promote crystallization and formation of a fibrous mass. This was then washed with water to remove soluble impurities. The insoluble crystals thus obtained were used to provide nucleus for another melt cooled to 550–600°C. The ratio of Na_2O/P_2O_5 in these melts was about one and the fibrous mass could be obtained only after six attempts. The potassium metaphosphates were prepared by the method of Lamm and Malmgrem.[4]

Potassium 1 : 1 phosphate glass.—It was prepared by fusing KH_2PO_4 in a platinum dish, keeping it in a molten state for 4-5 hours and then cooling abruptly.

Potassium Kurrol salt.—It was prepared by heating KH_2PO_4 in a dish at 350–380°C. The same product was obtained by sintering KH_2PO_4 over a mecker burner for half an hour.

Potassium trimeta phosphate.—It was got by heating KH_2PO_4 (dehydrated) for ten hours at 170–180°C.

Preparation of solutions.—In case of insoluble metaphosphates the solutions were made by dissolving the powdered samples in minimum amount of 5% NH_4Cl solution. When completely dissolved, precipitation of the phosphate was effected by addition of alcohol to cold solution and allowing it to crystallize over night. The precipitated phosphate was then filtered, redissolved in water and reprecipitated by adding alcohol. The filtered sample was washed with alcohol, dried in vacuum and its solution in water used for viscosity determinations. Reprecipitation was necessary to eliminate the electrolytes in the presence of which viscosity is known to decrease a good deal. Malmgrem (*loc. cit.*) found that although the molecule becomes stable in Na CNS solution, the molecular size actually decreases and becomes stable at a lower value.

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[1] Partridge, *Chem. Engg. News.*, 1949, **27** 214.
Pascal, *Compt. Rend.*, 1923, **176**, 1398.

[3] Topley, B., *Q. Reviews*, 1949, **111**, 365.

[4] Lamm, O. and Malmgrem, H., *Z. Anorg. Chem.*, 1940, **245**, 103.

Apparatus.—An Ostwald type viscometer constructed according to the design of Fenske and Cannon [1] to eliminate drainage and tilting error was used. Since it had a flow time of about 220 seconds with water no kinetaic error correction was applied and kinematic viscosity could be obtained directly from the time of flow. The temperature of the bath was kept at 25°C. (±0·05°.)

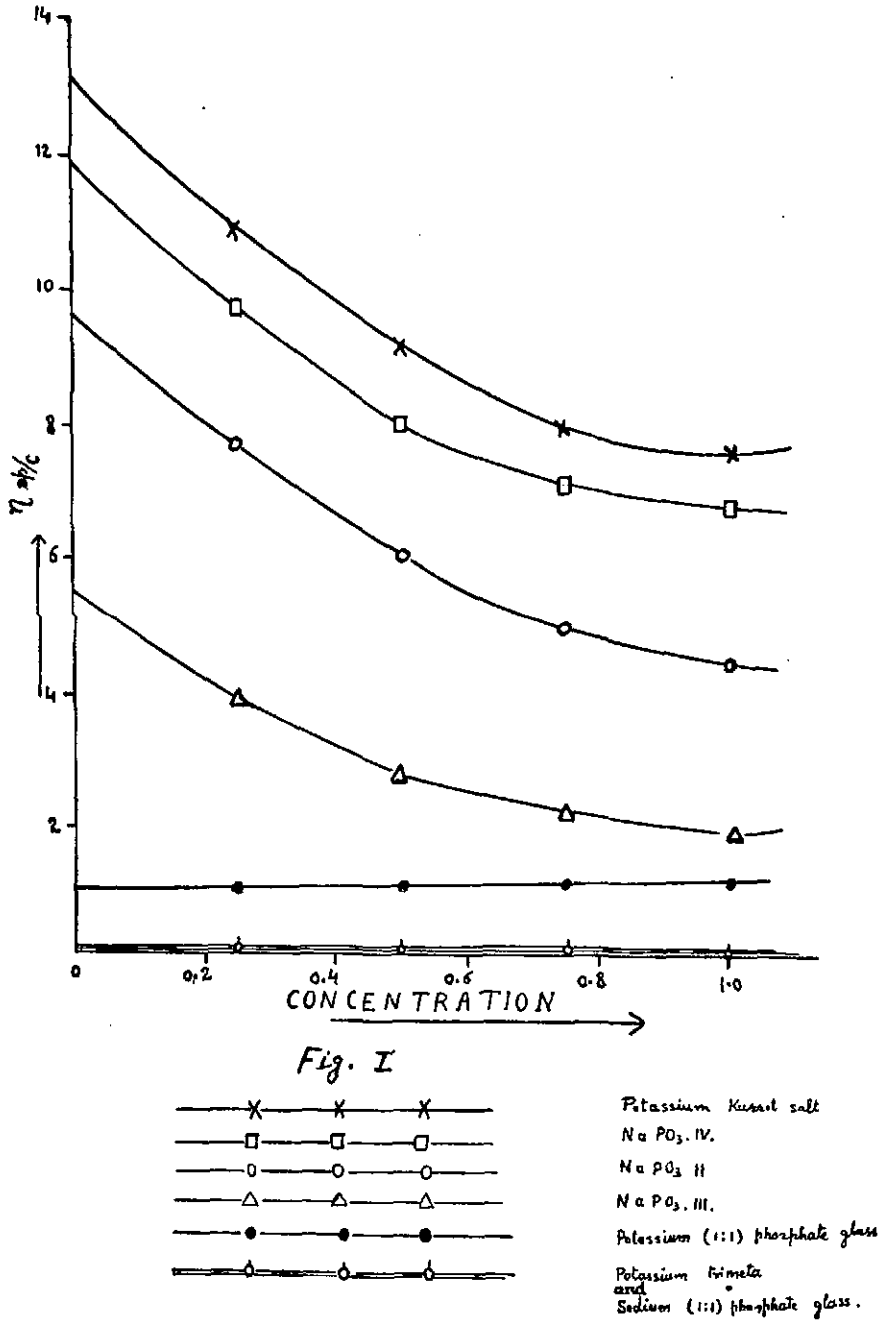

Fig. I

[1] Fenske and Cannon, *Ind. Engg. Chem. Analyst. Ed.*, 1938, **10**, 192.

Results and discussion.—The results have been tabulated in Table I. The relative viscosity, specific viscosity and the ratio of sp. visc. to concentration are shown against concentration of the solutions. In fig. I $\eta_{sp.}/C$ is plotted against C. Unlike organic polymers, slight curves are obtained. This is in accord with the results of earlier authors[1] who have worked with similar molecules. The curves have been extrapolated to infinite dilution to obtain the value of intrinsic viscosity. Substituting this in equation (1), the values of mol. wts., have been calculated and are given in Table II. The constants K and a in the equation (1) were calculated by substituting the values of molecular weights of sod. 1 : 1 *phosphate glass and potassium Kurrol as found by Malmgrem.*[2]

From the results it is noticed that all the phosphates which are insoluble in water show high mol. wts., e.g. greater degree of polymerization. The insolubility is probably due to the enormous size of the molecules. The values of mol. wts. can only be approximate due to the inherent character of the method that has been used and also due to the process of solvation of the samples during which the substances have sometimes been warmed with NH_4Cl solution. NH_4Cl has been selected preferentially for solvation due to the ease with which it can be estimated quantitatively. The rôle of NH_4Cl in bringing about the dissolution of the insoluble phosphates is under investigation.

TABLE I.

No.	Sample	% of solution C	Relative Visc. η_r	Sp. Visc. $\eta_{sp.}$	$\eta_{sp.}/C$
1	Sodium (1 : 1) phosphate glass	1·00	1·116	0·116	0·116
		0·75	1·085	0·085	0·113
		0·50	1·055	0·055	0·110
		0·25	1·028	0·028	0·112
2	$NaPO_3$ II	1·00	5·42	4·42	4·42
		0·75	4·69	3·69	4·92
		0·50	4·03	3·03	6·06
		0·25	3·20	2·20	8·80
3	$NaPO_3$ III	1·00	2·96	1·96	1·96
		0·75	2·66	1·66	2·20
		0·50	2·40	1·40	2·80
		0·25	1·99	0·99	3·96
4	$NaPO_3$ IV	1·00	7·80	6·80	6·80
		0·75	6·45	5·45	7·26
		0·50	5·02	4·02	8·04
		0·25	3·45	2·45	9·80
5	Pot. (1 : 1) phosphate glass..	1·00	2·19	1·19	1·19
		0·75	1·81	0·81	1·08
		0·50	1·50	0·50	1·00
		0·25	1·25	0·25	1·00
6	Pot. Kurrol salt ..	1·00	8·61	7·61	7·61
		0·75	7·06	6·05	8·06
		0·50	5·67	4·67	9·35
		0·25	3·75	2·75	11·02
7	Pot. trimeta phosphate ..	1·00	1·171	0 171	0·171
		0·75	1·162	0·162	0·121
		0·50	1·118	0·118	0·236
		0·25	1·073	0·0·3	0·292

[1] John, R. Van Wazer, *J. Amer. Chem. Soc.*, 1950, 72, No. 2, 906; Bawn, *Chem. of High Polymers*; Interscience Publishers, Inc., New York.

[2] Lamm and Malmgrem, *loc. cit.*; Malmgrem, H., *Acta. Chem. Scand.*, 1948, 2, 147–166.

TABLE II.

No.	Sample	(η)	Mol. wt.
1	Sodium (1 : 1) phosphate glass ..	0·111	13,000
2	$NaPO_3$ II	9·800	981,700
3	$NaPO_3$ III	5·560	566,200
4	$NaPO_3$ IV	11·820	1,175,000
5	Potassium (1 : 1) phosphate glass ..	0·980	124,500
6	*Potassium Kurrol salt*	*13·100*	*1,300,000*
7	Potassium trimeta phosphate ..	0·330	37,240

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 29 CHEMISTRY Pp. 51-53

June, 1953

ANTIMALARIALS IN QUINAZOLONE SERIES

by

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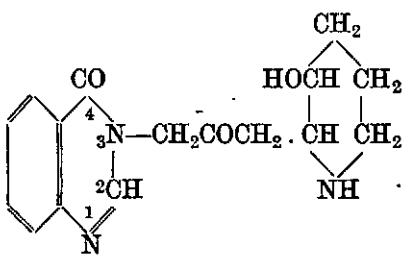
Edited for the East Panjab University by Vishwa Nath,
Department of Zoology, East Panjab University,
HOSHIARPUR

Price Re.0-11-0

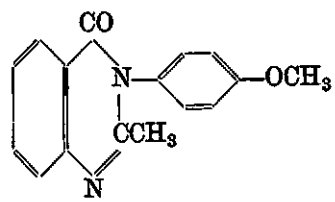
ANTIMALARIALS IN QUINAZOLONE SERIES

By Megh Kumar Jain *and* K. S. Narang, *Chemistry Department, Panjab University College, Hoshiarpur*

Febrifugine (I) is a 4-quinazolone having a basic side chain at 3. The remarkable antimalarial activity of febrifugine, namely 100 *Q—E*, led us to synthesize a few 3-substituted 4-quinazolones. In fact, the compound (II), synthesized already in these laboratories, has been found to be as active as quinine when tested against *P. gallinaceum* in chicks.



(I)



(II)

The present communication records the preparation of some 2, 3-substituted 4-quinazolones (Table I). Details will, however, be published later on.

Acknowledgement.

The authors wish to express their gratefulness to Dr. S. M. Mukherji for all the keen interest he showed in this work.

TABLE I

List of Quinazolone Derivatives

(Micro-analyses are by Drs. Weiler and Strauss, Oxford)

S. No.	Name	Formula	Solvent of Crystallization	m.p. °C.	Analysis Results	
					Found %	Required %
1	2-Methyl-3-(*p*-Acetylaminophenyl) Quinazolone-4	$C_{17}H_{15}N_3O_2$	Dil. Alcohol	260	C 69·43 H 5·12 N 14·20	C 69·62 H 5·12 N 14·33
2	2-Methyl-3-(*p*-Aminophenyl) Quinazolone-4 ..	$C_{15}H_{13}N_3O$	Dil. Alcohol	220	N 16·40	N 16·73
3	2-Methyl-3-(*p*-Chloroacetylamino phenyl) Quinazolone-4.	$C_{17}H_{14}N_3O_2Cl$	Alcohol	206	N 12·60	N 12·82
4	2-Methyl-3-(*p*-β-Chloropropionylamino phenyl) Quinazolone-4.	$C_{18}H_{16}N_3O_2Cl$	Alcohol	222	N 12·10	N 12·29
5	2-Methyl-3-(*p*-Piperidinoacetylamino phenyl) Quinazolone-4.	$C_{22}H_{24}N_4O_2$	Dil. Alcohol	140	C 69·78 H 6·18 N 14·66	C 70·21 H 6·38 N 14·80
6	2-Methyl-3-(*p*-Diethanolamino-acetylamino phenyl) Quinazolone-4.	$C_{21}H_{24}N_4O_4$	Alcohol	220	C 63·47 H 5·96 N 14·29	C 63·63 H 6·06 N 14·14
7	2-Methyl-3-(*p*-β-Piperidinopropionylamino phenyl) Quinazolone-4.	$C_{23}H_{26}N_4O_2$	Alcohol	200	N 14·20	N 14·36
8	3-(*p*-Acetylamino phenyl) Quinazolone-4 ..	$C_{16}H_{13}N_3O_2$	Gl. acetic acid/ethyl acetate	226	N 14·60	N 15·05
9	3-(*p*-Aminophenyl) Quinazolone-4 ..	$C_{14}H_{11}N_3O$	Alcohol	180	C 70·73 H 4·66 N 18·00	C 70·90 H 4·64 N 17·72
10	3-(*p*-Chloroacetylamino phenyl) Quinazolone-4 ..	$C_{16}H_{12}N_3O_2Cl$	Alcohol	230	N 13·60	N 13·39
11	3-(*p*-β-Chloropropionylaminophenyl) Quinazolone-4.	$C_{17}H_{14}N_3O_2Cl$	Alcohol	245	N 12·49	N 12·80
12	3-(*p*-β-Piperidinopropionylamino phenyl) Quinazolone-4.	$C_{22}H_{24}N_4O_2$	Alcohol	202	C 69·95 H 6·33 N 14·75	C 70·21 H 6·38 N 14·80

13	3-(*p*-*β*-Diethanolaminopropionylamino phenyl) Quinazolone-4.	$C_{21}H_{24}N_4O_4$	Alcohol	120	C 63·53 H 6·14 N 13·94	C 63·63 H 6·06 N 14·14
14	3-(4′-Methyl thiazolyl) Quinazolone-4 ..	$C_{12}H_9N_3OS$	Alcohol	184	N 17·10	N 17·28
15	2-Methyl-3-(4′-Methyl thiazolyl) Quinazolone-4 ..	$C_{13}H_{11}N_3OS$	Benzene	148	N 16·10	N 16·34
16	3-(4′, 5′-Dimethyl thiazolyl) Quinazolone-4 ..	$C_{13}H_{11}N_3OS$	Alcohol	202	N 16·29	N 16·34
17	2-Methyl-3-(4′, 5′-Dimethyl thiazolyl) Quinazolone-4.	$C_{14}H_{13}N_3OS$	Benzene	200	N 15·20	N 15·49
18	2-Mothyl-3-(4′-Ethyl thiazolyl) Quinazolone-4 ..	$C_{14}H_{13}N_3OS$	Benzene	201	N 15·10	N 15·49
19	3-(4′-Phenyl thiazolyl) Quinazolone-4 ..	$C_{17}H_{11}N_3OS$	Benzene	162	N 13·48	N 13·77
20	2-Methyl-3-(4′-Phenyl thiazolyl) Quinazolone-4 ..	$C_{18}H_{13}N_3OS$	Benzene	144	N 12·90	N 13·16
21	3-(4′-*p*-Chlorophenyl thiazolyl) Quinazolone-4 ..	$C_{17}H_{10}N_3OSCl$	Benzene/pet. ether	172	N 12·00	N 12·04
22	2-Methyl-3-(4′-*p*-Chlorophenyl thiazolyl) Quinazolone-4.	$C_{18}H_{12}N_3OSCl$	Ethyl acetate	204	N 11·70	N 11·80
23	3-(4′-*p*-Methoxyphenyl thiazolyl) Quinazolone-4..	$C_{18}H_{13}N_3O_2S$	Benzene/pet. ether	182	N 12·64	N 12·54
24	2-Methyl-3-(4′ *p*-Methoxyphenyl thiazolyl) Quinazolone-4.	$C_{19}H_{15}N_3O_2S$	Alcohol	160	C 65·27 H 4·60 N 12·20	C 65·32 H 4·30 N 12·03
25	2-Methyl-3-(4′-Methyl 5′-carbethoxy thiazolyl) Quinazolone-4.	$C_{16}H_{15}N_3O_3S$	Alcohol	235	C 58·10 H 4·76 N 12·48	C 58·35 H 4·56 N 12·76
26	2-Methyl-3-(α-1-Piperidyl-*o*-cresol) Quinazolone-4	$C_{21}H_{23}N_3O_2$	Alcohol	214	C 71·8 H 6·35 N 12·00	C 72·20 H 6·59 N 12·03

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 30 CHEMISTRY Pp. 55–66

July, 1953

POTENTIOMETRIC STUDIES IN OXIDATION-REDUCTION REACTIONS

Part XVI. Oxidation with Chloramine-T. Determination of Organic Substances

by

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Price Rs.1-12-0

POTENTIOMETRIC STUDIES IN OXIDATION-REDUCTION REACTIONS

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By BALWANT SINGH, APAR SINGH *and* MOHAN SINGH

Noll (1924) described the use of chloramine-T as an oxidizing reagent. The solid salt has the composition

$$p\text{-}CH_3.\,C_6H_4.\,SO_2.\,N\begin{matrix}Na\\Cl\end{matrix}.\,3H_2O$$

In solution it behaves like a stable hypochlorite

$$CH_3.\,C_6H_4.\,SO_2.\,N\begin{matrix}Na\\Cl\end{matrix}+H_2O = CH_3.\,C_6H_4.\,SO_2.\,NH_2+NaCl+O$$

Rupp (1925, 1928) used chloramine-T for the determination of trivalent antimony, and later for the titration of stannous tin.

Tomicek and Sucharda (1932) used it as a reagent for the potentiometric determination of tervalent arsenic and antimony; bivalent tin and iron and the ferrocyanide and iodide ions; and for the visual titration of tervalent arsenic and antimony, using methyl red as an indicator.

Komarovskii, Filonova and Korenman (1933, 1934) have shown that chloramine-T can be used as a volumetric reagent to replace the more expensive iodine and the less stable hypochlorite in the estimation of ferrocyanide, hydrazine and hypophosphite. B. Singh and A. Rehmann (1940) have used chloramine-T for the determination of hydroquinone, quinhydrone, hydrazine hydrochloride, potassium iodide, sodium bisulphite and sodium nitrite by a potentiometric method. Thomas Lee, Holzmann, Swift and Neimann (1948) used chloramine-T in the quantitative estimation of various organic sulphides.

B. N. Afanas'ev (1949, 1950) has shown that chloramine-T is a suitable reagent for determining many organic compounds by oxidative volumetric procedures, using methyl red or indigo carmine as an indicator.

In the present investigation chloramine-T has been used for the potentiometric estimations of vanilin, *p*-hydroxy benzaldehyde, benzaldehyde, salicylaldehyde, acetaldehyde, furfural, urea, oxalic acid, thio-urea, glycerol and mannitol.

The aldehydes are oxidized with chloramine-T in a strongly alkaline medium, urea in a weak alkaline medium, and the rest in an acidic medium. These compounds are oxidized according to the following equations:—

1. *VANILIN.*

$$CH_3.\ C_6H_4SO_2N\begin{matrix}Na\\Cl\end{matrix} + CH_3O.\ (OH).\ C_6H_3CHO + NaOH$$
$$= CH_3.\ C_6H_4SO_2NH_2 + CH_3O.\ (OH).\ C_6H_3COONa + NaCl$$

2. *p-HYDROXY BENZALDEHYDE.*

$$CH_3C_6H_4SO_2N\begin{matrix}Na\\Cl\end{matrix} + HO.\ C_6H_4CHO + NaOH$$
$$= CH_3.\ C_6H_4SO_2NH_2 + HO.\ C_6H_4COONa + NaCl$$

3. *BENZALDEHYDE.*

$$CH_3.\ C_6H_4SO_2N\begin{matrix}Na\\Cl\end{matrix} + C_6H_5CHO + NaOH$$
$$= CH_3.\ C_6H_4SO_2NH_2 + C_6H_5COONa + NaCl$$

4. *SALICYLALDEHYDE.*

$$CH_3.\ C_6H_4SO_2N\begin{matrix}Na\\Cl\end{matrix} + C_6H_4(OH)\ CHO + NaOH$$
$$= CH_3.\ C_6H_4SO_2NH_2 + C_6H_4(OH)\ COONa + NaCl$$

5. *ACETALDEHYDE.*

$$CH_3.\ C_6H_4SO_2N\begin{matrix}Na\\Cl\end{matrix} + CH_3CHO + NaOH$$
$$= CH_3.\ C_6H_4SO_2NH_2 + CH_3COONa + NaCl$$

6. *FURFURAL.*

$$CH_3.\ C_6H_4SO_2N\begin{matrix}Na\\Cl\end{matrix} + C_4H_3(O)\ CHO + NaOH$$
$$= CH_3.\ C_6H_4SO_2NH_2 + C_4H_3(O)\ COONa + NaCl$$

7. *UREA.*

$$3\ CH_3.\ C_6H_4SO_2N\begin{matrix}Na\\Cl\end{matrix} + H_2O + OC(NH_2)_2$$
$$= 3\ CH_3.\ C_6H_4SO_2NH_2 + 3\ NaCl + N_2 + CO_2$$

8. *OXALIC ACID.*

$$CH_3.\ C_6H_4SO_2N\begin{matrix}Na\\Cl\end{matrix} + \begin{matrix}COOH\\|\\COOH\end{matrix} = CH_3.\ C_6H_4SO_2NH_2 + NaCl + 2\ CO_2$$

9. *THIOUREA.*

$$7\,CH_3.\,C_6H_4SO_2N\begin{matrix}Na\\Cl\end{matrix} + 6\,H_2O + SC(NH_2)_2$$
$$= 7\,CH_3.\,C_6H_4SO_2NH_2 + 7\,NaCl + N_2 + CO_2 + H_2SO_4$$

10. *GLYCEROL.*

$$7\,CH_3.\,C_6H_4SO_2N\begin{matrix}Na\\Cl\end{matrix} + 3\,H_2O + C_3H_8O_3$$
$$= 7\,CH_3.\,C_6H_4SO_2NH_2 + 7\,NaCl + 3\,CO_2$$

11. *MANNITOL.*

$$13\,CH_3.\,C_6H_4SO_2N\begin{matrix}Na\\Cl\end{matrix} + 6\,H_2O + C_6H_{14}O_6$$
$$= 13\,CH_3.\,C_6H_4SO_2NH_2 + 13\,NaCl + 6\,CO_2.$$

Experimental

Standardization of Chloramine-T Solution.

25 c.c. of the solution were acidified with 5 c.c. of 2*N* hydrochloric acid and 10 c.c. of ten per cent potassium iodide solution were added to it.

$$CH_3.\,C_6H_4SO_2N\begin{matrix}Na\\Cl\end{matrix} + 2\,HI = CH_3.\,C_6H_4SO_2NH_2 + NaCl + I_2$$

The liberated iodine was titrated against standard sodium thiosulphate solution.

Potentiometric Titrations.

A known weight of an aldehyde was dissolved in aqueous alcohol and the solution was made alkaline with sodium hydroxide. This was titrated against standard chloramine-T solution. The alcohol did not interfere in the oxidation. B. N. Afanas'ev has shown that methyl alcohol and ethyl alcohol are not oxidized by chloramine-T under any conditions.

A known weight of urea was dissolved in water and was titrated against chloramine-T solution in the presence of sodium bicarbonate.

Aqueous solutions of glycerol, mannitol, oxalic acid and thiourea were acidified with sulphuric acid and titrated against chloramine-T.

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.

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 0·1140 gms. of vanilin mixed with 63 c.c. of aqueous alcohol and 0·096 gms. of NaOH, against 0·0456 M chloramine-T.

Chloramine-T c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)	Chloramine-T c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)
0·00	−0·1038				12
		9	16·50	−0·0027	
1·00	−0·0950				12
		8	16·60	−0·0015	
3·00	−0·0790				4
		8	17·00	0·0000	
6·00	−0·0556				2
		6	18·00	0·0020	
10·00	−0·0334				2
		4	20·00	0·0054	
13·00	−0·0217				2
		4	23·00	0·0101	
15·00	−0·0140				1
		4	27·00	0·0149	
16·00	−0·0102				2
		6	30·00	0·0194	
16·30	−0·0084				1
		32	32·00	0·0219	
16·35	−0·0068				1
		44	35·00	0·0252	
16·40	−0·0046	(maximum)			1
		26	40·00	0·0302	
16·45	−0·0033				

TABLE II

Titration of 0·1647 gms. of p*-hydroxy benzaldehyde mixed with 113 c.c. of aqueous alcohol and 0·172 gms. of NaOH, against 0·0453 M chloramine-T.*

Chloramine-T c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)	Chloramine-T c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)
0·00	−0·0773				30
		6	29·70	0·0121	
3·00	−0·0586				50
		4	29·75	0·0146	(maximum)
6·00	−0·0466				28
		3	29·80	0·0160	
10·00	−0·0346				10
		3	30·00	0·0180	
15·00	−0·0224				1
		2	30·50	0·0187	
20·00	−0·0115				1
		2	31·00	0·0193	
24·00	−0·0055				1
		2	32·00	0·0205	
29·00	+0·0029				1
		8	35·00	0·0225	
29·30	0·0052				1
		12	38·00	0·0255	
29·50	0·0076				1
		16	42·00	0·0287	
29·60	0·0092				1
		28	46·00	0·0313	
29·65	0·0106				1
			50·00	0·0340	

TABLE III

Titration of 0·0797 gms. of benzaldehyde mixed with 39 c.c. of aqueous alcohol and 0·0480 gms. of NaOH, against 0·0453 M chloramine-T.

Chloramine-T c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)	Chloramine-T c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)
1·00	−0·0621				16
		14	16·65	0·0469	
3·00	−0·0345				14
		8	16·70	0·0476	
5·00	−0·0179				12
		7	17·00	0·0513	
7·00	−0·0045				4
		5	18·00	0·0550	
9·00	0·0063				2
		4	21·00	0·0610	
12·00	0·0170				2
		3	24·00	0·0659	
15·00	0·0265				2
		7	26·00	0·0692	
16·00	0·0334				2
		12	28·00	0·0726	
16·30	0·0370				2
		15	30·00	0·0760	
16·50	0·0400				2
		46	32·00	0·0792	
16·55	0·0423				2
		76	35·00	0·0852	
16·60	0·0461	(maximum)			1
			40·00	0·0900	

TABLE IV

Titration of 0·04274 gms. of salicylaldehyde mixed with 29 c.c. of aqueous alcohol and 0·044 gms. of NaOH, against 0·0476 M chloramine-T.

Chloramine-T (c.c.)	E.M.F. (volts)	E/C (m. volts/c.c.)	Chloramine-T (c.c.)	E.M.F. (volts)	E/C (m. volts/c.c.)
0·00	−0·0693				88
		46	7·45	0·0865	
1·00	−0·0236				30
		31	7·50	0·0880	
2·00	0·0069				13
		19	7·70	0·0905	
3·00	0·0255				11
		15	8·00	0·0937	
4·00	0·0401				5
		10	8·50	0·0961	
5·00	0·0501				2
		11	9·00	0·0971	
6·00	0·0606				2
		5	10·00	0·0995	
7·00	0·0656				5
		22	11·00	0·1047	
7·20	0·0700				2
		24	12·00	0·1064	
7·30	0·0724				2
		78	13·00	0·1081	
7·35	0·0763				1
		116	14·00	0·1091	
7·40	0·0821	(maximum)			1
			15·00	0·1103	

TABLE V

Titration of 0·0487 gms. of acetaldehyde mixed with 57 c.c. of water and 0·070 gms. of NaOH, against 0·0476 M chloramine-T.

Chloramine-T (c.c.)	E.M.F. (volts)	E/C (m. volts/c.c.)	Chloramine-T (c.c.)	E.M.F. (volts)	E/C (m. volts/c.c.)
0·00	−0·0803				
		23	23·20	0·0722	
1·00	−0·0574				76
		12	23·25	0·0760	
3·00	−0·0327				46
		10	23·30	0·0783	
6·00	−0·0021				11
		5	23·50	0·0805	
10·00	0·0176				3
		4	23·70	0·0812	
15·00	0·0381				3
		2	24·00	0·0820	
19·00	0·0465				2
		3	26·00	0·0860	
22·00	0·0559				1
		4	28·00	0·0888	
22·50	0·0581				1
		9	30·00	0·0910	
23·00	0·0627				1
		10	32·00	0·0932	
23·10	0·0637				1
		44	36·00	0·0974	
23·15	0·0659				1
		126 (maximum)	40·00	0·1012	

TABLE VI

Titration of 0·0336 gms. of furfural mixed with 18 c.c. of aqueous alcohol and 0·0220 gms. of NaOH, against 0·0476 M chloramine-T (Temp. 20°C.).

Chloramine-T (c.c.)	E.M.F. (volts)	E/C (m. volts/c.c.)	Chloramine-T (c.c.)	E.M.F. (volts)	E/C (m. volts/c.c.)
0·00	−0·1000				44
		15	7·45	−0·0072	
1·00	−0·0847				38
		12	7·50	−0·0053	
2·00	−0·0724				19
		12	7·70	−0·0015	
3·00	−0·0603				15
		11	8·00	0·0029	
4·00	−0·0496				6
		10	8·50	0·0060	
5·00	−0·0396				5
		10	9·00	0·0083	
6·00	−0·0295				4
		6	10·00	0·0125	
6·50	−0·0265				5
		10	11·00	0·0179	
7·00	−0·0217				5
		16	12·00	0·0230	
7·30	−0·0170				5
		70	13·00	0·0279	
7·35	−0·0135				5
		82 (maximum)	14·00	0·0325	
7·40	−0·0094				4
			15·00	0·0361	

TABLE VII

Titration of 0·0270 gms. of urea mixed with 27 c.c. of water and excess of $NaHCO_3$, against 0·0615 M chloramine-T (Temp. 20°C.).

Chloramine-T (c.c.)	E.M.F. (volts)	E/C (m. volts/c.c.)	Chloramine-T (c.c.)	E.M.F. (volts)	E/C (m. volts/c.c.)
1·00	0·4917				388
		76	21·90	0·6880	(maximum)
3·00	0·6435				42
		24	21·95	0·6859	
5·00	0·6804				26
		8	22·00	0·6846	
7·00	0·6964				3
		3	22·20	0·6852	
10·00	0·7048				3
		2	22·50	0·6862	
14·00	0·7130				2
		1	23·00	0·6872	
16·00	0·7114				3
		1	24·00	0·6900	
18·00	0·7134				3
		2	26·00	0·6956	
21·00	0·7186				3
		6	28·00	0·7020	
21·50	0·7156				5
		17	31·00	0·7170	
21·80	0·7106				2
		64	34·00	0·7227	
21·85	0·7074				1
			37·00	0·7248	

TABLE VIII

Titration of 0·0441 gms. of oxalic acid mixed with 7 c.c. of water and 7 c.c. of 8N H_2SO_4 against 0·0556 M chloramine-T (Temp. 80°C.).

Chloramine-T (c.c.)	E.M.F. (volts)	E/C (m. volts/c.c.)	Chloramine-T (c.c.)	E.M.F. (volts)	E/C (m. volts/c.c.)
1·00	1·0275				18
		6	6·35	1·0148	
2·00	1·0333				16
		5	6·40	1·0140	
3·00	1·0382				15
		2	6·50	1·0125	
4·00	1·0405				2
		1	7·00	1·0137	
5·00	1·0414				2
		1	8·00	1·0152	
5·50	1·0421				1
		7	9·00	1·0138	
5·80	1·0399				1
		18	10·00	1·0124	
6·00	1·0363				1
		31	11·00	1·0114	
6·10	1·0332				2
		55	12·00	1·0096	
6·20	1·0277				1
		72	13·00	1·0086	
6·25	1·0241				1
		168	14·00	1·0075	
6·30	1·0157	(maximum)			

TABLE IX

Titration of 0·01195 gms. of thiourea mixed with 22 c.c. of water and 22 c.c. of 8N H_2SO_4, against 0·0615 M chloramine-T (Temp. 80°C.).

Chloramine-T (c.c.)	E.M.F. (volts)	E/C (m. volts/c.c.)	Chloramine-T (c.c.)	E.M.F. (volts)	E/C (m. volts/c.c.)
5·00	1·0724				60
		11	17·95	1·0875	(maximum)
7·00	1·0950				14
		1	18·00	1·0868	
9·00	1·0976				4
		1	19·00	1·0827	
11·00	1·1000				3
		1	20·00	1·0799	
13·00	1·0981				1
		1	22·00	1·0777	
15·00	1·0955				1
		1	24·00	1·0760	
17·00	1·0942				1
		2	27·00	1·0731	
17·50	1·0934				1
		3	30·00	1·0700	
17·80	1·0926				1
		16	33·00	1·0675	
17·85	1·0918				1
		26	36·00	1·0648	
17·90	1·0905				1
			40·00	1·0610	

TABLE X

Titration of 0·01445 gms. of glycerol mixed with 22 c.c. of water and 22 c.c. of 8N H_2SO_4, against 0·0556 M chloramine-T (Temp. 80°C.).

Chloramine-T (c.c.)	E.M.F. (volts)	E/C (m. volts/c.c.)	Chloramine-T (c.c.)	E.M.F. (volts)	E/C (m. volts/c.c.)
3·00	0·9750				140
		17	19·75	0·9690	(maximum)
6·00	1·0253				60
		3	19·80	0·9660	
9·00	1·0155				10
		1	20·00	0·9640	
12·00	1·0120				5
		3	20·50	0·9615	
15·00	1·0045				2
		1	21·00	0·9605	
18·00	1·0005				3
		5	24·00	0·9505	
19·00	0·9953				3
		14	27·00	0·9430	
19·30	0·9910				4
		30	30·00	0·9320	
19·50	0·9850				2
		37	33·00	0·9270	
19·60	0·9813				1
		48	36·00	0·9240	
19·65	0·9789				1
		58	40·00	0·9200	
19·70	0·9760				

TABLE XI

Titration of 0·0070 gms. of mannitol mixed with 10 c.c. of water and 10 c.c. of 12N H_2SO_4. against 0·0500 M chloramine-T (Temp. 85°C.).

Chloramine-T (c.c.)	E.M.F. (volts)	E/C (m. volts/c.c.)	Chloramine-T (c.c.)	E.M.F. (volts)	E/C (m. volts/c.c.)
1·00	1·0926				
		7	10·05	1·0111	356 (maximum)
3·00	1·0792				86
		3	10·10	1·0068	
5·00	1·0716				62
		2	10·20	1·0006	
7·00	1·0676				10
		4	10·50	0·9977	
8·00	1·0635				8
		4	11·00	0·9938	
9·00	1·0600				3
		15	12·00	0·9907	
9·50	1·0525				5
		15	13·00	0·9862	
9·80	1·0480				4
		57	15·00	0·9782	
9·90	1·0423				4
		106	17·00	0·9708	
9·95	1·0370				4
		162	19·00	0·9638	
10·00	1·0289				2
			20·00	0·9617	

DISCUSSION

It is evident from the foregoing tables that there was a sharp break in E.M.F. in each potentiometric titration at the equivalence-point. From the amount of chloramine-T 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 XII

Vanillin.			*p*-Hydroxy Benzaldehyde.		
Vanilin taken.	Chloramine-T used.	Vanilin found.	*p*-Hydroxy Benzal-dehyde taken.	Chloramine-T used.	*p*-Hydroxy Benzal-dehyde found.
(gms.)	(gms.)	(gms.)	(gms.)	(gms.)	(gms.)
0·0532	0·09730	0·0526	0·0427	0·09848	0·0427
0·0760	0·14070	0·0760	0·0610	0·14050	0·0609
0·1140	0·21018	0·1135	0·0915	0·21000	0·0911
0·1672	0·30960	0·1672	0·1342	0·30860	0·1338
0·2052	0·37890	0·2047	0·1647	0·37904	0·1643
0·2508	0·46500	0·2509	0·2013	0·46390	0·2011

TABLE XIII

Benzaldehyde.			Salicylaldehyde.		
Benzaldehyde taken.	Chloramine-T used.	Benzaldehyde found.	Salicylaldehyde taken.	Chloramine-T used.	Salicylaldehyde found.
(gms.)	(gms.)	(gms.)	(gms.)	(gms.)	(gms.)
0·0372	0·09905	0·0373	0·04274	0·09895	0·0428
0·0531	0·14130	0·0532	0·06106	0·14110	0·0611
0·0797	0·21080	0·0795	0·09160	0·21150	0·0916
0·1169	0·31050	0·1169	0·13430	0·31100	0·1344
0·1435	0·38140	0·1436	0·16480	0·38140	0·1651
0·1754	0·46600	0·1755	0·20143	0·47600	0·2019

TABLE XIV

Acetaldehyde.			Furfural.		
Acetaldehyde taken.	Chloramine-T used.	Acetaldehyde found.	Furfural taken.	Chloramine-T used.	Furfural found.
(gms.)	(gms.)	(gms.)	(gms.)	(gms.)	(gms.)
0·01550	0·09923	0·01553	0·0336	0·09885	0·0337
0·02215	0·14163	0·02214	0·0480	0·14085	0·0480
0·03323	0·21260	0·03323	0·0720	0·21000	0·0717
0·04870	0·31060	0·04860	0·1056	0·31090	0·1057
0·05967	0·38146	0·05972	0·1296	0·38020	0·1296
0·07309	0·46720	0·07304	0·1584	0·46480	0·1585

TABLE XV

Urea.			Oxalic acid.		
Urea taken.	Chloramine-T used.	Urea found.	Oxalic acid taken.	Chloramine-T used.	Oxalic acid found.
(gms.)	(gms.)	(gms.)	(gms.)	(gms.)	(gms.)
0·0070	0·0973	0·0069	0·0441	0·0980	0·0439
0·0100	0·1407	0·0100	0·0630	0·1399	0·0626
0·0150	0·2091	0·0149	0·0945	0·2109	0·0944
0·0220	0·3095	0·0220	0·1386	0·3086	0·1382
0·0270	0·3786	0·0269	0·1701	0·3799	0·1700
0·0330	0·4653	0·0330	0·2079	0·4634	0·2075

TABLE XVI

Thiourea.			Glycerol.		
Thiourea taken.	Chloramine-T used.	Thiourea found.	Glycerol taken.	Chloramine-T used.	Glycerol found.
(gms.)	(gms.)	(gms.)	(gms.)	(gms.)	(gms.)
0·00380	0·0983	0·00379	0·00460	0·0981	0·00458
0·00543	0·1407	0·00543	0·00657	0·1405	0·00656
0·00813	0·2092	0·00806	0·00985	0·2109	0·00984
0·01195	0·3102	0·01196	0·01445	0·3087	0·01441
0·01465	0·3788	0·01462	0·01774	0·3800	0·01774
0·01791	0·4653	0·01786	0·02169	0·4645	0·02169

TABLE XVII

Mannitol.

Mannitol taken.	Chloramine-T used.	Mannitol found.
(gms.)	(gms.)	(gms.)
0·00490	0·0986	0·00491
0·00700	0·1410	0·00701
0·01050	0·2113	0·01051
0·01540	0·3119	0·01542
0·01890	0·3802	0·01891
0·02301	0·4646	0·02301

The above results show that vanilin, *p*-hydroxy benzaldehyde, benzaldehyde, salicylaldehyde, acetaldehyde, furfural, urea, oxalic acid, thiourea, glycerol and mannitol can be determined quantitatively by the potentiometric method, using chloramine-T as an oxidizing agent.

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Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN

OF THE

EAST PANJAB UNIVERSITY

No. 31 ZOOLOGY Pp. 67–72

August, 1953

SPERMATELEOSIS IN THE DRAGON-FLY *SYMPETRUM HYPOMELAS* (SELYS)

by

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Price Rs.1-8-0

SPERMATELEOSIS IN THE DRAGON-FLY *SYMPETRUM HYPOMELAS* (SELYS)

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Introduction

In November, 1952, S. R. Bawa, Panjab University Research Scholar working in this Department, gave to one of us (R.R.) a paraffin block of the testicular material of the local dragon-fly, fixed in Flemming—without —acetic. On cutting the block and staining with 0·5% iron-haematoxylin it was observed that the material abounded in spermatids and sperms, but, to our great surprise, the mitochondrial material was conspicuous by its complete absence—not to talk of the typical insect nebenkern, which one comes across in the spermateleosis of the Hemiptera, Lepidoptera, Coleoptera, Orthoptera and even of such wingless primitive insects as the silver-fish, *Lepisma*.

In a very recent publication Nath and Bhatia (1953) have attempted to solve the very controversial homologies of the *Lepisma* sperm, and have demonstrated the complete degeneration of the mitochondrial nebenkern *in this atypical sperm. But in the dragon-fly, Sympetrum hypomelas*, which forms the subject of the present investigation, the mitochondrial material seems to be completely absent even in the spermatogonia and spermatocytes. This material is also unusually favourable for the demonstration of the direct origin of the acrosome from the acroblast, which is formed by the fusion of a number of Golgi granules.

Subsequently more material was fixed in F.W.A., and sections stained with 0·5% iron-haematoxylin; but throughout winter the material consistently lacked in dividing spermatogonia and spermatocytes.

Our thanks are due to Dr. Rattan Lal of the Indian Agricultural Research Institute, New Delhi, for having identified the dragon-fly.

Observations

Spermateleosis

In the earliest spermatid (Pl. I, Figs. 1 and 2) the nucleus lies at one pole of the cell, and its chromatin content is lightly stained. The mitochondrial substance is conspicuous by its absence, but a few prominent, darkly-staining Golgi bodies are invariably met with in the cytoplasm.

In the next stage the chromatin is condensed and the nucleus becomes darkly staining. When the chromatin starts condensing the nucleus loses its spherical form and appears irregular (Pl. I, Fig. 3), but later on it again becomes spherical (Pl. I, Fig. 4). The nucleus which, to begin with, is in

close contact with the cell wall now comes to lie almost in the middle of the cell.

Meanwhile the Golgi bodies start fusing. From three or four separate bodies they form two Golgi masses first, and eventually their fusion is completed by the formation of a single mass of the Golgi material (Pl. I, Figs. 3 and 4). At this stage the Golgi mass lies away from the nucleus as a distinct spherical body (Pl. I, Fig. 4).

This Golgi mass now moves towards one side of the nucleus (Pl. I, Fig. 6), but, sooner or later, it places itself on the anterior aspect of the nucleus, where it applies itself firmly to the nuclear membrane (Pl. I, Figs. 7 and 8).

The earlier history of the centriole could not be traced, but once the axial filament has put in its appearance, the centriole can be very clearly made out as a darkly staining granule at the base of the nucleus (Pl. I, Fig. 8).

A remarkable feature of the axial filament is that it never emerges out of the cytoplasm of the maturing spermatid; hence it remains short (Pl. I, Figs. 13 to 15). Indeed the tail of the ripe sperm is ridiculously short as compared with the length of the nucleus (Pl. II, Figs. 37 and 38).

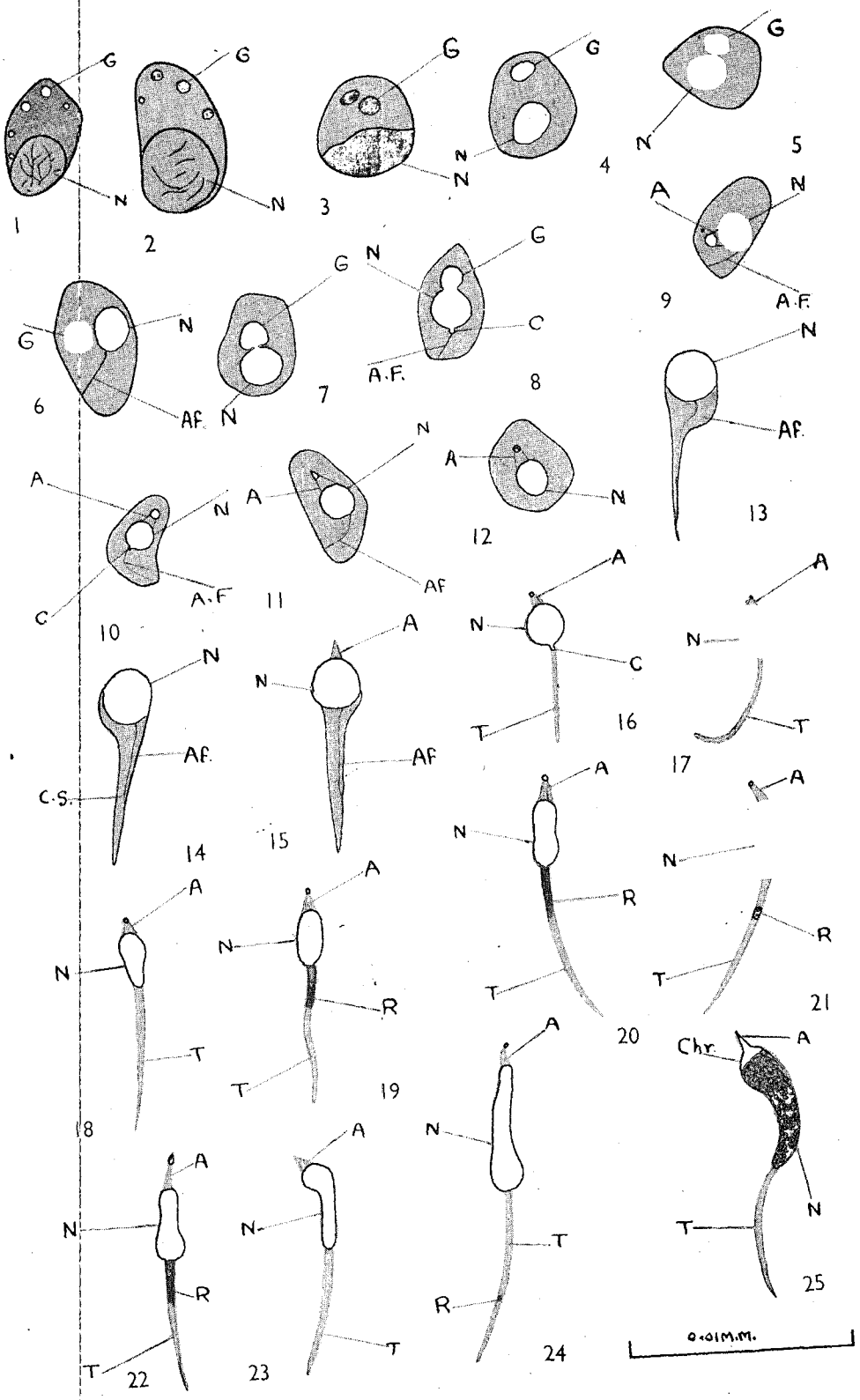
Meanwhile the Golgi mass, lying at the anterior end of the nucleus, is bodily transformed into the acrosome of the ripe sperm. The first step in this transformation is the appearance of a very lightly staining triangular area, the Golgi mass, now reduced in size, lying at the apex of the triangle (Pl. I, Figs. 9 to 12). The acrosomal triangle never grows to a considerable size, but it invariably shows a tiny granule, perched at its free tip: this is *the Golgi mass, which has undergone progressive reduction in its size* (Pl. I, Figs. 16 to 22 and Pl. II, Figs. 37 and 38).

When the formation of the acrosome is almost complete the cell starts elongating along the axial filament (Pl. I, Figs. 13 to 15). During this stage the axial filament can be clearly made out with the cytoplasmic sheath round it, but in subsequent stages, when the tail gets more attenuated, the axial filament as such completely disappears from view.

We left the spermatid nucleus at a stage when it becomes spherical and stains deeply and uniformly with haematoxylin (Pl. I, Fig. 4). After the acrosome has been differentiated from the Golgi mass at the anterior end of the nucleus, the latter begins to elongate, passing through pyriform, ovoid, dumbell-shaped, and even club-shaped forms (Pl. I, Figs. 17 to 24).

During the process of its elongation the nucleus is darkly stained. In the over-differentiated elongating sperms, however, the nucleus appears highly vacuolated (Pl. I, Fig. 25 and Pl. II, Fig. 26). The vacuolar appearance of the nucleus depends largely on the extent to which the stain has been extracted. These vacuoles are very small and closely packed together. They give an appearance of a net-work (Pl. II, Fig. 26). In some cells chromatin is condensed at the two poles of the nucleus with a central vacuolated region (Pl. II, Figs. 26 and 28). Sometimes, however, the chromatin is aggregated at one pole only (Pl. II, Fig. 27).

At a still later stage a prominent rod is seen traversing the nucleus. It joins the two chromatin masses (Pl. II, Fig. 28). At first the rod is rather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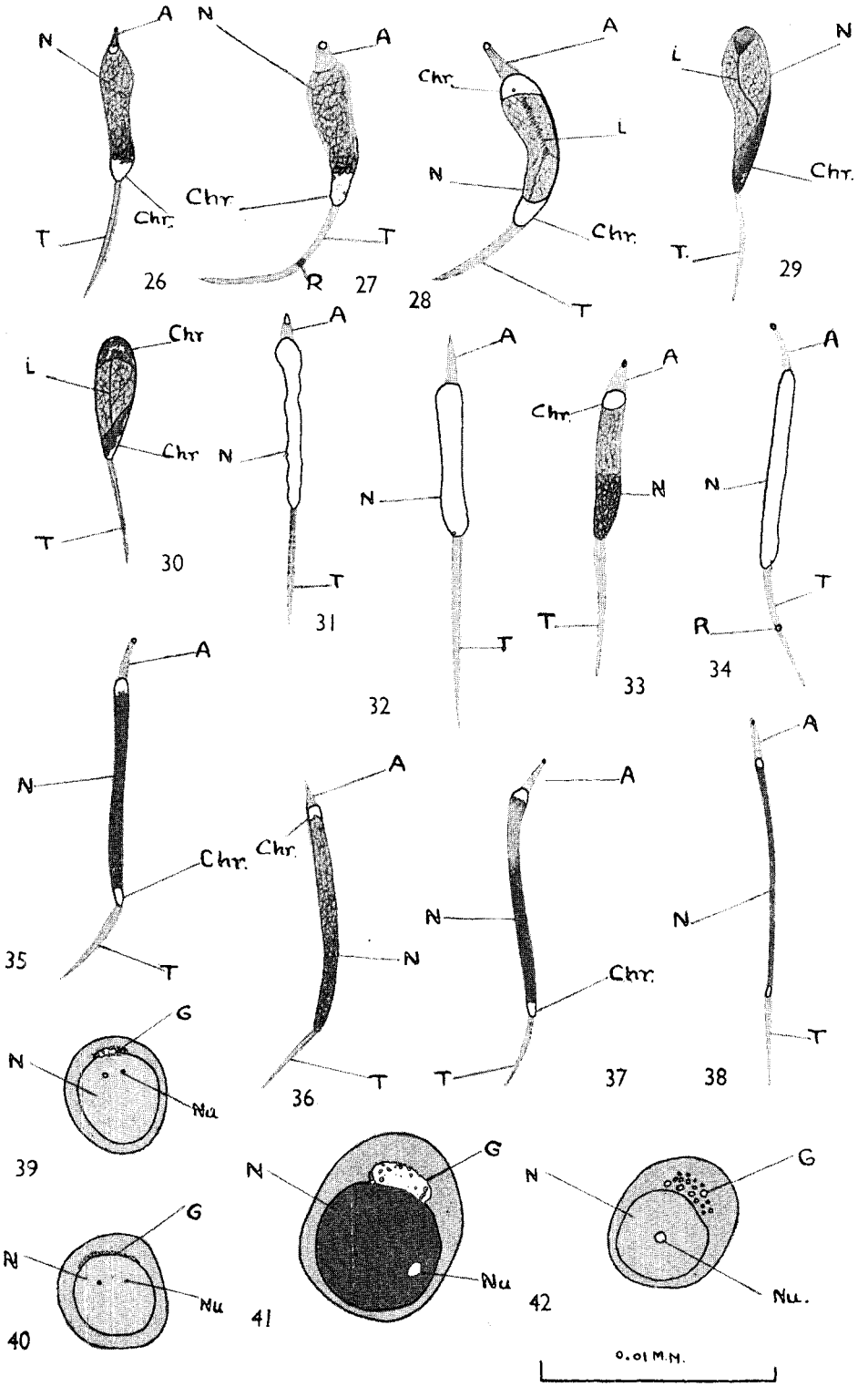
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kinky, and it seems that it has been formed by the condensation of the chromatin reticulum in the central region (Pl. II, Fig. 28). Gradually it smoothens up into a more or less wavy outline (Pl. II, Fig. 29), but eventually it straightens up to form a straight axial core of the sperm nucleus (Pl. II, Fig. 30).

A certain amount of faintly staining material can be observed in the maturing sperm-tail near the base of the nucleus (Pl. I, Figs. 19, 20 and 22). It soon condenses to form more darkly stained granules, which travel from the base of the nucleus to the posterior tip of the tail (Pl. I, Figs. 21 and 24 and Pl. II, Figs. 27 and 34). They undoubtedly represent some residual material, which is ultimately sloughed off. In view of the fact that there are no mitochondria and *all* the Golgi granules fuse to form the acrosome, it is conjectured that the residual substance thrown out along the tail is nuclear in origin. This view is strengthened by the important observation that these remnants first of all appear near the base of the nucleus and then travel downwards.

The club-shaped form of the nucleus gradually leads to the rod form (Pl. II, Figs. 31 and 32). Even at this stage, if the sperm nucleus is over differentiated, it shows a vacuolated condition (Pl. II, Fig. 33).

Meanwhile the acrosome elongates to its maximum length but even now a granule can be observed at its tip, which is in the form of a minute speck. In the final stage the nucleus attains a considerable length by further elongation and becomes a narrow rod (Pl. II, Figs. 34 to 37).

In the ripe sperm the acrosome is very prominent with a distinct minute granule at its tip. The nucleus is long and narrow and is now less darkly staining than before. The tail is very small as compared to the length of the head (Pl. II, Fig. 38).

Early Stages

As mentioned previously, the material was fixed in winter months (November to March) at different times. In all the preparations the earlier stages, i.e. the spermatogonia and the spermatocytes, were very rare. The mitotic and meiotic stages were completely absent.

Spermatogonia

The earliest spermatogonia of the dragon-fly are large, more or less, rounded, or oval cells with comparatively large nuclei. The nucleus, which reveals inside it one or two darkly staining chromatin granules, is surrounded by a thin layer of cytoplasm. The cytoplasm on one side of the nucleus appears to be denser and very often it is possible to make out a few darkly staining granules in this area. These are the Golgi bodies and they lie aggregated in a juxta-nuclear mass (Pl. II, Figs. 39 and 40).

Spermatocytes

The resting primary spermatocytes are definitely larger cells than the spermatogonia, their nuclei also being larger (Pl. II, Fig. 41). In the cyto-

plasm there is a juxta-nuclear mass of closely packed dark granules, which are the Golgi bodies.

Each resting secondary spermatocyte is similar to the primary, but it is definitely smaller than the latter (Pl. II, Fig. 42). The nucleus contains inside it a deeply staining nucleolus-like body. The Golgi granules are still lying in a juxta-nuclear mass, but now they are not so closely packed, so that many Golgi granules of various sizes can be made out.

It is notable that the mitochondria are conspicuous by their absence even in the early stages.

Discussion

Undoubtedly the most remarkable feature of the process of sperm formation in *Sympetrum hypomelas* is the complete absence of mitochondrial material right from the earliest spermatogonia to the ripe sperm. On the contrary a prominent Golgi body is formed in the maturing spermatid by the fusion of a few discrete Golgi elements. This Golgi body is directly transformed into a triangular acrosome, which invariably shows a tiny granule perched at the apex of the triangle.

In a recent publication Nath and Bhatia (1953) have reported the complete paling off of the mitochondrial nebenkern of the sperm of *Lepisma domestica*. The mitochondrial nebenkern in this insect is formed in a typical manner, and as is usual in insect spermatogenesis, it consists in the early spermatids of a central chromophilic mass and an outer chromophobic cortex. But, surprisingly enough, the mitochondrial nebenkern does not develop further. On the contrary, it begins to pale off, and is transformed into a pale, fusiform, hyaline structure, which places itself on the surface of the nucleus with the centrosome at its anterior end and the acrosome at its posterior end!! Gradually the mitochondrial nebenkern completely disappears from view and does not contribute at all to the formation of the cytoplasmic sheath of the axial filament of the ripe sperm.

But in the sperm of *Sympetrum hypomelas* the climax is reached inasmuch as the mitochondrial material is never differentiated at all—not to talk of the formation of a mitochondrial nebenkern.

The complete absence of the mitochondrial material in the sperm of *Sympetrum hypomelas* leads us to the conclusion that this material is not essential in the final make-up of the sperm.

In the sperm of the spider *Plexipus paykulli*, as described by Sharma (1950), the mitochondrial material is completely sloughed off. Indeed in this spider Sharma has reported that the nucleus and the axial filament completely wriggle out of the cytoplasm of the cell, which contains the entire mitochondrial content.

A complete absence of mitochondrial material has also been described from the egg of *Culex fatigans* by Nath (1929).

These facts fit into the theory of Hirsch (1939), according to whom there is a primordial lipoidal material in all undifferentiated cells, and gradually with the differentiation of the cell the mitochondria and the Golgi elements, which are allied substances, are differentiated from the

primordial substance. It is, therefore, not surprising if in some cases the primordial lipoidal substance is differentiated into the Golgi elements only.

Another remarkable feature of the sperm of *Sympetrum hypomelas* is the ridiculously small size of the tail of the sperm. This small size is directly related to the fact that the axial filament never emerges out of the cytoplasm of the maturing spermatid.

Lastly there can be no doubt that the acrosome in the sperm of *Sympetrum hypomelas* is formed directly from the Golgi material as described by Nath in several cases of spermatogenesis.

It will be recalled that in the sperm of *Sympetrum hypomelas* we have reported an intra-nuclear rod and two darkly staining areas, one at the anterior and the other at the posterior end of the nucleus. We propose to defer a detailed consideration of these structures to a later stage, when we have completed our observations on the living material under the Phase Contrast Microscope.

Summary

1. The spermateleosis of *Sympetrum hypomelas* has been worked out.

2. In the early spermatids the Golgi bodies are in the form of discrete granules, and in later stages they fuse together to form a prominent, spherical Golgi body. This Golgi body directly forms the acrosome. A prominent granule is invariably seen at the apex of the triangular acrosome.

3. The axial filament of the maturing spermatid never emerges out of the cytoplasm of the cell, with the result that the sperm tail is ridiculously small as compared with the sperm nucleus.

4. In the elongating nucleus of the sperm a vacuolized condition of the ground substance can be observed in suitably differentiated cells.

5. A prominent intra-nuclear rod can be observed in the over-differentiated ripening sperms.

6. Mitochondria are conspicuous by their absence throughout spermatogenesis.

7. In the spermatogonia and spermatocytes the Golgi elements are in the form of granules and are arranged in a mass on one side of the nucleus.

Explanation of Plates

All the figures have been drawn with camera lucida at the table level with Spencer 10 × eyepiece using an oil immersion objective. They are subsequently enlarged two times, thus giving an approximate total magnification of 3,400 times. Figures 3 to 12 and 39 to 42 have been selected from the sectioned material fixed in Flemming-without-acetic acid followed by 0·5% iron haematoxylin. All the others have been drawn from smears fixed in Flemming-without-acetic acid, diluted with an equal quantity of distilled water and stained with 0·5% iron-haematoxylin.

Lettering

A—Acrosome; *AF*—Axial filament; *C*—Centrosome; *Chr*—Chromatin; *Cs*—Cytoplasmic sheath; *G*—Golgi body; *I*—Intra-nuclear rod; *N*—Nucleus; *Nu*—Nucleolus; *R*—Remnant; *T*—Tail.

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Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 32 ZOOLOGY Pp. 73-91

September, 1953

THE LOUSE SPERM

by

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Price Rs.2-2-0

THE LOUSE SPERM

By G. P. Sharma *and* A. P. Malik, *Department of Zoology, Panjab University College, Hoshiarpur*

Introduction

Sharma (1944) referred to the work on the louse sperm, carried out by Daljit Singh Sarkaria in the Zoological laboratories of the Government College, Lahore (now in Pakistan). Sarkaria worked out fully the spermatogenesis of the cattle-louse, *Haematopinus tuberculatus*, obtained from buffaloes of Lahore and published the results of his investigations in the form of a short abstract in the Proceedings of the 31st Indian Science Congress held at Delhi in 1944. In this communication he confirmed the earlier work of Doncaster and Cannon (1920) on the spermatogenesis of the louse (*Pediculus corporis* and *Pediculus capitis*). He could not, however, publish a detailed account of his investigations as the manuscript and the slides prepared by him were all left in Lahore and could not be brought to this side of the border on account of the partition of the country. Consequently one of us (A. P. M.), at the suggestion of Prof. Dr. Vishwa Nath, prepared some slides of the testicular material obtained from the two species of louse, viz., *Pediculus capitis* and *Pediculus corporis* and these were studied independently as well as jointly by both of us. The present paper is an account of these studies.

Previous Work

Although the spermatogenesis in insects has been the subject of investigation for a number of years, yet we find very few papers dealing with the sperm of louse. The reason for this seems to be the very small size of the germ cells in all the lice examined so far by the various workers.

The earliest work on the sperm of louse is that of Foot (1919), who worked out the spermatogenesis of *Pediculus vestimenti*. According to her the number of chromosomes in the somatic as well as the spermatogonial cells is ten. She also noticed an unequal bivalent in the primary spermatocytes. Although the division of the secondary spermatocytes was not observed by her, yet she assumed that this division was similar to that in corresponding mitoses in the other species of Hemiptera. Strange as it would appear, Foot has not mentioned or figured so conspicuous a structure as the mitochondrial nebenkern in her paper.

Doncaster and Cannon (1920) also worked out the spermatogenesis of two species of *Pediculus*, viz., *corporis* and *capitis* and noted some very interesting facts about the sperm formation in these lice. According to them the somatic number of chromosomes is twelve but the spermatogonial division stages show only six. This haploid number of chromosomes at

the spermatogonial stage has been ascribed by them to premature pairing. They further state that in *Pediculus* only one maturation division is met with and that too is very unequal so far as the cytoplasmic components are concerned. A small nucleus with a little amount of cytoplasm is nipped off from the relatively very large cell which retains the most of the cytoplasm, the whole of the mitochondrial nebenkern and the acroblast. Doncaster and Cannon were, however, not sure of the origin and nature of the acroblast but they rightly suspected that it belonged to the category of the Golgi apparatus. They have also demonstrated very clearly that the acroblast is directly transformed into the acrosome. The spermatid, according to them, has two centrosomes and from each an axial filament is given out. The mitochondrial nebenkern (their mitosome) has been shown by them to have a plate-work structure.

Doncaster and Cannon (1920) make no reference to Foot (1919) as they had not seen her paper. Later on, however, when Cannon studied this paper and found her account differing so much from the one given by Doncaster and himself he decided to make a thorough examination of the testicular material of three more species of louse, viz., *Lignognathus piliferus, Haematopinus asini* and *Haematopinus consobrinus*. As a result of these fresh investigations Cannon (1922) published another paper in which he not only criticized Foot (1919) but also confirmed the earlier account of spermatogenesis given by Doncaster and himself for the two species of louse, viz., *Pediculus corporis* and *Pediculus capitis*.

Material and Method

Both the species of the genus *Pediculus*, viz., *capitis* (the head louse) and *corporis* (the body louse) are found in abundance on a person with unclean habits. The head lice are found mostly on the scalp, clinging to the roots of the hair. The body lice, on the other hand, may be found on the skin of those parts of the body which are covered over with clothes, but a great majority of them are seen hiding in the seams of the inner clothing.

In the young stages, when the external sexual characters are not developed, the male lice cannot be distinguished externally from the females. However, the testes in these very young and small lice are very well developed while the ovaries in the females of the corresponding stages are not so.

For fixing the testes a live louse is first placed in a drop of saline water on a slide under a mounted lens. The thorax is held in position by means of a needle with one hand and the tip of the abdomen is cut with the other, using a sharp scalpel or another needle. The contents of the abdomen are then squeezed out by slight pressure and the testes, which are in the form of white pear-shaped bodies, can be easily spotted. There are two pairs of these pear-shaped bodies, a pair situated on each side. In each pair the two pear-shaped structures are placed with their broader ends facing each other. From the broad inner end of each lobe of the testis is given off a vas deferens. The two vasa deferentia soon unite to form a common vas

deferens on each side. It is always better to take out the testes along with the vasa deferentia as, by doing so, it becomes easier to handle them during the later processes of fixation, washing, dehydration and embedding, etc.

Out of the several fixatives tried, Champy's and Flemming's—without acetic acid—gave the best results so far as the cytoplasmic inclusions are concerned. Flemming's—without acetic acid—was used with various modifications, but with 75% of water it gave excellent revelations of the Golgi apparatus and the mitochondria.

The material fixed in Flemming's and in Bouin's fluid particularly, gave us the best preparations for the study of the chromatin contents especially during the division stages. The presence of the two centrosomes and the two axial filaments in the spermatid was also confirmed by sections obtained from the material fixed in Bouin's fluid.

The sections were mordanted in 4% iron alum, stained in 0·5% haematoxylin, differentiated in 2% iron alum, dehydrated as usual and were mounted finally in Canada balsam.

Observations

Spermatogonia

In both the species of the genus *Pediculus*, viz., *capitis* and *corporis* the spermatogonia fill up practically the whole of the space in the testes of a younger louse. As the louse, however, matures the spermatogonia occupy lesser and lesser space and ultimately become confined only to the distal narrow ends of the testes and a little way down along the sides of the testes.

The spermatogonia are small and round cells, when lying free, but they may appear as many as five- or six-sided, when seen in a compact follicle.

Each spermatogonium has a comparatively large nucleus, occupying generally a central position. The nucleus invariably contains a big darkly staining nucleolus which is surrounded by a clear area. The nuclear membrane at this stage is not very conspicuous (Plate I, Fig. 1). The cytoplasm in the spermatogonia stains uniformly and does not show any prominent structure like the Golgi apparatus or the mitochondria. The spermatogonial wall is very delicate as is typical of the animal cells.

In the testes of *Pediculus* the division stages of the spermatogonia were rarely seen. The spermatogonia, however, divide a number of times as is evident from the increasing size of the testes which are very much larger in the fully grown animal but are still filled up with spermatogonial cells only. When a spermatogonium prepares to divide the first structure to appear in its cytoplasm is the centrosome in the form of a small granule which soon divides into two (Plate I, Fig. 2). The nuclear membrane round the clear area of the nucleus also becomes prominent at this stage. Small chromatin granules or prochromosomes now make their appearance and they arrange themselves on the inside of the nuclear membrane (Plate I, Figs. 3, 4 and 5). These prochromosomes gradually grow larger and finally obliterate the entire clear space inside the nucleus. The nuclear membrane at this stage is very prominent (Plate I, Fig. 6). Most of these

prochromosomal granules are double from the very beginning and the maximum number of chromosomes during the present studies has been found to be six. With the growth of the prochromosomes the nucleolus becomes less prominent and finally disintegrates completely, probably contributing to the formation of the chromosomes. During the metaphase stage of the spermatogonial divisions the chromosomes arrange themselves in the form of a ring on the equator of the spindle and their double structure can be clearly seen in the polar as well as the sideviews of the spermatogonial spindles (Plate I, Figs. 7 and 8). Each spermatogonial spindle is a fusiform area completely devoid of any fibrillar structure. At each pole of the spindle is a conspicuous darkly staining centrosome which is also devoid of astral rays.

Spermatocytes

In *Pediculus corporis* the mitochondria put in their appearance for the first time just when the growth phase begins in the spermatocytes (Plate I, Figs. 9 to 11). In *Pediculus capitis*, however, they appear at a little later stage of growth in the spermatocytes (Plate I, Fig. 12). In the former case, the mitochondria, to begin with, occupy the whole of the cytoplasmic space available around the nucleus (Plate I, Fig. 9) while in the latter they are seen lying scattered loosely on one side of the nucleus (Plate I, Fig. 12). The mitochondria, at the time of their first appearance, are in the form of granules but due to their very small size and their readiness to take up the stain intensely it often becomes difficult to demonstrate their exact structure.

Like the spermatogonia each spermatocyte also possesses a nucleus with a very prominent nucleolus which is surrounded by a clear space and a very faint nuclear membrane. The spermatocyte may grow as much as about four times the size of a spermatogonium. With its growth the mitochondria tend to collect together in one half, while the nucleus occupies the other half of the spermatocyte (Plate I, Figs. 12 and 13). At this stage the mitochondrial granules may be arranged in a number of ways. They may be lying either in the form of one compact mass, or in a semi-circle or in the form of a complete ring (Plate I, Fig. 13).

The small mitochondrial granules gradually fuse with each other to form bigger and fewer granules. These may now be termed as the mitochondrial vesicles because each one of them seems to be made up of a chromophilic cortex and a chromophobic core (Plate I, Fig. 14). Later on, however, these mitochondrial vesicles lose their identity as such and this results from their further fusion with each other. The mitochondrial mass, which may now be called the mitochondrial nebenkern, appears at this stage in the form of an anastomosing network (Plate I, Fig. 15). This reticulate formation seems to be made up of the chromophilic portion of the mitochondrial vesicles while the spaces in it are filled up with the chromophobic material. This mitochondrial mesh-work has larger chromophobic spaces near its periphery than in the centre (Plate I, Fig. 15). As the condensation of the mitochondrial nebenkern proceeds further the chromophilic mass is gradually withdrawn from the peripheral portion

of the reticulum till we get a few large chromophobic cavities which are still provided with thick chromophilic walls surrounding the central chromophilic mass in a more or less irregular fashion (Plate I, Fig. 16). Most of the chromophilic material from the walls of the peripheral chromophobic cavities is further retracted centrally with the result that they become perfectly chromophobic except for the thin chromophilic rims only (Plate I, Figs. 17 and 18, and Plate II, Fig. 19). In properly differentiated sections the central chromophilic area always shows a number of lines traversing it. These mark the boundaries of the alveoli which are still present in this area. If, however, the stain is not properly extracted the central chromophilic mass appears structureless. Later on the number of alveoli in the central chromophilic mass is gradually reduced till only a few lines can be seen traversing this area (Plate I, Fig. 18, and Plate II, Fig. 19). We have now reached a stage in which the mitochondrial nebenkern possesses a few prominent peripheral chromophobic cavities surrounding a central chromophilic mass.

During all the above-mentioned changes in the mitochondrial material i.e., from the small mitochondrial granules to a star-shaped mitochondrial nebenkern (Plate I, Figs. 9 to 18, and Plate II, Fig. 19) the spermatocytes do not exhibit any other structure beside it, except the nucleus with a prominent nucleolus which is surrounded by a clear area.

In *Pediculus capitis*, further differentiation of the mitochondrial nebenkern in the spermatocyte leads to the formation of a very beautiful pattern. In an optical section this looks something like a set of four rings of different sizes, made up of the chromophilic material (Plate II, Figs. 20 to 23). This mitochondrial nebenkern appears differently in different sections, the shape depending on the angle a section is cut. The chromophobic material still surrounds the chromophilic rings of the mitochondrial nebenkern and also fills up the spaces enclosed by the various rings. In reality these rings are thick-walled shells which are made up of the chromophilic material. One of them is larger than the others which are invariably enveloped by it (Plate II, Fig. 23). In all these stages the mitochondrial nebenkern occupies a central position in the cell, while the nucleus is pushed towards one side (Plate II, Figs. 20 to 23).

Before the division of the spermatocyte a number of characteristic changes take place in the cell. Firstly the chromophilic portion of the mitochondrial nebenkern fades away gradually, with the result that only a shaded outline marks its existence during meiosis. The chromophobic substance, however, still envelops the very lightly but uniformly stained chromophilic portion of the mitochondrial nebenkern, separating it from the rest of the cytoplasm (Plate II, Figs. 24 to 28).

Secondly there appears for the first time a darkly staining and perfectly spherical structure, about the size of the nucleolus, in the cytoplasm of the spermatocyte. This is the Golgi body which could not be observed in any of the earlier stages of spermatogenesis (Plate II, Figs. 24 to 28).

In all these pre-division stages of the spermatocytes the nucleolus is the only structure which can be made out in the nucleus. But a few small

granules, known as the prochromosomes, now put in their appearance just underneath the nuclear membrane (Plate II, Fig. 24). The nucleolus also soon disappears probably contributing to the growing chromosomes (Plate II, Fig. 25). These chromosomes are clearly double even when they are in the making. Figures 26 and 27, in Plate II, depict the anaphase stages of the spermatocyte division in which the chromosomes have moved quite apart on the spindle but the mitochondrial nebenkern and the Golgi body show no signs of division.

The meiotic spindle, like the one seen during spermatogonial mitosis, is a fusiform area completely devoid of any fibrillar structure. At each pole of the spindle, there is a conspicuous darkly staining centrosome which again is devoid of astral rays. Corresponding to the position of the nucleus in the early stages of the spermatocytes the spindle also lies eccentrically in the cell throughout the meiotic division.

In figure 28, Plate II, is shown a late telophase stage of the meiosis in which the two sets of chromosomes have already given rise to two daughter nuclei. One of these nuclei is seen lying in a finger-like outgrowth of the dividing cell, while the main body of the cell has retained not only the other nucleus but also the whole of the mitochondrial nebenkern and the Golgi body. This spermatocyte division is thus extremely unequal so far as the cytoplasm and its inclusions are concerned. The smaller cell containing one of the daughter nuclei and a little amount of cytoplasm gets cut off from the main body of the cell just like a polar body from a maturing ovum. This small cell does not undergo any further development as it is very often seen as such, lying along with the maturing spermatids (Plate III, Fig. 33).

There has not been observed any other division of the spermatocyte.

Spermateleosis

Even in the earliest spermatids of both the species, viz., *Pediculus capitis* and *Pediculus corporis* we find two centrosomes lying in close proximity to the nucleus (Plate II, Figs. 29 and 30). This fact clearly proves that the originally single centrosome soon divides into two in preparation for the second meiotic division which, however, does not take place due to some unknown reasons. The nucleus has resumed its shape which is so typical of the resting stages, i.e., there is only a small nucleolus with a clear space around it.

After the meiosis, the next change that the mitochondrial nebenkern undergoes in the spermatid, is characterized by the appearance of a new chromophilic mass in place of the previous chromophilic material of the mitochondrial nebenkern. This new substance is in the form of big vesicles but differs from the old chromophilic material in being not so deeply stainable (Plate II, Figs. 29 and 30). This new chromophilic substance corresponds to the 'Central substance' of Bowen (1922) or 'sheath substance' of Nath (1925) as it ultimately forms the sheath of the tail of the ripe sperm. The number of vesicles observed in the newly appeared 'sheath substance' is quite large in the beginning (Plate II, Fig. 29), but later on these vesicles seem to coalesce with each other gradually till only

one big spherical structure is formed (Plate II, Fig. 31, and Plate III, Fig. 32). In sections of the late spermatids the 'sheath substance' appears in the form of a ring of chromophilic material enclosing a chromophobic substance inside. The latter substance also completely envelops the chromophilic sphere, separating it from the rest of the cytoplasm.

An axial filament now grows out from each of the two centrosomes lying beneath the nucleus. Considering the nuclear end of the spermatid as the anterior, the two axial filaments grow backwards towards the posterior end of the cell (Plate II, Fig. 31). In some such cells one of the two axial filaments appears to divide the mitochondrial nebenkern into two symmetrical halves (Plate III, Figs. 33 and 37). The other axial filament seems to be a bit thicker than it but as it lies in close proximity to the mitochondrial nebenkern it is not always possible to discern it.

The growing axial filaments sometimes protrude a little out of the cell at the posterior end, carrying at their free outer ends small knob-like distal centrosomes. These resemble very much the antennæ of a butterfly (Plate III, Fig. 32). But that is not the end, because the axial filaments still grow further, carrying a little amount of cytoplasm along with them, till they attain a length which is roughly double that of the cell they are lying in (Plate II, Fig. 31). The two axial filaments very often get twisted over each other outside the main body of the cell.

The single spherical Golgi body may be lying anywhere in the cytoplasm in the early stages of spermateleosis (Plate III, Fig. 33), but mostly it is found in the anterior region of the cell. As the spermatid begins to elongate the Golgi body comes to lie very near the posterior end of the nucleus, quite often overlapping one of the centrosomes (Plate II, Fig. 31, and Plate III, Figs. 32 and 35 to 37).

In the elongating spermatids the nucleolus disappears and the nucleus becomes solid, the whole of it getting packed up with the deeply staining chromatin contents (Plate III, Figs. 32 and 33).

As regards the elongation of the spermatids a slight difference has been noted between the two species. In *Pediculus corporis* the head region of the spermatid, consisting of the nucleus, Golgi body, centrosomes and a little cytoplasm takes a lead in the process of elongation. This region is pushed out like a small bulb-like off-shoot from the main body of the cell and remains connected with it by means of a broad neck (Plate III, Fig. 35). In *Pediculus capitis*, on the other hand, the process of elongation is accomplished rather uniformly. As is shown in Figures 36 and 37, Plate III, the cell is narrowed down as a whole with the cytoplasm preceding the nucleus and the other structures anteriorly.

Next and the most important step in spermateleosis is the movement of the Golgi body from the posterior to the anterior end of the nucleus. This process is clearly illustrated in Figures 39 to 42, Plate III. As has already been said the Golgi body is generally found near the nucleus in the elongating spermatids. Later on it gets applied to the posterior end of the nucleus (Plate III, Fig. 32) over which it spreads like a cap on one side of it (Plate III, Figs. 39 to 41). Finally the whole of the substance of the Golgi body, after having crept over the nuclear membrane is accumulated

in front of the nucleus (Plate III, Figs. 42 to 44). This spherical Golgi body, now lying at the anterior end of the nucleus, is practically of the same size as at the time when it started its journey over the nuclear membrane. It is, therefore, quite clear that no part of the Golgi body is left behind or cast off. For some time now the lengthening of this Golgi body to form the acrosome is postponed till the nucleus has elongated considerably.

It has been mentioned before that the nucleus in the early stages of spermateleosis takes up a uniform and intense stain. But when the Golgi body starts moving up along one side of the nucleus its staining capacity is once again lost. At this stage the nucleus is generally quite clear (Plate III, Figs. 39 to 42) but sometimes it reveals inside it a deeply staining nucleolus-like body which is so characteristic of the early stages of spermatogenesis (Plate III, Fig. 38).

The nucleus which has so far been almost perfectly spherical now begins to elongate, carrying at its tip the button-like Golgi body. As a result of this elongation the nucleus may attain a length which is approximately three or four times its original diameter (Plate III, Figs. 43 and 44).

With the narrowing of the spermatid, the centrosomes come to lie so close to each other as to look like one whole mass and the same is the case with the axial filaments. When the spermatids have spun out considerably the separate entity of the axial filaments cannot be made out (Plate III, Figs. 43 and 44).

The middle portion of the spermatid thins out comparatively sooner than the anterior and the posterior regions with the result that the bulk of the 'sheath substance' gets accumulated in the posterior region which appears as a spindle-shaped swelling (Plate III, Fig. 43).

After this the cell attenuates enormously. The head consisting of the nucleus behind and the Golgi body in front tapers anteriorly from its comparatively broad base. Like the nucleus the Golgi body also spins out anteriorly to form a long flagellum-like acrosome (Plate III, Fig. 45). With further elongation of the cell, the spindle-shaped swelling in the tail region disappears as it seems to spread out uniformly along the whole length of the sperm tail. Consequently the discrimination between the two substances, i.e., the cytoplasm and the 'sheath substance' of which the spindle-shaped swelling is made, is done away with in the mature sperm.

Discussion

(a) *Golgi apparatus and Acrosome*

Doncaster and Cannon (1920) state that in *Pediculus* the acroblast, which is really the Golgi apparatus, may 'sometimes be double at the time of its first appearance, but is always a single spherical body later'. Similarly in the horse-louse, *Haematopinus asini*, Cannon (1922) records that the acroblast 'is usually, but not always, double and remains so until the spermatid has formed and is elongated'. On the other hand, in the two species of *Pediculus*, viz., *capitis* and *corporis*, investigated by us, the Golgi apparatus, from the very beginning, is in the form of a single, spherical

and darkly staining body. It appears for the first time in the cytoplasm of the spermatocyte. It could not be observed in the spermatogonia and in the spermatocyte too it appeared quite late, just at the time when the nucleus had started preparing for the division. It takes up the haematoxylin stain rather sharply and homogeneously. In good preparations its outline is always evenly circular and it is generally surrounded by a small lightly staining cytoplasmic area. In size it is equal to that of the nucleolus of the resting stages. Doncaster and Cannon (1920) and Cannon (1922) were doubtful about the origin and nature of the acroblast but judged from the roll that it plays in the formation of the acrosome there seems to be no doubt at all about its original nature that it belongs to the category of the Golgi apparatus. Actually in Mann-Kopsch preparations of the dog-louse, *Lignognathus piliferus* there are, according to Cannon himself (1922), indications of the acroblast arising from two or three scattered granules which may be the true Golgi bodies.

From its first appearance to the time till it forms an acrosome there takes place no change of any kind in the single spherical Golgi body. In other words it remains as such during the only maturation division and goes as a whole to one daughter cell only. In the earliest spermatid, thus formed, the Golgi body may be lying anywhere in the cytoplasm. In the late spermatids, however, it shows a distinct tendency to be near the nuclear or the anterior end. As the spinning out process to form the ripe sperm is taking place the Golgi body comes to lie very close to the nucleus and gets applied to its membrane at the posterior end. It then begins to creep over the surface of the nucleus and in doing so it forms a sort of cap over its one side. Gradually the whole of the substance of the Golgi body, after having crept over the nuclear membrane, is accumulated in front of the nucleus. The size of this Golgi body, now lying at the anterior end of the nucleus, is practically the same as at the time when it started its journey over the nuclear membrane. It is, therefore, quite clear that no fragment of the Golgi body is left behind or cast off. Some similar observations have been made by Doncaster and Cannon (1920) on *Pediculus*. But in the horse-louse, *Haematopinus asini* Cannon (1922) records 'that during spermateleosis the double acroblast loses one of its halves, which passes away from the nucleus and disappears'. This apparently useless half of the acroblast can be easily homologized with the Golgi remnant recorded in so many animals.

Further development of the Golgi body takes place only after the nucleus has elongated considerably. Then by a spinning out process the Golgi body is transformed directly into a long flagellum-like acrosome which is narrower in front and a little broad behind. This whole structure gets so much blended in general appearance with the likewise elongated nucleus that in the mature sperm it becomes increasingly difficult to discriminate one from the other.

These observations regarding the direct origin of the acrosome from a single spherical Golgi body completely confirm the earlier accounts of Doncaster and Cannon (1920) and Cannon (1922). Recently in this laboratory Nath and Bhatia (1953) have also clearly shown that in *Lepisma*

the acrosome is formed by the direct fusion of the Golgi vesicles and is not a secretory product thereof, as described by Bowen (1924). For the direct origin of the acrosome from the Golgi bodies reference may also be made to Sharma (1944 and 1950) and Nath (1932 and 1942).

(*b*) *Mitochondria*

According to Cannon (1922) the cytoplasm in the earliest spermatogonia of the dog-louse, *Lignognathus piliferus* is completely filled up with vacuolated mitochondria. In *Pediculus*, however, Doncaster and Cannon (1920) were of the opinion that some of the earliest spermatogonia showed granular mitochondria. In contrast to these observations the mitochondria could not be demonstrated at all in the spermatogonia of both the species of *Pediculus*, viz., *capitis* and *corporis* investigated by us. They appear for the first time in the spermatocytes at a time when the growth phase has started. As there is a very large number of these mitochondria, to begin with, they just look like a black cloud in an overstained preparation. But in a carefully differentiated cell each mitochondrium appears as a small granule, which takes up the haematoxylin stain intensely. These mitochondrial granules have a distinct tendency to come together and consequently bigger mitochondrial granules, which are fewer in number, are formed. These may now be termed as mitochondrial vesicles because each one of them seems to have, at this stage, a chromophilic cortex and a chromophobic core. By the running together of these mitochondrial vesicles, is formed a rather compact mitochondrial nebenkern which generally gives an impression of soap bubbles or of an anastomosing network, if there is a lot of stain on it.

The next step in the process of condensation of the mitochondrial nebenkern is that the most of the chromophilic material from the alveoli lying at the periphery is progressively withdrawn, with the result that an irregular envelope of large chromophobic alveoli surrounding a central darkly staining chromophilic mass is formed. In properly differentiated sections the central chromophilic area always reveals a number of lines traversing it and these represent the boundaries of the alveoli which are included in this area. If, however, the stain is not properly removed the central chromophilic mass appears structureless.

With further retraction of the chromophilic substance towards the centre of the mitochondrial nebenkern there are formed a few very large alveoli at its periphery, each with a large chromophobic area enclosed by only a thin chromophilic wall. At this stage the central chromophilic mass of the mitochondrial nebenkern clearly reveals a plate-work structure as shown earlier by Doncaster and Cannon (1920) and Cannon (1922) and not a spireme as urged by Gatenby (1917). This plate-work consists of a number of spherical or ovoid shells with thick walls of a chromophilic material and containing in them a chromophobic substance. These shells are of different sizes and the biggest of them seems to envelop all the others. In optical sections these shells appear as rings which look different in different views, depending upon the angle a cell is cut. The maximum number of these rings, observed in a section, is four.

As the spermatocyte prepares for the only meiotic division the central chromophilic part of the mitochondrial nebenkern gradually grows dim and ultimately fades away altogether. Consequently only a lightly staining sphere, surrounded by a clear area, marks the existence of the mitochondrial nebenkern during the division stages. Doncaster and Cannon (1920) have also clearly shown in their figures this fading away of the chromophilic part of the mitochondrial nebenkern but they make no reference to it in their text. In their explanation of plate, however, they mention in the form of a note that the acroblast (our spherical Golgi body) and the mitochondrial body (our mitochondrial nebenkern) are not preserved in their figures 11 to 13 which depict the division stages of the spermatocyte.

The mitochondrial nebenkern does not divide at all during the spermatocytic division but, like the Golgi body, it is simply passed on as such to one of the two daughter cells only.

In the spermatid the mitochondrial nebenkern is always lying almost in the centre of the cell, the two centrosomes lying between it and the nucleus. A new chromophilic substance now makes its appearance in place of the old one. This new substance in the central part of the mitochondrial nebenkern differs from the old chromophilic material inasmuch as it does not take up the haematoxylin stain intensely. In this paper it has been homologized by us to the 'central substance' of Bowen (1922) or the 'sheath substance' of Nath (1925) as it ultimately forms the sheath of the tail of the ripe sperm. The number of vesicles observed in this newly appeared 'sheath substance' is quite large, to begin with, but later on these vesicles seem to coalesce with each other gradually, till only one big spherical structure is formed. In sections of the late spermatids the 'sheath substance' appears in the form of a ring of chromophilic material enclosing a chromophobic substance inside. The latter substance also envelops it completely from all sides, separating it from the rest of the cytoplasm.

When the axial filaments start growing out, the mitochondrial nebenkern appears to be divided into two equal halves by the impressing of one of the axial filaments on the envelope of this body. The symmetrical disposition of the two parts can be clearly made out in the cross-sections of the spermatids at this stage.

As the spinning out of the spermatid takes place during the process of sperm formation the mitochondrial nebenkern is also drawn out along with the cytoplasm. In the penultimate stage the bulk of the sheath-forming substance of the mitochondrial nebenkern gets accumulated in a swelling in the posterior region of the attenuated spermatid. Finally, however, when the sperm has drawn out to its full length, the sheath-forming substance of the mitochondrial nebenkern is also spread out uniformly, forming the sheath of the tail of the ripe sperm.

It will thus be seen from the foregoing account that a mitochondrial nebenkern which is so typical of the insect spermatogenesis is also formed in the two lice examined by us by the usual process of running together of the mitochondrial vesicles. The only difference is that in the lice the

process of condensation of the mitochondrial nebenkern starts as early as in the spermatocyte and not in the spermatid as in the other insects.

(*c*) *Nucleus*

In the spermatogonia of both the species of *Pediculus*, viz., *capitis* and *corporis* the nucleus is the only structure to be found besides comparatively little cytoplasm in which it is placed almost in the centre. In the resting stages of both the spermatogonia and the spermatocytes the nucleus always reveals a prominent darkly staining nucleolus surrounded by a clear area and a very faint nuclear membrane. The spermatocyte nucleus is almost of the same size as that of the spermatogonium, but it now lies towards one side of the cell and is not situated in the centre as was the case in the spermatogonia. In both the spermatogonia and the spermatocytes the nuclear membrane becomes conspicuous only when the nucleus is preparing for the division.

Just before each division, whether mitotic or meiotic, small prochromosomal granules appear in the clear space around the nucleolus. The latter, however, disappears, probably contributing to the growing chromosomes which finally fill up the whole of the available space in the nucleus. In *Pediculus* there is not formed in the nucleus any structure like a spireme. In the dividing spermatocyte, corresponding to the position of the nucleus in the resting stages, the meiotic spindle also lies eccentrically in the cell.

Another interesting fact about *Pediculus* is that unlike the normal state of affairs during meiosis, the division of the nucleus fails to induce the other cell components to divide into two parts. The result is that one small daughter nucleus along with a little amount of cytoplasm is nipped off from the main cell which retains not only the single spherical Golgi body but also the whole of the mitochondrial nebenkern. The small polar-body-like cell does not undergo any further development. A similar single spermatocyte division has also been observed by Meves (1907) and Mark and Copeland (1906) in the hive-bee.

After this only meiotic division the nucleus in the early spermatid resumes its normal shape, typical of the resting stages, but with this difference that it is now considerably smaller. As the spermatid begins to elongate the chromatin gets distributed evenly throughout the clear space in the nucleus which now stains intensely and uniformly. In the late stages of spermatcleosis, however, when the single spherical Golgi body is moving towards the anterior side along one side of the nucleus, it loses its staining capacity and becomes clear once again. In some such cells a deeply staining nucleolus-like body was also seen in the centre of the nucleus but it disappeared quickly. On the other hand, Doncaster and Cannon (1920) have described a deeply stained oval body which lies within the nucleus at the posterior end of more elongated heads and this seems to persist till the formation of the ripe sperm.

During the transformation of the spermatid into the sperm the spherical nucleus of the earlier stages is lengthened out along a line continuous with the axis of the tail sheath. As a result of this, the nucleus

thins out anteriorly leaving its posterior region comparatively broad. This elongated nucleus carries at its tip the spherical Golgi body which is also now spun out directly into a long flagellum-like acrosome. The elongated nucleus and the acrosome together at this stage form a needle-like head for the ripe sperm.

(*d*) *Centrosomes and Axial filaments*

In both the species of *Pediculus*, viz., *capitis* and *corporis* the centrosome is not visible during the resting stages of the spermatogonia and the spermatocytes. When a spermatogonium prepares to divide the centrosome is the first new structure to put in its appearance in the cytoplasm of the cell and it soon divides into two. This structure is very conspicuous in the preparations made from the material fixed in Bouin's fluid but it could not be made out with certainty in the sections of the material fixed in Flemming's—without acetic acid. During the division stages of both the spermatogonia and the spermatocytes the centrosomes behave as usual, i.e., they are found at the apices of the spindle as darkly staining granules. They have neither the astral rays nor any clear area round them.

The spindle, whether it is mitotic or meiotic, is a fusiform area completely devoid of any fibrillar structure. The characteristic feature of the meiotic spindle is that throughout the division period it remains lying quite eccentrically in the cell.

Even in the earliest spermatid there are found two centrosomes. It is, therefore, quite evident that the originally single centrosome divides immediately into two preparatory to the second maturation division of the spermatocyte which, however, does not take place in *Pediculus* due to some unknown reasons.

The two centrosomes in the spermatid are always lying beneath the nucleus in close proximity to it. Later an axial filament grows out from each centrosome and proceeds to the posterior side. These growing axial filaments may sometimes be seen to bear at their free ends the distal centrosomes. The two axial filaments grow extensively and outside the cell they get twisted round each other. One of the axial filaments appears to divide the mitochondrial nebenkern into two symmetrical halves and the other seems to be a bit thicker than it.

In some of the elongating spermatids one of the centrosomes is lost to view as it becomes covered over by the single spherical Golgi body which now takes its position at the posterior end of the nucleus to make its forward journey along one side of the nucleus. In the late stages of spermateleosis the two centrosomes come to lie so near each other that their separate entities cannot be made out. Similarly the two axial filaments also appear as one at this stage.

Doncaster and Cannon (1920) have also described a double centrosome and two axial filaments in the elongating spermatids. They are quite definite that both the axial filaments pass outside the mitochondrial nebenkern. According to them the bilateral symmetry of the mitochondrial nebenkern is possibly due to the line of junction of the two outer vacuoles running over its surface. On the other hand, we have clearly

shown that it is due to the impressing of one of the axial filaments upon the envelope of this body. Similar observations have also been made by Gatenby (1917).

Summary

1. In this paper the spermatogenesis of two species of louse, viz., *Pediculus capitis* and *Pediculus corporis* is described.

2. Each spermatogonium contains a large nucleus with a prominent nucleolus in the centre and comparatively very little cytoplasm.

3. The mitochondria appear for the first time in the growing spermatocytes in the form of a dense cloud of numerous small granules. These gradually fuse to form a fewer and bigger granules which may now be termed as the mitochondrial vesicles, each having a chromophilic cortex and a chromophobic core.

4. By the running together of these mitochondrial vesicles, is formed a typical mitochondrial nebenkern the condensation of which starts as early as in the spermatocyte. The structure of the chromophilic part of the mitochondrial nebenkern is that of a plate-work.

5. The Golgi apparatus also appears in the spermatocyte at a time when it is preparing for the division. It is in the form of a single, darkly staining, spherical body.

6. There is only a single spermatocyte division during which the single spherical Golgi body and the whole of the mitochondrial nebenkern are passed on as such to only one daughter cell. The other daughter cell containing a small nucleus and a little amount of cytoplasm degenerates.

7. During the only meiotic division the chromophilic part of the mitochondrial nebenkern disappears and in its place a new chromophilic substance appears in the spermatid. This forms the sheath of the tail of the ripe sperm.

8. During spermateleosis the spherical nucleus attenuates anteriorly leaving its posterior end comparatively broad.

9. The single spherical Golgi body of the early spermatid first moves from the posterior to the anterior end of the nucleus and is later on transformed directly into a long flagellum-like acrosome during the late stages of spermateleosis.

10. There are two centrosomes in the spermatid from the very beginning and from each one of these is given out an axial filament. In the ripe sperm, however, it is as difficult to make out their separate entities as it is to distinguish the acrosome from the nucleus.

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Explanation of Lettering in the Plates

A—Acrosome; *Af*—Axial filament; *C*—Centrosome; *Ch*—Chromosomes; *Ch'*—Prochromosomes; *G*—Golgi body; *M*—Mitochondria; *M.n.*—Mitochondrial nebenkern; *N*—Nucleus; *N'*—Nucleolus.

Explanation of Plates

All figures have been drawn with a Spencer Camera lucida at the stage level, using Spencer 10× eyepiece and an oil immersion objective. They were subsequently enlarged 16 times, thus giving a total magnification of 16,000 times. Unless otherwise mentioned in the text all figures have been selected from preparations of *Pediculus capitis*.

Plate I

Fig. 1. Earliest spermatogonium showing only a nucleolus with a clear space around it and a little cytoplasm.

Fig. 2. A spermatogonium in which a double centrosome and a prominent nucleolus are seen.

Figs. 3 to 6. Spermatogonia showing a prominent nuclear membrane. The prochromosomes are becoming larger gradually.

Fig. 7. Polar view of a spermatogonial metaphase.

Fig. 8. Side view of a spermatogonial metaphase.

Figs. 9 to 11. Early spermatocytes showing numerous mitochondrial granules in the cytoplasm.

Figs. 12 and 13. Spermatocytes after the growth period showing mitochondria accumulated in one-half of the cell while the other half is occupied by the nucleus.

Fig. 14. Spermatocyte in which the small mitochondrial granules have fused to form a few big mitochondrial vesicles.

Fig. 15. Spermatocyte in which a network-like structure is formed by the fusion of mitochondrial vesicles.

Figs. 16 to 18. Late spermatocytes showing condensation of the mitochondrial nebenkern.

Plate II

FIGS. 19 to 23. Late spermatocytes showing condensation of the mitochondrial nebenkern.

FIGS. 24 and 25. Spermatocytes preparing for the division, showing faded mitochondrial nebenkern. Prochromosomes are seen developing into the nucleus. The Golgi apparatus has also appeared in these cells in the form of a single spherical body.

FIGS. 26 and 27. Anaphase stages of the only spermatocyte division. The single spherical Golgi body and the faded mitochondrial nebenkern are not dividing.

FIG. 28. Late telophase stage of the only spermatocyte division. One of the daughter nuclei lying in a finger-like outgrowth of the cell.

FIGS. 29 and 30. Early spermatids showing two centrosomes lying underneath the nucleus. The newly appeared 'sheath substance' can also be seen in the mitochondrial nebenkern.

FIG. 31. Elongating spermatid with two long axial filaments forming a tail-like structure.

Plate III

FIG. 32. Spermatid in which the two axial filaments along with their distal centrosomes are seen protruding out of the cell like the antennæ of a butterfly.

FIGS. 33 and 34. Cross-sections of the elongating spermatids through the anterior and posterior regions respectively.

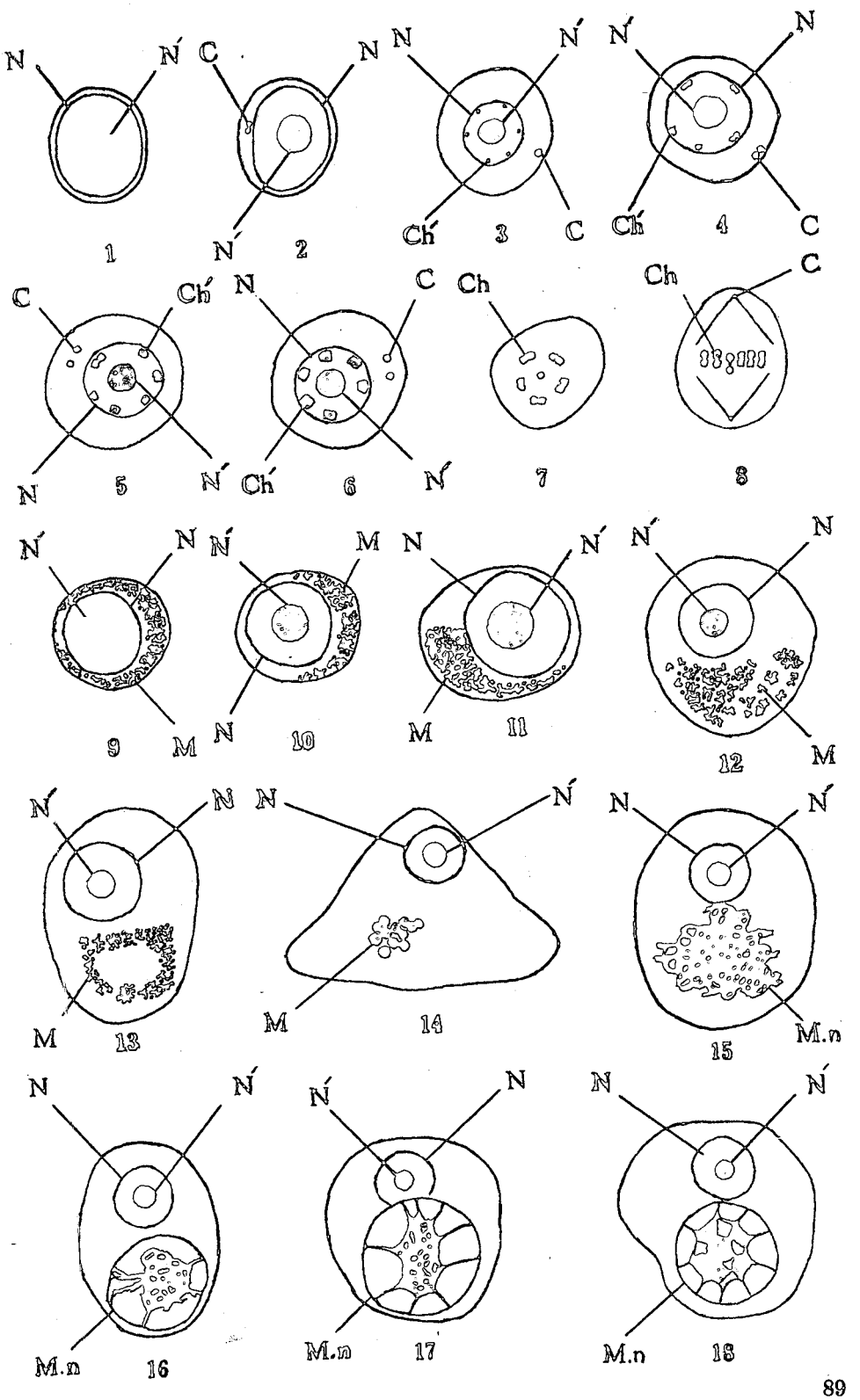
FIGS. 35 to 38. Elongating spermatids.

FIGS. 39 to 42. Anterior ends of the elongating spermatids showing the creeping up of the Golgi body along one side of the nuclear membrane.

FIG. 43. A late spermatid showing a cone-shaped nucleus with a button-like Golgi body situated at its anterior end. The bulk of the sheath-forming substance is accumulated in the spindle-shaped swelling in the tail region.

FIG. 44. A very late spermatid showing the process of attenuation.

FIG. 45. A nearly ripe sperm showing the needle-like acrosome lying in front of the elongating nucleus.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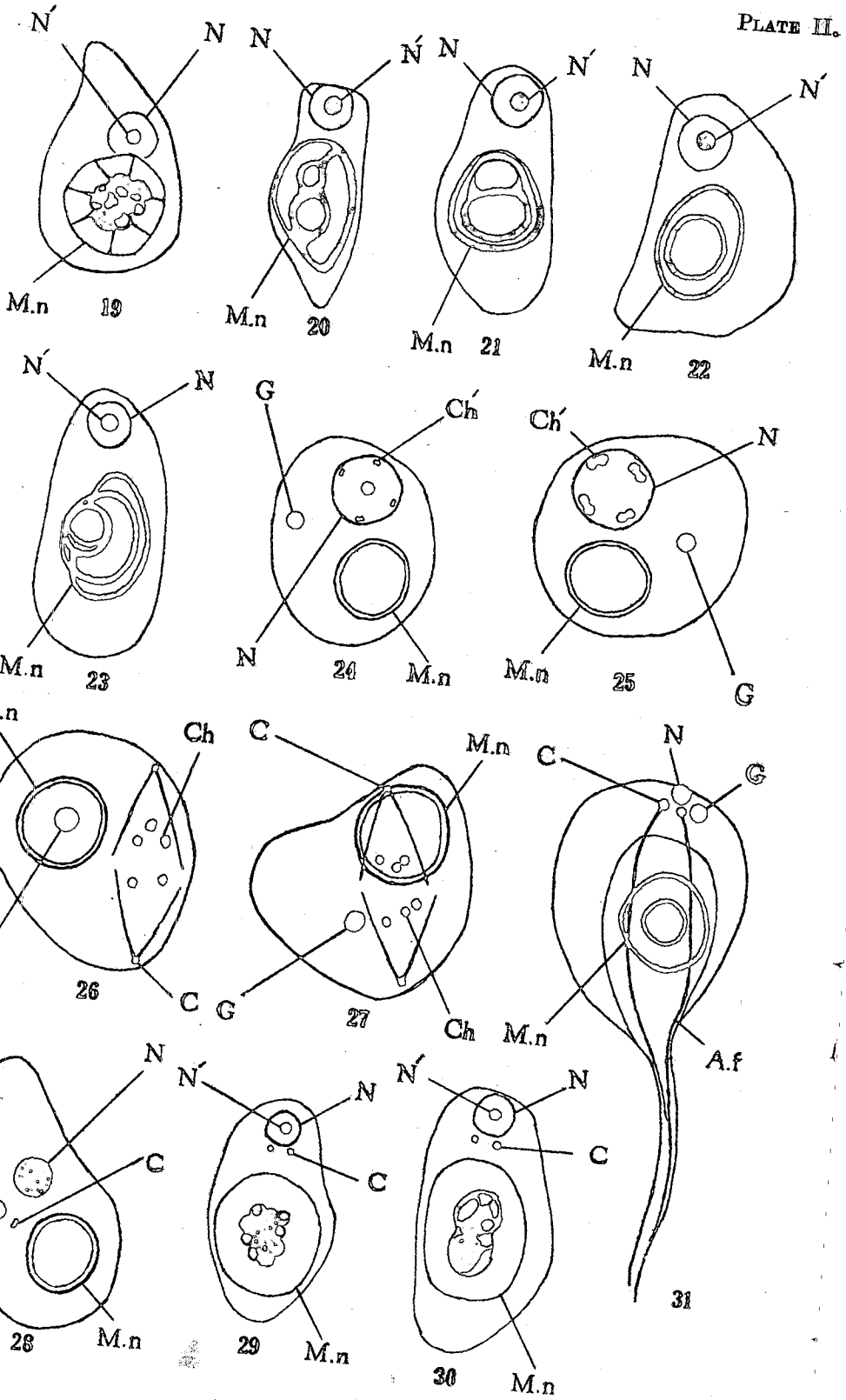
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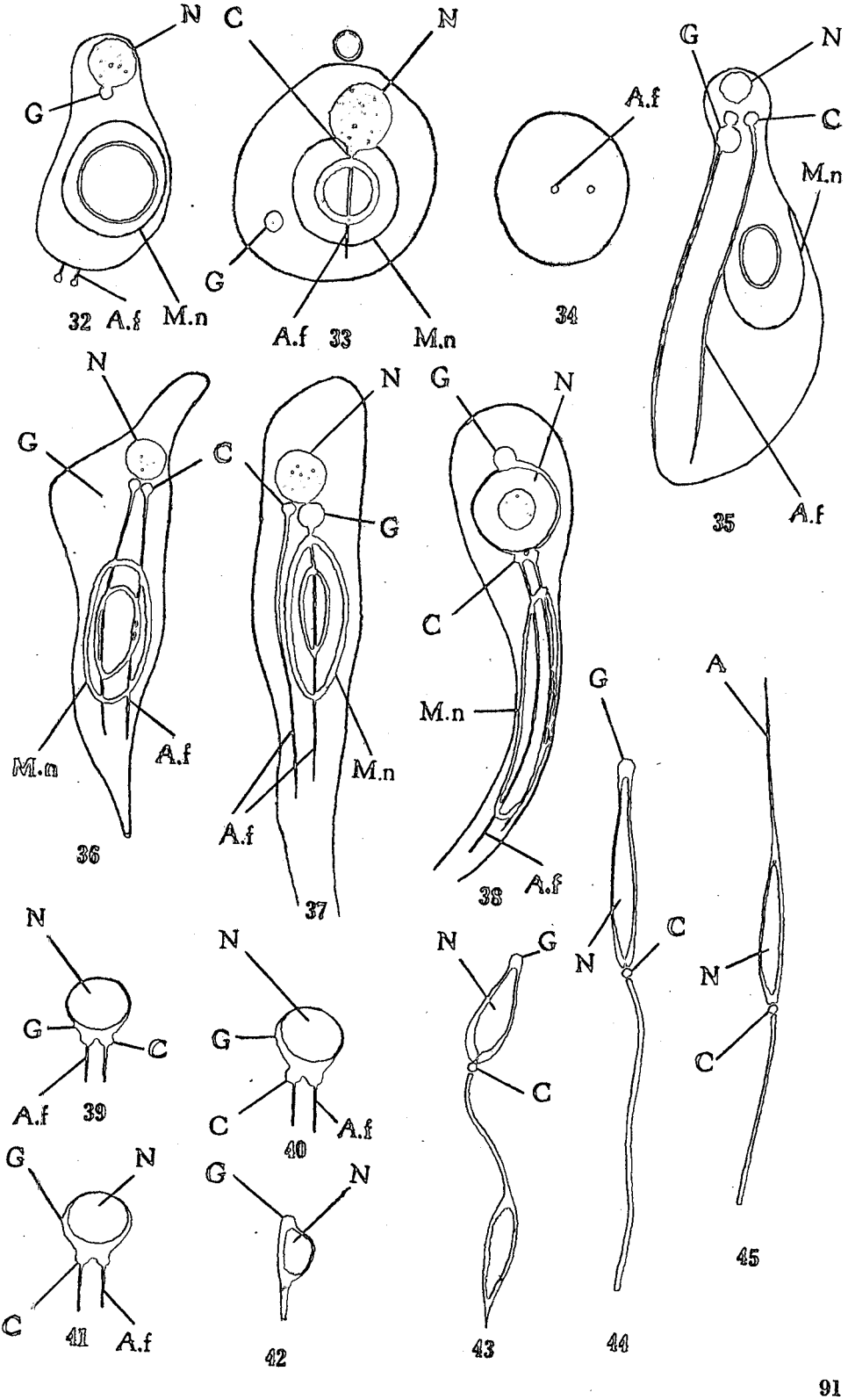
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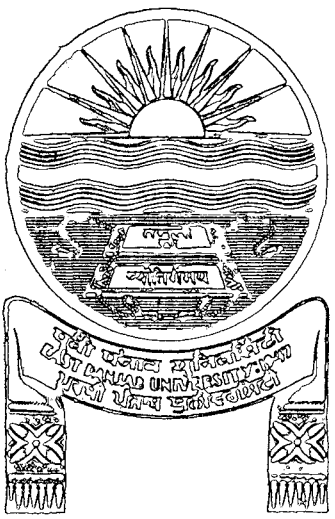
Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 33 CHEMISTRY Pp. 93-105



September, 1953

POTENTIOMETRIC STUDIES IN OXIDATION-REDUCTION REACTIONS

Part XVIII. Oxidation with Alkaline Potassium Permanganate

by

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Edited for the East Panjab University by Vishwa Nath,
Department of Zoology, East Panjab University,
HOSHIARPUR

Price Rs.1-14-0

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Part XVIII. Oxidation with Alkaline Potassium Permanganate

By Balwant Singh, Apar Singh *and* Miss Raj Kumari Nahan

Holluta (*Z. Phys. Chem.*, 1922, **102**, 32 and 276) has shown that in the reaction between permanganate and formate the reduction of permanganate in alkaline solution proceeds in two stages:—

$$MnO_4^- + e = MnO_4^{--} \quad .. \quad .. \quad .. \quad .. \quad .. \quad .. \quad (1)$$

$$MnO_4^{--} + 2\,H_2O + 2e = MnO_2 + 4\,OH^- \quad .. \quad .. \quad .. \quad .. \quad (2)$$

The first of these two reactions proceeds much more rapidly than the second. This is in conformity with the observations of Sackur and Taegener (*Zeit. Elecktrochem.*, 1912, **18**, 718). The difference of the velocities of the two partial reactions increases with rising hydroxyl ion concentration.

According to Stamm's procedure (*Z. angew. chem.*, 1934, **47**, 191; 1935, **48**, 710) the oxidation with alkaline permanganate solution involves only the first partial reaction, i.e., the conversion of permanganate to manganate and the slow conversion of manganate to manganese dioxide is not allowed to occur. To attain the highest possible oxidation velocities, the substance to be determined is added to an excess of alkaline permanganate solution. After the oxidation is finished, the excess of permanganate is titrated back with sodium formate.

$$2\,MnO_4^- + 3\,OH^- + HCO_2^- = 2\,MnO_4^{--} + CO_3^{--} + 2\,H_2O.$$

To prevent the formate from carrying the reduction of the manganate on to manganese dioxide, which would occur as soon as about ninety per cent of the permanganate originally present had changed to manganate, a barium salt is added to the reaction mixture before beginning the addition of the formate. Barium permanganate is soluble in water, while barium manganate is insoluble. The manganate ions produced by the reduction consequently are bound by the barium ions so rapidly that they are effectively removed from any further action of the reducing agent.

P. A. Heredia (*Arch. farm. bioquim. Tucuman*, 1947, 3, 173) found the method of Stamm suitable in the volumetric analysis of potassium iodide, potassium iodate, potassium cyanide, potassium phosphite, methyl alcohol, formaldehyde and formic acid.

In the present investigation alkaline potassium permanganate has been used as an oxidizing agent and the Stamm's procedure employed in the potentiometric estimations of Acetaldehyde, Formaldehyde, Benzaldehyde, Mandelic acid, Cinnamic acid, Tartaric acid, Malic acid, Isoamyl

alcohol, Benzyl alcohol, Ethyl alcohol, *n*-Propyl alcohol, *n*-Butyl alcohol. These compounds *react with alkaline permanganate* according to the following equations:—

1. $2\,MnO_4^- + 3\,OH^- + CH_3CHO = 2\,MnO_4^{--} + CH_3COO^- + 2\,H_2O$
2. $4\,MnO_4^- + 6\,OH^- + H.CHO = 4\,MnO_4^{--} + CO_3^{--} + 4\,H_2O$
3. $2\,MnO_4^- + 3\,OH^- + C_6H_5CHO = 2\,MnO_4^{--} + C_6H_5COO^- + 2\,H_2O$
4. $4\,MnO_4^- + 7\,OH^- + C_6H_5CHOH.COOH$
$= 4\,MnO_4^{--} + CO_3^{--} + C_6H_5COO^- + 5\,H_2O$
5. $10\,MnO_4^- + 15\,OH^- + C_6H_5CH.CH.COOH$
$= 10\,MnO_4^{--} + 2\,CO_3^{--} + C_6H_5COO^- + 9\,H_2O$
6. $8\,MnO_4^- + 14\,OH^- + \begin{vmatrix} CHOH.COOH \\ CHOH.COOH \end{vmatrix}$
$= 8\,MnO_4^{--} + 10\,H_2O + 2\,CO_3^{--} + \begin{vmatrix} COO^{--} \\ COO \end{vmatrix}$
7. $12\,MnO_4^- + 20\,OH^- + COOH.CH_2.CHOH.COOH$
$= 12\,MnO_4^{--} + 4\,CO_3^{--} + 13\,H_3O$
8. $4\,MnO_4^- + 5\,OH^- + (CH_3)_2CH_2CH.CH_2OH$
$= 4\,MnO_4^{--} + (CH_3)_2CH_2.CH..COO^- + 4\,H_2O$
9. $4\,MnO_4^- + 5\,OH^- + C_6H_5.CH_2OH$
$= 4\,MnO_4^{--} + 4\,H_2O + C_6H_5COO^-$
10. $4\,MnO_4^- + 5\,OH^- + C_2H_5OH = 4\,MnO_4^{--} + CH_3COO^- + 4\,H_2O$
11. $4\,MnO_4^- + 5\,OH^- + CH_3.CH_2CH_2OH$
$= 4\,MnO_4^{--} + CH_3.CH_2.COO^- + 4\,H_2O$
12. $4\,MnO_4^- + 5\,OH^- + CH_3.CH_2.CH_2.CH_2OH$
$= 4\,MnO_4^{--} + CH_3.CH_2.CH_2.COO^- + 4\,H_2O.$

Experimental

A known weight of the substance was added to a known excess of standard potassium permanganate solution to which 10 c.c. to 30 c.c. of 30% sodium hydroxide solution had already been added. The mixture was allowed to stand for ten minutes at 45°C. About 10 c.c. to 30 c.c. of 30% barium chloride solution was then added to the mixture and the excess of potassium permanganate solution titrated back potentiometrically against standard sodium formate solution. In these titrations the mixture was kept stirred by a mechanical stirrer.

The oxidation-reduction electrode which consisted of a bright platinum foil immersed in a solution to be titrated, was coupled with a saturated calomel electrode through an agar-agar potassium chloride bridge. The cell was placed in a water bath, the temperature of which was maintained at 45°C. E.M.F. of the cell was read on a potentiometer.

A series of potentiometric titrations were performed with different amounts of each substance. The titrations, one for each substance, are recorded in the following tables:—

TABLE I.—*Titration of 0·1058 gm. of acetaldehyde in presence of 15 c.c. 30% sodium hydroxide, 20 c.c. 30% barium chloride and 40 c.c. 1·0101 N potassium permanganate against 0·1 M sodium formate.*

Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)	Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)
1·00	0·6191				668
		1	16·20	0·4804	
3·00	0·6166				2154 (Max.)
		2	16·25	0·3727	
5·00	0·6131				440
		3	16·30	0·3507	
7·00	0·6076				282
		3	16·35	0·3366	
9·00	0·6015				220
		4	16·40	0·3256	
11·00	0·5942				85
		5	16·50	0·3171	
13·00	0·5835				14
		7	17·00	0·3100	
15·00	0·5698				7
		3	18·00	0·3028	
15·50	0·5567				6
		3	20·00	0·2918	
16·00	0·5442				4
		144	23·00	0·2803	
16·10	0·5298				3
		320	27·00	0·2671	
16·15	0·5138				1
			30·00	0·2650	

TABLE II.—*Titration of 0·0543 gm. of formaldehyde in presence of 15 c.c. 30% sodium hydroxide, 20 c.c. 30% barium chloride and 48 c.c. 1·0105 N potassium permanganate against 0·1 M sodium formate.*

Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)	Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)
1·00	0·6105				254
		4	12·75	0·4855	
3·00	0·6032				310
		4	12·80	0·4700	
5·00	0·5957				4096 (Max.)
		5	12·85	0·2652	
8·00	0·5810				214
		6	12·90	0·2545	
10·00	0·5690				63
		16	13·00	0·2482	
10·50	0·5610				13
		19	13·50	0·2419	
11·00	0·5517				12
		21	14·00	0·2361	
11·50	0·5413				3
		27	16·00	0·2296	
12·00	0·5278				2
		41	18·00	0·2262	
12·30	0·5155				1
		49	21·00	0·2226	
12·50	0·5058				2
		38	24·00	0·2167	
12·70	0·4982				

TABLE III.—*Titration of 0·0450 gm. of benzaldehyde in presence of 10 c.c. 30% sodium hydroxide, 15 c.c. 30% barium chloride and 1·0204 N 20 c.c. potassium permanganate against 0·1 M sodium formate.*

Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)	Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)
1·00	0·6445		16·10	0·3576	2704 (Max.)
		1			
4·00	0·6416		16·15	0·3464	224
		2			
6·00	0·6377		16·20	0·3384	160
		3			
8·00	0·6316		16·25	0·3300	168
		5			
10·00	0·6221		16·30	0·3234	132
		6			
12·00	0·6160		16·50	0·3162	36
		10			
14·00	0·5891		17·00	0·3100	12
		15			
15·00	0·5745		18·00	0·3016	8
		53			
15·50	0·5481		20·00	0·2932	4
		53			
15·80	0·5322		22·00	0·2808	6
		118			
16·00	0·5085		25·00	0·2800	3
		314			
16·05	0·4928				

TABLE IV.—*Titration of 0·1853 gm. of mandelic acid in presence of 20 c.c. 30% sodium hydroxide, 25 c.c. 30% barium chloride and 46 c.c. 1·0105 N potassium permanganate against 0·1 M sodium formate.*

Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)	Sodium formate c.c.	E.M.F (volts)	E/C (m. volts/c.c.)
5·00	0·6182		22·00	0·4640	95
		3			
9·00	0·6077		22·05	0·4385	510
		4			
13·00	0·5925		22·10	0·2545	3680 (Max.)
		4			
17·00	0·5763		22·15	0·2335	420
		7			
19·00	0·5628		22·20	0·2190	290
		6			
20·00	0·5568		22·30	0·2142	48
		44			
20·50	0·5350		22·50	0·2100	21
		23			
21·00	0·5235		23·00	0·2060	8
		28			
21·30	0·5150		24·00	0·2016	4
		43			
21·50	0·5065		26·00	0·1965	3
		55			
21·70	0·4955		29·00	0·1923	1
		125			
21·80	0·4830		34·00	0·1866	1
					1
			39·00	0·1815	

TABLE V.—*Titration of 0·0551 gm. of cinnamic acid in presence of 10 c.c. 30% sodium hydroxide, 15 c.c. 30% barium chloride and 29 c.c. 0·9806 N potassium permanganate against 0·1 M sodium formate.*

Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)	Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)
0·00	0·5831				402
		5	10·00	0·2150	
1·00	0·5783				198
		5	10·05	0·2051	
3·00	0·5686				194
		5	10·10	0·1954	
5·00	0·5584				99
		8	10·20	0·1855	
7·00	0·5432				12
		10	11·00	0·1757	
9·00	0·5241				10
		44	12·00	0·1719	
9·40	0·5064				24
		102	13·00	0·1658	
9·60	0·4859				12
		205	15·00	0·1420	
9·70	0·4654				3
		153	18·00	0·1336	
9·85	0·4424				1
		1268	21·00	0·1312	
9·90	0·3790				1
		2878 (Max.)	24·00	0·1294	
9·95	0·2351				1
			30·00	0·1271	

TABLE VI.—*Titration of 0·0714 gm. of tartaric acid in presence of 10 c.c. 30% sodium hydroxide, 15 c.c. 30% barium chloride and 34 c.c. 0·9801 N potassium permanganate against 0·1 M sodium formate.*

Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)	Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)
1·00	0·5698				208
		2	16·00	0·4458	
3·00	0·5665				688
		1	16·05	0·4114	
5·00	0·5650				1636 (Max.)
		1	16·10	0·3296	
7·00	0·5624				814
		3	16·15	0·2889	
9·00	0·5564				410
		4	16·20	0·2684	
11·10	0·5476				132
		6	16·30	0·2552	
14·00	0·5300				39
		13	16·50	0·2474	
15·00	0·5169				11
		24	17·00	0·2419	
15·50	0·5049				5
		77	18·00	0·2374	
15·70	0·4895				2
		98	21·00	0·2316	
15·80	0·4797				1
		131	25·00	0·2260	
15·90	0·4666				1
			30·00	0·2200	

TABLE VII.—*Titration of 0·0350 gm. of malic acid in presence of 22 c.c. 30% sodium hydroxide, 22 c.c. 30% barium chloride and 30 c.c. 1·0309 N potassium permanganate against 0·1 M sodium formate.*

Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)	Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)
5·00	0·5958				2402 (Max.)
		2	15·50	0·3534	
7·00	0·5928				586
		3	15·55	0·3241	
10·00	0·5830				312
		5	15·60	0·3085	
12·00	0·5726				450
		7	15·65	0·2860	
14·00	0·5587				192
		19	15·70	0·2764	
14·50	0·5493				25
		14	16·00	0·2690	
15·00	0·5421				4
		180	17·00	0·2650	
15·20	0·5061				3
		107	19·00	0·2593	
15·30	0·4954				4
		108	22·00	0·2478	
15·35	0·4900				4
		132	25·00	0·2355	
15·40	0·4834				2
		198	28·00	0·2283	
15·45	0·4735				3
			30·00	0·2214	

TABLE VIII.—*Titration of 0·1507 gm. of isoamyl alcohol in presence of 20 c.c. 30% sodium hydroxide. 20 c.c. 30% barium chloride and 50 c.c. 1·064 N potassium permanganate against 0·1 M sodium formate.*

Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)	Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)
1·00	0·5865				2190 (Max.)
		1	19·05	0·3380	
3·00	0·5842				910
		1	19·10	0·2885	
6·00	0·5802				380
		2	19·15	0·2695	
9·00	0·5730				158
		3	19·20	0·2616	
12·00	0·5635				15
		5	19·50	0·2571	
15·00	0·5492				7
		9	20·00	0·2536	
16·00	0·5398				3
		10	21·00	0·2508	
17·00	0·5296				2
		14	23·00	0·2468	
18·00	0·5154				1
		42	28·00	0·2418	
18·50	0·4946				1
		46	32·00	0·2365	
18·90	0·4761				1
		286	40·00	0·2321	
19·00	0·4475				

TABLE IX.—*Titration of 0·0650 gm. of benzyl alcohol in presence of 10 c.c. 30% sodium hydroxide, 15 c.c. 30% barium chloride and 20 c.c. 1·064 N potassium permanganate against 0·1 M sodium formate.*

Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)	Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)
3·00	0·6225				520
		5	9·50	0·4745	
5·00	0·6131				1480 (Max.)
		4	9·55	0·4005	
6·00	0·6088				854
		15	9·60	0·3578	
6·50	0·6012				450
		10	9·65	0·3353	
7·10	0·5955				306
		14	9·70	0·3200	
7·50	0·5900				110
		13	10·00	0·2870	
8·00	0·5835				14
		16	10·50	0·2800	
8·50	0·5755				9
		23	11·00	0·2755	
8·80	0·5685				4
		42	12·00	0·2715	
9·00	0·5602				3
		49	14·00	0·2651	
9·20	0·5505				2
		194	17·00	0·2592	
9·40	0·5118				1
		226	22·00	0·2520	
9·45	0·5005				

TABLE X.—*Titration of 0·0797 gm. of ethyl alcohol in presence of 25 c.c. 30% sodium hydroxide, 25 c.c. 30% barium chloride and 55 c.c. 1·0104 N potassium permanganate against 0·1 M sodium formate.*

Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)	Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)
1·00	0·5986				159
		1	21·40	0·4368	
3·00	0·5968				3988 (Max.)
		1	21·45	0·2374	
5·00	0·5958				306
		2	21·50	0·2221	
9·00	0·5868				122
		4	21·55	0·2160	
13·00	0·5741				108
		4	21·60	0·2106	
16·00	0·5628				23
		6	21·80	0·2060	
18·00	0·5499				21
		14	22·00	0·2018	
19·00	0·5363				10
		20	22·50	0·1972	
19·50	0·5265				4
		22	23·00	0·1950	
20·00	0·5153				3
		23	24·00	0·1925	
20·50	0·5037				1
		33	28·00	0·1878	
21·00	0·4874				1
		94	32·00	0·1841	
21·20	0·4686				

TABLE XI.—*Titration of 0·0682 gm. of* n-*propyl alcohol in presence of 15 c.c. 30% sodium hydroxide, 20 c.c. 30% barium chloride and 40 c.c. 1·0104 N potassium permanganate against 0·1 M sodium formate.*

Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)	Sodium formate c.c.	E.M.F (volts)	E/C (m. volts/c.c.)
0·00	0·6314				1842
		18	17·55	0·3744	
1·00	0·6138				2156 (Max.)
		3	17·60	0·2666	
3·00	0·6083				218
		2	17·65	0·2526	
7·00	0·5994				124
		4	17·70	0·2464	
10·00	0·5885				92
		5	17·75	0·2418	
12·00	0·5793				62
		7	17·80	0·2387	
14·00	0·5662				23
		11	18·00	0·2342	
15·00	0·5555				4
		12	18·50	0·2322	
16·00	0·5436				4
		14	19·00	0·2302	
16·80	0·5328				1
		76	20·00	0·2290	
17·00	0·5176				1
		53	25·00	0·2254	
17·40	0·4965				1
		300	30·00	0·2230	
17·50	0·4665				

TABLE XII.—*Titration of 0·0718 gm. of* n-*butyl alcohol in presence of 15 c.c. 30% sodium hydroxide, 20 c.c. 30% barium chloride and 40 c.c. 1·0204 N potassium permanganate against 0·1 M sodium formate.*

Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)	Sodium formate c.c.	E.M.F. (volts)	E/C (m. volts/c.c.)
0·00	0·6575				92
		2	20·70	0·5132	
3·00	0·6503				262
		1	20·80	0·4870	
5·00	0·6475				3036 (Max.)
		2	20·85	0·3352	
9·00	0·6385				414
		4	20·90	0·3145	
13·00	0·6243				110
		5	21·00	0·3035	
16·00	0·6095				29
		7	21·50	0·2890	
18·00	0·5960				16
		11	22·00	0·2810	
19·00	0·5850				9
		22	23·00	0·2725	
19·50	0·5742				5
		22	25·00	0·2620	
20·00	0·5632				4
		40	28·00	0·2500	
20·30	0·5513				3
		99	32·00	0·2375	
20·50	0·5315				2
			36·00	0·2297	

Discussion

In these titrations with the addition of standard sodium formate, the E.M.F. decreased steadily till the equivalence point. At the equivalence point there was a sharp break in the E.M.F. in each case. For the addition of 0·05 c.c. of the titrant, the inflection potential was of the order of 106, 157, 114, 195, 157, 86, 167, 106, 102, 172, 134 and 156 m. volts for Acetaldehyde, Formaldehyde, Benzaldehyde, Mandelic acid, Cinnamic acid, Tartaric acid, Malic acid, Isoamyl alcohol, Benzyl alcohol, Ethyl alcohol, *n*-Propyl alcohol and *n*-Butyl alcohol respectively.

From the volume of sodium formate used, corresponding to the equivalence point in each titration, the amount of each substance was calculated. The results are given in the following tables:—

Table I.—*Acetaldehyde.*

Substance taken	$KMnO_4$ added	0·1 *M* HCOONa used for excess $KMnO_4$	Excess $KMnO_4$	$KMnO_4$ used for the substance	Substance found
(gms.)	(gms.)	(c.c.)	(gms.)	(gms.)	(gms.)
0·1058	1·2767	16·220	0·5125	0·7642	0·1064
0·0966	1·2767	18·663	0·5897	0·6870	0·0956
0·0874	1·2767	20·390	0·6447	0·6320	0·0880
0·0782	1·2767	22·477	0·7102	0·5665	0·0788
0·0460	0·9575	19·826	0·6265	0·3310	0·0460

Table II.—*Formaldehyde.*

Substance taken	$KMnO_4$ added	0·1 *M* HCOONa used for excess $KMnO_4$	Excess $KMnO_4$	$KMnO_4$ used for the substance	Substance found
(gms.)	(gms.)	(c.c.)	(gms.)	(gms.)	(gms.)
0·0543	1·5326	12·820	0·4051	1·1275	0·0535
0·0495	1·5326	15·777	0·4985	1·0341	0·0491
0·0425	1·5326	21·841	0·6901	0·8425	0·0399
0·0354	1·2772	16·972	0·5363	0·7409	0·0351
0·0307	1·3762	23·074	0·7291	0·6471	0·0307

TABLE III.—*Benzaldehyde.*

Substance taken	$KMnO_4$ added	0·1 *M* HCOONa used for excess $KMnO_4$	Excess $KMnO_4$	$KMnO_4$ used for the substance	Substance found
(gms.)	(gms.)	(c.c.)	(gms.)	(gms.)	(gms.)
0·0720	0·6440	13·541	0·4278	0·2162	0·0725
0·0450	0·6440	16·071	0·5078	0·1362	0·0456
0·0360	0·3220	6·758	0·2135	0·1085	0·0363
0·0333	0·4830	11·958	0·3778	0·1054	0·0352
0·0270	0·4830	12·629	0·3990	0·0840	0·0281

TABLE IV.—*Mandelic Acid.*

Substance taken	$KMnO_4$ added	0·1 *M* HCOONa used for excess $KMnO_4$	Excess $KMnO_4$	$KMnO_4$ used for the substance	Substance found
(gms.)	(gms.)	(c.c.)	(gms.)	(gms.)	(gms.)
0·2074	1·7242	26·960	0·8519	0·8723	0·2097
0·1853	1·4688	22·072	0·6974	0·7714	0·1855
0·1152	0·9579	14·980	0·4738	0·4846	0·1165
0·0768	0·6386	10·130	0·3201	0·3185	0·0765
0·0614	0·5747	10·063	0·3179	0·2568	0·0617

TABLE V.—*Cinnamic Acid.*

Substance taken	$KMnO_4$ added	0·1 *M* HCOONa used for excess $KMnO_4$	Excess $KMnO_4$	$KMnO_4$ used for the substance	Substance found
(gms.)	(gms.)	(c.c.)	(gms.)	(gms.)	(gms.)
0·0551	0·8985	9·912	0·3132	0·5853	0·0547
0·0493	1·0535	16·791	0·5305	0·5230	0·0489
0·0435	0·9295	15·181	0·4797	0·4498	0·0421
0·0377	0·8056	12·769	0·4035	0·4021	0·0376
0·0290	0·5267	6·677	0·2109	0·3158	0·0295

TABLE VI.—*Tartaric Acid.*

Substance taken	$KMnO_4$ added	0·1 *M* HCOONa used for excess $KMnO_4$	Excess $KMnO_4$	$KMnO_4$ used for the substance	Substance found
(gms.)	(gms.)	(c.c.)	(gms.)	(gms.)	(gms.)
0·1092	1·4556	20·538	0·6490	0·8066	0·1071
0·0840	0·9291	9·550	0·3017	0·6274	0·0833
0·0714	1·0530	16·077	0·5080	0·5450	0·0724
0·0504	0·7433	11·337	0·3582	0·3851	0·0511
0·0252	0·3724	6·020	0·1902	0·1822	0·0242

TABLE VII.—*Malic Acid.*

Substance taken	$KMnO_4$ added	0·1 *M* HCOONa used for excess $KMnO_4$	Excess $KMnO_4$	$KMnO_4$ used for the substance	Substance found
(gms.)	(gms.)	(c.c.)	(gms.)	(gms.)	(gms.)
0·0233	0·6515	10·350	0·3244	0·3271	0·0231
0·0350	0·9774	15·487	0·4893	0·4881	0·0344
0·0466	1·3032	20·667	0·6530	0·6502	0·0459
0·0583	1·6290	25·550	0·8236	0·8054	0·0568
0·0700	1·9548	30·777	0·9725	0·9823	0·0693

TABLE VIII.—*Isoamyl Alcohol.*

Substance taken	$KMnO_4$ added	0·1 *M* HCOONa used for excess $KMnO_4$	Excess $KMnO_4$	$KMnO_4$ used for the substance	Substance found
(gms.)	(gms.)	(c.c.)	(gms.)	(gms.)	(gms.)
0·1507	1·6800	19·050	0·6020	1·0780	0·1500
0·1292	1·3440	12·873	0·4067	0·9373	0·1304
0·0818	1·0752	15·368	0·4856	0·5896	0·0820
0·0606	1·0080	18·072	0·5710	0·4370	0·0608
0·0430	0·6720	11·373	0·3593	0·3127	0·0435

TABLE IX.—*Benzyl Alcohol.*

Substance taken	$KMnO_4$ added	0·1 *M* HCOONa used for excess $KMnO_4$	Excess $KMnO_4$	$KMnO_4$ used for the substance	Substance found
(gms.)	(gms.)	(c.c.)	(gms.)	(gms.)	(gms.)
0·1495	1·5456	29·483	0·9316	0·6140	0·1048
0·1300	1·3440	19·139	0·6047	0·7393	0·1262
0·1170	0·9408	13·836	0·4372	0·5036	0·0860
0·0845	0·8736	13·250	0·3871	0·4865	0·0830
0·0650	0·6720	9·531	0·3011	0·3709	0·0633

TABLE X.—*Ethyl Alcohol.*

Substance taken	$KMnO_4$ added	0·1 *M* HCOONa used for excess $KMnO_4$	Excess $KMnO_4$	$KMnO_4$ used for the substance	Substance found
(gms.)	(gms.)	(c.c.)	(gms.)	(gms.)	(gms.)
0·0797	1·7556	21·40	0·6762	1·0794	0·0784
0·0458	1·2129	19·00	0·6004	0·6125	0·0445
0·0338	1·0853	19·95	0·6304	0·4549	0·0331
0·0374	0·8299	9·65	0·3049	0·5250	0·0381
0·0199	0·7022	13·70	0·4329	0·2693	0·0196

TABLE XI.—n-*Propyl Alcohol.*

Substance taken	$KMnO_4$ added	0·1 *M* HCOONa used for excess $KMnO_4$	Excess $KMnO_4$	$KMnO_4$ used for the substance	Substance found
(gms.)	(gms.)	(c.c.)	(gms.)	(gms.)	(gms.)
0·0873	1·2768	10·95	0·3460	0·9308	0·0883
0·0682	1·2768	17·60	0·5530	0·7238	0·0683
0·0382	0·8937	15·45	0·4882	0·4055	0·0384
0·0273	0·6384	11·00	0·3476	0·2908	0·0275
0·0220	0·5107	8·80	0·2781	0·2326	0·0220

TABLE XII.—n-*Butyl Alcohol.*

Substance taken	$KMnO_4$ added	0·1 *M* HCOONa used for excess $KMnO_4$	Excess $KMnO_4$	$KMnO_4$ used for the substance	Substance found
(gms.)	(gms.)	(c.c.)	(gms.)	(gms.)	(gms.)
0·0945	1·6100	24·90	0·7868	0·8232	0·0963
0·0718	1·2800	20·85	0·6588	0·6212	0·0726
0·0604	1·2558	22·75	0·7189	0·5369	0·0628
0·0491	0·9660	17·10	0·5403	0·4257	0·0498
0·0302	0·6440	11·90	0·3760	0·2680	0·0313

From the above results, it is evident that Acetaldehyde, Formaldehyde, Benzaldehyde, Mandelic acid, Cinnamic acid, Tartaric acid, Malic acid, Isoamyl alcohol, Benzyl alcohol, Ethyl alcohol, *n*-Propyl alcohol and *n*-Butyl alcohol can be determined potentiometrically by using alkaline potassium permanganate as an oxidizing agent.

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN

OF THE

EAST PANJAB UNIVERSITY

No. 34 CHEMISTRY Pp. 107-115

October, 1953

CATIONOTROPIC REACTIONS WITH ETHYLENIC CARBONYL COMPOUNDS

I. Convenient synthesis of some methoxy Naphthalene Derivatives

by

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Edited for the East Panjab University by Vishwa Nath,
Department of Zoology, East Panjab University,
HOSHIARPUR

Price Rs.1-2-0

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I. Convenient synthesis of some methoxy Naphthalene Derivatives

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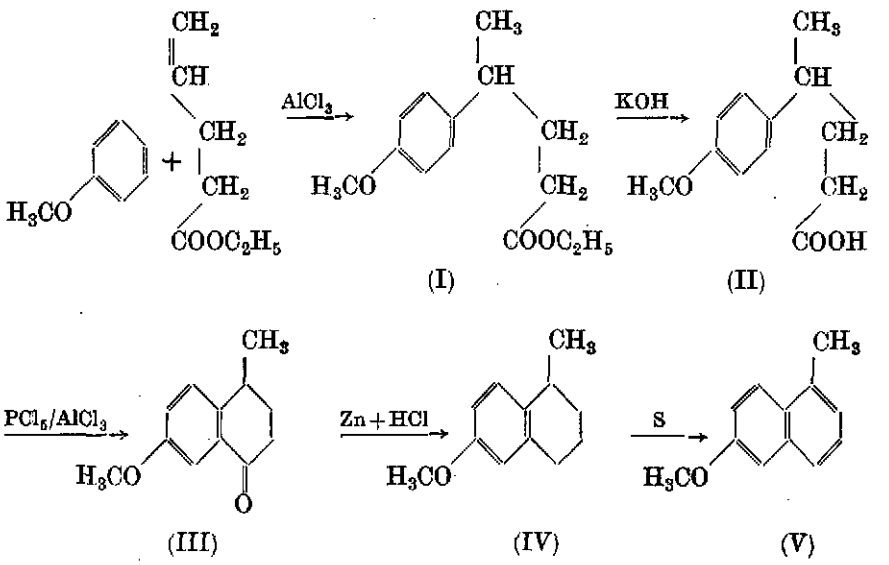
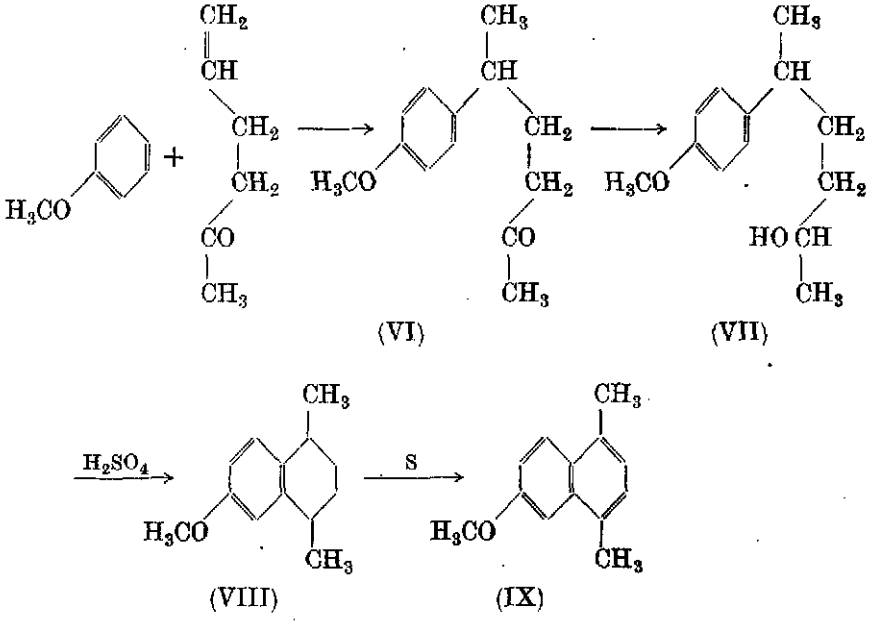
The method developed by Mukherji and co-workers (1, 4, 5, 9–12, 14) was extended to a study of the behaviour of anisol when subjected to aluminium chloride-catalysed reaction at 0–5° with ethyl allylacetate and allylacetone. Friedel-Crafts' alkylation of anisol is rather well known (13) and the para-oriented products are the major components, although in the case of condensation of anisol with cyclo-hexene (2), the ortho-isomer was reported to be greater in proportion. However, when anisol was subjected to aluminium chloride-catalysed reaction at 0–5° with ethyl allylacetate, 4-(*p*-methoxyphenyl)-valerate (I) was obtained. This ester (I) on hydrolysis gave a quantitative yield of the corresponding acid (II). That the product (I) was para-orientated was proved by alkaline potassium permanganate oxidation of the acid (II) to *p*-anisic acid. Furthermore, the acid (II) was cyclized by Johnson's inverse cyclization process (8) to furnish the ketone (*III*) which was smoothly reduced by Clemmensen method (Martin's modification) to the tetralin derivative (IV) followed by dehydrogenation with sulphur to give 1-methyl-6-methoxy-naphthalene (V).



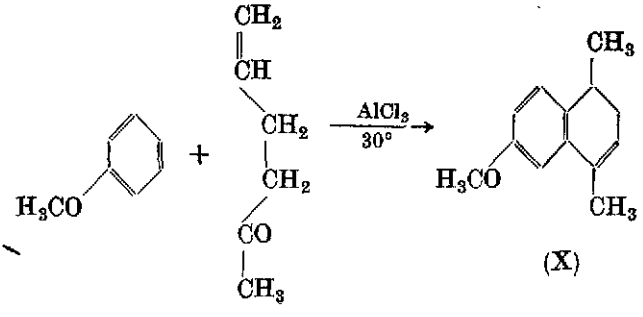
As the yield in the Friedel-Crafts' condensation step was not satisfactory, attempts were made to alkylate anisol under more vigorous temperature

conditions with a view to improving the yield, but extensive demethylation was observed (15).

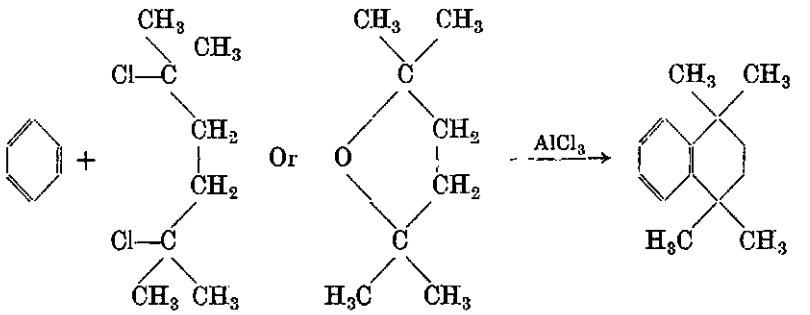
Similarly, anisol was condensed with allylacetone under the comparable conditions when 5-(*p*-methoxyphenyl)-hexan-2-one (VI) was obtained. The orientation in this case was established by sodium hypoiodite oxidation of the ketonic product (VI) to give the same acid (II) as identified through the mixture melting point of their S-benzyl-iso-thiuronium salts. However, the ketone (VI) was reduced with sodium and moist ether to the corresponding carbinol (VII) which was cyclized to the tetralin derivative (VIII) by means of concentrated sulphuric acid. The tetralin derivative was then dehydrogenated to afford a good yield of 1 : 4-dimethyl-6-methoxy-naphthalene (IX).



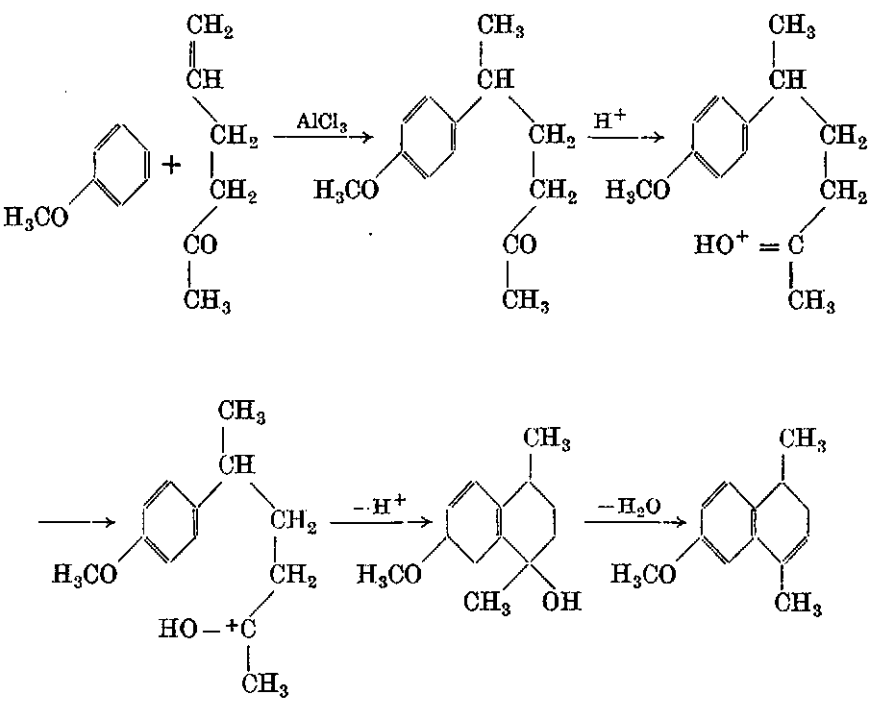
In one of our experiments with anisol and allylacetone, the temperature of the reaction mixture was not controlled so that the temperature rose as high as 30°. It was found that the product from this lot failed to give the semicarbazone. Examination of this product revealed that the initial Friedel-Crafts' reaction at the double bond of allylacetone was accompanied in situ by cyclodehydration to give the dihydro derivative (X).



This was confirmed by dehydrogenation of the product (X) by sulphur to give 1 : 4-dimethyl-6-methoxy-naphthalene. The optimum condition found suitable for the one step synthesis of (X) is described in the experimental section. Although it appears to be very similar with the process of 'cyclialkylation' (3) as under:



our observation may well be explained on the basis of initial Friedel-Crafts' addition of the *p*-methoxyphenyl to the double bond followed by cyclodehydration (6) according to the following scheme:



This observation seems to offer possibility for the development of a still simpler method for the synthesis of naphthalene derivatives in two steps. This possibility encouraged us to extend this method to benzene, toluene, *m*-xylene and naphthalene, which would form the basis of future communications.

Experimental

Melting points and boiling points are uncorrected.
Micro-analyses by Drs. Weiler and Strauss, Oxford.

Ethyl-4-(p-methoxyphenyl)-valerate (I):

Freshly distilled anisol (75 c.c.) and anhydrous aluminium chloride (10 g.) out of a total of 30 g. were taken in a three necked flask (500 c.c.) fitted with a (i) mercury sealed mechanical stirrer, (ii) dropping funnel, and (iii) guard tube and a thermometer. The flask was placed in an ice-bath and the stirrer was started. When the temperature of the resulting mixture came down to 5°, the dropwise addition of ethyl allylacetate was commenced. When half (7 g.) of the total amount was added, its further addition was stopped. About 2-3 g. of anhydrous aluminium chloride was added, followed by the dropwise addition of the remaining ethyl allylacetate (8 g.). The remaining aluminium chloride was then added in small instalments, maintaining the temperature between 5–10° throughout the addition. The addition was complete in an hour and the stirring was continued for another two hours. The reaction mixture was then decomposed by pouring it into iced hydrochloric acid and extracted four times with ether. The ethereal extract was washed once with water, twice with 5% sodium bicarbonate solution and again with water till neutral to litmus. After drying over anhydrous sodium sulphate and removing ether, the liquid was distilled under reduced pressure to give 18 g. (65·2%) of ethyl-4-(*p*-methoxyphenyl)-valerate, b.p. 152–154°/10 mm.

4-(p-methoxyphenyl)-valeric acid (II):

Hydrolysis of the ester (I; 13 g.) was effected by refluxing it with alcoholic potassium hydroxide (potassium hydroxide 7·5 g.; distilled water 5 c.c.; rectified spirit 165 c.c.) for fourteen hours on the water-bath. The alcohol was partly removed and the remaining liquid diluted sufficiently with distilled water. It was extracted twice with ether to remove any unchanged ester (I) and then acidified with dilute sulphuric acid. The liberated oil was taken up in ether. The ethereal extract was washed twice with water and dried over anhydrous sodium sulphate. The solvent being removed, the residue was distilled under reduced pressure, when 10 g. (87%) of 4-(*p*-methoxyphenyl)-valeric acid was obtained as a colourless oil, b.p. 171–173°/6 mm.

S-benzyl-iso-thiuronium derivative:

The acid (II; 1 c.c.) was taken in 10 c.c. of distilled water and two drops of phenolphthalein were added, followed by the addition of a few drops of sodium hydroxide solution. The solution was neutralized with hydrochloric acid indicated by the disappearance of the pink colour. It was warmed and mixed with the reagent prepared by dissolving 2 g. of the same in 10 c.c. of distilled water. Immediate separation of the acid derivative was observed. The acid derivative was crystallized from 50% ethyl alcohol, m.p. 125-126°.

Analysis : Calculated for $C_{20}H_{26}O_3N_2S$: N, 7·48; S, 8·55; C, 64·17; H, 6.95
Found: N, 7·70; S, 8·131; C, 63·94; H, 6·75.

4-methyl-7-methoxy-1-tetralone (III):

Phosphorus pentachloride (11 g.; 1·1 mol) was placed in a 100 c.c. distillation flask fitted with a guard tube on its side arm and covered with 25 c.c. thiophene-free dry benzene. The acid (II) (10 g.; 1 mol) dissolved in dry thiophene-free benzene (20 c.c.) was then added. The reaction mixture was shaken when a violent reaction set in. After the vigorous reaction had subsided, benzene and phosphorus oxychloride were removed under reduced pressure at a temperature not exceeding 90°. Anhydrous aluminium chloride (7·5 g.; 1·1 mol) was placed in a 250 c.c. conical flask carrying a calcium chloride guard tube and immediately covered with petroleum ether (80–100°; 40 c.c.) and the mixture cooled in an ice-salt bath at −10°. The acid chloride was taken up in 20 c.c. thiophene-free dry benzene and cooled in an ice-bath. The thoroughly cooled acid chloride solution was added all at once to the aluminium chloride-petroleum ether mixture and swirled. On warming to room temperature (25°), the reaction started with vigorous evolution of hydrochloric acid gas. When the vigour of the reaction had subsided, the mixture was just warmed on the water-bath at 50° and immediately taken out and the reaction allowed to proceed as such; this was repeated for an hour. The mixture was dark brown in colour. A jelly like complex was obtained which was decomposed by crushed ice and hydrochloric acid. The cyclized compound was extracted with benzene, the extract washed thrice with 5% sodium carbonate solution, then with water and dried over anhydrous sodium sulphate. The residual liquid, left after the removal of the solvent, was distilled under reduced pressure, when 4 g. (44%) of the ketone (III) was obtained, b.p. 102–105°/4 mm.

The semicarbazone derivative was prepared in the usual way and crystallized as colourless crystals from ethanol, m.p. 199-200°.

Analysis : Calculated for $C_{13}H_{17}O_2N_3$: N, 17·00
Found: N, 17·50

The 2 : 4-dinitro phenylhydrazone was prepared in the usual way and was crystallized as orange red needles from ethyl acetate, mp. 214-215°.

Analysis : Calculated for $C_{18}H_{18}O_5N_4$: N, 15·13
Found: N, 15·50.

1-Methyl-6-methoxy-1 : 2 : 3 : 4-tetrahydro-naphthalene (IV):

Preparation of amalgamated zinc: 17 g. of granulated zinc, 2 g. of mercuric chloride, 1 c.c. pure hydrochloric acid and 28 c.c. of distilled water were stirred for about five minutes in a beaker. The aqueous solution was decanted off and the amalgamated zinc washed with distilled water.

Amalgamated zinc (17 g.) was taken in 250 c.c. flask fitted with a reflux condenser. To this was added 3·5 g. of ketone (III), 30 c.c. toluene

20 c.c. water, 1 c.c. glacial acetic acid, 30 c.c. hydrochloric acid and the mixture refluxed in the oil-bath at 130–140° for thirty-six hours. After every six hours, 5 c.c. of pure hydrochloric acid was added. The reaction mixture was cooled and extracted with toluene; the toluene extract washed and dried over anhydrous calcium chloride. The solvent was removed and the residual liquid was distilled under reduced pressure, when 2·5 g. (78%) of (IV) was obtained as a clear oil, b.p. 120–122°/5 mm.

1-Methyl-6-methoxy-naphthalene (V):

The above product (2·5 g.) was heated with 1·1 g. of sulphur in a sulphuric acid bath for ten hours. The dehydrogenated product was then submitted to steam distillation. The distillate was taken up in ether and worked up in the usual way, when 1·6 g. (66·6%) of 1-methyl-6-methoxy-naphthalene was obtained, b.p. 135°/10 mm.

The picrate of (V) was prepared in the usual manner and crystallized as orange red needles from 80% ethyl alcohol, m.p. 144-145° (shrinkage at 136°).

Analysis: Calculated for $C_{12}H_{12}O$, $C_6H_3N_3O_7$: N, 10·47
Found: N, 10·30.

Oxidation of the acid (II) with alkaline permanganate:

The acid (II; 2·5 g.) was oxidized with alkaline potassium permanganate solution (prepared from 15 g. of potassium permanganate, 300 c.c. of distilled water and 2-3 pallets of sodium hydroxide) by refluxing for eight hours. The manganese dioxide precipitate was removed by filtration. The colourless filtrate was reduced to a small bulk by evaporation on the water-bath and then acidified with hydrochloric acid. *p*-Anisic acid was obtained as crystalline precipitate. It was recrystallized as colourless crystals from water, m.p. 182-183° decom. (lit. 7) m.p. 184° decomp.

5-(p-Methoxyphenyl)-hexan-2-one (VI):

A mixture of freshly distilled anisol (100 c.c.) and anhydrous aluminium chloride (12 g. out of total required quantity of 36 g.) was placed in a three necked flask (500 c.c.) carrying (i) a mercury sealed stirrer, (ii) a thermometer and a calcium chloride guard tube, and (iii) a dropping funnel. The mixture was stirred vigorously and cooled in ice-bath. As soon as the temperature of the reaction mixture came down to 2°, about 10 g. of allylacetone out of the total of 20 g. were added dropwise to the contents of the flask. A small amount of anhydrous aluminium chloride was then added, followed by the dropwise addition of the remaining 10 g. of allylacetone maintaining the temperature between 0–5°. The rest of aluminium chloride was added in portions under controlled conditions of temperature. The addition was completed in an hour and the stirring was continued for another two hours in the ice-bath. The reaction mixture was kept overnight in an ice chamber and then decomposed in the usual way (vide *supra*), when 13 g. (31%) of 5-(*p*-methoxyphenyl)-hexan-2-one was obtained, b.p. 136–140°/7 mm.

The semicarbazone derivative was prepared in the usual way and crystallized from dilute ethanol, m.p. 151–152°.

Analysis: Calculated for $C_{14}H_{21}N_3O_2$: C, 63·87; H, 7·98; N, 15·96.
Found: C, 63·32; H, 8·30; N, 15·72.

5-(p-Methoxyphenyl)-hexan-2-ol (VII):

The ketone (VI; 6 g.), 100 c.c. of ether and 5 c.c. of water were taken in a flask fitted with a condenser. Metallic sodium (5·5 g.; 8 atoms) was cut into pieces and slowly added to the ketone. Whenever the reaction with sodium subsided, 1-2 c.c. of water was added to facilitate the reaction. After the addition of the required amount of sodium, the product was taken up in ether, washed with water and dried over anhydrous sodium sulphate. After removing the ether, the viscous liquid was distilled under reduced pressure, when 4 g. (66·6%) of 5-(*p*-methoxyphenyl)-hexan-2-ol was obtained, b.p. 135-136°/4 mm.

1: 4-Dimethyl-6-methoxy-tetralin (VIII):

The above alcohol (4 g.) was placed in a conical flask (100 c.c.) fitted with a guard tube and cooled in ice. Sulphuric acid (8 g.; 1·84 d) was added gradually with constant shaking within fifteen minutes and shaking was continued in the bath for another thirty minutes. The contents were then allowed to attain the room temperature. The resulting mixture was decomposed by pouring into iced water and extracted with ether. The ethereal extract was washed with water, 5% sodium carbonate solution, again with water and dried over anhydrous sodium sulphate. After removing the solvent, the residue was distilled under reduced pressure, when 2·5 g. (69·5%) of the tetralin derivative (VIII) was obtained, b.p. 195–200°/12 mm.

1: 4-Dimethyl-6-methoxy-naphthalene (IX):

The product (VIII; 2 g.) was heated with 0·900 g. of sulphur in a sulphuric acid bath for ten hours at 180–190°. The dehydrogenated product was obtained by steam distillation. The yellow coloured oily drops in the distillate were taken up in ether and worked in the usual way, when 1: 4-dimethyl-6-methoxy-naphthalene, b.p. 156–158°/12 mm., was obtained in 52·6% yield.

The picrate of (IX) was prepared in the usual manner and crystallized from ethanol, m.p. 107-108°.

Analysis: Calculated for $C_{13}H_{14}O$, $C_6H_3N_3O_7$: C, 54·94; H, 4·09; N, 10·12
Found: C, 54·36; H, 4·22; N, 9·7.

Oxidation of 5-(p-methoxyphenyl)-hexan-2-one with sodium hypoiodite:

The ketone (VI; 3 g.) was dissolved in 60 c.c. of dioxan and 80 c.c. of 10% sodium hydroxide solution. The mixture was well stirred. Some iodine solution (prepared in the ratio of KI : I_2: H_2O: : 2: 1: 4) was added when a yellow colour developed. The addition of iodine solution was

continued till the dark red colour of iodine persisted in the cold as well as on warming the mixture on the water-bath (60°) for 2-3 minutes (about 120 c.c. of iodine solution was required). The excess of iodine was removed by adding a few drops of dilute sodium hydroxide solution. The reaction mixture was allowed to stand when iodoform, formed during the oxidation, separated at the bottom. The aqueous layer was decanted off and it was extracted with ether to remove any unchanged ketone (VI). The ethereal extract was rejected. The aqueous layer was acidified with dilute sulphuric acid and the liberated iodine was discharged with sodium bisulphite. The oily product was taken up in ether, washed and dried over anhydrous sodium sulphate. The liquid, left after the removal of ether, was distilled in vacuum, when 4-(*p*-methoxyphenyl)-valeric acid (II) was obtained, b.p. 178°/7 mm.; yield: 1·65 g. (55%).

The S-benzyl-iso-thiuronium derivative was prepared in the usual way and crystallized from dilute ethyl alcohol, m.p. 124–125°, undepressed when mixed with the sample previously prepared.

Friedel-Crafts' Reaction at Higher Temperature:

Between anisol and allylacetone:

1: 4-Dimethyl-6-methoxy-1 : 2-dihydro-naphthalene (X):

Freshly distilled anisol (50 c.c.) was placed in a three necked flask having (i) a mercury sealed stirrer (ii) a dropping funnel, (iii) a thermometer and a calcium chloride guard tube. 6 g. of anhydrous aluminium chloride was added to anisol and the contents of the flask were cooled in an ice-bath. The stirrer was started and, as soon as the reaction mixture attained the desired temperature (5°), dropwise addition of allylacetone was commenced. When about 5 g. of allylacetone out of the total quantity of 10 g. was added, its further addition was stopped. About 3 g. of aluminium chloride was added, followed by the dropwise addition of the remaining 5 g. of allylacetone. Then the remaining aluminium chloride was added in five equal instalments, maintaining the temperature between 5–10° (forty-five minutes). The ice-bath was removed and the stirring was continued for another one and a half hours at room temperature (25°). The reaction mixture was then heated on the water-bath at 50° for thirty minutes without stirring and then kept overnight at room temperature. It was then decomposed and worked up in the usual way, when the product (X) was obtained in 52·3% yield, b.p. 194–198°/12 mm.

1: 4-Dimethyl-6-methoxy-naphthalene:

The product (X; 5 g.) was dehydrogenated with sulphur (1·04 g.) and the dehydrogenated product was worked up in the usual manner already described, when 2·5 g. (51·1%) of 1: 4-dimethyl-6-methoxy-naphthalene, b.p. 156–160°/12 mm., was obtained.

The picrate was prepared in the usual manner and crystallized from 80% ethanol, m.p. 107°, undepressed when mixed with the previous sample of the product (IX).

Summary

The aluminium chloride-catalysed reactions between anisol and ethyl allylacetate, and allylacetone have been studied leading to convenient syntheses of some naphthalene derivatives such as 1-methyl- and 1, 4-dimethyl-6-methoxy-naphthalene. Preliminary observations on one-step Friedel-Crafts' condensation accompanied by cyclodehydration giving rise to dihydronaphthalene derivatives have also been recorded.

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Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN

OF THE

EAST PANJAB UNIVERSITY

No. 35 ZOOLOGY Pp. 117–138

October, 1953

STUDIES ON THE PROTOZOA OF INDIAN FISHES

I. The Morphology and the Systematic Position of the Ciliates inhabiting the Intestine of Fish *Mystus cavasius* (Hamilton)

by

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Edited for the East Panjab University by Vishwa Nath,
Department of Zoology, East Panjab University,
HOSHIARPUR

Price Rs.3-4-0

STUDIES ON THE PROTOZOA OF INDIAN FISHES

I. The Morphology and the Systematic Position of the Ciliates inhabiting the Intestine of Fish *Mystus cavasius* (Hamilton).

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Preface

Investigation on the Protozoa of Indian Fishes was carried out by the author at the suggestion and under the supervision of the undersigned. The author submitted a thesis in 1951 on the 'Studies of the Protozoa of Indian Fishes' for the award of the M.Sc. degree (Honours School) of the Panjab University, which was assessed by Dr. H. N. Ray, Officer-in-charge, Section of Parasitology, Indian Veterinary Research Institute, Mukteswar. Dr. Ray also conducted the viva voce, and subsequently helped the author in the preparation of the manuscript for publication.

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and
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Dated 24-4-1953.

1. Introduction

Work on the Ciliates from the gut of fish is very scanty, though quite a lot of work has been done on Myxosporidia from this host. Schouten (1940) described *Nyctotherus mackinoni* from the faeces of a fish—*Pimelodus clarias*; while some have worked on the ectoparasitic *Trichodina* of fishes. With a view to make a survey of the protozoal fauna of the food fishes of the State of the Panjab, a start was made by examining the intestinal contents of the available local fishes at Ludhiana. During the course of these studies two new Holotrichous Ciliates were encountered in the gut of *Mystus cavasius*; and the object of this paper is to describe in detail the morphology of these new Ciliates.

It will appear from the description, given below, that a new family *Nathelliidae* [1], with the new genus *Nathella*, had to be created to embrace the two new species, viz., *N. rayii* [2], and *N. Kumarii* [3].

2. Material and Methods

The greatest difficulty was encountered in procuring the material for study. I had been examining the intestine of the common mud-fish, *Ophiocephalus punctatus*, at Hoshiarpur, for about six months (from November, 1949 to March, 1950), but none was found to be infected. In the month of April *O. punctatus*, from Ludhiana ponds, were also examined with negative results. From a village Jassian (Ludhiana) pond I got a specimen of *Mystus cavasius* (Hamilton), vernacular, *Tengra* (Plate 1, Fig. 1), which was found to be infected with Ciliates. Being encouraged by this finding, attention was then solely directed towards this fish, for procuring the required material for study.

From time to time this particular fish was collected and the Ciliates found in its intestine were examined both in their living condition as well as in fixed and stained preparations. In order to study the morphology in detail, the infected portions of the intestine were fixed in Bouin's fluid for 12 hours, Flemming's without acetic acid or Champy's fluid for 24 hours in each, and then cut into thin sections (3–5 μ thick).

It was observed that the infected fish used to die within a period of a few hours when brought out from its normal environments. Various methods were adopted to carry the living fish from Ludhiana to Hoshiarpur, but only the infected ones used to die on the way, while the uninfected ones could survive the journey and remain alive for months together. This is highly suggestive of this Ciliate playing a pathogenic rôle, although confirmatory experimental proof is still lacking. Most of the Ciliates were found to be moving freely in the lumen of the intestine, while a few were seen in close contact with the intestinal epithelium. The presence of trichocysts, however, indicates that this Ciliate is non-parasitic in habit. It will, therefore, be of great interest to study the relationship of this Ciliate to its host.

Infected fish were found to be very rare. As the Ciliates could be seen with the naked eye, instead of carrying them to the laboratory, I could dissect the fish at the source (*Budha Nala*) and keep the infected intestine in normal saline. The stomach proved to be uninfected. Behind the stomach, a little portion of the intestine, i.e., about one-fourth of the entire intestine, was found to be rarely infected. After this region the infection

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[1] The family is named after Prof. Vishwa Nath, under whose supervision the entire work was planned and carried out in the Zoology Department, Panjab University, at Hoshiarpur.

[2] This new species is named after Dr. H. N. Ray, the Protozoologist, Indian Veterinary Research Institute, Mukteswar, Kumaon (U.P.).

[3] This new species is named after Dewan Anand Kumar, Vice-Chancellor of the Panjab University.

increased proportionately towards the posterior region. The uninfected intestine was found to be very narrow and contained very little food material, but the intestine of the infected ones was invariably very highly distended and filled with food material, consisting of Gastropods, insects, Crustaceans and other aquatic organisms. In a collection, females outnumbered the males and it was observed that the infection was mostly confined to them. From the observations so far made it cannot, however, be definitely asserted that the Ciliates have any particular preference for the sex of the host.

It was very interesting to observe that other carnivorous fishes, living side by side with *Mystus cavasius*—a purely carnivorous fish—were found to be entirely free from this Ciliate, although trematodes, nematodes and tape-worms were found to infect them. The Jassian pond, which is about three miles from Budha Nala, contained many species of carnivorous fishes, but none of them except *M. cavasius* was infected with these Ciliates.

Why these Ciliates prefer the gut of *M. cavasius* and not the other carnivorous fishes, living in the same environment, is a problem which requires elucidation.

Other carnivorous fishes that were found negative for Ciliates are:—

1. *Wallagonia attu* (Block & Schneider) .. Mullee.
2. *Mastacembelus armatus* (Lacepede) .. Bam or Groj or Samp machli.
3. *Ophiocephalus marulius* (Hamilton) .. Saul.
4. *Nandus nandus* (Hamilton) .. Khotah or Gadha.
5. *Colisa fasciata* (Block & Schneider) .. Chidu or Kangee.
6. *Colisa lalius* (Hamilton) .. .. Chhoti Kangee.
7. *Callichrous bimaculatus* (Block) .. Pallu or Pafta.

Their food habit was apparently the same as that of *M. cavasius*. Even the other species of *Mystus* (*M. vittatus* (Block), *Kengar*) and (*M. aor* (Hamilton) *Shinghari*), were found to be uninfected. More than 20 specimens of each of these fishes were examined from different localities but none of them showed any infection.

Fixatives Used:—

Both histological and cytological fixatives were used in these studies.

1. *Osmic acid Preparation.*—The Ciliates were uniformly spread on a cover glass that was previously besmeared with a thin layer of Mayer's egg-albumen. This cover glass was then placed on the mouth of the bottle which contained 2 per cent osmic acid. The osmic vapour was allowed to act for a varying period (from 5 seconds to 10 minutes) followed by washing in 50 per cent alcohol and dehydration (one minute in each dehydrating alcohol) and then mounted in Canada Balsam. Films fixed with osmic vapours were also stained with Giemsa's stain or Heidenhain's iron haematoxylin, or crystal violet. For the latter, the fixed material was kept in 0·005 per cent crystal violet for 18–24 hours, at a temperature of 22°–24°C. Differentiation was carried out with clove oil, under the microscope, and subsequently mounted in Canada balsam after washing in xylol.

2. *Bouin's Fluid.*—The material was fixed in Bouin's fluid from 10 minutes to 2 hours. This fixative produces shrinkage. Its action on the lipoidal material was also observed which was dissolved by the fluid. Portions of the infected intestine were also fixed in Bouin's fluid.

Smear preparations as well as the sections were stained with Delafield's haematoxylin, Ehrlich's haematoxylin, Haedenhain's iron-haematoxylin and Mallory's triple stain.

3. *Kolatchev's Method.*—The material was fixed in Champy's fluid for 24 hours and washed in running tap-water for about the same period. Hot fixative was found to be effective in sticking the animalcules to the cover-slip. The material was then placed in 2 per cent osmic acid at a temperature of 30°–37°C., in the dark, for 3–6 days. Washing was again done for some hours, prior to dehydration, and subsequent mounting.

4. *Da Fano's cobalt nitrate modification.*—The material was fixed for 2 hours in the fixative containing 100 c.c. of a 1 per cent cobalt nitrate solution in distilled water and 15 c.c. of formalin. After fixation, it was washed quickly in distilled water and placed in one per cent silver nitrate solution in the dark for 12–48 hours. To remove the excess of silver, the material was given a quick wash in distilled water. For the reduction of silver nitrate, Cajal's hydroquinone formalin (reducing)[1] mixture was used for 12–48 hours.

5. *Flemming's fluid without acetic acid (F.W.A.).*—Fixation was done in F.W.A. for 24 hours. Time for washing the smears was reduced to 2–4 hours, but for sections the material was washed for 24 hours.

6. *Sudan IV Technique.*—The material was fixed in 4 per cent formalin for 24 hours; washed in distilled water for 5 minutes. The specimens were then transferred, fairly quickly, from 30 per cent, 50 per cent, 70 per cent alcohols to Sudan IV solution for 7–10 minutes. This solution was freshly made in 70 per cent alcohol to saturation. After keeping the material in the stain for 7–10 minutes, the smears were passed through 50 per cent, 30 per cent alcohols to distilled water and mounted in glycerine, the cover-glass being sealed with paraffin wax or gold-size.

7. *Schaudinn's sublimate alcohol.*—Fixation was carried out from 10 minutes to 1 hour. This is the best fixative for the Ciliates. Almost every stain was tried on the organisms after fixation in this fluid.

8. *Zenker's acetic fluid.*—Fixation was carried out from 4–6 hours and then the material was washed in tap-water for 24 hours.

9. *Carnoy's fixative.*—Films were fixed from 10–30 minutes. Washing was done in rectified (several changes) from 6–12 hours.

10. *Concentrated solution of hot corrosive sublimate.*—A drop containing the organisms was spread over a cover-slip and before the preparation was completely dried, the cover-slip was inverted over hot corrosive sublimate solution. The animalcules could, by this way, be made to stick to the

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[1] Hydroquinone .. .. 1 gram.
Distilled water .. .. 85 c.c.
Formaldehyde (neutralized) 40 per cent .. .. 15 c.c.
Sodium sulphite (anhydrous) .. .. .. 0·15 gram.

cover-glass very firmly. No Mayer's egg-albumen was used for making the smear.

11. *Iodine vapour.*—A few crystals of iodine were put in a test tube. The Ciliates were spread on a slide in a drop of water. The test tube, with iodine crystals, was heated and the brownish green vapour that came out was allowed to pass over the Ciliates on the slide for a few minutes. A drop of water was added and the slide was warmed a little to remove the excess of iodine.

12. *Klein's dry silver method.*—The smear on the cover-slip was dried and then put in 2 per cent silver nitrate solution, in the dark, for 15–30 minutes. It was rinsed in distilled water and exposed to diffuse sunlight, from time to time, watching under the microscope till the ciliary lines were clearly visible. The cover-slip was then dipped in a very weak solution of hyposulphite of soda (one or two crystals in 100 c.c. of distilled water) for a very short period (one or two dips). The smear was finally dried in air and mounted in Canada balsam.

Stains used:—

The following stains were used:—

1. *Borax carmine.*—Half an hour was sufficient for the stain to penetrate. Differentiation was done in acid alcohol.

2. *Delafield's haematoxylin and Ehrlich's haematoxylin.*—These were used to stain the individual Ciliates in smears and in mass. Sections were also stained with these stains. Differentiation was carried out with 1 per cent hydrochloric acid in 70 per cent alcohol. The material was then counterstained with Eosin or Chromotrop 2 R.

3. *Heidenhain's iron haematoxylin.*—The material fixed in Schaudinn's, Bouin's, F.W.A., Zenker's or Carnoy's, was stained with iron haematoxylin. After mordanting with 4 per cent aqueous solution of iron alum for 6 hours, and, washing in distilled water, the specimens in smears or in sections were placed overnight in 0·5 per cent solution of haematoxylin in distilled water. Differentiation was done with 1 per cent iron alum. Rapid method of staining was also tried in the case of sections. Counterstaining was done with water soluble Eosin or in saturated solution of Chromotrop 2 R in absolute alcohol.

4. *Dobell's iron haematein.*—After fixation in Bouin's or Schaudinn's the material was taken to 70 per cent alcohol and then transferred to 1 per cent solution of iron alum in 70 per cent alcohol for ten minutes (the solution was made by dissolving 1 gram of iron alum in 23 c.c. of warm distilled water and adding 77 c.c. of 90 per cent alcohol). After mordanting, the material was rinsed in 70 per cent alcohol. Preparations were subsequently stained in 1 per cent solution of haematein in 70 per cent alcohol for 2–5 minutes. After rinsing the material in 70 per cent alcohol, differentiation was carried out in the original iron alum solution.

5. *Haemalum and picro-carmine.*—Haemalum solution was diluted with distilled water and the specimens, after fixation in Schaudinn's were kept in it for about five minutes and then taken to picro-carmine solution for 10–15 minutes.

6. *Mallory's triple stain.*—The smears, as well as sections, were stained, after fixation in Bouin's or Schaudinn's fluid in 0·5 per cent aqueous solution of acid Fuchsin in distilled water for 5–10 minutes and then transferred to the second solution for 4–6 minutes. Differentiation was carried out in tap-water.

7. *Bresslau's method of staining with Opal blue.*—To a drop of water containing a large number of organisms a drop of colloidal aniline blue stain (soluble in absolute alcohol) was mixed. The film was dried rapidly by swinging in the air and then mounted in Canada balsam.

8. *Giemsa's stain.*—The specimens were stained for one hour in stock solution. Red and Blue stains were controlled by 0·02 per cent NaOH and 0·1 per cent acetic acid solution respectively.

In order to study the morphology in details, the infected portions of the intestine were fixed in Bouin's fluid and F.W.A. and then 3 μ to 5 μ thick sections were cut.

Photomicrographs were taken with a special Spencer's vertical camera, the source of illumination being an incandescent lamp specially meant for this purpose.

3. (*a*) Observations on Living Ciliates

Infected fish, though rare, gave an abundant supply of Ciliates for making various observations. Observations on the living organisms were made in a 0·75 per cent sodium chloride solution in distilled water, under a cover-slip, usually in a small cavity slide. The shape of the organism remains like that of a broad cigar with the anterior end more tapering than the posterior.

In the freshly cut fish, the Ciliates could be seen with the naked eye as white specks even through the intestinal wall.

The organisms move about very actively among the slimy contents of the gut. The active movement, coupled with the thickness of the body, renders it difficult to observe the Ciliates in their living condition.

When the movements of the Ciliates were slowed with the help of a solution of Ispaghul seeds, the movement of the cilia could be seen very clearly. In free movement the Ciliate used to move forward along the longitudinal axis of the body, as other Ciliates, with body cilia moving in the clockwise direction. The Ciliates never used to turn by the side, but they used to change their position dorsoventrally. When the movements were very brisk, they even used to move in a revolving manner round their own longitudinal axis. When the movements slowed down a bit, they exhibited a longitudinal groove along the surface, called the dorsal surface of the Ciliates. The pellicle, at the anterior end, is thick and resembles a shield which is characteristic of these Ciliates. The opening of the mouth is situated towards the posterior end.

At the anterior end, from where the groove starts, there is seen a type of circling ciliary movement and many minute particles can be seen moving in the clockwise direction in this place. This type of circular movement of the liquid was also seen at the end of the groove where the mouth is located.

In their active movements, they avoid solid particles, which come in their way, by squeezing the extremely pliable body. They can also easily squeeze through narrow spaces by changing the form of the body, becoming more or less elongated, and afterwards assuming the same normal shape. The progress is further facilitated by the rigid, pointed shield-like anterior end, which pushes the debris out of the way.

The movements of the undulating membrane in the groove are very peculiar. At intervals, the undulating membrane moves in a wavy manner antero-posteriorly.

(*b*) Observations on Ciliates in whole mounts and sections

The whole body is clothed with uniform, short and very fine cilia which are arranged in longitudinal rows. The ciliary lines are very close to each other (Plate 1, fig. 2, *Cl.*; Plate 2, fig. 6, *Cl.*; Plate 3, fig. 13, *Bc.*, fig. 17, *Cl.*; Plate 5, fig. 25, *Cl.*).

The peristomial groove starts from the anterior end, i.e., from the centre of the anterior shield-like portion. In *N. rayii* the groove in the beginning turns a little to the left side of the body, but later on it turns to the right and ends in the middle of the posterior region; while in *N. kumarii*, it keeps itself to the left hand side of the body and dips to the right (Plate 2, fig. 6, *Pgdv.*; Plate 3, fig. 15, *Pgdv.*; Plate 4, fig. 21, *Pgdv.*) and turns upwards on the ventral surface, ending almost in the centre of the posterior region (Plate 2, fig. 6, *Pgvs.*; Plate 3, fig. 15, *Pgvs.*; Plate 4, fig. 21, *Pgvs.*). The anterior one-fourth region in *N. rayii* and one-sixth in *N. kumarii* is a little bit dilated than the remaining portion, which is uniform throughout its length (Plate 1, fig. 2, *Pg.*; Plate 2, fig. 6, *Pg.*; Plate 4, fig. 19, *Pg.*; Plate 6, figs. 33, 34). The peristomial groove is about 3-4 μ deep, on the surface of the Ciliates, and disappears in 3-4 μ thick serial sections (Plate 3, fig. 17 (complete peristomial groove); Plate 4, fig. 18, *Pg.d*). The undulating membrane (Plate 2, fig. 8, *Um.*; Plate 3, figs. 14, 17, *Um.*; Plate 5, figs. 23, 25, *Um.*; Plate 6, figs. 35, 36) is seen arising from a closely set row of basal granules (Plate 2, fig. 8, *bgum.*; Plate 3, fig. 14, *bgum.*). On the outside, just besides these closely set row of basal granules, there is a row of peristomial fibres (Plate 3, figs. 13, 14, *Pf.*). On the opposite side of the undulating membrane, along the right border of the groove, there is a row of lamellae directed towards the anterior end (Plate 2, fig. 8, *L.*; Plate 3, figs. 13, 14, 17, *L.*; Plate 5, figs. 23, 24, 25, *L*). The outer ends of the lamellae are joined with one another by means of small septa (Plate 3, figs. 13, 14, *S*). These lamellae are thicker than the body cilia and the cilia present on the left side of the groove. The undulating membrane occupies about half the breadth of the groove. In front of the undulating membrane there is a row of small papillae forming a ridge (Plate 2, fig. 8, *R.*; Plate 3, figs. 13, 14, 17, *R.*; Plate 5, fig. 23, *R.*). Each papilla is like an inverted *U*, the function of which is almost the same as that of an undulating membrane. The arms of the papilla, which form inverted *U*-shaped structure, arise from the basal granules (Plate 2, fig. 10, *bgr.*; Plate 3, fig. 14, *bgr.*). The undulating membrane and the ridge stop short in front of the mouth.

The lamella on the right margin and cilia on the left continue. On the left outer side of the groove, there is a row of very long and fine cilia (longer than the body cilia) (Plate 2, fig. 8, *olc.*; Plate 3, figs. 13, 14, 17, *olc.*; Plate 5, fig. 23, *olc.*) arising from the basal granules situated close to the inwardly directed fine cilia. On the inner left hand side of the groove, there is a row of very fine cilia touching the papillae of the ridge (Plate 2, fig. 8, *ifc.*; Plate 3, figs. 13, 14, 17, *ifc.*; Plate 5, fig. 23, *ifc.*). The basal granules of these cilia are rod-shaped (Plate 2, fig. 10, *rsbg.*; Plate 3, fig. 14, *rsbg.*) and so closely arranged that they form the pseudolamellae. Amongst the longer outer cilia are attached a few crescent-shaped trichocysts provided with an opening (Plate 4, fig. 22, *otr.*), towards the anterior end of the ciliates.

At the posterior end of the groove is the mouth which is protected by a guard cirrus (Plate 5, fig. 24, *Cr.*). The mouth, then, opens into the body (Plate 5, fig. 26, *Mo.*) through the cytostomal groove (Plate 5, fig. 24, *Cg.*).

The anterior portion of the groove has got a 'comma'-shaped cirrus (Plate 4, figs. 19, 20, *Cr.*).

The macronucleus (Plate 1, fig. 2, *Mn.*; Plate 2, fig. 6, *Mn.* See Tables 1, 2, 3, 4) is seen in the living ciliate as a clear granular area usually near the middle of the body, but sometimes it may be placed anteriorly or posteriorly. The outline of the macronucleus is nearly always variable due to the food-vacuoles and lipoid inclusions pressing against it. In fixed and stained specimens, it is seen to be surrounded by a delicate thin membrane, which, in sections, becomes very obvious. Within this thin nuclear membrane, the granular nuclear material (Plate 5, fig. 28, *Gm.*) is closely packed. The macronuclear material is typically contracted away from the membrane in fixed individuals. The chromatin appears to be distributed in the form of numerous small granules. The size of the macronucleus ranged between 49·5μ–82·8μ in length and 19·8μ–41·4μ in breadth as determined by measuring 12 living specimens, the average being 65·7μ by 29·79μ; and 26·4μ–82·5μ in length and 13·2μ–33·0μ in breadth as determined by measuring 54 fixed specimens, the average was 53·07μ by 21·61μ in *N. rayii*. In *N. kumarii*, the size of the macronucleus ranged between 46·2μ–75·9μ in length, and 19·8μ–33·0μ in breadth as determined by measuring 10 living specimens, averaging 60·06μ by 27·29μ; and 36·3μ–82·5μ in length and 9·9μ–28·0μ in breadth as determined by measuring 42 fixed specimens, the average was 50·0μ by 21·19μ. The long axis of the macronucleus in relation to the long axis of the organism may be transverse, oblique or parallel.

The spherical micronucleus, which is very small, lies close to the macronucleus, frequently in a depression on the surface of the latter (Plate 1, fig. 2, *Mn.*; Plate 2, figs. 6, 11, *mn.*). It is heavily stained with Heidenhain's iron haematoxylin, following fixation in some strong fixative. In such preparations the chromatin mass is contracted away from the micronuclear membrane.

Micronucleus, as usual, takes a very active part in division. When it just starts to divide, the internal granular material contracts, and gets collected in the middle and then becomes lengthened (Plate 5, figs. 28, 29, 30). On division two micronuclei are formed which remain connected with each other by a very minute spindle (Plate 5, fig. 31, *Sp.*).

The following Tables I–IV will give an analysis of measurements, etc., of a number of individuals.

There is one contractile vacuole present in the posterior region of the body (Plate 1, fig. 2, *Cv.*; Plate 2, fig. 6, *Cv.*; Plate 4, fig. 21, *Cv.*; Plate 6, figs. 33, 34). The contractile vacuole situated in the posterior part of the body opens to the exterior by a small canal (Plate 1, figs. 4, 5). Sometimes there is one lateral contractile vacuole also. The portion of the body is a little bit pushed outside due to its opening. The size of the contractile vacuole is very small. In living individuals, the contractile vacuole is emptied at intervals of 5–10 minutes. After systole, several small vacuoles appear at the same spot which quickly coalesce to form a new contractile vacuole.

The contractile vacuole has got small auxiliary vacuoles (Plate 1, fig. 3, *aw.*), which open into the central bigger contractile vacuole (Plate 1, fig. 3, *oa.*). These auxiliary vacuoles, after pouring their contents into the main contractile vacuole, reappear again at the same place after sometime (Plate 1, fig. 3, *av*).

The body is clothed with longitudinal rows of short, fine and closely set uniform cilia. The cilia are seen to arise from the inner thinner layer and penetrate through the pellicle, which, in whole mounts, looks to be very thick, but in sections, basal granules are clearly visible, situated quite close to one another (Plate 2, fig. 8, *Bg.*; Plate 3, fig. 16, *Bg.*; Plate 4, figs. 18, 20, *Bg.*; Plate 6, fig. 37). The cilia vary from 1·6μ–4·9μ in length, averaging 4·3μ in *N. rayii*, and 3·3μ–6·6μ in length, averaging 4·75μ in *N. kumarii*. The cilia on the peristomial groove are quite different from the body cilia.

The trichocysts are arranged along the left hand border of the groove in between the longer cilia. Some of the trichocysts are scattered throughout the body (Plate 2, fig. 9, *Tr.*; Plate 3, figs. 18, 22, *Tr.*; Plate 5, fig. 27, *Tr.*), but most of them are found clustered near the anterior end (Plate 4, fig. 22). They are crescent shaped or appear as bent or curved needles with one end drawn out into a filament (Plate 2, fig. 7). In the section of the Ciliates, they are seen in different shapes and positions; some are straight while others are curved. The organism is provided with a power to use these trichocysts, whenever needed and so some of them are thrown out at one time into the surrounding medium. In the sections of the intestine, they were found to be scattered, in the disintegrated intestinal epithelium.

Myonemes are of two types:—(*a*) Longitudinal, (*b*) Circular.

Longitudinal myonemes, are placed slightly on one side of the anterior portion of the groove and run downwards in the cytoplasm. Their direction is more or less oblique (Plate 2, fig. 8, *Lm.*; Plate 4, figs. 19, 20, 22, *Lm.*). Anteriorly, at their origin, they occupy a larger space, but gradually they go on decreasing. Ray (1932) described in *Balantidium sushilii* three or four fibres on the left hand side of the peristome. Chakravarty (1935)

TABLE I.

Measurements and some other Observations on the Living Specimens of *Nathella rayii*.

Size in Microns.	Size of the macro-nucleus in microns.	Position of the macro-nucleus.	Shape of the macro-nucleus.	Length of the cilia in microns.	Length of the Peristomial groove in microns.	Situation of the macronucleus to the long axis of the body.
250·8 × 138·6	66·0 × 26·4	Anterior	Kidney-shaped	3·3	231·0	Parallel.
244·2 × 138·6	49·5 × 19·8	Central	do.	4·9	194·7	Longitudinal.
277·2 × 151·8	66·0 × 33·0	Central	do.	3·3	247·5	do.
231·0 × 165·0	82·5 × 33·0	Anterior	do.	4·9	221·0	Oblique.
280·5 × 184·8	82·6 × 41·4	Posterior	Mango-Shaped	4·9	264·0	do.
250·8 × 125·4	72·6 × 33·0	Central	Oval	3·3	214·5	Parallel.
287·1 × 141·9	52·8 × 33·0	Anterior	U-shaped	4·9	Not taken	do.
330·0 × 181·4	82·8 × 36·3	Anterior—a little above the centre	Almost flask-shaped	4·9	do	Oblique.

TABLE II.

Measurements and some other Observations on the Fixed Specimens of *Nathella rayii*.

Size in *microns*.	Size of the macro-*nucleus* in microns.	Position of the *macro*-nucleus.	Shape of the *macronucleus*.	Length of the cilia in microns.	Length of the Peristomial groove in microns.	Situation of the macronucleus to the *long* axis of the body.
244·2 × 178·2	52·8 × 19·8	Anterior	Hook-shaped	3·3	217·8	Transverse.
217·8 × 145·2	39·6 × 19·8	Central	More or less kidney-shaped	4·9	188·1	do.
204·6 × 148·5	47·8 × 18·1	Anterior	Elongately oval	3·3	161·7	Oblique.
231·0 × 138·6	59·4 × 19·8	Anterior	Saucer-shaped	3·3	198·0	Parallel.
260·7 × 151·8	66·0 × 26·4	do.	Kidney-shaped	1·6	231·0	Transverse.
313·5 × 189·7	62·7 × 19·8	Central	Flat	1·6	270·6	Parallel.
301·9 × 150·1	59·4 × 23·1	Anterior	Flat	1·6	273·9	Oblique.
141·9 × 89·1	36·3 × 13·2	do.	Oval	3·3	105·6	Transverse.
237·6 × 118·8	42·9 × 36·3	Central	Roundish	4·9	201·3	do.
303·6 × 151·8	56·1 × 33·0	Posterior	Egg-shaped	3·3	264·0	Parallel.

TABLE III.

Measurements and some other Observations on the Living Specimens of *Nathella kumarii*.

Size in microns.	Size of the macronucleus in microns.	Position of the macronucleus.	Shape of the macronucleus.	Length of the cilia in microns.	Situation of the macronucleus to the long axis of the body.
240·9 × 132·0	75·9 × 29·7	Central	Kidney-shaped	6·6	Parallel.
145·2 × 105·6	49·5 × 19·8	Central	Flat	4·9	do.
188·1 × 118·8	46·2 × 19·8	Posterior	Irregular	4·1	do.
264·1 × 89·1	66·0 × 33·0	do.	Kidney-shaped	4·9	Oblique.
201·3 × 99·0	49·5 × 33·0	Central	Oval	3·3	Parallel.

TABLE IV.

Measurements and some other Observations on the Fixed Specimens of *Nathella kumarii*.

Size in microns.	Size of the macronucleus in microns.	Position of the macronucleus.	Shape of the macronucleus.	Length of the cilia in microns.	Situation of the macronucleus to the long axis of the body.
184·8 × 115·5	67·6 × 23·1	Anterior	Kidney-shaped	1·6	Oblique.
214·5 × 105·6	41·8 × 19·8	Central	Irregular	1·6	Parallel.
237·6 × 92·4	66·0 × 26·4	Central	Embryo-shaped	3·3	do.
161·7 × 125·4	82·5 × 16·5	do.	U-shaped	3·3	Oblique.
165·0 × 102·3	36·3 × 13·2	Anterior	Flat	2·4	Transverse
273·9 × 165·0	62·7 × 23·1	Posterior	Slipper-shaped	3·3	Parallel.
211·2 × 128·7	59·4 × 13·2	Anterior	Flat	1·6	Oblique
277·2 × 158·4	56·1 × 28·0	do.	Oval	3·3	Transverse.
161·7 × 89·1	44·6 × 9·0	do.	Flat	3·3	Oblique.
151·8 × 99·0	37·9 × 16·5	do.	Egg-shaped	1·6	do.

saw them in *B. depressum* (Ghosh) as three or four fibres, arising from the left side of the peristome, but in this ciliate there are numerous fibres running downwards. Posteriorly also there is a bundle of fibres near the groove directed upwards and running in the cytoplasm in the same manner as the anteriorly situated fibres (Plate 4, fig. 21, *Lm*). In *N. kumarii* there are a few additional myonemes provided to keep the groove in its position (Plate 3, figs. 12, 15, *Alm.*; Plate 4, fig. 21, *Alm.*).

Circular myonemes are present throughout the entire inner layer of the body (Plate 2, fig. 8, *Cm.*; Plate 3, fig. 16, *Cm.*; Plate 4, figs. 20, 22, *Cm.*; Plate 6, fig. 37). These myonemes are connected with the body cilia and are responsible for the flexibility of the body. They also help in the active movement of the cilia. These circular myonemes according to the nomenclature of Ten Kate, may be called Morphonemes, for they maintain the body form.

In sections of the infected intestine, the Ciliates could be seen scattered throughout the lumen (Plate 6, fig. 32), but it cannot be said definitely whether they are parasitic or non-parasitic to the host. Presence of trichocysts proves them to be entirely non-parasitic, but mortality amongst the infected fishes only, as mentioned earlier, suggests that they may exert some adverse influence on their host. Further studies are, however, needed to settle this question.

Besides the micronucleus another irregular mass of protoplasm is seen to lie near the macronucleus (Plate 6, figs. 33, 34) which takes a faint nuclear stain. The exact nature of this mass of protoplasm has not been determined yet.

No cysts or conjugants could be encountered through the course of the study.

4. Systematic Position

From their superficial appearance the Ciliates resemble *Nicollella* and *Collinella* Chatton and Perard and *Buxtonella* Jameson. For instance, the peristomial groove in *N. rayii* resembles that of *Nicollella*, while in *N. kumarii*, it resembles that of *Collinella*, but on closer examination of their morphology one finds ample grounds for creating a new family for receiving the Ciliates described in this article. For example the presence of membranellae in both *N. rayii* and *N. kumarii* separates them both from *Nicollella* or *Collinella*. Again in *Nicollella* and *Collinella*, the mouth is situated at the anterior end while in these new Ciliates, it is at the posterior end. The presence of a thick pellicle at the anterior margin and the construction of the groove makes it differ from *Buxtonella*. The species of *Nicollella*, *Collinella* and *Buxtonella* have all been described from mammalian hosts, while the Ciliates described here are from the fish. The morphological features of this new Ciliate also differ in many respects from hitherto known families of Ciliates described under the order Holotricha and suborder Hymenostomata.

Order: HOLOTRICHA

Sub-order: HYMENOSTOMATA

Family: Nathelliidae. Fam. N.

The Ciliates, which are quite new, have recently been discovered from the intestine of fish—*Mystus cavasius*.

The Ciliates belonging to this family are Holotrichous Ciliates with body uniformly covered with short and fine cilia arranged in longitudinal rows. The peristomial groove which is always kept on the dorsal surface, while in movement, runs from the anterior region, where the pellicle is thick and shield-like, to the posterior region where either it ends on the dorsal surface, or turns towards the ventral surface. The mouth, which is provided with a cirrus, is situated at the end of the groove. The groove has got an undulating membrane on the right border with a row of lamellae on the outside, and cilia on the inner left border, with rod-shaped basal granules, arranged very closely, so as to form the pseudolamellae. The other cilia are to the outside, and these are longer than the body cilia. The ridge which lies between the inner fine cilia and the undulating membrane is provided with papillae. The anterior portion of the groove is slightly dilated. The undulating membrane and the ridge continue from the anterior to the posterior region, but stop short in front of the mouth. The macronucleus varies in size and shape. The position of the macronucleus in the body is variable. The micronucleus is spherical and is placed in a depression on the surface of the macronucleus. One or two contractile vacuoles may be seen lying at the posterior end of the body. Trichocysts are present, amongst the longer cilia, on the left border of the groove and are also scattered in the body. They are crescent-shaped with one end drawn out into a filament. Longitudinal and circular myonemes are present.

Genus *Nathella*. N. gen.

Characters of the family. The genus embraces two new species, viz., *N. rayii*, n.sp., and *N. kumarii* n. sp.

DIAGNOSIS

Nathella rayii n.sp.

This is Holotrichous ciliate with broadly elliptical body. Body cilia are uniform, fine and arranged along longitudinal rows. Ciliary lines are very closely set. Body cilia vary from $1{\cdot}6\mu$–$4{\cdot}9\mu$ in length. The groove starts from the anterior region and ends posteriorly on the dorsal surface bearing the mouth with a cirrus. Undulating membrane is present throughout the right margin of the peristomial groove. On the anterior region about one-fourth of the peristomial groove is slightly dilated. The undulating membrane stops short a little above the posterior portion of the groove where it just closes encircling the mouth, while the lamellae on the outside continue to the end of the groove on the right border. Length of the

peristomial groove varies from 197·0μ–264·0μ according to the variation in the size of the body. Contractile vacuole usually on the posterior end opens to the outside by a canal and is provided with auxiliary vacuoles. Macronucleus is granular and varies greatly in shape. The spherical micronucleus is situated in a depression on the surface of the macronucleus. Nuclear dimensions range between 49·5μ–82·8μ in length and 19·8μ–41·4μ in breadth. The average being 65·70μ by 29·79μ. The position of the macronucleus in the body is variable. The long axis of the macronucleus is oblique, transverse or parallel to the long axis of the body. Longitudinal myonemes are present at the anterior and posterior regions of the body. Circular myonemes (morphonemes) are present throughout the inner region of the body.

Dimensions: Size very variable. 198·0μ–330·0μ × 125·4μ–181·4μ, averaging 252·68μ × 151·07μ.

Habitat: Intestine of fish *Mystus cavasius*.

Locality: Ludhiana.

Nathella kumarii n.sp.

This is also a Holotrichous Ciliate usually with an elongated body but shows variety of shape and form. Body cilia are short, fine and uniform, arranged along the closely set longitudinal rows. Cilia vary from 3·3–6·6μ in length. The groove starts from the anterior region and passes over the entire dorsal surface, dips to the ventral surface and curves upwards making the shape of *U*. At the end of the peristomial groove is the mouth with cirrus. Undulating membrane is present throughout the length of the groove. About one-sixth of the anterior portion of the groove is slightly dilated. The groove is longer than the length of the body. Contractile vacuole usually on the posterior end opens to the outside by a canal and is provided with auxiliary vacuoles. Macronucleus is granular and very variable in shape. On its surface lies the spherical micronucleus, more or less, sunk in a depression on the surface. Nuclear dimensions range between 46·2μ–75·9μ in length, 19·8μ–33·0μ in breadth, the average being 60·06μ by 27·29μ. The long axis of the macronucleus is oblique, transverse or parallel to the long axis of the body. The macronucleus is anterior, central or posterior in position in the body. Longitudinal myonemes are present at the anterior and posterior region of the body near the start and at the end of the groove. There are myonemes also between the two arms of the groove, obliquely situated, which help to keep the two arms of the U-shaped curvature in their position and also help to keep them at a certain distance. The posterior region of the groove bears a very minute triangular keel or guard to keep the groove in position (Plate 3, figs. 12, 15, *K*). Circular myonemes (morphonemes) are present lining the inner surface of the body throughout.

Dimensions: Size very variable. 145·2μ–297·0μ × 89·0μ–148·5μ averaging 218·79μ by 126·05μ.

Habitat: Intestine of fish *Mystus cavasius*.

Locality: Ludhiana.

5. Acknowledgement

I am deeply indebted to Dr. Vishwa Nath, Ph.D. (Cantab.), F.N.I., Head of the Panjab University Zoology Department and Principal, University College, Hoshiarpur, who suggested to me this probelm and encouraged me in every way in conducting my researches both at Ludhiana and Hoshiarpur, and offering his valuable criticism in the preparation of the manuscript.

I wish to thank Prof. D. R. Puri, Head of the Zoology Department, Government College, Ludhiana, who very kindly permitted me to carry on my research work in his laboratories.

I am deeply indebted to Dr. H. N. Ray, M.Sc. (Cal.), Ph.D. (London), F.N.I., Officer-in-Charge, Section of Parasitology, Indian Veterinary Research Institute, Mukteswar, Kumaon (U.P.), who very kindly examined my preparations and helped me in the identification of the Ciliates, and took the photomicrographs illustrating this article.

I am also thankful to Dr. S. Datta, D.Sc., M.R.C.V.S., D.T.V.M., F.R.S. (Edin.), F.N.I., Director, Indian Veterinary Research Institute, Mukteswar, Kumaon (U.P.), for kindly permitting me to work in the Protozoology laboratory of his Institute.

I also wish to thank the officers of the Fisheries Department, Ludhiana, who made the collection of the fish possible.

6. Summary

The morphology of the two new Ciliates from the intestine of a carnivorous fish—*Mystus cavasius*—(mostly from Budha Nala and often from Jassian Pond, Ludhiana) are described. The morphological characters are identical with the Ciliates belonging to the sub-order Hymenostomata, but a new family *Nathelliidae* and a new *Genus Nathella*, with two new species *Nathella rayii* n.sp. and *Nathella kumarii* n.sp., have been described in detail.

The peristomial groove starts from the anterior end, and finishes just at the posterior end in *N. rayii*, while in *N. kumarii* it continues throughout the posterior surface, dips to the right, and turns upwards on the ventral region. The end of the groove bears the mouth in both. The groove bears the undulating membrane on the right side with lamellae on the outside. The morphology of the groove is the same in both the species. The only difference is that about one-fourth of the anterior portion of the groove in *N. rayii* and one-sixth of the anterior portion of the groove in *N. kumarii*, is dilated, because the groove in the latter is longer, though the length of the dilated portion of the groove is nearly the same in both. The body cilia is shorter in *N. rayii* than those in *N. kumarii*. The macronucleus in *N. rayii* is longer than that of *N. kumarii*. In shape, latter's macronucleus is more complicated than the former's. The macronucleus in *N. rayii* is larger than that in *N. kumarii*. Both have got contractile vacuoles at their posterior ends. These are provided with auxiliary vacuoles. Both have got longitudinal and circular myonemes, but *N. kumarii* has got another bundle of myonemes, obliquely placed, on the dorsal and ventral surface of the arms of the groove. The groove is also provided with a keel

or guard at the posterior end, where the groove just dips to the ventral surface. This keel or guard keeps the groove in position and is present only in *N. kumarii*.

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7. Explanation of Figures

N.B.—Figures were drawn with the aid of camera lucida with the magnification given below.

Plate 1

Fig. 1. *Mystus cavasius*—the host fish, highly infected with Ciliates. ×3/4.

Fig. 2. *Nathella rayii*, from a smear fixed in Bouin's fluid and stained in Heidenhain's iron haematoxylin. ×480.

Fig. 3. Contractile vacuole of *N. rayii* in the entire specimen with no opening to the outside. ×1000.

Fig. 4. Contractile vacuole of *Nathella kumarii* in the entire specimen provided with an opening. ×1000.

Fig. 5. Contractile vacuole of *N. kumarii* in section. ×1000.

PLATE 2

FIG. 6. *N. kumarii*, from a smear fixed in Schaudinn's fluid and stained in Heidenhain's iron haematoxylin. ×480.

FIG. 7. Crescent-shaped trichocysts of *N. rayii* and *N. kumarii*. ×1200.

FIGS. 8, 9, 10. Transverse sections (4 microns) of *N. rayii* showing the structure of the peristomial groove. ×600.

FIG. 11. Transverse section (4 microns) of *N. kumarii* after fixation with F.W.A. for 24 hours before the effect of the turpentine oil. ×500.

PLATE 3

FIG. 12. Transverse section (3 microns) of the posterior portion of the peristomial groove of *N. kumarii*, showing the keel or the guard. ×1000.

FIG. 13. Posterior end of the peristomial groove of *N. rayii*. ×2400.

FIG. 14. A portion of the peristomial groove of *N. rayii*. ×4800.

FIG. 15. Posterior portion of the peristomial groove of the entire specimen of *N. kumarii*, showing the keel or the guard. ×1900.

FIG. 16. Transverse section (3 microns) of *N. kumarii* showing the arrangement of the circular myonemes. ×500.

FIG. 17. First serial longitudinal section (3 microns) of *N. rayii* passing on the surface of the peristomial groove. ×1000.

PLATE 4

FIG. 18. Second serial longitudinal section (3 microns) of *N. rayii* passing on the surface of the peristomial groove. ×1000.

FIG. 19. Anterior end of *N. kumarii*, showing the peristomial groove dilated anteriorly, with a cirrus situated at its origin. ×600.

FIG. 20. Longitudinal section (3 microns) through the anterior region of *N. rayii*. ×1000.

FIG. 21. Posterior portion of the peristomial groove in *N. kumarii*. ×480.

FIG. 22. Transverse section (3 microns) of *N. rayii* through the anterior region showing the opening for the trichocysts. ×1000.

PLATE 5

FIGS. 23, 24. First two serial transverse sections (3 microns) through the right hand side of the peristomial groove of *N. rayii*. ×1000.

FIGS. 25, 26, 27. First three serial transverse sections (4 microns) of *N. rayii* from the posterior portion of the peristomial groove. ×1000.

FIG. 28. Macro- and micro-nuclei in section (4 microns) of *N. rayii* showing the granular cytoplasm uniformly distributed. ×1000.

FIGS. 29, 30. Macro- and micro-nuclei in section (4 microns) of *N. kumarii* showing the granular cytoplasm collected at both ends before the division. ×1000.

FIG. 31. Macro- and micro-nuclei in section (4 microns) of *N. rayii* with the micronucleus after the division attached with each other with a spindle. ×1000.

PLATE 6

(*Photomicrographs*)

FIG. 32. Transverse section (4 microns) through the intestine 56, showing the Ciliates scattered in the lumen of the gut.

FIG. 33. *Nathella rayii*. The Ciliate shows on the anterior region a thick shield-like pellicle with the peristomial groove starting from its centre: the groove reaching up to the posterior region, bearing the undulating membrane and ending on the dorsal surface bearing the mouth. Contractile vacuole posteriorly placed at the tip. Macronucleus almost in the middle of the body and parallel to the long axis of the body. Shapeless mass of protoplasm near the macronucleus. ×190.

FIG. 34. *Nathella kumarii*. Peristomial groove dipping towards the ventral side. Macronucleus kidney-shaped and anteriorly placed and longitudinal to the long axis of the body. Shapeless mass of protoplasm near the macronucleus. ×190.

FIG. 35. A portion of *N. rayii* in whole mount showing the details of the peristomial groove. ×700.

FIG. 36. Longitudinal section (3 microns) through the posterior end of the peristomial groove in *N. rayii* showing the mouth, undulating membrane with longer cilia on the left margin of the groove. Trichocysts scattered in the body. ×700.

FIG. 37. Transverse section (3 microns) through *N. kumarii* showing the basal granules, body cilia, arrangement of the circular myonemes and the macronucleus. ×400.

ABBREVIATIONS

A.—Anal fin.
Alm.—Additional longitudinal myonemes.
av.—Collapsed auxiliary vacuole.
aw.—Filled auxiliary vacuole.
B.—Thin layer of skin on the dorsal surface of the body of the host fish.
Br.—Brownish globules or lipoids.
Bc.—Body cilia.
Bg.—Basal granules.
bgr.—Basal granules of the ridge.
bgum.—Basal granules of the undulating membrane.
c.—Caudal fin.
Cg.—Cytostomal groove.
Cl.—Ciliary lines.
Cm.—Circular myonemes.
Cr.—Cirrus.
Cv.—Contractile vacuole.
D.—Dorsal fin.
Gm.—Granular material.
ifc.—Inner fine cilia.
K.—Keel or guard.
L.—Lamellae.
lm.—Longitudinal myonemes on the posterior end.
Lm.—Longitudinal myonemes.
M.—ventral cirrus.
Mn.—Macronucleus.
mn.—Micronucleus.
Mo.—Mouth.
N.—Nasal cirri.
O.—Oral cirri.
oa.—Opening of the auxiliary vacuole.
oc.—Opening of the contractile vacuole.
olc.—Outer longer cilia.
otr.—Opening for the trichocysts.
P.—Pelvic fin.
Pf.—Peristomial fibres.
Pg.—Peristomial groove.
Pg.d.—Peristomial groove disappearing.
Pgdv.—Peristomial groove dipping towards the ventral surface.
Pgvs.—Peristomial groove on the ventral surface.
R.—Ridge.
rsbg.—Rod-shaped basal granules.
s.—Septa.
Sp.—Spindle.
Tr.—Trichocysts.
Um.—Undulating membrane.
V.—Ventral fin.

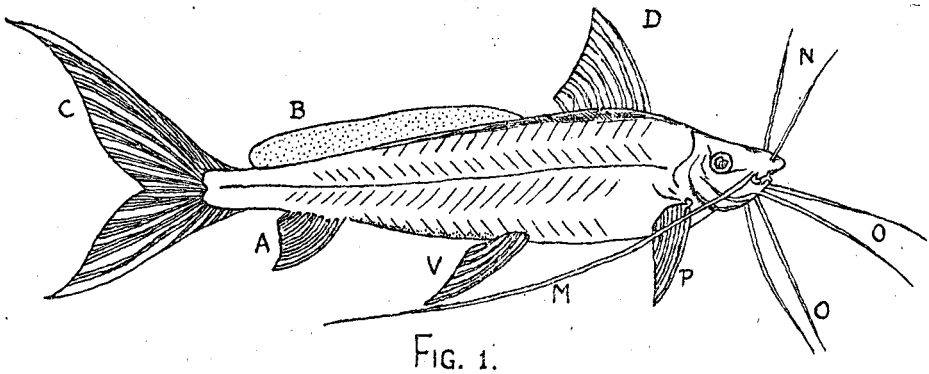


FIG. 1.

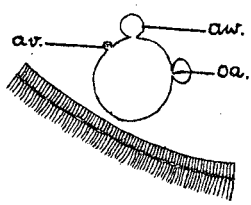


FIG. 3.

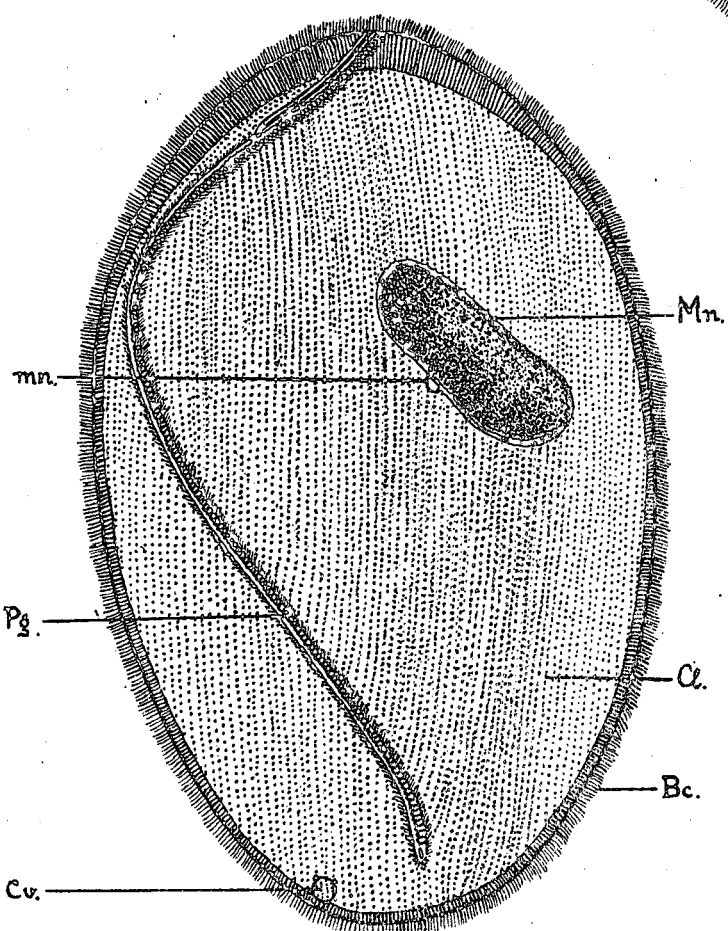
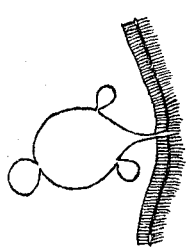


FIG. 2.



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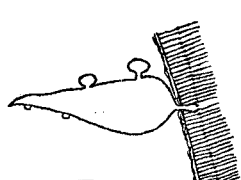
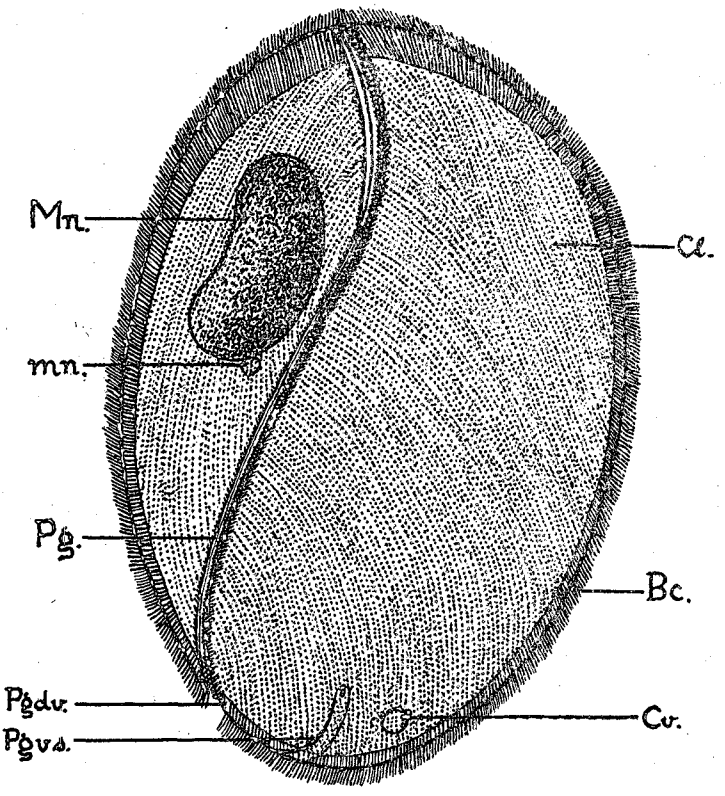


FIG. 5.



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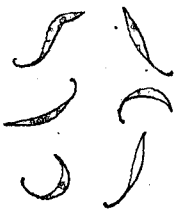

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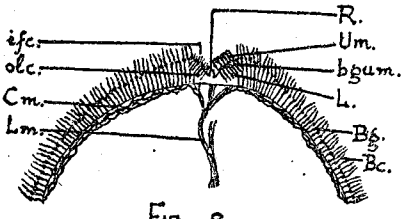

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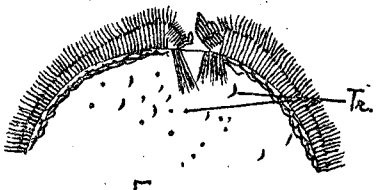

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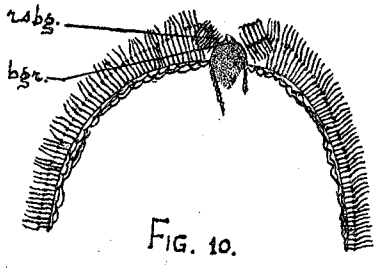

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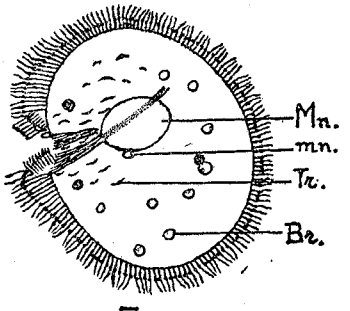

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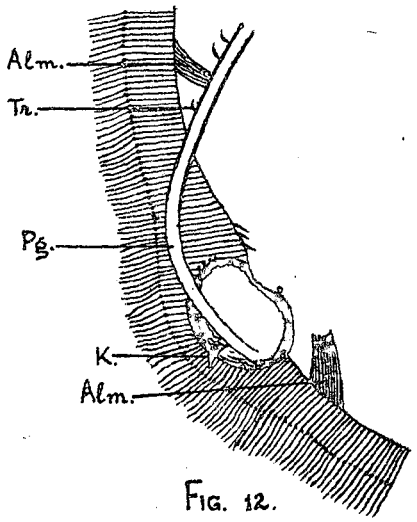

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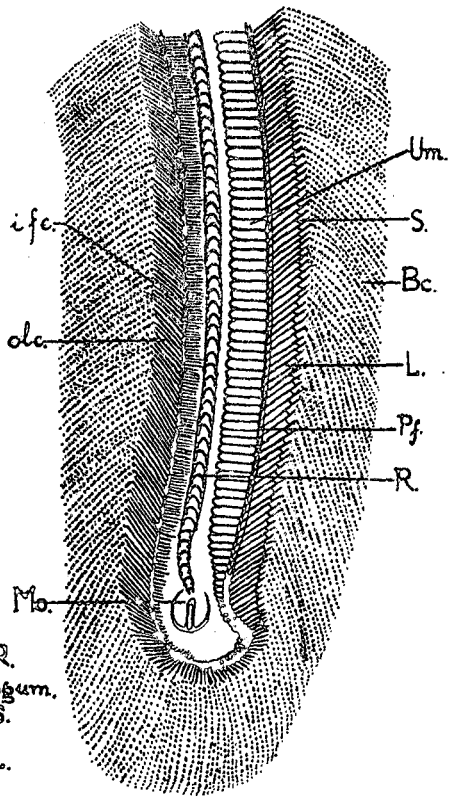

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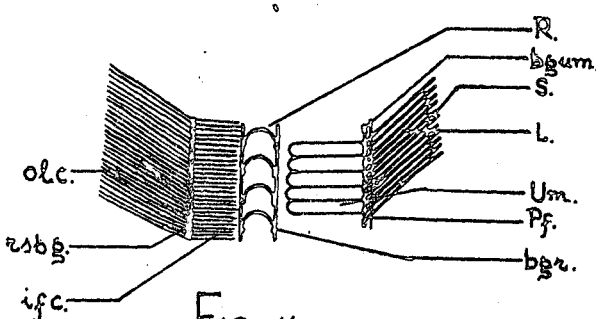

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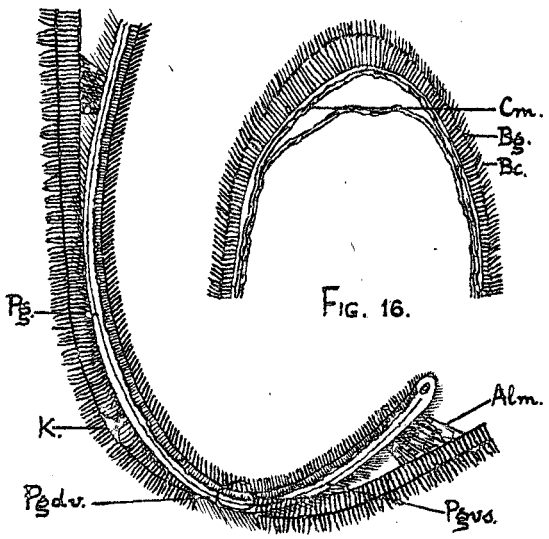
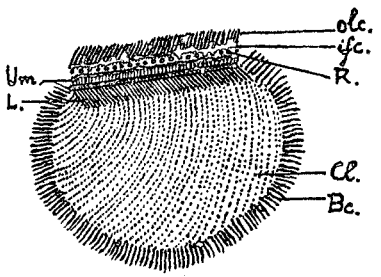


FIG. 15.

FIG. 16.



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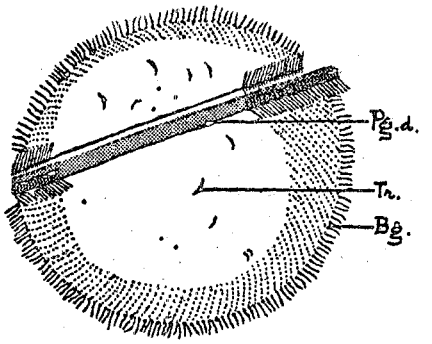

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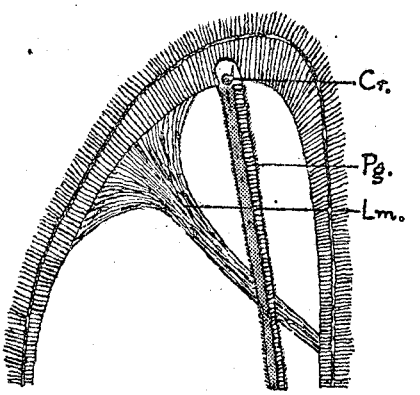

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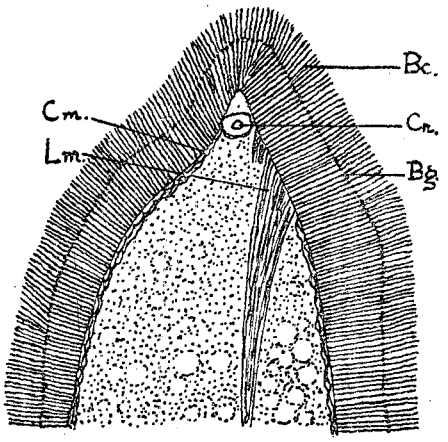

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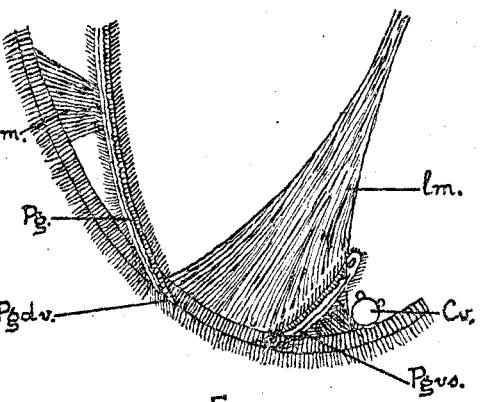

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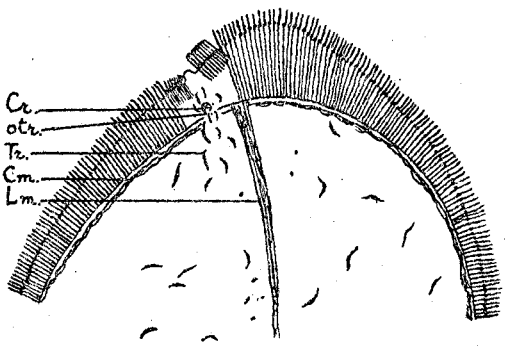

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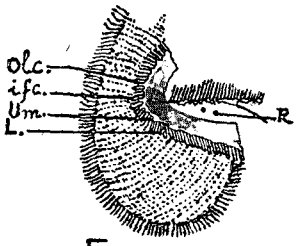

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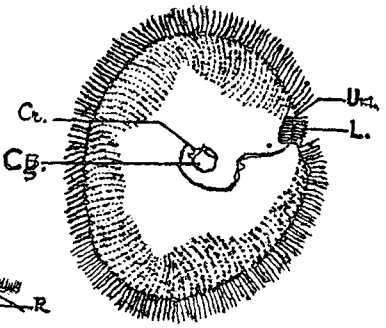

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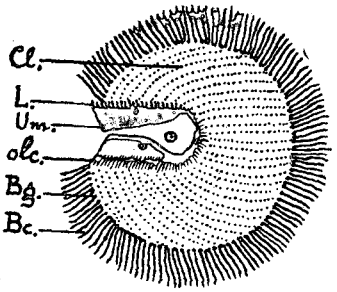

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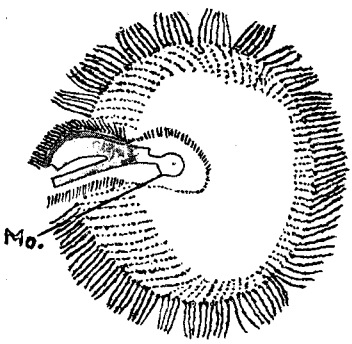

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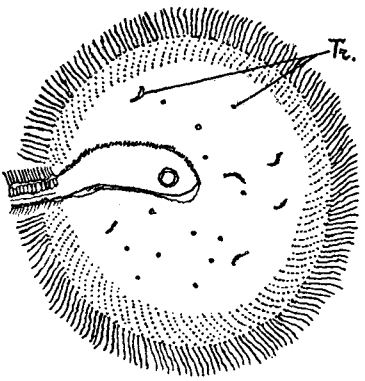

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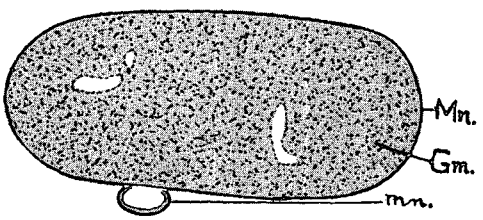

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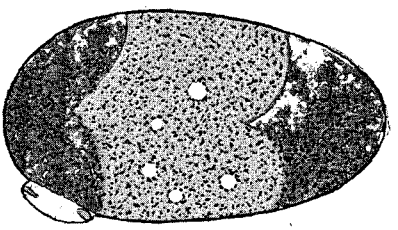

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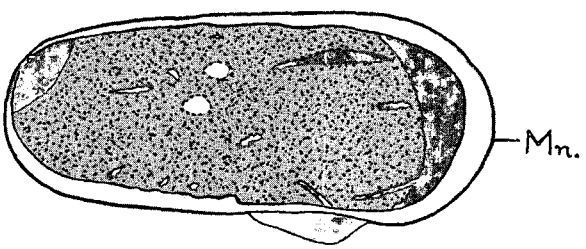

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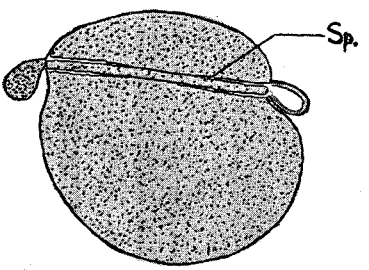


FIG. 31.

PLATE 6.

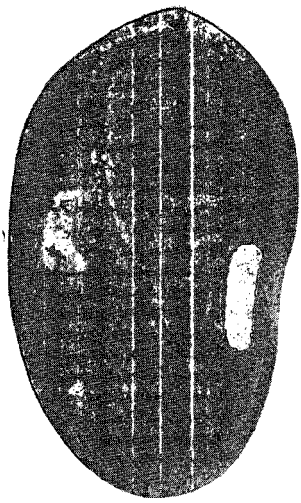
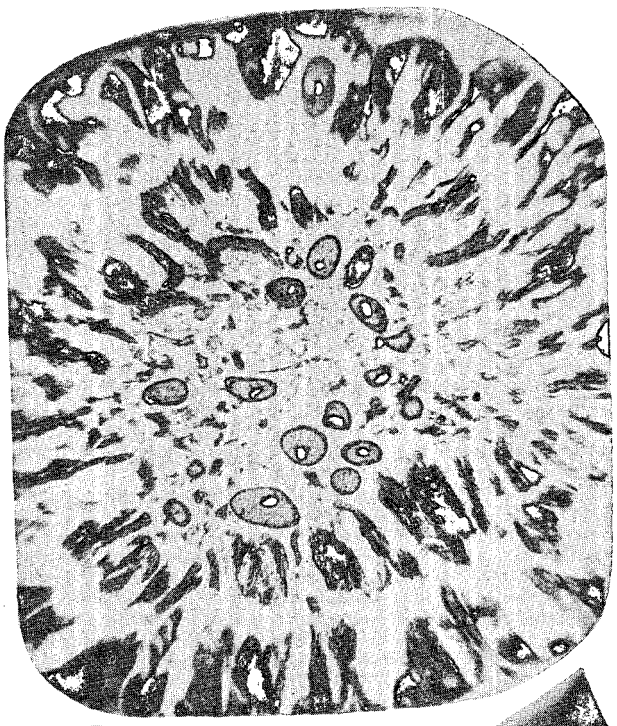


FIG. 33.



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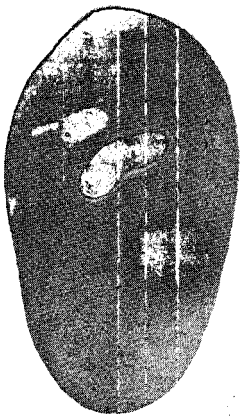

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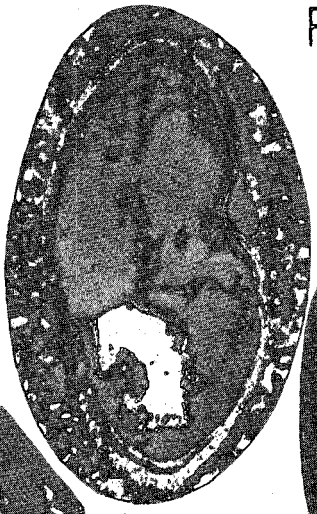

FIG. 37.

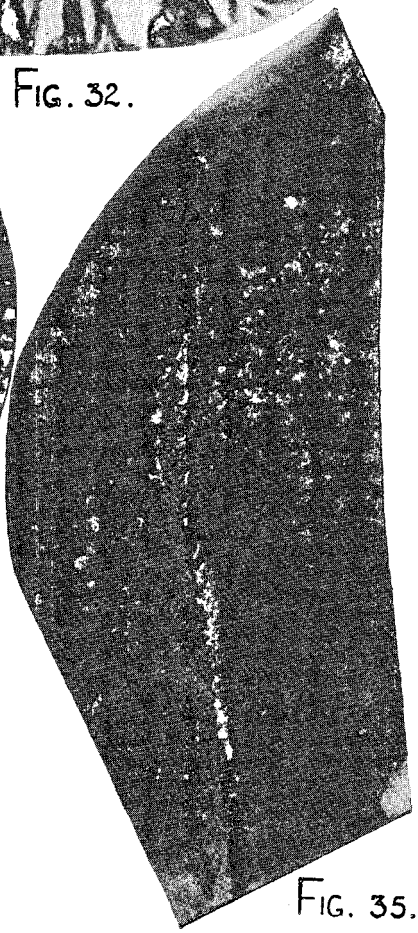

FIG. 35.

FIG. 36.

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RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 36 CHEMISTRY Pp. 139-144

November, 1953

THIOPEGAN DERIVATIVES. PART II

by
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Edited for the East Panjab University by Vishwa Nath,
Department of Zoology, East Panjab University,
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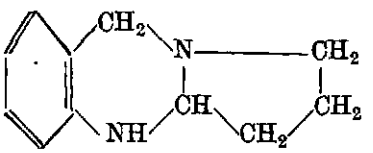
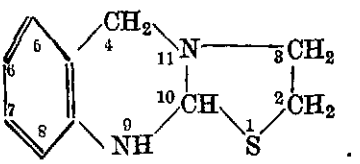
THIOPEGAN DERIVATIVES. PART II.

By M. S. Dhatt *and* K. S. Narang (*Department of Chemistry, Panjab University College, Hoshiarpur, East Panjab, India*).

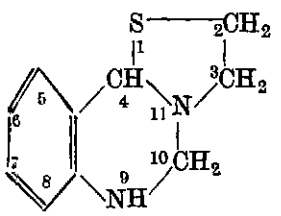
Since Pyrrolidino-Tetrahydro Quinazoline (I) has been named Pegan by Späth,[1] the authors[2] have suggested the name Thiopegan for the structure (II). Since compounds of the Isomeric structures (III) and (IV) are closely related to (II) it is considered desirable to finalize the system of nomenclature in the three compounds. The original nomenclature Thiopegan could be extended to (III) and (IV) by showing the positions in the Quinazoline ring to which the Thiazolidino ring is fused in each of the three isomers. (II), (III) and (IV) would, therefore, be named as 10 : 11 Thiopegan ; 4 : 11 Thiopegan and 9 : 10 Thiopegan, respectively.



(I)



(II)



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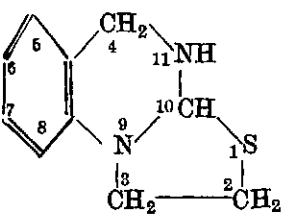
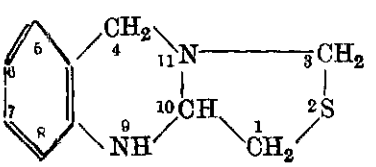
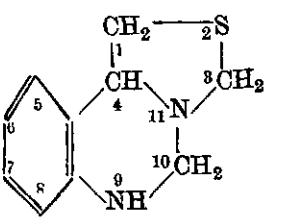


(IV)

The ring systems (V), (VI) and (VII) with S in position 2 could then be named as 2 Iso-10 : 11 Thiopegan ; 2 Iso-4 : 11 Thiopegan and 2 Iso–9 : 10 Thiopegan, respectively. The ring systems (VIII), (IX) and (X) with S in position 3 could be named as 3 Iso-10 : 11 Thiopegan ; 3 Iso-4 : 11 Thiopegan and 3 Iso–9 : 10 Thiopegan, respectively.



(V)



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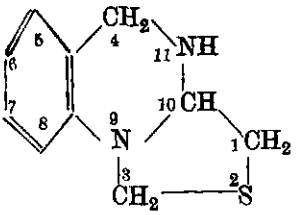
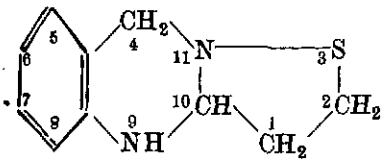


(VII)



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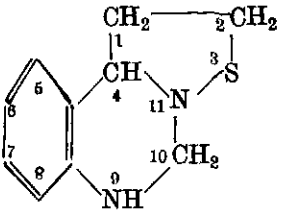

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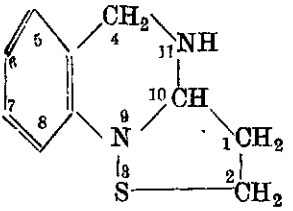
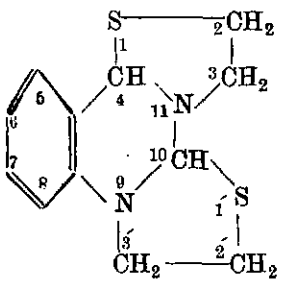


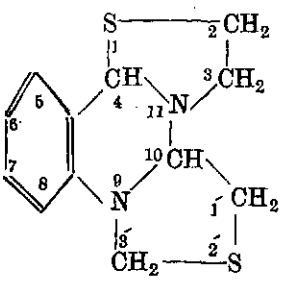
(X)

The ring systems (XI)–(XIX) could be named with reference to one of the Thiopegans or Iso-Thiopegans as shown.



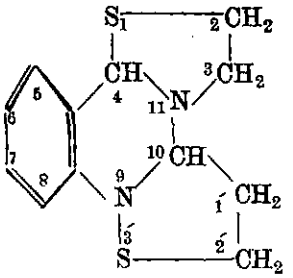
(XI)

1́ Thiazolidino 4 : 11 Thiopegan.



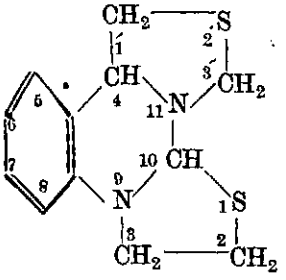
(XII)

2́ Thiazolidino 4 : 11 Thiopegan.



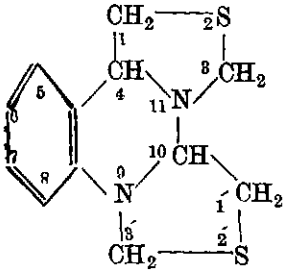
(XIII)

3́ Iso-Thiazolidino 4 : 11 Thiopegan.



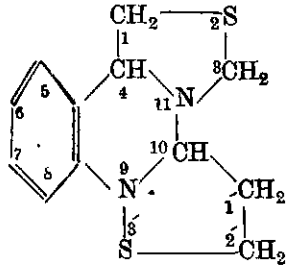
(XIV)

2́ Thiazolidino 9 : 10 Thiopegan.



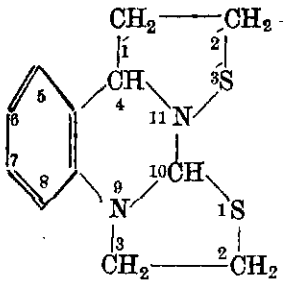
(XV)

2́ Thiazolidino 2 Iso-4 : 11 Thiopegan.



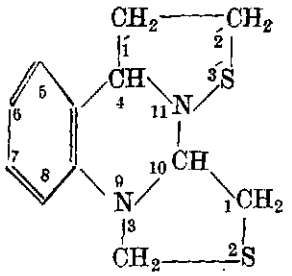
(XVI)

3́ Iso-Thiazolidino 2 Iso-4 : 11 Thiopegan.



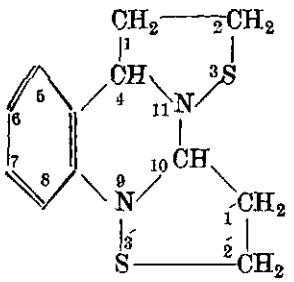
(XVII)

3 Iso-Thiazolidino 9 : 10 Thiopegan.



(XVIII)

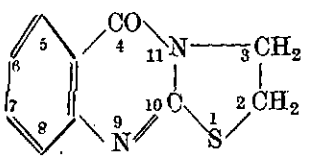
3 Iso-Thiazolidino 2 Iso-9 : 10 Thiopegan.



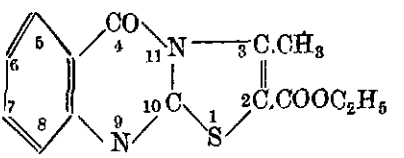
(XIX)

3 Iso-Thiazolidino 3 Iso-4 : 11 Thiopegan.

Apart from the intrinsic interest in the chemistry of these ring systems, their physiological activity is likely to be of some value due to the combined presence of Thiazolidine and Quinazoline rings. The high antimalarial activity shown by 10 : 11 Thiopegen-9, 4-one. (XX) against *P. Gallinaceum* in chicks and prevention of the growth of Mycrobacterium Tuberculosis in a dilution of 1 : 1,000 by 2-carbethoxy-3 methyl 10 : 11 Thiopega-2 : 9 diene-4-one (XXI) have justified the above expectation.

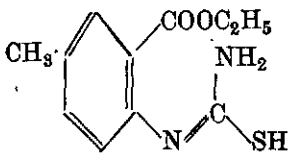


(XX)

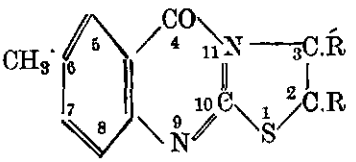


(XXI)

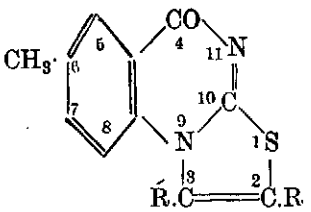
The present communication records the preparation of some Thiopegadiene derivatives (Table I) by the condensation of 2-carbethoxy-4-methyl phenyl thiourea (XXII) with appropriate α-chloro or α-bromo ketones. The products have been tentatively given the general structure (XXIII), but these could also be given the general structure (XXIV). Confirmation of the exact structures by independant synthesis is in progress.



(XXII)



(XXIII)



(XXIV)

R and R̄ are various substituents.

Besides the compounds enlisted below, 2-carbethoxy-4-methyl phenyl thiourea has also been condensed with chloroacetone, α-bromo ethyl acetoacetate and α-chloro-ethyl methyl ketone. The analytical results of the products are awaited

Acknowledgment

The authors are grateful to Dr. S. M. Mukherji, Head and Professor of Chemistry Department, Hoshiarpur, for his kind interest in the work and to Col. Jaswant Singh, Director, Malaria Institute of India, Delhi, for kindly carrying out antimalarial tests.

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TABLE I

No.	Condensation of 2-carbethoxy-4-methyl phenyl thiourea with the particular ketones	Name of the product	Solvent of *crystallization*	M.P. °C.	Formula	Analytical Results	
						Found (%)	Required (%)
1.	ω-Bromo Acetophenone	3-phenyl-6-methyl 10 : 11 Thiopega-2: 9-diene-4-one.	Benzene	264	$C_{17}H_{12}ON_2S$	N: 9·51	N: 9·60
2.	ω-Bromo P-methyl Acetophenone ..	3-(P-Tolyl)-6-methyl 10 : 11 Thiopega-2: 9-diene-4-one.	Ethyl Acetate	254	$C_{18}H_{14}ON_2S$	N: 9·21	N: 9·13
3.	ω-Bromo P-methoxy Acetophenone ..	3-(P-methoxy phenyl)-6-methyl 10 : 11 Thiopega-2: 9-diene-4-one.	50% dilute ethyl alcohol	244	$C_{18}H_{14}O_2N_2S$	N: 8·66	N: 8·69
4.	ω-Bromo P-chloro Acetophenone	3-(P-chloro phenyl)-6-methyl 10 : 11 Thiopega-2: 9-diene-4-one.	Ethyl alcohol	326	$C_{17}H_{11}ON_2SCl$	C: 62·54 H: 3·35	C: 62·48 H: 3·37

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 37 ZOOLOGY Pp. 145–155

December, 1953

SPERMATOGENESIS OF *ACHETA DOMESTICUS* LINN. *(GRYLLUS DOMESTICUS)* WITH OBSERVATIONS UNDER THE PHASE CONTRAST MICROSCOPE

by

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Edited for the East Panjab University by Vishwa Nath,
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SPERMATOGENESIS OF *ACHETA DOMESTICUS* LINN. (*GRYLLUS DOMESTICUS*) WITH OBSERVATIONS UNDER THE PHASE CONTRAST MICROSCOPE

By Vishwa Nath *and* Bachittar Singh Bhimber, *Department of Zoology, Panjab University College, Hoshiarpur*

Introduction

Cytologists have long found insecta to be a very fertile field for investigation. Although much work has been done on the cytoplasmic inclusions in the germ cells of Lepidoptera (Gatenby, 1917; Bowen, 1922), Hemiptera (Bowen, 1922-23; Chickering, 1927), Coleoptera (Bowen, 1924; Nath, Bawa, Bhardwaj and Gupta, 1951; Mickey, 1935) and Aptera (Charlton, 1921; Bowen, 1924; Gatenby and Mukerji, 1929; and Nath and Bhatia, 1953), the Orthoptera have received very little attention.

Bowen has done some work on the Orthoptera to which he has made occasional references in his papers on Lepidoptera (1922) and Hemiptera (1922-23). Recently Chang-Chun Wu (1940) has worked out the spermatogenesis of an Orthopteran form, *Diestrammena* sp. with special reference to Golgi bodies and mitochondria. Earlier Payne (1916-17) published his paper on *Gryllotalpa borealis* and *G. vulgaris* and Baumgartner (1902) on 'Spermatid Transformations in Gryllus, etc.'

One of us (B.S.B.) started investigations on the spermatogenesis of *Acheta domesticus* Linn. (*Gryllus domesticus*) in 1952. The material was found to be exceptionally favourable for cytological studies. All preparations have been checked by the senior author, who has also prepared the manuscript for the press. All the figures have been drawn by the junior author.

Studies under the phase contrast microscope were made jointly by us; and they have been remarkably profitable.

Material and Technique

Specimens of *Acheta domesticus* Linn. (*Gryllus domesticus*) were collected from kitchens and storehouses. The male can be distinguished from the female by the absence of an ovipositor.

Specimens were dissected by cutting across the posterior end of the abdomen by a pair of scissors, and the contents were squeezed out in a watch glass containing normal saline solution. Small pieces of the testis not exceeding two mm. in thickness were then transferred to the fixatives.

The material was mostly fixed in Flemming-without-acetic acid for varying periods, and then washed in running tap water or distilled water for the same period. It was then dehydrated and embedded in paraffin wax. Sections were cut five microns thick and were stained in 0·5% iron

haematoxylin after usual mordanting in 4% iron alum. Differentiation was done in 2% iron alum. Some material was also fixed in Champy's Fluid. Bouin's fluid was used for control. Smears for the study of the mature sperms were also prepared. A portion of the testis was pressed between the two slides, which were then placed in F.W.A. diluted with an equal quantity of distilled water for one or two hours. After washing for the same period slides were stained in 0·5% iron haematoxylin. Best results were obtained from the material fixed for 36 hours in F.W.A. and for 12 hours in Champy's fluid.

Observations on the fresh material were also made under the phase contrast microscope.

Observations

Spermatogonium

The earliest spermatogonium (Fig. 62, Pl. IV) of *Acheta domesticus* Linn. (*Gryllus domesticus*) appears as a more or less rounded cell in the living condition under the phase contrast microscope. It contains a comparatively big nucleus, which reveals fine chromatin granules connected by fibres forming a network. One or two nucleoli may also be present in the nucleus. The nucleus of the spermatogonium is so big in some cases that the cytoplasm can be scarcely made out. A few very dark granules are present in the cytoplasm, juxta-nuclear in position.

In sectioned and stained material the spermatogonium shows the same form and structure as in the living condition (Fig. 1, Pl. I), except that the juxta-nuclear mass shows two types of granules, viz., a mass of very faintly-staining, greyish granules, in which are embedded a few bigger and darkly-staining spherical bodies. The former are the mitochondria and the latter the Golgi elements.

During the spermatogonial metaphase (Fig. 2, Pl. I) chromosomes arrange themselves at the equator of the spindle. A centriole is present as a fine granule at each pole of the spindle. The fine, faintly-staining mitochondria cannot be seen at this stage, as they are uniformly distributed in the cell, but a few Golgi granules can be easily detected in the cells as they are bigger in size and stain more deeply with iron haematoxylin.

In polar views of the metaphase plate (Fig. 3, Pl. I) 21 chromosomes can be easily counted; one of these is U-shaped and is the sex chromosome.

Primary spermatocyte

The earliest primary spermatocyte (Fig. 4, Pl. I) shows an oval nucleus, which does not reveal any structure, save for the presence of one or two nucleoli. The mitochondria form one or two deeply-staining, cloudy masses close to the nucleus. Each mass is so dense and compact that it is very difficult to make out the individual granules. In some cases they appear to be composed of threads, which are most probably formed by the alignment of granules. In some cases Golgi spheres are also met with, with only the chromophobic area present, while in others the usual characteristic duplex structure of the Golgi sphere can be easily made out.

At a later stage (Fig. 5, Pl. I) the mitochondria appear as fine granules, which have started spreading around the nucleus. The nucleus is again oval here, and does not show any structure except one or two nucleoli. Golgi bodies are present in the form of crescents, and spheres which show only the chromophobic medulla.

At a still later stage (Fig. 6, Pl. I) the mitochondria become vesicular. They now appear as small vesicles, which are ovoid or spherical. Each vesicle consists of a droplet of some substance, which has no affinity for the stain. This material is enclosed in a delicate envelope of a substance, which takes the haematoxylin stain rather sharply. After some time these vesicles begin to lengthen out and form hollow tubes closed at both the ends. These may be straight or a bit curved (Fig. 7, Pl. I). These tubes further elongate, and their cavities become obliterated. Thus thick mitochondrial threads are formed, which cover the nucleus on all sides. Gradually they are converted into fine threads (Fig. 8, Pl. I).

The primary spermatocytes have been studied in the living condition under the phase contrast (Fig. 63, Pl. IV). As thus studied, the primary spermatocyte is a rounded cell having a spherical nucleus with one or two nucleoli. Mitochondria are present in the form of numerous delicate threads, which form a circum-nuclear ring. Golgi bodies are conspicuous in the cytoplasm in the form of dark granules.

Fig. 64, Pl. IV, has been drawn from a living cell as seen under the phase contrast. The metaphase I chromosomes, the mitochondrial threads forming a mantle round the spindle, a centriole at each pole of the spindle and lastly the Golgi bodies in the form of dark granules can be easily observed. An important point may be noted that the fibres in the spindle area are conspicuous by their absence at this stage. This is fully confirmed by the study of fixed and stained cells (Fig. 9, Pl. I).

In the late telophase stage (Fig. 11, Pl. I) of the first maturation division the Golgi bodies and the mitochondria have been distributed to the two daughter cells. The spindle fibres and mid-body granules are now conspicuous.

Secondary spermatocyte

It may be recalled that the mitochondria, which are granular in the spermatogonia first become vesicular and then thread-like in the primary spermatocytes during the growth period. But in the earliest secondary spermatocyte the mitochondria once more assume the vesicular form (Fig. 12, Pl. I). Soon after, however, the vesicles are converted into threads (Fig. 13, Pl. I), which can be observed very clearly in the living cell also studied under the phase contrast (Fig. 65, Pl. IV).

Metaphase II stages are very rare in our preparations, although one polar view of such a stage has been figured by us (Fig. 15, Pl. I). Telophase II stages, however, are very common in both the fixed preparations as well as in the living cells as studied under the phase contrast (Figs. 14 and 16, Pl. I; and Fig. 67, Pl. IV). In Fig. 16, Pl. I, and in Fig. 67, Pl. IV, the spindle fibres seem to be disappearing; at any rate, they cannot be made out as such from the thick mitochondrial filaments. But since the mid-

body granules are present at this stage (Fig. 67, Pl. IV) it can be safely assumed that the spindle fibres are masked by the mitochondrial threads.

Spermatid and Spermateleosis

The earliest spermatid (Fig. 17, Pl. I) shows a small nucleus, which reveals a comparatively lightly-stained portion, filled with chromatin granules and a small very darkly-staining portion. These portions of the nucleus, i.e. darkly- and lightly-staining, are separated by a narrow clear space. Prominent thread-like mitochondria taking a very dark stain are present. Golgi bodies are present in the form of granules. At a later stage the darkly-staining portion of the nucleus becomes constricted off from the lightly-staining portion (Fig. 18, Pl. I). At a still later stage (Fig. 19, Pl. I) the deeply-staining portion of the nucleus is completely cut off from the lightly-staining portion and starts degenerating. Ultimately it disappears altogether.

After a part of the nucleus has been constricted off, an important change comes over the spermatid nucleus. It now starts staining very feebly and homogeneously (Fig. 20, Pl. I).

After this stage the mitochondria come close together to form the mitochondrial nebenkern, which is characteristic of insect spermatogenesis. The mitochondria pass through various stages of condensation, until the final differentiation of the nebenkern into an outer chromophobic and an inner chromophilic area is established.

In the living condition under the phase contrast, the mitochondria in the earlier stages of condensation form a cloudy mass very close to the nucleus (Fig. 68, Pl. IV). The nucleus is spherical and shows a nucleolus and chromatin granules. Golgi granules are present close to the mitochondrial nebenkern.

In the sectioned and stained preparations, the mitochondrial nebenkern shows a vacuolated structure in its earlier stages (Fig. 21, Pl. II). Contrary to our observations on the living cells, most of the Golgi bodies appear in the form of crescents, although a few Golgi granules may also be present.

The nebenkern now starts rounding up (Fig. 22, Pl. II) till it becomes completely spherical. At the same time it begins to stain homogeneously (Fig. 23, Pl. II). In between the nucleus and the mitochondrial nebenkern a few Golgi crescents can be observed close together. It is most probable that they will fuse together to form the acroblast, but the actual fusion has not been observed by us.

At a later stage, when the acroblast is fully formed, the mitochondrial nebenkern starts differentiating and passes through a series of internal changes. Firstly it becomes spherical and shows vacuoles (Figs. 24 and 26*a*, Pl. II), which are separated by chromophilic plates. At the points where these plates meet, prominent chromophilic thickenings are present in the form of granules. The nebenkern at this stage represents a beautiful artistic pattern.

At a further stage in the condensation of the nebenkern, its cortical portion becomes uniformly thick all round and begins to stain darkly with

iron haematoxylin. The medulla of the nebenkern reveals a vacuolated appearance, the vacuoles being separated by chromophilic plates, which also reach the outer chromophilic cortex (Fig. 26*b*, Pl. II). Prominent granules are present on these plates towards the centre. Generally there is a large granule in the centre of the nebenkern, surrounded by other granules forming a single layer around it.

In the next stage (Fig. 26*c*, Pl. II) the differentiation of the nebenkern into an outer chromophobic and an inner chromophilic zone has started. The cortex becomes thicker than before. Its one half has become chromophobic, while the other half is still chromophilic. Chromophilic plates separating the vacuoles meet as usual the chromophilic part of the cortex, but have been withdrawn from the chromophobic half. Gradually the chromophilic portion of the cortex also becomes chromophobic, and thus a typical insect nebenkern with an outer chromophobic and an inner chromophilic area is formed (Figs. 26*d* and 25, Pl. II). Vacuoles disappear completely from the medullary region. A distinction between the chromophilic and chromophobic portions of the nebenkern could not be observed in the living spermatids (Fig. 69, Pl. IV).

By this time a fully formed acroblast has been formed between the nucleus and the nebenkern (Figs. 24 and 25, Pl. II; Fig. 69, Pl. IV) presumably by the fusion of the Golgi bodies observed in an earlier stage (Fig. 23, Pl. II). Sometimes the acrosomal granule appears in the interior of the acrosomal vesicle (acroblast) almost simultaneously with the first appearance of the acroblast (Fig. 70, Pl. IV). This figure is from a living cell studied under the phase contrast, and it will be noticed that here the acroblast appears in the form of a vesicle and not a crescent. There seems to be hardly any doubt that the crescents are optical sections of spheres. A few Golgi bodies (*G*) do not co-operate in the formation of the acroblast, and they are ultimately sloughed off down the tail.

After the acroblast has been fully formed, an axial filament can be seen arising from the centriole, which is attached to the nucleus (Fig. 25, Pl. II). The axial filament sooner or later comes to lie over the mitochondrial nebenkern and lengthens towards the posterior end (Fig. 27, Pl. II). The spermatid also elongates along its future long axis.

Living spermatids with elongating nebenkerns were frequently observed under the phase contrast microscope. In such a cell the centriole is prominent (Fig. 71, Pl. IV), and in some cases the axial filament can also be seen projecting beyond the free end of the mitochondrial nebenkern (Fig. 72, Pl. IV).

After the mitochondrial nebenkern gets attached to the centriole, it starts elongating along the future long axis of the sperm. It shows the usual chromophobic and chromophilic areas (Fig. 28, Pl. II). Sooner or later the chromophobic area is divided into two halves (Fig. 28*a*, Pl. II). But sometimes the mitochondrial nebenkern begins to elongate before the final distinction into an outer chromophobic and an inner chromophilic area is fully established (Fig. 29, Pl. II).

Shortly after the nebenkern has started elongating a new substance makes its appearance in the outer chromophobic area (Fig. 30, Pl. II). It

appears in the form of very fine granules, which are so disposed that they look like cross lines. This new substance is identical with the 'central substance' of Bowen (1922) or the 'sheath substance' of Nath (1925). The chromophilic area of the nebenkern is now pushed to the posterior end. Golgi remnants (*G*) are seen going back. The nebenkern goes on elongating; its central substance becomes more and more prominent; and its chromophilic area is progressively reduced (Fig. 32, Pl. II) till it ultimately disappears (Fig. 32, Pl. II). With the final disappearance of the chromophilic area the nebenkern divides completely into two right and left halves, which enclose the axial filament between them (Figs. 32 and 33, Pl. II).

The two halves of the mitochondrial nebenkern now spin out into threads. A series of bleb-like swellings appear along each half of the nebenkern, and the intermediate portions become correspondingly thinned out (Fig. 35, Pl. II). A few large swellings first formed (Fig. 34, Pl. II) are sub-divided as the sheaths spin out; and as the sheaths spin out the individual swellings become smaller but much more numerous. Bleb-like swellings sooner or later disappear completely (Fig. 37, Pl. II; and Fig. 38, Pl. III).

During all these stages, i.e. up to the formation of the mitochondrial sheath of the sperm tail, the centriole always remains at the posterior aspect of the nucleus. But after the sheath is formed, it starts moving towards the anterior end of the nucleus. While it moves anteriorly the centriole takes along with it some portion of the mitochondrial nebenkern in the form of blebs (Fig. 36, Pl. II; and Figs. 40, 41, 44 and 46, Pl. III).

Reverting to the subject of acrosome-formation, a prominent granule, the acrosomal granule, is secreted by the acroblast and lies attached to its anterior end (Figs. 40 and 41, Pl. III). This granule is deposited on the nuclear membrane at its posterior aspect. After the acrosomal granule is deposited on the nucleus (Fig. 42, Pl. III) the acroblast starts moving backwards, and is finally sloughed off along with other Golgi bodies (Figs. 45 and 46, Pl. III). Simultaneously, the acrosomal granule itself starts moving forward along the periphery of the nucleus, and deposits itself at its anterior aspect. At the same time the centriole, which formerly had acquired an anterior position, moves backward and reassumes its posterior position. These shiftings of the centriole and the acrosomal granule seem to be partly due to the rotation of the nucleus (Fig. 43, Pl. III).

The acrosomal granule grows in size (Fig. 43, Pl. III), and is then differentiated into the *acrosomal triangle.* This consists of 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* (Figs. 47, 49 and 52, Pl. III). In Figs. 46 and 48, Pl. III, the shelf of the acrosomal triangle, as seen from above, has been represented. Gradually the shelf of the acrosomal triangle breaks up into fine granules, which later disappear from view, along with the apical granule of the triangle (Figs. 53–58, Pl. III).

The time at which the acrosomal granule is differentiated into the acrosomal triangle varies considerably. This differentiation may take place when the acrosomal granule is at the posterior end of the nucleus, i.e., just after its deposition (Fig. 47, Pl. III); or on its way to the anterior

end (Figs. 44 and 50, Pl. III); or again when the acrosomal granule has reached the anterior end (Fig. 45, Pl. III). Generally this differentiation takes place before the acrosomal granule has reached the anterior aspect of the nucleus.

After the acrosome is formed, the cytoplasm surrounding the nucleus and the axial filament gets attenuated rather suddenly (Figs. 48, 49 and 50, Pl. III). The centriole, which was hitherto only a small granule, increases in size and becomes conical in form (Fig. 49, Pl. III). The nucleus shows a small chromatin granule in its interior. At this stage the nucleus stains feebly, but soon after it starts staining deeply (Figs. 51 and 52, Pl. III).

The nucleus, which was hitherto spherical, now starts elongating. Its posterior region elongates first, and the nucleus thus becomes pear-shaped (Figs. 53-55, Pl. III). The anterior end of the nucleus now also starts elongating and the centriole can no longer be distinguished from the nucleus (Fig. 56, Pl. III). The nucleus further elongates and becomes rod-like with the acrosome at one end and the axial filament at the other (Fig. 57, Pl. III). At a later stage the central portion of the nucleus does not stain as darkly as the peripheral, so that a clear medullary space appears in sections (Fig. 58, Pl. III).

As the nucleus further elongates a deeply-staining spherical body appears at its anterior end, and a similar body appears at a short distance in front of its posterior end. Both these bodies stain deeply with haematoxylin. They are continuous with the chromatin of the nucleus, which is in the form of a spirally arranged coil. This chromatin coil stains deeply at the base of the nucleus, but its staining capacity decreases as we proceed from the base to the apex (Fig. 59, Pl. III).

In a fully ripe sperm the anterior deeply-staining body completely disappears from view, but the posterior one persists. The nucleus is thin and shows chromatin in the form of a spiral thread. This chromatin thread runs for a short distance behind the posterior spherical body. The acrosome in the ripe sperm is small, but the axial filament is very long (Fig. 60, Pl. III).

Fig. 74, Pl. IV, represents a living sperm, which has not yet become fully ripe, as studied under the phase contrast microscope. The chromatin spiral has not yet been fully formed; nor has the anterior nuclear body yet disappeared. It was possible to see many sperms moving under the phase contrast microscope. Movements of the sperm are rhythmical. A wave starts at the anterior end of the sperm and passes backwards. This wave is responsible for the forward movement of the sperm.

Discussion

Perhaps the most important conclusion arrived at in this communication is that the Golgi body is either granular in form or, when it grows, it assumes a vesicular appearance with a chromophilic cortex and a chromophobic medulla. Studies of the fresh material under the phase contrast miscroscope always revealed this spherical form of the Golgi body. The

conclusion, therefore, is inevitable that the crescents met with in sectioned material are the optical sections of the Golgi vesicles.

During the last twenty years or so, Nath has been laying stress on the vesicular nature of the Golgi element (vide Nath, 1944). These views have been confirmed by Baker (1949), who employed the technique of phase contrast microscopy, describing the Golgi element as consisting 'of separate bodies, spheroid in shape'.

Another point, which is worthy of special notice, is that the acrosomal granule, which is the fore-runner of the acrosome, is deposited in the chromophobic interior of the Golgi vesicle or the acroblast (Fig. 70, Pl. IV).

It may also be noted that there is a well-marked process of chondriokinesis inasmuch as each mitochondrial filament is cut across during both the meiotic divisions.

With regard to the spermatid nucleus, it is of special interest to note that it cuts off a small portion, which degenerates in the spermatid cytoplasm (Figs. 17–19, Pl. I). A somewhat similar phenomenon has been described by Bowen (1922, 1922-23, 1924) in the Lepidoptera, Hemiptera and Coleoptera respectively. This phenomenon is obviously related to the condensation of the nuclear contents during spermateleosis.

Lastly mention may be made of two spherical, deeply-staining bodies, one at the anterior end and the other a short distance in front of the posterior end of the nucleus of the maturing sperm (Fig. 59, Pl. III; and Fig. 74, Pl. IV). Soon the anterior body disappears completely, but the posterior one becomes a permanent feature of the ripe sperm (Fig. 60, Pl. III).

Summary

1. The spermatogenesis of *Acheta domesticus* Linn. (*Gryllus domesticus*) has been worked out in this paper.

2. In the spermatogonium Golgi bodies and mitochondria are present in the form of granules. In the primary spermatocyte the mitochondria first appear in the form of one or two cloudy masses. They then become granular and later vesicular, and finally they are converted into threads, which surround the spindle area during the first maturation division.

3. The secondary spermatocyte is like the primary except for its small size. The mitochondria are first in the form of vesicles and later they become thread-like.

4. In the spermatid the mitochondria fuse to form a mitochondrial nebenkern with an outer chromophobic and an inner chromophilic area. The nebenkern elongates, and with the complete disappearance of the chromophilic area, it divides into two halves with the axial filament passing between them. The 'central substance' appears in the chromophobic area.

5. Observations made under the phase contrast microscope have convinced us that the real form of the Golgi body is either granular or vesicular. The acroblast also has been seen in the living cell as a large vesicle, with the acrosomal granule developing within its interior.

6. The nucleus of the ripe sperm is spiral in structure; and a nuclear body at the posterior end of the nucleus is a permanent feature of the ripe sperm.

Explanation of Lettering in the Plates

A.—Acrosome; A_1.—Acroblast; *A.f.*—Axial filament; *A.g.*—Acrosomal granule; *C*.—Centriole; *Ch*.—Chromosome; *C.s.*—Central substance; *G*.—Golgi body; *M*.—Mitochondria; *M.n.*—Mitochondrial nebenkern; *M.b.*—Mid-body; *N*.—Nucleus, N_1.—Nucleolus; *S.f.*—Spindle fibres.

Explanation of Figures

All the figures of the first three plates have been drawn with camera lucida at the table level with 10× eyepiece and oil immersion objective, giving approximately a magnification of 1,700 times, and they are all from fixed preparations. Figures 57, 59 and 60 are from smears. Figures of Plate IV are from the living material studied under the phase contrast microscope.

Plate I

Fig. 1. Spermatogonium showing granular Golgi bodies and mitochondria.
,, 2. Spermatogonial metaphase.
,, 3. Polar view of the spermatogonial metaphase.
,, 4. Very early primary spermatocyte.
,, 5. Early primary spermatocyte showing granular mitochondria.
,, 6. Primary spermatocyte showing vesicular mitochondria.
,, 7. Primary spermatocyte showing vesicular mitochondria elongating.
,, 8. Primary spermatocyte showing thread-like mitochondria.
,, 9. Metaphase I.
,, 10. Polar view of metaphase I.
,, 11. Telophase I.
,, 12. Early secondary spermatocyte showing vesicular mitochondria.
,, 13. Secondary spermatocyte showing thread-like mitochondria.
,, 14. Early telophase II.
,, 15. Polar view of Metaphase II.
,, 16. Late telophase II.
,, 17. Early spermatid.
,, 18. Spermatid showing darkly-staining portion of the nucleus being separated from the lightly-staining portion.
,, 19. Darkly-staining portion completely cut off and degenerating.
,, 20. Full grown spermatid.

Plate II

Fig. 21. Spermatid showing the formation of the mitochondrial nebenkern.
,, 22. Spermatid at a later stage.
,, 23. Spermatid showing Golgi crescents between the nucleus and the mitochondrial nebenkern.
,, 24. Spermatid showing a fully formed acroblast.
,, 25. Spermatid showing fully formed mitochondrial nebenkern, acroblast and axial filament.
,, 26. Four stages in the formation of the mitochondrial nebenkern.
,, 27. Spermatid showing axial filament passing over the mitochondrial nebenkern.
,, 28. Spermatid with elongating nebenkern
,, 28*a*. Cross-section of the nebenkern showing division of the chromophobic area into two halves.

FIG. 29. Spermatid showing the elongation of the mitochondrial nebenkern before its final differentiation is fully established.

„ 29*a*. Cross-section of the mitochondrial nebenkern at above stage.

„ 30. Spermatid showing the appearance of the central substance.

„ 31. Further elongation of the mitochondrial nebenkern.

FIGS. 32-33. Spermatids showing complete disappearance of the chromophilic area of the mitochondrial nebenkern.

FIG. 34. Spinning out of the two halves of the mitochondrial nebenkern.

„ 35. Elongated spermatid showing bleb-like swellings along the axial filament.

„ 36. Spermatid showing the forward movement of the centriole.

„ 37. Spermatid showing complete disappearance of blebs.

PLATE III

FIGS. 38-39. Complete disappearance of blebs.

„ 40-41. Acrosomal granule attached to the acroblast and the nucleus.

FIG. 42. Acroblast going back after the deposition of the acrosomal granule on the nucleus.

„ 43. Acrosomal granule going forward and centriole moving backward.

„ 44. Differentiation of the acrosomal granule into the acrosomal triangle with a shelf at its base.

„ 45. Acrosomal granule at the anterior aspect of the nucleus.

„ 46. Spermatid showing the shelf of the acrosomal triangle, as seen from above.

„ 47. Differentiation of the acrosomal granule into a triangle just after its deposition.

„ 48. Spermatid after the complete disappearance of cytoplasm.

„ 49. Spermatid showing acrosomal triangle. Centriole is large and conical.

„ 50. Spermatid showing acrosomal triangle half way between the anterior and posterior ends of the nucleus.

FIGS. 51-52. Nucleus of the spermatid taking stain.

FIG. 53. Elongation of the nucleus and breaking up of the shelf of the acrosomal triangle into granules.

FIGS. 54-55. Disappearance of the granules and further elongation of the nucleus.

FIG. 56. Acrosome fully formed: centriole is not distinguishable from the nucleus.

„ 57. Nucleus has become rod-shaped.

„ 58. Sperm nucleus showing a clear medulla and a peripheral staining portion in a section.

„ 59. Early sperm showing chromatin in the form of a spiral. Two deeply-staining spherical bodies are present, one at the anterior end of the nucleus and the other a short distance in front of its posterior end.

„ 60. A fully ripe sperm. Anterior spherical body has disappeared.

„ 61. A portion of the tail of the spermatid showing Golgi remnants moving down the tail.

PLATE IV

FIG. 62. Spermatogonium.

„ 63. Primary spermatocyte showing thread-like mitochondria.

„ 64. Metaphase I.

„ 65. Secondary spermatocyte.

„ 66. Early Telophase II.

„ 67. Late telophase II.

„ 68. Spermatid showing the formation of nebenkern.

„ 69. Spermatid showing fully formed mitochondrial nebenkern and acroblast.

„ 70. Spermatid showing acrosomal granule within the acroblast.

„ 71. Spermatid showing the elongation of the nebenkern.

„ 72. Axial filament present at the end of the elongating mitochondrial nebenkern

FIG. 73. Secondary spermatocyte showing two spermatid nuclei. Cytoplasmic cleavage is delayed.

„ 74. Sperm just before maturity. Two spherical bodies in the nucleus are clearly visible.

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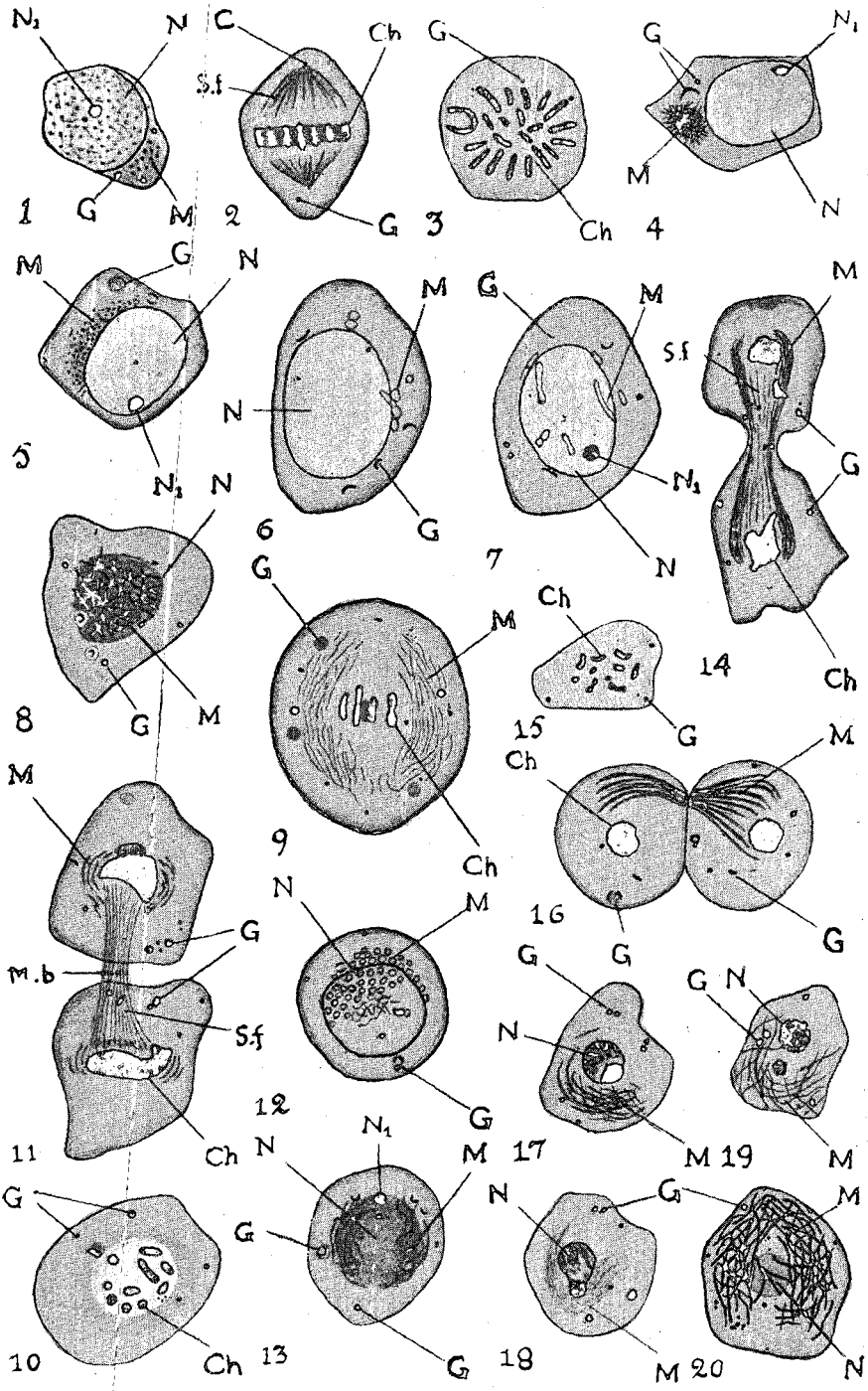
Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.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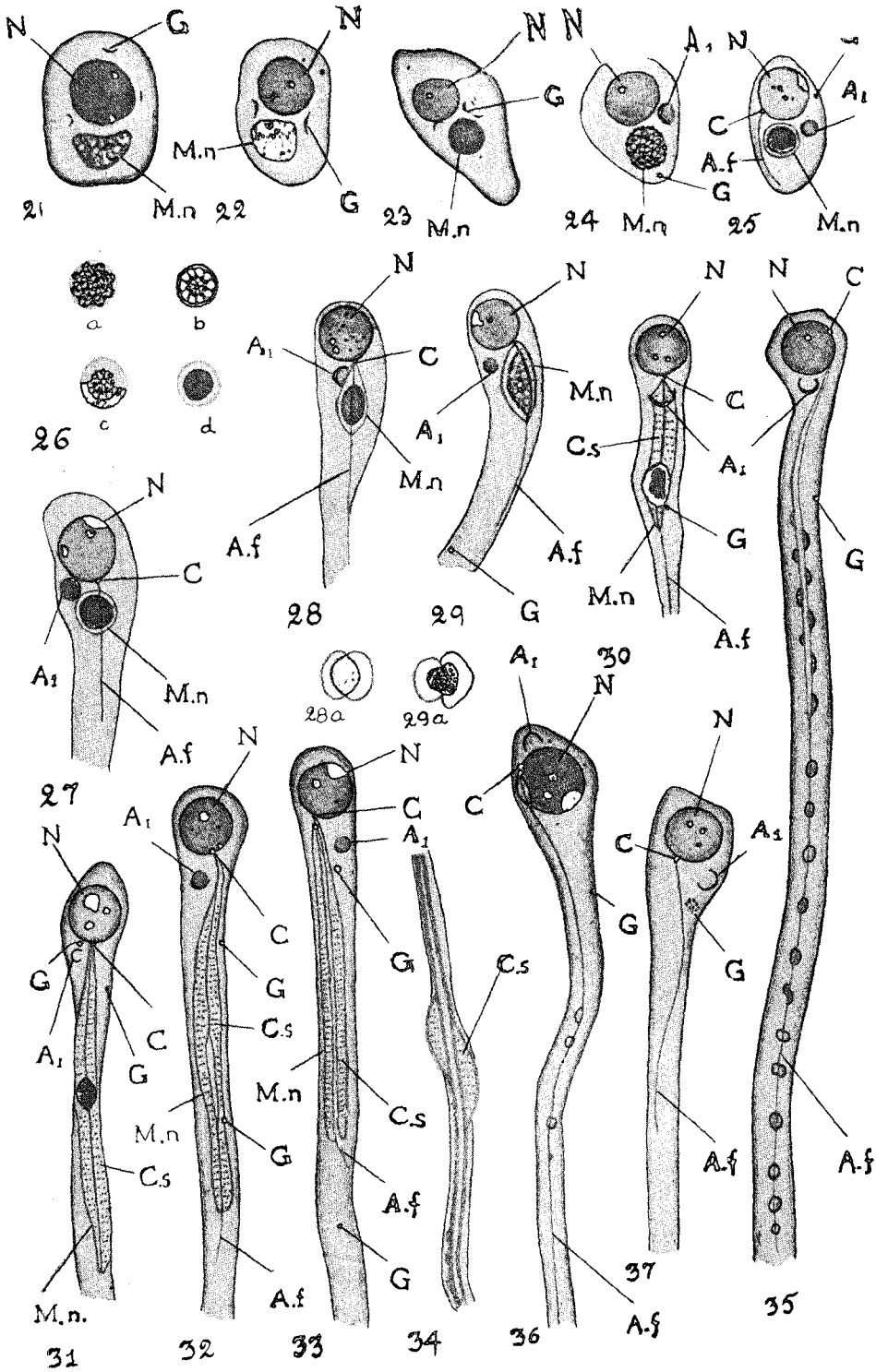
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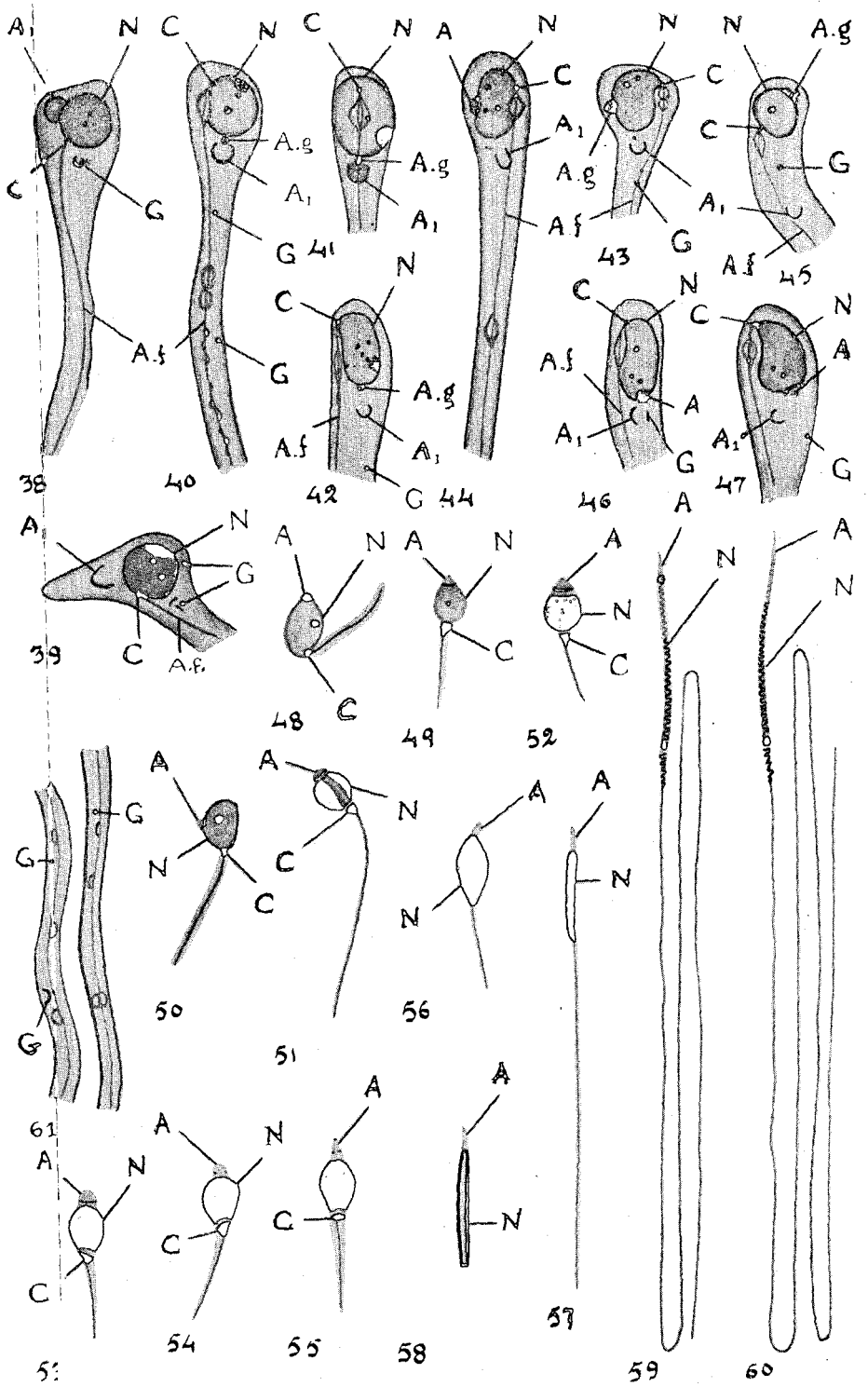
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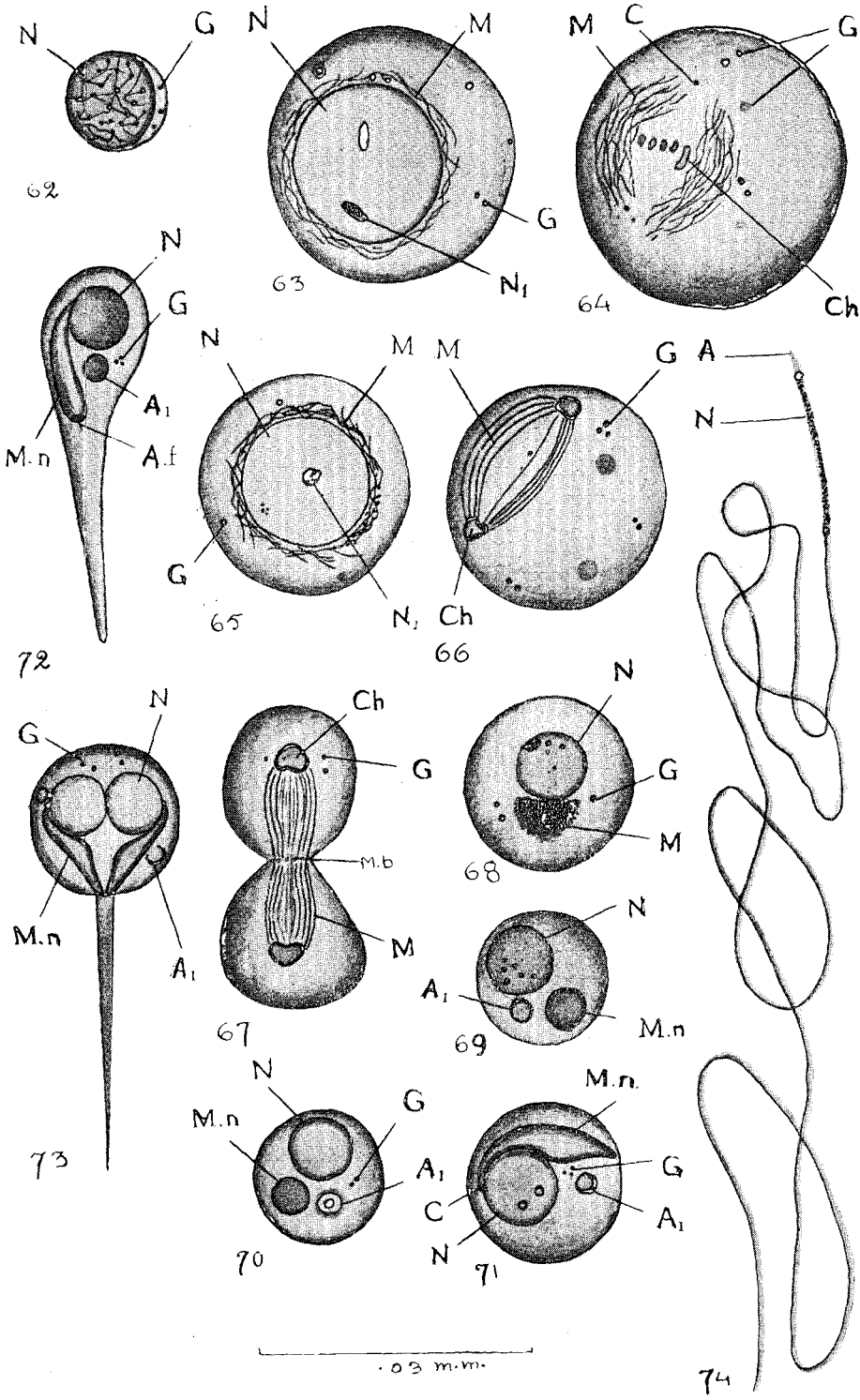
PLATE IV.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RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 38 ZOOLOGY Pp. 157-180

December, 1953

THE GUINEA-PIG SPERM

by

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Edited for the East Panjab University by Vishwa Nath,
Department of Zoology, East Panjab University,
HOSHIARPUR

Price Rs.3-8-0

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THE GUINEA-PIG SPERM

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Introduction

In spite of the fact that mammalian spermatogenesis has been the subject of considerable research for more than half a century, there still remain many controversial points. In most of the available literature *numerous bizarre descriptions of the morphology and functions of certain* cytoplasmic inclusions, especially of the Golgi apparatus, have been given.

The idiosome of the spermatocytes and spermatids aroused a great interest in many workers like Meves (1899) who was indeed much ahead of his times in describing clear granules, each enclosed in a vesicle, embedded in the idiosome. The workers following him, however, ignored this classical work and tried to attribute various shapes to the Golgi elements which are associated with the idiosome. By employing techniques which are far from being satisfactory there has been a good deal of confusion about the form and function of the various cytoplasmic inclusions.

The introduction of a new category of structures, like the 'Post-nuclear granules' of Gatenby and Wigoder (1929) has also stimulated research in the line of tracing the specific action of silver salts on certain cytoplasmic inclusions.

In view of the conflicting and varied descriptions of spermatogenesis in the same animal (*Cavia*) it was felt necessary to reinvestigate the various points in the light of modern work and to arrive at some definite conclusions.

This work was, therefore, started in October, 1942, by one of us (G. C. C.) in the laboratories of the Government College, Lahore (now in Pakistan), to investigate the exact nature of the post-nuclear body and the formation of the acrosome. The results of these investigations were published in the form of an abstract in the Proceedings of the 31st Indian Science Congress held at Delhi in 1944. Before, however, the detailed paper could be published partition of the country took place and all of us had to flee to this side of the border. Some years later, after we had established ourselves at Hoshiarpur, one of us (V. S. S.) prepared some more slides of the testicular material of the Guinea-pig obtained from the Medical College, Amritsar, and these were thoroughly studied by him and the senior author (G. P. S.) independently as well as jointly. The present paper is an account of the studies made both at Lahore and at Hoshiarpur.

Previous work

As such a lot of work has already been done on the spermatogenesis of mammals, it will not be possible for us to give here a complete account

of each and every paper available on the subject. An attempt will, however, be made to review as much of the literature as is relevant for this work.

Meves (1899), in his classical paper on the spermatogenesis of the guinea-pig has given a detailed review of the previous work on this subject. He describes a number of small granules (Kornchen) which are irregularly scattered in the idiosome (Idiozome) of the spermatocytes and the spermatids. This idiosome with granules lies adjacent to the nucleus. Each of these granules becomes enclosed in a vesicle (Blaschen) in the spermatid. Meves differs from Moore (1906) in that these vesicles do not necessarily arise before the appearance of granules in the idiosome. In the later spermatids the granules in the idiosome fuse together and ultimately give rise to a single bead which gets differentiated into two regions: the inner intensely black and the outer pale yellow in colour. This single differentiated bead grows to form the acrosome (Spitzenknopf) of the ripe sperm. When the outer and inner zones of the acrosome appear, the idiosome moves away and ultimately goes behind the nucleus. It then disappears or is sloughed off with the residual cytoplasm.

Meves has also given an extensive account of a complicated system of the central apparatus. He states that the centrosome, to begin with, is a dumb-bell-shaped body, situated in the idiosome of the primary spermatocytes. This body then comes out of the idiosome and consists of two distinct centrosomes in the spermatids. The proximal centrosome remains more or less connected with the nucleus, whereas the distal one moves towards the periphery of the cell. The ring-like centrosome, which is the product of the distal centrosome, moves down the axial filament and ultimately fuses with the base of the middle-piece of the ripe sperm. As a matter of fact the central apparatus is more complicated in the late spermatid than it is in the ripe spermatozoon.

A spindle-shaped vacuole has also been shown lying on the axial filament of the late spermatids. It moves downwards and ultimately disappears without performing any function. A 'Schwanzmanchette', which arises as a basket of threads in the region of the centrosomes, has also been described. This becomes a membranous tube which ultimately disappears apparently without serving any purpose.

Meves also mentions the chromatoid accessory body (Chromatoiden nebenkörper) which stains light red with Ehrlich's triple stain. This body lies in the cytoplasm on the side of the nucleus and near the idiosome. Meves differs from Moore (1906) who believes it to be a derivative of the extruded nuclear chromatin. Von Ebner's granules appearing in the cytoplasm of the later stages of spermateleosis are described as of fatty and osmiophilic nature.

In the mature spermatozoon, the tail is shown to be inserted on one side of the head and it has a protoplasmic bead on it. The posterior half of the nucleus in the ripe sperm is brighter than the anterior half.

Papanicolaou and Stockard (1918) have also traced the development of the idiosome in the germ cells of the male guinea-pig. According to them the idiosome in the primary spermatocytes is differentiated into two

regions: an outer blue-staining 'idioectosome' and an inner purple-staining 'idioendosome'. During the preparation for the first meiotic division the 'idioectosome' disappears and during the division itself the 'idioendosome' becomes scattered throughout the cytoplasm in the form of small granules which are named as 'idiogranulomes'. In the secondary spermatocyte appears a new 'idioectosome' containing the 'idiogranulomes' which, however, during the second meiotic division, are again scattered throughout the cytoplasm. In the reformed 'idioectosome' of the spermatid there is seen a clear vacuole, 'idiogranulotheca' surrounding each 'idiogranulome'. By rapid fusion the 'idiogranulomes' form a single large red-staining 'idiosphaerosome' enclosed in a large vacuole, the 'idiosphaerotheca' which in turn is formed by the fusion of the 'idiogranulothecae'. The 'idioectosome' which now begins to move away is renamed as the 'idiophthartosome'. In the meantime the 'idiosphaerosome' secretes a crescentic blue-staining 'idiocalyptosome' and is itself known from now onwards as the 'idiocryptosome'. In the ripe sperm the 'idiophthartosome' disappears along with the cytoplasm. The 'idiocryptosome' and the 'idiocalyptosome' together form a double cap, 'spermiocalyptra' for the sperm head. The 'idiosphaerotheca' persists through all the later stages and develops into a membranous cover for the cap and the head of the sperm and it is then known as the 'spermiocalyptrotheca'.

Gatenby and Woodger (1921) traced the origin of the Golgi apparatus on the middle-piece of the ripe sperm of *Cavia* and the development of the acrosome. Their description is mainly based on the preparations made by formalin-silver nitrate techniques of Cajal and Da Fano and long osmication process of Mann-Kopsch.

According to them the Golgi apparatus consists of an inner core of archoplasm and a cortical region of curved plates and rods called the dictyosomes. The appearance of the apparatus is, however, said to be sometimes reticular and sometimes homogeneous. Spherical mitochondria are scattered in the cytoplasm of the spermatocytes and the spermatids. A chromatoid body, similar to the one described by Meves (1899), has also been shown by them in the spermatocytes, spermatids and the division stages.

During the growth stages of the spermatocyte numerous tiny spheres known as the proacrosomic granules are differentiated within the archoplasm and Gatenby and Woodger believe that the Golgi elements or dictyosomes are associated with this differentiation. As already claimed by Papanicolaou and Stockard (1918) they also think, although they have not been able to establish it themselves, that the proacrosomic granules do not disintegrate during the division stages but retain their individuality and become scattered throughout the cytoplasm. These are subsequently distributed to the daughter cells and eventually come to lie within the re-formed archoplasm of the spermatid. Each proacrosomic granule, at this stage, is said to have a liquid-filled sphere around it and thus comes to lie in an archoplasmic vacuole. These proacrosomic granules lying in their respective archoplasmic vacuoles now come together and fuse gradually to form a fewer and larger granules. Finally, by this process of fusion,

is formed one large granule the proacrosome which is surrounded by a single archoplasmic vacuole. An inner darkly-staining area and a paler cortical zone are soon differentiated in this proacrosome. The Golgi apparatus complex now moves up and becomes applied to the anterior end of the spermatid nucleus. The proacrosome itself moves through the archoplasm, comes into close contact with the nuclear membrane and is now known as the acrosome. The archoplasmic vacuole still surrounds it except on one side where it is in contact with the nucleus and thus separates it from the archoplasm and the Golgi elements. The acrosome grows rapidly while the Golgi elements with the archoplasmic remains are drifting away from it, and develops by itself to attain its final form. The Golgi apparatus and the mitochondria now lie posterior to the nucleus and when the spermatid is elongating the former buds off a small part which ultimately takes up its position in the protoplasmic bead lying on the middle-piece of the mature sperm. Most of the mitochondria are grouped around the axial filament in the middle-piece. The remaining mitochondria, the main part of the Golgi apparatus and almost the entire cytoplasm are sloughed off the maturing sperm.

Ludford and Gatenby (1921) have shown that the 'Golgi apparatus in *Mus* and *Cavia* is a mere cortical layer plastered upon the surface of the centrosphere (archoplasm), separate dictyosomes or rods of any kind being apparently absent'. It has also been shown that the process of dictyokinesis is generally haphazard, at least in *Mus* and *Cavia* and is merely an unprecise sorting out of the Golgi elements between the two daughter cells.

During the dictyokinesis of *Mus*, Ludford and Gatenby have described the Golgi elements breaking away from the archoplasm and becoming scattered in the cytoplasm, while the archoplasm itself undergoes the process of fission. When the chromosomes are sorted out to the two daughter cells, the dictyosomes assemble together again to become attached to the archoplasm. The cause of dispersal of the Golgi elements is said to be the division of the centrosome.

During the early phases in the development of the spermatocyte of *Cavia*, on the other hand, the archoplasm to which the dictyosomes are attached, is shown to have remarkable frothing. Small bubbles appear on the periphery of the archoplasm and these disappear at the end of the diakinetic stage of the chromosomes. The dictyosomes are seen as distinct semilunar rods attached peripherally to the archoplasm, after the frothing disappears. The rest of the process of dictyokinesis is the same as in *Mus*.

Along with the Golgi elements the chromatoid body is also said to divide in equal parts during the process of dictyokinesis and each spermatid is shown to have a single chromatoid body, directly derived from that of the spermatogonium.

Rau and Brambell (1925), working on the living male germ cells of *Cavia* (also of rat and mouse) by Lewis method and with Janus green-staining, have confirmed the account of Gatenby and Woodger (1921). They have demonstrated the Golgi rods and dictyosomes but have failed to show the proacrosomic granules inside the archoplasm.

In 1929 Gatenby published a paper on the Golgi apparatus and vacuolar system of *Cavia*, *Helix* and *Abraxas* by intra-vital methods. This writer has shown that in the male germ cells there are a number of neutral red-staining vacuoles lying near, but not attached to, the Golgi dictyosomes. In some cases the vacuoles are in the archoplasmic area, at the periphery of which the Golgi dictyosomes lie, while in others they are extra-archoplasmic. These vacuoles are distinct from the Golgi dictyosomes, not only in the resting cells but also during the meiotic divisions when they are sorted out roughly into two equal parts. According to Gatenby these vacuoles are not consistently argentophilic, whereas the dictyosomes, from which these vacuoles are derived, are. Gatenby also gives an account of the work of Hirschler, Monne and Voinov on the male germ cells of lizard and grasshopper. These authors, as quoted by Gatenby, have also shown a vacuolar system, lying near, but distinct from the Golgi dictyosomes.

In the same year Gatenby and Wigoder (1929) recognized a new category of structures, the 'post-nuclear bodies' in the spermatogenesis of *Cavia cobaya* and other animals. It may be remarked that Gatenby (1918) had already made a mention of such bodies in the male germ cells of certain molluscs. In the ripe sperm of *Cavia cobaya*, Gatenby and Wigoder show an argentophilic band in the posterior region of the nucleus and they mention that 'no method except Da Fano picks out these bands in such an extraordinary manner'.

They have tried to trace back the history of this argentophilic band to the spermatocyte where the post-nuclear granules are shown to form the cortex of a vacuole in the vicinity of the Golgi apparatus but later on the vacuole disappears. In the spermatid these granules are grouped behind the nucleus and are described as 'elongate, hollow structures, which seem to expand till they touch and finally unite to form the solid covering behind the nucleus'. 'This expands further till it forms the cup-like structure, depicted in the spermatozoon'. They have, however, failed to give any test which can distinguish these granules from the other cytoplasmic inclusions like the Golgi bodies, mitochondria or the centrosomes.

Technique

It is a well-known fact that the morphology of a cell depends mainly on the technique employed. The different forms under which the Golgi apparatus of the same material has been described can be attributed to the different methods used. A good deal of time was, therefore, spent in trying almost all the techniques recommended by modern cytologists.

Chloroform was used as a light anaesthetic. The testes were quickly removed from the body of the animal and cut into small pieces with a razor blade in normal saline. These pieces were placed in different fixatives as quickly as possible.

The fixatives tried were those of Bouin, Champy, Da Fano, Flemming, Flemming-without-acetic, Kolatchev, Regaud and Zenker. The chilled Da Fano method recommended by Gatenby and Wigoder (1929) was also tried by cooling down the capsules containing fixative from 3° to 6° C.

Smears of the testicular material were also made in Flemming, Flemming-without-acetic, Bouin's and Da Fano's fixatives.

The mordant used was 4% or 5% iron-alum and the stain 0·5% hæmatoxylin. F.W.A. and Champy fixed sections were treated according to Kull's modification and stained with acid fuchsin. Da Fano's smears were at times toned in Manns' methyl blue eosin. Unstained F.W.A. and Champy sections were also mounted.

Feulgen's reaction was tried on sections and smears fixed in Flemming, Flemming-without-acetic, Champy and Zenker's fluids. For this reaction Flemming's fluid was found to be the best.

Breinl's preparations for the differentiation of the nucleus from the acrosome were also made. Both the Feulgen and Breinl preparations were found useful for determining the exact nature of the post-nuclear body.

As a result of our experiments with different techniques it was found that Flemming-without-acetic and Champy preserve all the cell inclusions perfectly.

Fresh material, teased out in a drop of 0·9% normal saline, on sterilized slides, was observed under oil immersion lens for short periods, both in reduced light and with full aperture.

Slightly osmicated material was also studied either in normal saline or in 2% osmic acid solution.

The manuscript of this paper was almost ready for the press when one of us (G. P. S.) had an opportunity to study the male germ cells of the guinea-pig under the phase contrast microscope. In general, this study also confirmed the results obtained from the fixed material.

Observations

Spermatogonia: The spermatogonia in the guinea-pig are fairly large cells, usually found near the periphery of the follicles (Plate I, Fig. 1). In each of them there is a vesicular nucleus which contains inside it not only fine chromatin granules but also big chromatin masses. Distributed all over the cytoplasm are fine granules which appear pale yellow with iron-hæmatoxylin stain. These are the mitochondria. The Golgi elements exist in the form of a juxta-nuclear mass of darkly-staining granules which are definitely bigger in size than the mitochondria. In the fully grown spermatogonia the Golgi elements also become spread throughout the cytoplasm (Plate I, Fig. 2).

During the spermatogonial metaphase (Plate I, Fig. 3) the chromosomes arrange themselves at the equator of the spindle which is a fibrillar structure. At each pole of the spindle there is a sharply-staining granule, the centrosome, but it is completely devoid of the astral rays. The cytoplasm reveals fine granules which are the mitochondria. It has not, however, been possible to demonstrate the Golgi elements with certainty during the spermatogonial divisions.

Primary spermatocytes: The primary spermatocytes are definitely larger cells than the spermatogonia, their nuclei also being larger (Plate I, Fig. 4). In this particular cell the chromosomes have already been

differentiated in the nuclear area. The mitochondria, which are scattered throughout the cytoplasm, are small and granular. The Golgi elements, at this stage, have a distinct tendency to come together to form bigger granules. Some of these big Golgi granules have already collected together to be in the localized condition which is so prominent in the secondary spermatocytes and the spermatids.

The primary spermatocytes in metaphase are quite common (Plate I, Fig. 5). The spindle area stands out from the general cytoplasm by being less darkly stained than the latter and is presumably of a different consistency. The spindle fibres are also clearly visible. At each pole of the spindle is a small but distinct centrosome. The Chromosomes appear at the equator of the spindle as a deeply-staining mass in which the individual elements cannot be clearly made out. The finely granular mitochondria remain scattered throughout the cytoplasm. Two Golgi granules (one big and the other small) are also seen separating in the cytoplasm.

In the telophase I (Plate I, Fig. 6) the chromosomes are grouped at the two poles of the spindle. The centrosomes are hardly distinguishable in these stages. The mitochondria still remain spread throughout the cytoplasm but some of the Golgi elements can now be clearly seen within the spindle area also. As the cell divides both the mitochondria and the Golgi elements are almost evenly distributed to the two daughter cells.

Secondary spermatocytes: Each resulting secondary spermatocyte is again smaller than the primary (Plate I, Fig. 7). While the mitochondria in it remain distributed throughout the cytoplasm, most of the Golgi elements have collected together to be in the typical localized condition. At this stage one can see a number of small osmiophilic Golgi granules embedded in a spherical or ovate osmiophobic area which appears to be nothing but a denser portion of the cytoplasm. This is the idiosome. The rest of the Golgi granules still remain scattered in the cytoplasm and some of these have been referred to as the chromatoid bodies by the previous workers.

During the second meiotic division the chromatin of the nucleus gives rise, as usual, to the regular chromosomes. The centrosomes become active once again and they take up their position at the two poles of the spindle which reveals faintly staining spindle fibres (Plate I, Fig. 8). Like the mitochondria, the Golgi elements also get dispersed in the cytoplasm at this stage. Some of the Golgi elements can even be seen lying on the spindle fibres between the two groups of chromosomes (Plate I, Figs. 8 and 9). After the process of nuclear reconstruction the cell divides and we thus get two daughter cells—the spermatids. During this division also the mitochondria and the Golgi elements are sorted out, more or less evenly, to the two spermatids thus formed.

It is important to note that some of the secondary spermatocytes do not divide for a long time even after the nuclei of the two daughter spermatids have been completely formed in them. One such example is illustrated in Figure 10, Plate I.

Spermatid: The earliest spermatid is a comparatively small cell with a compact nucleus (Plate I, Fig. 11). Most of the Golgi elements in it have

once again come to lie in the special chromophobic and denser area in the cytoplasm known as the idiosome. The rest of the Golgi elements, just like the mitochondria, remain scattered throughout the cytoplasm.

Spermateleosis: The process of spermateleosis is heralded by a distinct tendency of the Golgi elements to come together. This results in the formation of a fewer and bigger Golgi granules not only in the idiosome but also in the cytoplasm (Plate I, Figs. 11 and 12). The fusuion of the Golgi granules in the idiosome, however, continues till only a single big Golgi granule is produced (Plate I, Figs. 13 to 17). This is situated almost in the centre of the idiosome (Plate I, Fig. 17). Soon after its formation it becomes surrounded by a clear vacuole which gradually increases in size as the idiosome moves towards one side (Plate I, Figs. 18 to 21). The single Golgi granule now moves through the vacuole and comes in intimate contact with the nuclear membrane (Plate I, Fig. 22). This Golgi granule may, at this stage, be termed as the acrosome. To begin with, it is a small structure (Plate I, Fig. 22), but it increases in size gradually (Plate I, Figs. 23 and 24). The nucleus which had been almost perfectly spherical till this stage now grows under the vacuole in the form of a conical projection with the acrosome situated at its tip (Plate II, Fig. 25). The acrosome in all these stages has been a big darkly-staining granule but from now onwards it becomes differentiated into two regions—an inner darkly-staining and an outer lightly-staining (Plate II, Figs. 26 to 31).

Simultaneously when the single Golgi granule, which ultimately gives rise to the acrosome, is being formed in the idiosome another equally big Golgi granule appears to pass out of the idiosome into the cytoplasm (Plate I, Figs. 18 and 21). This seems to be the homologue of the accessory body described in a number of mammals by Gresson and Zlotnik (1945). From this it appears to us that all the Golgi elements in the idiosome are not used up in the formation of the acrosome. This is further proved by the presence of the Golgi granules in a small portion of the idiosome which is separated from the main mass of the idiosome as depicted in Figure 19, Plate I. It is also quite likely that the small portion of the idiosome with Golgi elements in it may be the equivalent of that portion of the Golgi apparatus complex which, according to Gatenby and Woodger (1921), gives rise to the Golgi apparatus on the middle-piece of the ripe sperm. On the other hand, the portion of the idiosome without Golgi granules may be the subspherical body which, according to Gresson and Zlotnik (1945), separates at this stage from the Golgi apparatus complex of the Rabbit. In any case, after the acrosome has been formed, the remains of the idiosome with or without the Golgi elements drift backwards from the anterior end of the nucleus and in this study they have all been labelled as the Golgi remnants (Plate I, Figs. 19 to 24 and Plate II, Figs. 25 to 33).

At this stage one can also see an axial filament growing out from a small granule lying in the cytoplasm. This granule is the proximal centrosome (Plate II, Figs. 31 to 33 and 35 to 37). Soon afterwards the distal centrosome also makes its appearance just near the proximal and it is in the form of a ring which encircles the axial filament (Plate II, Fig. 38) It appears that, when the spermatid begins to elongate, the originally

single centrosome of the early spermatid divides into two—one forming the granular proximal centrosome and the other ring-like distal centrosome. The latter now begins to travel down the axial filament (Plate II, Fig. 39 and Plate III, Fig. 40) till ultimately it takes up its position at the posterior end of the sperm middle-piece. In the meanwhile a somewhat darkly-staining area of the cytoplasm is differentiated immediately behind the elongating nucleus. This is the 'manchette' which forms the lateral boundaries of the middle-piece while the two centrosomes form its anterior and posterior boundaries (Plate II, Fig. 37 and Plate III, Figs. 40 to 42). The mitochondrial granules which are included in this area now become darkly-staining (Plate III, Fig. 41). It seems that by a process of fusion they form bigger and prominent mitochondrial granules which ultimately arrange themselves around the axial filament to form the middle-piece of the sperm (Plate III, Figs. 43 to 45 and 47 to 49). The rest of the mitochondria along with the Golgi remnants and also those Golgi granules which did not assemble in the idiosome in the earlier stages remain lying in the residual cytoplasm (Plate III, Figs. 43 to 45). All these apparently useless inclusions will now either degenerate or will be sloughed off with the residual cytoplasm during the final maturation of the sperm. Some of the Golgi elements at this stage are, according to a number of previous workers, converted into fat but we are not in a position to say anything definitely about it as no special tests were performed for detecting fat in our preparations.

During all these changes the acrosome which had earlier taken its position at the anterior end of the elongating nucleus can be clearly seen spreading itself gradually like a cap along the sides of the nucleus till ultimately it covers roughly more than half of the sperm nucleus (Plate II, Figs. 34 to 39 and Plate III, Figs. 40 to 42).

Spermatozoon: The sperm of the guinea-pig (Plate III, Figs. 46 to 50) reveals almost the same structure as that of the typical mammalian sperm. It has a broad head and a long tail with a small but distinct neck in between them.

The head consists of a nucleus and an acrosome. The acrosome which covers roughly more than half of the sperm nucleus appears to be in the form of a broad cap-like structure in surface views (Plate III, Figs. 48 to 50) but in side views it seems to be produced into a conical projection (Plate III, Figs. 46 and 47). It is often differentiated into two regions which were also noted earlier during spermateleosis. The outer of these is lightly-staining and the inner deeply-staining. The nucleus is more or less an oval body with a flattened posterior border. Its anterior half which is covered over by the acrosomal cap appears to be darker than the posterior uncovered portion (Plate III, Figs. 48 and 49).

The tail is attached to the posterior end of the nucleus. The neck which comes in between the head and the tail is bounded on both sides by two fine threads (Plate III, Figs. 47 to 49). On the middle-piece is very often seen a small portion of the cytoplasm containing the Golgi elements (Plate III, Fig. 46). This is the protoplasmic bead which seems to travel down the middle-piece till it is finally lost during the passage of the sperms

through the sperm ducts. Distinct mitochondria can also be clearly seen forming the middle-piece of the sperm (Plate III, Figs. 43 to 45 and 47 to 49).

The so-called post-nuclear body shown at the posterior end of the nucleus of the ripe sperm of *Cavia cobaya* by Gatenby and Wigoder (1929) was never seen in any of the numerous preparations made by us. In Feulgen's and Breinl's preparations it has been clearly seen that the post-nuclear body of Gatenby and Wigoder (1929) is in reality the dense posterior region of the nucleus itself and is not a separate body as urged by them (Plate III, Fig. 50). In some of our Da Fano's smears, however, silver is deposited on the posterior part of the nucleus of the ripe sperm and this may correspond to the so-called post-nuclear body of Gatenby and Wigoder (1929).

During the study of the living material it was observed that the sperms move by means of the lashing movement of the tail and they maintain their motility for a considerably long time. They often move in groups of three or four with their heads one above the other. These sperm aggregates give the impression as if they constitute the conjugate sperms. Sperms in side views reveal that the heads are convex on one side and concave on the other.

Discussion

Golgi elements: It will be recalled that in the earliest spermatogonia of the guinea-pig the Golgi elements exist in the form of a juxta-nuclear mass of darkly-staining granules which are definitely bigger in size than the mitochondria. In the fully grown spermatogonia, however, the Golgi elements, like the mitochondria, become spread throughout the cytoplasm. In the primary spermatocytes the Golgi elements reveal a distinct tendency to come together to form bigger granules. Some of these big Golgi granules now collect together to be in the localized condition which is so prominent in the secondary spermatocytes and the spermatids.

In its localized form the Golgi apparatus has very often been described as a network. In fact it was the general opinion of Golgi, his pupils and many other workers that the Golgi apparatus in the cell was characterized by its reticulate form. That the reticulate or network-like appearance of the Golgi apparatus is produced as a result of certain fixatives is admitted even by Gatenby and Woodger (1921), according to whom, the Golgi apparatus in *Cavia* with formalin-silver nitrate techniques 'either appears as a reticulum, or the whole cortex of the apparatus reduces the silver, and then appears as homogeneous: with Mann-Kopsch techniques the individual dictyosomes are often very clearly marked'. In regard to the Golgi nets it will be refreshing to quote what Nath said in 1944. 'For many years since the discovery of the 'apparato reticolare interno' by the silver method, nobody seems to have questioned the validity of Golgi networks. The various modifications of the original silver method employed by Golgi were followed by the long osmication methods, which are, at best, only slightly less unsatisfactory. There is no doubt that the Golgi material has a marked affinity for silver and osmium, and further

it has been made abundantly clear that the Golgi elements are often held in chains or are closely aggregated in a juxta-nuclear position. When silver nitrate or osmium tetroxide is reduced to metallic silver or osmium respectively, the reduced particles seem to come down like a shower and are deposited not only on the surface of and in the interior of the Golgi elements, but also in the narrow spaces between them. The result is a network! . . .'

Nath (1944) continues—'It is pertinent to ask: Have ever Golgi nets been demonstrated by methods not involving reduction of silver nitrate or of osmic acid for long periods? To the best of my knowledge they have never been. On the contrary, various miscellaneous methods (e.g., Champy or Flemming-without-acetic and iron-hæmatoxylin) have invariably demonstrated discrete Golgi elements in a large variety of cells. I have never observed any nets in the course of my studies on male and female germ cells of various animals, even in silver and osmium preparations; and I have always doubted the validity of the reticulate structure of the Golgi apparatus.'

Similarly Bourne (1951) says that ' . . . grains of reduced silver seem to be composed fundamentally of a network structure. The granules of silver in a photographic emulsion even under the high power of the ordinary microscope appear to be homogeneous, but when they are examined with an electron microscope, magnifying them by 25,000 diameters, they may be seen to be networks, made up of threads, some of which are only five atoms thick (Plate II, Fig. 2). The type of network can be varied according to the developer used. Hydroquinone produces coarse threads, whereas metol produces fine threads. With some other developers the network becomes a sort of fluffy mass. The form of the Golgi network also varies according to the time of development and the type of developer used.'

It is now generally believed that the localized Golgi apparatus consists of discrete Golgi elements which are associated with a slightly deep-staining body known by the name of 'idiosome' or 'archoplasm'. The Golgi material of the spermatocytes and the spermatids of *Cavia*, according to Gatenby and Woodger (1921) is in the form of curved plates and rods called the dictyosomes which surround the archoplasm. These observations have also been confirmed by Rau and Brambell (1925) who carried out investigations on the living spermatocytes and early spermatids of the guinea-pig by the Lewis method and with Janus green-staining. On the other hand, we have clearly shown that the Golgi elements in the male germ cells of the guinea-pig are in the form of small osmiophilic granules, most of which become embedded in the idiosome of the secondary spermatocytes and the early spermatids. Even under the phase contrast microscope, while we could easily see these Golgi granules embedded in the idiosome, we could not make out any such cortex of banana-shaped bodies or the dictyosomes round the idiosome as has been described by the above-mentioned authors. Our preparations made with Flemming-without-acetic and stained with 0·5% iron hæmatoxylin show clearly that the large dictyosomes figured by Gatenby and Woodger (1921) at the

periphery of the idiosome are artifacts produced by Cajal and Mann-Kopsch techniques employed by these authors. As already pointed out, in the silver-nitrate or long osmication techniques, on account of the deposition of the reduced particles of silver or osmium not only on the Golgi granules but also in the narrow spaces between them, the Golgi granules situated at the periphery of the idiosome may join together to form rods, crescents and dictyosomes. Indeed Meves (1899) in his classical work on this material has actually figured granules and not rods in the idiosome.

The granular Golgi elements have also been described in dog by Bell (1929) who at no stage was able to see them in the shape of crescents or dictyosomes. He has clearly mentioned that 'these granules are undoubtedly the homologue of the Golgi apparatus described for the guinea-pig by Gatenby and Woodger (1921) but at no time do they assume a crescentic shape like those of *Cavia*'. Even Gatenby and Beams (1935), working with the human material, admit that a cortex of osmiophile or argentophile material is found only in those preparations which are made by the Golgi apparatus methods. They further state that 'sometimes the osmiophile material is smooth, sometimes crenated, sometimes raised into beads and sometimes even batonette-like in arrangement'. Gresson (1942) 'concludes that the Golgi material of the developing male germ cells of the mouse is in the form of rods and granules. The Golgi elements are, however, small and closely packed together around the archoplasm, making observation of their shape extremely difficult'. Some similar observations have also been made by Gresson and Zlotnik (1945 and 1948). In our opinion, however, the dictyosomes, batonettes and rods, etc., are all artifacts produced by the action of certain fixatives. The real form of the Golgi apparatus is just granular. In fact the proacrosomic granules which, according to Gatenby and Woodger (1921), are differentiated within the archoplasm during the later growth stages of the spermatocyte are really the Golgi elements.

Idiosome: It has been shown earlier that most of the Golgi granules in the secondary spermatocytes and the early spermatids of the guinea-pig become embedded in a slightly deep-staining, rounded or oval body lying near the nucleus. This body has been referred to as idiosome by Papanicolaou and Stockard (1918), idiozome by Meves (1899), sphäre by Niessing (1896), accessory corpuscle by Brown (1885), archiplasm by Benda (1896-7) and archoplasm by Moore (1906) and Gatenby and Woodger (1921).

Formerly it was thought that the idiosome was a structure distinct from the classical Golgi material (osmiophilic) though the two are usually closely associated with each other. But it is now generally believed that this body forms a definite part (osmiophobic) of the classical Golgi apparatus (osmiophilic), the latter forming the wall and the former filling up the interior.

Gatenby and Woodger (1921), Gatenby and Beams (1935), Gresson (1942) and Gresson and Zlotnik (1945) have all described the idiosome and its cortex as respectively the chromophobic and chromophilic parts of the Golgi apparatus. As, however, we have already shown that the curved

plates and rods which, according to these authors, form the chromophilic cortex of the idiosome are artifacts produced by the techniques employed by them, it appears to us that the granules embedded in the idiosome represent the chromophilic or osmiophilic part and the idiosome itself represents the chromophobic or osmiophobic part of the Golgi apparatus.

A reference may also be made here to the 'Pre-substance Golgi system' theory of Hirsch (1939) according to whom the Golgi bodies of the cell are not always duplex in structure. He states that there is some 'pre-substance' of the Golgi apparatus, probably of mitochondrial origin, which does not show differentiation into osmiophilic and osmiophobic substances and is able to build up nets. Each piece of the 'pre-substance' gradually develops the double structure by the absorption and concentration in its interior of water and water soluble substances and it is then referred to as a 'Golgi system'.

It is significant to note that the presence of a juxta-nuclear mass of Golgi granules in association with the mitochondria in the spermatogonia of the guinea-pig lends support to Hirsch's theory.

Dictyokinesis: There is no unanimity about the details of the division of the Golgi elements during the meiotic stages. The process of division which is usually known as dictyokinesis is generally haphazard. It does not require any sort of fission of the individual elements as is seen during karyokinesis but is merely an unprecise sorting out of the Golgi elements among the daughter cells.

Duesberg (1920) mentions that the Golgi material in the opossum does not break up into granules until the anaphase, when it forms one or more clumps situated between the chromosomes. Similarly Gresson (1942) states that in the mouse 'the Golgi material remains in a single clump and is separated passively into two smaller groups with the division of the cell'. On the other hand, the whole of the localized Golgi apparatus of *Mus* and *Cavia*, according to Ludford and Gatenby (1921), divides into two parts which pass with the centrioles to opposite poles of the spindle and break up into separate Golgi bodies or dictyosomes. These scatter through the cell during the metaphase and again aggregate into a localized mass in the daughter cells.

Papanicolaou and Stockard (1918) claim that the idiogranulomes, which are really the Golgi elements, retain their individuality during the cell division and ultimately become sorted out to the two daughter cells. Meves (1899), Niessing (1896) and Moore (1906) have all failed to follow these granules during the division stages but they believe that these granules soon become visible after the idiosome is reformed in the daughter cells. Gatenby and Woodger (1921) also follow the account of Papanicolaou and Stockard (1918), though they have not observed it themselves. They have given a diagrammatic figure illustrating the phenomenon of mitosis according to their own view. Around each mitotic aster they have shown groups of approximately half of the Golgi elements or dictyosomes which break away from the archoplasm by its disintegration in the earlier stages.

According to Gatenby and Beams (1935) the dictyokinesis during the spermatogenesis of man 'was of a somewhat unexpected type' because the idiozome or archoplasm during the early prophases broke up and the osmiophile cortex separated into a cloud of granules and not into curved rods or batonettes. This certainly supports our claim that the form of the Golgi apparatus is essentially granular and that the batonettes and curved plates, etc., are mere artifacts.

During the meiotic divisions of the guinea-pig we have clearly shown that the Golgi granules become dispersed and lie not only in the vicinity of the spindle but also in the spindle area itself. It is presumed that the idiosome breaks up in the early stages of division and the individual Golgi elements in it then become spread all over to be sorted out almost equally to the two daughter cells where they again become localized in the reformed idiosome. The Golgi material during these stages was never observed in the form of clumps as described by Gresson (1942). It is believed that such clumps may be due to faulty technique. The remarkable frothing described in the idiosome of the spermatocytes of *Cavia* by Ludford and Gatenby (1921) was also not observed.

Acrosome: As has been said above most of the Golgi granules, after the two meiotic divisions, again become embedded in the idiosome of the early spermatid. At this stage the Golgi granules reveal a distinct tendency to come together and this results in the formation of a fewer and bigger Golgi granules. The process of fusion of the Golgi granules in the idiosome, however, continues till only a single big Golgi 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 gradually 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. Henceforth this Golgi granule is termed as the acrosome which is a small structure, to begin with, but increases in size gradually. It then becomes differentiated into two regions—an inner darkly-staining and an outer lightly-staining. As the spermatid begins to elongate the two regions of the acrosome become spread over the anterior end of the nucleus like a cap which, in the ripe sperm, can be seen covering more than half of the elongated nucleus.

As early as 1899 Meves also recognized a number of granules (Kornchen) embedded in the idiosomic body. He showed that the clear vesicles round the granules develop later and not before the appearance of the granules as described by Moore (1906). Duesberg (1908) like Moore (1906) shows the vacuoles arising earlier than the appearance of the granules in the idiosome. On the other hand, we could not make out any such vacuole round each Golgi granule at this stage.

Duesberg (1908) traces the origin of the acrosome of rat to the granules which develop in the vacuoles. Papanicolaou and Stockard (1918) also derive the acrosome by the fusion of the granules in vacuoles (their idiogranulomes in idiogranulothecae) which are present in the idiosome (their idioectosome). Gatenby and Woodger (1921) describe the formation of the acrosome by the fusion of the proacrosomic granules which, according

to them, are differentiated in the archoplasm during the later growth stages of the spermatocyte.

It, therefore, becomes an admitted fact that the acrosome owes its origin to the Golgi apparatus. There is, however, difference of opinion as to whether the Golgi apparatus secretes the acrosome or itself gets transformed into it. Gatenby (with Woodger, 1921 and with Beams, 1935) believes that the acrosome is formed by the fusion of the proacrosomic granules which, in their turn, are differentiated in the archoplasm perhaps under the influence of the surrounding dictyosomes. Gresson (1942), though he could not demonstrate the proacrosomic granules, also seems to believe in the secretory hypothesis as he states that 'there is little doubt that the acrosome is formed under the influence of the Golgi material. It is probable that it arises from proacrosomic granules which run together in a similar manner to those of the guinea-pig'.

Gresson and Zlotnik (1945) further show that the Golgi material is not only associated with the formation of the proacrosome but 'is also concerned with the secretion of a substance inside the archoplasmic vacuole, which at a later stage takes part in the formation of the acrosome'.

On the other hand, the observations of Bell (1929) on the spermatid of dog coupled with our own findings in the guinea-pig definitely suggest that the proacrosomic granules of Gatenby and Woodger (1921) are the Golgi elements themselves and not the secretory products of the Golgi dictyosomes which have been shown by us to be artifacts (vide supra). Indeed the figures of Meves (1899) for the spermateleosis of the guinea-pig clearly show that the acrosome is directly formed by the fusion of the Golgi granules which cannot be the secretions at all as there are no dictyosomes shown in his figures. Our observations on the guinea-pig are strongly supported by the recent work of Nath and Bhatia (1953) on *Lepisma* sperm, according to whom, the acrosome is formed by the direct fusion of the Golgi vesicles and is not a secretory product thereof, as described by Bowen (1924). Similarly Sharma and Malik (1953) have also shown clearly that in the lice, *Pediculus capitis* and *Pediculus corporis* there is a single Golgi granule, to begin with, which during the late stages of spermateleosis is directly transformed into the acrosome. For the direct origin of the acrosome reference may also be made to Nath (1932 and 1942) and Sharma (1944 and 1950).

Accessory or *Chromatoid body*: Gatenby and Beams (1935) were the first to describe an accessory body in the human spermatocyte. They have neither recorded its behaviour during meiosis nor its presence in the spermatid. They, however, felt that many of these accessory bodies were simply parts of the spermatogonial Golgi elements which failed completely to assemble together. Gresson (1942) also recognized a Golgi granule in the neck region of very late spermatids and ripe spermatozoa. 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) believe that the accessory body has been seen in the male

germ cells of a number of mammals and has been described as a chromatoid body. According to them the accessory bodies originate from the localized Golgi material of the spermatocytes and the spermatids. These do not divide during the meiotic divisions but each spermatid receives at least one. Finally an accessory body is included in the neck region of the ripe sperm. It will be remembered that we 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 fore-runner of the acrosome and we compared it to the accessory body described by Gresson and Zlotnik (1945 and 1948). We further feel that some of those Golgi granules also which do not come together to be in the localized condition and remain dispersed throughout the cytoplasm of the spermatocytes and the spermatids may have been labelled as chromatoid bodies by the earlier workers. That the chromatoid body, described by Bowen (1924), Gatenby and Mukerji (1929) and Mukerji (1929), is only that part of the Golgi apparatus which does not merge into the acrosome has also been recently proved in this laboratory by Nath and Bhatia (1953). See also Nath (1932, 1937 and 1942).

Middle-piece protoplasmic bead: Retzius (1909) figures a small bead of protoplasm on the middle-piece of the spermatozoa of several mammals including *Cavia*. His figures show that the bead is not always present in the same position in different animals and that in some it is absent altogether. Gatenby and Woodger (1921) show it immediately behind the nucleus. According to them a small part of the Golgi material with archoplasm becomes separated from the main mass in the elongating spermatid and it ultimately takes up its position in the protoplasmic bead of the middle-piece. As pointed out by Rau and Brambell (1925) we also believe that the bud from the Golgi remnant is given off at an earlier stage than that described by Gatenby and Woodger (1921) and that it becomes separated from the Golgi remnant before it moves away from the vacuole surrounding the big Golgi granule which is destined to form the acrosome (Plate I, Fig. 19). It is, however, very difficult to say that the Golgi granules noted in the protoplasmic bead are derived from the same bud, as urged by Gatenby and Woodger (1921) and later confirmed by Gresson (1942). In our opinion, the protoplasmic bead is merely a cytoplasmic structure. When the residual cytoplasm is being sloughed off a small part of it remains sticking to the middle-piece of the ripe sperm. As the residual cytoplasm contains not only the Golgi remnants but also those Golgi granules which did not assemble together in the earlier stages, it is quite likely that some of those Golgi granules also become included in the protoplasmic bead along with the small part of the residual cytoplasm forming it. Gresson and Zlotnik (1945) have shown that the Golgi material after the formation of the acrosome moves to the posterior part of the spermatid. There it breaks up into two parts one of which gives rise to the argentophil granules contained in the protoplasmic bead. They further show that the protoplasmic bead moves down the middle-piece when the sperms are in the epididymis and is finally eliminated. Some similar observations have also been made in this country by Mukherjee and Bhattacharya (1949), according to whom, the maximum number of spermatozoa with protoplasmic

bead in the neck region were found in the caput epididymis of all the species of farm animals studied by them. The cauda epididymis contained the maximum number of spermatozoa with protoplasmic bead on the middle-piece while in the ampullæ the percentages of spermatozoa with protoplasmic beads were minimum and hardly exceeded 2%. They also believe that the spermatozoa with protoplasmic beads are still immature.

Post-nuclear body: Gatenby and Wigoder (1929) were the first to describe the post-nuclear granules in the male germ cells of *Cavia*. According to them, these granules, to begin with, can be seen in the neighbourhood of the Golgi apparatus of the spermatocytes forming the cortex of a vacuole which, however, disappears later on. In the spermatid these granules are situated behind the nucleus and have been described as 'elongate, hollow structures, which seem to expand till they touch and finally unite to form the solid covering behind the nucleus'. 'This expands further till it forms the cup-like structure depicted in the spermatozoon.' In the ripe sperm they show an argentophile band (the post-nuclear cup) on the posterior part of the nucleus, stressing that 'no method except Da Fano picks out these bands in such an extraordinary manner'.

Gatenby and Beams (1935), though they have not been able to follow the development of these granules through the different stages of spermatogenesis, claim that the human testicular material also contains the post-nuclear bodies. They have depicted these structures only in their diagrammatic figures and none of their diagrams drawn from actual preparations shows these structures except their Fig. 26 in which they have given just an indication of the post-nuclear cap. They admit that 'both the origin and the subsequent history of these sharply impregnated structures are somewhat difficult to make out . . .' They further state —'The earliest stage we can trace the rudiment back is shown in Fig. 9, Pl. 2, where in many cases a very sharp membrane (PNC) is found at the back of the nucleus. Between the stages in Figs. 9 and 10, Pl. 2, we have studied many examples of this growing membrane, but in other cells it is not demonstrable (Fig. 28, Pl. 4)'. Even in the absence of the requisite stages Gatenby feels convinced that the final form of these bodies 'is brought about by a creeping up and growth of such material, probably of lipoidal nature'. Gatenby and Beams (1935), however, confess that they are not certain 'whether it is ever a separate rudiment in the cytoplasm of the spermatid, or merely a thickening on the nuclear membrane'. This is attributed by them to the fact that they did not have any Da Fano silver nitrate preparation and that 'it is in this material that the post-nuclear bodies of *Cavia* are clearest'.

Zlotnik (1943) described a nuclear ring surrounding the nucleus of the late spermatid and the sperm of the dog and the cat. He believed that the post-nuclear cap was formed by its widening posteriorly. Gresson and Zlotnik (1945), after a thorough examination by one of them (I.Z.) of the sperms of the animals in which the post-nuclear cap is clearly visible, conclude 'that the widening of the nuclear-ring never reaches the extreme posterior end of the sperm-head; a narrow light space being present between the wide band which comes down from the nuclear-ring and the

black posterior edge of the head. This argentophil posterior edge of the head is traced back to a time when the nuclear-ring is more conspicuous than at any other stage, and a black granular ring, often interrupted, forms the posterior end of the nucleus of the late spermatid. This new structure is called the posterior ring, and it is suggested that it may be formed from argentophil granules scattered around the posterior region of the nucleus at an earlier stage. The origin of these granules is not clear; it is probable that they may be identical with Gatenby's and Woodger's post-nuclear body, on the other hand some of the granules at least may originate from the Golgi material. The granules make their first appearance in the dog and cat spermatids very soon after the Golgi material moves away from the posterior part of the already elongate nucleus, while in the rabbit they are seen at a much earlier stage, at the time when the archoplasmic vacuole begins to lift up the Golgi material. The suggestion is made that not all of these granules form the posterior ring, but only those which are attached to the nuclear membrane'.

Regarding the silver methods (by the application of which the so-called post-nuclear body is said to be clearly demonstrated) it may be mentioned that one should not depend merely on these for deciding the nature of any particular structure. The draw back in these and long osmication methods is that they blacken mitochondria, nuclear membrane and several other structures in the cell which are not related to the Golgi apparatus at all. It, therefore, appears that the granules of silver and osmium can settle down on many kinds of surfaces. Friend (1936), while working on the sperms of the British Muridae, discovered an asymmetrical deeply-staining area in the posterior region of each sperm nucleus he examined, and it gave the characteristic Feulgen's reaction. He named it as the 'dense posterior region' and remarked that in position it agreed with Gatenby's 'post-nuclear body'. By employing Feulgen's reaction on smears of the testicular material of the guinea-pig it has been confirmed by us that the so-called post-nuclear body is in reality the dense posterior region of the nucleus itself and not a separate body (Plate III, Fig. 50). Neither in Da Fano's (chilled) nor in any other preparation were the post-nuclear granules observed at any stage of spermatogenesis of the guinea-pig. In some of our Da Fano's smears, however, silver was deposited on the posterior part of the nucleus of the ripe sperm. This is identical with the post-nuclear cup of Gatenby and Wigoder (1929) and it clearly explains how these authors, depending mainly on the capricious silver nitrate technique, have been led to establish a new category of structures. In view of the characteristic Feulgen's reaction we are inclined to the view that the post-nuclear body is nothing but a part of the nucleus, probably containing most of the dense chromatin material.

Recently Nath and Bhatia (1953) have also confirmed that the 'post-nuclear body' of Gatenby and Mukerji (1929) and Mukerji (1929) in *Lepisma* is in reality the acrosome as shown earlier by Bowen (1924).

Centrosomes: The centrosomes embedded in the idiosome, as described by some of the earlier workers, were never observed in the present study. They were clearly seen at the time of divisions in the form of small granules

situated one at each pole of the spindle. In some of the spermatocytes and early spermatids a small darkly-staining granule surrounded by a clear area was also seen and this was most probably the centrosome. As the spermatid begins to elongate an axial filament can be seen growing out from a darkly-staining granule situated at the posterior end of the nucleus. This is the proximal centrosome. The distal centrosome, which soon appears near it, is in the form of a ring encircling the axial filament. It appears that the originally single centrosome of the early spermatid divides into two but we have never observed this division. Gatenby and Beams (1935) believe that the axial filament of the human sperm grows out jointly from the two bodies which are formed as a result of the division of the single centrosome. While the proximal granular centrosome remains in the vicinity of the posterior pole of the nucleus, the distal ring-like centrosome travels down the axial filament and ultimately takes up its position at the distal end of the middle-piece.

Wodsedalek (1913) has described that the ring centriole in the spermatid of the pig sometimes divides but usually becomes spherical and is finally lost with the residual cytoplasm. We have not, however, seen the distal or proximal centrosome dividing or being lost and thus our observations are in complete accord with those of Gatenby and Beams (1935) and Gresson and Zlotnik (1945 and 1948).

Mitochondria: According to Gatenby and Woodger (1921) the mitochondria in the spermatogonia of the guinea-pig surround the archoplasm, to begin with, but become dispersed throughout the cytoplasm later on. Gatenby and Beams (1935) also claim that in man these are situated mostly near the Golgi apparatus. Gresson (1942), however, states that in the mouse there are very many mitochondria near the nucleus, the periphery of the cell being almost clear. According to Gresson and Zlotnik (1945) the mitochondria in the pig and the sheep are dispersed throughout the cytoplasm, in the dog they form a horse-shoe and in the other animals studied by them they are in plenty in the neighbourhood of the nucleus. Gresson and Zlotnik (1948) state that the mitochondria in the spermatogonia of bull surround the nucleus and are most numerous near the Golgi material. In the primary spermatocytes the mitochondria, according to most of the previous workers, are found near the Golgi material, to begin with, but become spread throughout the cytoplasm gradually. In contrast to these observations we have noticed in our present investigations that the mitochondria remain dispersed throughout the cytoplasm not only in the spermatogonia but also in the spermatocytes and the spermatids. During the mitotic as well as meiotic divisions these are distributed almost equally to the two daughter cells. In the late spermatids the mitochondrial granules which are included in the area enclosed by the manchette become darkly-staining. It appears that by a process of fusion they form much 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 which remain lying in the residual cytoplasm either degenerate or are sloughed off with the residual cytoplasm during the final maturation of the sperm.

Manchette: In the guinea-pig the manchette was first seen in the late spermatid as a somewhat darkly-staining area of the cytoplasm immediately behind the elongating nucleus. As stated above the mitochondria also in this area become darkly-staining and these, by a process of fusion, form much bigger mitochondrial granules which are so conspicuous in the middle-piece of the ripe sperm. During the late stages of spermateleosis the manchette forms the lateral boundaries of the middle-piece while its anterior and posterior boundaries are formed by the proximal and distal centrosomes respectively. These observations are strongly supported by the works of Lenhossek (1898), Mollé (1910) and Gresson and Zlotnik (1945 and 1948). Some other previous workers like Leplat (1910), Duesberg (1911), Branca (1924) and Gatenby and Beams (1935), are, however, of the opinion that the manchette appears and disappears in the late spermatids without any apparent function.

Summary

1. In this paper the spermatogenesis of the guinea-pig has been worked out with special reference to the form of the Golgi elements, the formation of the acrosome and the nature of the so-called post-nuclear body.

2. The mitochondria are in the form of fine lightly-staining granules, scattered throughout the cytoplasm, from the early spermatogonia to the late spermatids. During the final stages of sperm formation, those mitochondrial granules which are included in the 'manchette' become darkly staining. It seems probable that by a process of fusion they 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 along with the residual cytoplasm.

3. The Golgi elements in the early spermatogonia exist in the form of a juxta-nuclear mass of darkly-staining granules which are definitely bigger than the mitochondria. In the fully grown spermatogonia, however, these become spread throughout the cytoplasm. In the primary spermatocytes they begin to show a distinct tendency to come together to form bigger granules. Some of these big Golgi granules now collect together to be in the localized condition which is so prominent in the secondary spermatocytes and the spermatids. During the division stages, however, 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.

4. As the process of spermateleosis begins the idiosome becomes closely applied to the nucleus. Most of the Golgi granules in it now begin to coalesce gradually till ultimately a single big Golgi granule is produced. This is situated almost in the centre of the idiosome. Soon after its formation it becomes surrounded by a clear vacuole which gradually 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 which is a small structure, to

begin with, but increases in size gradually and also becomes differentiated into two regions—an inner darkly-staining and an outer lightly-staining.

5. As the acrosome grows in size the remains of the idiosome with or without the Golgi elements drift back to the posterior side of the nucleus to be sloughed off with the residual cytoplasm.

6. From the staining reactions and the behaviour, the so-called chromatoid body appears to be merely a part of the unassembled Golgi elements. Similarly the accessory bodies also seem to us to be those Golgi elements which are not consumed in the formation of the acrosome.

7. Neither in Da Fano's (chilled) nor in any other preparation, were the post-nuclear granules observed in any stage of spermatogenesis. In some of our Da Fano's smears, however, deposit of silver was noticed on the posterior part of the nucleus of the ripe sperm. In position, this posterior part of the nucleus is identical with the post-nuclear body of Gatenby and Wigoder (1929). This is, in reality, the dense posterior region of the nucleus itself and not a separate body. It has also been confirmed by employing Feulgen's reaction on smears and sections and the results obtained are in harmony with those of Friend (1936) on the sperms of the British Muridae.

8. A single protoplasmic bead, containing also a few Golgi elements, is present on the middle-piece of the ripe sperm. This is, however, lost while the sperm is within the epididymis.

9. The proximal and the distal centrosomes do not divide. The proximal remains granular but the distal becomes ring-like and travelling down the axial filament marks the posterior boundary of the middle-piece of the ripe sperm.

10. The manchette is a purely cytoplasmic structure in origin. Since it marks the lateral boundaries of the middle-piece it does not seem to disappear without any apparent function, as is urged by some of the previous workers.

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Explanation of Lettering in Plates

A—Acrosome; A_1—Inner Zone of Acrosome; A_2—Outer Zone of Acrosome; *A.f*—Axial filament; *C*—Centrosome; C_1—Proximal Centrosome; C_2—Distal Centrosome; *Ch*—Chromosomes; *G*—Golgi elements; *G'*—Golgi remnants; *I*—Idiosome; *M*—Mitochondria; *Ma*—Manchette; *N*—Nucleus; *P.b*—Protoplasmic bead; *R.c*—Residual cytoplasm; *V*—Vacuole.

Further explanation of figures will be found in the text.

Explanation of Plates

All figures except Fig. 50 have been selected from the sectioned testicular material of the guinea-pig, fixed in Flemming-without-acetic and stained with 0·5% iron-hæmatoxylin and have been drawn with a camera lucida at the stage level, using Leitz 6× eye-piece and an oil immersion objective. They were subsequently enlarged four times, thus giving a total magnification of approximately 2,400 times.

Figure 50 has been selected from a smear preparation, made for Feulgen's reaction after fixation in strong Flemming diluted with an equal quantity of water. It was also drawn with a camera lucida, at the table level, using Beck 10× eye-piece and an oil immersion objective, giving a magnification of about 1,700 times.

Plate I

Fig. 1. An early spermatogonium.
„ 2. Fully grown spermatogonium.
„ 3. Spermatogonial metaphase.
„ 4. Primary spermatocyte.
„ 5. Metaphase I.
„ 6. Telophase I.
„ 7. Secondary spermatocyte.
„ 8. Anaphase II.
„ 9. Telophase II.
„ 10. Secondary spermatocyte in which the nuclei of the two daughter spermatids have been completely formed and yet no division of the cytoplasm has taken place.
„ 11. An early spermatid.
„ 12. Spermatid showing a fewer and bigger Golgi granules not only in the idiosome but also in the cytoplasm.
Figs. 13 to 17. Spermatids showing the gradual fusion of the Golgi granules in the idiosome till only a single big Golgi granule is formed.

FIG. 18. Spermatid showing the appearance of a clear vacuole round the big Golgi granule formed in the centre of the idiosome. Another big Golgi granule may be seen coming out of the idiosome.

FIGS. 19 to 21. Spermatids showing the growth of the vacuole round the big Golgi granule as the idiosome moves towards one side. In Fig. 19 a small portion of the idiosome containing Golgi granules is seen separating from the main mass of the idiosome. In Fig. 21 a big Golgi granule is seen coming out of the idiosome.

FIG. 22. Spermatid in which the big Golgi granule has come in intimate contact with the nuclear membrane to form the acrosome.

FIGS. 23 and 24. Spermatids showing the growth of the acrosome.

PLATE II

FIG. 25. Spermatid showing the acrosome situated at the tip of a conical projection of the nucleus.

FIGS. 26 to 30. Spermatids showing the differentiation of the acrosome into two regions—an inner darkly-staining and an outer lightly-staining.

,, 31 to 33. Spermatids in which an axial filament can be seen growing out from a small granule lying in the cytoplasm. This granule is the proximal centrosome.

,, 34 to 37. Spermatids showing the growth of the acrosome. In Figs. 35 to 37 an axial filament can be seen growing out from the proximal centrosome. In Fig. 37 manchette has also made its appearance just behind the nucleus.

FIG. 38. Spermatid showing the elongated nucleus with the acrosome covering its anterior end. The ring-like distal centrosome has also made its appearance just near the proximal. It is seen encircling the axial filament.

,, 39. Spermatid in which the ring-like distal centrosome has travelled down the axial filament.

PLATE III

FIGS. 40 to 42. Elongating spermatids showing the growth of the acrosome over the anterior end of the nucleus. The manchette can also be seen at the posterior end of the nucleus.

FIG. 43. Elongating spermatid in which the middle-piece of the ripe sperm has already been formed by the mitochondria included in the region of the manchette earlier.

FIGS. 44 and 45. Nearly ripe spermatozoa with the residual cytoplasm still sticking on to the middle-piece.

FIG. 46. The side view of a nearly ripe spermatozoon showing the protoplasmic bead, containing the Golgi elements, situated on the middle-piece.

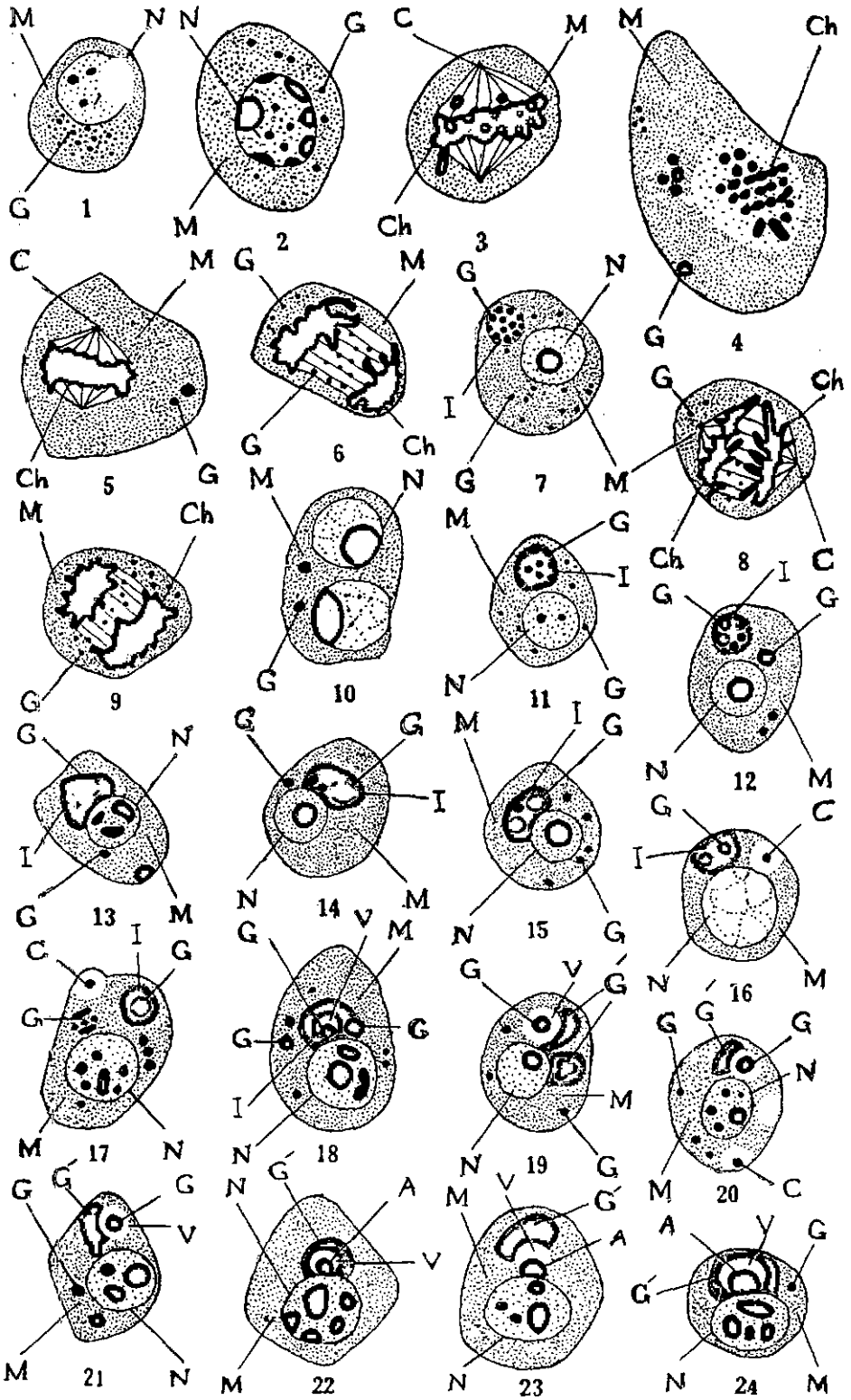
,, 47. A nearly ripe spermatozoon (side view).

,, 48. The anterior end of a nearly ripe spermatozoon (surface view).

,, 49. A nearly ripe spermatozoon (surface view).

,, 50. Ripe sperm (surface view).

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.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G I G N M 12
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G C I N M 16
C I G G M N 17
G V M G I N 18
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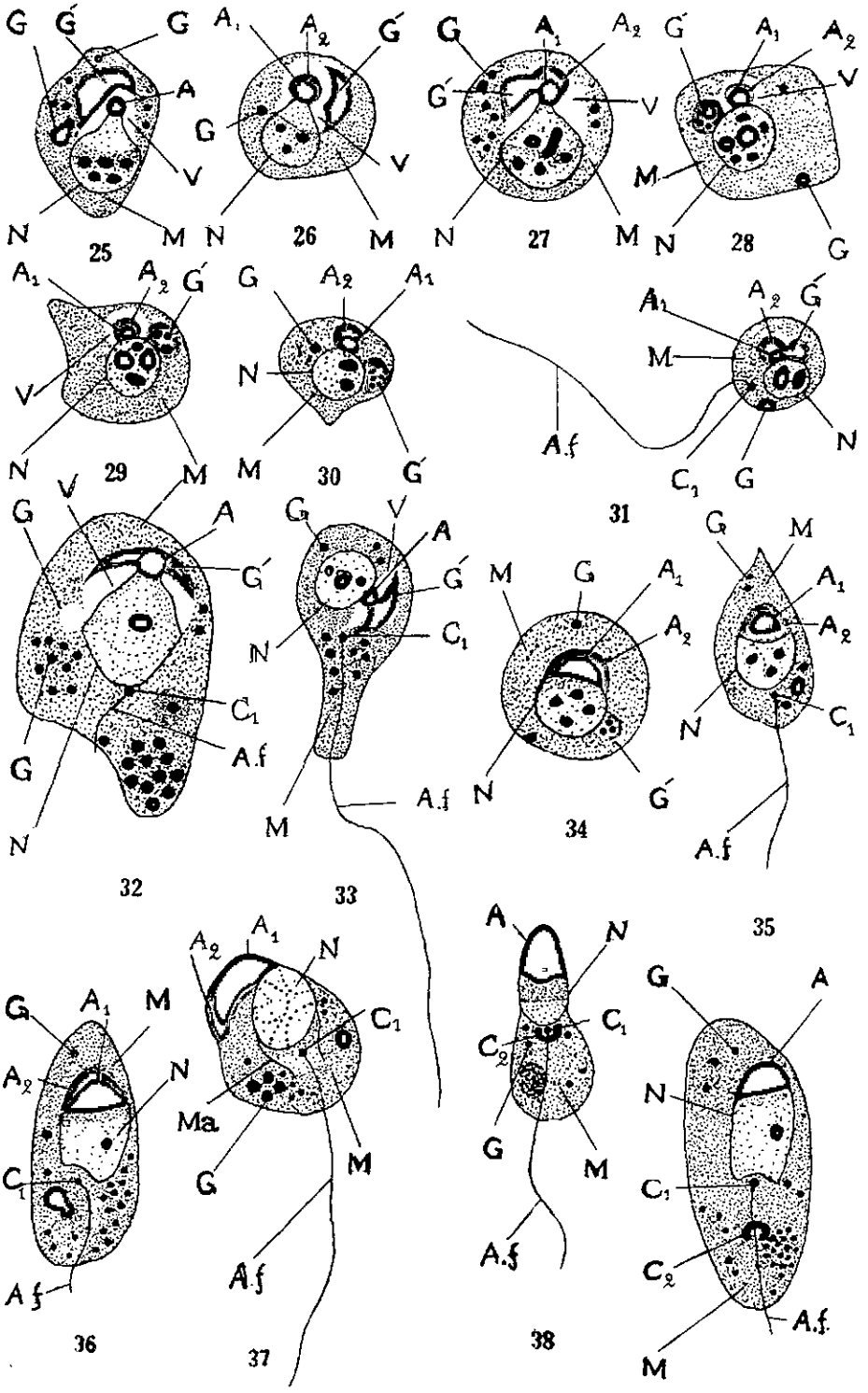
PLATE II.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G V M A G' C1 A.f G N 32
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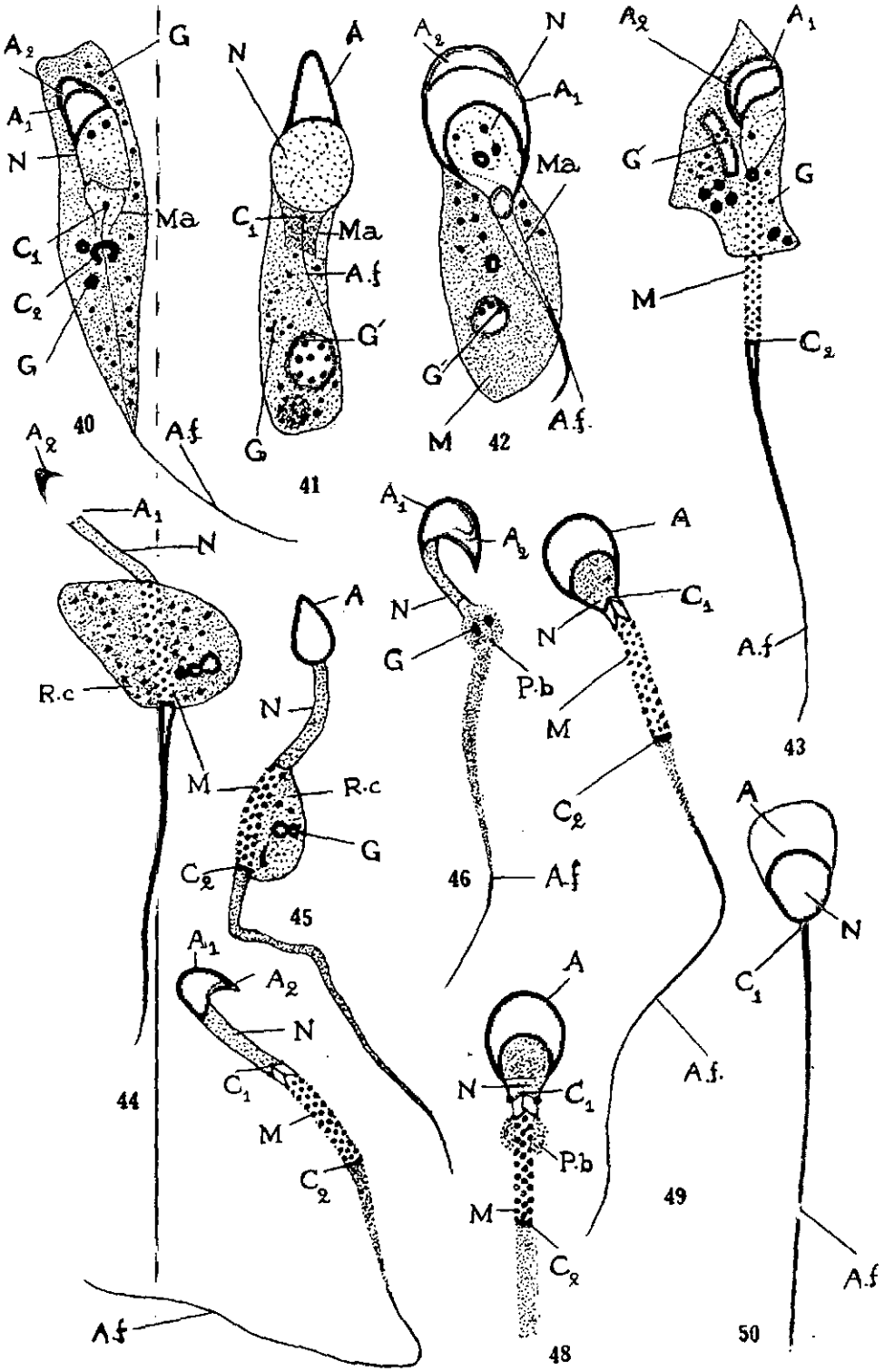
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RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 39 ZOOLOGY Pp. 181–192

December, 1953

STUDIES ON INSECT SPERMATOGENESIS

I. Hemiptera-Heteroptera

The Sex Chromosomes and Cytoplasmic Inclusions in the Male Germ Cells of *Laccotrephes maculatus* Fabr. and *Sphaerodema rusticum* Fabr.

by

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Department of Zoology, East Panjab University,
HOSHIARPUR

Price Rs.3-2-0

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STUDIES ON INSECT SPERMATOGENESIS

I. Hemiptera-Heteroptera

The Sex Chromosomes and Cytoplasmic Inclusions in the Male Germ Cells of *Laccotrephes maculatus* Fabr. and *Sphaerodema rusticum* Fabr.

By Sukhdev Raj Bawa, *University Research Scholar, Department of Zoology, Hoshiarpur, Panjab (I.)*

I. Introduction

To the best of my knowledge Gatenby (1917) was the first worker to study the cytoplasmic inclusions in the male germ cells of Lepidoptera by modern cytological technique, as almost all the previous workers confined their attention mainly to the chromosomes and mitotic figure.

Subsequently there appeared a series of publications by Bowen (1920, 1922, 1924) on Hemiptera, Lepidoptera, Orthoptera, Coleoptera and Aptera. In his studies Bowen conceives the Golgi body in the form of a chromophilic crescent to which is attached a chromophobic sphere. According to him, the acrosome is a secretory product of the Golgi material. The accounts of Payne (1927), Doyle (1933), Pollister (1930), Mickey (1935), Gresson (1936) and of few others are in accord with Bowen.

On the other hand, it has been suggested by Hirsch (1939) and Nath (1944) that the Golgi bodies are essentially granular or vesicular in form. They grow in size and very often reveal a duplex structure, having an outer deeply-staining chromophilic cortex and an inner lightly-staining chromophobic core. Nath's earlier observations have been confirmed by Baker (1949), (who made use of the Phase Contrast Microscope), Nath, Bawa, Bhardwaj and Gupta (1951), and Nath and Bhatia (1953). Moreover, the acrosome arising directly by the transformation of the Golgi bodies has been described by Nath (1942), Doncaster and Cannon (1920), Cannon (1922), Richter (1940), Nath and Bhatia (1953), Nath and Rishi (1953), and Sharma and Malik (1953).

In view of the conflicting interpretations of different authors on the structure of the Golgi bodies and the mode of acrosome formation, I undertook the present study to survey the process of sperm formation in various orders of insects with particular reference to the Golgi bodies and acrosome formation. The need for such investigations was considered all the more urgent as very little has been reported on Insect spermatogenesis since Bowen's last paper of 1924. Moreover, we possess hardly any knowledge of some insect groups, so far as the fate of the Golgi material and mitochondria in spermatogenesis is concerned. The investigations recorded in this paper—the first of the proposed series of studies on Insect

spermatogenesis—were undertaken in the male germ cells of *Laccotrephes maculatus* and *Sphaerodema rusticum*.

My findings furnish a rather striking confirmation of many of the observations of Bowen (1922) on Pentatomids with respect to the acrosome and mitochondrial nebenkern.

The nuclear changes, especially the behaviour of the sex-chromosomes, are also very carefully studied in both the species. Some of the observations made on fixed preparations of *L. maculatus* have been confirmed under the Phase Contrast Microscope.

II. Acknowledgments

The present work was carried out in the department of Zoology, Panjab University, Hoshiarpur. My thanks are due to Professor Vishwa Nath for granting me necessary research facilities, for the interest he has taken in the progress of my work and for correcting the manuscript of this paper. My thanks are also due to Dr. Ganpati Parshad Sharma for many useful suggestions and his constant advice. I am also indebted to the Entomological section of the Pusa Research Institute, New Delhi, for the identification of the insects.

III. Material and Methods

Observations were made on the germ cells of adults of *Laccotrephes maculatus* (water-scorpion) and *Sphaerodema rusticum* (water-bug). The water scorpions are rather sluggish in their movements, feeding on small insects and aquatic animals found creeping at the bottom of the ponds during spring and summer. The water bug, *Sphaerodema rusticum*, however, is very active in its movements. Both the species were collected in large numbers from 'Budha Nala' (a small stream) near Ludhiana, Panjab (I.).

The testes were dissected from insects in normal saline and were then transferred to Flemming-without-acetic acid, which gave very good results. Bouin's fluid, which was used as control, gave excellent results for the study of chromosomes. After the usual process of dehydration of the testicular material, sections were cut 5–7 μ thick. They were stained with 0·5% iron haematoxylin and mounted in canada balsam. For the study of mature sperm, smears of the vasa deferentia of the testes were made in Flemming-without-acetic acid diluted with an equal quantity of distilled water and stained in 0·5% iron haematoxylin.

IV. Observations

Since the process of sperm formation is more or less similar in the two species of Hemiptera-Heteroptera described here, it has been considered best to give a comparative account of both the species to avoid unnecessary repetition.

(i) *Spermatogonia*

In the resting spermatogonia of *Laccotrephes maculatus* there can be distinguished a juxta-nuclear mass of greyish mitochondrial granules. A few darkly-staining Golgi elements, which are slightly bigger than the mitochondria, can also be easily made out in this mass (Pl. I, Fig. 1). But in *Sphaerodema rusticum* no Golgi elements can be detected in the mitochondrial mass (Pl. III, Fig. 1).

The spermatogonial nucleus reveals a prominent rounded nucleolus and fine faintly-staining chromatin granules in *L. maculatus*, while the nucleolus is very often irregular in outline in *S. rusticum* (Pl. I, Fig. 1; and Pl. III, Fig. 1). Dividing spermatogonia were not observed in *L. maculatus*. On the other hand, a large number of counts of the chromosomes in the spermatogonial metaphase plates were made in *S. rusticum* in which the polar views consistently reveal 28 well-defined chromosomes (Pl. III, Fig. 2). These chromosomes are of various sizes, but the variation is not great, and only two small chromosomes could be differentiated from the rest. The sex-chromosomes cannot be distinguished as such at this stage. During spermatogonial mitosis a spindle makes its appearance with a crescentic centriole at each pole (Pl. III, Fig. 3) and the mitochondrial granules form a sort of palisade round the spindle. These granules seem to be sorted out into two roughly equal parts to the two daughter cells (Pl. III, Fig. 4).

(ii) *Spermatocytes*

In the primary spermatocytes of *Laccotrephes maculatus* and *Sphaerodema rusticum* the mitochondria are small and vesicular in form, while the Golgi bodies, which appear for the first time at this stage in *S. rusticum*, are like crescents, distributed amongst the mitochondria (Pl. I, Fig. 2; and Pl. III, Fig. 5). The Golgi crescents are most probably the optical sections of the Golgi spheres, as distinct Golgi *spheres* and vesicular mitochondria have been observed in the living spermatocytes of *L. maculatus* under the Phase Contrast Microscope. At no stage Golgi *crescents* observed in sectioned material were seen in the living cells of *L. maculatus* (Pl. V, Fig. 1).

As the spermatocytes grow, the Golgi elements as well as the mitochondrial granules increase in size (Pl. I, Fig. 3; and Pl. III, Figs. 6 and 7). Very often both these cell-components reveal a duplex structure with a chromophilic, darkly staining cortex, enclosing a lightly staining chromophobic substance. However, the Golgi elements are bigger in size than the mitochondria and are generally embedded in the mitochondria which form a compact juxta-nuclear frothy mass (Pl. I, Figs. 4, 5 and 7; and Pl. III, Figs. 9–12). The mitochondria seem to reach their maximum size prior to the appearance of the meiotic spindle (Pl. I, Figs. 8 and 9; and Pl. III, Fig. 13). As the nuclear wall breaks down, the mitochondria get scattered in the cytoplasm and are distributed almost evenly to the two secondary spermatocytes (Pl. I, Figs. 10–13; and Pl. III, Figs. 15–18; and Pl. IV, Fig. 19). The Golgi elements also are distributed more or less evenly to the daughter cells during first meiotic division in *L. maculatus*, but I could not trace the Golgi elements at this stage in *S. rusticum*.

A resting stage between the two meiotic divisions does not occur. So far as the mitochondria and the Golgi elements are concerned, the second meiotic division is a repetition of the changes observed during the first division (Pl. I, Figs. 15, 16 and 18–21; and Pl. IV, Figs. 20–22).

In Pl. I, Fig. 2 and Pl. III, Fig. 7 are shown primary spermatocytes in leptotene stage. The fine chromatin threads are so numerous and are arranged in such a way that it is impossible to count the autosome threads. However, the sex-chromosomes have already condensed and stain deeply The process of gradual condensation of the sex-chromosomes can be clearly seen in preleptotene stages in *S. rusticum* (Pl. III, Figs. 5 and 6). The sex-chromosomes can be seen often lying in close contact with the nuclear wall. After the usual process of pairing of the chromatin threads, the chromosomes condense into diplotene threads (Pl. I, Figs. 3–5; and Pl. III, Figs. 8 and 9). The nucleus then enters into a diffuse stage in *S. rusticum*, while no such stage occurs in *L. maculatus* (Pl. III, Fig. 10). During the process of diakinesis, chromosomes further contract, and with the appearance of a split in each chromosome, which will function during the second meiotic division only, dumb-bell shaped tetrads now place themselves at the equator of the spindle (Pl. I, Figs. 6–8; and Pl. III, Figs. 11–14). At each pole of the spindle, which appears fibrillar in the fixed preparations of *L. maculatus*, there is a deeply-staining conspicuous crescentic centrosome (Pl. I, Fig. 10; and Pl. III, Figs. 16 and 17). The crescent-shaped centrioles can be very clearly made out in fresh cells of *L. maculatus* examined under the Phase Contrast Microscope (Pl. V, Fig. 3). In this figure it may be noted that the spindle is not fibrillar; it simply appears as a hyaline fusiform area.

The chromosomal plates at metaphase I in polar view show twenty-two and fifteen distinct pairs of homologus chromosomes in *L. maculatus* and *S. rusticum* respectively (Pl. I, Fig. 9; and Pl. III, Fig. 15). These 22 pairs of chromosomes of *L. maculatus* have been clearly seen and counted in the fresh cells examined under the Phase Contrast (Pl. V, Fig. 2).

The sex-chromosomes during metaphase I cannot be distinguished from the autosomes. During late anaphase I in *S. rusticum* *X* and *Y* chromosomes seem to be connected temporarily by means of granular spindle (Pl. III, Fig. 17). In the late telophase I the spindle usually gives rise to a mid-body at the junction of the two daughter cells (Pl. I, Fig. 12; and Pl. IV, Fig. 19).

Actual formation of the mid-body by the deposition of small granules at the equator of the telophase I spindle has been studied in fresh material of *L. maculatus* under the Phase Contrast (Pl. V, Fig. 4).

Meiosis II immediately follows telophase I without the intervention of any resting stage. In Pl. I, Figs. 14 and 15, and Pl. IV, Fig. 20 are shown metaphase II plates of *Laccotrephes maculatus* and *Sphaerodema rusticum* with the reduced number (haploid) of twenty-two and fourteen chromosomes respectively. In the side views of second meiotic spindles can be seen *X* and *Y* chromosomes lying apposed to each other in *S. rusticum*, while 2 *X* chromosomes and a *Y* chromosome form a pseudo-trivalent almost in the central zone of the spindle in *L. maculatus* (Pl. I, Fig. 17; and Pl. IV, Fig. 21). It will be noted that the reductional division for the

sex-chromosomes is the second meiotic division, during which the autosomes divide equationally.

(iii) *Spermateleosis*

In both the species, *Laccotrephes maculatus* and *Sphaerodema rusticum*, vesicular mitochondria derived from the second spermatocyte, aggregate to form a mitochondrial nebenkern in the spermatid. As revealed by studies of fresh material under the Phase Contrast the earliest spermatid of *L. maculatus* possesses a vesicular nucleus having a hyaline appearance and showing no structure whatsoever within its interior, a mass of closely aggregated mitochondria and a vesicular acroblast (Pl. V, Figs. 5 and 6).

The earliest stages in the process of condensation of the mitochondrial nebenkern have not been traced as the material in both the species does not seem to be favourable for such a critical study. It may be that the earliest condensation stages are so short-lived that they have escaped notice. Similar difficulty was experienced by Bowen (1922) in Pentatomids. The mitochondrial nebenkern, when fully condensed, shows a chromophobic envelope through which pass a few strands from the central chromophilic core (Pl. II, Figs. 22–24; and Pl. IV, Figs. 25–27). Within the chromophilic core can be seen many vacuoles, which can be demonstrated only if the nebenkern is suitably destained. The transverse strands in the chromophobic area of the nebenkern subsequently disappear. The mitochondrial nebenkern now reveals a clear space surrounding the chromophilic core (Pl. II, Figs. 25–27; and Pl. IV, Fig. 28). As the process of nebenkern differentiation proceeds further, the chromophobic substance increases, while there is corresponding decrease of the chromophilic core. During this process of gradual obliteration of the chromophilic core, a number of greyish granules appear in the chromophobic area of the nebenkern. These granules later get dispersed throughout the chromophobic area, while no trace of the chromophilic core is left whatsoever (Pl. II, Figs. 28–33; and Pl. IV, Figs. 29–32). These granules seem to form the sheath of the tail of the mature sperm and correspond to the 'central substance' of Bowen (1922) and 'Sheath substance' of Nath (1925). The axial filament springs up from the centriole and divides the mitochondrial nebenkern into almost two equal halves. The nebenkern elongates and gets constricted into a number of vesicles, which finally seem to be sloughed off (Pl. II, Figs. 34–37; and Pl. IV, Figs. 33 and 36).

The series of changes involved in the gradual disappearance of the chromophilic core and the appearance of the 'central substance' granules in the chromophobic area of the nebenkern have been studied under Phase Contrast in the fresh material of *L. maculatus* (Pl. V, Figs. 7–14).

When the spermatid is formed, the Golgi elements reappear as crescent-shaped bodies and tend to aggregate between the nucleus and the nebenkern. The Golgi bodies finally fuse to form a single acroblast which soon becomes vesicular in nature (Pl. II, Figs. 22 and 23; and Pl. IV, Figs. 23–25). The acroblast further gets differentiated and comes to have a brightly-staining acrosomal granule at its periphery. This granule from

the very beginning of its formation remains in contact with the nuclear wall (Pl. II, Figs. 24–27; and Pl. IV, Figs. 26 and 27). During the process of elongation of the spermatid, the acroblast accompanied with the acrosomal granule travels round the spermatid nucleus for a fairly long time. The acrosomal granule which according to Bowen's terminology is the acrosome, grows in size at the expense of the acroblast. When the acrosomal granule has considerably increased in size it gets disassociated from the acroblast. The acrosome, later on, travels forward in front of the nucleus, while the acroblast seems to be moving back into the tail region and finally disappears (Pl. II, Figs. 28–37; and Pl. IV, Figs. 28–37).

Various changes involved in the formation of the acrosome from the acroblast have been studied very carefully under Phase Contrast in the fresh material of *L. maculatus*. The movement of the acroblast accompanied with the acrosomal granule round the spermatid nucleus in living material of *L. maculatus* was most fascinating to watch (Pl. V, Figs. 6–14).

In Pl. II, Figs. 38–41, and Pl. IV, Figs. 39–41, are shown progressive changes in the position of the acrosome. In the beginning, the acrosome is a button-like structure closely attached to the nuclear wall; later it gets transformed into a triangular area in front of the nucleus, and finally in the mature sperm, the acrosome is seen as a fine thread right at the tip of the sperm nucleus (Pl. II, Fig. 42; Pl. IV, Fig. 42).

The nuclear changes involved throughout the process of spermateleosis need some mention also. In *Laccotrephes maculatus*, to start with, the chromatin is spread out along the periphery of the nucleus enclosing a central area (Pl. II, Figs. 22 and 23). Very soon this demarcation of the nucleus into two areas is lost and the spermatid nucleus now stains very feebly, showing no structure whatsoever within its interior (Pl. II, Figs. 24–29; and 34–37). In a very few cases in *L. maculatus* some faintly-staining granules can be seen along the periphery of the spermatid nucleus (Pl. II, Figs. 31–33).

In *Sphaerodema rusticum* the spermatid nucleus does not reveal any differentiation into chromophilic and chromophobic areas in any stage during spermateleosis. In few of the early stages, however, sometimes there is seen a brightly-staining granule in the interior of the spermatid nucleus. This may be one of the sex chromosomes, which finally loses its identity in later stages of spermateleosis. An important point in the maturing nucleus of the spermatid of both the species of Hemiptera under consideration may be noted that the almost universal process of reversal of the chromophilic cortex and the chromophobic medulla, which accompanies the condensation of the vesicular spermatid nucleus into a compact homogeneously staining sperm head, is conspicuous by its absence.

In addition to the cell components mentioned above a brightly-staining granule is very frequently observed in the spermatids of *Sphaerodema rusticum* (Pl. IV, Figs. 25–27, 32, 33, 36–38). As the spermatid elongates, this granule grows in size and is finally seen moving backwards into the tail region. This granule corresponds to the 'chromatoid body' of Bowen (1924). It is probably one of the Golgi bodies, which does not take part in the formation of acroblast, and gets sloughed off.

V. Discussion

My work on *Laccotrephes maculatus* and *Sphaerodema rusticum* furnishes a striking confirmation of many of the observations of Bowen (1922) on the Pentatomids, regarding the nature and the behaviour of the Golgi elements in the process of spermatogenesis.

According to Bowen, each Golgi body is a rod composed of two substances—a deeply-staining outer rim (chromophilic) nearly surrounding, a more lightly-staining 'Idiosome' (chromophobic).

Nath (1944), on the other hand, is of the view that the Golgi bodies in the form of rods described by Bowen are really the optical sections of *discrete Golgi spheres*.

Nath's views about the granular or vesicular nature of the Golgi body have been fully confirmed by Baker's studies under the Phase Contrast (1949).

It is important to note that at no stage in the process of spermatogenesis, have I observed any crescents or rods in the living testicular material of *Laccotrephes maculatus*, studied thoroughly under the Phase Contrast. The Golgi bodies thus appear as beautiful spheres, each of which shows in sections an outer brightly coloured chromophilic cortex and an inner chromophobic medulla.

It is now universally held that the acrosome owes its origin to the Golgi bodies (Gatenby, 1917, Bowen, 1920, 1922, and 1924, and Nath, 1942, 1944, 1951, 1953). According to Bowen, the Golgi bodies in the hemipteran spermatid fuse to form a single acroblast, which is sloughed off after it has secreted the acrosome. My observations on *L. maculatus* and *S. rusticum* are fully in accord with Bowen's on Pentatomids in this respect.

Payne (1927) in *Gelastocoris* describes the acrosome arising from the fusion of a large number of 'pro-idiosomal spheres' met with in the late growth period of the spermatocytes. These spheres fuse to form an idiosome. The author shows the Golgi bodies clustering round the 'idiosome', but he is unable to say whether they contribute anything to idiosome formation or not. He is also doubtful regarding the origin of the 'spheres'.

Chickering (1927) in Belostomatidae agrees fully with the observations of Bowen (1922), but his account is incomplete as there is no reference to acrosome formation at all.

Pollister (1930) in *Gerris* also agrees with Bowen as regards acrosome formation, but shows the acrosome to consist of a sheath and a core each of which shows further differentiation. I am unable to agree with Pollister regarding these details of the structure of the acrosome.

The mitochondria are present as a juxta-nuclear, granular mass in the spermatogonia of *Laccotrephes maculatus* and *Sphaerodema rusticum*. During growth of the spermatocytes, these granules increase in size, become vesicular and reveal a chromophilic covering, enclosing a chromophobic substance in its interior. The vesicular mitochondria seem to be sorted out into two sub-equal halves in the two meiotic divisions. During spermateleosis, these vesicles coalesce to form a compact nebenkern, which

later on gets differentiated into an outer chromophobic and an inner chromophilic core. Gradually the chromophilic core disappears and a 'central substance' of (Bowen) appears in the form of numerous granules in the chromophobic area. These 'central substance' granules seem to form the sheath of the mature sperm. A number of tail vesicles are also observed along the entire length of the maturing spermatid. Bowen (1920, 1922) was the first worker to study the mitochondria in spermatogenesis with modern cytological technique. My observations are in full accord with those of Bowen.

VI. Summary

1. In this paper, the behaviour of the cytoplasmic elements and the sex-chromosomes in the male germ cells during the course of spermatogenesis has been worked out in two species of Hemiptera-Heteroptera, viz., the water scorpion *Laccotrephes maculatus* Fabr. and the water bug *Sphaerodema rusticum* Fabr.

2. There is multiple sex-chromosome mechanism consisting of $2X$ and a Y chromosome in *L. maculatus*; while there is a single X and a Y chromosome in *S. rusticum*.

3. In both the species, the sex-chromosomes divide equationally in meiosis I, and reductionally in meiosis II.

4. The mitochondria are granular in the spermatogonia and become vesicular during the growth of the spermatocytes. They coalesce to form a nebenkern in the spermatid in both the species.

5. The Golgi bodies are complete spheres revealing a duplex structure in both the species.

6. The Golgi bodies and the mitochondria seem to be equally divided in the two meiotic divisions.

7. There is a single acroblast in the spermatid formed by the fusion of the Golgi bodies. The acrosome gets differentiated from the acroblast; the latter finally disappears in the tail region of the spermatid.

8. The transformation of the spermatid nucleus into the mature sperm nucleus is unusually simple—there being no reversal of the chromophilic and chromophobic substances of the nucleus during the process of spermateleosis.

9. The centrioles in *L. maculatus* and *S. rusticum*, are crescentic.

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VIII. Explanation of Lettering in Plates

A.—Acroblast; *A'* or A_1.—Acrosome; *A.Ch.*—Sex-chromosomes; *A.g.*—Acrosomal granule; *A.f.*—Axial filament; *C.*—Centrosome; *C.B.*—Chromophobic area; *Ch.*—Chromosomes; *C.L.*—Chromophilic area; *C.s.*—Central substance; *G.*—Golgi elements; *M.*—Mitochondria; *M.n.*—Mitochondrial nebenkern; *M.B.*—Mid-body; *N.*—Nucleus; N_1.—Nucleolus; *S.f.*—Spindle fibres; *V.*—Tail vesicle.

IX. Explanation of Figures in the Plates

All figures of the Plates I–IV have been drawn with a camera lucida at the table level with spencer 10× eyepiece and oil immersion objective giving approximate magnification of 1,700 times. All the figures of the Plates I–IV except Figs. 6, 14 and 17, Pl. I, and Figs. 2, 8 and 14, Pl. III have been selected from sectioned material fixed in Flemming-without-acetic acid followed by 0·5% iron haematoxylin.

Fig. 42, Pl. II, and Fig. 42, Pl. IV are from smear preparations made by Flemming-without-acetic acid diluted equally with water and stained in 0·5% iron haematoxylin.

All figures of Plate V are from the fresh material of *Laccotrephes maculatus* studied under the Phase Contrast Microscope.

Plate I (*Laccotrephes maculatus*)

Fig. 1. Resting spermatogonia.
„ 2. Primary spermatocyte showing leptotene nucleus, mitochondria and Golgi elements.
„ 3. Primary spermatocyte showing the sex-chromosomes condensed into a deeply-staining body.
„ 4. Primary spermatocyte showing scattered Golgi bodies, mitochondria and the nucleus revealing nucleolus, sex-chromosomes and pachytene threads.
„ 5. Primary spermatocyte. Diplotene stage.
„ 6. Primary spermatocyte showing tetrads. Two crescentic centrioles in the cytoplasm are very clear (Bouin's).
Figs. 7 and 8. Primary spermatocytes showing condensation of the tetrads into dumb-bell chromosomes.
Fig. 9. Polar view of metaphase I showing 22 chromosomes.
„ 10. Primary spermatocyte showing two centrioles located at the apices of the meiotic spindle. The mitochondria are arranged on either side of the spindle.
„ 11. Early anaphase I.
„ 12. Telophase I showing mid-body granules at the equator of the dividing cell.
„ 13. Polar view telophase I.
„ 14. Polar view metaphase II showing 22 chromosomes (Bouin's).
„ 15. Polar view metaphase II showing 22 chromosomes.
„ 16. Side view metaphase II showing the crescentic centrioles at the two poles of the spindle and scattered Golgi bodies.
„ 17. Side view metaphase II showing two *X* chromosomes going towards one pole and a *Y* chromosome to the opposite polo (Bouin's).
„ 18. Early anaphase II.
Figs. 19–21. Telophase II.

Plate II (*Laccotrephes maculatus*)

Figs. 22 and 23. Early spermatids. The Golgi bodies aggregate in the neck between nucleus and mitochondrial nebenkern. Deeply-staining chromatin is seen along the periphery of the nucleus.
Fig. 24. Spermatid showing homogeneously staining nucleus; vacuolar chromophilic core of the nebenkern and an acroblast with acrosomal granule.
Figs. 25–27. Spermatids showing dissolution of transverse strands of the nebenkern. The axial filament is springing from the centriole.
Figs. 28 and 29, and 31–33. Spermatids showing gradual obliteration of the chromophilic core of the nebenkern and the movement of the acroblast with the acrosomal granule round the nucleus.
Fig. 30. T.S. tail region of the spermatid showing small chromophilic core and numerous ' central substance ' granules in the chromophobic area.
Figs. 34 and 35. Spermatids. The nebenkern elongates and gets constricted into tail vesicles.
Figs. 36 and 37. Spermatids showing disassociation of the acrosome from the acroblast.
Figs. 38–41. Progressive stages in the elongation of the sperm nucleus.
Fig. 42. Mature sperm showing deeply-staining nucleus with an acrosome at its tip and a long tail posteriorly (F.W.A. smear).

PLATE III (*Sphaerodema rusticum*)

FIG. 1. A group of spermatogonia. Each spermatogonium showing a nucleus, an irregular nucleolus and a few mitochondrial granules in the cytoplasm.

„ 2. Polar view spermatogonial metaphase showing 28 distinct chromosomes (Bouin's).

„ 3. Side view spermatogonial metaphase.

„ 4. Spermatogonial telophase. The mitochondria are forming a palisade round the spindle.

FIGS. 5 and 6. Primary spermatocytes showing mitochondria and deeply-staining Golgi bodies. Two distinct sex-chromosomes in the preleptotene nuclei can be seen also.

FIG. 7. Primary spermatocyte showing leptotene nucleus.

„ 8. Primary spermatocyte showing pachytene nucleus (Bouin's).

„ 9. Primary spermatocyte showing diplotene chromosomes. The Golgi bodies and vesicular mitochondria are juxta-nuclear.

„ 10. Primary spermatocyte showing diffuse nucleus.

FIGS. 11–13. Primary spermatocytes showing diakinesis.

FIG. 14. Primary spermatocyte showing 14 bivalents (Bouin's).

„ 15. Polar view metaphase I showing 15 chromosomes.

„ 16. Side view metaphase I. The two crescentic centrioles can be seen at the two poles of the spindle.

„ 17. Early anaphase I.

„ 18. Polar view anaphase I.

PLATE IV (*Sphaerodema rusticum*)

FIG. 19. Telophase I.

„ 20. Polar view metaphase II.

„ 21. Side view metaphase II.

„ 22. Telophase II.

FIGS. 23-25. Spermatids showing coalescence of mitochondria and the fusion of the Golgi bodies.

FIGS. 26 and 27. Spermatids showing nucleus with a deeply-staining granule in its interior; mitochondrial nebenkern, acroblast with an acrosomal granule.

FIGS. 28–31. Spermatids showing progressive differentiation of the nebenkern and the growth of the acrosomal granule.

FIGS. 32–34. Spermatids showing the elongation of the mitochondrial nebenkern.

FIGS. 35–37. Late spermatids. The acrosomal granule separates from the acroblast which moves down in the tail of the spermatid.

FIG. 38. Tail vesicle of the spermatid showing a sloughed off Golgi body in its interior.

FIGS. 39-41. Late spermatid heads showing deeply-staining acrosome and a uniformly staining nucleus.

FIG. 42. Mature sperm (F.W.A. smear).

PLATE V (*Laccotrephes maculatus*)

FIG. 1. Primary spermatocyte showing nucleus, spherical Golgi bodies and vesicular mitochondria.

„ 2. Polar view metaphase I showing 22 chromosomes.

„ 3. Side view metaphase I. The Golgi and the mitochondria are forming a palisade round hyaline spindle area. Two crescentic centrioles are also seen.

„ 4. Telophase I showing mitochondria and the deposition of the mid-body granules at the equator of the dividing cell.

„ 5. An early spermatid showing the compact mass of vesicular mitochondria and an acroblast.

FIG. 6. Spermatid showing uniformly grey nucleus, acroblast and mitochondrial nebenkern differentiated into outer vacuolar chromophobic area and inner solid chromophilic core.

,, 7. Spermatid showing the axial filament dividing the mitochondrial nebenkern into two halves.

,, 8. Spermatid showing the differentiation of the acroblast into acrosomal granule.

,, 9. Two spermatids enclosed in a common cell wall showing nucleus; acroblast accompanied with an acrosomal granule and 'central substance' granules in the chromophobic area of the mitochondrial nebenkern.

,, 10. Spermatid showing centriole lying on the surface of the nucleus from which springs the axial filament dividing the nebenkern into two halves.

FIGS. 11-13. Progressive stages of the elongation of the spermatid.

FIG 14. Very late spermatid showing nucleus, attached to which is the acrosome.

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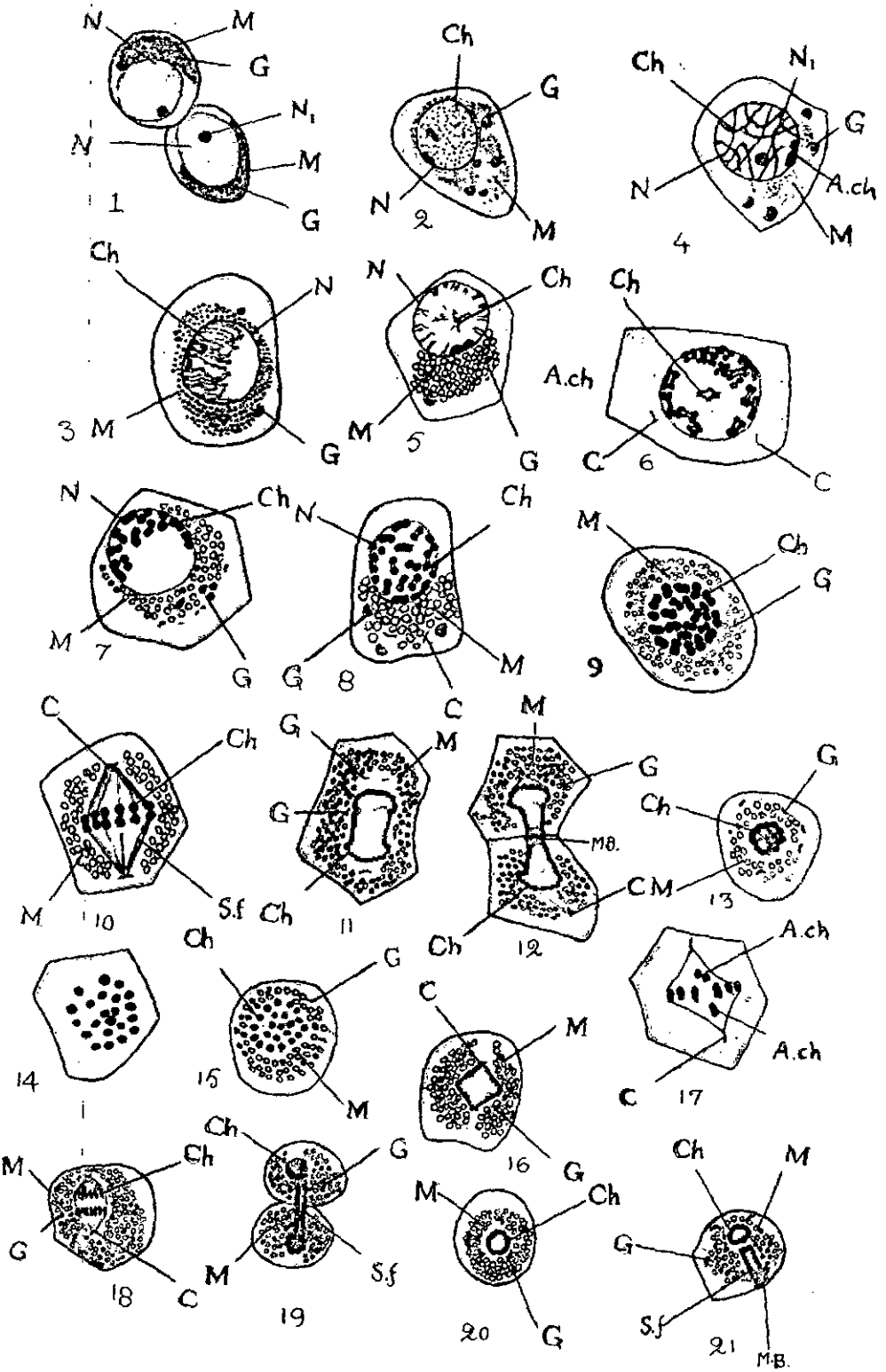
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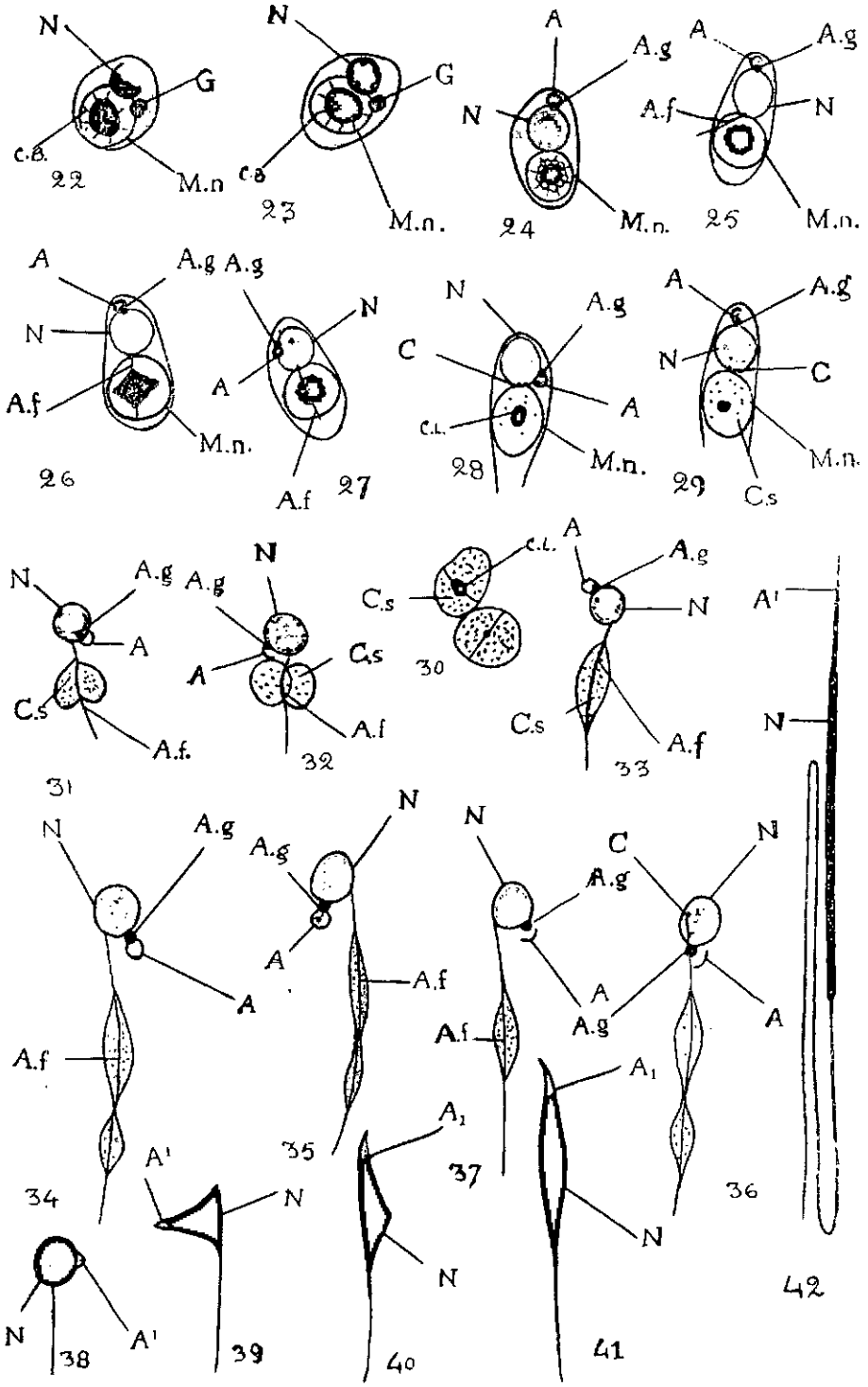
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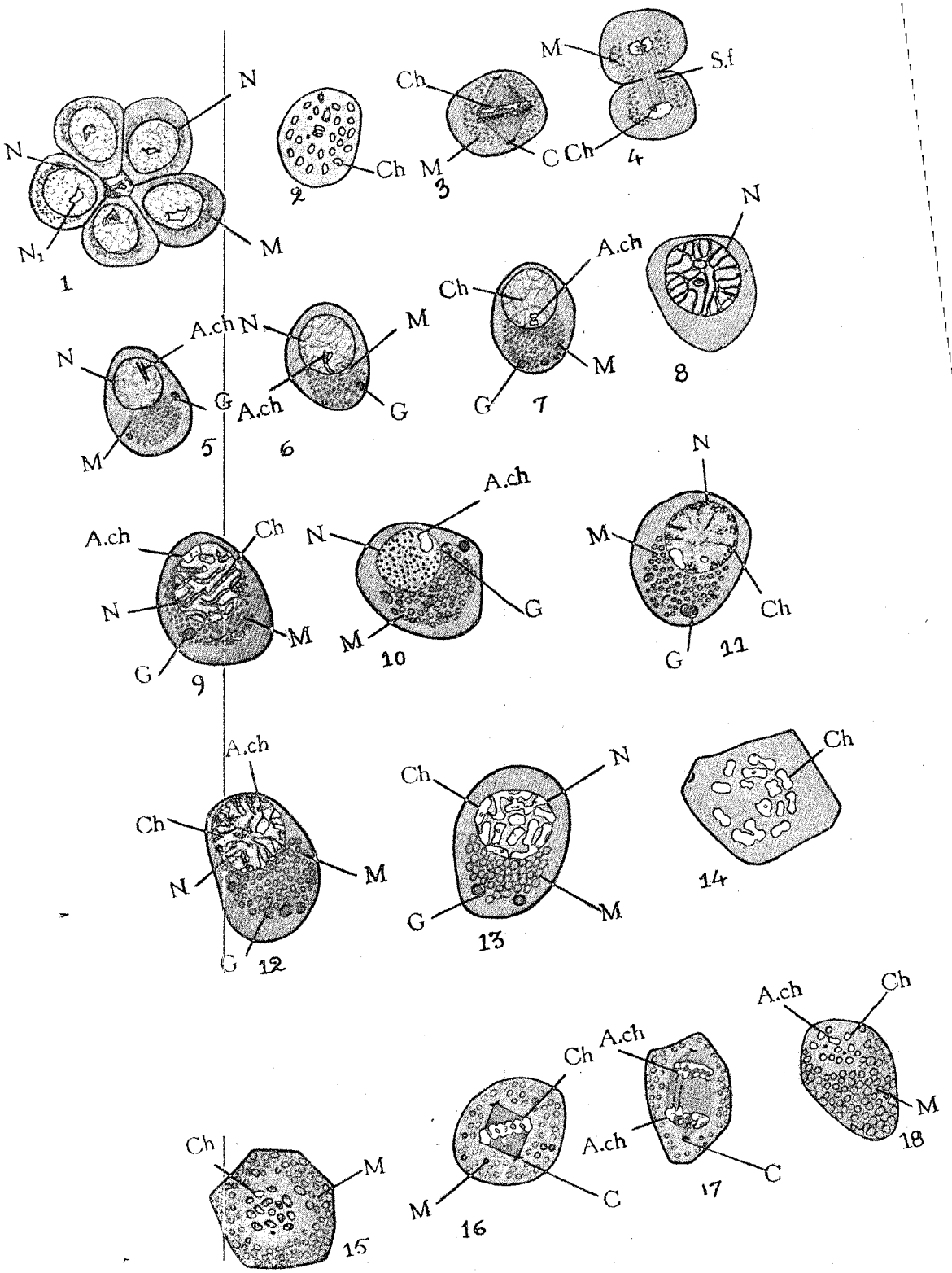
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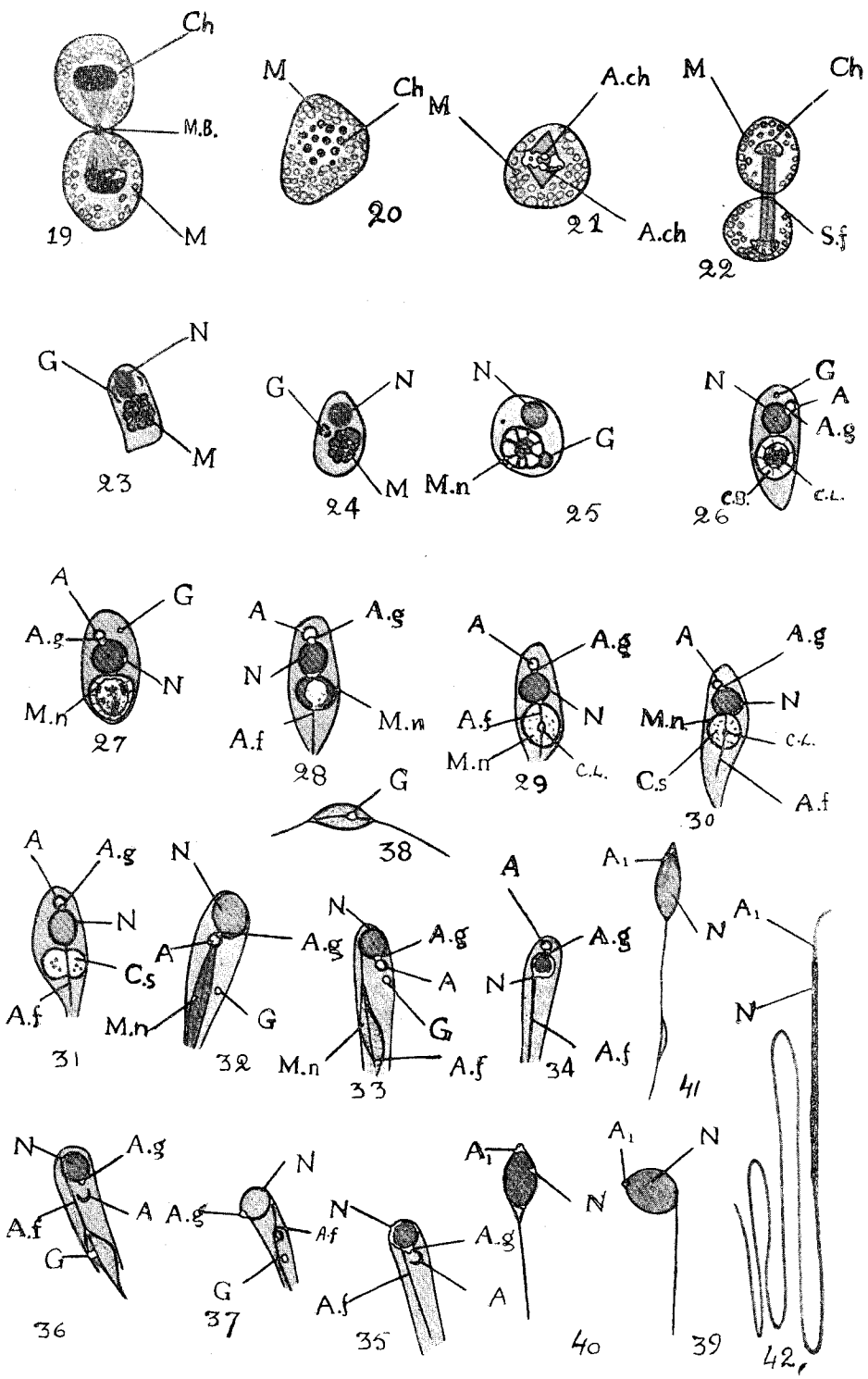
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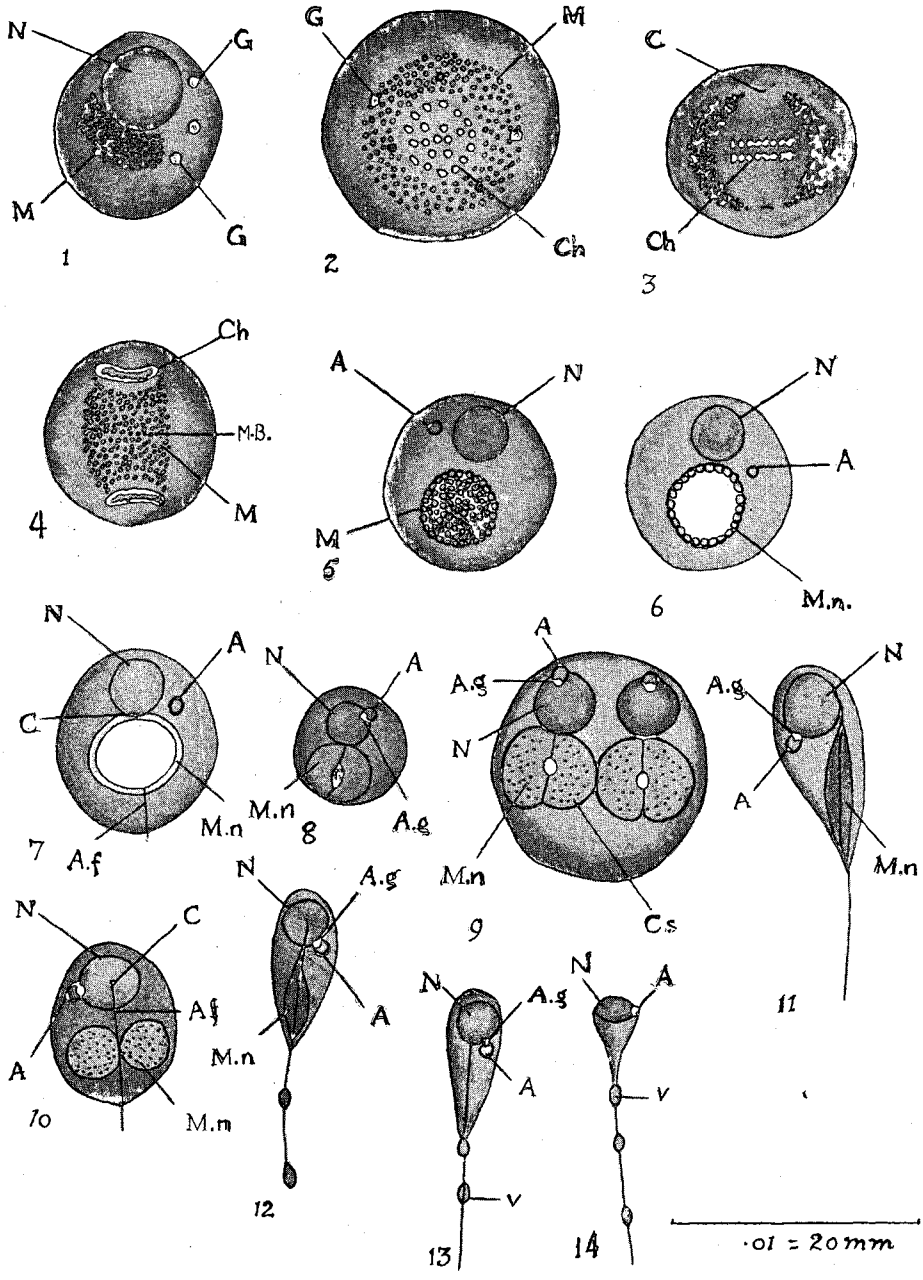
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RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 40 ZOOLOGY Pp. 193–204

December, 1953

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

I. Spermatogenesis of Elasmobranchs (Hypotremata)

by

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Edited for the East Panjab University by Vishwa Nath,
Department of Zoology, East Panjab University,
HOSHIARPUR

Price Rs.3-14-0

All correspondence in connection with this Bulletin should be addressed to Dr. Vishwa Nath, University Professor of Zoology, and Editor, Research Bulletin (Science), Panjab University, Hoshiarpur (Panjab-India).

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

I. Spermatogenesis of Elasmobranchs (Hypotremata)

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

Introduction

In recent years the cytoplasmic inclusions in the germ cells of a large number of animals have been the subject of investigation, but, strange as it would appear, fish spermatogenesis has received very little attention.

To the best of my knowledge Hermann (1882) was the first worker, who gave an exhaustive account of the sperm formation in Selachians. A year later Jensen (1883), and Swaen and Masquelin (1883) published two independent papers on the same subject. Ballowitz (1890) published a paper on tail-formation in fish sperm. Cunningham (1892) worked on the spermatogenesis of a cyclostome, *Myxine glutinosa*. Moore (1895) worked mainly on the nuclear changes in different sharks and rays, e.g. *Scyllium canicula, Scyllium catulus, Pristiurus, Torpedo, Raja macrorhynchus* and *Raja maculata*. During the course of his studies Moore describes an 'archoplasmic vesicle' in the cytoplasm, which as will appear later, should be interpreted as the acroblast or pro-acrosome. Likewise the phenomenon of 'cytoplasmic condensation' must be interpreted as mitochondrial aggregation. Suzuki (1899) published a note on the middle-piece and axial filament of fish sperm. Retzius (1909) also worked on the sperm development of fish.

Since all these workers unfortunately used fixatives containing strong fat-solvents, they could not have been expected to give an account of the Golgi bodies and the mitochondria even if these cell components had been discovered or known sufficiently at that time.

Jean Vaupel (1929) demonstrated the mitochondria and the Golgi bodies in a teleost, *Lebistes reticulatus*, for the first time, by making use of a fixative without a fat-solvent. She has shown a complete degeneration of the Golgi bodies in spermateleosis with the result that the acrosome is conspicuous by its absence. Ratnavathy (1941) worked out the spermatogenesis of the shark, *Chiloscyllium griseum*.

In these series of papers it is intended to publish an exhaustive account of the spermatogenesis of cartilaginous as well as bony fishes, with particular reference to the Golgi bodies and the mitochondria. Unfortunately, this material is very unfavourable for the study of chromosomes, which have a marked tendency to agglutinate.

Material and Technique

The spermatogenesis of nine Elasmobranch species has been worked out in this paper. The names of these species appear below. They all belong to the order Rajiformes, except *Torpedo marmorata*, which belongs to the order Torpediniformes. (1) *Aetomyleus maculatus*, (2) *Aetomyleus nichofii*, (3) *Aetobatus narinari*, (4) *Rhynobatus obtusus*, (5) *Rhynobatus granulatus*, (6) *Dasyatis zugei*, (7) *Dasyatis kuhlii*, (8) *Dasyatis sephen*, and (9) *Torpedo marmorata*.

I started these investigations in 1951. In 1952 I visited different fishing grounds in the suburbs of Bombay during the early hours of the day. The fishing was done with the help of barrier nets. Generally I used to dissect the fish on the spot, but sometimes it was felt necessary to take the catch to the laboratory.

The male genital organs of all the rays and skates studied, except *Torpedo marmorata*, are built up on the same plan as described in *Scoliodon* by Thillayampalam (1928). The testes are a pair of white elongated structures with a pair of vasa deferentia opening to the outer world through the grooves in the claspers. These claspers are only present in the male Elasmobranchs. The testes are situated below the kidneys, extending from the base of the liver to the rectal gland and are attached to the body wall by a fold of peritoneum. In *Torpedo marmorata* the testes are small bean-shaped structures situated at the anterior end of the kidneys. Immediately after the testes were taken out they were put in normal saline, cut into small pieces of not more than one millimeter in diameter, and were transferred to 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. Amongst all the fixatives tried, Flemming-without-acetic acid proved to be the best. This was followed by 0·5% iron haematoxylin. Best results were obtained after fixation in F.W.A. for 24 or 48 hours. The material was washed in running water for the same period as it was kept in the fixative. Sections were cut 5 μ thick.

Acknowledgements

My thanks are due to the staff and the curator, Taraporewala Aquarium, Bombay, and in particular to Dr. S. B. Setna, Director of Bombay Fisheries, for the help, which they all rendered ungrudgingly. I am also grateful to Dr. Vishwa Nath for suggesting this problem to me and for correcting the manuscript of this paper. My thanks are also due to Mr. Ashok Nair for the translation of some French publications.

Observations

As the spermatogenesis of the nine Elasmobranch species described here is more or less similar, it has been considered best to give a compara-

tive account, with occasional references to the existing variations especially in the process of spermateleosis.

Spermatogonia

The earliest spermatogonia in all the nine rays and skates studied are small rounded cells with vesicular nuclei. The nucleus contains chromatin granules, delicate chromatin threads and a distinct darkly-stained nucleolus. The nucleus is large and central in position, with an attenuated layer of cytoplasm. The cytoplasm is clear and does not contain any granules in it (Pl. I, Fig. 1; Pl. II, Fig. 31; Pl. III, Fig. 57; Pl. IV, Figs. 83 and 109; and Pl. VII, Fig. 164). As the spermatogonium grows in size a few dust-like granules make their appearance in the cytoplasm. These are the mitochondrial granules (Pl. I, Fig. 2; Pl. II, Fig. 32; Pl. V, Fig. 129; and Pl. VII, Fig. 177). These granular mitochondria are grey in colour. In *Rhynobatus obtusus*, however, the mitochondria are vesicular (Pl. IV, Figs. 84 to 86). It is further observed that one or more granules, which are comparatively larger and which stain more sharply also make their appearance amongst the mitochondria (Pl. I, Fig. 2; Pl. III, Fig. 58; Pl. IV, Figs. 85 and 110; Pl. VI, Fig. 143; and Pl. VII, Figs. 165 and 177). These are the Golgi bodies. In *R. obtusus*, in a comparatively more grown-up cell, the Golgi bodies clearly appear as vesicles and crescents (Pl. IV, Fig. 86).

Primary Spermatocyte

The primary spermatocyte is the largest cell in the testis. It has a vesicular nucleus, generally containing a nucleolus. During the growth period the mitochondria grow in size appreciably into darkly-staining granules (Pl. I, Figs. 3 to 7; Pl. II, Figs. 33 to 35; Pl. III, Figs. 59 to 61; Pl. IV, Figs. 111 to 114; Pl. V, Figs. 130 to 132; Pl. VI, Figs. 144 and 145; and Pl. VII, Figs. 166, 167 and 178), except in *R. obtusus*, where the mitochondria appear as feebly-staining vesicles (Pl. IV, Figs. 87 to 90). In this species it is seen that the mitochondria, which were vesicular even in the spermatogonia, continue to remain so except that they have now grown in size. In *Aetobatus narinari* at a little advanced stage the mitochondria also become vesicular (Pl. III, Figs. 62 and 63).

It is very difficult to spot out the Golgi elements in the majority of the primary spermatocytes as they are mixed up with the darkly-staining mitochondria. This, however, is not the case in all the cells. A few of the spermatocytes have distinct Golgi bodies. The Golgi bodies do not lie in a particular position, but are scattered amongst the mitochondria. The Golgi bodies are generally very few in number as compared with the mitochondria, and are more often than not in the form of crescents. In *Rhynobatus obtusus* where the mitochondria are vesicular from the very beginning, the Golgi elements are very clear and are sometimes seen to occupy a juxta-nuclear position (Pl. IV, Fig. 89).

A few division stages (meiosis I) in *Aetomyleus maculatus*, *Aetomyleus nichofii* and *Dasyatis kuhlii* have been observed and figured by me. The

Golgi bodies and the mitochondria are probably sorted out, more or less evenly, to the two secondary spermatocytes. During the division of the cell, a well-defined spindle can be noticed. Two prominent sharply-stained centrosomes appear, one at each pole of the spindle. During division stages, the chromosomes always appear as a deeply-stained compact mass (Pl. I, Fig. 8; Pl. II, Fig. 36; and Pl. VI, Fig. 146). During anaphase I, the single chromosome mass splits into two, one mass moving towards one pole and the other to the opposite pole (Pl. I, Fig. 9; and Pl. VI, Fig. 147). During telophase I, two daughter nuclei are formed and the centrosomes disappear from the scene (Pl. I, Fig. 10; Pl. II, Fig. 37; and Pl. VI, Fig. 148). Cytoplasmic cleavage then follows (Pl. II, Fig. 38).

Secondary Spermatocyte

The secondary spermatocytes are smaller than the primary spermatocytes, but bigger than the spermatogonia (Pl. I, Figs. 11 and 12; Pl. II, Figs. 39 and 40; Pl. III, Fig. 64; Pl. IV, Figs. 91, 92 and 115; Pl. V, Figs. 116 and 133; Pl. VI, Fig. 149; and Pl. VII, Figs. 168 and 179). There is a definite interkinetic resting stage, when the nucleus of the secondary spermatocyte is re-formed (Pl. I, Fig. 12; Pl. II, Figs. 39 and 40; Pl. III, Fig. 64; Pl. IV, Figs. 91 and 92; Pl. V, Figs. 116 and 133; Pl. VI, Fig. 149; and Pl. VII, Figs. 168 and 179), and in some cases all traces of chromosomes are lost (Pl. II, Fig. 40). The secondary spermatocytes have the usual complement of mitochondria and Golgi bodies.

In *Aetomyleus maculatus, Aetomyleus nichofii* and *Dasyatis kuhlii* metaphase II shows the chromosomes jumbled up in a single mass (Pl. I, Fig. 13; Pl. II, Fig. 41; and Pl. VI, Fig. 150). This single chromatic mass lies at the equator of the spindle, while at each pole there is a prominent centrosome. The mitochondria are either vesicular or granular. The Golgi bodies and the mitochondria are evenly scattered in the cell. In Pl. I, Fig. 14 is shown a cell in anaphase II. In Pl. II, Fig. 42, and Pl. VI, Fig. 151, are shown two early telophase II stages.

Spermateleosis

Since there is no essential difference in the spermateleosis of the nine Elasmobranch species under discussion, it has been thought best to give a comparative account of this process as has been done in the case of spermatogonia and spermatocytes.

The earliest spermatid reveals in almost all cases, a very darkly-staining excentric nucleus, a few Golgi bodies, juxta-nuclear mitochondria and two centrosomes. The mitochondria are either still granular or have become vesicular (Pl. I, Fig. 15; Pl. II, Figs. 43 to 45; Pl. III, Fig. 65; Pl. IV, Figs. 93 and 94; Pl. V, Figs. 121 to 123; Pl. VI, Fig. 152; and Pl. VII, Figs. 169 and 180).

When the transformation of the spermatid begins, either a single Golgi body advances towards the nucleus to form the acrosome as in *Aetomyleus nichofii, Dasyatis zugei, Dasyatis kuhlii* and *Torpedo marmorata*

(Pl. II, Figs. 47 to 51; Pl. V, Figs. 134 to 136; Pl. VI, Figs. 153 and 154; and Pl. VII, Fig. 181), or a few Golgi bodies come close together and ultimately fuse to form the fore-runner of the acrosome (pro-acrosome) as in *Aetomyleus maculatus, Rhynobatus obtusus, Rhynobatus granulatus* and *Dasyatis sephen* (Pl. I, Figs. 17, 21 and 22; Pl. III, Figs. 66 and 67; Pl. V, Figs. 121 and 122; and Pl. VII, Figs. 169 and 170). The pro-acrosome travels forward along the side of the nucleus and ultimately fuses with its anterior aspect. Sooner or later this darkly-staining pro-acrosome is gradually transformed into a semi-transparent pear-shaped acrosome (Pl. I, Figs. 23 to 30; Pl. II, Figs. 52 to 56; Pl. III, Figs. 72 to 82; Pl. IV, Figs. 97 to 108; Pl. V, Figs. 124 to 128 and 137 to 141; Pl. VI, Figs. 155 to 163; and Pl. VII, Figs. 171 to 176 and 182 to 187).

It must be noted carefully that all the Golgi bodies present in the cell do not take part in the formation of the pro-acrosome. A few, which are left unused, remain as the Golgi remnant and are sloughed off along with the residual cytoplasm (Pl. II, Figs. 49 to 55; Pl. III, Fig. 74; Pl. IV, Figs. 97, 99, 101, 102 and 104; Pl. V, Figs. 125, 127, 136, 137 and 140; Pl. VI, Figs. 155, 160, 161 and 162; and Pl. VII, Figs. 171, 172, 174, 181, 182, 183 and 185).

While the Golgi bodies are taking part in the formation of the acrosome the mitochondria are becoming vesicular and are coming close to one another. These compactly arranged mitochondria lie on one side of the nucleus and are traversed by the axial filament. It appears that during the later stages of spermateleosis, the mitochondria lose their staining capacity. Nevertheless, most of them form a compact sheath of the axial filament in the region of the middle-piece, whereas a few are sloughed off along with the residual cytoplasm, which also contains the Golgi remains. Although mitochondria as such cannot be observed in the middle-piece of the ripe sperm, there is no doubt that the sheath of the axial filament in this region is mitochondrial in nature (Pl. III, Figs. 80 and 81; Pl. IV, Figs. 105 and 106; Pl. V, Fig. 140; and Pl. VII, Figs. 185 and 186).

Concomitant with these changes the two centrosomes of the early spermatid move apart, and there appears an intra-cellular filament between them (Pl. I, Fig. 19; Pl. II, Fig. 46; Pl. III, Figs. 68 and 69; and Pl. VII, Fig. 170). The two centrosomes are named according to their position with respect to the nucleus. The anterior one, which is nearer the nucleus, is called the proximal centrosome, and the posterior one the distal. The proximal centrosome soon moves more anteriorly and comes in touch with the nucleus. It is visible only for a short time as it gets covered by the nucleus at a later stage (Pl. I, Figs. 21 to 23; Pl. II, Figs. 47, 51 and 52; Pl. III, Figs. 72 to 77; Pl. IV, Figs. 98 to 108; Pl. V, Figs. 125 to 127 and 135 to 141; Pl. VI, Figs. 156 and 157; and Pl. VII, Figs. 172, 174 to 176 and 182 to 185). The proximal centrosome does not by any means fuse with the nucleus but remains separate as will be clear from the study of many figures (Pl. I, Fig. 24; Pl. II, Figs. 46, 49 and 50; Pl. III, Figs. 78 and 80; Pl. VI, Figs. 159 and 162; and Pl. VII, Figs. 173 and 186). The granular distal centrosome abuts against the cell wall and seems to give rise to the extra-cellular filament, which forms the tail of the sperm. The proximal

and the distal centrosomes are situated at the anterior and posterior ends of the middle-piece respectively.

One finds that once the distal centrosome touches the cell wall, the cell begins to elongate, and simultaneously the intra-cellular axial filament also begins to lengthen out. With the elongation of the cell the nucleus also begins to elongate (Pl. I, Figs. 23 to 27; Pl. II, Fig. 51; Pl. III, Figs. 72 to 74; Pl. IV, Figs. 100 to 104; Pl. VI, Figs. 156 to 159; and Pl. VII, Figs. 173 to 175 and 183). In *Aetobatus narinari*, the elongating nucleus, which is horizontal in position at first, rotates to a vertical position (Pl. III, Figs. 72 to 75). In *Rhynobatus obtusus* the nucleus is at first obliquely placed, and it then assumes a vertical position. Sometimes a spermatid nucleus is curved due to exigencies of space (Pl. IV, Figs. 100 to 104).

As the nucleus and the acrosome are ripening, the residual cytoplasm containing the Golgi bodies and the mitochondria is pushed down the axial filament in the form of blebs (Pl. III, Fig. 80; Pl. IV, Figs. 105 and 106; Pl. VI, Fig. 162; and Pl. VII, Fig. 186).

In *Dasyatis zugei* after the formation of the middle-piece there appears a constriction at a point of location of the distal centrosome. The piece behind the distal centrosome is the residual cytoplasm, which soon gets separated from the area in front and starts moving backward (Pl. V, Figs. 138 to 140).

The fully ripe sperm has a long cylindrical nucleus with a short acrosome in front, a middle-piece between the two centrosomes behind the head and an axial filament forming the tail or flagellum.

Discussion

As has already been pointed out all the previous workers on the fish sperm used fixatives containing fat-solvents, except Jean Vaupel (1929) and Ratnavathy (1941). But apart from this the fish material is rather refractory so far as fixation is concerned. At any rate, it is not a favourable material for cytological studies. Reference to the difficulties of fixation has also been made by Jean Vaupel and Ratnavathy.

Jean Vaupel carried out her investigation on the spermatogenesis of *Lebistes reticulatus*, a bony fish. Since I am publishing a paper on the sperm of bony fish at a subsequent stage, it is not my intention to review her work here.

Before taking up the work of Ratnavathy (1941) for discussion, it will be profitable to discuss briefly the work of earlier workers, who used exclusively fixatives containing fat-solvents and carried out their investigations at a time when the Golgi bodies and the mitochondria had not even been discovered.

Hermann (1882), the earliest worker on the subject of Elasmobranch sperm, nevertheless, gave a faithful account of what he saw in his preparations. Hermann describes a body, 'nodule cephalique', which is, later on, applied to the surface of the 'kernel' (nucleus) of the 'spermatoblast' (spermatid). The 'nodule cephalique' forms, according to him, the 'cephalic point', which undoubtedly is the acrosome in modern terminology.

The greasy granules of Hermann, which are said to unite to form the 'nodule cephalique', are undoubtedly the Golgi bodies, which have been distorted very much.

Jensen (1883), who studied some fresh Elasmobranch material also describes two refringent bodies in fresh spermatids slightly osmicated. These bodies always take a very deep colouring in osmic acid. They fuse together and form one voluminous body, which ultimately takes a position in a depression at the anterior aspect of the nucleus. Here the voluminous body becomes cylindrical. There is no doubt that the refringent bodies are the Golgi elements, as the Golgi elements being lipoidal are definitely known to blacken with osmic acid. Nath (1933) has employed this technique exhaustively and has demonstrated the Golgi elements in fresh oocytes slightly osmicated. Likewise we have no doubt that Jensen's voluminous body is transformed into the acrosome of the ripe sperm.

Swaen and Masquelin (1883), while working on some Elasmobranch sperms, describe a problematic body, which gradually comes near the anterior aspect of the nucleus and ultimately comes in contact with it. Here also it can safely be concluded that the problematic body of these authors corresponds to my pro-acrosome.

Moore (1895) describes an 'archoplasmic vesicle', which becomes first flattened and then elongates out, together with the nuclear chromatin, forming a definite 'cephalic point to the spermatozoon head'. The 'archoplasmic vesicle' of Moore and his 'cephalic point' obviously correspond to my pro-acrosome and acrosome respectively.

Ratnavathy (1941), working on the spermatogenesis of the shark *Chiloscyllium griseum*, gives an account of acrosome formation. It will be profitable to describe this process in her own words—'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 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 the sperm formation as a thin short apical filament, until in the metamorphosed spermatozoon it presents the appearance of a spirally twisted filament'. I have myself completed investigations on the spermatogenesis of this shark and I hope

to send my paper to the press shortly. I agree with Ratnavathy that the acrosome in *Chiloscyllium* 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' of Ratnavathy. The process of the formation of the acrosome from the Golgi bodies in the shark is exactly like the process described in rays and skates in this paper. In the shark *Chiloscyllium* the earliest spermatid has a few Golgi bodies in the form of granules, which soon come together to form a pro-acrosome, the fore-runner of the acrosome. But there is no indication, whatsoever, of either the 'acroblast' or the 'acrosomal vesicle'.

Summary

1. In this paper the spermatogenesis of nine species of Elasmobranch fishes, i.e. *Aetomyleus maculatus*, *Aetomyleus nichofii*, *Aetobatus narinari*, *Rhynobatus obtusus*, *Rhynobatus granulatus*, *Dasyatis zugei*, *Dasyatis kuhlii*, *Dasyatis sephen* and *Torpedo marmorata*, has been described.

2. The Cytoplasmic inclusions have been thoroughly studied in the spermatogonia, spermatocytes and in the process of spermateleosis.

3. The earliest spermatid has an excentrically placed nucleus, a few Golgi elements coming near one another, vesicular mitochondria and two small centrosomes.

4. During spermateleosis, a pro-acrosome is formed directly from one or more Golgi bodies. This fuses with the nucleus and is transformed into an acrosome.

5. There is a prominent middle-piece in the ripe sperm, having a mitochondrial sheath. The two centrosomes, proximal and distal, are situated at the anterior and posterior ends of the middle-piece respectively.

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

7. The ripe sperm has an elongated nucleus with a short acrosome in front, a prominent middle-piece and a long flagellum.

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Abbreviations

A—Acrosome; *A'* or A_1—Pro-acrosome; *A.f*—Axial filament; *C*—Centrosome; *C.s*—Centrosomes; C_1—Proximal centrosome; C_2—Distal centrosome; *C.G*—Chromatin granules; *Ch*—Chromosomes; *G*—Golgi body; *M*—Mitochondria; *M.p*—Middle-piece; *N*—Nucleus; N_1—Nucleolus; *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× eye-piece and oil immersion objective giving a magnification of approximately 1,700 times. All figures (except Pl. I, Figs. 27, 29 and 30; Pl. II, Figs. 55 and 56; Pl. III, Figs. 75 to 82; Pl. IV, Figs. 103, 105 and 108; Pl. V, Figs. 128 and 138 to 141; Pl. VI, Figs. 161 to 163; and Pl. VII, Figs. 176, 183 and 185 to 187 which are drawn from smears) have been drawn from sectioned material fixed in Flemming-without-acetic acid followed by 0·5% iron haematoxylin.

Plate I (*Aetomyleus maculatus*)

Fig. 1. Earliest spermatogonium.
,, 2. Late spermatogonium showing the mitochondria and the Golgi apparatus.
Figs. 3, 4 and 5. Primary spermatocytes.
,, 6 and 7. Primary spermatocytes showing big granules of mitochondria.
Fig. 8. Metaphase I.
,, 9. Anaphase I.
,, 10. Telophase I.
Figs. 11 and 12. Secondary spermatocytes.
Fig. 13. Metaphase II.
,, 14. Early anaphase II.
Figs. 15 and 16. Early spermatids.
Fig. 17. A late spermatid showing the vesicular mitochondria and the Golgi elements in a group.
,, 18. Spermatid showing two centrosomes.
,, 19. Spermatid showing an axial filament.
Figs. 20 and 21. The Golgi bodies are moving in front in a group.
Fig. 22. Pro-acrosome is formed.
Figs. 23 and 24. Pro-acrosome is transformed into an acrosome.
,, 25 to 27. Spermatids showing the nuclei and the cells elongating.
Fig. 28. Sperm from the sections.
,, 29. Early sperm with the residual cytoplasm.
,, 30. Fully formed sperm.

Plate II (*Aetomyleus nichofii*)

Fig. 31. Early spermatogonium.
,, 32. Spermatogonium showing the mitochondria in the cytoplasm.
Figs. 33 to 35. Primary spermatocytes showing mitochondria and Golgi bodies.

FIG. 36. Metaphase I.
„ 37. Telophase I.
„ 38. Late telophase I.
FIGS. 39 and 40. Secondary spermatocytes.
FIG. 41. Metaphase II.
„ 42. Telophase II.
FIGS. 43 and 44. Early spermatids.
FIG. 45. Nucleus of the spermatid taking more stain due to condensation. Note the two centrosomes in addition to the Golgi bodies and mitochondria.
FIGS. 46 and 47. Late spermatids.
„ 48 to 50. The pro-acrosome is formed.
FIG. 51. Spermatid. Note the elongating nucleus.
FIGS. 52 to 55. Pro-acrosome is transformed into an acrosome.
FIG. 56. Mature sperm.

PLATE III (*Aetobatus narinari*)

FIG. 57. Earliest spermatogonium.
„ 58. Late spermatogonium.
FIGS. 59 to 61. Primary spermatocytes.
„ 62 and 63. Late primary spermatocytes showing vesicular mitochondria and Golgi bodies.
FIG. 64. Secondary spermatocyte.
„ 65. Early spermatid.
„ 66. Spermatid showing a distinct tendency of the Golgi elements to come together to form a pro-acrosome.
„ 67. The pro-acrosome is attached to the nucleus.
FIGS. 68 to 70. Spermatids showing a filament between two centrosomes.
FIG. 71. Spermatid showing the intra- as well as extra-cellular filament.
FIGS. 72 and 73. Acrosome is formed. Note the nucleus in the horizontal position.
FIG. 74. The nucleus has rotated to a vertical position.
FIGS. 75 and 76. The nucleus is seen wriggling out of the cytoplasm.
„ 77 and 78. The late spermatids showing the mitochondria on both sides of the axial filament.
„ 79 to 81. Middle-piece of the sperm is formed and the residual cytoplasm is shown going back.
FIG 82. Complete sperm.

PLATE IV (*Rhynobatus obtusus*)

FIG. 83. Early spermatogonium.
„ 84. Spermatogonium showing the appearance of faint vesicular mitochondria.
FIGS. 85 and 86. Late spermatogonia showing the Golgi elements and mitochondria.
„ 87 and 88. Primary spermatocytes.
„ 89 and 90. Primary spermatocytes. Note the Golgi bodies in a group near the nucleus.
„ 91 and 92. Secondary spermatocytes.
FIG. 93. Early spermatid showing a deeply-stained nucleus, the Golgi apparatus and two centrosomes.
„ 94. Spermatid showing pro-acrosome and two centrosomes.
FIGS. 95 and 96. Pro-acrosome has touched the nucleus.
„ 97 to 99. Acrosome is advancing forward and the axial filament is formed.
„ 100 to 104. Spermatids. Nuclei have started elongating.
FIG. 105. Early sperm.
FIGS. 106 and 107. Note the residual cytoplasm going back.
FIG. 108. Fully developed sperm.

PLATE IV (*Rhynobatus granulatus*)

FIG. 109. Early spermatogonium showing a nucleolus in the vesicular nucleus.
,, 110. Spermatogonium showing the mitochondria and the Golgi apparatus.
FIGS. 111 and 112. Primary spermatocytes.
,, 113 and 114. Primary spermatocytes showing the fully grown mitochondria and the Golgi elements.
FIG. 115. Secondary spermatocyte.

PLATE V (*Rhynobatus granulatus*)

FIG. 116. Secondary spermatocyte.
,, 117. Early spermatid.
FIGS. 118 and 119. In the spermatids the nucleus has started showing condensation.
FIG. 120. Spermatid showing a condensed nucleus and a centrosome near the nucleus.
FIGS. 121, 122 and 123. The pro-acrosome is shown on one side of the nucleus.
FIG. 124. Spermatid showing acrosome.
,, 125. Spermatid with an acrosome and an axial filament.
FIGS. 126 and 127. Spermatid with an acrosome and an axial filament, except that the nucleus has rotated along with the acrosome.
FIG. 128. Complete sperm.

PLATE V (*Dasyatis zugei*)

FIG. 129. Spermatogonium.
,, 130. Primary spermatocyte.
FIGS. 131 and 132. Primary spermatocytes showing the grown-up mitochondria.
FIG. 133. Secondary spermatocyte.
,, 134. Spermatid. Note the pro-acrosome.
FIGS. 135 and 136. Pro-acrosome is still not transformed into an acrosome and the axial filament is seen.
FIG. 137. Pro-acrosome is transformed into an acrosome.
FIGS. 138 to 140. The early sperm showing the blebs of residual cytoplasm.
FIG. 141. Fully mature sperm.

PLATE VI (*Dasyatis kuhlii*)

FIG. 142. Spermatogonium.
,, 143. Spermatogonium, showing the mitochondria and the Golgi apparatus.
FIGS. 144 and 145. Primary spermatocytes showing an excentric nucleus with a nucleolus, the mitochondria and the Golgi bodies.
FIG. 146. Metaphase I.
,, 147. Anaphase I.
,, 148. Telophase I.
,, 149. Secondary spermatocyte.
,, 150. Metaphase II.
,, 151. Early Telophase II.
,, 152. Spermatid.
,, 153. Spermatid. Note the centrosome and the pro-acrosome.
,, 154. Pro-acrosome has come in touch with the nucleus.
,, 155. Pro-acrosome is transformed into an acrosome. Note the two centrosomes.
,, 156. Axial filament is formed in the spermatid.
,, 157. The nucleus is elongating.
,, 158. Note the vesicular mitochondria arranged on both sides of the axial filament, and the proximal and the distal centrosome.
,, 159. The nucleus is shown wriggling out of the cytoplasm.
FIGS. 160 and 161. The cytoplasm is shown receding back.
FIG. 162. Spermatid. Note the residual cytoplasm.
,, 163. Sperm.

PLATE VII (*Dasyatis sephen*)

FIG. 164. Early spermatogonium.

,, 165. Spermatogonium with a vesicular nucleus containing nucleolus and chromatin threads. Mitochondria and the Golgi apparatus is also shown.

FIGS. 166 and 167. Primary spermatocytes.

FIG. 168. Secondary spermatocyte.

,, 169. Spermatid showing a deeply-stained nucleus and the Golgi bodies have come close together.

,, 170. Pro-acrosome is formed. Note an intra-cellular filament between the two centrosomes.

,, 171. Pro-acrosome is transformed into an acrosome.

FIGS. 172 to 174. Spermatids showing the cells and the nuclei elongating.

FIG. 175. The cytoplasm is receding back. Note the Golgi body in the residual cytoplasm.

,, 176. Fully formed sperm.

PLATE VII (*Torpedo marmorata*)

FIG. 177. Spermatogonium.

,, 178. Primary spermatocyte.

,, 179. Secondary spermatocyte.

,, 180. Early spermatid showing a darkly-stained excentric nucleus, a few Golgi bodies, vesicular mitochondria and two centrosomes.

,, 181. Note the pro-acrosome.

,, 182. Pro-acrosome is transformed into an acrosome. Note the axial filament and the unutilized Golgi bodies.

FIGS. 183 and 184. The nucleus and the cell has started elongating.

FIG. 185. Early sperm.

,, 186. Early sperm. Note the bleb of residual cytoplasm.

,, 187. Mature sperm. It has an elongated nucleus with a short acrosome in front, a prominent middle-piece and a long flagellum.

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.

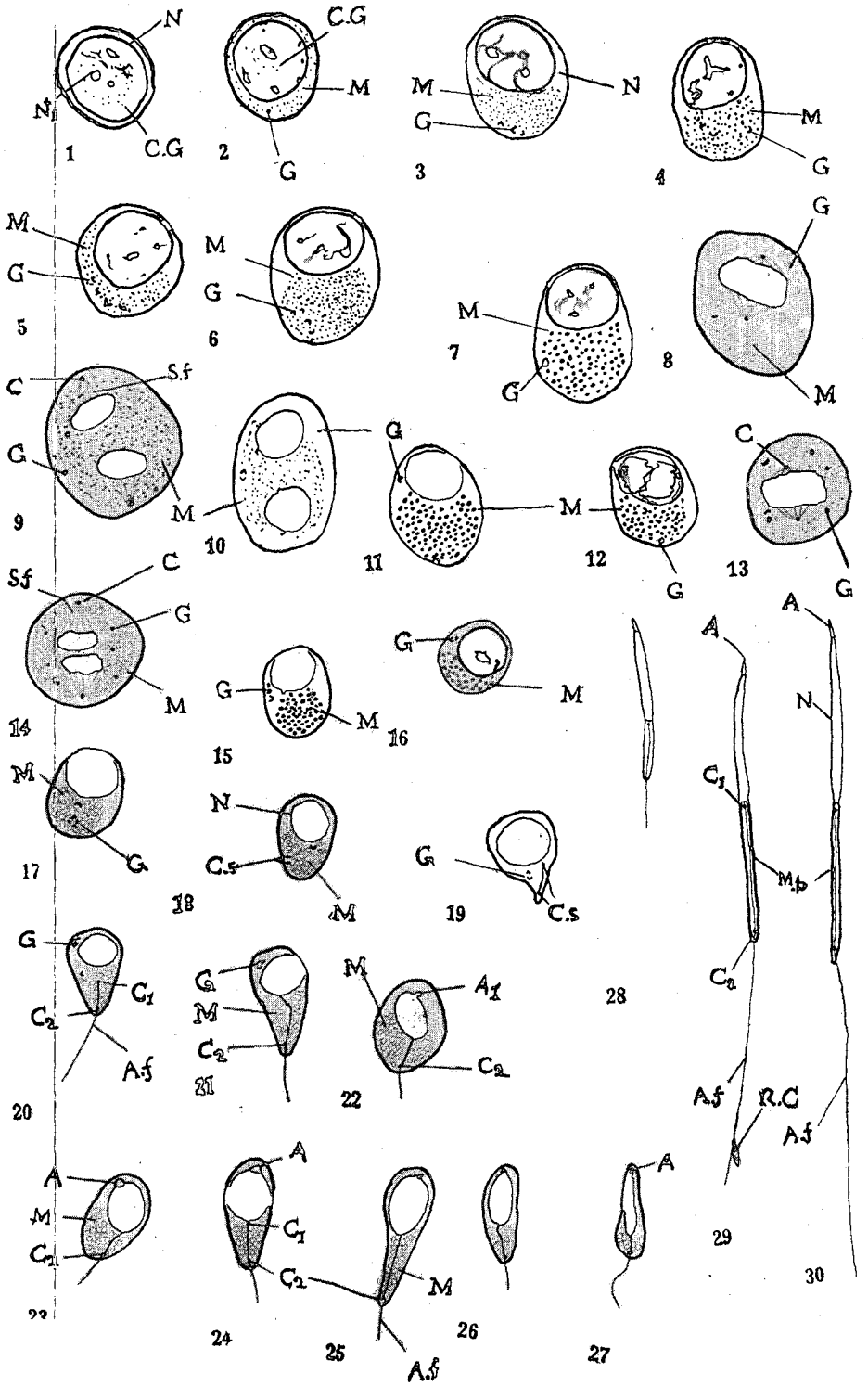
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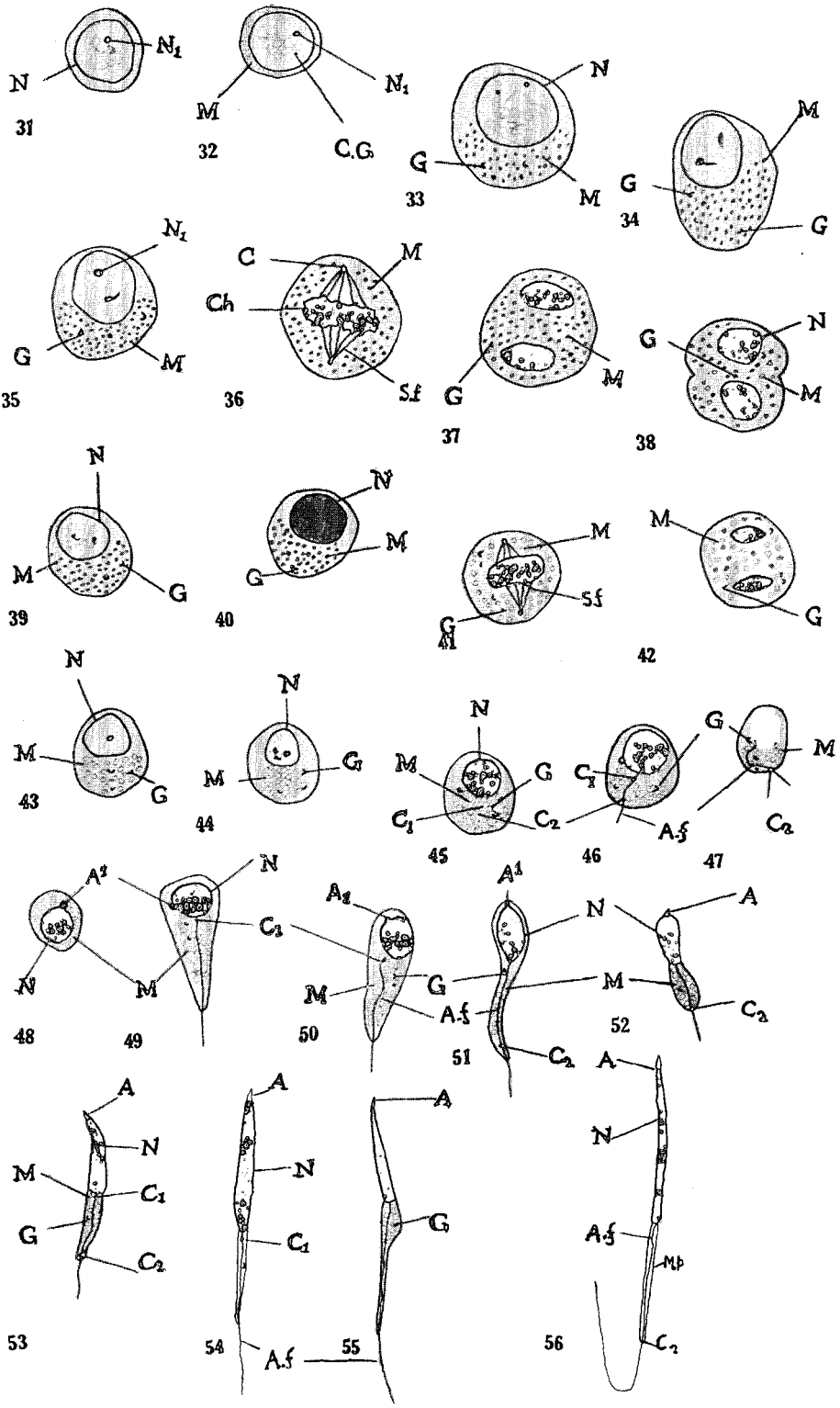
PLATE II.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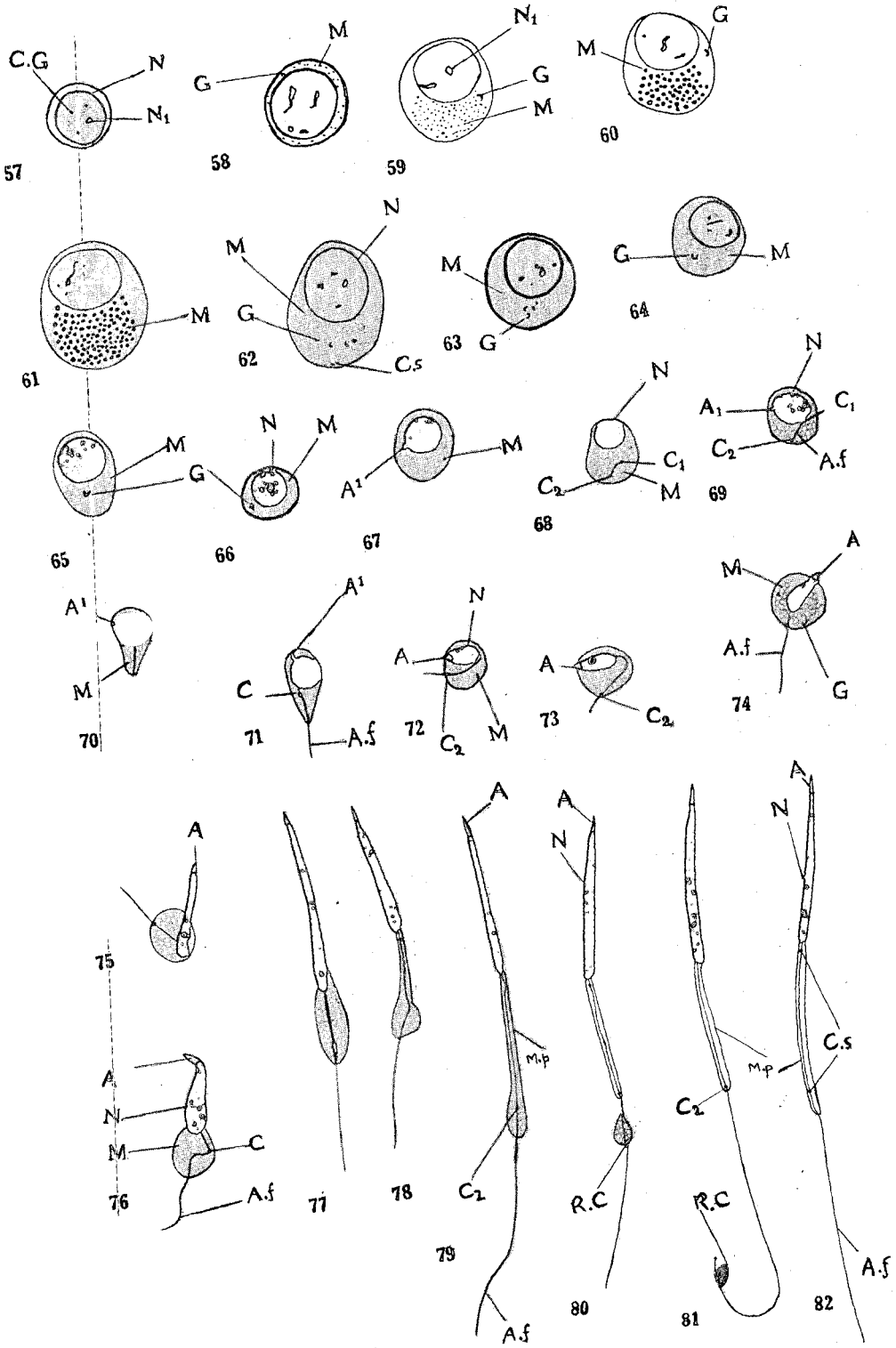
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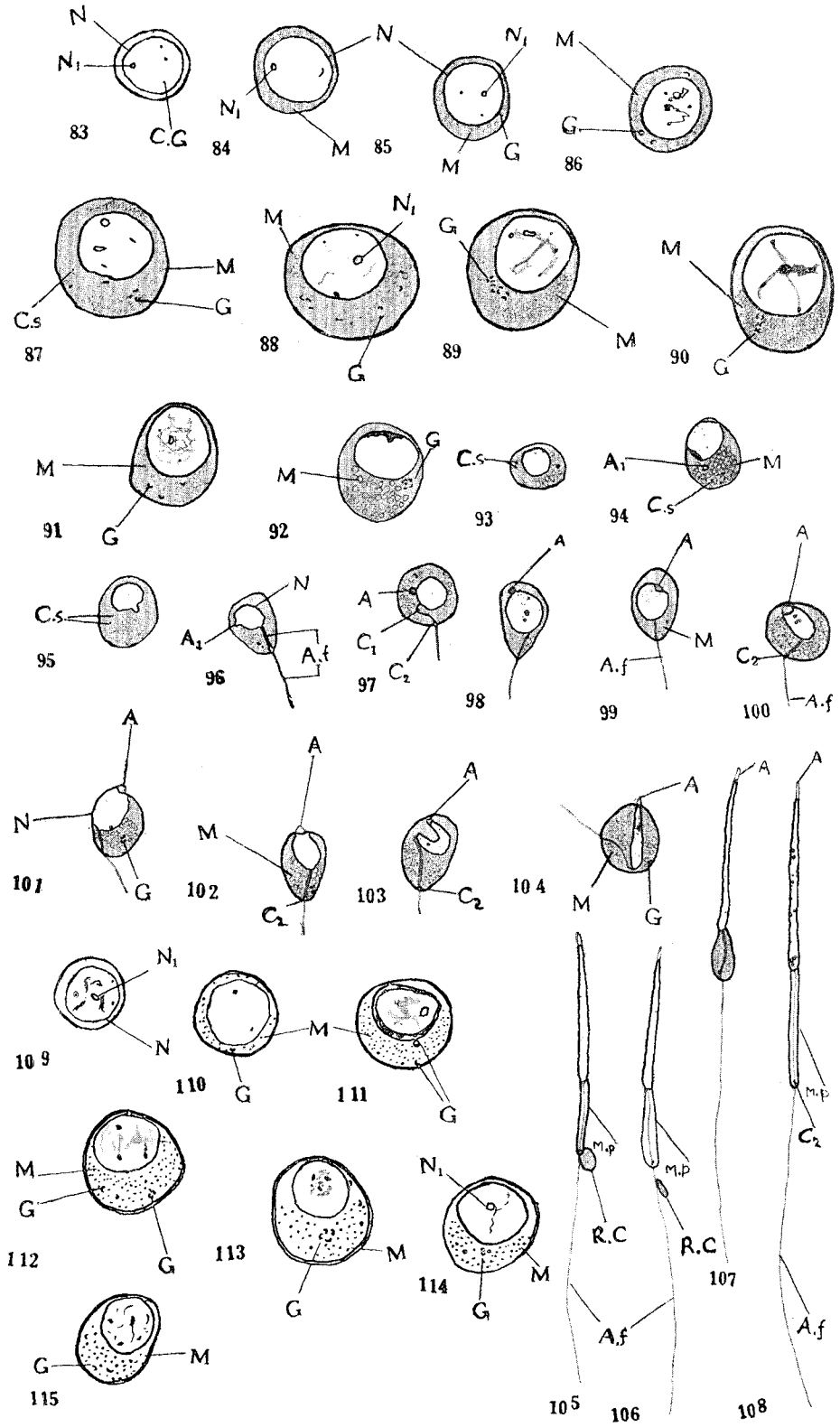
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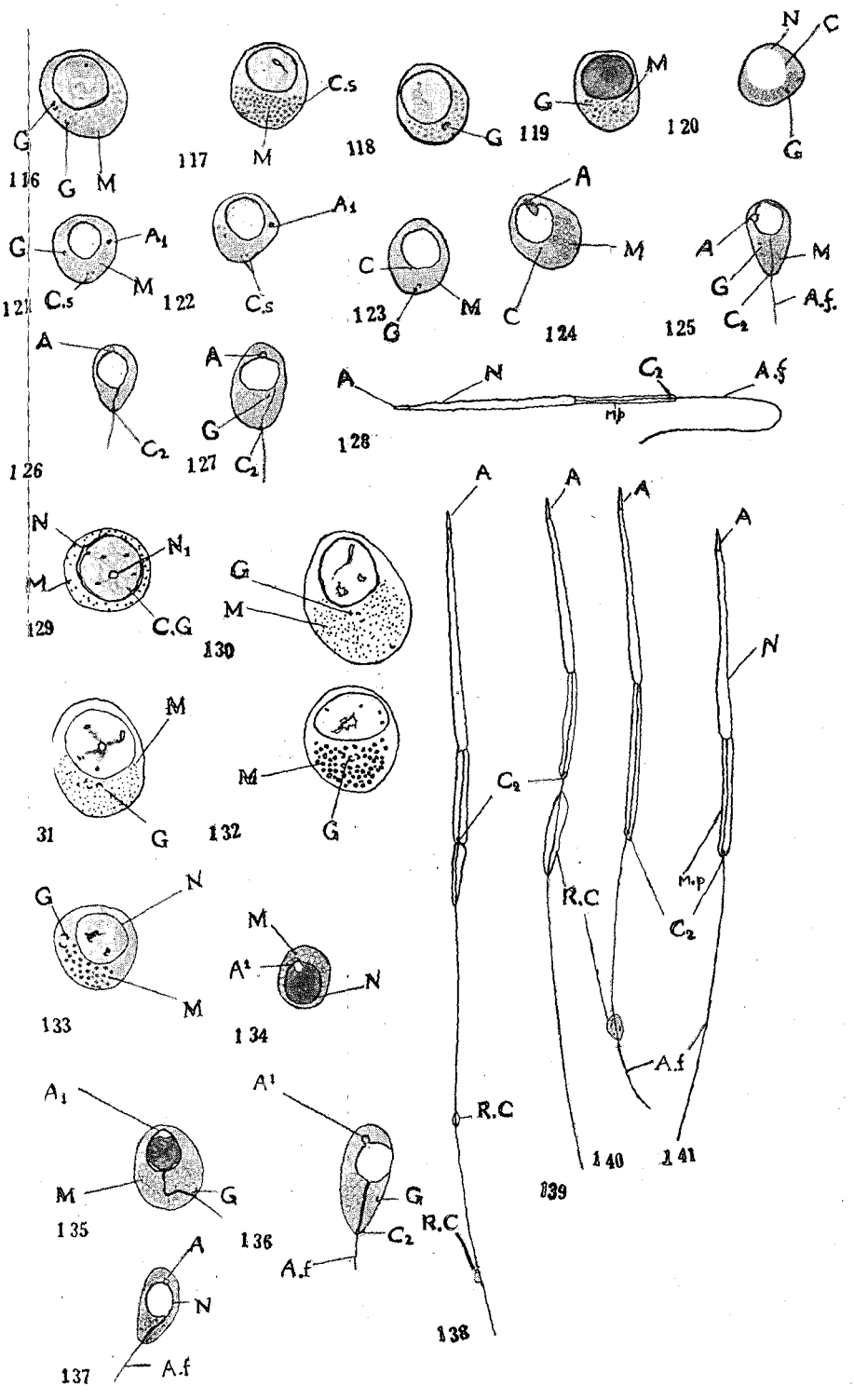
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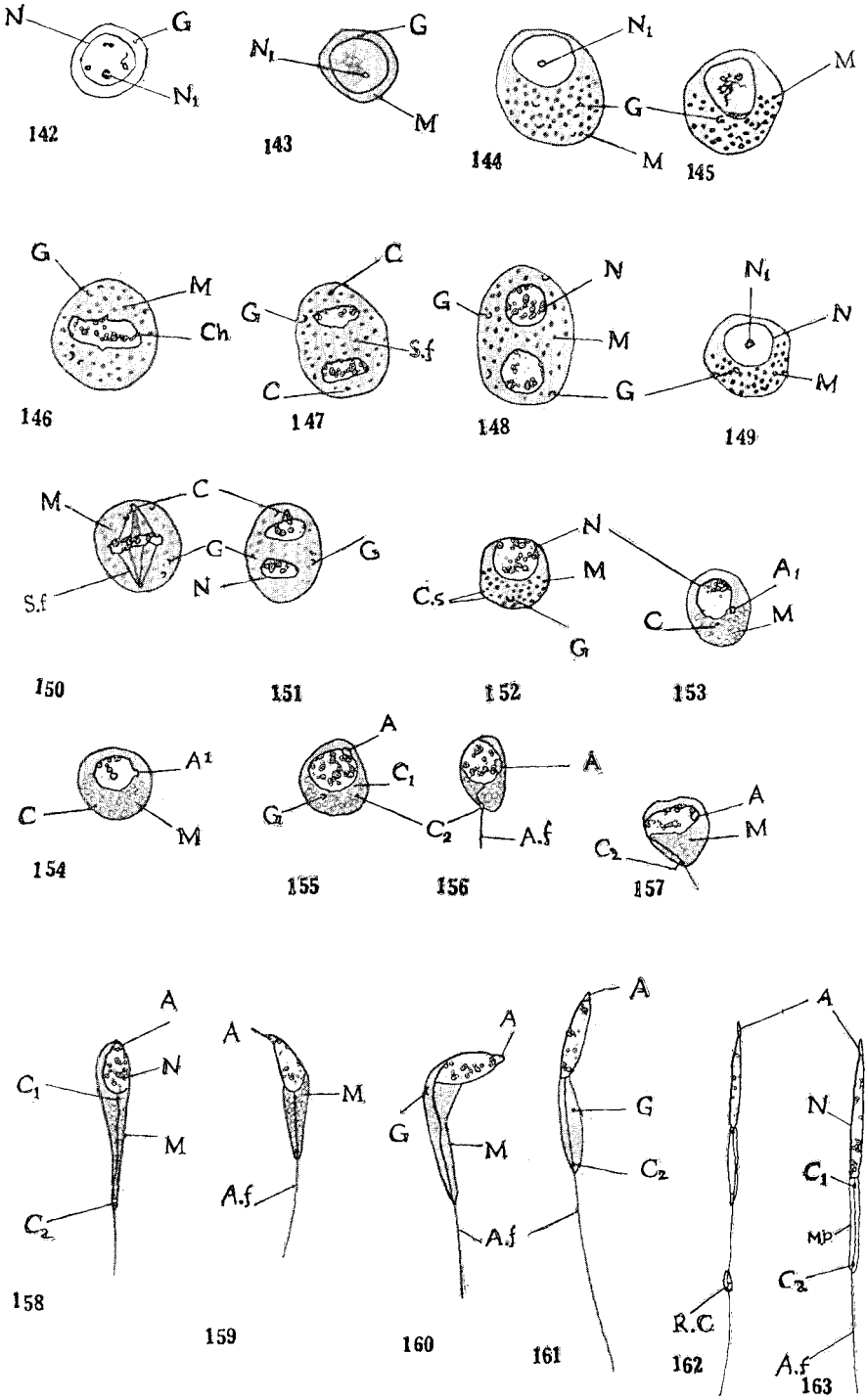
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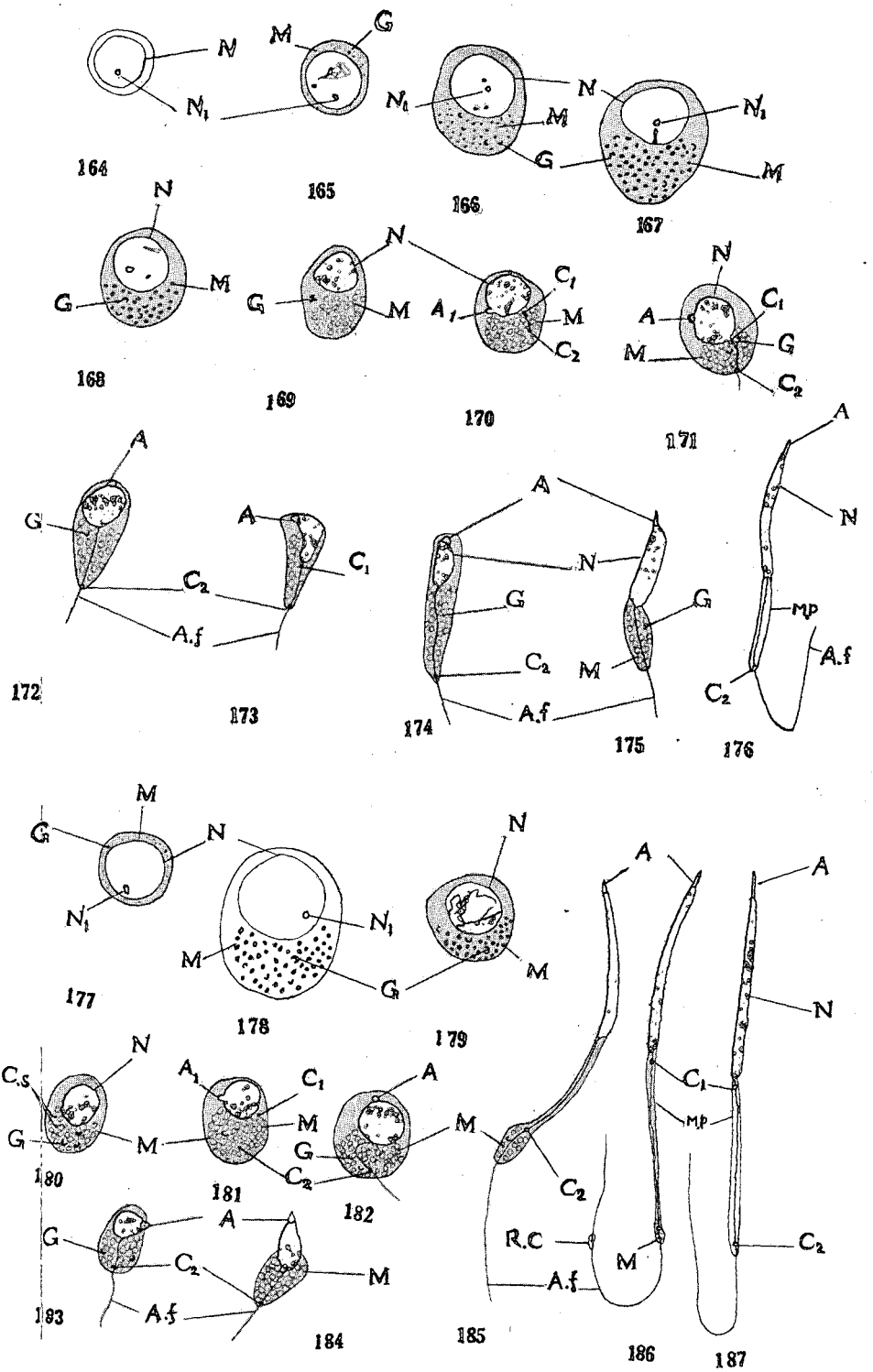
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RESEARCH BULLETIN
OF THE
EAST PANJAB UNIVERSITY

No. 41 CHEMISTRY Pp. 205–210

December, 1953

OXIDATION WITH CHLORAMINE-B

Volumetric Determination of Metals by Oxine Method

by

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HOSHIARPUR

Price Re.1-0-0

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OXIDATION WITH CHLORAMINE-B

Volumetric Determination of Metals by Oxine Method

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Various metals under specified conditions of *p*H yield crystalline precipitates with 8-hydroxyquinoline (oxine). These precipitates have the general formula $M(C_9H_6ON)_n$, where n is the valency of the metal M. On treatment of the oxinate with dilute hydrochloric acid, the oxine is liberated. It reacts with bromine to give 5 : 7-dibromo-8-hydroxy-quinoline (Arthur I. Vogel, 1948; and H. Diehl and G. F. Smith, 1952).

$$C_9H_7ON + 2\ Br_2 = C_9H_5ON\ Br_2 + 2\ HBr$$

The bromine is obtained by the addition of standard chloramine-B and excess of potassium bromide to the acid solution.

$$C_6H_5SO_2N\ ClNa + 2\ KBr + 2\ HCl = C_6H_5SO_2NH_2 + NaCl + 2\ KCl + Br_2$$

Experimental

A known quantity of a metallic salt was dissolved in water, about 5 grams of ammonium acetate and acetic acid were added and the solution warmed to 60° C. An excess of two per cent solution of oxine in alcohol was added in all cases except in solutions of aluminium, magnesium and uranyl salts where two per cent solution of oxine in 2*N* acetic acid was added to precipitate the metallic oxinates.

After the addition of oxine, ammonium hydroxide was added slowly to make the solution faintly alkaline for the complete precipitation of magnesium oxinate.

The mixture was boiled and allowed to cool. The oxinate precipitate was filtered through a sintered glass crucible, washed with hot water and dissolved in warm hydrochloric acid. About one gram of potassium bromide and a known excess of standard chloramine-B solution were added and the solution largely diluted with 2*N* hydrochloric acid. One gram of potassium iodide was then added to the solution and the liberated iodine was titrated against standard sodium thiosulphate, using starch as an indicator.

From the sodium thiosulphate used in these titrations, the amount of bromine required to form 5 : 7-dibromo-8-hydroxy-quinoline and the amount of chloramine-B used to liberate the bromine and hence the quantity of metal present in the metallic compound was calculated. The results are recorded in the following tables.

TABLE I

Potassium Aluminium sulphate $K_2SO_4 . Al_2(SO_4)_3 . 24H_2O$

Substance taken.	0·102*N* chloramine-B added.	0·100*N* $Na_2S_2O_3 . 5H_2O$ used.	0·102*N* chloramine-B used.	Substance found.	Aluminium calculated.
(gm.)	(c.c.)	(c.c.)	(c.c.)	(gm.)	(gm.)
0·03953	20·00	10·40	9·80	0·03952	0·002236
0·05535	25·00	10·45	13·77	0·05552	0·003142
0·07511	30·00	11·65	18·58	0·07492	0·004190
0·09883	35·00	10·70	24·51	0·09883	0·005543
0·12650	45·00	13·85	31·42	0·12669	0·007169
0·15823	50·00	10·90	39·31	0·15851	0·008970

1 c.c. of 0·102*N* chloramine-B ≡ 0·004032 gm. of $K_2SO_4.Al_2(SO_4)_3.24H_2O$.
≡ 0·0002282 gm. of Aluminium.

TABLE II

Copper sulphate ($CuSO_4 . 5H_2O$)

Substance taken.	0·1073*N* chloramine-B added.	0·100*N* $Na_2S_2O_3 . 5H_2O$ used.	0·1073*N* chloramine-B used.	Substance found.	Copper calculated.
(gm.)	(c.c.)	(c.c.)	(c.c.)	(gm.)	(gm.)
0·03122	20·00	11·45	9·33	0·03125	0·007954
0·04370	25·00	12·80	13·07	0·04377	0·011143
0·05931	25·00	7·80	17·73	0·05938	0·015116
0·07804	30·00	7·25	23·24	0·07783	0·019813
0·09989	40·00	10·80	29·93	0·10024	0·025517
0·12486	45·00	8·30	37·26	0·12478	0·031766

1 c.c. of 0·1073*N* chloramine-B ≡ 0·003349 gm. of $CuSO_4 . 5H_2O$.
≡ 0·0008526 gm. of Copper.

TABLE III

Zinc sulphate ($ZnSO_4 . 7H_2O$)

Substance taken.	0·115*N* chloramine-B added.	0·100*N* $Na_2S_2O_3 . 5H_2O$ used.	0·115*N* chloramine-B used.	Substance found.	Zinc calculated.
(gm.)	(c.c.)	(c.c.)	(c.c.)	(gm.)	(gm.)
0·02516	15·00	10·23	6·10	0·02521	0·005733
0·04313	20·00	10·97	10·46	0·04324	0·009830
0·05392	20·00	8·00	13·04	0·05390	0·012255
0·06111	25·00	11·75	14·79	0·06114	0·013900
0·08267	30·00	11·53	19·97	0·08254	0·018768
0·10783	35·00	10·20	26·13	0·10701	0·024557

1 c.c. of 0·115*N* chloramine-B ≡ 0·004133 gm. of $ZnSO_4 . 7H_2O$.
≡ 0·0009398 gm. of Zinc.

TABLE IV

Cadmium acetate ($Cd(CH_3COO)_2 . 2H_2O$)

Substance taken.	0·127*N* chloramine-B added.	0·100*N* $Na_2S_2O_3 . 5H_2O$ used.	0·127*N* chloramine-B used.	Substance found.	Cadmium calculated.
(gm.)	(c.c.)	(c.c.)	(c.c.)	(gm.)	(gm.)
0·02333	20·00	18·35	5·55	0·02349	0·009907
0·03333	20·00	15·35	7·91	0·03348	0·014120
0·05666	20·00	8·30	13·46	0·05698	0·024027
0·07666	27·00	11·30	18·10	0·07662	0·032310

1 c.c. of 0·127*N* chloramine-B ≡ 0·004332 gm. of $Cd(CH_3COO)_2 . 2H_2O$.
≡ 0·001785 gm. of Cadmium.

TABLE V

Cobalt chloride ($COCl_2 . 6H_2O$)

Substance taken.	0·127*N* chloramine-B added.	0·100*N* $Na_2S_2O_3 . 5H_2O$ used.	0·127*N* chloramine-B used.	Substance found.	Cobalt calculated.
(gm.)	(c.c.)	(c.c.)	(c.c.)	(gm.)	(gm.)
0·02082	10·00	5·70	5·51	0·02081	0·005156
0·02974	15·00	9·03	7·89	0·02980	0·007383
0·03569	17·00	9·60	9·44	0·03565	0·008822
0·04462	17·00	6·50	11·88	0·04499	0·011116
0·05057	20·00	8·40	13·39	0·05058	0·012529
0·06841	25·00	8·75	18·11	0·06841	0·016945

1 c.c. of 0·127*N* chloramine-B ≡ 0·003777 gm. of $COCl_2 . 6H_2O$.
≡ 0·0009358 gm. of Cobalt.

TABLE VI

Nickel chloride ($NiCl_2 . 6H_2O$)

Substance taken.	0·115*N* chloramine-B added.	0·100*N* $Na_2S_2O_3 . 5H_2O$ used.	0·115*N* chloramine-B used.	Substance found.	Nickel calculated.
(gm.)	(c.c.)	(c.c.)	(c.c.)	(gm.)	(gm.)
0·02080	15·00	10·23	6·10	0·02084	0·005140
0·03565	20·00	14·00	10·43	0·03554	0·008789
0·04457	20·00	8·05	13·00	0·04442	0·010955
0·05051	25·00	11·75	14·79	0·05055	0·012464
0·06834	30·00	11·45	20·04	0·06846	0·016888
0·08914	35·00	10·20	26·13	0·08918	0·022020

1 c.c. of 0·115*N* chloramine = B ≡ 0·003417 gm. of $NiCl_2 . 6H_2O$.
≡ 0·0008427 gm. of Nickel.

TABLE VII

Uranyl nitrate ($UO_2(NO_3)_2 . 6H_2O$)

Substance taken.	0·109*N* chloramine-B added.	0·100*N* $Na_2S_2O_3 . 5H_2O$ used.	0·109*N* chloramine-B used.	Substance found.	Uranium calculated.
(gm.)	(c.c.)	(c.c.)	(c.c.)	(gm.)	(gm.)
0·04185	20·00	11·82	9·16	0·04177	0·01981
0·05859	20·00	7·80	12·84	0·05857	0·02777
0·07961	30·00	13·75	17·38	0·07928	0·03758
0·10462	30·00	7·75	22·89	0·10441	0·04950
0·13391	40·00	11·70	29·27	0·13351	0·06330
0·16739	50·00	14·60	36·61	0·16699	0·07917

1 c.c. of 0·109*N* chloramine-B ≡ 0·004561 gm. of $UO_2(NO_3)_2 . 6H_2O$.
≡ 0·002163 gm. of Uranium.

TABLE VIII

Magnesium sulphate ($MgSO_4 . 7H_2O$)

Substance taken.	0·1073*N* chloramine-B added.	0·100*N* $Na_2S_2O_3 . 5H_2O$ used.	0·1073*N* chloramine-B used.	Substance found.	Magnesium calculated.
(gm.)	(c.c.)	(c.c.)	(c.c)	(gm.)	(gm.)
0·03081	20·00	11·45	9·33	0·03084	0·003043
0·04313	25·00	12·80	13·07	0·04321	0·004263
0·05854	30·00	13·25	17·65	0·05835	0·005757
0·07703	35·00	12·55	23·30	0·07703	0·007600
0·09859	40·00	10·90	29·84	0·09865	0·009733
0·12324	45·00	8·30	37·26	0·12318	0·001215

1 c.c. of 0·1073*N* chloramine-B ≡ 0·003306 gm. of $MgSO_4 . 7H_2O$.
≡ 0·0003262 gm. of Magnesium.

From these results it is concluded that aluminium, copper, zinc, cadmium, cobalt, nickel, uranium and magnesium can be determined volumetrically by the oxine method using chloramine-B as an oxidizing agent.

SUMMARY

Aluminium, copper, zinc, cadmium, nickel, cobalt, uranium and magnesium are precipitated as oxinates which dissolve in hydrochloric acid liberating oxine. An excess of potassium bromide and a known volume of

standard chloramine-B are added to the acid solution. The potassium bromide is oxidized by the chloramine-B to bromine which reacts with the oxine to form 5 : 7 dibromo-8-hydroxy-quinoline. The excess of bromine is determined by adding potassium iodide to the solution and titrating the liberated iodine against standard sodium thiosulphate solution. From the thiosulphate used in the titrations, the amount of bromine required to form 5 : 7 dibromo-8-hydroxy-quinoline and the amount of chloramine-B used to liberate the bromine and hence the amount of metal present in the metallic compound is calculated.

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Diehl, H., and Smith, G. F. (1952). Quantitative Analysis, New York, p. 397.
Vogel, Arthur I. (1948). Quantitative Inorganic Analysis, London, p. 452.

Published by the East Panjab University, Hoshiarpur, and Printed by Norman A. Ellis, Baptist Mission Press, 41A Lower Circular Road, Calcutta.