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XXII.—Studies on the Gametogenesis of *Stenophylax stellatus*, Curt. (Trichoptera).—Oogenesis. By **R. A. R. Gresson**, Ph.D.

(From the Department of Zoology, University of Edinburgh.) *Communicated by* Professor J. H. Ashworth, F.R.S. (With One Group Text-figures (1–14) and Two Plates.)

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I. Introduction and Previous Work.

Present knowledge of the oogenesis of caddis-flies is scanty. Marshall's paper (17) on the ovary of *Platyphylax designatus* is the only detailed account of the growth and history of the ovarian elements during the larval and pupal stages, but he does not deal with the cytoplasmic inclusions.

According to Marshall, the ovarioles of the youngest larva examined consisted of a terminal filament and an oval portion. The cells of the terminal filament have an elongate nucleus and a single small nucleolus; chromatin granules are present which are connected by achromatic strands. The cells of the oval part vary according to their position in the tube—those at the sides being similar to the cells of the terminal filament, while those situated in the axial region of the anterior portion contain an oval or spherical nucleus, an "achromatic nucleolus," chromatin granules, and achromatic threads. In the nuclei of the cells situated at the posterior end of the ovarioles the threads have a beaded appearance, but chromatin granules are not present.

In the middle portion of the ovarian tubes of an older larva many of the nuclei are in synapsis; the threads have a beaded structure, are closely packed together and difficult to make out. The nucleolus is still present, but does not always occur at the pole towards which the threads have contracted. After this stage the threads become distributed through the nucleus; later, they are shorter and thicker, and each thread gives rise to two long chromosomes.

In an ovary from a slightly older larva oocytes and nurse-cells could be identified; the former contain paired chromosomes which lie in an achromatic material which is identified as the remains of the threads; the "achromatic nucleolus" is still present. The chromosomes of the nurse-cells form tetrads and later fragment to form numerous granules which are at first arranged in distinct groups but ultimately spread out through the nucleoplasm.

Marshall states that the ovarian tubes of a young pupa contained five groups of oocytes and five groups of nurse-cells. Chromosomes are not present in the older oocytes, but masses of achromatic material surround small chromatin granules or rods. The nucleolus of the most highly developed cells is vacuolated, while unstained bodies, some of which contain "a distinct chromatin spot or spots," occur scattered through the nucleus. The two anterior oocytes have a blunt process arising from the anterior pole passing between the nurse-cells of the adjoining chamber.

In the late pupæ two large oocytes filled with yolk are situated at the posterior end of the tubes. The yolk-spheres are not figured, and are not described beyond stating that the yolk is composed of round bodies "which lie in a granular network."

Marshall does not give the chromosome number for either the oogonia or oocytes. Lutman, however, in a paper on the spermatogenesis of *Platyphylax designatus* (16), states that the haploid number is thirty, and that an examination of the oogonia gave counts of from fifty-five to sixty chromosomes.

Hirschler and Hirschlerowa (10), in a short note on certain large cells which they believe are somatic in origin, situated in the terminal filament of the ovary of *Phryganea grandis*, state that the Golgi elements are present as round or oval bodies, the elements of the vacuome as numerous grains which stain with neutral red, and the mitochondria as small granules which stain with Janus green.

According to Hirschlerowa (11), in a brief account of the cytoplasmic inclusions of the ovary of *Phryganea grandis*, the majority of the elements of the vacuome are present, in the oocyte, as granules which adhere to the Golgi bodies. The latter are figured as rings, and after prolonged treatment are stained by neutral red, while the vacuome is blackened by osmic acid. Mitochondria are present as small grains which sometimes form dense masses. The Golgi elements and the vacuome of the follicle-cells and nurse-cells are similar to those of the oocyte.

Hosselet (15) states that the young nurse-cells of *Setodes tineiformis* have a spherical nucleus which contains small masses of chromatin and an irregularly shaped nucleolus. As the nucleus increases in size the nucleolus begins to break up into fragments which are extruded from the nucleus. The fragments are often surrounded by a basophil material, and it is suggested that the latter, which corresponds to a plasmosome, may be capable of forming communicating bridges by means of which the nucleolar fragments pass to the cytoplasm.

In material fixed in Zenker-formol and subsequently treated with potassium bichromate, and in osmic acid preparations, small networks and irregular meshes occur at one pole of the nucleus of the young nurse-cells; these structures Hosselet identifies as the "chondriome." In the later cells the networks are reduced to two or three meshes and to some chondriosomes.

In the older nurse-cells, groups of two or three rings occur; the periphery of these bodies is at first regular, but later becomes thickened in places so that granules appear to be formed on the edges of the rings. During the time that these changes are taking place the centre becomes clear. Later, the central vacuole is clearer and the "chondriome" finally disappears. A few elements do not take part in this process and are present as isolated granules.

Hosselet does not deal at any length with the oocyte of *Setodes tineiformis*. The "chondriome," in the form of a dense filo-reticular mass, occurs in contact with the nucleus; in the part of the cell next to the nutritive chamber, however, it is more dispersed and occurs in groups of individual elements situated between the nucleus and the neighbouring nurse-cells; some of these elements are ring-shaped. A projection from the oocyte usually extends between two adjacent nurse-cells.

From the above account it will be seen that comparatively little work has been carried out on the oogenesis of caddis-flies. Marshall's paper, on *Platyphylax designatus*, contains the only description of the prophase of the first maturation division; Marshall, however, was chiefly concerned with the growth and history of the ovarian elements, and consequently does not give a detailed description of the prophase stages. Although a certain amount of work has been carried out on the cytoplasmic inclusions of nurse-cells, follicle-cells, and oocytes, there appears to be no detailed account of the history of the Golgi elements and mitochondria during all stages of the development of the oocyte.

II. Material and Methods.

The material for this paper was obtained from larvæ and pupæ of *Stenophylax stellatus* collected from the Braid Burn, near Edinburgh, in the summer and autumn of 1932, and from larvæ collected in the spring of 1933.

For the study of the growth of the ovarioles, ovaries were fixed in Flemming's mixture and in Bouin's picro-formol. For the preservation of late oocytes, filled with yolk-spheres, Smith's fixative was found to be the most satisfactory, and this fixative also gave good results for the younger cells in the ovaries of pupæ.

Certain ovaries were treated according to Feulgen's technique for the identification of chromatin. The material was fixed in corrosive-acetic mixture; light green was used as a counter-stain.

For the demonstration of the mitochondria, material fixed in Flemming (without acetic) and by the Mann-Kopsch method was found to be satisfactory. The Golgi bodies were studied in material treated according to the method of Mann-Kopsch.

For the demonstration of the vacuome, ovaries were dissected out in saline solution and immediately placed in a weak solution of neutral red.

My thanks are due to Professor J. H. Ashworth, F.R.S., for help and advice, and to Mr Martin E. Moseley, of the Natural History Museum, South Kensington, for identifying specimens of *Stenophylax stellatus*. I wish to express my thanks for a grant in aid of this work from the Earl of Moray Endowment of the University of Edinburgh.

III. Observations.

1. *The Growth of the Ovarioles.*

In the ovarioles of the youngest larva examined three regions can be identified. At the anterior end the cells are small and are, in most cases, approximately equal in size; each nucleus contains a small deeply stained nucleolus and minute deeply stained chromatin granules which are arranged on a faintly stained network (Pl. I, fig. 1). In the middle region of the ovarioles the majority of the cells have increased in size, and the nucleolus and chromatin granules are larger and more deeply stained than those present in the earlier cells. The nuclei of some of the cells in this region contain large masses of material, while others are so deeply stained that they resemble homogeneous bodies. Their appearance strongly suggests that they are undergoing degenerative changes (Pl. I, fig. 1). At the posterior end of the ovarian tubes the cells are of about the same size as those in the middle region; the nucleolus is deeply stained, but in most cases the chromatin granules are more faintly stained than those of the slightly younger cells (Pl. I, fig. 1). In these young ovarioles it is not possible to distinguish between oocytes and nurse-cells. Small cells are situated towards the sides of the posterior region; it is probable that, at a later stage, they give origin to the cells of the follicular epithelium.

In ovaries from more advanced larvæ, cells in corresponding stages of development to those of the younger ovarioles are situated in the anterior and middle part of the tubes. The succeeding cells are larger and their nuclei are in the early prophase of the first maturation division, while at the posterior end of the ovarioles paired chromosomes and a deeply stained nucleolus are present in each nucleus. Large deeply stained granules are present in the cytoplasm of the older cells (Pl. I, fig. 2). It is not possible to distinguish with certainty between oocytes and nurse-cells, but some of the cells, situated at the posterior end of the ovarian tubes, are larger than their neighbours and are, apparently, oocytes which have not yet become clearly differentiated from the other cells.

An examination of ovaries from young pupæ showed that the anterior and middle regions of the ovarioles are occupied by the same type of cells as are present in the late larvæ, while immediately posterior to the middle region it is possible to distinguish between oocytes and nurse-cells. Some of the cells have increased in size and contain a large nucleolus and paired chromosomes. These cells are identified as the early oocytes, and are surrounded by smaller cells; the latter are, at a later stage, transformed into nurse-cells. Both types of cells contain large deeply stained cytoplasmic granules (Pl. I, fig. 3). The next stage in the differentiation of the nurse-cells is shown by slightly older cells situated below the very early oocytes and nurse-cells described above. The chromosomes of these nurse-cells fragment into granules which at first remain in tetrads and small groups in or near the position previously occupied by the chromosomes from which the granules originated. As these changes are taking place the nucleolus becomes irregular in shape and some of its substance appears to be passed into the nucleoplasm in the form of nucleolar buds or extrusions (Pl. I, fig. 4). In slightly older cells (situated at the posterior end of the ovarioles) the chromosomes have fragmented into numerous granules which are situated, for the most part, round the nucleolus and extend in clumps from the nucleolus to the periphery of the cell. In some cases the nucleolus is breaking up to form buds, but in others, although irregular in shape, has not yet entered upon a process of fragmentation (Pl. I, fig. 5). In the nucleus of the oocyte situated in contact with the older nurse-cells the chromosomes are paired and the nucleolus is breaking up into several smaller bodies. In both oocyte and nurse-cells deeply stained cytoplasmic granules occur close to or in contact with the outside of the nuclear membrane (Pl. I, fig. 5).

In a slightly more advanced pupa the posterior ends of the ovarioles are occupied by a large oocyte and a group of nurse-cells (Pl. I, fig. 6). Paired chromosomes are present in the nucleus of the oocyte and the nucleolus has fragmented to form two or more deeply stained bodies. The nuclei of the nurse-cells have increased in size and become less regular in outline; the nucleolus is deeply stained but in most cases has broken up into several pieces; numerous scattered chromatin granules are present. The cytoplasm of both oocyte and nurse-cells contains granules, and in many of the oocytes these granules tend to form a chain at the posterior pole of the cell (Pl. I, fig. 6). The high degree of nuclear activity (as evidenced by the fragmentation of the nucleolus of oocyte and nurse-cells) and the presence of granules on the outside of the nuclear membrane indicate that nucleolar material is extruded to the cytoplasm where it becomes visible in the form of deeply stained granules. Definite follicle-cells occur at the posterior end of the ovarioles of the young pupa described above, but oocyte and nurse-cells have not yet been separated into their respective chambers.

In slightly older pupæ two groups of oocytes and nurse-cells are present in the posterior part of the ovarian tubes. The nurse-cells of the more highly developed group are now much larger than the adjacent oocyte, while the latter has increased in size and become irregular in shape so that its anterior end projects between the adjoining nurse-cells (Pl. I, fig. 7). Paired chromosomes occur scattered through the nucleus and the nucleolus is differentiated into two parts—a large faintly stained spherical body and a darkly stained mass of material (Pl. I, fig. 7). In some cases the deeply stained part is very large and almost completely covers the lightly stained portion. Small granules occur distributed through the nucleoplasm and in some cases are in contact with the nucleolus; in all probability they arise from the latter as nucleolar buds or extrusions. Cytoplasmic granules are present and are most numerous in the vicinity of the nucleus and in the posterior part of the cell. The nuclei of the nurse-cells contain numerous small granules and large irregularly shaped bodies; the former have arisen by the fragmentation of the chromosomes and the latter are derived from the nucleolus of the younger cells. Owing to the large number of nucleoli present it is evident that they grow and divide after their liberation from the original nucleolus.

In the ovarioles of late pupæ nine oocytes are present. The nuclei of the first and second oocytes, situated at the anterior end of the tubes, contain paired chromosomes and a deeply stained nucleolus. This region is followed by four groups of oocytes and nurse-cells which are in stages of development comparable with those of the younger ovarian tubes described above. The follicle-cells in association with the early nurse-cells are similar to those of the adjoining oocyte; in the seventh group, however, they are elongate, but have not yet formed a partition between the nutritive chamber and the oocyte. The seventh oocyte is larger, but in other respects is similar to the sixth.

The nucleus of the eighth oocyte contains a lightly stained vacuolated nucleolus and paired chromosomes. Large vacuoles are present in the ooplasm, and towards the periphery yolk-spheres are situated amongst the vacuoles (Pl. I, fig. 8).

The nuclei of the nurse-cells have increased in size and become very irregular in shape. Several nucleoli and numerous chromatin granules are present; the latter are greater in number and the nucleoli larger than in the younger cells (Pl. I, fig. 10). Granules are situated in the cytoplasm. The nutritive chamber is separated from the adjoining oocyte by a single layer of elongate follicle-cells.

The ninth oocyte has increased greatly in size, the yolk-globules are larger, have become slightly more numerous and now extend towards the central region of the cell (Pl. I, fig. 9). Chromosomes and a vacuolated nucleolus are present. The nurse-cells are similar to those of the eighth nutritive chamber.

In late pupæ, which have darkened preparatory to emergence of the imago, the last two oocytes are completely filled with yolk-globules. A cup-shaped depression is present at the anterior end of each oocyte, and into this concavity projects the group of neighbouring nurse-cells. The layer of follicle-cells separating the oocyte from the nutritive chamber, owing to the elongation of the cells, has become very thin. The chromosomes are faintly stained and clumped towards the middle of the egg nucleus; a nucleolus was not observed. The nurse-cells and their nuclei are identical in structure with those of the eighth and ninth nutritive chambers of the younger pupæ.

The ovarioles of certain pupæ were treated according to Feulgen's technique for the identification of chromatin. The chromatin granules and network of the undifferentiated cells and the prophase threads and chromosomes of the later cells gave a positive reaction; the chromosomes of the fully formed oocytes, however, were stained less brightly than those of the younger cells. The history of the chromatin of the nurse-cells was found to agree with the account based on the examination of other material

—thus the nuclei of the early nurse-cells contain paired chromosomes and a nucleolus which stains with light green; later, the chromatin is represented by groups of granules which increase in number and finally are distributed singly and in groups throughout the nucleus. In the nuclei of the older cells the groups of granules are connected with one another by threads which give the chromatin reaction; chromatin granules occur at intervals along the threads. In some cases, at least, it is probable that the appearance of threads is due to the close proximity of chromatin granules arranged in a line stretching from one group to another. In other cases there is little doubt as to the presence of chromatin threads. In the larger nuclei chromatin granules are situated on the inside of the nuclear membrane (text-fig. 1).

Spherical and irregularly shaped bodies were observed among the chromatin granules of the older cells; these bodies do not contain chromatin but stain faintly with light green and are identified as the nucleolar fragments described on p. 327. The chromatin granules often closely surround the nucleoli so that the latter are difficult to observe.

A nucleolus which stains with light green, chromatin granules, and a chromatin network were observed in the nuclei of the follicle-cells (text-fig. 2).

Although oocytes and their accompanying nurse-cells were carefully examined at all stages of development up to the appearance of yolk-globules, granules which give the chromatin reaction were not observed in the cytoplasm of the nurse-cells or in the ooplasm.

Certain nuclei situated among the undifferentiated cells appear as homogeneous masses of material which give the chromatin reaction, but are darker in colour than the chromatin of the surrounding cells (text-fig. 3). These nuclei are identical with those observed in sections stained in iron hæmatoxylin and identified as the nuclei of cells undergoing degenerative changes (p. 325). The whole nucleus appears to be filled with chromatin and no other structures are visible. The cytoplasm of the degenerating cells contains several bodies or granules which give the chromatin reaction, and are, apparently, small masses of chromatin which have been extruded from the nuclei during the degenerative changes (text-fig. 3).

2. *The Prophase of the First Maturation Division.*

At the stage of development of the undifferentiated cells shown in Pl. I, fig. 2, the chromatin granules become more faintly stained and give rise to thin leptotene threads; large granules of chromatin persist for some time (text-fig. 4). The threads now contract towards one pole of the nucleus to form a dense synizetic knot from which a few individual threads extend towards the opposite pole (text-fig. 5). Owing to the closeness of the knot it is impossible to make out any structure; a deeply stained area, however, observed in certain nuclei, is probably a nucleolus. In slightly older cells thick pachytene threads and a large nucleolus are present (text-fig. 6). The pachytene threads rapidly contract and give rise to the chromosomes of the diplotene stage, the members of a pair lying side by side. As already described (p. 326), the chromosomes of the nurse-cells fragment while those of the oocytes are present in the nuclei of the oldest cells examined. In the younger oocytes the chromosomes tend to lie towards the periphery of the nucleus, but in the fully developed cells they form a clump, usually in the vicinity of the nucleolus.

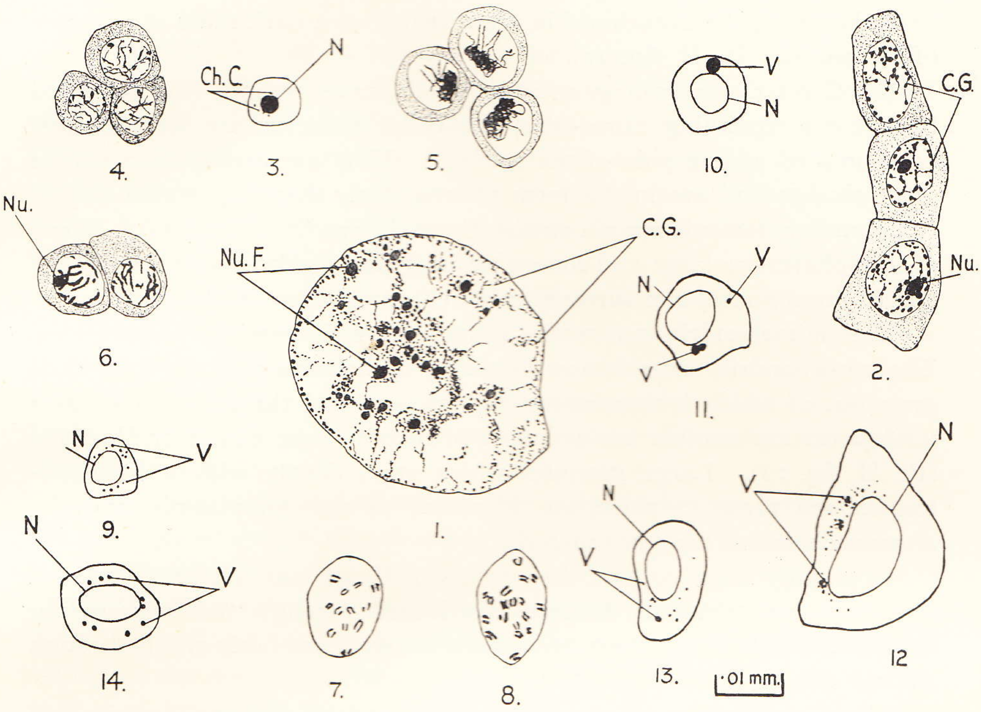
Owing to the presence of the nucleoli and nucleolar fragments which take up stain similar to the chromosomes, it was found difficult to obtain accurate counts of the chromosome number in material stained in iron hæmatoxylin. The oogonia contain about sixty chromosomes, but the smallness of the cells and of the chromosomes rendered counts unsatisfactory. Ovarioles treated according to Feulgen's technique were examined and a large number of oocytes gave counts of sixty chromosomes (text-figs. 7 and 8). In order to check these findings, sections of the testes of larvæ were examined and the haploid number was found to be thirty.

A nucleolus was not always observed in the Feulgen material during the leptotene stage; when visible, it was slightly stained with light green. The sections did not contain many nuclei in synizesis, but an examination of those present revealed the nucleolus stained green and closely surrounded by the chromatin threads. During the pachytene stage the nucleolus was identified with ease, and its subsequent behaviour agrees with the findings recorded on pp. 326–8. The nucleolus does not contain chromatin at any stage in the history of the oocyte.

3. *The Golgi Bodies and Mitochondria.*

(a) *Undifferentiated Cells.*—In material treated according to the Mann-Kopsch technique the Golgi elements of the undifferentiated cells, situated at the anterior end of the ovarioles of larvæ and early pupæ, are in the form of granules and small rings distributed round the nuclear membrane. In older cells the majority of the Golgi bodies form one or more clumps at one pole of the nucleus. Some of the larger spheres consist of a chromophilic rim and a chromophobic central substance (Pl. II, fig. 23).

Mitochondria could not be identified with certainty in this material,



TEXT-FIGS. 1–14.

LETTERING.

Ch.C, chromatin granules in cytoplasm; C.G, chromatin granules; N, nucleus; Nu, nucleolus; Nu.F, nucleolar fragments; V, elements of the vacuome.

FIG. 1.—Nucleus of nurse-cell. Pupa. Feulgen.
FIG. 2.—Follicle-cells. Pupa. Feulgen.
FIG. 3.—Degenerating cell. Pupa. Feulgen.
FIG. 4.—Leptotene stage. Early pupa. Bouin.
FIG. 5.—Synizesis. Early pupa. Bouin.
FIG. 6.—Pachytene stage. Early pupa. Bouin.
FIG. 7.—Nucleus of young oocyte, showing twenty-four chromosomes. Pupa. Feulgen.
FIG. 8.—The next section of the same nucleus, showing thirty-six chromosomes. Feulgen.
FIG. 9.—Undifferentiated cell. Larva. Neutral red.
FIG. 10.—Undifferentiated cell. Larva. Neutral red.
FIG. 11.—Later stage. Larva. Neutral red.
FIG. 12.—Young oocyte. Pupa. Neutral red.
FIG. 13.—Nurse-cell. Pupa. Neutral red.
FIG. 14.—Follicle-cell. Pupa. Neutral red.

partly, no doubt, owing to the occurrence of the Golgi material in that part of the cell in which the mitochondria are situated. In Flemming preparations, however, the mitochondria are revealed as a dark mass at one pole of the nucleus (Pl. II, fig. 21).

(b) *Oocytes.*—In young oocytes which have recently differentiated from the surrounding nurse-cells, the Golgi elements are for the most part situated at one pole of the nucleus. They are arranged in one or more clumps and are in the form of irregularly shaped granules and of rings; a few elements remain outside these clumps (Pl. II, fig. 11). Many of the spheres are large and somewhat irregular in shape, and consist of a deeply impregnated rim surrounding a chromophobic substance.

The mitochondria are well shown in the Flemming preparations. The mitochondrial mass is now breaking up and many of the individual granules, of which it was composed, are scattered through the ooplasm while a certain number are arranged in rows to form chains or filaments (Pl. II, fig. 22). Large granules which stain deeply with iron hæmatoxylin are present; these are identified as the cytoplasmic granules already described for other material.

In slightly older oocytes the clumps of Golgi material break up and the individual elements become distributed through the surrounding ooplasm. At this stage the mitochondria are scattered fairly evenly through the cell (Pl. II, fig. 12).

The Golgi bodies increase in number and in size and are situated throughout the ooplasm, in many cases they are less numerous in the posterior part of the cell. At this stage two or three Golgi elements are often closely applied to one another to form a larger body, and the substance of some of the ring-shaped Golgi bodies becomes broken up to form several small granules which surround an area which is not blackened by osmic acid. The mitochondria, as in the slightly younger oocytes, are scattered throughout the cell (Pl. II, fig. 14).

In older oocytes, in which a few yolk-globules occur, the majority of the Golgi bodies are situated towards the periphery and in the posterior part of the cell. Many of the Golgi bodies are large and consist of two or more closely associated rings or granules; several are deeply impregnated and are almost as large as the yolk-globules. Appearances indicate that the Golgi material is transformed into fatty yolk (Pl. II, fig. 15). Mitochondria are present but are less numerous in the neighbourhood of the nucleus.

In a later oocyte, in which fatty yolk-formation is well advanced, the Golgi bodies are situated chiefly towards the periphery, and in many cases two or more elements are associated to form a large body. The mitochondria in the form of single granules and chains are scattered through the ooplasm; they are less numerous in the vicinity of the nucleus (Pl. II, fig. 17).

In the fully formed oocytes the ooplasm is packed with yolk-globules so that observation of the Golgi elements and mitochondria is rendered very difficult. Small deeply osmophil granules and rings are present between the yolk-spheres, and are identified as Golgi bodies, but many of the smaller granules may possibly be mitochondria (Pl. II, fig. 20). Large bodies with chromophilic rims similar to those of the younger cells were not observed in the late oocytes.

(c) *Nurse-cells.*—The Golgi elements of the early nurse-cells are situated chiefly towards one pole of the nucleus; a few bodies may occur in other parts of the cytoplasm. They are in the form of granules and rings (Pl. II, fig. 11). As in the early oocytes, the mitochondrial mass breaks up and the individual granules become distributed throughout the cell.

In the more advanced nurse-cells the Golgi bodies are situated round the nucleus, while the mitochondria are fairly evenly scattered through the cytoplasm (Pl. II, fig. 13).

As the nurse-cells increase in size the Golgi bodies spread out to the periphery and are usually absent from the cytoplasm immediately surrounding the nucleus. The distribution of the mitochondria is similar to that of the slightly younger cells (Pl. II, fig. 16).

An examination of nurse-cells in association with fully formed oocytes showed that the Golgi material and mitochondria occupy the same positions as in the preceding stage. The Golgi bodies are, however, smaller and are granular in form; many of these granules arise from the Golgi rings in a similar manner to those of the late oocytes.

(d) *Follicle-cells.*—In the young follicle-cells, situated towards the anterior end of the ovarioles, the Golgi elements occur close to the nuclear membrane, and in many cases appear to be most numerous at the pole of the nucleus next to the adjoining oocyte. Mitochondria as a few small granules are present in the neighbourhood of the nucleus (Pl. II, fig. 18).

In follicle-cells surrounding fully formed oocytes and nurse-cells the Golgi bodies are more numerous, and occur singly and in clumps round the nucleus and also in parts of the cytoplasm towards the periphery (Pl. II, fig. 19). Some of the larger bodies consist of a chromophilic rim and a central clear substance. The mitochondria are more numerous than in the early cells; they are scattered through the cytoplasm but do not extend to the periphery of the cell.

4. *The Vacuome.*

The elements of the vacuome of the young undifferentiated cells were present as granules which stained after a few minutes' treatment with neutral red (text-fig. 9). In older cells the vacuome is represented by a single large spherical body situated close to the nuclear membrane (text-fig. 10). In certain preparations of cells at this stage the vacuome did not stain homogeneously but consisted of several deeply stained granules situated in a large spherical body which was stained but lightly by neutral red. An examination of slightly larger cells showed that the majority contain two or three bodies stained by neutral red, and in many cases appearances indicate that these bodies arise by division from the single element of the earlier cells (text-fig. 11).

In young oocytes and nurse-cells the vacuome is present as granules which stain brightly with neutral red, and, in size, closely resemble those of the early undifferentiated cells. They are more numerous in the oocytes than in the nurse-cells (text-figs. 12 and 13). The late oocytes and nurse-cells did not always give good results with neutral red; in some cases the elements of the vacuome could not be identified, while in others they were shown as small bodies similar to those of the young oocytes and nurse-cells, but more numerous and distributed throughout the cell. Late oocytes, in which yolk-globules were present, contained no structure stainable by neutral red. The elements of the vacuome of the follicle-cells are situated round the nucleus (text-fig. 14).

During the investigation of living cells stained with neutral red, refractile bodies were observed in the cytoplasm of oocytes and nurse-cells. These bodies are, in all probability, the Golgi elements; they were not stained by neutral red.

5. *Yolk-formation.*

The eighth oocyte in the ovarioles of advanced pupæ was the youngest cell in which yolk-globules were identified. At this stage the ooplasm stains but faintly (iron hæmatoxylin) and contains numerous vacuoles, while deeply stained granules are present towards the periphery; these granules are identified as fatty yolk-spheres (Pl. I, fig. 8). The spheres increase in size and spread towards the central region of the cell, so that in the ninth oocyte they occur throughout most of the cytoplasm (Pl. I, fig. 9). In late oocytes from mature pupæ the fatty yolk-globules have increased greatly in size and in number, and are closely packed together throughout the entire egg.

The details of fatty yolk-formation were revealed by an examination of ovarioles treated according to the Mann-Kopsch technique. The Golgi elements of the late oocytes become distributed through the ooplasm. Many of the Golgi bodies increase in size while others become clumped in groups consisting of two or more elements. Small yolk-spheres make their appearance and are, at first, about the same size or are slightly larger than the bigger Golgi bodies. These spheres are deeply impregnated with osmic acid and are the fatty yolk-globules (Pl. II, fig. 15). In a later oocyte the majority of the Golgi elements are situated at the periphery, while yolk-globules occur scattered among them and, to a less extent, in the cytoplasm towards the central region of the egg (Pl. II, fig. 17).

In the fully formed oocytes of mature pupæ most of the fatty yolk-spheres are large and in some cases contain a clear vacuole; they are not very numerous and are distributed fairly evenly throughout the ooplasm. Albuminous yolk-globules are now present; they are numerous and vary in size but are always smaller than the fatty yolk-spheres (Pl. II, fig. 20). Small Golgi elements and minute granules, which may be mitochondria, occur among the yolk-spheres and appear to be especially numerous towards the periphery.

There appears to be little doubt that the fatty yolk is formed from the Golgi elements of the late oocyte. The fatty yolk-globules arise in that part of the cell in which the Golgi elements are most numerous, and when formed are about the same size as the larger Golgi bodies. Additional evidence in favour of this view was produced by an examination of the larger Golgi rings. As already mentioned, the substance of some of the Golgi rings becomes broken up into granules which surround a clear area; appearances indicate that these granules coalesce and in some cases are joined by other Golgi elements, and are finally converted into fatty yolk-globules. In other cases the Golgi rings do not form granules, the central area becomes blackened with osmic acid and the whole structure gives rise to a fatty yolk-sphere. It is significant that Golgi elements are not numerous in mature oocytes, while those present are always small (Pl. II, fig. 20).

The albuminous yolk-globules were not observed to arise in connection with any of the cytoplasmic inclusions. The nucleolar extrusions disappear or become less numerous prior to the beginning of yolk-formation. In all probability they are dissolved in the ooplasm and their substance utilised in the formation of the albuminous yolk.

The mitochondria are less numerous in the late oocytes; their small size and the large number of yolk-globules present rendered their identification very difficult. They are numerous in oocytes in which fatty but not protein yolk is being formed (Pl. II, figs. 15 and 17). It is possible, therefore, that the mitochondria may play some part in the formation of the albuminous yolk.

IV. Discussion.

The general account of the growth of the ovary of *Stenophylax stellatus* agrees fairly closely with Marshall's findings for *Platyphylax designatus* (17); the behaviour of the nucleoli of both oocytes and nurse-cells is given in more detail in the present paper, and the application of Feulgen's technique throws further light on the nature of the nucleoli, and of the granules and network of the early undifferentiated cells.

Marshall could not determine whether the numerous nucleoli of the late nurse-cells arose from the original nucleolus or were formed from other material. An examination of the nurse-cells of *Stenophylax stellatus* shows that the nucleolus of the young cells fragments into several pieces. The large number of these bodies present in the fully formed nurse-cells indicates that they cannot all have arisen from the nucleolus of the earlier cells and that the fragments grow and multiply at the expense of some substance derived from the nucleus, or that bodies, which stain in a similar manner to the nucleoli, are formed independently from some other nuclear material. While it was impossible to determine this matter with certainty, examination of the nucleolar fragments, many of which seem to be breaking up to form smaller bodies, supports the first view.

Hosselet (15) records fragmentation of the nucleolus and the occurrence of nucleolar extrusions from the nurse-cell nuclei of *Setodes tineiformis*, and believes that the "chondriome" is regenerated at the expense of these bodies. The present writer was unable to find any evidence that the nucleolar extrusions of *Stenophylax stellatus* take part in the regeneration of either the mitochondria or Golgi elements.

Marshall records the presence of deeply stained granules in the cytoplasm of the growing oocytes of *Platyphylax designatus*; he makes no suggestion as to their origin, but believes that they represent some product of the metabolism of the cell. During the present investigations similar bodies were observed in the ooplasm. In the young oocytes they are large and are in contact with the nuclear membrane; in the older cells they are smaller, and while a few may still be adjacent to the nucleus, the majority are scattered through the cytoplasm. These cytoplasmic bodies, although a few may be present in the very early cells, are most numerous at the time when the nucleolus is breaking up into two or more pieces. In all probability they are formed from nucleolar material which is passed in solution through the nuclear membrane. This appears to be the only recorded case of the occurrence of nucleolar extrusions in the eggs of caddis-flies. It is worthy of note that the extrusion of nucleolar material from the nucleoli of the spinning-gland cells of caddis-flies has been recorded by Nakahara (19), by Beams and Wu (1), by Hosselet (15), and by Wajda (31).

The darkly stained tracts of material mentioned by Marshall as extending from the neighbourhood of the nuclei of the nurse-cells to the adjoining oocytes of *Platyphylax designatus* were not observed during the present investigation.

Marshall records the occurrence of degenerating cells in the ovarioles of *Platyphylax designatus*. The present findings throw further light on degeneration in *Stenophylax stellatus*. In the early stages the nucleus is occupied by large masses of dark material (in iron hæmatoxylin preparations), while later the entire nucleus is deeply stained. The Feulgen preparations did not contain nuclei in the early stages, but the nuclei of cells in an advanced stage of degeneration were filled with material which gave the chromatin reaction, and granules of chromatin were observed in the cytoplasm. It is evident that the beginning of nuclear degeneration is marked by the formation of large chromatin masses, which later increase in size and fill the whole nucleus, while some of the chromatin is extruded to the cytoplasm where it is visible as granules.

Nuclei in an advanced stage of degeneration are usually considerably smaller than those of the surrounding normal cells; this may account in part for the fact that they are completely filled by the chromatin. Later stages of degeneration were not observed; it is probable, therefore, that the cells may persist until very late in oogenesis.

The behaviour of the chromatin threads and chromosomes during the prophase of the maturation division of *Stenophylax stellatus* agrees fairly closely with Marshall's account for *Platyphylax designatus*. Marshall, however, found that chromosomes were not present in the older oocytes, while chromosomes, although stained faintly in Feulgen material, were identified in fully formed eggs during the present investigations. A nucleolus was not visible in the oldest eggs of late pupæ; this agrees with Marshall's statement that nucleoli were absent from the fully developed oocytes.

The presence of chromosomes, as demonstrated by Feulgen's method, throughout the growth phase and stages of yolk-formation is an unusual condition for insect oogenesis. Thus the present writer working on Tenthredinids (7), and on *Periplaneta* (8), and Mukerji on *Apanteles* (18) record the absence of chromatin from the later oocytes, while Sanderson in a paper on a Tenthredinid (26) states that chromatin is present during the growth period as a kidney-shaped mass.

The present findings that the nucleolus, nucleolar buds, and extrusions of both oocytes and nurse-cells do not contain chromatin agree with the writer's former observations (7 and 8).

Steopoe (29) states that the nucleolus of the spermatocytes of *Nepa cinerea* and of *Naucoris cimicoides* gave a negative reaction with Feulgen's method.

Owing to the small size of the chromosomes of the oocytes, counts were difficult to obtain. The majority of counts gave the diploid number as sixty chromosomes, while in a few cases the nuclei appeared to contain only fifty-eight or fifty-nine chromosomes. An examination of the spermatocytes showed clearly that the haploid number is thirty. It is concluded, therefore, that the diploid number is sixty. Lutman (16), working on the oogonia and spermatogonia of *Platyphylax designatus*, obtained counts of from fifty-five to sixty chromosomes.

The structures called Golgi elements or Golgi bodies in the present contribution appear to be closely similar to the "chondriome" described by Hosselet (15) as occurring in the nurse-cells and oocytes of *Setodes tineiformis*; the present writer finds, however, that the juxta-nuclear masses consist of individual elements which are closely approximated in the early cells. Hosselet states his belief that the Golgi apparatus is an artifact; the structures often present in material treated by methods for the demonstration of the Golgi bodies he calls the "chondriome golgiesque." The present writer believes that the rings and large granules observed in the cells of the ovary of *Stenophylax stellatus* are identical with the material described as Golgi bodies by most workers, and, furthermore, that these bodies, as shown by osmic methods, are distinct from the mitochondria, which in the present investigations were revealed as minute granules. Both Golgi elements and mitochondria have previously been identified by Hirschler and Hirschlerowa (10), and by Hirschlerowa (11) in the ovarioles of the caddis-fly, *Phryganea grandis*.

As previously mentioned (p. 324), Hosselet describes the formation of a vacuole in the interior of the ring-shaped elements of the "chondriome" present in the older nurse-cells. The vacuole increases in size and finally the "chondriome" disappears with the exception of a few elements which do not take part in this process and remain as isolated granules. An examination of nurse-cells in association with fully formed oocytes of *Stenophylax stellatus* showed that the Golgi elements are still present, but are, for the most part, smaller than those of the earlier cells; vacuoles were not present. In slightly younger nurse-cells the substance of some of the ring-shaped Golgi bodies was observed to have become separated into a number of granules arranged round a clear area in a similar manner to that described by Hosselet for the osmophil granules of *Setodes*. In *Stenophylax* these granules do not disappear, but, apparently, are distributed through the cytoplasm to form the numerous granular Golgi elements of the late nurse-cells.

The ring-shaped Golgi elements of the oocytes, nurse-cells, and follicle-cells of *Stenophylax*, although consisting of an osmophil ring surrounding a central clear substance, are not identical in structure with the vesicular Golgi bodies recently recorded as occurring in the cells of the ovary of certain insects by Nath and his co-workers (21, 22, and 23), by Bhandari and Nath (3), by the present writer (6 and 8), and by Payne (25). The Golgi vesicles described by Nath and his collaborators and by the present writer increase in size, fat is deposited in the chromophobic central area and they are converted into fatty yolk-spheres. Payne finds that the Golgi bodies take no part in yolk-formation.

Many of the Golgi rings in the eggs of *Stenophylax* become broken up into a number of granules which, at first, surround the original clear central area, but later run together to form a body which increases in size, impregnates deeply with osmic acid, and is identified as a fatty yolk-sphere. In other cases the Golgi material does not appear to break up, the central area impregnates with osmic acid and the whole structure is converted into a fatty yolk-globule; two or more rings may become associated to form a globule. The actual substance of the Golgi bodies seems to be converted into fatty yolk, but owing to the rapid growth of the spheres it is highly probable that other material, derived from the ooplasm, plays an important part in the formation of fatty yolk.

Recent conclusions as to the participation of the cytoplasmic inclusions and nucleolar extrusions in yolk-formation in insects may be suitably summarised by means of the following table. In some instances the authors have not recorded the presence of fatty yolk, or have not determined its origin; in these cases the space under "fatty yolk" is left blank. The letter "G" under "Technique" indicates that methods suitable for the demonstration of the Golgi bodies have been employed.

Insect Studied and Author.	Protein Yolk.	Fatty Yolk.	Technique.
Periplaneta americana. Hogben (13).	Nucleolar extrusions.	. .	. .
Periplaneta americana. Nath and Mohan (23).	" "	Golgi bodies.	G.
Periplaneta orientalis. Gresson (8).	" "	" "	G.
Gryllotalpa vulgaris. Voinov (30).	. .	" "	G.
Anisolobis annulipes. Payne (25).	Not from Golgi bodies or mitochondria.	Not from Golgi bodies or mitochondria.	G.
Oecanthus quadripunctatus. Payne (25).	Golgi bodies take no direct part in yolk-formation.	. .	G.
Nepa cinerea. Spaul (27).	Nucleolar extr.	. .	. .
Nepa cinerea. Steopoe (28).	Golgi bodies.	. .	G.
Dysdercus cingulatus. Bhandari and Nath (3).	Nucleolar extr.	Golgi bodies.	G.
Galastocoris oculatus. Payne (25).	Not from Golgi bodies.	Not from Golgi bodies.	G.
Libellula depressa (*Anax* and *Plathemis*). Hogben (14).	Interaction of nucleolar extr., mitochondria and Golgi bodies.	. .	G.
Anopheles maculipennis. Nicholson (24).	Rosette cells and oocyte nucleus.	. .	. .
Culex fatigans. Nath (20 and 21).	Material derived from nurse-cells and from surrounding fluid.	Some of the Golgi vesicles contain fat.	G.
Culex pipiens. Hosselet (15).	First stages not examined.	Elaborated by the "chondriome."	G.
Dicranomya sp. Hosselet (15).	Close connection between "chondriome" and yolk-spheres.	. .	G.
Chironomus plumbicornis. Hosselet (15).	Arise in connection with "chondriome." Nurse-cells produce substance used in yolk-formation.	. .	G.

Insect Studied and Author.	Protein Yolk.	Fatty Yolk.	Technique.
Luciola gorhami. Nath and Mehta (22).	Nucleolar extr.	Golgi bodies.	G.
Formica rufa (*Synergus*). Hogben (12).	Secondary nuclei.	..	..
Apanteles glomeratus. Gatenby and Woodger (4).	Interaction of secondary nuclei and mitochondria.	None.	G.
Thrinix macula. Gresson (6).	Nucleolar extr.	Golgi bodies.	G.
Thrinix mixta. Gresson (6).	,, ,,	,, ,,	G.
Allantus pallipes. Gresson (6).	,, ,,	,, ,,	G.

An examination of the table on yolk-formation shows that the Golgi bodies, in a large number of cases in which they have been identified in the insect egg, are connected with the formation of fatty yolk. There is considerable disagreement as to the origin of protein yolk, but in several cases and in widely separated groups it is believed that the yolk-spheres are formed, in part at least, from nucleolar extrusions.

Payne (25) examined the eggs of other insects besides those noted in the table but does not state the method of yolk-formation. In conclusion he mentions that the Golgi bodies seem to decrease in number during fat and yolk-formation, but there is no evidence that Golgi elements or mitochondria take part in this process. Payne states that the Golgi vesicles of *Oecanthus quadripunctatus* probably disintegrate and give rise to chemical substances which are used in other synthetic processes.

Hosselet (15) believes that, during yolk-formation, the "chondriome" acts as a catalytic agent. The "chondriome" disappears when the yolk-spheres are formed, and it is suggested that the elements fuse with the spheres.

In *Stenophylax* the Golgi bodies appear to be transformed into a fatty substance, and the latter together with other material derived from the ooplasm form the fatty yolk-spheres. With reference to the direct conversion of the Golgi elements into fat, it is of interest that Bell (2) has recently described the origin of neutral fats from the Golgi material of the spermatid of the dog, and that the present writer (9) believes that the Golgi bodies in atretic eggs of the mouse are transformed into fat-globules.

As already stated (p. 335), it is probable that the nucleolar extrusions take part in the formation of the protein yolk. Nucleolar extrusions have been described in the oocytes of several insects, and have been recorded by Nath and his co-workers (22 and 23), by Bhandari and Nath (3), and by the present writer (5, 6, and 8) as giving rise to albuminous yolk-globules.

Hirschlerowa (11), in a short note on the oocytes of *Phryganea grandis*, states that the elements of the vacuome are present as granules which adhere to the Golgi bodies, and that the vacuome of the nurse-cells and of the follicle-cells is similar to that of the oocytes. In the older oocytes the vacuome and Golgi bodies occupy similar positions. The present contribution shows that the elements of the vacuome of *Stenophylax stellatus* are at first numerous, and are situated round the nuclear membrane of the early undifferentiated cells. At this stage the Golgi bodies are distributed in a closely similar manner. At a later stage, corresponding to the localisation of the Golgi material, the vacuome is represented by a single spherical body situated at one pole of the nucleus. In the young oocytes and nurse-cells the elements of the vacuome and the Golgi bodies are again distributed through the cytoplasm. There is, therefore, a close similarity between the distribution of the vacuome and the Golgi material during the early stages of oogenesis. During the present investigations it was not determined with certainty if the elements of the vacuome adhere to the Golgi bodies as described by Hirschlerowa. It is of interest that Hosselet (15) finds that the vacuome of the young ovarian cells of *Culex pipiens* occurs in contact with the localised "chondriome"; later, the vacuome becomes distributed with the elements of the "chondriome."

Payne (25), working on the oocytes of certain insects, identified vacuoles which are stained by neutral red but are not elements of the vacuome. He believes that in insects there is no vacuome in the sense of a substance common to all cells. Nath (21), Nath and Mehta (22), and Nath and Mohan (23) do not record the presence of the vacuome in the eggs of the insects upon which they worked, and the present writer could not find structures which stained with neutral red in the oocytes and nurse-cells of Tenthredinids (6) or in the oocytes of *Periplaneta orientalis* (8). It would appear, therefore, that vacuoles or granules which stain with neutral red are absent from the ovarian cells of certain insects, or that in some cases they are very difficult to identify. During the present investigations the vacuome of the young cells was usually brightly stained after a few minutes' treatment with neutral red.

There is a certain amount of disagreement as to whether the neutral red vacuoles are definite pre-existing cell structures or are formed after treatment with the stain. This matter has been discussed fully in recent literature and need not be reviewed here. The present findings show that in *Stenophylax* the vacuoles are not identical with the Golgi elements, and that their distribution resembles that of the latter. Their definite distribution in cells at different stages of development would appear to support the supposition that they represent some pre-existing cell structure.

V. Summary.

1. The present account of the growth and history of the ovarian elements of *Stenophylax stellatus* agrees fairly closely with Marshall's findings (17) for *Platyphylax designatus*. The fragmentation of the nucleoli and the occurrence of nucleolar extrusions in the cytoplasm of the nurse-cells agrees with Hosselet's observations on *Setodes tineiformis*. The present writer found no evidence in support of Hosselet's statement that the "chondriome" is regenerated at the expense of the nucleolar extrusions. The presence of nucleolar extrusions in the ooplasm is recorded in the eggs of caddis-flies.

2. The application of Feulgen's technique has thrown further light on the nature of the granules and threads in the nuclei of the undifferentiated cells, nurse-cells, and follicle-cells, and on the nature of the nucleoli. The absence of chromatin from the nucleoli of the oocytes and nurse-cells agrees with former findings for other insects. The presence of chromosomes throughout the growth period of the oocyte appears to be unique in insect oogenesis. The presence of degenerating cells in the ovarioles of caddis-flies has previously been recorded by Marshall (17): in the present paper it is shown that the nuclei of these cells are completely filled with chromatin. Some of the chromatin is extruded to the cytoplasm where it is visible as granules which give the chromatin reaction.

3. The chromosome number of *Stenophylax stellatus*, as determined by counts in oocytes and spermatocytes, is sixty.

4. The structure and behaviour of the Golgi bodies and mitochondria of the undifferentiated cells, nurse-cells, follicle-cells, and oocytes are described. The material called Golgi bodies or Golgi elements in the present contribution corresponds with the "chondriome" described by Hosselet in the ovary of *Setodes tineiformis*. The present paper describes the behaviour of the mitochondria and Golgi elements during the growth of the oocyte and stages of yolk-formation.

5. It is suggested that material derived from nucleolar extrusions is utilised in the oocyte in the formation of the protein yolk. Mitochondria and substances derived from the ooplasm may also take part in this process. The origin of the fatty yolk-globules from the Golgi elements is described;

it is probable that material derived from the ooplasm is added to the growing globules. The findings of other workers on yolk-formation in insects are discussed.

6. Vacuoles which are stained by neutral red are present in oocytes, nurse-cells, and follicle-cells. Their distribution resembles that of the Golgi elements.

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DESCRIPTION OF PLATES.

A.Y., protein yolk; Ch., chromosomes; C.G., chromatin granules; De., degenerating cells; F.C., follicle-cell; F.Y., fatty yolk; G.E., Golgi element; M., mitochondria; M.C., mitochondrial mass; N.C., nurse-cell; Nu., nucleolus; Nu.B., nucleolar bud; Nu.E., nucleolar extrusion; Nu.F., nucleolar fragment; Oo., oocyte; Pr., nuclei in prophase.

Plate I.

Fig. 1. Ovariole from young larva. Bouin.

Fig. 2. Ovariole from older larva; cells in middle region are in early prophase; chromosomes present in nuclei at posterior end. Bouin.

Fig. 3. Early oocyte and neighbouring nurse-cells. Young pupa. Bouin.

Fig. 4. Nurse-cells showing granules formed by fragmentation of chromosomes; nucleolus beginning to break up. Young pupa. Bouin.

Fig. 5. Older oocyte and nurse-cells. Young pupa. Bouin.

Fig. 6. Oocyte and two nurse-cells from posterior end of ovariole of young pupa. Bouin.

Fig. 7. Oocyte and two nurse-cells from posterior end of ovariole of older pupa. Bouin.

Fig. 8. Part of oocyte to show early stages of fatty yolk-formation. Pupa. Smith.

Fig. 9. Part of oocyte to show slightly later stage of fatty yolk-formation. Pupa. Smith.

Fig. 10. Nurse-cell, showing chromatin granules and nucleolar fragments in nucleus, and granules in cytoplasm. Pupa. Smith.

Plate II.

Fig. 11. Early oocyte and nurse-cells from anterior region of ovarioles of pupa. Mann-Kopsch.

Fig. 12. Older oocyte. Pupa. Mann-Kopsch.

Fig. 13. Older nurse-cell. Pupa. Mann-Kopsch.

Fig. 14. Oocyte showing dispersal of Golgi elements and mitochondria. Pupa. Mann-Kopsch.

Fig. 15. Oocyte; a few fatty yolk-globules are present. Pupa. Mann-Kopsch.

Fig. 16. Nurse-cell. Pupa. Mann-Kopsch.

Fig. 17. Late oocyte, showing fatty yolk-globules, Golgi elements, and mitochondria. Pupa. Mann-Kopsch.

Fig. 18. Follicle-cells, showing Golgi elements and mitochondria. Pupa. Mann-Kopsch.

Fig. 19. Older follicle-cells. Pupa. Mann-Kopsch.

Fig. 20. Part of fully formed oocyte. Pupa. Mann-Kopsch.

Fig. 21. Undifferentiated cells. Larva. Flemming.

Fig. 22. Young oocyte. Larva. Flemming.

Fig. 23. Undifferentiated cells. Larva. Mann-Kopsch.

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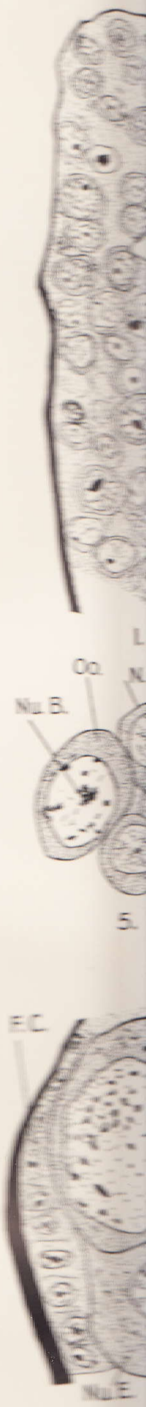

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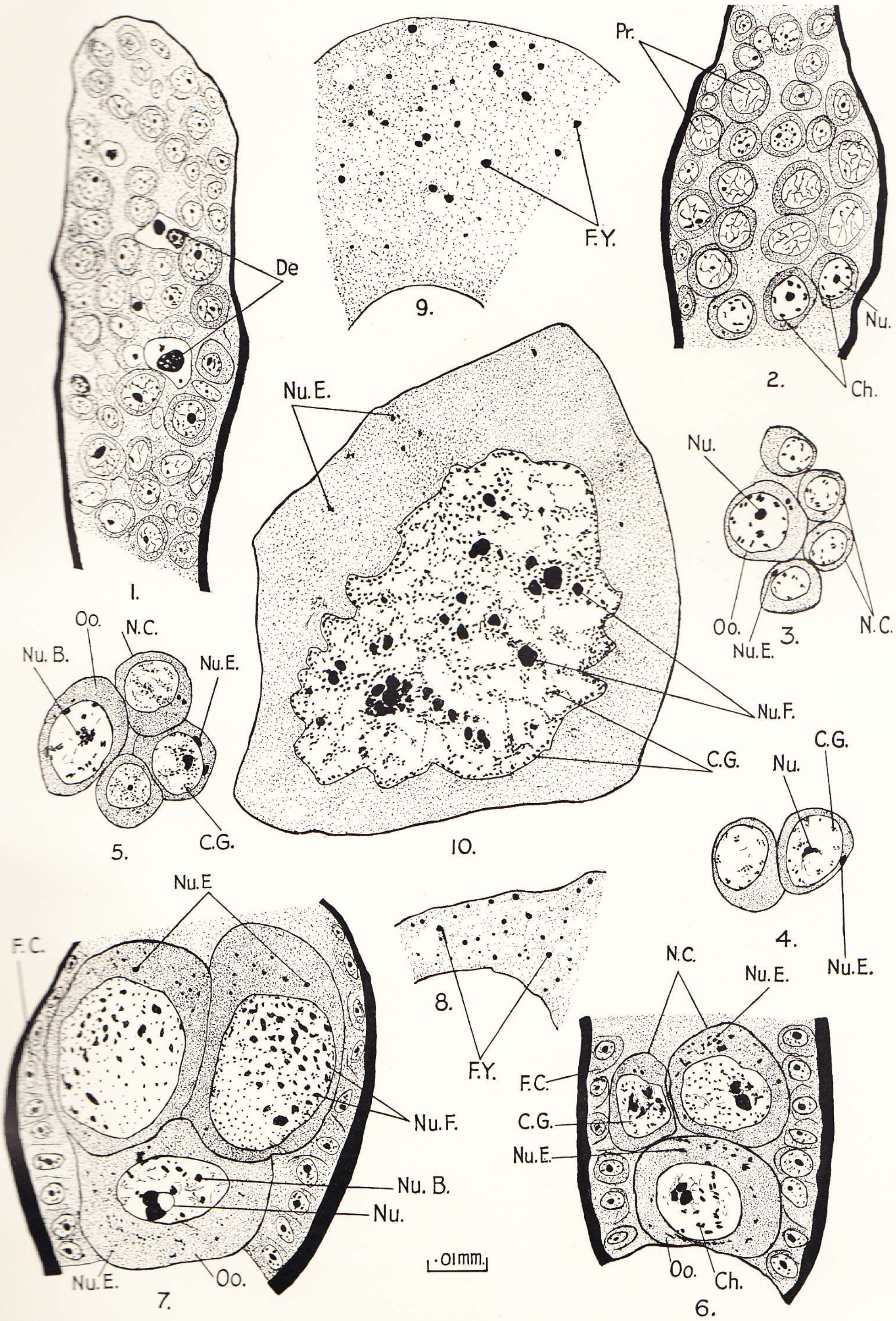

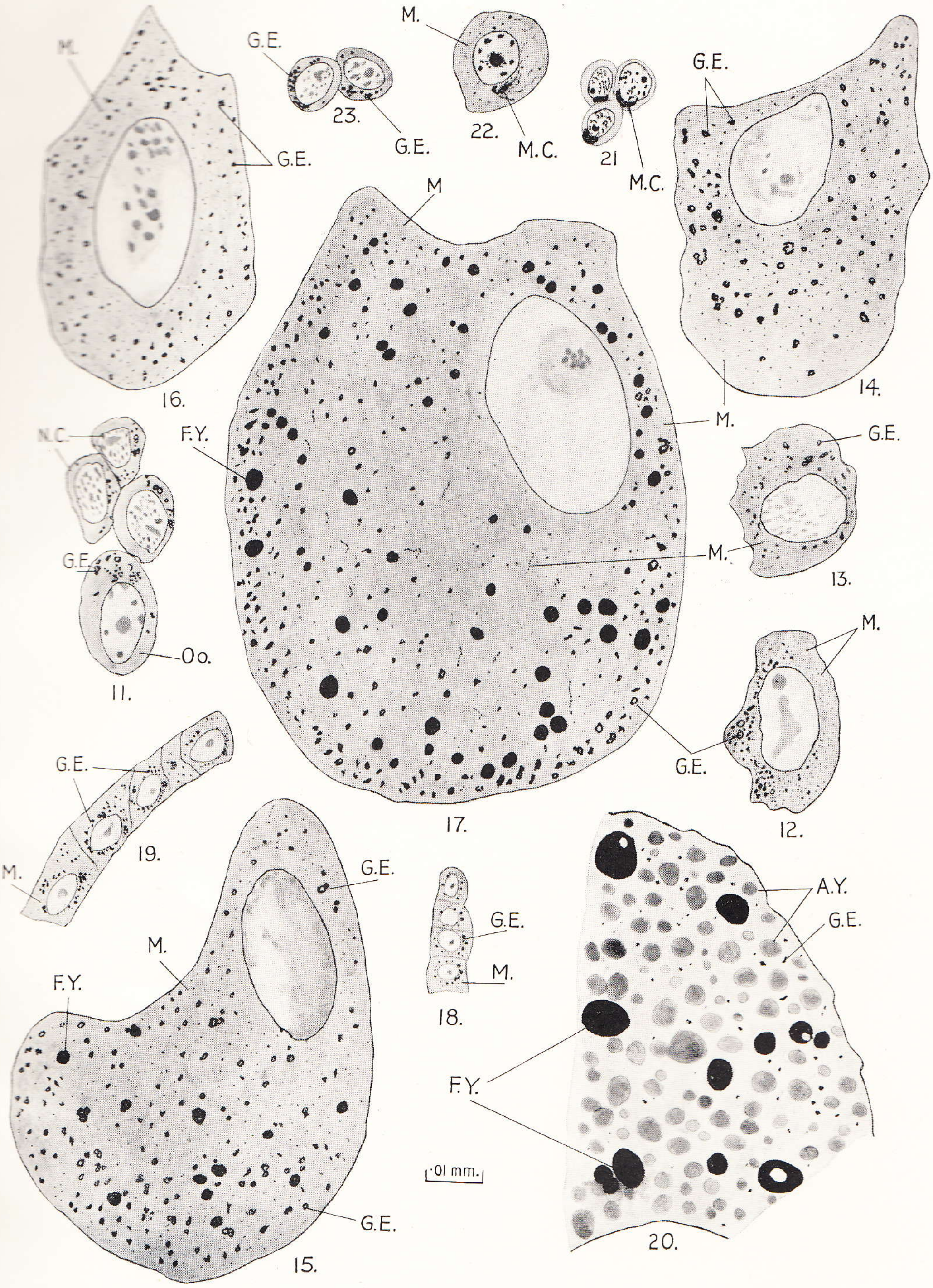
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