

STUDIES ON GERM CELLS

IV. PROTOPLASMIC DIFFERENTIATION IN THE OOCYTES OF CERTAIN HYMENOPTERA

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NINETY-EIGHT FIGURES (THIRTEEN PLATES)

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I. THE DIFFERENTIATION OF THE OOCYTES AND NURSE CELLS IN THE OVARIES OF THE HONEY-BEE, *APIS MELLIFICA*

As the writer has recently pointed out (Hegner '14 c), there are in many animals two definite periods in the germ-cell cycle during which germ cells and somatic cells arise from the same mother cells. One period occurs during embryonic development when the primordial germ cells are segregated. This segregation takes place at different stages of development in different species. For example, in the midge, *Chironomus*, one of the first four cleavage cells gives rise to all of the germ cells (Hasper '11); in the paedogenetic fly, *Miastor*, the primordial germ cell is differentiated at the eight-cell stage (Kahle '08; Hegner '14 a) but in most cases where a very early segregation has been observed, one cell at the thirty-two-cell stage is the primordial germ cell, as in *Ascaris* (Boveri '92), in *Cyclops* (Haecker '97;

Amma '11), and in *Sagitta* (Elpatiewsky '09, '10; Buchner, 10 a, '10 b; Stevens '10). The other period is that of the differentiation of the oocytes and nurse cells in the female and, at least in man, of the differentiation of the spermatocytes and Sertoli cells in the male (Montgomery '11; Winiwarter '12). These two periods seem rather distantly removed from each other, since we ordinarily begin our ontogenetic studies after the eggs are laid, but in reality they are very close together in the germ-cell cycle since the oocytes and nurse cells often become differentiated shortly before the deposition of the eggs, and the primordial germ cells are segregated shortly after cleavage begins.

This contribution deals entirely with the second period described above and the data have been derived from a study of the cellular elements in the ovaries of the queen honey-bee, *Apis mellifica*. Bees of three ages were employed: (1) those still within their pupal cells, (2) virgin queens three days old, and (3) virgin queens shortly before the deposition of eggs. The ovaries were dissected out in Ringer's solution and fixed in five different fluids: (1) Towers', (2) Carnoy's, (3) Bouin's, (4) Altmann's, and (5) Meves' modification of Flemming's solution.¹

¹ The formulae are as follows:

- | | |
|--|----------|
| (1) Tower's solution. | |
| Saturated sol. HgCl ₂ in 35 per cent alcohol..... | 95 vols. |
| Glacial acetic acid..... | 2 vols. |
| Nitric acid, c.p..... | 3 vols. |
| (2) Carnoy's solution. | |
| Absolute alcohol..... | 1 vol. |
| Glacial acetic acid..... | 1 vol. |
| Chloroform..... | 1 vol. |
| HgCl ₂ to saturation | |
| (3) Bouin's solution | |
| Picric acid, sat. aqueous sol..... | 75 vols. |
| Formol..... | 25 vols. |
| Glacial acetic acid..... | 5 vols. |
| (4) Altmann's solution | |
| Bichromate of potash, 5 per cent..... | 1 vol. |
| Osmic acid, 2 per cent..... | 1 vol. |
| (5) Meves' solution | |
| Osmic acid, 2 per cent..... | 100 cc. |
| Chromic acid..... | 0.5 cc. |
| NaCl..... | 1 gram. |
| Glacial acetic (just before using)..... | 30 drops |

The general structure of the ovary and ovarioles was obtained from in toto preparations and from thick sections. Detailed studies were made of sections 4μ thick and stained in the following ways: (1) Heidenhain's iron hematoxylin; (2) Rubaschkin's mitochondrial method;² (3) Altmann's acid fuchsin; (4) Benda's crystal violet; and (5) Delafield's hematoxylin. These methods of fixation and of staining were selected in order that both cytoplasmic and nuclear bodies could be studied.

The ovaries of insects consist of a number of tubes, the ovarioles, which are attached at the anterior end by means of terminal threads and open at the posterior end into the oviduct. The variations in the structures of the ovarioles are due principally to the presence or absence of nurse cells and the distribution of these when present. Certain ovaries, for example, those of the Orthoptera and Aptera, are not provided with nurse cells. In others the nurse cells may remain within a terminal chamber and supply the growing oocytes through a nutritive strand, as in certain Hemiptera and Coleoptera; or a rather definite number of nurse cells may become separated from the terminal chamber and accompany each oocyte, as in the Neuroptera, Hymenoptera, Diptera, and Lepidoptera. The result of the last named method of nutrition is the formation of ovarioles which resemble rows of beads.

In the bee each oocyte is accompanied by a group of nurse cells. An outline of a single ovariole is shown in figure 1. The terminal filament (*t*) consists of a row of long slender cells which extend entirely across the filament. Following this is a region occupying about half of the entire ovariole which is characterized by rosettes of cells (*r*). These cells are apparently all alike

² Rubaschkin fixes tissues in Meves' modification of Flemming's solution for one or two days. Sections are treated as follows:

Potassium permanganate, $\frac{1}{4}$ per cent.....	1 minute
Wash in water	
Oxalic acid and potassium sulphate, $\frac{1}{4}$ per cent.....	1 minute
Wash in running water.....	15 minutes
Ferric alum, 4 per cent.....	24 hours
Weigert's hematoxylin.....	2-3 days
Differentiate in ferric alum, 2 per cent	

and those in a single rosette have descended from a single mother cell which may be called the oogonial mother cell. The actual differentiation of the oocytes and nurse cells occurs in a much shorter part of the ovariole (*d*). After the oocytes are definitely established, they move down the ovariole, become arranged in a single row (*o*) and are gradually separated from each other by groups of nurse cells (*n*) which lie in nurse chambers just above them. The elements within ovarioles of different ages differ, of course, both in their stage of development and in their distribution.

The three kinds of cells within the ovaries of insects are the oocytes, nurse cells, and epithelial cells. These three kinds of cells arise differently in different groups of insects. Thus the nurse cells and epithelial cells in the paedogenetic fly, *Miastor* (Kahle '08; Hegner '14 a) are of mesodermal origin and the germ cells give rise only to oocytes. In the Hymenoptera on the other hand Korschelt ('86) in *Bombus*, Pauleke ('01) in *Apis*, and Marshall ('07) in *Polistes* agree that the three cellular elements within the ovaries arise from one sort of cells, the germ cells. I have been unable to determine the origin of the epithelial cells in the ovarioles of the bee because of the lack of young ovaries, but that nurse cells and oocytes arise from oogonia there can be no doubt.

Part of the rosette region of an ovariole is shown in figure 2. Two kinds of cells are present, (1) Those that make up the rosettes (*r*); and (2) the epithelial cells (*e*) among the rosettes. The ground substance within the ovariole in this region appears to be a loose cytoplasmic reticulum containing a few scattered nuclei. These nuclei are rather irregular in shape, and contain a clear matrix in which may be seen one or two large chromatin masses and a very delicate reticulum. No epithelial cell boundaries could be observed in this part of the ovariole and it seems probable that the rosettes are imbedded in a syncytium. There seems to be no regular arrangement of the rosettes; they do not crowd one another, but the cells in each are closely united, hence it is a very simple matter to distinguish the separate rosettes in an ovariole even with low magnification. It seems strange

because of this perfect distinctness that Paulcke ('01) failed to observe these rosettes.

The evidence for the statement that all of the cells in a single rosette have descended from a single mother cell is irrefutable. In figure 2 the cells of the rosette to which the guide line(*r*) extends are grouped about a branching strand which stains black in iron hematoxylin. A similar rosette is shown enlarged in figure 3. One branch of the black strand extends into the cytoplasm of each cell. These strands consist of the spindle fibers remaining after previous mitotic divisions, and, as will be pointed out later, such strands are not uncommon in either the ovaries or the testes of insects. A section through one end of a rosette at right angles to that shown in figure 3 is illustrated in figure 4. The spindle remains form a sort of axis about which the strands from the most recent divisions are radially arranged. The entire rosette is therefore oblong and may be sectioned longitudinally or transversely. The number of cells in each of the rosettes figured is sixteen, indicating that four divisions had occurred since the oogonial mother cell was established. No evidence was obtained which indicated the presence of amitosis in these ovarioles, and very few mitotic division figures were observed. Those that were found were invariably restricted to the cells in single rosettes (fig. 5), thus indicating that the cells in a rosette divide synchronously.

A critical examination of both the cytoplasm and nuclei of the cells in the rosettes failed to reveal any constant differences among the cells of any particular rosette. Giardina ('01) discovered a difference in the nuclei of certain rosette cells in the ovarioles of *Dytiscus*, and Kern ('12) has reported a difference in the cytoplasm of similar cells in *Carabus*, but no such distinguishing marks were found in the bee. This indicates that all of the cells at this stage in the oogenesis of the bee are probably potentially alike. At any rate no visible differences were discovered in material fixed and stained so as to bring out to the best possible advantage both nuclear and cytoplasmic bodies.

The rosette zone in the ovariole is followed by the zone of differentiation (fig. 1, *d*). Certain of the cells increase in size

and are recognizable as oocytes (fig. 6, *o*). This is brought about by an increase in the amount of cytoplasm and by the enlargement of the nucleus. The arrangement of the chromatin within the nucleus changes during this differentiation; that of the nurse cells (fig. 6, *n*) retains the condition characteristic of the rosette stage (fig. 2), whereas in the newly formed oocytes the chromatin forms threads which are scattered about irregularly within the nucleus (fig. 6, *o*). The connecting strands, so noticeable in the rosettes (fig. 3), either disappear at this time or lose their staining capacity since they are apparently absent from this stage on. Nevertheless it is very easy to determine which cells have descended from a single mother cell since a dark double ring remains where the strands passed from one cell to another (fig. 7). These rings are quite conspicuous but were completely overlooked by Paulcke ('01).

The change from the rosette zone to the zone of differentiation in the ovariole of the bee is an abrupt one—a fact which makes a study of the differentiation of the oocyte difficult, since no intermediate stages can be studied unless material in just the proper condition is obtained. In several cases which will be described later, investigators have found that a single rosette gives rise to one oocyte and a group of nurse cells. This is certainly not true in the bee, since the oocytes in the zone of differentiation are much too numerous, compared with the number of rosettes, and many instances were observed of two or more oocytes which had been directly connected by spindle remains as indicated by the presence of double rings between them (fig. 7).

If all of the cells in a single rosette are potentially alike the question arises, what causes some of the cells to become oocytes and others nurse cells? Three explanations have occurred to me: (1) There may be differential changes during the mitotic divisions in rosette formation as in *Dytiscus* (Giardina '01) resulting in one or more cells (oocytes) which differ in content from the others (nurse cells). No visible changes of this sort were observed. (2) The polarity of the rosette may influence the cells in such a way that those near the center of the ovariole and closest to the zone of differentiation tend to develop into

oocytes. (3) Those cells of the rosette which reach the zone of differentiation first are stimulated to become oocytes and by their growth and differentiation prevent the other cells of the rosette from similar changes. It would be futile to argue on the basis of known facts in favor of any of these hypotheses.

The arrangement of the oocytes and nurse cells within the ovariole resulting in a linear series of oocytes which alternate with groups of nurse cells takes place a short distance back of the zone of differentiation (fig. 1, *n*). Paulcke ('01) has satisfactorily described and figured the formation of the epithelium around the oocytes and the structure of the nurse chamber, but, as stated above, he failed to see the intercellular rings which indicate the descent of the cells concerned. A group of nurse cells about an oocyte is shown in figure 8. This oocyte is connected with at least three nurse cells. One of the nurse cells (*a*) lies below the oocyte in the ovariole; since this is never true in later stages it is probable that such a cell would either degenerate or become separated from the oocyte and forced over to one side. This has evidently happened in the case of the oocyte illustrated in figure 9, since a ring is present here at the lower end (*a*), but it does not connect the oocyte with a nurse cell. The relation between the oocyte and its accompanying nurse cells is shown in figure 10. All of the nurse cells are not included, since this is a camera drawing of a section. It illustrates, however, the way in which the nurse cells form into rows converging toward the oocyte.

The descent of the cells within the zone of differentiation would be impossible to determine if it were not for the presence of the rings between them. These rings continue to connect the nurse cells with the oocyte, even in late stages in the growth of the latter (fig. 11) and many of them may also persist between the nurse cells after the nurse chamber is fully formed, as in the stage illustrated in figure 12. Kern ('12) also finds these rings connecting the nurse cells with the oocytes of *Carabus*, and claims that nutritive material passes through them during the growth of the egg.

As soon as the oocytes are differentiated, numerous granules of various sizes appear within their cytoplasm; in the earlier

stages these lie mostly near the nucleus (figs. 7-8), but later (fig. 9) become scattered throughout the cytoplasm. These granules stain best in iron hematoxylin after fixation in Meves' modification of Flemming's solution. No evidence was obtained that they are of nuclear origin, although their early position near the nucleus indicates that they may have arisen in this way; or if not directly from the nucleus, at least through its influence. On the other hand, their sudden appearance within the cytoplasm indicates that they are cytoplasmic bodies which have resulted either from the aggregation of smaller pre-existing bodies of a similar nature or from the synthesis of other substances under the stimulus of the metabolic processes set up at the inauguration of the growth period. Duesburg ('08) has recognized granules in the peripheral layer of cytoplasm in the full grown egg of the bee, especially near the nucleus in the thickened area which Petrunkevitch ('01) has called the 'Richtungsplasma,' and considers them to be mitochondrial in nature. It seems probable that the bodies we have observed are the 'mitochondria' of Duesberg at an earlier stage. Paulcke ('01) failed to observe them.

Discussion. The differentiation of the cellular elements in the ovaries of insects and the relations of the oocytes to the nurse cells has interested students of histology and cytology for three quarters of a century. Mayer, as early as 1849, expressed the opinion that the nurse cells are abortive eggs. The connections between them and the oocytes were observed by Huxley ('58) in oviparous aphids, and were considered by him a nutritive canal for the conduction of food material from the nurse cells to the growing egg—a conclusion concurred in by Lubbock ('60) and Claus ('64). Balbiani ('70), however, proved this 'nutritive canal' to be a protoplasmic strand, but, as Wielowiejski ('85) has pointed out, he was in error when he stated that the terminal chambers of the ovarioles of aphids contain a large central cell which gives rise to both the oocytes and nurse cells (abortive eggs). He nevertheless established the fact of a protoplasmic cellular bridge between these two kinds of cells.

Protoplasmic bridges between the cells of Metazoa are not uncommon and may exist in all tissues. As a rule, they are delicate strands which pass through pores in the cell walls. The cellular elements in a syncytium, such as occurs during the cleavage of the insect egg, must be even more closely united physiologically, since here the cytoplasm forms a continuous network. Cellular bridges similar to those described above in the queen bee, have been observed in the germ glands of a number of other animals, especially insects, but mostly during spermatogenesis. Thus Platner ('86) found in Lepidoptera that often two neighboring spermatocytes, and sometimes three, were connected by intercellular ligaments which were attached to an intracytoplasmic body in each cell. The latter were considered 'Nebenkerne.' Similar conditions were discovered by Prenant ('88), Zimmerman ('91) and Lee ('95) in the male germ cells of Gastropoda. Lee, in his work on *Helix*, recognized the true origin of the intercellular bridges and their significance. They were found to be the remains of the spindle fibers following a mitotic division. The term 'pont fusorial' was applied by Lee to the bridge itself and 'moignons fusoriaux' to the ramification of the fibers within the cytoplasm of the cells. Similar intercellular ligaments were observed by Henneguy ('96) in the seminal cells of *Caloptenus*; by Erlanger ('96, '97) in both the testes and ovaries of the earthworm; by Wagner ('96) in the male germ cells of spiders; by Meves ('97) in both the testes and ovaries of the salamander; by Giardina ('01), Debaisieux ('09) and Günthert ('10) in the ovaries of *Dytiscus*; by Marshall ('07) in the ovarioles of *Polistes*; by Kern ('12) in the ovarioles of *Carabus*; by Govaerts ('13) in the ovarioles of *Carabus* and *Cicindela*; by Maziariski ('13) in the ovarioles of *Vespa*; and by Hegner ('14 a) in the testes of *Leptinotarsa*.

By far the most interesting results are those obtained by Giardina and confirmed by Debaisieux ('09) and Günthert ('10). Giardina proved conclusively that a single oogonium in the ovary of *Dytiscus* undergoes four divisions, thus producing sixteen cells, one of which is the oocyte and the remaining fifteen nurse cells. The processes of differentiation in this genus are partic-

ularly interesting, because they include a separation of the chromatin of the mother cell into two masses. One of these masses of chromatin forms an 'anello cromatico;' the other gives rise to forty chromosomes which divide equally, half of each passing to each daughter cell. The chromatic ring remains undivided and becomes situated entirely in one of the daughter cells. At each of the three succeeding divisions the chromatic ring is segregated entirely in one cell; this cell is the oocyte, whereas the other fifteen which have a common origin with it are nurse cells.

Since the publication of Giardina's observations many investigators have attempted to discover similar visible differentiations in the ovaries of other insects, but without much success. Thus Govaerts ('13) made detailed studies of beetles of the genera *Carabus*, *Cicindela*, and *Trichisoma* but was unable to find anything resembling the chromatic ring which occurs in *Dytiscus*. He found however that the spindle fibers ('residu fusorial') persist after the daughter cells are formed during the differential divisions, just as they do in *Dytiscus*, and that a definite polarity is marked by the position of these spindle remains. The conclusion is reached that something more fundamental than the unequal division of chromatic elements is responsible for the differential divisions and decided in favor of a 'polarite pre-differentielle.' No explanation is offered, however, as to the origin of this polarization.

A brief account of the oogenesis in carabid beetles has also been published by Kern ('12), who finds that during the differential mitoses, the oocyte mother-cell may be distinguished by the presence of certain intracytoplasmic granules which he describes as follows:

Befinden sich die Zellen der Zellrosetten in Teilung, so findet man mitunter in einer Zelle neben der Teilungsfigur eine Anhäufung von färbbaren Körnchen, ähnlich denjenigen, die in späteren Stadien in der jungen Eizelle im Cytoplasma gefunden werden. Es liegt nahe, an einen *Diminutionsvorgang*, ähnlich demjenigen, welchen Giardina bei *Dytiscus* beschrieben hat, oder auch an einen Vergleich mit den *Ectosomen* bei *Cyclops* zu denken; doch gelang es mir bisher nicht, alle Einzelheiten festzustellen. Die Körnchen im Cytoplasma junger Eizellen werden nach und nach aufgelöst.

The origin of these granules was not determined, and although Kern is inclined to consider them similar to the chromatic-ring substance in *Dytiscus*, there is a possibility that they may be mitochondrial in nature or may consist of some other cytoplasmic material.

The presence of intercellular bridges is important, since it makes it possible to determine the relationship of the groups of cells in the ovarioles. But in the queen bee these bridges do not persist to any considerable extent after the zone of differentiation has been reached. Here, however, as shown in figures 7 to 12, there are well defined rings between the cells which indicate their relationship. It might be argued that these rings may arise where two cells happen to come into contact, if it were not for the fact that all stages between the fully developed bridges and the presence of clearly defined rings have been observed. There are no doubt the persisting mid-bodies or 'Zwischenkörper' which remain between the cells after division. They have been noted especially by Giardina ('01) in *Dytiscus*; by Marshall ('07) in *Polistes*; by Kern ('12) in *Carabus*; and by Maziarski ('13) in *Vespa*.

Summary of Part I. 1. Four rather definite regions may be recognized in the ovariole of the queen honey bee (fig. 1): (a) the terminal filament; (b) a rosette region; (c) a zone of differentiation; and (d) the posterior part in which the oocytes are arranged in a linear series and separated from each other by groups of nurse cells.

2. The rosette region is filled with rosette-like groups of cells, each group consisting of the descendants of a single mother oogonium. The cells of a rosette are united by strands which are the persisting spindle fibers from earlier mitoses (fig. 3). The cells in a rosette divide synchronously (fig. 5).

3. Oocytes and nurse cells are both derived from the oogonia. Their differentiation occurs in the zone of differentiation (fig. 1, d). One or more cells of each rosette enlarges and becomes an oocyte, whereas the others retain more of their earlier characteristics and become nurse cells. Although the strands which connected the cells in a rosette disappear, the descendants of a single oogonium

may still be determined, because of the presence of deeply staining rings between the cells (figs. 7-12).

4. The causes of differentiation could not be definitely determined, but several hypotheses are mentioned (p. 500).

5. Granules appear near the nucleus of oocytes shortly after their differentiation. Later they become distributed throughout the egg cytoplasm. These granules appear to be mitochondrial in nature and to arise from, or under the influence of the nucleus.

II. THE BACTERIA-LIKE RODS AND SECONDARY NUCLEI IN THE OOCYTES OF *CAMPONOTUS HERCULEANUS* VAR. *PENNSYLVANICA* DEG.

The important contributions by Blochmann ('84, '86) upon the growth of the oocytes in ants seem to be the only reports that have ever been made on this subject. Blochmann discovered two very interesting facts regarding these oocytes: (1) the presence of rod-shaped bodies almost completely filling the growing egg which he considered symbiotic bacteria, and (2) the formation of nuclear-like bodies around the oocyte nucleus. Recently Tanquary ('13) has described, in the freshly laid eggs of the carpenter ant, a body which he calls a cleavage nucleus, but which resembles very closely bodies that have been discovered in the eggs of other animals and to which I have applied the term keimbahn- or germ-line determinants. The observations recorded in the following pages were made in order to trace the genesis of the eggs of ants with special reference to the origin, distribution, and fate of the bacteria-like bodies, nuclear-like bodies, and the germ-line determinants.

The material used for these studies consisted of the ovaries of the carpenter ant, *Camponotus herculeanus* var. *pennsylvanica* DeGeer. A large number of virgin queens were obtained from a dying apple tree on April 3, 1914, and some of them were kept alive until June 9, 1914. The ovaries were dissected out in Ringer's solution and immediately fixed in the same manner as were those of the honey bee (page 496). Ovaries were preserved at intervals of a few days during the period of two

months. In this way oocytes in all stages of growth were obtained up to almost the period of deposition. Sections were cut and stained as in the queen bee.

The ovaries of the carpenter ant resemble those of the queen bee in general structure and the ovarioles are likewise similar. The youngest ovaries obtained had already passed the period when the oocytes and nurse cells are differentiated, so there was no opportunity to study the events that occur during this differentiation. Four regions may be distinguished in the ovarioles as shown in figure 13. There is a terminal filament (*t*) at the anterior end. This is followed by a region which we may call the terminal chamber (*t.c.*) containing oocytes, nurse cells, and epithelial cells without any special arrangement. The next part of the ovariole is short and contains oocytes which have grown considerably but have not yet taken a position in the axis of the tubule. This we may call the first zone of growth (*g*). The rest of the ovariole consists of a linear series of oocytes (*o*) each with its accompanying group of nurse cells (*n*). Each oocyte is larger than the one anterior to it and the nurse cells gradually become grouped into a definite nurse chamber (*n.c.*). The bacteria-like bodies discovered by Blochmann are present only in the last described zone. The first signs of nuclear-like bodies around the oocyte nucleus also appear here. For the sake of convenience oocytes in the various stages which need to be referred to have been drawn in outline and to scale as shown in figures 14 and 15.

The posterior end of the terminal filament (*t*) and anterior end of the terminal chamber (*t.c.*) are shown in outline in figure 16. The cells of the terminal filament are long and slender and extend entirely across it. One is shown enlarged in figure 17. Within the terminal chamber are two kinds of cells, oocytes and nurse cells. The oocytes, as indicated in figure 18, are the youngest to be found in the ovarioles at this time and I have regarded them as Stage A (fig. 14). The cell walls of the nurse cells are not very distinct. Their nuclei (fig. 19) are much smaller than those of the oocytes and contain a single irregular mass of chromatin granules. The structure of the oocytes and nurse cells is similar throughout the entire terminal chamber.

The terminal chamber is separated from the first zone of growth (fig. 20) by what appears to be a distinct membrane (*m*). The condition of all of the oocytes is similar throughout this zone (Stage B, fig. 14). The oocytes have grown considerably and their nuclei (fig. 21) contain a few clumps of chromatin granules lying near the nuclear membrane. Outside of the nucleus (fig. 21) is a layer of darkly staining substance which resembles chromatin in some respects and may represent chromatin which has passed through the nuclear membrane into the cytoplasm. The nurse cells now have definite cell walls (fig. 22) and are also characterized by a layer of darkly staining material lying around the nucleus. Among the oocytes and nurse cells are a few epithelial cells (fig. 23); these have no definite cell walls, and their nuclei are rather irregular in shape and contain a single mass of chromatin.

Whether or not the first zone of growth is definitely separated from the remaining part of the ovariole could not be determined with certainty, but its limit is conspicuously marked by the abrupt appearance of the bacteria-like bodies of Blochmann. This is indicated in figure 24, which shows the posterior portion of the first zone of growth and the anterior part of the rest of the ovariole. In the upper part of this figure is a single oocyte in Stage B and a number of nurse cells. These are apparently embedded in a loose reticulum of cytoplasm. Further down the ovariole the spaces surrounding the nurse cells and epithelial cell nuclei are filled with more or less wavy rods which Blochmann considered symbiotic bacteria. These rods extend throughout the ovariole in all directions, being represented by distinct spherical granules where cut across.

From this point on, the oocytes are arranged in a linear row in the central axis of the ovariole (figs. 13 and 25). The cytoplasm of the oocytes increases rapidly in amount, but the nuclei enlarge very little. The nurse cells (fig. 25, *n*) become arranged more or less definitely into rows which radiate toward the upper end of the oocyte. Those nurse cells closest to the oocyte increase more rapidly in size than do the others. Compare, for example, that lettered *a* in figure 25 with its companions, and

those accompanying the upper oocyte with those of the lower oocyte. Surrounding the oocytes, nurse cells, and epithelial cell nuclei are the groups of bacteria-like bodies.

The transition of the oocyte from Stage C (fig. 14, *C*; fig. 25, *C*₂) to Stage D (fig. 14, *D*, fig. 26) is accompanied by an invasion of the oocyte cytoplasm by the bacteria-like rods. Some of these rods form almost perfect circles, resulting in what at first sight appear to be vacuoles. Some of the epithelial-cell nuclei are in very close contact with the oocyte but these were not observed actually within the oocyte cytoplasm.

The principal difference between an oocyte in Stage D (fig. 26) and one in Stage E (fig. 14, *E*; fig. 27) is the sudden appearance of nuclear-like bodies around the nucleus, which I shall call secondary nuclei. The nucleus itself is about equal in size to that of the preceding stage (fig. 26). The chromatin, which in younger oocytes (figs. 24-26) has gradually migrated from the periphery toward the center of the nucleus where it formed an irregular clump, has again become scattered, being represented by a few smaller and widely separated masses. In the illustration (fig. 27) three secondary nuclei are shown lying below but in contact with the oocyte nucleus. These likewise contain a delicate reticulum and from one to three chromatin masses. No intermediate stages between the nucleus of Stage D (fig. 26) and that of Stage E (fig. 27) were discovered, and it was thus impossible to determine with certainty the origin of these secondary nuclei. If, however, the oocyte nucleus continued to increase in size at the same rate as indicated in Stage C (fig. 25) and in Stage D (fig. 26) it would be about the size of that in figure 27 *after* having given rise to the secondary nuclei by the method of budding or in some other way. This subject will be discussed more in detail later.

During the interval between Stage E (fig. 14, *E*; fig. 27) and Stage F (fig. 14, *F*, fig. 28) the oocyte enlarges until it extends almost across the ovariole, and the epithelial cell nuclei become arranged in a single layer around it, forming a follicle. At this time (fig. 28) the cytoplasm of the oocyte and that surrounding the nurse cells and epithelial-cell nuclei is crowded full of the

bacteria-like rods. The secondary nuclei also increase in number around the oocyte nucleus; the nucleus itself does not increase in size. Both the oocyte nucleus and the secondary nuclei are sometimes irregular in shape, a condition that may be due to the effects of fixation, or that may represent a stage in budding or in amitotic nuclear division (page 518).

The next phase of the growth period (Stage G, fig. 14, *G*, fig. 29) witnesses the lengthening of the oocyte and the further arrangement of the nurse cells to form a compact group, which becomes surrounded by epithelial cells, thus producing a definite nurse chamber. The bacteria-like bodies increase in number as the oocyte grows and continue to fill it completely with bundles of rods. The secondary nuclei near the oocyte nucleus also increase slightly in number.

Shortly after this condition is reached the oocyte is invaded just beneath the nurse chamber by an influx of cytoplasm elaborated by the nurse cells (fig. 30, *c*). This cytoplasm is free from the bacteria-like bodies and it seems very probable that it either forces these rods out of its path or else dissolves those which it encounters. There is evidence that, from this stage on, the number of bacteria-like rods does not increase, the rods gradually lose their compact grouping and become further separated from one another, the spaces between them probably being occupied by the cytoplasm added to the oocyte by the nurse cells. The oocyte nucleus by this time (fig. 30) is completely surrounded by secondary nuclei from which it differs in appearance. The secondary nuclei contain a rather dense reticulum and one or several large chromatin granules, whereas the oocyte nucleus is very irregular in shape and contains a delicate reticulum which causes it to appear clearer. The irregular shape of the oocyte nucleus is probably due to the pressure upon it of the secondary nuclei which surround it. Its decrease in size is also noticeable and one cannot but suspect that this decrease is directly related to the increase in the number of secondary nuclei. A transverse section through an oocyte near the nurse chamber is shown in figure 31.

The nurse chamber is now completely formed (fig. 13, *n.c.*). The nurse cells are still free from the bacteria-like rods and their nuclei, as pointed out by Blochmann ('86), possess very thick membranes (fig. 30, *n*). Part of one of these nuclei greatly enlarged is shown in figure 32. The membrane contains, in a homogeneous matrix, a number of vacuoles and a great many granules of various sizes which appear in material fixed and stained by a number of different methods. Their reactions all indicate that they are chromatic in nature and their position suggests that they may have migrated into the membrane from inside of the nucleus and are on their way into the cytoplasm. It could not be definitely determined, however, whether this is a true case of chromatin emission or simply a condition due to the action of the fixing solutions used.

A further increase in the amount of cytoplasm within the oocyte is evident when Stage H (fig. 15, *H*; fig. 33) is reached. Here an opening (*a*) is present in the follicle connecting the oocyte directly with the nurse chamber. The small plug of cytoplasm filling this channel is no doubt homologous with the nutritive string present in the ovarioles of insects whose oocytes are not accompanied by a group of nurse cells, but are connected with the terminal chamber by a protoplasmic thread. In this stage the oocyte nucleus (*o*) is still closely pressed by the secondary nuclei (*s*) surrounding it and the entire group lies within the cytoplasmic zone. Such a group is shown enlarged in figure 34, in which the oocyte nucleus may be distinguished from the secondary nuclei by its irregular shape, central position, and clearness.

The succeeding stages in the growth of the oocyte (fig. 15, *I*, *J*, *K*, *L*; figs. 35-39) are characterized by a decrease in the number of bacteria-like rods, by the formation of yolk globules, and by the increase in number and the scattering of the secondary nuclei. Part of a section through an oocyte of Stage I (fig. 15, *I*) is shown in figure 35 which represents a portion extending from a point midway between the two poles out to the middle of the oocyte. Just within the follicular epithelium (*e*) is the suggestion of a clear layer (*k*) which later becomes the 'Keimhautblastem.' The

black spherical bodies are yolk globules (*y*) which appear to originate near the periphery and gradually to migrate into the central region. The bacteria-like rods are still present but they are widely scattered and faintly staining.

By the time the next stage is reached (fig. 15, *J*; fig. 36) the bacteria-like rods have completely disappeared everywhere except near the periphery, around the lower part of the oocyte. According to Blochmann ('86) they are still present in this region after the eggs are laid, and they are also mentioned by Tanquary ('13) in the freshly deposited eggs. The latest oocyte studied by the writer is Stage L (fig. 15, *L*), which is considerably younger than the fully grown egg. A few faintly staining rods still exist at this stage near the posterior end.

The compact group of secondary nuclei which surround the oocyte nucleus up to this stage now breaks up, and the individual nuclei become scattered throughout the entire egg near the periphery. Quite a number of them still appear near the anterior pole of an oocyte in Stage K (fig. 15, *K*; fig. 37) where they surround the opening into the nurse chamber. At some distance back of this pole the secondary nuclei are imbedded in the cytoplasm especially near the periphery. They retain at this time (Stage K) the characteristics noted in early stages; i.e., they are more or less spherical, filled with a reticulum, and contain one or several large chromatin granules (fig. 38). Later (Stage L, fig. 15, *L*; fig. 39) they seem to be more numerous and a single egg must contain hundreds of them. In this, the last stage examined, these secondary nuclei have changed in appearance as indicated in figure 40. The chromatin granules have become aggregated into a few irregular strands, a condition which may be a phase of degeneration or, as Loyez ('08) believes, a stage in the formation of yolk globules. The fate of the secondary nuclei was not discovered and, so far as I know, none of the investigators who have described similar bodies has been able to determine with certainty what becomes of them.

The posterior ends of the older oocytes in my series were carefully examined with a view to tracing the origin of the body which Tanquary ('13) observed near the posterior pole of freshly

laid eggs of *Camponotus herculeanus* var. *ferrugineus* Fabr. and called the cleavage nucleus (fig. 41, *n*). This body is obviously not a cleavage nucleus since it is not in the usual position occupied by this nucleus, and does not possess the characteristics of a cleavage nucleus. Furthermore, it persists during the early cleavage stages at the posterior end, whereas the cleavage cells (nuclei) are shown by Tanquary in their proper position near the anterior pole (fig. 42, *cc*). It seems probable therefore that this body belongs to the class of substances which have been found in the eggs of many different kinds of animals and which later become part of the material within the primordial germ cells—substances to which I have applied the term 'Keimbahn or germ-line determinants.' This seems all the more probable since it persists at least until a late cleavage stage (fig. 43, *rn*.) and later there is present a group of cells (fig. 44, *kc*.) which Tanquary describes as a group of small cells applied to the posterior end of the inner peripheral protoplasm," and which further research will doubtless prove to be germ cells. No bodies were discovered in my material that could be recognized as an early stage in the formation of the 'cleavage nucleus' described and figured by Tanquary.

Discussion. No opportunity was afforded by the material in my possession for determining the differentiation of the oocytes from the nurse cells, since these two sorts of cells are established in ovarioles younger than those in the virgin queens collected in the spring. The problem of the separation of germ cells (oocytes) from somatic cells (nurse cells) therefore could not be solved. The most interesting phenomena exhibited by the ovarioles are (1) the presence of the bacteria-like rods and (2) the formation and distribution of the secondary nuclei.

The bacteria-like rods. Blochmann ('84, '86) was the first to observe the bacteria-like rods in the ovarioles of two species of ants, *Camponotus ligniperda* and *Formica fusca*. Recently Tanquary ('13) has observed these bodies in the cytoplasm at the posterior end of freshly laid eggs of *Camponotus herculeanus* var. *ferrugineus*. Blochmann found those in the eggs of *Camponotus* to be from 10 to 12 μ in length, whereas those in *Formica*

were only from 4 to 5 μ long and were not so regularly arranged in bundles as in the former species. He at first supposed these bacteria-like bodies to be cytoplasmic structures, but, after observing them in various stages of division, expressed the opinion that they are symbiotic bacteria.

Bodies of a similar kind have been observed in many other insects. Those that occur in the cockroaches most closely resemble the bacteria-like rods in the ovarioles of ants. These likewise were first discovered by Blochmann ('87, '92) in *Periplaneta orientalis*. They occurred not only in the eggs but also among the blastoderm cells and in the spaces formed by the liquefaction of the yolk in the embryos. Later they were observed in the anlage of the fat body where they persist in the adult stage. Wheeler ('89) described them in the 'Keimhautblastem' of *Blatta germanica* as "minute rod-shaped bodies so numerous in the surface protoplasm as to make it appear reticulate. They look like bacillar micro-organisms and stain deeply."

Mercier ('07) has subjected these bacteria-like rods in *Periplaneta orientalis* to careful study. He agrees with Blochmann regarding their distribution and confirms Blochmann's statement that they multiply by division. Mercier was able to cultivate the rods and concludes that they are true bacteria and thinks them to be of a symbiotic nature although he was unable to suggest any advantage that the host receives because of their presence. They are given the name *Bacillus cuenoti* by Mercier.

Many other investigators have reported bacteria within the eggs or tissues of insects. Blochmann ('87) observed them in *Pieris*, *Musca*, and *Vespa*; Stuhlmann ('86) shows them in many of his figures, and Forbes ('91) found them in the caecal glands of various Heteroptera. The 'green or yellow granular mass' described by Leydig ('50) in the embryos of viviparous aphids and later called the 'pseudovitellus' by Huxley ('58) and the 'green body' by Witlaczil ('84) is considered now to be due to symbiotic organisms. Of particular importance are the contributions of Mercier ('07) on the cockroach and of Sule ('06, '10), Pierantoni ('10), and Buchner ('12) on the Hemiptera. Buchner ('12) has given a full historical discussion of the subject

besides adding considerable new material, and any one desiring a comprehensive review of the present state of our knowledge of these symbiotic organisms is referred to his paper. Thirty-four species are described and figured by Buchner. Some of them are bacteria, such as those in the cockroach, but others are more like yeasts. The infection of the egg, which reminds one of the infection of the egg of the Texas fever tick by *Piroplasma bigeminum*, may be diffuse, as in the cockroach, or localized, as in the aphids. Buchner decides that these organisms are symbiotic, but, like Mercier, was unable to discover any advantage to the insect host from the relationship.

The secondary nuclei. One of the most interesting features of the growth of the oocyte in certain insects is the formation of small nuclear-like bodies around the oocyte nucleus. Bodies of this sort were first described by Blochmann ('84, '86) in Hymenoptera. Since then they have been observed in insects belonging to this order by Stuhlmann ('86) and Marshall ('07) and similar bodies were noted by Korschelt ('86) near the nuclei of both the oocyte and nurse cells of the fly, *Musca vomitoria*. Korschelt was unable to determine the origin, function, and fate of these 'helle Bläschen' but noted their resemblance to those discovered by Blochmann.

The two ants, *Camponotus ligniperda* and *Formica fusca*, and the wasp, *Vespa vulgaris*, were all found by Blochmann to be very much alike so far as the growth of their oocytes is concerned. The origin of the nuclear-like bodies is described in *Camponotus* as follows:

Bei etwas älteren Eiern beginnt nun an der Oberfläche des Kernes ein Knopfungsprocess, der schliesslich zur Entstehung einer grossen Anzahl kleiner Kerne führt. Man bemerkt als erste Andeutung dieses Processes kleine, helle, rundliche Gebilde, die dicht an der Oberfläche des Kernes anliegen, und die ich in meiner vorläufigen Mittheilung als 'knötchenförmige Verdichtungen' bezeichnet hatte. Ich neige jetzt zu der Ansicht, dass es von vornherein kleine Vacuolen sind, da die Kernmembran sich meist etwas färbt und stets sehr scharf erscheint, während ich an diesen kleinen Gebilden bei ihrem ersten Auftreten keine derartige Membran unterscheiden könnte. Bald tritt in diesen Vacuolen ein kleines mit Pikrocarmin sich färbendes Körnchen auf diese Vacuolen oder, wie ich sie jetzt nach dem Auftreten des Chro-

matinkörnchens nennen will, Nebenkerne, nehmen allmählich an Grösse zu, wobei sie dann eine sehr deutliche Membran an ihrer Oberfläche erkennen lassen, zugleich nimmt der Inhalt an festen, färbbaren Substanzen zu. Diese treten theils als kleine, rundliche Nucleolen, oder als feine, wenig sich färbende Fädchen auf.

Da nach und nach immer mehr solche Nebenkerne entstehen, finden wir bei etwas weiter in der Entwicklung fortgeschrittenen Eiern in der Region der Eiröhre, wo bereits Eifächer und Nährzellenfächer deutlich abgegrenzt sind, die Oberfläche des Eikernes von einer ganzen Schicht solcher Nebenkerne von verschiedener Grösse bedeckt, die sich gegenseitig berühren. So bleiben die Verhältnisse auch in noch etwas älteren Eiern. (pp. 144-145).

These Nebenkerne, according to Blochmann, after multiplying by self-division become scattered within the yolk where they degenerate, none being present in the ripe egg.

Among the other investigators who have observed similar bodies in the oocytes of insects are the following: Will ('84) and Ayers ('84) observed them in Hemiptera and considered them follicular epithelial cells which contributed to the formation of the yolk. Stuhlmann ('86) has described them in many insects, including *Musca*, *Periplaneta*, *Locusta*, *Pieris*, *Aphrophora*, *Sphinx*, and certain Coleoptera and Hymenoptera. They were called 'Reifungsballen' by him and were thought to be similar to the polar bodies which at that time had not yet been observed in insects. The 'Reifungsballen' appear at different stages of the growth period in different species and also have different fates; some of them fuse to form a large 'Dotterkern' which lies near the posterior end of the egg and resembles what I called 'Keimbahn-determinants,' and others become widely distributed and disappear in the yolk. The possible origin of the 'Ballen' from epithelial cells is suggested but not considered probable. Korschelt ('89) from a study of them in *Bombus*, concludes, as did Will and Ayers, that they are derived from epithelial cells.

In *Blatta germanica* and *Leptinotarsa decemlineata*, Wheeler ('89) has described as 'maturation spheres' a number of globular bodies which appear after the egg nucleus migrates to the periphery and prepares for maturation. In *Blatta* several of these spheres may be present. In *Leptinotarsa* a number of oval

hyaline masses likewise occur which are considered the equivalents of the 'maturation spheres' in *Blatta* and homologous to the 'Reifungsballen' of Stuhlmann. No chromatin masses were observed in any of these spheres, but in *Leptinotarsa* the wandering of part of the chromatin into the yolk, where it disappears, is described. As Stuhlmann pointed out, these spheres may appear in different species at different stages in the growth period and it seems therefore possible that the 'Nebenkerne' of Blochmann, the 'Reifungsballen' of Stuhlmann and the 'maturation spheres' of Wheeler may be homologous, although the first two contain chromatin whereas the 'maturation spheres' do not. Lameere ('90) was able to confirm Blochmann's account regarding the origin of the Nebenkerne in *Camponotus* and Henneguy ('04) found them in both the wasp and the honey-bee. In the former they appear around the germinal vesicle and disappear very early, but in the bee they seem to be derived from follicular epithelial cells and persist until a later developmental stage. None of these bodies could be found in the oocytes of the honey-bee which I have studied.

Marshall ('07) made a careful study of the secondary nuclei in *Polistes*, but, like previous investigators, was unable to determine definitely regarding their origin and fate. He agrees with Blochmann that they probably arise from the germinal vesicle by budding, but was unable to find any stages in such a process. Concerning their function Marshall was likewise unable to come to a definite conclusion, but suggests that they may act upon the nurse-cell substance making it available for the oocyte.

As described on page 509, the secondary nuclei of *Camponotus* make their appearance at Stage E (fig. 14, *E*; fig. 27) in the growth of the oocyte. From this stage on the size of the primary nucleus does not increase but actually decreases and the number of secondary nuclei becomes greater as the oocyte enlarges (compare figs. 28, 31 and 33). At first the oocyte nucleus always lies very close to the center of the anterior pole of the oocyte and the secondary nuclei form a single layer in contact with the opposite wall of the oocyte nucleus (figs. 27, 28, 29), but in later

stages (figs. 30, 33) the group of nuclei is more often near one side, at the anterior pole, and the oocyte nucleus is entirely surrounded by secondary nuclei, the latter sometimes being several layers in thickness (fig. 34). Hundreds of such groups were carefully examined, beginning with oocytes in Stage D (fig. 14, *D*; fig. 26), but in no case could the origin of the secondary nuclei be definitely determined. As the latter increase in number, they, as well as the oocyte nucleus, tend to lose their spherical shape and become oblong, or indented, or more or less irregular (figs. 28, 29, 34). This may be due to the action of the fixing solution, or to the pressure of one upon another, but many of them present shapes very suggestive of budding, or of more or less equal constriction into two. Some groups selected from the large number examined are shown in outline in figure 45. Frequently the space produced by the indentation of one of the nuclei is perfectly clear and resembles a vacuole. This suggests the possibility that the irregularity of the nucleus may be due to the escape of material from it which occupies the space formed by the caving in of the nuclear membrane. If this material were then to become surrounded by a nuclear membrane a secondary nucleus would be the result.

Two other theories have been suggested to account for the formation of secondary nuclei. According to Will ('84) in Hemiptera, Korschelt in *Bombus*, Henneguy in the honey-bee, and Brunelli ('04) in Hymenoptera they appear to come from follicular epithelial cells. Brunelli thinks they are attracted around the germinal vesicle by chemical action. Gross ('03) likewise believes from his studies on *Bombus* and other Hymenoptera that they are true nuclei, but that they originate from the epithelial cells which are situated among the nurse cells. This cannot of course be true in forms such as *Camponotus* where the secondary nuclei appear before a follicle and nurse cells are acquired. The other theory is that advanced by Stuhlmann who says "Ich wiederhole noch einmal, dass ich diese Kerne nur für 'Dotterconcretionen' halte."

The investigations of Loyez ('08) upon the 'noyaux de Blochmann' are the most thorough yet published. She studied these

secondary nuclei or 'pseudo-noyaux' as she calls them, in four species of *Bombus*, two species of *Vespa*, and one species of *Xylocopa*. They were found to resemble true nuclei in their fully developed condition, but all stages were observed between these and the very small vacuole-like bodies from which they apparently arise. The theories of their origin by budding off from the germinal vesicle and by the emigration of epithelial cells are considered by Loyez to be untenable. The conclusion is reached that they originate from the germinal vesicle, follicular epithelial cells, and nurse cells not by budding or the emigration of entire nuclei "mais résultent d'une coagulation de substances venues du dehors à l'état fluide ou granuleux et modifiées par le cytoplasme de l'oeuf." (p. 100). In old oocytes the secondary nuclei were found to change in structure so that they resemble nuclei which are undergoing synapsis, and, since all stages between the typical secondary nucleus and a homogeneous globule were observed, Loyez decides that they transform into deutoplasmic spheres.

The presence of these secondary nuclei in certain insects and not in others can be regarded as a sort of precocious diminution of nuclear substances. The loss of chromatin by passage through the nuclear membrane and its identification as chromidia in the cytoplasm has been reported by a number of investigators as taking place in the nuclei of many different kinds of cells during what is known as the resting stage. During ordinary mitosis only a part and sometimes the smaller part of the nuclear chromatin is concerned in the formation of the spireme, the rest being cast out into the cytoplasm with the other nuclear contents when the membrane breaks down. These substances become scattered and dissolved in the cytoplasm. Just before the maturation divisions occur, it is customary for the germinal vesicle to liberate into the cytoplasm a considerable part of its contents, including granules or small masses of chromatin which become scattered amid the yolk globules and disappear. The diminution of nuclear substance therefore seems to be a widespread process. That the formation of at least part of the secondary nuclei in the oocytes of certain insects is likewise a nuclear

diminution process also seems probable. Each secondary nucleus contains masses of chromatin and in every way resembles a true nucleus, but, as in other cases of diminution, this chromatin and the other contents of the secondary nuclei are lost in the general egg substance. The elimination of this material simply occurs in these species at an earlier stage than in the oocytes of other animals.

The function and fate of the secondary nuclei cannot be stated with any degree of certainty. We have seen that they cease to form a compact group in the older oocytes and become distributed throughout the egg, especially near the periphery (figs. 37 and 39). Later they undergo a process which appears to be degenerative, and, according to those who have studied later stages, finally disappear altogether. The writer suggested a few years ago (Hegner '09) that secondary nuclei of this sort might migrate to the posterior pole and take part in the formation of the germ-line-determinants, but thus far no actual evidence that this occurs has been obtained. Marshall ('07) has expressed the opinion that they may make the substances provided by the nurse cells available for the oocyte. It also is possible, as Loyez claims, that these secondary nuclei may have some function in the formation of yolk.

Summary of Part II. 1. The ovarioles of *Camponotus* consist of four distinct regions (fig. 13), (a) a terminal filament, (b) a terminal chamber, (c) a zone of growth free from bacteria-like rods, and (d) the posterior part in which the oocytes are arranged in a linear series, are accompanied by nurse cells, and are surrounded and later invaded by the bacteria-like bodies.

2. The bacteria-like rods occupy definite regions of the ovariole. They are absent entirely from the terminal filament, terminal chamber and first zone of growth. In the rest of the ovariole they occur everywhere except in the nurse cells (fig. 25). The oocyte is at first free from them (fig. 25) but later is invaded (fig. 26) and almost completely filled with them (fig. 29). The rods are arranged at first in bundles (figs. 25, 29), but later become scattered (figs. 35, 36). As the oocyte increases in size and

yolk formation proceeds, they gradually disappear until none are visible except near the periphery in the posterior region.

3. Secondary nuclei appear near the oocyte nucleus at an early stage of growth (fig. 27). They increase in number, finally completely surrounding the germinal vesicle (figs. 33, 34). They later become distributed throughout the oocyte especially near the follicular epithelium (figs. 37, 39). Their origin by budding from the oocyte nucleus, or by the immigration of epithelial cells seems improbable. The conclusion is reached that the oocyte nucleus gives off materials into the cytoplasm which become enclosed by a membrane and develop into nuclear-like bodies. The fate of the secondary nuclei was not determined.

III. HISTORY OF THE NUCLEI AND GERM-LINE DETERMINANTS IN THE OOCYTES OF CERTAIN PARASITIC HYMENOPTERA AND HYMENOPTEROUS GALL-FLIES

1. *Copidosoma gelechiae*

In June, 1914, I published a short account of the growth of the oocyte in *Copidosoma gelechiae* with special reference to the origin of the germ-line-determinants. Since then two other accounts have appeared on the same subject, one by Martin ('14) on *Ageniaspis (Encyrtus) fuscicollis*, and the other by Silvestri ('14) on *Copidosoma buyssoni*. I have also been able to obtain and study a new lot of material. This makes it possible for me to add to my previous account and to clear up certain points about which differences of opinion have arisen. These poly-embryonic Hymenoptera are interesting principally because of the peculiarities in their embryonic development. We shall refer in this paper to two of these; (1) the history of the egg nucleus and (2) the origin and fate of the germ-line-determinants.

Silvestri ('06-'08) has shown that a body which he considered the nucleolus of the germinal vesicle is present near the posterior end of the eggs of certain parasitic Hymenoptera. During embryonic development this body is segregated in a single cleavage cell until the seven-cell stage is reached; then, having disintegrated, its substance is divided between two cells. These,

according to Silvestri, are the parents of all of the germ cells, a conclusion that seems justified, since a similar body in monembryonic parasites has been definitely traced until it becomes distributed among the germ cells.

In my preliminary report on *Copidosoma gelechia* I pointed out the improbability of the origin of the 'nucleolo' of Silvestri from the nucleolus of the germinal vesicle, and concluded from the material I then possessed that this body consists of all of the chromatin from the oocyte nucleus which had formed into a compact mass. To explain the presence of both this body and an egg nucleus it was suggested that two oocytes might fuse end to end, the posterior one furnishing the 'nucleolo' and the other the nucleus. The eggs of these insects, when ready to be laid, are long, with a very slender bent portion between the two thicker ends, as shown in figure 54. My material consisted only of serial sections cut 2 and 4 μ thick, and, as Silvestri ('14) has pointed out, I considered sections through the anterior and posterior ends of an oocyte as sections of complete oocytes. This is a mistake that I now wish to acknowledge, but is one that could hardly be avoided without good *in toto* preparations. With the aid of such preparations I have been able to confirm Silvestri's account in most respects. My conclusion, however, that the 'nucleolo' of Silvestri is not the nucleolus of the oocyte nucleus is correct, and my account of the history of the oocyte nucleus up to its change into an oval mass of chromatin is also correct, as indicated by the study of new material, and by the confirmatory account by Martin in *Ageniaspis*. I am indebted to Dr. R. W. Glaser for this new material.

Martin ('14) records the presence of three kinds of cytoplasmic inclusions in the growing eggs of *Ageniaspis*: (1) a cloud of granules near the posterior end, (2) a 'nucleolus' also near the posterior end, and (3) a few chromatin granules cast out by the nucleus. The 'nucleolus' is of particular interest to us, since it is undoubtedly a body similar to the 'nucleolo' of Silvestri. Martin was able to trace this 'nucleolus' from the young oocytes to the three-cell stage in the cleavage of the developing eggs. It appears first as a small group of granules lying in the midst

of the cloud of granules mentioned above. It gradually increases in size, reaching its maximum dimensions about the time the egg reaches its full size. Then it becomes vacuolated and loses some of its affinity for stains. When the first cleavage division occurs, it passes entire into one of the two blastomeres. This blastomere does not divide as quickly as the other and a three-cell stage thus results, one cell containing the 'nucleolus' and the other two lacking this body. At this point the 'nucleolus' breaks down and can not be traced further. Regarding the origin of the 'nucleolus' Martin is not certain. He agrees with me that it is not derived from the nucleolus of the germinal vesicle, since, when it first appears, it is at the opposite end of the oocyte. Apparently it is built up by the aggregation of the deeply staining granules among which it lies, but where these granules originate was not determined.

The history of the oocyte nucleus. The ovaries of *Copidosoma* consist of a number of ovarioles, each of which contains a row of oocytes in various stages of growth, the oldest being situated near the posterior end. It is thus possible to find without much difficulty all stages in the growth period. We shall begin our account with an oocyte (Stage A, fig. 46) which has already acquired an epithelium and is accompanied by a nurse chamber. A close examination of such an oocyte (fig. 55) reveals a very large nucleus, containing an irregular, homogeneous mass of chromatin. A very thin layer of cytoplasm surrounds the nucleus.

The nucleus does not increase much in size during the growth period, but the oocyte enlarges rapidly because of the accumulation of cytoplasm. During the interval between Stages A and B (figs. 46, 47) both the oocyte and the oocyte nucleus become larger and oval. The chromatin now consists of what appears to be a long much coiled thread (fig. 56) and one is led to believe that the homogeneous mass in Stage A is really the same thread much more compactly coiled; in other words, in the condition as synezeisis. By the time Stage C (fig. 48) is reached the nucleus has again regained a spherical shape and the chromatic spireme has become spread out as shown in figure 57. Up to

this time the cytoplasm appears to be homogeneous throughout. Martin finds in *Ageniaspis* at this stage a cloud of granules in the posterior region (fig. 70) but nothing of the sort is present in my preparations, nor were such granules observed by Silvestri in *Copidosoma bussyoni*. Silvestri ('14), however, thinks he has discovered a group of granules at the posterior end of the nucleus at about this stage (fig. 69) which he suggests may lead to the formation of the oosoma (formerly called by him the 'nucleolo' and designated by me as a keimbahn or germ-line-determinant).

The nuclear phenomena are of considerable interest from this time on. The spireme becomes more and more open (Stages D, E, figs. 49, 50) and finally breaks up into thin, chromosomes of irregular shape (Stage F, fig. 51). These chromosomes then become shorter and thicker and appear to unite near their ends (Stage G, fig. 52). At first the pairs are scattered about within the nucleus (fig. 58) but they soon straighten out and become arranged in a parallel series with their points of union lying in the equator (Stage H, figs. 53, 59, 60). Spindle fibers could be seen, but apparently no centrosomes or asters are present. The number of pairs of chromosomes as indicated by cross sections of spindles of this sort seem to be twelve, the same number recorded by Silvestri for *C. bussyoni*, but several very clear sections contain only eleven (fig. 61).

Soon after the parallel arrangement of the chromosome pairs occurs, the egg reaches its full growth and attains its definite shape (Stage I, fig. 54). The mitotic figure then passes through the stages of condensation, as described in my preliminary report (Hegner '14 b). The chromosomes gradually get closer together and become shorter and thicker (fig. 62). Where their ends meet at the equator a ridge appears, which causes the complex to resemble a maltese cross (fig. 63). Soon the spaces between the chromosomes are entirely obliterated (fig. 64) and a homogeneous mass of chromatin results (fig. 65).

Silvestri has noted the parallel arrangement of these chromosome rods, but has evidently failed to observe their condensation. Martin, however, has reported a similar phenomenon in *Agenias-*

pis, although in this form the rods which condense seem to consist of single instead of double chromosomes (fig. 66). The history of the nucleus as recorded by Martin is as follows:

The chromatin in the very young oocyte is aggregated at the posterior side of the nucleus. As the oocyte grows, it spreads throughout the nucleus, forming numerous granules which are distributed upon a reticulum. Chromosomes are then formed and soon become arranged on a spindle, which becomes more and more compact until a single mass of chromatin results (fig. 67). This mass divides in polar body formation (fig. 68) apparently without the presence of spindle fibers or asters.

The germ-line-determinants in Copidosoma. The 'nucleolo' or germ-line-determinant appears in my material at about Stage D (fig. 49) at which time it is perfectly distinct, staining a deep black in iron hematoxylin. From this stage on it is invariably present, increasing in size until Stage F (fig. 51) is attained. Five methods of origin have been suggested for this body. (1) Silvestri's ('06) first idea that it consists of the nucleolus of the germinal vesicle was shown to be incorrect in my preliminary report (Hegner '14 b) and Silvestri has admitted his error (Silvestri '14). (2) My conclusion that it arises from the chromatin of the oocyte nucleus has on the other hand been disputed by Silvestri and I wish here to acknowledge the truth of his observations. (3) In his latest report Silvestri ('14) coins a new name for this body, calling it the 'oosoma,' and thinks that it may possibly arise from a heap of granules at the posterior end of the nucleus (fig. 69). (4) Martin accepts Silvestri's term 'nucleolus' for the body, but claims that in *Ageniaspis* it is gradually built up by the aggregation of granules which appear in the cytoplasm of the posterior region of the egg (fig. 70). (5) Since I have been unable to confirm with my material either of the methods of origin suggested by Silvestri and Martin and since this germ-line-determinant appears suddenly at about Stage D (fig. 49), I wish to propose another theory as to its genesis. In Part II of my series of "Studies on germ cells" (Hegner '14 a) I have expressed the following conclusion, after collecting and discussing all the literature on the origin and history of the germ-line-determinants in animals.

The most plausible conclusions from a consideration of these observations and experiments are that every one of the eggs in which Keimbahn-determinants have been described, consists essentially of a fundamental ground substance which determines the orientation; that the time of appearance of Keimbahn-determinants depends upon the precociousness of the egg; that the Keimbahn-determinants are the visible evidences of differentiation in the cytoplasm; and that these differentiated portions of the cytoplasm are definitely localized by cytoplasmic movements, especially at about the time of maturation.

This conclusion still seems to me the only tenable one at the present time and applies, I believe, to the germ-line-determinants in *Copidosoma*, as well as to those in other animals.

2. *Apanteles glomeratus*.³

Another Hymenopteron that resembles *Copidosoma* in some respects is *Apanteles*, a parasite of the larva of the cabbage butterfly. An abundance of material was obtained in the month of August, 1914. The pupae and recently emerged adults were liberated from the cocoons, and their abdomens were fixed either in Bouin's picro-formol solution or Carnoy's mixture. As in *Copidosoma*, the ovaries contain oocytes in all stages of growth and hence their history could be traced without much difficulty.

The history of the oocyte nucleus. Oocytes at a very early stage (fig. 71) acquire an epithelium (*e*) and are accompanied by a group of nurse cells (*n*). The chromatin is large in amount and massed into an irregular homogeneous body. As growth proceeds (fig. 72) this chromatin-mass expands, revealing the spireme of which it consists. Soon the entire nucleus is filled with a network of chromatin threads (figs. 73-76), a condition that persists for a considerable part of the growth period. When the oocyte has reached its definitive size (fig. 77), the chromatin threads contract into chromosomes which apparently unite in pairs, as in *Copidosoma* (fig. 60), and become arranged side by side upon an asterless spindle (fig. 78). This stage is followed by the condensation of the chromosomes, as shown in figure 79. No later stages in the nuclear history were present in my material,

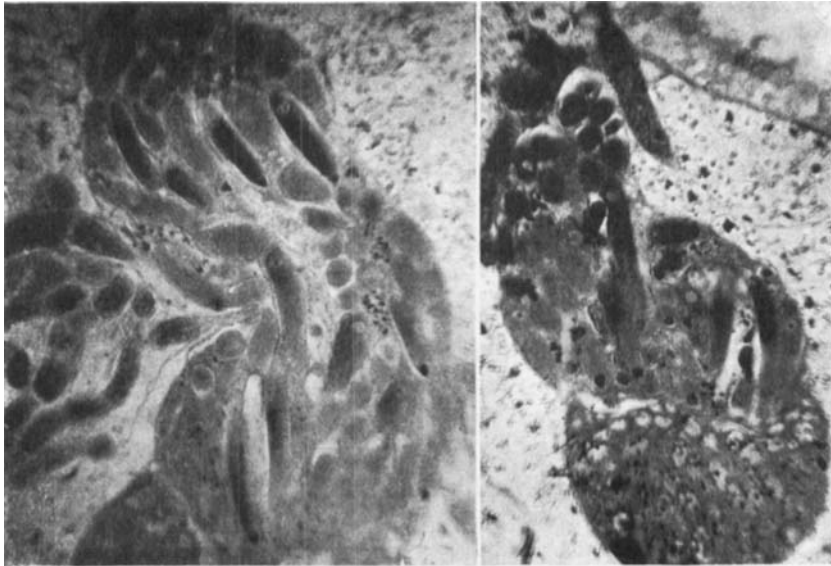
³ I am indebted to Dr. H. L. Viereck for the identification of this parasite.

but it is safe to assume that a further condensation occurs resulting in an oval, homogeneous mass as in *Copidosoma* (fig. 65).

The secondary nuclei When a stage about like that shown in figure 75 is reached, there appears within the cytoplasm of the anterior one-half of the oocyte a great number of spherical bodies which are arranged as in figure 75, and which resemble small nuclei. Figure 76 is an enlarged drawing of the anterior end of the section shown in figure 75. The secondary nuclei vary considerably in size. The substance within them stains like chromatin and is in the form of one or several small masses from which a few strands of chromatin granules radiate toward the membrane. These secondary nucleoli are present for only a brief period, having all disappeared by the time the chromosomes are formed (fig. 77). Their origin and function are problematical, but it seems hardly possible that they can arise from the germinal vesicle by budding, and hence we are forced to the same conclusions already stated in the case of *Camponotus* (p. 371).

The germ-line-determinants. The fully grown oocytes of *Apanteles* contain the most conspicuous germ-line-determinants yet described (fig. A). Although its history during embryonic development is not known, the probability that it plays an important rôle in the formation of the primordial germ cells is so great that it seems safe to include it among the bodies to which the term *keimbahn* or germ-line-determinant has been applied.

The first indication of this body occurs in a half-grown oocyte (fig. 73). Here a triangular area at the extreme posterior end may be distinguished from the rest of the egg by a slightly greater staining capacity (somewhat exaggerated in fig. 73). This affinity for basic stains increases as the oocytes grow older, and a network appears (fig. 74) which very much resembles the 'netzapparat' described by many writers both in germ cells and somatic cells (Duesberg '11). In succeeding stages this network condenses into a solid mass (fig. 75), but cavities soon appear again (figs. 77, 80), and the threads become thinner (fig. 81). Finally a condition is reached (fig. 82) in which the threads break



Text fig. A Microphotographs of longitudinal sections through the abdomen of *Apanteles* showing various stages in the growth of the oocytes. The germ-line determinants appear as distinct triangular black bodies near the posterior ends of certain of the larger oocytes.

up into a large number of irregular masses, suspended in a homogeneous substance. Near the germ-line-determinant in later stages (fig. 75) are a number of large widely scattered granules which are probably separated off from the main body.

The resemblance between this body and the pole-plasm in the egg of *Miastor* (Kahle '08; Hegner '12, '14 a, '14 c) is quite striking. The pole-plasm in *Miastor* appears just before the oocyte undergoes maturation, and apparently does not arise directly from the germinal vesicle, nurse cells, or follicular epithelium, but is a visibly differentiated portion of the cytoplasm that has become localized at the posterior end. What causes this differentiation is not known, but a discussion of the subject will be found in my previous contributions (Hegner '14 a, '14 c). In *Miastor* the pole-plasm never proceeds beyond the granular stage, but in *Apanteles* a rather definite series of conditions

ensues during which the granular stage (fig. 73) is succeeded by a heavy network (fig. 74); this is followed by condensation into a solid mass (fig. 75), the formation of a heavy network again (fig. 80), the thinning out of this network (fig. 81), and finally the breaking up of the threads into many large irregular granules (fig. 82).

3. *Hymenopterous gall-flies*

There is still much to be learned regarding the life-cycles of the gall-flies, but what we do know is enough to prove that these insects offer a very profitable field for research. Examinations of the growing oocytes of certain Hymenopterous gall-flies have revealed many interesting structures that have a bearing upon the conditions described in the preceding part of this contribution and, although much more work needs to be done, the data already obtained are included here to indicate the widespread occurrence of phenomena described above. The oogenesis in these insects is more difficult to study than in the parasitic Hymenoptera because fewer stages of growth are represented by the oocytes in a single individual.

The maturation spindle in the oak-knot gall-fly, Andricus punctatus. The oak-knot gall-fly lays a pear-shaped egg (fig. 83), from the anterior end of which extends a long, slender process with an expanded terminal portion. This process resembles those described by Korschelt ('87) in *Ranatra linearis*. The two long slender processes extending from the anterior end of the eggs of *Ranatra*, arise from a single bud-like protuberance at one side of the anterior end of the oocyte, and their place of origin alternates from one side to the other in the row of oocytes which lie in the lower part of the ovariole. When the eggs are laid, the processes are left extending freely out into the water from the decaying wood in which the rest of the egg is imbedded by the female.

The egg of the oak-knot gall-fly shown in figure 83, was taken from an adult which was just about to emerge from the gall. At one side near the anterior end could be seen a spindle-shaped body—the nucleus of the egg. Several stages in the develop-

ment of this body were found and they seem to indicate a condition similar to that described in *Copidosoma* and *Apanteles*. The earliest stage discovered (fig. 84) represents an asterless spindle bearing a number of pairs of chromosomes attached near their ends and drawn out so as to form a more or less parallel series. These pairs then condense, as shown in figures 85 and 86, and finally produce the pear-shaped body mentioned above (fig. 83). Apparently the chromosomes become completely fused in forming this body, since a high magnification (fig. 87) reveals nothing more than a vacuolated mass of chromatin. The nucleus in *Copidosoma* never seems to undergo vacuolization, nor does the similar body described in *Ageniaspis* by Martin ('14).

No body was found near the posterior end of the oocytes of the oak-knot gall-fly such as occur in those of *Copidosoma*, *Apanteles*, and the blackberry-knot gall-fly next to be described.

The maturation spindle and germ-line-determinants in the blackberry-knot gall-fly, Diastrophus nebulosus. The eggs of the blackberry-knot gall-fly (fig. 88) resemble in general shape and size those of the oak-knot gall-fly (fig. 83) and the nucleus is in a similar position. This nucleus forms a rather compact body, but not a homogeneous mass. The stage represented in figures 89, 90 and 91 may be one of a series ending in the production of such a mass, but no other stages were found. Figures 89 and 90 were drawn from longitudinal sections and show that the position of the oval nucleus may vary; figure 91 is from a transverse section.

At the posterior end of the egg (fig. 88) is a more or less spherical body to which we are justified, I believe, in applying the name, germ-line-determinant. This body stains black with hematoxylin and is filled with vacuoles (fig. 92). Weismann ('82) described a body near the posterior end of the eggs of *Rhodites rosae* which he called the 'Furchungskern,' but it is evident from his account and figures that this body is similar to the one I have just described and is not a cleavage nucleus. According to Weismann this body spreads out during cleavage and occupies

a large part of the posterior region; at this stage the term 'hinterer Polkern' is applied to it. Its later history was not followed.

It is worth mentioning that the follicle cells of the oocytes divide by mitosis (fig. 93) and not by amitosis as has been described in some insects.

Secondary nuclei in the oocytes of the mealy rose gall-fly Rhodites ignota. The eggs of this gall-fly (fig. 94) possess a very long anterior process, as in the two species already described, and the nucleus is similarly placed, but no body occurs at the posterior end. Of particular interest here is the presence of a large number of secondary nuclei at certain stages in the growth of the oocyte. These secondary nuclei were first observed near the periphery, as indicated in (fig. 95), which is part of a transverse section. They are very small and appear to consist of a single body that stains like chromatin, and are surrounded by a membrane. The occurrence of deeply staining granules without these membranes, and the various sizes of the secondary nuclei formed, lead to the conclusion that chromatin granules from the oocyte nucleus, from the nurse cells, or from the follicle cells, migrate into the cytoplasm and become the center of origin of the secondary nuclei. In later stages these nuclei are all larger and form a layer a slight distance from the periphery of the oocyte (fig. 96). They vary greatly in size as shown in figure 97, but exhibit all the characteristics of true nuclei. No secondary nuclei could be found in older oocytes, but what becomes of them was not determined.

*Summary of Part III.*⁴ *A. Copidosoma gelechiae.* 1. The chromatin in the oocyte nucleus forms chromosomes at an early stage in the growth period (fig. 51). These chromosomes unite near their ends in pairs (figs. 52 and 58) and then become arranged in a parallel series upon an asterless spindle (figs. 53, 59, 60). Condensation then occurs and an apparently homogeneous oval-shaped mass of chromatin is formed (figs. 54 and 62-65). The number of pairs of chromosomes is eleven (fig. 61) or twelve. The nuclear history is essentially as described in my preliminary

⁴ Summaries of Parts I and II will be found on pages 505 and 520.

report (Hegner '14 b) and similar to that described by Martin ('14) in *Agenaspis*.

2. The germ-line-determinant is not the chromatin from an oocyte nucleus, as stated in my preliminary paper, but it appears to be a differentiated part of the protoplasm which arises at an early stage (fig. 49) near the posterior end of the oocyte.

B. Apanteles. 1. The oocyte nucleus has a history similar to that described for *Copidosoma*. Chromosomes are formed at an early period, fuse in pairs, become arranged upon an asterless spindle (figs. 77-78), and undergo condensation (fig. 79). Whether or not they finally form a homogeneous mass could not be determined because of the lack of late stages.

2. Secondary nuclei make their appearance in the almost fully grown oocytes. They are distributed throughout the anterior half of the oocyte (figs. 75-76), but are entirely absent in later stages (fig. 77). Their origin and fate were not determined.

3. The deeply staining substance at the posterior end of the older oocytes is probably a germ-line-determinant. It first appears in a partially grown oocyte as a dark granular mass, which probably represents a differentiated part of the protoplasm (fig. 73). Later it passes through the stages described on pages 527-529 and illustrated in figures 74, 75 and 80 to 82.

C. Gall-flies. 1. The history of the oocyte nucleus of the oak-knot gall-fly resembles very closely that of *Copidosoma* and *Apanteles* (figs. 84-87).

2. The oocytes of the blackberry-knot gall-fly contain a chromatin body (figs. 88-91) which probably results from the condensation of chromosomes as in the other forms described above. A conspicuous germ-line-determinant is also present near the posterior end (figs. 88, 92); the follicle cells divide by mitosis (fig. 93).

3. The half-grown oocytes of the mealy rose gall-fly are provided with hundreds of secondary nuclei (fig. 97) which are situated in a single layer equidistant from the periphery at all points (fig. 96). In younger oocytes (fig. 95) these secondary

nuclei appear to arise near the periphery from granules which stain like chromatin. These granules may be extruded by the oocyte nucleus, the follicle cells or the nurse cells.

February 19, 1915

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PLATE 1

EXPLANATION OF FIGURES

Apis mellifica

- 1 Outline of an ovariole showing the terminal filament, *t*, a zone containing rosettes of cells, *r*, a zone of differentiation, *d*, a region containing a linear series of oocytes, *o*, and nurse cells, *n*, accompanying an oocyte. × 110.
- 2 Part of the rosette region of an ovariole; *r*, a rosette, the cells of which are held together by deeply staining strands; *e*, an epithelial cell nucleus. × 650.
- 3 A single rosette in longitudinal section. × 1900.
- 4 A single rosette in transverse section. × 1900.
- 5 Synchronous division of the cells in a rosette. × 1900.
- 6 Part of the zone of differentiation of an ovariole; *o*, oocyte; *e*, epithelial cell nuclei; *n*, nurse cell. × 430.
- 7 Two neighboring oocytes from the zone of differentiation showing the double rings connecting them with each other and with surrounding nurse cells. × 1900.

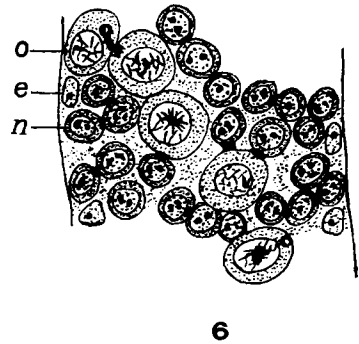
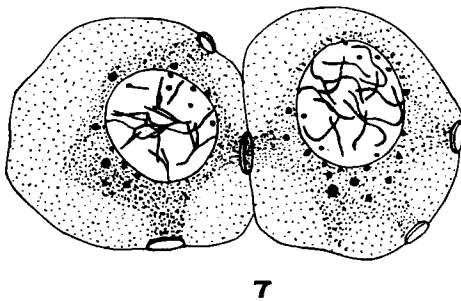
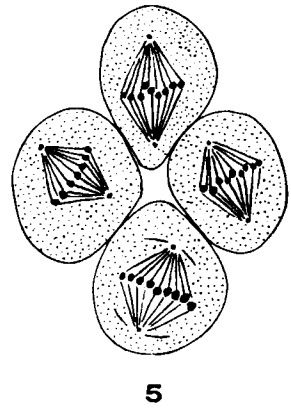
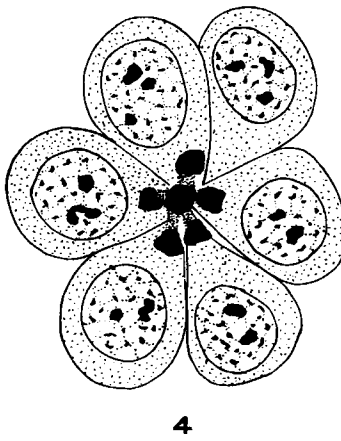
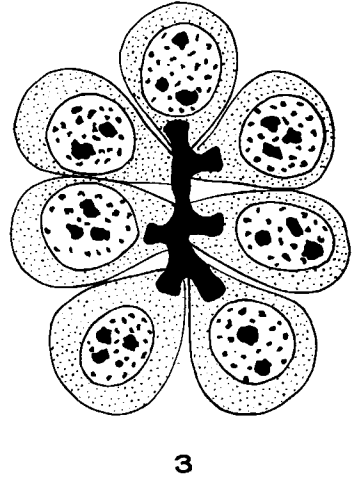
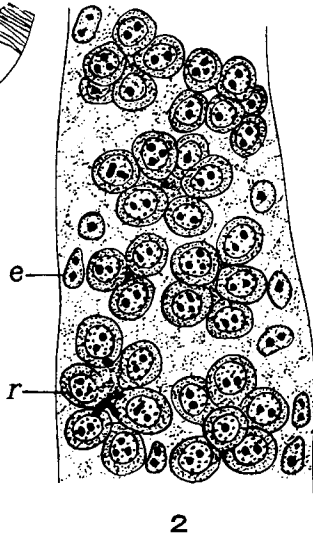
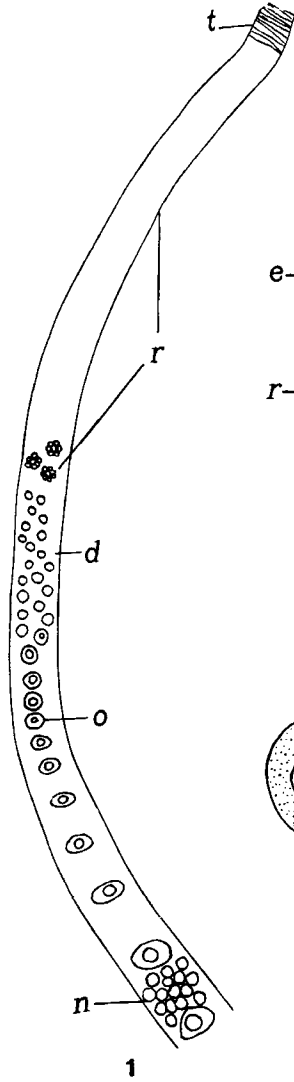
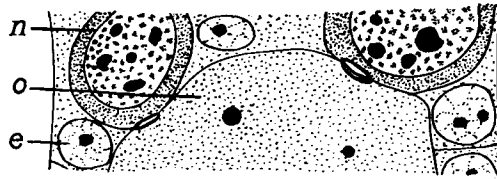
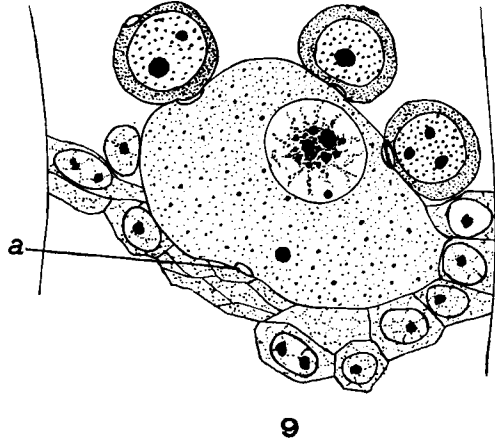
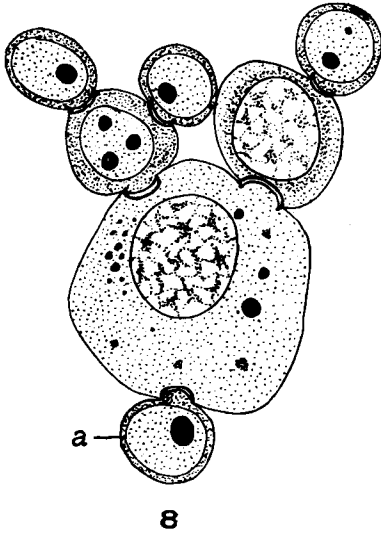


PLATE 2

EXPLANATION OF FIGURES

Apis mellifica

- 8 A group of nurse cells surrounding an oocyte. × 1900.
- 9 An older oocyte with nurse cells and epithelial cells. × 1250.
- 10 An outline showing the arrangement of an oocyte and its accompanying nurse cells. × 1250.
- 11 Part of a rather old oocyte, *o*, still connected with nurse cells, *n*, by means of rings, *e*, epithelial cell. × 1250.
- 12 An outline of an older oocyte showing the rings between the nurse cells and oocyte and between neighboring nurse cells. × 430.



11

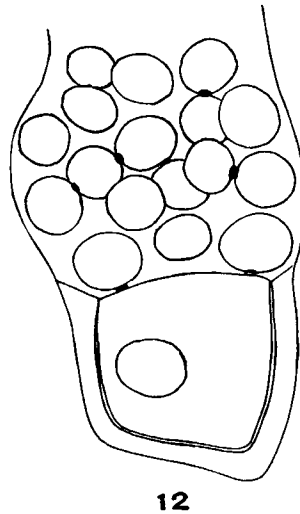
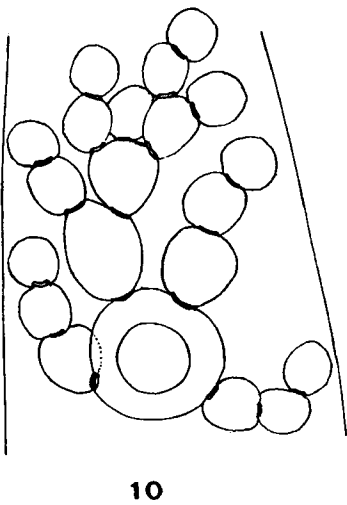


PLATE 3

EXPLANATION OF FIGURES

Camponotus herculeanus var. *pennsylvanica*

13 Outline of an ovariole showing the terminal filament, *t*, terminal chamber, *t.c.*, first zone of growth, *g*, and later growth zone containing oocytes, *o*, and nurse cells, *n* and *nc*. × 170.

14 Outlines of oocytes in Stages A to G. × 110.

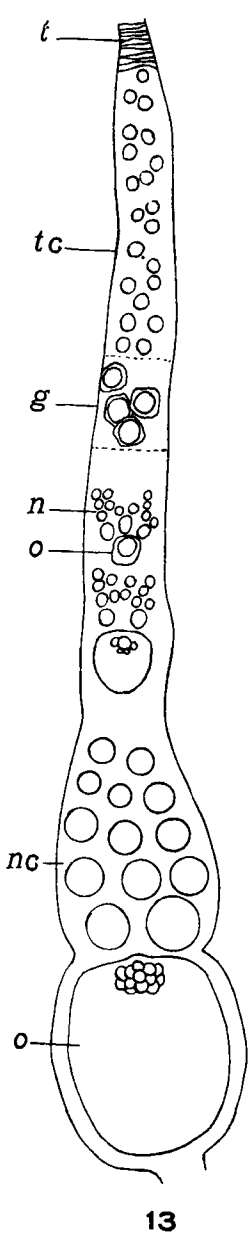
15 Outlines of oocytes in Stages H to L. × 110.

16 Outline of part of the terminal filament, *t*, and terminal chamber, *tc*. The numbers 17, 18 and 19 refer to cells shown enlarged in figures 17, 18 and 19. × 620.

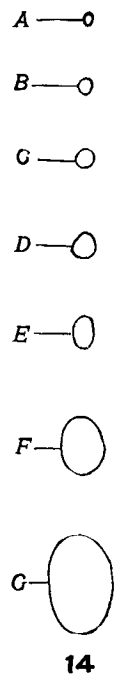
17 A single cell from the terminal filament. × 3300.

18 An oocyte from the terminal chamber. × 3300.

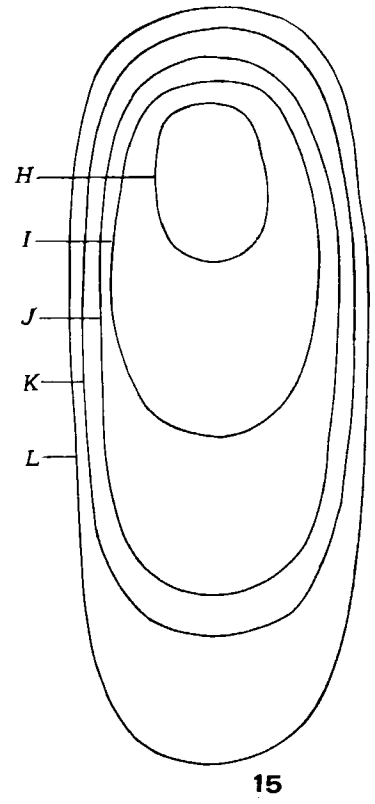
19 A nurse cell nucleus from the terminal chamber. × 3300.



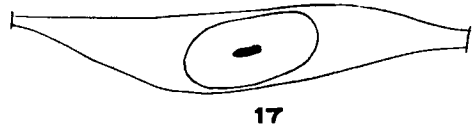
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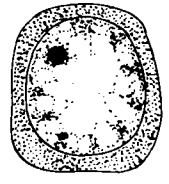
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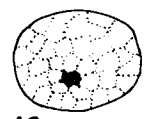
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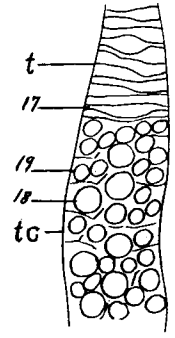
17



18



19



16

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PLATE 4

EXPLANATION OF FIGURES

Campanotus herculeanus var. *pennsylvanica*

20 Outline of the first zone of differentiation, showing the membrane, *m*, separating it from the terminal chamber, and the oocytes, *o*, nurse cells, *n*, and epithelial cells, *e*. The numbers 21, 22 and 23 refer to cells shown enlarged in figures 21, 22 and 23. × 620.

21 An oocyte from the first zone of growth. × 3300.

22 A nurse cell from the first zone of growth. × 3300.

23 An epithelial cell nucleus from the first zone of growth. × 3300.

24 The posterior portion of the first zone of growth and the anterior portion of the rest of the ovariole containing bacteria-like rods. *e*, epithelial cell nucleus; *n*, nurse cell; *o*, oocyte. × 1250.

25 Part of an ovariole showing two oocytes, C_1 and C_2 , in Stage C. *n*, nurse cell. × 1250.

26 An oocyte in Stage D. The bacteria-like rods have invaded the cytoplasm of the oocyte. × 1250.

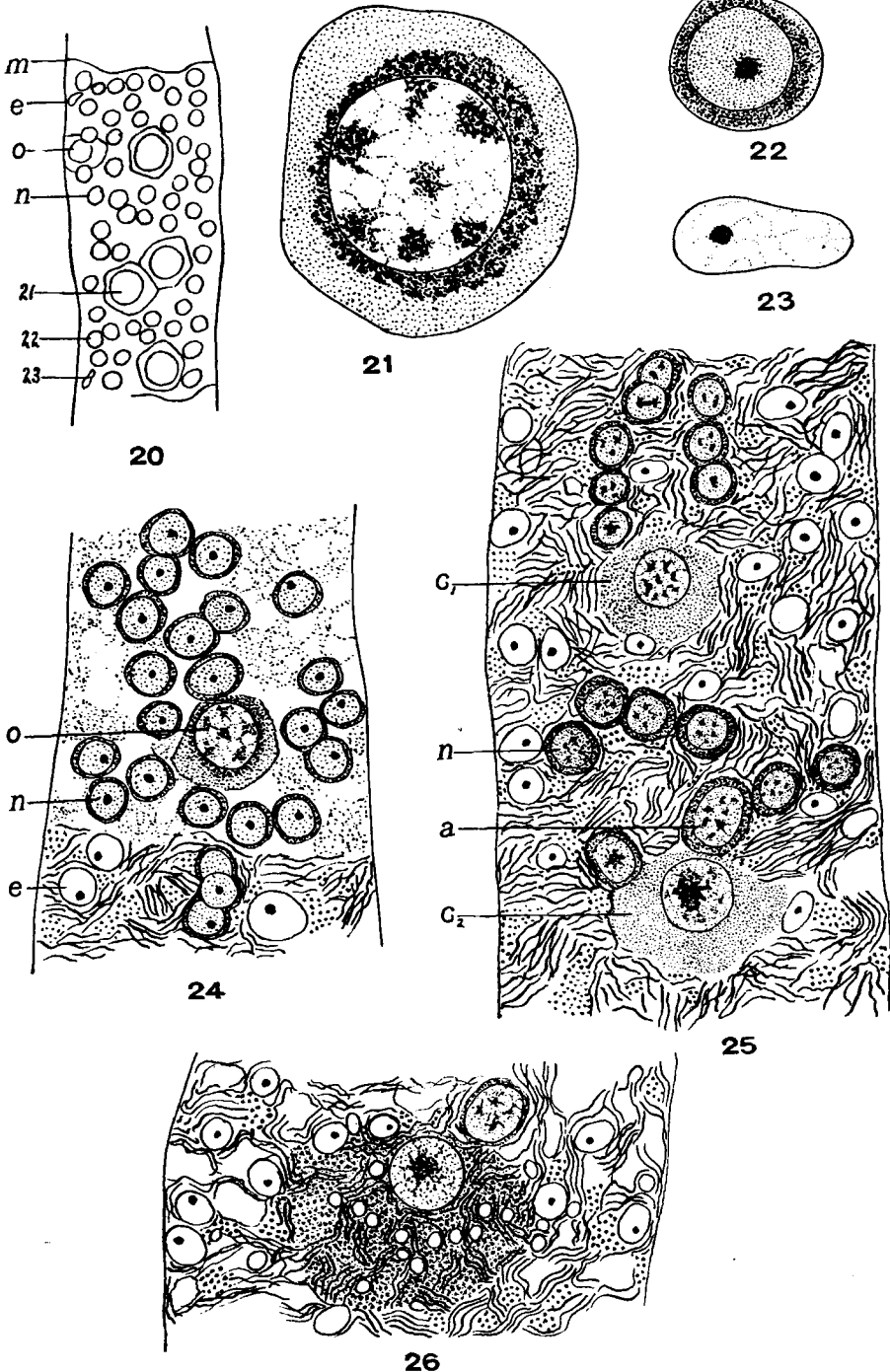


PLATE 5

EXPLANATION OF FIGURES

Camponotus herculeanus var. *pennsylvanica*

- 27 An oocyte in Stage E showing three secondary nuclei, *s*, near the oocyte nucleus, *o*. *e*, epithelial cell nucleus; *n*, nurse cell. × 1250.
- 28 An oocyte in Stage F. Lettering as in figure 27. × 620.
- 29 An oocyte in Stage G. Lettering as in figure 27. × 620.
- 30 Part of an oocyte and two nurse cells, *n*. Cytoplasm, *c*, elaborated by the nurse cells is present near the nurse chamber. *o*, oocyte nucleus; *s*, secondary nuclei. × 430.
- 31 Transverse section through the anterior end of an oocyte. × 620.
- 32 Part of the nucleus of a nurse cell showing vacuoles and deeply staining granules in the thick nuclear membrane. × 3300.

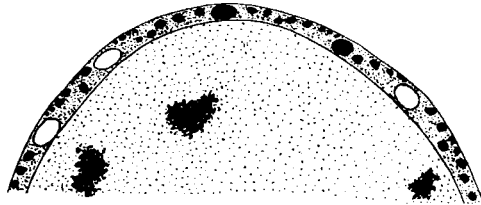
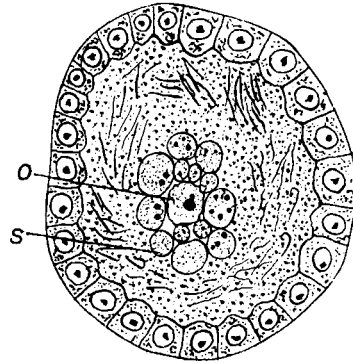
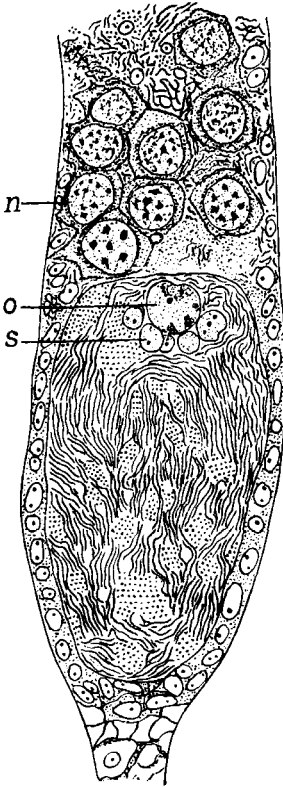
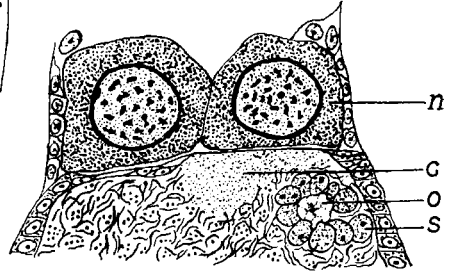
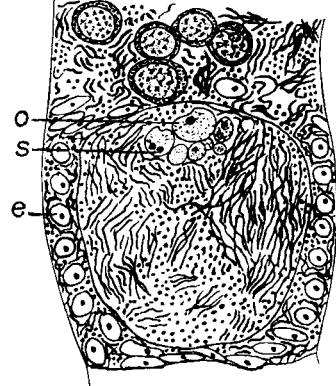
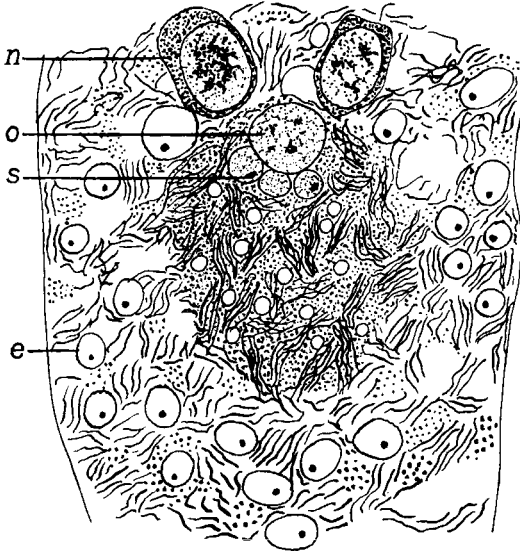


PLATE 6

EXPLANATION OF FIGURES

Camponotus herculeanus var. *pennsylvanica*

33 Part of an oocyte in Stage H showing its connection, *a*, with the nurse chamber; *o*, oocyte nucleus; *s*, secondary nuclei. × 430.

34 An oocyte nucleus surrounded by secondary nuclei from an oocyte in Stage H. × 1250.

35 Part of an oocyte in Stage I. *e*, follicular epithelium; *k*, 'Keimhautblastem'; *y*, yolk globules. × 430.

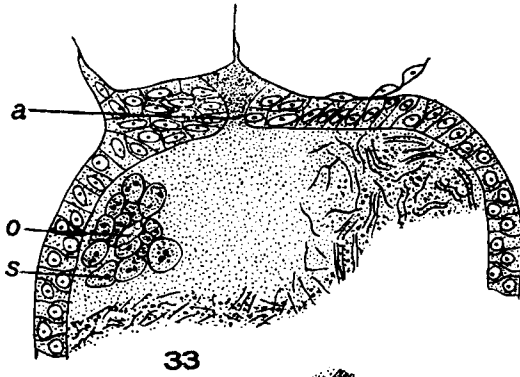
36 Part of an oocyte in Stage J. × 430.

37 The anterior part of an oocyte showing the breaking up of the group of secondary nuclei, *s*. *a*, connection with nurse chamber. *c*, cytoplasm; *e*, follicular epithelium; *y*, yolk globules. × 430.

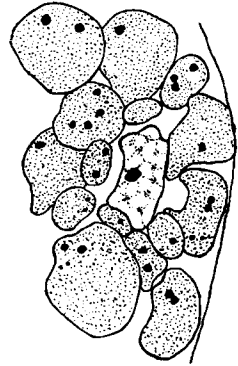
38 A single secondary nucleus and three yolk globules in Stage K. × 1900.

39 Part of the edge of an oocyte in Stage L showing the follicular epithelium and the distribution of secondary nuclei and yolk globules. × 430.

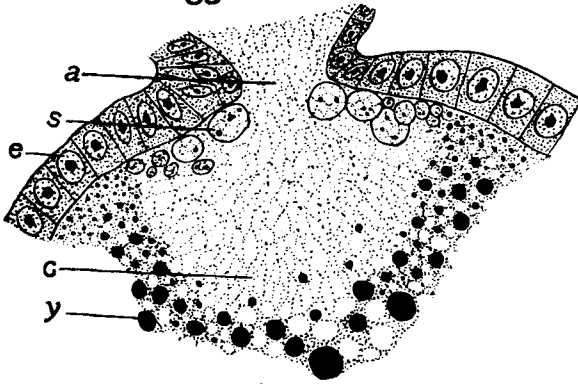
40 Two secondary nuclei and two yolk globules, enlarged, from an oocyte in Stage L. × 1900.



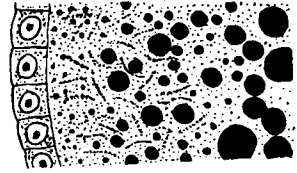
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34



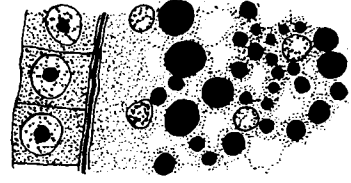
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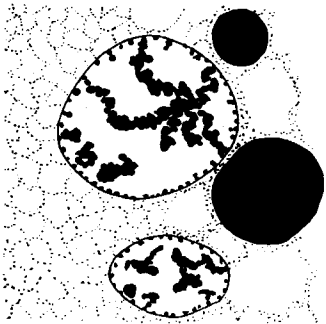
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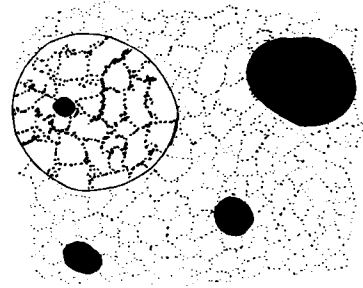
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39



40



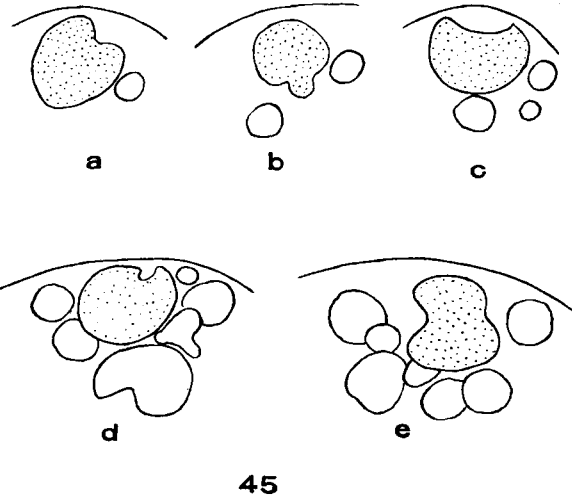
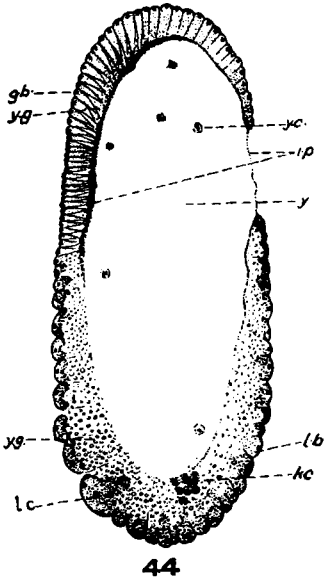
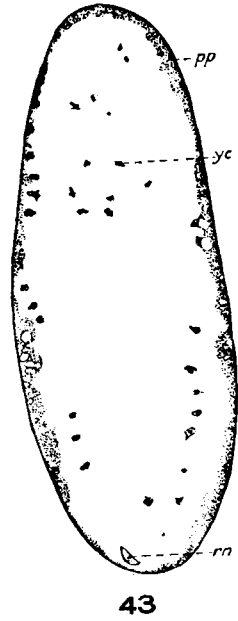
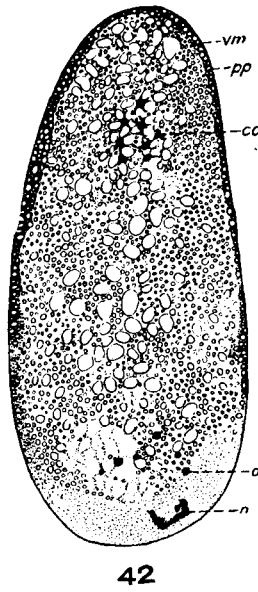
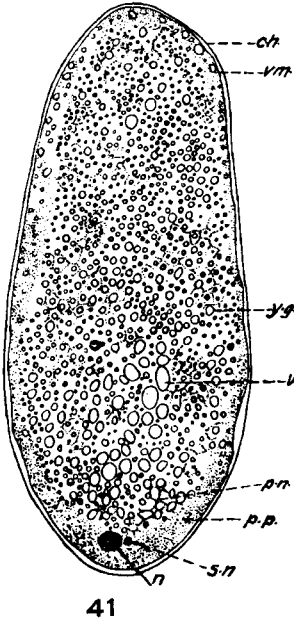
38

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PLATE 7

EXPLANATION OF FIGURES

- 41 Longitudinal section through an egg of *C. herculeanus* var. *ferrugineus* one hour old. $\times 52$ (from Tanquary).
- 42 Ditto, twenty hours old. $\times 52$ (from Tanquary).
- 43 Ditto, slightly older. $\times 52$ (from Tanquary).
- 44 Ditto, two days old. $\times 52$ (from Tanquary).
- 45 Outlines of oocyte nuclei (dotted in) with their accompanying secondary nuclei of *C. herculeanus* var. *pennsylvanica*. $\times 1250$.



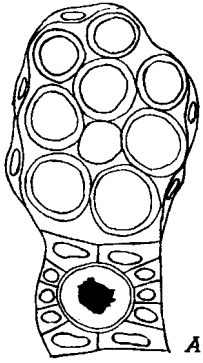
H. W. HEGNER, *del.*

PLATE 8

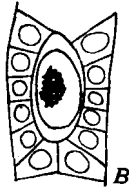
EXPLANATION OF FIGURES

Copidosoma gelechia

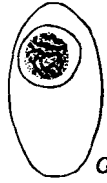
- 46 Outline of a young oocyte in Stage A surrounded by a follicular epithelium and accompanied by a group of nurse cells. $\times 1250$.
- 47 Outline of an oocyte in Stage B. The follicular epithelium is shown, but the nurse cells have been omitted. $\times 1250$.
- 48 Outline of an oocyte in Stage C. $\times 1250$.
- 49 Outline of an oocyte in Stage D. First appearance of the germ-line-determinant near the posterior end. $\times 1250$.
- 50 Outline of an oocyte in Stage E. $\times 1250$.
- 51 Outline of an oocyte in Stage F. Single chromosomes are present. $\times 1250$.
- 52 Outline of an oocyte in Stage G. The chromosomes have united near their ends to form pairs. $\times 1250$.
- 53 Outline of an oocyte in Stage H. The pairs of chromosomes are arranged in a parallel series. $\times 1250$.
- 54 Outline of an oocyte in Stage I. $\times 1250$.



46



47



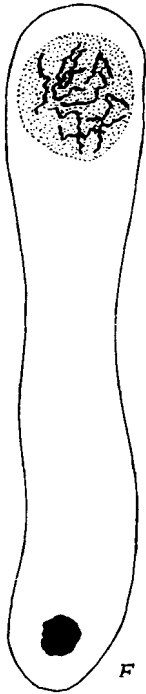
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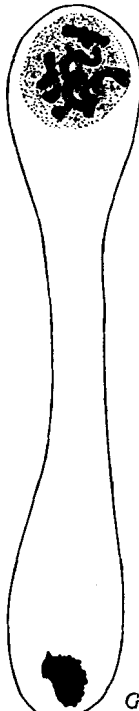
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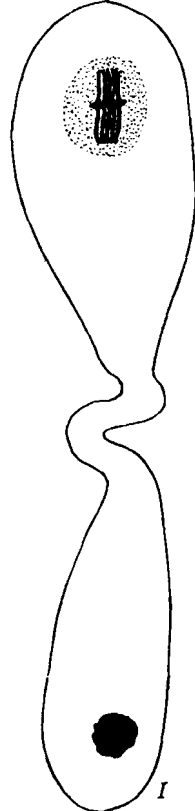
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52



53



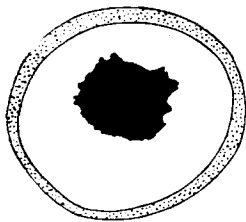
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PLATE 9

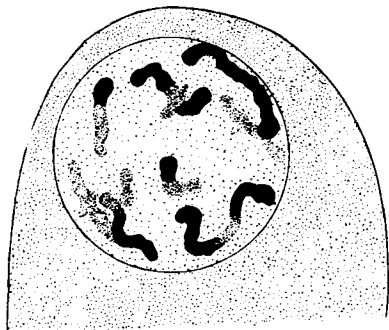
EXPLANATION OF FIGURES

Copidosoma gelechia

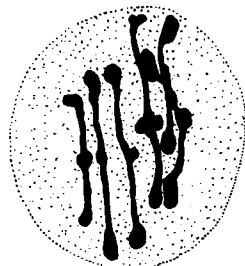
- 55 An oocyte in Stage A (see fig. 46). × 3300.
- 56 An oocyte in Stage B (see fig. 47). × 3300.
- 57 An oocyte in Stage C (see fig. 48). × 3300.
- 58 The anterior portion of an oocyte in Stage G (see fig. 52). × 3300.
- 59. The nucleus of an oocyte in Stage H (see fig. 53). × 3300.
- 60 The nucleus of a slightly older oocyte. × 3300.
- 61 A transverse section through a nucleus in a similar condition. × 3300.
- 62 to 65 Successive stages in the condensation of a spindle like that shown in figure 60. × 800.



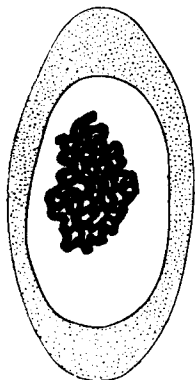
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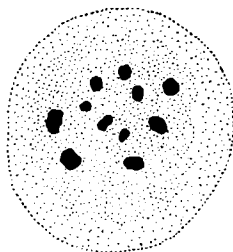
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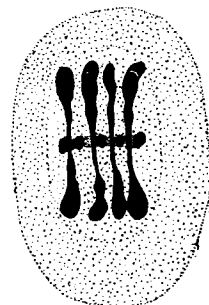
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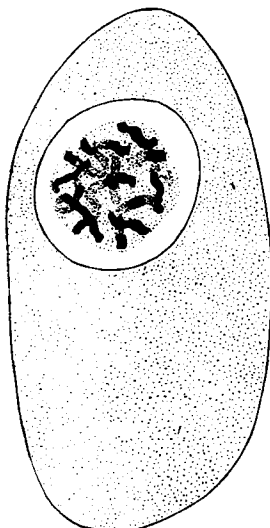
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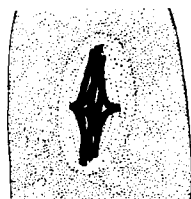
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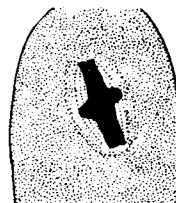
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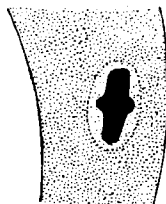
57



62



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64



65

PLATE 10

EXPLANATION OF FIGURES

66 *Ageniaspis*; the anterior portion of an oocyte showing the arrangement of the chromosomes on the spindle (after Martin).

67 *Ageniaspis*; a later stage showing the mass of chromatin resulting from the condensation of the chromosomes (after Martin).

68 *Ageniaspis*; the first maturation division of the egg (after Martin).

69 *Copidosoma*; a young oocyte showing a group of granules near the posterior end of the nucleus (after Silvestri).

70 *Ageniaspis*; a young oocyte containing a cloud of granules in the posterior portion and a larger body, the 'nucleolus' (after Martin).

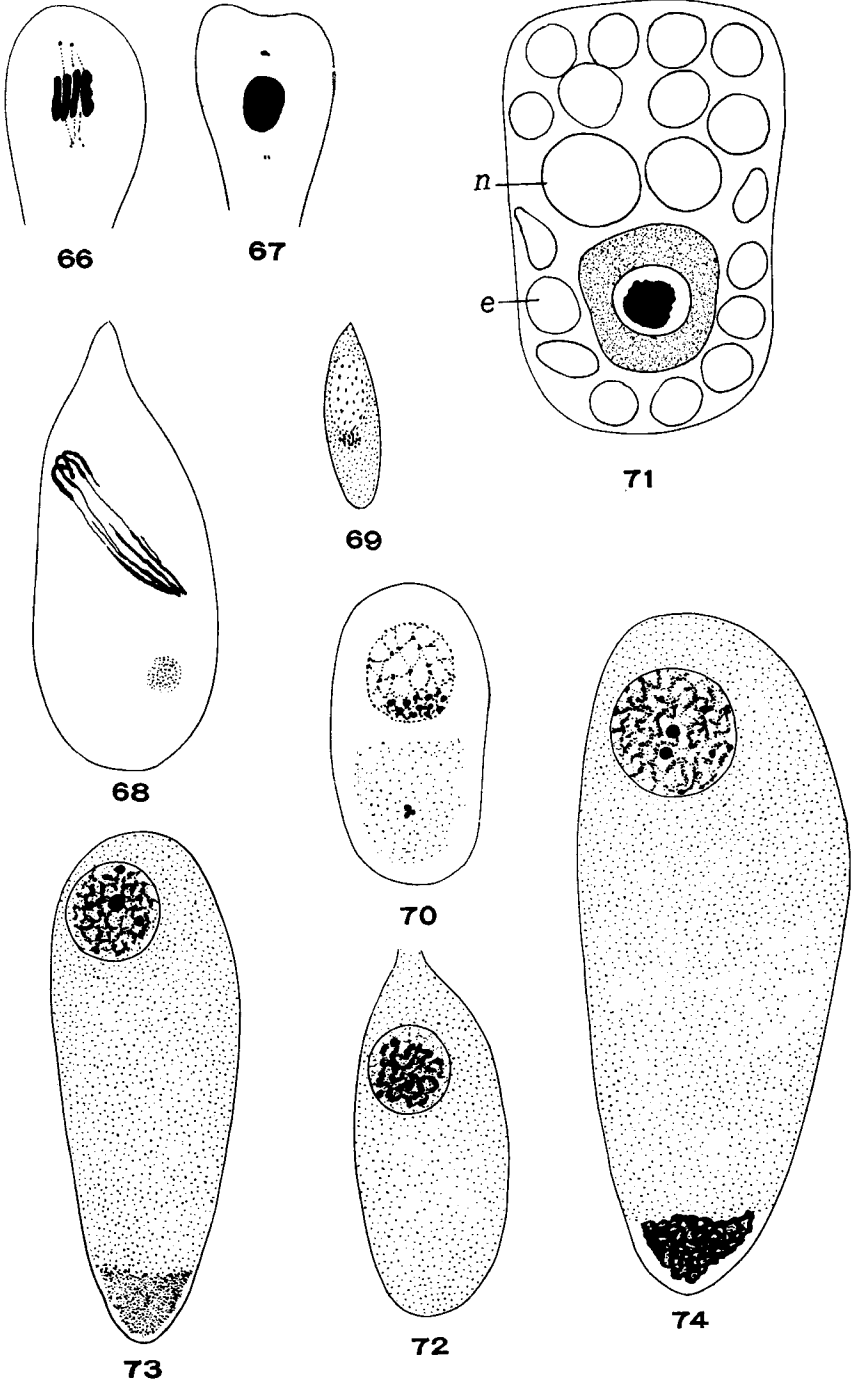
71-74 *Apanteles*.

71 A young oocyte surrounded by epithelial cells, *e*, and accompanied by nurse cells, *n*. $\times 1900$.

72 An older oocyte. $\times 1900$.

73 An older oocyte showing the first appearance of the germ-line-determinant. $\times 1900$.

74 A still older oocyte. $\times 1900$.



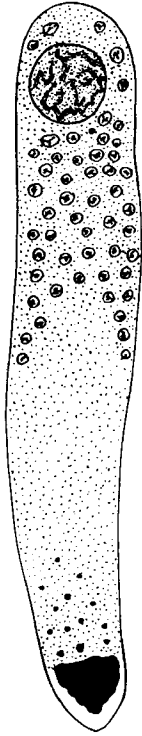
R. W. HEGNER, *del.*

PLATE 11

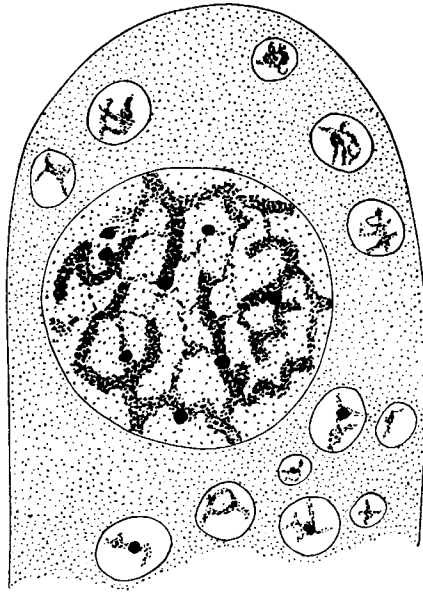
EXPLANATION OF FIGURES

Apanteles

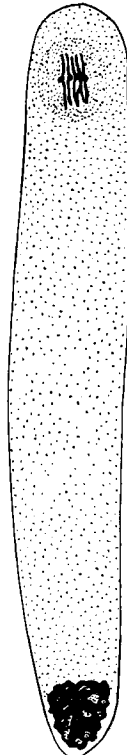
- 75 An oocyte containing many secondary nuclei. $\times 850$.
76 Part of the oocyte shown in figure 75 $\times 3300$.
77 An older oocyte showing the parallel arrangement of chromosomes. $\times 850$.
78 Nucleus enlarged from figure 77. $\times 3300$.
79 A later stage in the history of the nucleus. $\times 3300$.
80-82 Successive stages in the history of the germ-line-determinant. $\times 1900$.



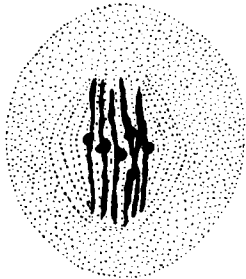
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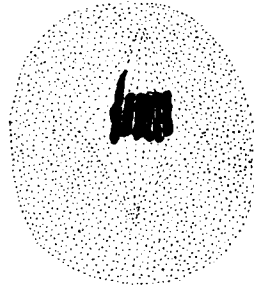
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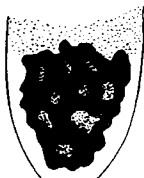
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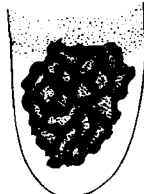
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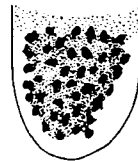
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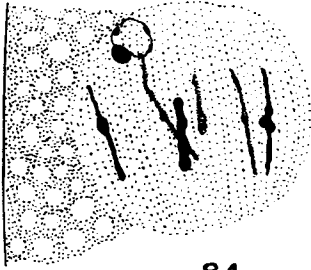
82

PLATE 12

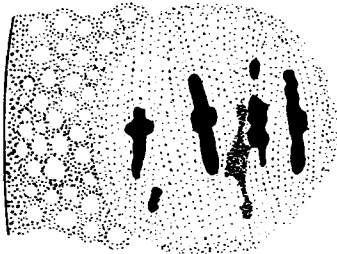
EXPLANATION OF FIGURES

Andricus punctatus

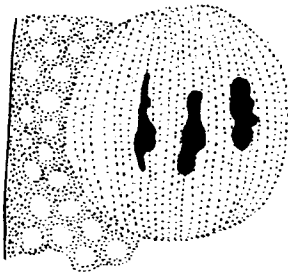
- 83 An egg ready to be laid. \times 430.
84-86 Nuclei showing stages in the condensation of the chromosomes.
 \times 1900.
87 The chromatin mass resulting from the condensation of the chromosomes.
 \times 1900.



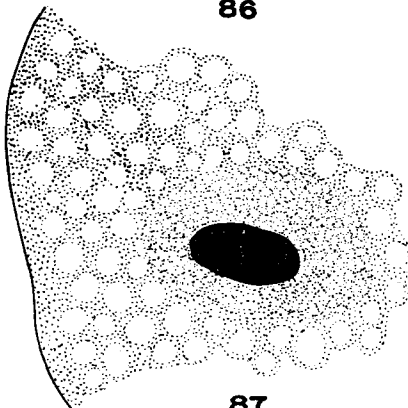
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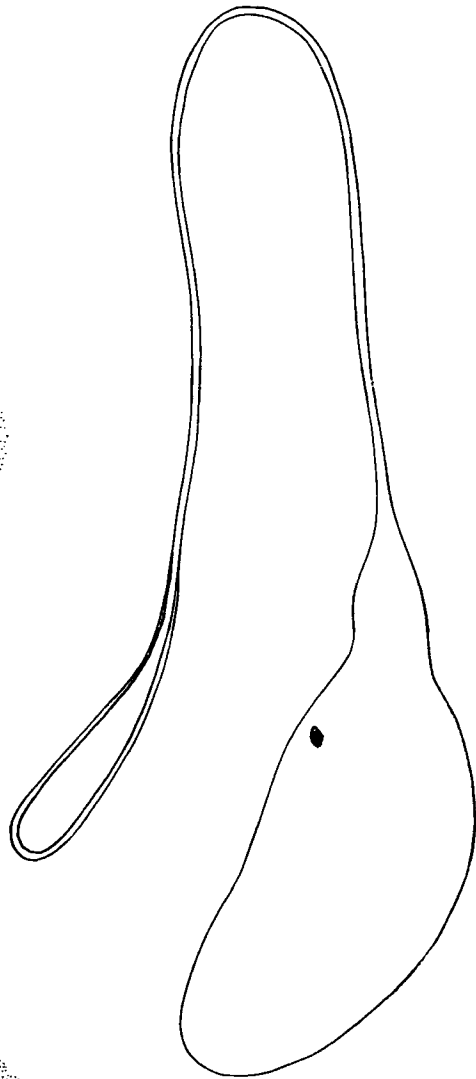
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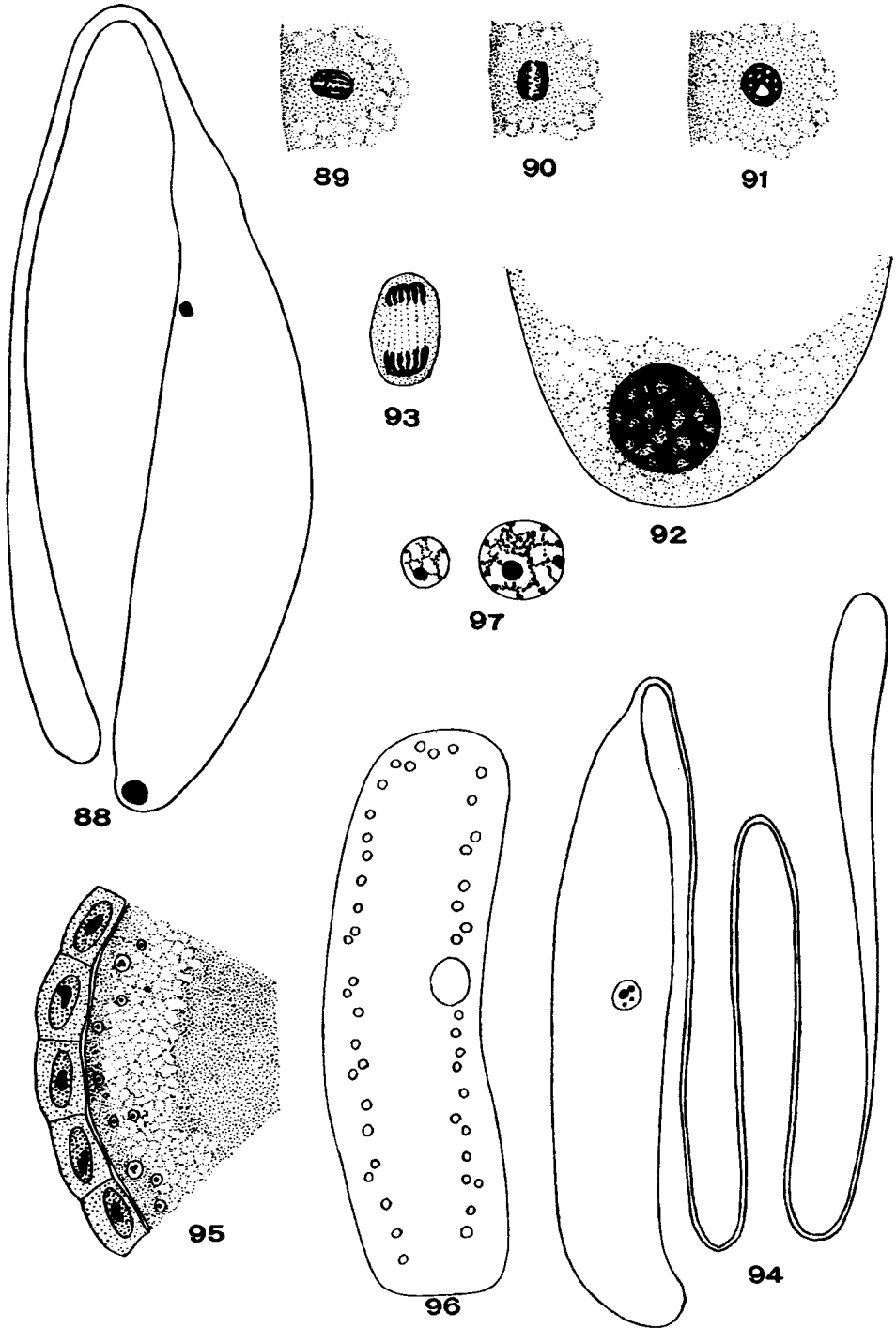


83

PLATE 13

EXPLANATION OF FIGURES

- 88-93 *Diastrophus nebulosus*.
88 An egg ready to be laid. $\times 430$.
89-90 Longitudinal sections through the nucleus of such an egg. $\times 1900$.
91 Transverse section through a nucleus in the same stage. $\times 1900$.
92 The germ-line-determinant near the posterior end. $\times 1900$.
93 The mitotic division of a follicular epithelial cell. $\times 1900$.
94-97 *Rhodites ignota*.
94 An egg ready to be laid. $\times 430$.
95 Part of an oocyte showing stages in the formation of secondary nuclei.
 $\times 1900$.
96 An older oocyte showing the arrangement of secondary nuclei. $\times 620$.
97 Two secondary nuclei much enlarged. $\times 2500$.



R. W. HEGNER *del.*