

# Contributions towards the Embryology and Anatomy of *Polistes pallipes*.

## II. The Early History of the Cellular Elements of the Ovary.

By

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With Plate XII—XIV.

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The material for the following paper was obtained in the vicinity of Madison, Wisconsin, U. S. A. The work was done in the Zoological Laboratory of the University of Berlin whose director, Professor F. E. SCHULZE, I desire to thank for extending to me the courtesies of the laboratory.

In gathering the material, the embryos, larvae and pupae were removed from the cells of the nest after returning to the laboratory; but the mature wasps were caught in its vicinity after it was first disturbed. The ovaries of all but the embryos and youngest larvae were generally dissected out in the preserving fluid which was to be used. A larva, for instance, would be pinned down in a small dish containing weak FLEMMING's Solution; it was then cut open and the ovaries, being cut out, would be at once placed in the solution in the dish. From this they were quickly removed to a dish of FLEMMING of the strength used in preserving them. The same was done with most of the other solutions. With GILSON's or TOWER's it was found much easier to see and remove the ovaries of larvae which were first injected, (by means of a small hypodermic syringe), with the solution used. They were then thrown into a dissecting-dish filled with the same fluid, allowed to remain here five or ten minutes, and then opened; the ovaries were removed and placed in another dish of fresh preserving fluid.

The different preserving fluids used were FLEMMING's, weak and

strong, HERMANN'S, TOWER'S, GILSON'S and hot water followed by corrosive sublimate. The stains I made use of were FLEMMING'S triple stain, EHRLICH'S, HEIDENHAIN'S iron-haematoxylin, MAYER'S haemalum, borax-carmine, alum-carmine, acid-fuchsin, and safranin alone. Of these, the two found most satisfactory were the iron-haematoxylin and the safranin. In the very youngest stages I found MAYERS haemalum and haematoxylin to give the best results.

### Historical.

The origin of the three kinds of cells within the insect ovary has already been worked out for the Hymenoptera and my work does not in this respect differ from what was already known. What KORSCHOLT (18) found for *Bombus* differs but slightly from what is here given for *Polistes*. He says, »Die gleichartigen Elemente des Endfadens gehen direkt in die der Endkammer über. In deren oberen Abschnitt differenzieren sich aus ihnen die Keimzellen, die am Grunde der Endkammer in die Eizellen übergehen und nur allein diese liefern. Die Kerne der Nährzellen entstehen an der Basis der Endkammer aus den kleinen Kernen, welche weitaus die größte Masse von deren Inhalt bilden. Die in großer Anzahl übrig bleibenden kleinen Kerne werden zu den Kernen des Follikelepithels. Alle drei Zellenarten entstammen demnach den gleichartigen Elementen, wie sie sich noch jetzt im obersten Abschnitt der Endkammer finden.«

PAULCKE'S (25) results are also the same as mine; both he and KORSCHOLT (18) failed to study the young stages and he was unable to ascertain that the two kinds of cells he starts with had come from similar cells. He says: »Zu Beginn der formalen Differenzierung haben wir also zweierlei Elemente; erstens undifferenziert bleibende Kerne, welche später dem Follikelepithel den Ursprung geben, und zweitens als Keimkerne oder Ureikerne zu bezeichnende Elemente, welche sich weiterhin, wie wir sehen werden, nach Bildung eines Zelleibes, zu Eizellen und Nährzellen differenzieren.«

In many of the papers on the anatomy of insect ovaries there is given an historical account of the subject; in most of these are found the different views which have arisen in regard to the origin of the three cellular elements; oöcyte, nurse-and epithelial cell, of the ovarian tubule. To give here such an historical account and trace, step by step, what has been done, would be only to repeat what can all or in part be found in a number of papers, LUDWIG (23), BRANDT (5), KORSCHOLT (18), GROSS (10), etc., or in HENNEGUY'S (14)

text-book. In most of the papers dealing with this subject one finds that several insects have been the subject of the work and it is only in recent papers PAULCKE (25), GIARDINA (8), that one finds the work restricted to a single species. As the present paper treats only of a single insect, it is thought best to omit a more general historical account and review the principal papers dealing, especially histologically, with the ovaries of Hymenoptera. Lately more attention has been given to cytological detail and we have the papers of GRÜNBERG (12), WIELOWIEJSKI (32) and GROSS (11) but especially those by GIARDINA (8 and 9).

Several of the older papers treat of the ovary of the Hymenoptera, but in them there is very little or nothing bearing on the present work. LEYDIG (20), working with *Osmia bicornis*, mentions that the terminal filament is filled with clear cells which have large round nuclei. In the following part of the tubule, he found cells whose nuclei contained nucleoli, distinguishing between these, and those with a single nucleolus; these latter developed into the egg. LUDWIG (23), observed that in *Megachile fusca* the egg sent a process up between the nurse cells; also that the epithelial cells did not entirely divide the egg and nurse chambers into two separate parts but that an opening was left between them.

In 1866 two important papers appeared; one by BLOCHMANN (3) and the other by STUHLMANN (27). BLOCHMANN (3) worked with three Hymenoptera; *Camponotus ligniperda*, *Formica fusca* and *Vespa vulgaris*. He found that the three showed but slight variations and his account is almost entirely restricted to the first species. His account begins after the oöcytes are arranged, one behind the other, in the middle of the tubule and were easily recognized by the greater size of their nucleus and their darker staining cytoplasm. He found the oöcyte nucleus large, without a nucleolus and with but little chromatin; it contained a central mass from which strands passed to the nuclear membrane. Oöcytes, when a little older, begin to bud and this process results in the formation of many small nuclei »Nebenkerne«; these first appear as small vacuoles lying near the nucleus. A little later small staining granules appear in them, they grow and acquire a distinct membrane. The contained matter increases and finally forms small nucleoli and fine threads. The »Nebenkerne« increase in number and proportionately the regular nucleus decreases. In the regular nucleus almost the entire contents stain; but in the »Nebenkerne«, only the nucleolus and the strands. Some of these



nucleoli he saw connected by a thin thread as if they were dividing. The nurse-cell nuclei he figures with a very thick membrane; each contains many nucleoli and a thick network. Between the nurse-cells and at the margin of their chamber he noticed a number of small cells which he concluded corresponded to the follicle cells around the oöcyte. The process the oöcyte sends up between the nurse cells was seen. He describes the nucleus of the oöcyte as wandering to its distal margin and with this, begins the formation of the deutoplasm. Division was observed in the follicle cells during the growth of the oöcyte.

No one has worked with so many different Hymenoptera as STUHLMANN (27); in his paper he describes, often very briefly, *Vespa germanica* and *Vespa media*, *Bombus terrestris*, *Troglus lutorius*, *Branchus fulvipes*, *Pimpla* sp. (?), *Anomalon circumflexum*, *Lampyris* sp. (?), *Ophion ventricosum* and *Ophion luteum*, *Ephialtes lituratus* and *Ephialtes* sp. (?). Of none of these does he give a very full account and there is a great deal of similarity in what he says of the different ones. He describes, for all of them in the early stages of the oöcyte, the formation of the small nuclear-like bodies. In all, the nucleus of the oöcyte goes to the periphery at the anterior end; there around it the small bodies are formed, but not for all at the same age. He says: »Bei den untersuchten Hymenopteren kann also der Austritt der „Ballen“ zu sehr verschiedenen Zeiten stattfinden, entweder bei ganz jungen Eiern oder bei ziemlich viel älteren.« These »Ballen« may fuse to form a large »Dotterkern« which lies at the lower pole of the egg; or, as »diffuse Dotterkerne« they may remain separate and generally later become lost or dissolved in the egg.

What he found, for the first two species of *Vespa*, was so similar that he gives a single description for both. In the youngest oöcyte he studied, the nearly central nucleus had a nucleolus; the small bodies near it contained something, but he was doubtful whether or not it was chromatin. He opposes BLOCHMANN's view as to these being »Nebenkerne« saying: »Ich wiederhole noch einmal, daß ich diese Kerne nur für „Dotterconcretionen“ halte.« He agrees with BLOCHMANN (3) that deutoplasm is first formed at the periphery and the lower pole of the oöcyte, »am oberen Pol noch eine Plasmamasse bleibt«. At later stages the oöcyte nucleus stains darker than at earlier ones; it remains at the upper pole until its function here, »die Ausstoßung der Ballen« is ended and then moves towards

the center. In the older oöcytes he could not find any trace of the nucleus.

In *Bombus*, STUHLMANN found that some of the »Dotterkerne« appeared very similar to the regular nucleus; others do not. The opening between egg- and nurse chamber was observed and once he saw three of the »Ballen« in this passageway »welche einen Übergang zwischen den Kernen des Follikelepithels zu den Dotterkernen zu bilden scheinen«. He suggested the possibility of these being cells which had passed from the outside into the egg but did not think it at all probable. The »Ballen« at the upper pole of the oöcyte remain but those at the lower pole are dissolved, the latter being the older, disappear first.

KORSCHOLT (18) found nuclei with nucleoli in the terminal filament but was unable to distinguish cell boundaries. Proximal to this the nuclei became larger and in the terminal chamber he found that some grew more rapidly than others; in this region he found cell boundaries. At this place »zwischen den Kernen der Keimzellen und den der jüngsten Eianlagen ist kein Unterschied zu bemerken«. Later he could distinguish the oöcytes by their lighter appearance and larger size. In the oöcyte nucleus the chromatin forms in large masses. Distally in the end chamber the nuclei have the same structure as those in the terminal filament but they were larger. The large nuclei become those of the oöcyte, the smaller ones surrounding these form the nurse-cells. By *Dytiscus* and *Musca* he found these »Keimzellen«, differentiating into oöcytes and nurse-cells but in *Bombus* to oöcytes only. The nurse-cells group themselves first at the side and then distal to the oöcyte; they increase in number and their nuclei are larger than those of the oöcytes.

PAULCKE (25) found in the bee no external or internal boundary between the terminal filament and the end chamber. In the upper part of the former are many elongated nuclei lying across the tubule (LEYDIG [20]), which are embedded in a mass of protoplasm. At the place where these small nuclei become larger there is a marked transverse striation in the tubule; in this region cell boundaries appear and nucleoli in the nuclei. These are now either oogonia or they remain unchanged and become the epithelial cells. In the former the chromatin in the nucleus becomes thicker, at first it forms a mass with two nucleoli which later disappear. He describes both a synapsis zone and a zone of differentiation. Proximal to the former zone small cells (nurse-cells) very much like the undifferentiated

nuclei appear and little further down larger cells (oöcytes) in whose nuclei the chromatin is massed in an excentric ball. He believes that synapsis has something to do with division but found no dividing cells. Other cells at this region have the chromatin separated into two parts. The oöcytes grow; at first lying two or three together, they later, separate. They now lie in the median part of the tubule with their long axis across it. Cell boundaries which have disappeared now appear again. The nucleus of the nurse-cells becomes larger and oval in form, the chromatin finely divided and one or two nucleoli are present. The oöcytes move further apart and the nurse-cells which were arranged in rows, get in between them and begin the chamber formation. The oöcytes may either appear along the margin or in the central part of the tubule. After the synapsis zone the epithelial nuclei appear, at first pointed, but later becoming rounder. In these nuclei one or two nucleoli are often present, and, at the beginning of the chamber formation cell boundaries are formed. He distinguishes a zone of chamber formation. He found the opening between nurse and egg chamber and the process the oöcyte sends up between the nurse-cells. At the beginning of chamber formation the nurse-cell nucleus has a nucleolus and a finely granular mass which later becomes thicker. Nuclei become irregular and secretory activity begins. At this same time the oöcyte nucleus loses the characteristic thread structure it earlier had and the chromatin forms in masses in its central part. It now has a number of the small nuclear-like bodies near it. It loses its regular form. The nurse cells are finally emptied into the egg chamber.

He holds with KORSCHULT (18) that in the Hymenoptera all three kinds of cells come from similar elements of the terminal chamber. In and near the synapsis zone a multiplication of cells begins, they become differentiated and the oöcytes are first recognized as such. He did not see mitosis in the synapsis zone, nor could he find a nucleolus present. In this zone he believes but few mitotic divisions are present and these only for the origin of the oöcyte, the nurse-cells originating amitotically. We will not go further into his theoretical part except to give his origin of the different cells in which he agrees with KORSCHULT. The first differentiation comes in the terminal filament and separates the vegetative (epithelial) from the generative cells. A second differentiation later takes place in which the »Keimzelle« become separated into oöcytes and nurse cells.



GROSS (10) working with *Bombus terrestris* found in the distal part of the end chamber nuclei of the epithelial cells with very indistinct boundaries; these nuclei were oval in shape and contained a few chromatin granules. Further down were large, round cells, with a distinct nucleolus; these were the nurse-cells and between them lay the oöcytes with larger and lighter nuclei. Near the wall were many epithelial cells and he holds that these remain unchanged in structure, and that they divide mitotically at nearly any place. Synapsis, if present, he claimed was very short. In that part of the tubule where the oöcytes have a linear arrangement in its center, they are separated by epithelial cells. GROSS holds, against PAULCKE (25) that, in this region, the nurse cells do not divide. The egg and nurse chamber have an opening between them. In the nurse chambers are a number of epithelial nuclei; these in the older chambers collect in the central part and pass into the oöcyte: they have something to do with the formation of the deutoplasm. In *Vespa* he holds that the nurse-cell nuclei may divide amitotically.

### Observations.

The following paper has nothing to do with the development of the female reproductive organs as such, but only with the cells, oöcyte, nurse, and epithelial, which develop within the ovary. The oöcytes we have traced from their origin, through the formation of the nuclear-like bodies, until they have nearly reached their full size. Of the nurse-cells the entire history is given; starting from their first differentiation and following them until they become lost, as food for the developing oöcytes. The epithelial cells we have traced from their origin until a part of them, those within the nurse chamber, have no further history, either going as food to the oöcyte or remaining within the empty chamber. Of the other part, those forming the follicle of the oöcyte, we give the history until the beginning of the formation of the chorion.

It has been thought advisable to draw in outline an ovarian tubule of some of the stages described, not to trace its development, but only that the position of the cells about which we speak, may be more clearly indicated. We begin our account with the gonads of the embryo, finding at this stage, that each reproductive organ is a small mass of protoplasm, surrounded by one or more layers of cells, and containing a number of nuclei similar in structure. In this earliest stage is not only a differentiation into terminal filament,

ovary proper and oviduct not present, but the gonads do not show any trace of the tubules which later appear in the young larva. After the appearance of cells in the gonads of the young larvae, some of them begin to increase considerably in size; the presence of these large cells distinguishes the ovaries from the testes. At first each of the three ovarian tubules, connected at their base, is similar throughout its entire length, and, it is only in the older larvae, that we can distinguish the different parts.

When either embryos or larvae were of nearly the same size it was very difficult to determine their relative ages. In many cases we have used the length of embryo or larva, but cannot say that this means very much. The eggs of *Polistes* show a difference in size, and the length of embryos of the longer eggs would, at an equal age, be greater than there of the shorter ones. Amount of contraction by different preserving fluids might also make some difference. In many pupa, the ends of the tubules were twisted and bent, so that it often became difficult to give their exact length. The length of the tubule cannot always be used as a standard of relative age, some of equal lengths showing a difference in the development of the enclosed cells.

**Embryo.** The youngest embryo studied, the one with which we begin our account, was 1.35 mm in length; its gonads were each 0.04 mm wide and a little over twice as long. Each gonad is filled with protoplasm which shows a great similarity at all points, in some there are a few small vacuoles present, but the majority, did not contain them. Nuclei of the same structure are found throughout the entire gonad; in it they have no definite position and fail to show a regular epithelial arrangement; they do not lie at any regular distance from each other, some nearly touch adjacent nuclei, and others are a little distance apart. In *Chalicodoma* CARRIÈRE and BURGER (6) found that the young gonads contained a number of similar cells. A comparison of this embryo, with others which were a little longer, shows no difference in the size or arrangement of the contained nuclei, which have either a round or an oval form, and vary but little in size (Fig. 1). A few of the nuclei are irregular in shape, but an examination of all the sections shows that the round form predominates. We find here in *Polistes*, as has been described for a number of animals, that the reproductive organ in the youngest stage or stages, is a syncytium; in the different embryos which we



studied we were unable to find cell boundaries, or any indication of cell formation, present within the gonads.

The nuclei within this youngest gonad would average about 0.0045 mm in diameter; each contains one or two nucleoli, seldom a greater number, which are irregular in shape, but generally somewhat elongated; they are seldom angular, their surface as a rule being rounded (Fig. 2). When but a single nucleolus is present it is apt to be elongated, when two or more are present, they are generally more rounded. Each nucleus also contains a number of chromatin granules; these are much smaller than the nucleoli and they are mostly peripheral in position, a few only, lying at any distance from the nuclear membrane. The achromatin does not form a definite network, but appears as strands which connect the chromatin granules with each other, and with the nucleolei. Neither in this, nor in any other embryo studied, were dividing nuclei seen.

The nuclei, found in the embryonal gonads of *Polistes*, are very similar to those described and figured by GRÜNBERG (12) for *Bombyx mori*, and we judge that both were of about the same age. As just mentioned, we failed to find any cell boundaries at this stage.

It was very difficult to stain the nuclei of the gonads in these early stages and we obtained better results by using the commonest stain, such as MAYER's haemalum, than with the more complicated ones used for nuclear structures. With this stain just mentioned, the bodies we have called nucleoli, are not so darkly colored as are the chromatin granules; the same result was obtained by using HEIDENHAIN's iron-haematoxylin. With FLEMMING's triple stain no differentiation could be obtained; with safranin alone both nucleoli and chromatin granules stained equally, and the same result was obtained with acid-fuchsin.

Older embryos were very similar to the young ones; the reproductive organs were somewhat changed, but not the contained nuclei. With the growth of the embryo the nuclei increase somewhat in size, and it is noticeable, that within the gonad, they show a variation in this respect, some being larger than others. BESSELS (1) long ago observed that, in the ovary of Lepidoptera, the cells were at first all the same size but later, grew and differentiated. These larger nuclei have no definite position within the gonad, most of them were centrally situated but some were seen lying close to, or against, the wall. In an embryo 1,5 mm in length, one of these large nuclei measured 0.0075 mm in diameter; the average diameter

of all the nuclei would be about 0,0063 mm, not much larger than those of the youngest embryo.

**Larva.** In the youngest larvae the gonads are similar to what we found in the oldest embryo, the structure of the nuclei is the same, and no cell boundaries have as yet appeared. In a larva 2,1 mm long, we find that the three ovarian tubules have been partially developed; each tubule has a width of 0.035 mm, making the reproductive organ larger than in the embryo, where we found the entire gonad, but slightly larger than one of the tubules in this larva. A view of an ovarian tubule from an larva 2,6 mm long (Fig. 3), shows that the greatest change noticeable, is the appearance of the cells; a small amount of cytoplasm around many of the nuclei being marked off by a cell boundary, and this cytoplasm is darker than that which is not so enclosed. Whether or not all the nuclei lie in cells, is difficult to determine, but this is the condition by slightly older ones. The nuclei, 0,005 to 0,006 mm in diameter, found in the gonads of this larva, are still similar in structure (Fig. 4) to those of the embryo. In larvae of this age a very few mitotic figures were seen, but these were not abundant. In looking over sections of a few larvae, of this and of a slightly greater length, we would find but two or three dividing cells in an entire gonad and all seen were in the equatorial plate stage (Fig. 5).

**Ovary A.** In larvae somewhat larger than this last one it is easy to distinguish between ovaries and testes, either by dissection, or by a microscopic examination. During the early growth of the larva, we find that but slight changes take place in the structure of the nuclei or of the ovary, the nuclei have increased a little in size, but a greater change has taken place in the growth of the ovary itself. In a larva 7 mm long sexual differentiation has become very marked; the ovarian tubules are each 0,275 mm long, but they as yet, show none of the parts which can so easily be distinguished in older stages. The tubules are filled with a mass of cells which show a greater difference in size and shape, than in structure. In each tubule one can easily distinguish between a distal half, in which the nuclei of the cells are small and ovoid, and a proximal half, containing fewer cells but nearly all with larger nuclei. GRÜNBERG (12) noticed in *Lepidoptera* that at an early stage the oogonia all had their position in the distal, and the epithelial nuclei in the proximal, part

of the tubule, a condition we do not find in *Polistes*. Externally, one notices that the distal end of the tubule is pointed, but the proximal end is thicker and rounded (Fig. 6). In studying the larvae of this age, we worked both with ovaries which had been dissected from the body and then sectioned, and also with those which had been sectioned in toto.

As already mentioned, the distal half of the tubule is filled with cells the nuclei of which are ovoid, here and there, some are found with a nearly spherical nucleus; these latter, have no definite position within this part of the tubule but may be median, or near the wall. The nuclei are closely packed together and mostly restricted to the marginal part of the tubule, lying close to its membrane, this leaves a central portion in which but few nuclei are found. Each nucleus lies with its long axis across the tubule, LEYDIG (20), PAULCKE (25), the cells are generally elongated but, owing to the crowding of the nuclei together, the cell boundaries are difficult to distinguish. Each nucleus, 0,005 mm in length, contains from one to three irregular nucleoli which do not stain so darkly as the chromatin granules; these latter lie mostly, but not all, in the peripheral part of the nucleus (Fig. 7 *b*). Achromatin strands pass irregularly from one to another of these, and, in these small, ovoid nuclei, one or more of them generally extends across it from side to side.

Near the middle of the tubule, a marked change is noticeable in the shape, both of the cells, and of their nuclei; those, which in the distal half, are shown elongated and with ovoid nuclei, give place to larger and more rounded cells, which contain larger and nearly spherical nuclei. These larger cells occupy all of the proximal half of the tubule, although, here and there, in this same region a few of the smaller ovoid nuclei can be found. Owing to the larger size of the cells in the proximal half, and to their being less crowded together, we find here a much smaller number than in the distal half. The nuclei of the large cells (Fig. 7 *a*) are similar in structure to those we found in the embryo, or young larvae.

In *Dytiscus*, GIARDINA (8) has described the oöcyte and its nurse cells as remaining together and forming a group, the cells of which, increase in number as mitosis proceeds. Somewhat similar groups were found in the ovary of *Polistes* in many different stages of development, and it was thought the same arrangement occurred here as in *Dytiscus*. The explanation given by GIARDINA (8) seems very plausible, and we should hold a similar view for *Polistes*, were



it not for two things which it would be hard to reconcile to this view. It was easy to assume that all the cells of a group came from the repeated division of a single cell and that, after each division, the resultant cells remained attached to each other. When, however, in a larva 13 mm in length, groups of cells were found similar, except in size, to the groups found in the ovaries of older insects, I began to doubt as to their origin. Even in this young larva, some of the groups contained as many as seven cells (Fig. 8). The cells all come from a common central portion, each is pyriform, and they are all attached by the narrow stalk like part. In *Megachile*, LUDWIG (23) observed that the eggs have for some time a stalk, and earlier, somewhat similar appearances, had been observed by insects other than Hymenoptera, HUXLEY (17), CLAUS (7), LUBBOCK (22). We have already called attention to the very few dividing cells present in the gonads of embryos, or of young larvae; in the larva we now describe, but very few were noticed, and none of these occurred in groups, as we shall notice in older larvae and in pupae. In young larvae of *Polistes* we never found more than five or six dividing cells in any ovarian tubule; if all the cells in a group have come from repeated divisions of a single one, it is certain, that some of the numerous embryos and young larvae, would have shown many dividing nuclei. Then again if these groups originate as above mentioned, some dividing cells must be found near together, whereas, as we have already said, up to this age the very few dividing cells were scattered in the tubules. It might be said that the cells of these groups arise by amitotic division; GIARDINA (8) has shown in *Dytiscus* that the cells divide mitotically, and the few mitotic figures seen in *Polistes*, would prove that this is also here the method of division; that both mitosis and amitosis occur in the ovaries of these young larvae, I do not believe.

In *Dytiscus*, each group consists of one oöcyte and several nurse cells. In the group found in *Polistes*, not only here but in older larvae and in pupa, all the nuclei are similar, and there is no difference in the size of the cells. We shall learn that, during the growth of the oöcytes and the nurse-cells, they soon become easily recognizable as such, and if, in older larvae, each group contained one oöcyte and several nurse-cells, this would be apparent. We do not believe that these groups of cells originate the same way in *Polistes* as in *Dytiscus*, but cannot offer a very satisfactory explanation to account for their origin and persistence in the former insect. It

might be that the cells of a group are attached to the wall of the tubule near each other, and in some way remain so, or become in part fused with each other. The central portion from which they all come, or to which they are all attached, is, in older stages, of a darker cytoplasm than the rest of the cell (Fig. 16) and this common central portion is very distinct.

Ovary B. Passing to a somewhat older larva, one 16,5 mm long, we notice that changes have taken place, especially, in the growth of the tubules themselves (Fig. 9); each is in this larva 0,6 mm long. A single tubule shows a proximal part, very different from the rest, (Fig. 9 *odt*) which later develops into the oviduct. This same differentiation was also seen in younger larvae from 12 to 15 mm long. These parts would probably correspond to the »Endfaden, Keimfach und Ausführungsgang«, the three divisions given by HENKING (13) for the ovary of the larva of *Pyrrhocoris*. Within the proximal part, the cells are different from those found in the rest of the tubule; they are filled with large vacuoles which, under a low power, gives them a much lighter appearance than the others; their nuclei are smaller than those in the other two parts of the tubule, but differ very little in structure. The cells are long and narrow, and arranged across the tubule; no central lumen has as yet appeared in the future oviduct. The middle section of the tubule (Fig. 9 *ov*), shows a great similarity throughout, the nuclei are nearly all large and spherical, their cells also large, and generally rounded or polyhedral. These nuclei (Fig. 10), are similar to the last one figured; each has generally two nucleoli, a number of chromatin granules, and connecting achromatin strands. These nuclei are 0,0085 mm in diameter. The chromatin granules are slightly larger than in the last stage, and the achromatin enlarges to a small irregular mass around each one. Along the margin of this middle portion of the tubule are a number of small, ovoid nuclei, each having about the same structure (Fig. 11) as those in the distal part. In this latter part nearly all the nuclei have an oval form (Fig. 12), each with two to four, generally two, nucleoli, small chromatin granules and a reticulum, of which one or two strands can generally be traced across the nucleus from side to side. The cells are elongated and both they, and their nuclei, lie with the long axis across the tubule. One notices that the cells are closely crowded together. The nuclei lie mostly near the margin of the tubule, only a few being found in

its median part. A few of the nuclei are nearly spherical, these are more apt to be found in the center of the tubule than near its wall. Here and there are groups of cells similar to those described for our earlier stage.

Where the distal passes into the middle region of the tubule, the change is quite abrupt, the small, ovoid nuclei giving place to the larger, spherical ones; the cells also change from an elongated to a more rounded form. At this transition region the tubule becomes somewhat wider. But few mitotic figures were present in this stage, not more than four or five being found in any tubule of the ovary.

Ovary C. This is the youngest ovary we found in larvae after they had ceased feeding, and enclosed themselves in the cell of their nest. The larva was of course full grown, the tubules were each 1.44 mm in length. Distally, each tubule is long and narrow, and not straight throughout its entire length but terminally, it is bent. In this latter region, and for some little distance down the tubule, many of the cells extend entirely across it; sometimes they are equally broad throughout their entire length, but oftener pointed at one end (Fig. 14). The nuclei of all the cells in this part were very similar, any slight differences they showed being in the arrangement of their contents. Each nucleus has two, seldom more or less, nucleoli, quite large and irregular in shape and position; each one is surrounded by a thin layer of achromatin from which strands pass to the nuclear membrane. In these achromatin strand lies a number of chromatin granules, these do not stain evenly some being darker than others. The cytoplasm of these cells is quite irregular in appearance and distribution. Passing further down the tubule we find it widening and the cells becoming arranged in a fairly even layer along its wall. Next we notice that a few cells appear in the central part of the tubule — not in a lumen because none is present — so that, here and there, one can find three cells, instead of two, across the tubule. The nuclei remain unchanged, but a few of the centrally situated cells become more rounded. As we pass down the tubule these central cells become more and more abundant, and finally, in the median portion of the tubules (Fig. 13 *ov*), the long narrow cells have almost entirely disappeared.

At the distal end of this median portion, differentiation takes place more rapidly than at any other part i. e. the change of the nuclei from an oval to a round form, and the enlargement, and



rounding, of the cells. After this change the nuclei are for some time very similar in structure. A short distance proximal to this point some of the cells lie in groups; there is a central part, common to all, which is darker than the rest, and this darkened portion extends a short distance up into each cell. Often the connection between this portion, and that part protruding into the cell, is, on account of the position of the cell, lost, and the cell then appears to have an irregular or round, darkened body in its attached end (Fig. 16, lower cell).

Within the central part of the tubule (Fig. 13 *ov*) some variations in nuclear structure are noticed. At the distal end the nuclei are all nearly alike, very similar to what we have figured (Fig. 23). A little proximal to this the only change noticeable is an increase in size of the chromatin granules. Such nuclei (Fig. 16) may be found at any place in the proximal two-thirds of the median portion of the tubule. Scattered through this part are some slightly larger, irregular cells, with one, seldom two, large nucleoles, and having the small chromatin granules nearly all peripheral in position (Fig. 15). These we believe to be young oöcytes, but they nowhere, at this age, show any of the changes which we shall find at later stages. GRÜNBERG (12) has noticed for *Pieris*, that early, the nucleus of the oöcyte and of the nurse cells, were to be distinguished by the different distribution of the chromatin; all nuclei of the »Keimzellen« he found had a spireme thread stage. He could, in half grown larvae, distinguish between the three kinds of cells. BLOCHMANN (3) distinguished the young oöcytes by their great size and their darker staining cytoplasm, KORSCHOLT (18), the young oöcytes, by their lighter color; the earliest stages both these saw were however much older than the present one. A third form of nucleus which shows considerable variation in size, is found especially in the proximal half of this portion of the tubule. One of the smallest of these (Fig. 17), is seen to contain an irregular, loose achromatin mass, in which a few chromatin granules of varying size can be seen. Very similar but larger nuclei are present, these differ from the small ones, in having the strands of achromatin more definite, and the chromatin granules more numerous. All gradations between these small and larger nuclei can be found. These we hold to be the nuclei of primitive nurse-cells soon after division, and that they are, by increase in size and amount of chromatin, assuming a structure very similar to figure 16. A number of these nuclei are generally found together and we shall notice them in later stages.

This entire ovary showed very few dividing cells; one or two scattered mitotic figures were seen in the region where the distal changes into the median part of the tubule, and small groups of them, in the proximal part of this latter portion. HENKING (13) found in the larva of *Pyrrhocoris*, that the mitotic figures were present in two regions, in the upper part of the »Keimfach«, and just under it.

We see that in this stage it is hard to determine the oöcytes with as much certainty as the primitive nurse cells, and we fail to find, at any place in the ovary, the large cells which, from their size and structure, are, in later stages, so easily distinguished as oöcytes. We find that oöcytes are differentiated (Fig. 15), but that their development has not gone so far as that of some of the primitive nurse cells, many of which have at least gone through one division. Earlier, in ovary B, we saw a differentiation of cells with small, ovoid nuclei, to those with larger and rounder ones. Some of the cells with the small ovoid nuclei do not change, and can, in later stages (as epithelial cells), be found in both the distal and the median portions of the tubule; in the latter region division in these cells does not cease, and they increase in number long after division in the nurse cells has stopped. The cells with oval nuclei, which are found here in the distal part of the tubule, either remain as they are and may become epithelial cells or differentiate into oöcytes or primitive nurse cells.

We have found that the ovary from a larva which has enclosed itself in the cell of its nest is further developed than one from a free larva. This is naturally what would be expected and is in general true but we may find exceptions. Some free larva are found in which the ovary is further developed than is some of the enclosed ones. From this it can be seen that a study of the ovary alone, would not be a sure criterion as to whether it had been taken from an old, free larva, or from one which had just enclosed itself for pupation.

Ovary D. Passing to a wasp which has commenced to pupate we find that a general view of an ovarian tubule is similar to what we found in the last stage. At that point in the tubule where the widening is most marked (Fig. 18 *T*) we find a change taking place in the structure of the nuclei and in the form and size of the cells; at this place, if any, we find a zone of differentiation (PAULCKE), and of the first differentiation, which takes place. KORSCHOLT (18) found

in *Bombus*, that in the distal part of the endchamber the nuclei were similar in structure, but larger, than those in the terminal filament. Even before entering this part (Fig. 18 *od*) we may, here and there, find cells which are changing, and these may lie either in the center of the tubule (Fig. 19, point 20), or along its margin (Fig. 19, point 22). These cells have changed from the long narrow form to a rounder one but, still greater than this, is the change in the structure of their nuclei. Throughout the distal portion of the tubule the nuclei are all alike and in this region, those which are similar in structure, greatly predominate (Fig. 21). The nuclei of the other cells (Figs. 20 and 22) show a slight increase in size, and a marked difference in their shape. Their structure is also different, the chromatin granules are a little smaller, the achromatin reticulum more scattered and made up of finer fibrils not so distinct as in the other nuclei. In them there is also seen a fairly large chromatin body which we here notice for the first time, but of which we shall speak again. In this place, and also further down in the tubule, the cells contain a darkly staining body which, for want of a better name, we shall call a yolk-nucleus. It is sometimes an irregular body but, at this stage, more often has the form of a partially coiled, thick rod; it is always found lying within a clear space. In all sections this body was noticeable, but especially so in those stained with safranin.

In this part of the tubule (Fig. 19) there is an irregular transverse striation which stops before the middle part is reached (PAULCKE). These striae are due to strands of cytoplasm, which are more darkened than the rest; they begin at the wall and extend towards the middle of the tubule some being short and others reaching nearly across. These striae almost entirely obliterate the cell boundaries which, distal to this region, were very distinct.

As we pass towards the proximal part of this distal portion of the tubule (Fig. 19), we find that the nuclei lying along the margin, become less and less epithelial like in their arrangement, and finally, below the transverse striae, all trace of it entirely disappears. At this point the larger, rounded cells are more abundant, but as yet their nuclei show no change in structure (Fig. 23). A little further down a change does take place; we find (Fig. 18, point 24) many nuclei showing larger chromatin granules and that the achromatin mass has increased, its strands being thicker, and widening to form a small irregular mass around each chromatin granule (Fig. 24). One



or two nucleoli are, as in the younger nuclei, present, and in many of the cells a yolk-nucleus is seen. In the proximal third of the middle portion of the tubule we find, besides these last nuclei, others which are in general appearance lighter, due to their having but a few chromatin granules; these we will describe and figure from the next stage.

In this median portion of the tubule, especially in the proximal half, we find a few nuclei different from the others we have described in this ovary. These (Fig. 25), are large and irregular in shape, each has one or two large achromatin nuclei and small, very distinct chromatin granules. A comparison of these with some of the nuclei (Fig. 15) we found in the last stage will show a similarity between the two and we undoubtedly have here the young oöcytes. The structure of their nucleus at once separates them from the primitive nurse-cells, but does not, at this early stage, show any of the peculiar structures we later find so characteristic for the oöcyte.

In the distal portion of the tubule but few dividing nuclei are found and these do not occur in groups. In the median portion we notice quite a number, especially in the middle or proximal part, and find that they nearly all occur singly; in only one place was a group of dividing cells seen and here they failed to show any connection with each other.

Ovary E. In a slightly older stage, while the ovarian tubules show but little change from what we found in ovary D, we find the nuclei further advanced. That part of the tubule which becomes the oviduct (Fig. 26 *odt*) shows in part, a lumen. Throughout the entire distal portion of the tubule (Fig. 26 part *t*) no changes have occurred, its terminal part contains cells similar to those in the last stage and which, here and there, extend entirely across the tubule. Further down (Fig. 26 point 27) we notice that the nuclei of the cells are similar to what we found in those taken from nearly the same position in ovary D; they are oval in form, with one or two, seldom more, achromatin nucleoli, and a number of chromatin granules lying in strands which are quite distinct (Fig. 27). We find that, in the distal portion of the tubule, the cytoplasm of the cells is not so compact and dark as in cells from the median part. A few dividing cells can be found in this distal portion, but they are scattered, and do not all occur in any one region.

The first distinct changes in nuclear structure are found here in

the same relative place that they were in the last stage, namely, at the distal end of the median portion of the tubule. At this region cells occur abundantly in the middle of the tubule and a majority have larger and more spherical nuclei. Near this region, we find the first cells which we can surely determine as oöcyte or as primitive nurse-cells, although as yet the difference is not very marked. Both of these cells, or their nuclei (Fig. 28 *a* and *b*), are still quite similar to those we find throughout the terminal portion of the tubule, and are therefore not very distinctly separated from each other. Further down the tubule the differentiation becomes more marked; we find the nuclei of the primitive nurse-cells spherical, and with a round darkly staining chromatin body, while the oöcyte nucleus is larger, and of an irregular form, containing one or two rather large nucleoli and a few small, but distinct, chromatin bodies (Fig. 29 *a* and *b*). The latter nucleus appears lighter than the former, due to the smaller amount of matter in it, and most of this is on the periphery. Others have noticed the lighter appearance of the oöcyte nucleus even as long ago as CLAUS (7). This is hard to show in the drawings, but after one has studied these nuclei for some time, those of the oöcytes, can generally be distinguished by their lighter and clearer appearance.

We notice here a greater variation in the structure of the nurse-cell nuclei and find that, in general, there is some relation between these and their position in the tubule. Somewhat further down (Fig. 26 place 30), we find that the chromatin granules in many of the nuclei have become larger and that each is imbedded in a small, irregular mass of achromatin. Such nuclei may be found almost any place within the proximal three-quarters of the middle section of the tubule, but they are not found near its distal end. In the same region, but not quite so far distal, are a few nuclei which show a number of chromosomes; a stage undoubtedly preparatory to the division of the primitive nurse-cells. If such a stage is found before each division we cannot say, as it has been impossible to distinguish the different divisions from each other. The chromosomes are curved, beaded rods; besides these the nucleus contains a fine fibrillar achromatin substance, but, whether it connects the chromosomes to each other, we could not determine. In the proximal half of the middle portion of the tubule are a number of lighter nuclei, not large and irregular as are those of the oöcytes, but small and spherical, with an indistinct reticulum, in which lie a few chromatin granules

of varying sizes. These are the lighter nuclei we mentioned in the last stage, but which are, in this older ovary, much more abundant. Most of them are smaller than the other nuclei but some are of an equal size. Besides the variation in size, they show a considerable amount of difference in the number and size of the contained chromatin granules. These are the nuclei of the primitive nurse-cells just after division; they increase in size and in the amount of chromatin granules as they grow and prepare to divide again.

Dividing nuclei are found in any part of this ovary but are more abundant in its middle portion. They are not more numerous here than in the last stage. In the equatorial-plate stage a rather indistinct centrosome is often seen from which radiate a few fibres, but these are short and not abundant (Fig. 33). The same is true of a later stage (Fig. 34). After completion of division (Fig. 35) a few small mid-bodies are seen at the boundaries of the new cells. The nuclei of the daughter-cells are small, and their contents indistinct, but we can distinguish a reticulum and a few chromatin granules in each.

Along the margin of the tubule, in this middle part, are a few nuclei (Fig. 36) similar in structure to those in the long distal portion. These are the nuclei of the future epithelial cells; they do not here, as in the distal region, all lie with their long axis across the tubule.

Ovary F. As pupal development goes on, we find the ovary increasing in length, and the differentiation of the nuclei becoming more marked. The next we take, ovary F, is from a pupa the ovarian tubules of which are each 2.1 mm in length. The long distal portion remains the same as we have found in younger stages, but in the middle region, a considerable change has taken place, and we now, for the first time, find that the older oöcyte nuclei are entirely different from those of the nurse-cells.

A study of the different nuclei shows us that, proximally in the distal portion (Fig. 37, position 38), they are generally of an oval form (Fig. 38) but larger than corresponding nuclei of the younger stages. In this, and many neighboring cells, we find a round body which does not stain, but is darker and denser than the surrounding cytoplasm; it generally, as here (Fig. 38), occupies a position between the nucleus and the free end of the cell, but may also be on the opposite side.

Where the distal passes over into the middle portion of the tubule (Fig. 37  $x-x$ ) we find, as described in earlier stages, that a



change takes place in both the arrangement of the cells and in the structure of their nuclei. The epithelial like arrangement entirely disappears and the cells become irregularly scattered throughout the tubule. The ovoid nuclei are much less abundant, and larger and more rounded ones take their place. In the upper part of the middle portion we find that the first differentiation, as in the younger stage, can be seen where the oöcyte nuclei are slightly different from those of the primitive nurse-cells (Fig. 39 *a* and *b*). When these nuclei are compared with those we find in the same region of the last stage (Fig. 28 and 29), the similarity is at once apparent. Near this same region (Fig. 37, positions 40 and 41) we find exactly the same changes taking place in the nuclei of the primitive nurse-cells, that we found in an earlier stage. They obtain a definite spherical form, the strands of achromatin assume a net-like arrangement, and a yolk-nucleus appears in many of the cells (Fig. 40). The chromatin granules increase in size (Fig. 41) and around each one a small achromatin mass is formed. Further down in the tubule, the clearer nuclei we have already mentioned, are present (Fig. 42), and they show here, as in the earlier stages, a variation in size and in the amount of contained chromatin. Within the proximal part of this median portion a few nuclei with chromosomes were seen.

In this ovary we find that the nurse-cell nuclei are, in corresponding parts, similar to those we found in the last stage. In the oöcyte nuclei we notice that very important changes have taken place and that they are no longer the same from all parts of the tubule in which they are found. Distal in the median portion of the tubule, we still find cells with rather large, irregular nuclei (Fig. 39 *a*), which are similar in structure to the oöcyte nuclei of the last two stages (Figs. 25 and 29 *a*); when however we come to the older stages we notice that there has been a decided change. At a position in the tubule which we have represented (Fig. 37, point 43) we find a few nuclei which, from the size of both cell and nucleus, we know to be those of oöcytes; they show a few bent, beaded, rod-like chromosomes and also some achromatin matter (Fig. 43). The nature of this latter substance was hard to determine, it formed irregular strands which were not clear. Both in the oöcytes and in the primitive nurse-cells, the chromosome stage was less abundant than any of the other forms.

In the oöcytes the chromosomes next lengthen to threads in which a beaded structure is easily seen. Such nuclei, (Fig. 44), lie proximal

to those with chromosomes, and thus show that they are older. No division follows this stage in the oöcytes, but the threads later become arranged in synapsis. In *Polistes* we find that during synapsis the cell boundaries are very easily seen, but PAULCKE (25) for *Apis* and GRÜNBERG (12) in *Pieris* failed to find them. In the many ovaries of about this same age which we examined, the nuclei of the oöcytes always took this order, and never in the young pupa of *Polistes* were synapsis nuclei found at any place except in the proximal part of the median portion of the tubule; always in front of these were nuclei similar in structure to figure 44. Synapsis nuclei were found, two or three in a section, or often none at all; they showed a variation in structure. Some have a large irregular mass of achromatin in which lie many deeply staining chromatin granules, and from which nearly all trace of the threads have disappeared, others show a few threads in this mass which can be seen protruding from or running through it (Fig. 45 *a*). We failed to find such a regularity in structure and polarization as GIARDINA (9) found in the oöcytes of *Mantis*. In most of these nuclei a few odd pieces of the beaded threads were scattered through them opposite the large mass. After this stage the threads become evenly distributed throughout the nucleus; whether or not they are of equal length we are unable to say; in most nuclei they were of about the same length, but in many, at least one thread was present, which was equal in length to the diameter of the nucleus (Fig. 46 *a*). This last oöcyte is one of the oldest in this ovary and, as is shown by its position, (Fig. 37, position 46), lies just distal to the oviduct.

In the proximal part of the median portion of the tubule we find a new form of nurse-cell nucleus. In this (Figs. 45 *b* and 46 *b*) a single irregular achromatin mass lies at, or near, the center and on, or near it, a few chromatin granules; achromatin strands pass from this central part to the nuclear membrane, these and others form an irregular network the strands of which contain a number of chromatin granules. We have here the nucleus of a nurse cell after all its divisions have been completed; this same form will again be seen in the older ovaries.

Ovary G. We select, from a slightly older pupa, a few nuclei for description to show that the same order is found here as we have just observed in Ovary F. Near the boundary between the distal and the middle parts of the tubule, we notice the same two kinds of

nuclei; the oöcyte nucleus (Fig. 47 *a*) shows the same structure we have already described; it is also found a little distance down the tubule (Fig. 48 *a*). In the nucleus of the primitive nurse-cells we also find a similarity between this and the younger stages. A little proximal to the boundary we just mentioned, we find nuclei with the spherical chromatin body (Fig. 48 *b*) we have already noticed in younger ovaries.

In the nuclei of the primitive nurse-cells we find that the same changes, as already noted, take place, and that proximally, in the median portion of the tubule, are a few nuclei with chromosomes. In this region the light colored nuclei are also found and they show the same variation in size and in contents as in the earlier stages. Near the proximal end the nurse-cell nuclei show the structure we have already said was characteristic for them after division has ceased, and they become the permanent nurse-cells (Fig. 51 *b*).

The oöcyte nucleus also shows a chromosome formation but here, as in the nurse cells, this stage is not abundant (Fig. 50). Synapsis stages are found, and after these the beaded threads become evenly distributed throughout the nucleus (Fig. 51 *a*). In both this and the preceding stage, dividing cells could be found in nearly every part of the median portion of the tubule.

Ovary H. The next stage we will consider, an older pupa, is one in which the oldest oöcyte and its accompanying nurse-cells are arranged to form the first chamber. In older stages we shall show that chambers are not at first formed as egg- and nurse chamber, this separation coming later in the development, but that the oöcyte and its accompanying nurse cells are together in a common chamber. In any tubule of this ovary we find, proximally, an oöcyte and accompanying nurse cells lying in a chamber. Distal to this group lie many other oöcytes, but none show any indication of chamber formation, although the few oldest ones, have grouped around them those nurse-cells which will later form their nurse chamber (Fig. 52). In neither this nor other chambers could we find the regular arrangement of the nurse cells as described by PAULCKE (25) for the bee.

In the last stages, ovaries F and G, we noticed that the zone in which the synapsis nuclei lay, was near the proximal end of the middle portion of the tubule (Fig. 37, position 45); as development goes on this zone changes its relative position and moves toward the



distal end. In this stage, ovary H, the cells in synapsis must be sought much further up (Fig. 52 *Syn*). Following from this place the development of the oöcytes we find, as in the last stages, that after synapsis the long, beaded threads distribute themselves throughout the nucleus. This condition remains for some time unaltered but, by the growth of the nucleus, the threads are separated more from each other; each one is beaded, the chromatin granules do not stain deeply and only appear very dark at the ends of the threads which one sees in optical, or real, section. As the nucleus grows the threads become clearer and more distinct (Fig. 53) and we reach a place, (Fig. 52, position 54), where we find them growing quite irregular (Fig. 54), more ragged along their edges, and losing the regularity within the nucleus they earlier showed. The threads are still beaded and are the only contents of the nuclei. The slight irregularity which we here notice is the beginning of their disruption, it becomes more marked (Fig. 55) and, while the threads still show a slight beaded structure, they have entirely lost the regularity which they had in the younger oöcytes. The oldest oöcyte (Fig. 56) in this tubule shows even a greater irregularity and the chromatin granules have gathered in achromatin masses. PAULCKE (25) notices that in *Apis* this change takes place when the chambers are being formed. »Mit Beginn der Kammerbildung verliert das Keimbläschen seine vorher so charakteristische feinfädige, gerüstartige Chromatinstructur.« In each of the last two oöcyte nuclei an achromatin nucleolus has appeared which we failed to notice in any of the preceding stages.

In all the oöcytes which we have figured from this tubule a yolk-nucleus was present. It is a deeply staining, round or ovoid, body, lying in a clear space within the cell. This has been drawn only in the oldest oöcyte (Fig. 56).

Ovary I. Passing next to the study of an ovary from a wasp which has been but a few hours out of the nest, we notice that many changes have taken place. The oldest oöcyte already lies in its own chamber separated from the nurse cells, also in their own chamber, which lie distal to it. The oldest oöcyte is the only one which, externally, shows a complete chamber formation; in a longitudinal section, however, one notices that the separation has taken place in the three next oldest oöcytes (Fig. 57). No distinct terminal filament is noticeable, but for a distance of about 0,3 mm from the tip, many

disintegrating cells are seen within the tubule; more here than at earlier stages.

Just as we found, that, when larvae first enclosed themselves within the cells of their nest, they did not always show an equal development, so we now find that upon issuing from it, the ovaries are not all the same. We have selected a specimen which will represent the average development that the ovaries have reached in wasps which have been but a few hours out of the nest.

The distal portion of the tubule shows the changes we have already recorded for both oöcytes and nurse cells. We find a place in which the cells are as yet undifferentiated and, proximal to this region, the nuclei undergo the same changes we have already noticed. A certain zone contains all the synapsis nuclei, which are however not abundant; some sections show none at all, and never more than two or three. In taking up the history of the different cells it will be best to treat of each kind separately, and to omit several stages we have already described.

The epithelial cells and their nuclei undergo by far the least change of all; here and there along the margin of the tubule, we find nuclei similar in structure to those we have already noticed as belonging to epithelial cells. When the chambers are formed, we find, in the portion occupied by the oöcyte, that the epithelial cells form in part a layer around it. In the region occupied by the nurse-cells we find the same nuclei on its margin; they are here also scattered among the nurse-cells (Fig. 79) especially in the median part of the chamber (Gross [10]). The boundaries of these cells is hard to distinguish, the nucleus is ovoid, but much smaller than that of the nurse cells. In each, one or two, small, irregular, achromatin masses are found (Fig. 58), and a number of quite distinct achromatin strands, one or two of which can generally be traced across the nucleus from side to side. A few chromatin granules are present, mostly peripheral in position. As soon as the chambers become definitely formed the epithelial cells begin to build a continuous layer around the oöcyte, enclosing it at all points, except centrally where it lies next to its nurse cells, where, as already noticed by many others, an opening remains. At the two ends of the oöcyte the epithelial cells assume a regular columnar form and its margin has here a serrated appearance, each indentation as long as the follicle cell is wide (Fig. 59). The nucleus of each of these epithelial cells is similar to those within the nurse chambers. We find a number

of mitotic figures. With the increase in size of the oöcyte and its chamber, the number of follicle cells becomes greater.

In the last stage we noticed that the nurse-cell nuclei, after division has entirely ceased, assume a form which is, at this stage, characteristic. Far up in this tubule we find that the nurse-cell nuclei are similar to those we have already described; the stage where each possesses a number of large chromatin nucleoles, each lying in a mass of achromatin (Fig. 41), predominates. Near that part of the tubule in which synapsis nuclei are found (Fig. 57 *syn*), the nurse-cell nuclei have assumed their permanent form (Fig. 60), an irregular achromatin mass at or near the center, strands of achromatin forming a network, and containing a number of darkly staining chromatin granules. Proximal to this these same nurse-cell nuclei can be found, and for some distance down the tubule they do not change their form. At this place, or wherever this form of nucleus is found, mitosis among the nurse cells has ceased. PAULCKE (25) holds that the nurse-cells in *Apis* must divide amitotically, this we never found in *Polistes*. GROSS (10) finds mitosis in the nurse-cells of *Bombus*, but in *Vespa* describes the nuclei of the nurse-cells as dividing amitotically, figuring some cells with two or three nuclei. When we reach that part of the tubule (Fig. 57, position 61) in which the oöcytes begin to arrange themselves regularly in a row in its center, we notice that nearly all the nurse-cell nuclei have changed a little in structure, and, instead of the single nucleolus, there are now two or three. Passing down the tubule the cells have, except the epithelial, increased considerably in size, and in the nurse-cell nuclei there are now a number of nucleoli (Fig. 62); these are still of about the same size but, in proportion to the size of the nucleus, smaller. In this place (Fig. 57, position 62) the nurse cells are grouped in regular order distal to the oöcyte to which they belong; amongst them a difference in size relative to their position is noticed, distally the smallest, proximally the largest. As the chambers grow this arrangement becomes more and more prominent. They continue to increase in size, this being more noticeable than any increase in structure. Each nucleus (Fig. 63) now contains a number of the large irregular bodies, nucleoli, which differ very much in depth of color; this is not only due to different stains but with the same stain, and even within the same chamber, quite a variation in this respect may be present. They also differ very much in size in the different nuclei found within any chamber, many small ones is the commonest arrangement, yet



some nuclei contain but a few large ones. In each nucleus the achromatin network is still seen and in these strands are a number of small, lightly staining, bodies similar in appearance to chromatin granules. In the oldest chamber many of the nurse-cells show a layer of darkened cytoplasm lying around the nucleus (Fig. 63). This we judge is due to the activity of the nurse cells.

A little distal to the synapsis nuclei we find young oöcytes similar to those we have already described. The nucleus has one, sometimes two, large achromatin nucleoles, a cloudy and fibrillar arrangement of other achromatin, in which lie imbedded a few chromatin granules generally peripherally arranged (Fig. 64 *a*). The large nucleolus becomes lost and does not appear again for some time. The achromatin becomes arranged in threads (Fig. 64 *b*), more and more chromatin granules appear in these, and we finally find a number of beaded chromosomes within the nucleus (Fig. 64 *c*). This last stage is much scarcer than the others, no section ever showing more than two and a majority of them none at all. Proximal to these last nuclei lie those in synapsis. These show but a slight degree of regularity and nowhere such a regular polarity of the strands as GIARDINA (8 and 9) found in *Dytiscus* or *Mantis*, the threads in his figures also appearing more beaded. The greatest amount of substance in the nucleus is arranged in a large irregular mass in which lie a number of distinct chromatin granules. Within this mass could be seen parts of the beaded threads, these would in part protrude from it in all directions (Fig. 65). Just proximal to the synapsis nuclei, we find the threads again becoming equally distributed throughout the nucleus (Fig. 66). The nuclei are apt to become elongated in form and will often lie two or three together (PAULCKE); later on, the shape changes and they separate from each other. From here on for some little distance the threads retain their regularity, and then break up, a process we will describe in the next stage.

Passing down the tubule to the older oöcytes we find that the oldest has increased very much in size, and both it, and the next oldest, are elongated in the direction of the tubule. Besides the nucleus, we now notice in each oöcyte a number of small bodies, which, in their structure, are quite similar to the nucleus (BLOCHMANN, STUHLMANN, KORSCHOLT, PAULCKE, GROSS). These have already appeared in the third oldest oöcyte (Fig. 57), and here, in that which is next to the oldest (Fig. 57, place 67), they are seen to lie in a mass around the nucleus (Fig. 57); each has one or two small irregular

bodies (nucleoli), an irregular reticulum, in the strands of which are found a number of small, slightly staining bodies (chromatin granules). At first these nuclear-like bodies lie in a single layer around the nucleus but, as their number increases, this layer changes to an irregular mass. In older oöcytes one notices (Fig. 57, position 68) that some of these bodies leave the mass and wander to other parts of the oöcyte where they always take a peripheral position. BLOCHMANN (3) and STUHLMANN (27) have studied these bodies more thoroughly than any one else but, as I have already given the views of each one and what they found, in the historical part, there is no use of repeating them here. In the two oldest oöcytes we notice that the nucleus has changed greatly, the regular beaded threads which can be found any place between *Syn.* and position 61 in figure 57, have disappeared, the entire nucleus being filled with a fine irregular, net-like reticulum in which lie a number of small, lightly staining bodies. Gathered near the center of the nucleus, are a few irregular achromatin masses in which lie imbedded the chromatin granules we earlier saw in the threads; these stain darker and are much more distinct than those in the reticulum (Figs. 67 and 68). In the oldest oöcyte we find that the nucleus is of about the same size as that of the next youngest one, but, in proportion to the size of the oöcyte, it is very much smaller. The oöcyte has increased in size but not the nucleus. In these oldest stages, we notice for the first time since the very early ones, that a nucleolus is present, it is generally round, but may be irregular; it contains a number of vacuoles (Fig. 68).

We have already noted the fact that in *Polistes*, when the epithelial cells arrange themselves as a follicular layer around the oöcyte, there is left a passage way between it and its accompanying nurse-cells. In this stage the oldest oöcyte shows that towards this opening it has sent out a process (Fig. 57). This, which has been observed in many other insects, is found more often in older ovaries where there are more oöcytes of this and later stages, and we shall refer to it again.

Ovary J. The ovary which we next study, if viewed externally, does not appear much older than the last; we find however that it is, and that, a much greater number of chambers being formed, we can more easily follow the changes which take place in the oöcyte nuclei. The tubules as a whole deserve but little attention, the chamber formation is externally much more marked and the oviduct further

developed. The oldest oöcyte is still small as compared to the size of the mature egg. Distally the contents of the tubule show a further disintegration and the position in the tubule in which the very youngest stages can be found is further towards the tip. The youngest stages must be passed over as they are now restricted to a rather narrow zone, are more difficult to study, and show nothing new. Far distal in the tubule we find but few regular cells, and believe that this part has nothing further to do with the production of cells which are to play an active part.

While we have said that the early development of the cells passes rapidly in this stage, we find the nuclei of both oöcytes and primitive nurse-cells passing through the same stages we have already noted. The early differentiation of the cells is the same but either very rapid or partly abbreviated. Dividing cells, except the epithelial, are restricted to a zone in which they are very numerous, occur in all stages of mitosis, and may be found in groups (Fig. 70) or scattered singly among the other cells.

Before synapsis there takes place in the oöcyte nucleus the formation of a number of chromosome like threads (Fig. 71). After synapsis the threads distribute themselves throughout the nucleus. At first (Fig. 72) they are shorter and irregular, but soon one notices that the nuclei have clear spireme-threads running through them; some of these are fairly straight, while others, especially the longer ones, are curved (Fig. 73). From the greater size of the nucleus the threads are now more distinct and longer than before synapsis, whether these are all of an equal length, or parts of a number of long threads, I am unable to say; they nearly always show a variation in their length. Here and there nuclei are found in this position in the tubule which, from a surface view, might be taken for a nucleus with a single spireme-thread (Fig. 74). Nuclei similar to these few we have last described continue for some distance down the tubule, until just before we find the oöcytes arranging themselves in the middle. Then there occurs a breaking up of the threads, they become shorter and lose their regular form, and some begin to form small masses. As we pass from this stage to the older oöcytes we never again meet with this structure.

At that part of the tubule where we find the first indication of a chamber, a common one for both oöcyte and nurse cells, the beaded threads in the oöcyte nucleus have become, as such, entirely lost. The breaking up which we noticed in the previous stage has gone



on, and we now find a few very irregular achromatin masses in which are the chromatin granules. Throughout the nucleus are numerous delicate achromatin fibrils and a rather large nucleolus. This latter, in slides stained with safranin and then well washed out, is very lightly if at all, tinged, and with HEIDENHAIN'S iron-haematoxylin never stains so dark as the chromatin granules. The small irregular masses containing the dark chromatin granules begin to fuse with each other, their number decreases but the few found are larger. The achromatin fibrils become more and more distinct; finally they show a beaded structure, these granules not staining nearly so darkly as the chromatin granules within the masses (Fig. 77). The union of the masses goes on, and there finally may be but one large one alone, or one or two small ones with it.

We have already noted that in the chamber formation the oöcyte and the nurse-cells first group themselves together and there is a chamber common to all; only later do the two chambers, egg and nurse, become separated from each other. At first the nurse-cells arrange themselves, not only distal to the oöcyte, but also at its sides, so that, excepting the proximal end, they surround it (Fig. 78). We find that even here there is a gradation in the arrangement of the nurse-cells according to size, the smallest in each group are distal, the largest proximal. This arrangement we find present in all later stages. Epithelial cells are found in small groups at the sides of the oöcyte, and generally a few of them both distal and proximal to it; a few are also scattered in with the nurse-cells. The nucleus of the oöcyte is about the same as that we last figured (Fig. 77) and the nurse-cell nuclei are similar to those we described for an earlier stage (Fig. 62).

A little later (Fig. 79) we notice that, due to an increase in the size of the nurse-cells, they are closer together. More epithelial cells are scattered among them and these are found along the margin of the chamber, or just above the oöcyte, where they form a small group (GROSS). The epithelial cells around the oöcyte have begun to form a regular follicle but as yet, lie only as a narrow girdle around it; more of them have become columnar than in the last stage. The structure of the nuclei of all the cells remains unaltered.

If we now pass over two or three chambers and come to an older one we notice that one of the greatest changes is in the size of oöcyte and nurse-cells (Fig. 80) and that no special change has taken place in the structure of any of the nuclei. The epithelial

cells have nearly surrounded the oöcyte but the structure of their nuclei also remains unchanged (Fig. 81). In the nurse chamber, or what will later be such, a number of epithelial cells are seen along the margin. Whether or not they form a complete layer here is doubtful. In our drawing (Fig. 80) one sees such a layer, but it would be hard to duplicate this regularity in many of the sections. These marginal epithelial cells, as well as those scattered in among the nurse-cells, have nuclei similar in structure (Fig. 82) to those around the oöcyte.

In the later stages we will not as heretofore study different ovarian tubules but will give separately the history of each kind of cell.

The epithelial cells have been found present in both kinds of chambers; of those in the nurse chamber there is nothing further to say, they remain for a long time unchanged but finally stain much lighter. Part of them are left within the old chamber after all the nurse-cells have disappeared, and others very likely go as nourishment to the developing oöcyte. Those epithelial cells which form a follicle around the oöcyte have a further history but this can be better given if, as is hoped, we at some future time can take up the formation of the chorion.

For a considerable time as development goes on, the nurse-cells show no further change, remaining as we have described them (Fig. 63). They have already shown, in the cytoplasm around the nucleus, secretory activity, and this, as they grow older, becomes more apparent; later we notice, as has been described for the nurse cell nuclei of so many insects, that they become irregular in form sending out pointed processes into the cell. In *Polistes* this is never so marked as in many other insects. The darkened layer of cytoplasm around the nucleus becomes thicker and within it a number of vacuoles finally appear (Fig. 85). Between egg and nurse chamber an opening exists (Figs. 84, 86 and 88), and through this the oöcyte often sends a lobate process, this and the nearby cytoplasm of the oöcyte, is often seen to have a dark stringy appearance which is undoubtedly due to the activity of this process in the absorption of nourishment. Older nurse-cell nuclei loose, to a great extent, their irregular appearance and become again more normal in shape; finally, when much older (Fig. 86), the cells loose in part their outline, the chamber becomes emptier as if less cells were in it than formally. All the cytoplasm is now of the same appearance as was the layer

directly around the nucleus and vacuoles appear in any part (Fig. 87). The nuclear structure has changed but little, the achromatin is more irregular and less net-like, the nucleoli are still present but more broken up.

In older stages one can see that there is a gradual absorption of the contents of the nurse chambers and that those cells lying nearest the oöcyte are the first to go. In younger nurse chambers the cells lying nearest the oöcyte are the largest, but now we notice that, in this region, they become much smaller, due to their partial absorption into the oöcyte. The cells in the other part of the chamber have nearly all lost their boundaries, and the cytoplasm of most of them lies entirely distal to the nucleus, giving the appearance as if they were passing down towards the oöcyte to be absorbed (Fig. 88). When the more proximal nurse-cells have disappeared and, although all the remaining cells are broken up, the structure of the nuclei is but little changed. A mass of many small and a few large bodies, nucleoli, are scattered through it, of these, the large ones especially, stain darkly (Fig. 89). This is all opposed to the view of PAULCKE (25) and GROSS (10) who hold that the nurse cells pass into the oöcyte, swallowed as it were. Such I have not found to be the case.

The nurse chamber grows smaller and smaller as its cells disappear, and, by the growth of the oöcytes, they are pushed to one side, so that two neighboring egg chambers lie with their ends nearly touching. The nurse cell boundaries disappear and the cytoplasm also goes; just before the chamber is finally emptied we find in it some epithelial cells, two or three nurse-cell nuclei, and a small amount of cytoplasm (Fig. 90).

We would record an irregularity in the finding of two nurse chambers without any egg chamber between them (Fig. 91). Whether or not there ever was an oöcyte in the chamber, we do not know, but most probably it died and disappeared and its chamber was crushed between those of the nurse cells.

We have seen how the oöcytes, after their chambers are formed, change their shape, becoming elongated and lying with their long axis parallel to that of the tubule (Fig. 57); the large spherical nucleus loses its regular shape and a nucleolus appears in it, the beaded threads become broken up (Figs. 75, 76 and 77), and one or more irregular masses appear which contain darkly staining chromatin granules, achromatin strands are present in which are lightly staining granules. We have also seen how around the oöcyte nucleus a number of



small bodies appear which in structure resemble a nucleus (Fig. 83); how at first they all lie near the nucleus, but later, many move away and spread over the periphery of the oöcyte. We will first briefly take up these nuclear-like bodies and then the oöcyte.

The origin of these bodies is not clear; BLOCHMANN (3) described them as budding off from the nucleus, and STUHLMANN (27) as forming from small concretions near the nucleus; while BLOCHMANN's explanation appears very propable I am unable to prove it, not having, in the very many nuclei examined, ever seen any appearance of budding. During the formation of the bodies the membrane of the nucleus remains fairly regular until after most, if not all, of them have been formed. I see no other explanation for their origin it not being very probable that they come from the yolk-nucleus which is present in the young oöcyte, when these bodies are being formed (Fig. 78), but which is not seen in the older oöcytes. Any other explanation does not occur to me and I think, although no proof can be shown for it, that they originate from the nucleus.

In an oöcyte  $0,05 \times 0,08$  mm in size a great number of these bodies are found around the nucleus (Fig. 92); they all have the same structure but vary considerably in size. When they begin to spread over the surface of the oöcyte they are found only at its distal end (Fig. 57) but later can be seen at any place on the surface (Fig. 84). When such a distribution has taken place, many of them still remain near the nucleus. After the elongation of the oöcyte its nucleus, as has been found for other Hymenoptera, lies near its distal end which is the part nearest the nurse chamber. When the nucleus of the oöcyte becomes irregular in shape, the same is true for many of these bodies (Figs. 95, 96 and 98); their contents becoming less darkly stained. The one or two darkly staining nucleoli each contains, remains, but the strands and the chromatin granules loose their distinctness and in part disappear. These small bodies, as well as the oöcyte nucleus, are present in the oldest oöcyte examined, one  $0,24 \times 0,47$  mm.

Just as we are unable to give definitely the origin of these bodies, so are we unable to state positively what is their function; it may be that a further study of the oöcyte will make this clear. The only explanation I can offer for their presence might be more plausible if they were found in other than Hymenopterous insects, (KORSCHOLT [18] *Musca*?). When one studies a longitudinal section of an oöcyte of *Polistes* after a large amount of deutoplasm has

been formed, it will be noticed that it is not equally distributed but occupies the central portion. On the surface of the oöcyte there is a rather dense finely granular layer free from deutoplasm; at the distal end of the oöcyte there is a cone shaped mass of the same substance, the base of the cone is at the end of the oöcyte its apex extending down towards its middle and sometimes passing nearly through the oöcyte (Figs. 84, 86 and 88, dotted lines). The basal part of this cone corresponds to that part of the oöcyte which lies nearest the nurse-cells and where the greatest activity is taking place. Those parts of the oöcyte which are free from deutoplasm, peripheral part and distal cone, contain these small nuclear-like bodies and it may be they have something to do with this activity helping to change the contents of the nurse cells into a substance suitable for the oöcyte.

The nucleus from an oöcyte having a diameter of 0,07 mm contains a nucleolus, one or more masses of achromatin in which are many darkly staining chromatin granules and an irregular reticulum in which are small lightly staining bodies. Such an oöcyte nucleus (Fig. 93) taken from the ovary of a nearly mature wasp-not its oldest oöcyte- is similar to what we have already found in the oldest oöcyte of a younger stage (Fig. 77 and 80). The nucleus still retains its regular form (Fig. 93) as do also the small nuclear-like bodies near it. In older and larger oöcytes we notice an irregularity in form and a change in structure (Fig. 94). The achromatin mass in this nucleus, from an oöcyte  $0,12 \times 0,15$  mm, stains with HEIDENHAIN'S iron-haematoxylin very darkly so that no darker granules can be distinguished in it. The nucleolus is darker and often contains one or more vacuoles. With the growth of the oöcyte the form of its nucleus becomes irregular; the large irregular mass and the nucleolus remain, the irregular beaded strands break up and a fine fibrillar mass appears in the central part of the nucleus: here and there are short beaded rods, whether these latter are only a stage in the distintegration of the strands or new structures which may play a further part in the history of the egg, we are unable to say, as here our present investigation stops.

We have already noted the presence of a chromatin ring (GIARDINA [8]), in the cells. This occurs quite early in the development, being seen in larval stages, but disappear shortly after synapsis. It is a small, darkly staining ring which lies between two adjacent cells; two may be found between the same cells (Fig. 101) or the

single one often appears split (Fig. 50). We are unable to offer any explanation as to its origin or fate but merely record its presence in *Polistes*. In early larval stages it seemed that its origin could be traced from a solid body (yolk-nucleus?) the central part of which dissolving left the ring. Some bodies with lighter central parts were seen but not enough to give any surety of the development of the rings in this way. They appear to be present in the center of the cell (Fig. 100) but this is, we believe, due to the different view we have of them. Two dividing cells were also found which showed a ring between them (Fig. 99), and from one pole of each mitotic figure a fibril passed to the ring.

### Summary.

In the embryos and very early larvae each gonad is a syncytium with a number of nuclei similar in structure. Very early in larval life cell boundaries appear.

The ovarian tubules develop; they at first contain cells similar in structure but differing in size, the largest being in the proximal half of each tubule.

Each ovarian tubule when first formed, ovary A, has a distal half, in which the cells and their nuclei are elongated, and a proximal half, where they are larger and rounder. This distal part becomes proportionately smaller and smaller; the cells in it never show the variations in structure that are seen in those of the proximal part.

In older larvae, ovaries A and B, we find each tubule of three parts; a distal portion in which the cells are very similar in structure, a median portion, where are found the differentiating oöcytes and nurse-cells, and a proximal part, that becomes the oviduct. At the boundary, not real, between the first and second parts most of the cells change from undifferentiated ones; as growth goes on, this boundary is found nearer the distal end of the tubule, i. e. the relative size of the median part increases, of the distal part, decreases.

As the ovarian tubules grow, the cells in the distal part remain the same; those in the middle portion change in size and in nuclear structure and we can distinguish between oöcytes and primitive nurse-cells, ovaries C and D. The latter cells pass through a number of divisions. As growth goes on these two kinds of cells become more and more unlike.

After the repeated division of the primitive nurse-cells have ended, the nuclear structure of all the nurse-cells is similar. For



a time they do not change and then, as growth goes on, the nucleoli increase in number. They do not again change their structure until broken up and absorbed by the oöcytes.

The oöcytes pass through synapsis out of which comes long beaded threads. These break up, the contained chromatin granules remaining together in small achromatin masses. A nucleolus has appeared, which, after filling with vacuoles, becomes smaller.

Small nuclear-like bodies have earlier appeared around the nucleus. These increase in number and many of them pass to the periphery of the oöcyte.

Mitosis occurs in the epithelial and primitive nurse-cells. After the oöcytes have differentiated as such, they do not divide.

Berlin, im August 1906.

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### Explanation of Plates.

All figures drawn with a camera-lucida.

In many of the cells the boundaries have been drawn to show comparison in size but the cytoplasm has not been filled in.

Each ovarian tubule is divided into three parts; *t*, the most distal of the

three parts; *ov*, the middle piece; *odt*, the proximal portion which later develops into the oviduct. Numbers in the tubules represent positions from which correspondingly numbered cells or nuclei have been taken.

The arrow points towards the distal end of the tubule.

### Plate XII.

Fig. 1. Section showing part of a gonad, from an embryo 1,35 mm long. The reproductive organ is here a synctium with the contained nuclei all of the same structure.  $\times 1600$ .

Fig. 2. Enlarged view of a nucleus from the same gonad.  $\times 2500$ .

Fig. 3. The proximal half an ovarian tubule from a larva 2,6 mm long.  $\times 1600$ .

Fig. 4. One of the largest cells from the same tubule.  $\times 2500$ .

Fig. 5. Dividing cell from the gonad of a larva of about the same age as figure 3.  $\times 2500$ .

Fig. 6. Ovary A. Ovarian tubule from a larva 7 mm; length of tubule 0,275 mm.  $\times 100$ .

Fig. 7. Two cells from ovary A; *a*, cell from the proximal; *b*, cell from the distal part of the tubule.  $\times 1600$ .

Fig. 8. Group of six attached cells, from ovarian tubule of a larva 13 mm in length.  $\times 850$ .

Fig. 9. Ovary B. Ovarian tubule from a larva 16,5 mm long; length of tubule 0,6 mm.  $\times 100$ .

Fig. 10. Cell from position 10 in ovary B.  $\times 1600$ .

Fig. 11. Another cell from the same ovarian tubule, taken from place at number 11. Line to right represents wall of tubule.  $\times 1600$ .

Fig. 12. A third cell from the same tubule, taken from position 12.  $\times 1600$ .

Fig. 13. Ovary C. An ovarian tubule from a full grown larva which has just enclosed itself for pupation.  $\times 100$ .

Fig. 14. A small part of ovary C near the distal end, showing three cells which reach across the tubule; from place designated by number 14 in preceding figure.  $\times 1600$ .

Fig. 15. Young oöcyte nucleus, from point 15 in ovary C.  $\times 1600$ .

Fig. 16. Group of cells from same ovary, taken from place indicated by 16.  $\times 1600$ .

Fig. 17. A cell from position 17 in ovary C.  $\times 1600$ .

Fig. 18. Ovary D. The proximal portion of an ovarian tubule from a wasp after pupation has begun. Bracket *T*, represents that part which is enlarged in the following figure.  $\times 100$ .

Fig. 19. Portion of ovary D shown in preceding figure by bracket *T*. This is the proximal end of the distal portion of the tubule, and the distal end of the median portion.  $\times 500$ .

Fig. 20. A cell, median in position, from point 20 of figure 19.  $\times 2000$ .

Fig. 21. A nucleus from a nearby marginal cell, from place 21 in figure 19.  $\times 2000$ .

Figs. 22, 23 and 24. Three primitive nurse-cells all taken from figure 19 at places with corresponding numbers.  $\times 2000$ .

Fig. 25. Nucleus of a young oöcyte taken from position 25 in figure 18.  $\times 2000$ .



Fig. 26. Ovary E. Ovarian tubule from a young pupa. The long distal part, *t*, contains throughout most of its length undifferentiated cells.  $\times 100$ .

Fig. 27. Three cells from the terminal portion of Ovary E; taken from position 27 in preceding figure.  $\times 1600$ .

Fig. 28. Two nuclei, *a*, from oöcyte, *b*, from primitive nurse-cell. Taken from position 28 in figure 26.  $\times 1600$ .

Fig. 29. Same a little further down the tubule, position 29.  $\times 1600$ .

Figs. 30, 31 and 32. Three cells, the first a primitive nurse-cell and the other two developing nurse-cells. All from Ovary E at places represented by corresponding numbers.  $\times 1600$ .

Figs. 33, 34 and 35. Mitotic figures from developing nurse-cells; from the middle part, *oo*, of ovary E.  $\times 1600$ .

Fig. 36. Nucleus of an epithelial cell from position 36 in figure 26. Line to right represents wall of the tubule.  $\times 1600$ .

Fig. 37. Ovary F. Part of an ovarian tubule from a pupa; the lines *x—x* and *z—z* represent the boundaries of its middle portion, *ov*; only a small piece of the other two parts, distal and proximal is shown.  $\times 100$ .

Fig. 38. Cell from near the base of the distal portion of the tubule, from position 38 in ovary F.  $\times 2000$ .

Fig. 39. Two nuclei from the distal end of the median portion; from place 38 in figure 37. *a*, of oöcyte, *b*, of primitive nurse-cell.  $\times 1600$ .

Figs. 40, 41 and 42. Three primitive nurse-cells, the last one just after division. Taken from places in ovary F represented by corresponding figures.  $\times 1600$ .

Fig. 43. Young oöcyte with beaded chromosomes; from position 43 in figure 37.  $\times 1600$ .

Fig. 44. Same a little older, first before synapsis.  $\times 1600$ .

Fig. 45. Oöcyte and nurse-cells from position 45 in ovary F. *a*, oöcyte in synapsis; *b*, fully developed nurse-cell after division has entirely ceased.  $\times 1600$ .

Fig. 46. The oldest oöcyte and nurse-cell in ovary F. *a*, oöcyte, *b*, nurse-cell.  $\times 1600$ .

### Plate XIII.

Fig. 47. Ovary G. Two nuclei, *a*, oöcyte, *b*, primitive nurse-cell from older tubule; from position corresponding to figure 40 in ovary F.  $\times 1600$ .

Fig. 48. Oöcyte nucleus, *a*, and primitive nurse-cell, *b*, from position slightly proximal to the last.  $\times 1600$ .

Fig. 49. Primitive nurse-cell in chromosome stage; from ovary G.  $\times 1600$ .

Fig. 50. Oöcyte in chromosome stage; to the left part of another oöcyte, and between the two, a double chromatin ring.  $\times 1600$ .

Fig. 51. Oöcyte, *a*, and nurse-cell, *b*, from proximal end of the middle portion of ovary G.  $\times 1600$ .

Fig. 52. Ovary H. Part of the middle portion of an ovarian tubule in which the first chamber is being formed. Proximal to the oldest oöcyte, 56, is the beginning of the oviduct. *Syn*, region in which synapsis occurs.  $\times 200$ .

Fig. 53, 54 and 55. Three oöcyte nuclei from positions represented by same figures in ovary H.  $\times 1600$ .

Fig. 56. Oldest oöcyte in ovary H. Only a small amount of cytoplasm in the neighborhood of the yolk-nucleus has been drawn.  $\times 1600$ .

Fig. 57. Ovary I. An ovarian tubule from a wasp six or eight hours after it has left its cell. *Syn*, position of the synapsis nuclei. The nurse-cells of the oldest chamber only, are drawn in, all cells distal to this are oöcytes.  $\times 100$ .

Fig. 58. Nucleus of one of the epithelial cells which are found in the nurse chambers; from position 58 in preceding figure.  $\times 1600$ .

Fig. 59. Three follicle cells from oldest egg chamber, position 59 in figure 57. The three nuclei are not from adjacent cells but show a resting nucleus, one preparatory to division and one in mitosis. *x*, designates that surface of the cells which lies nearest to the oöcyte.  $\times 850$ .

Figs. 60, 61 and 62. Three nurse-cell nuclei from ovary I, at positions represented by corresponding numbers in figure 57.  $\times 1600$ .

Fig. 63. Half of a nurse-cell from proximal part of the oldest nurse chamber.  $\times 850$ .

Fig. 64. Three oöcyte nuclei *a*, *b* and *c*, and one nurse-cell nucleus, *d*, from place 64 in ovary I, just distal to the synapsis nuclei. The four nuclei are not so close together as in the drawing but were found within a very short distance of each other.  $\times 1600$ .

Fig. 65. Oöcyte nucleus in synapsis, from position *Syn*, in ovary I. Between this and an adjacent oöcyte is a chromatin ring; enough cytoplasm has been drawn to show the darkened portion within the ring.  $\times 1600$ .

Fig. 66. Oöcyte nucleus just proximal to those in synapsis.  $\times 1600$ .

Fig. 67. Part of oöcyte in ovary I, showing the nucleus and the small nuclear-like bodies.  $\times 850$ .

Fig. 68. Nucleus and three of the small bodies from the oldest oöcyte in ovary I.  $\times 850$ .

Fig. 69. Ovary J. Part of an ovarian tubule, from the undifferentiated cells to part where the cells are grouping preparatory to chamber formation, from an older ovary than the last.  $\times 400$ .

Fig. 70. Four dividing cells from position 70 in ovary J.  $\times 1600$ .

Fig. 71. Two oöcytes just distal to the synapsis nuclei. In one the contraction of the strands towards one side of the nucleus, has begun. Just enough cytoplasm has been drawn to show the darkened part in the chromatin ring. Ovary J.  $\times 1600$ .

Fig. 72. Oöcyte nucleus just proximal to those in synapsis. Ovary J.  $\times 1600$ .

Fig. 73. Two oöcytes, *a*, and one nurse-cell, *b*, further down the same tubule.  $\times 1600$ .

Fig. 74. Surface view of oöcyte nucleus. Ovary J.  $\times 1600$ .

Fig. 75. Oöcyte nucleus in which the beaded threads have commenced to break up; from position 75 in ovary J.  $\times 1600$ .

Fig. 76. Oöcyte nucleus showing nucleolus and the irregular masses containing the chromatin granules.  $\times 1600$ .

Fig. 77. Oöcyte nucleus from position 77 in ovary J.  $\times 1600$ .

Fig. 78. Oöcyte with accompanying nurse-cells at beginning of chamber formation. Among the nurse-cells are a number of epithelial; some of these are also around the oöcyte.  $\times 500$ .

Fig. 79. Chamber a little older than the last. The epithelial cells have formed a narrow girdle around the oöcyte.  $\times 500$ .

Fig. 80. A still older chamber.  $\times 500$ .

Fig. 81. Epithelial cell from position 81 in last figure.  $\times 2000$ .

Fig. 82. Nucleus of epithelial cell from position 82 in figure 80.  $\times 2000$ .

Fig. 83. Two of the small nuclear-like bodies from near the oöcyte nucleus of figure 80.  $\times 2000$ .

#### Plate XIV.

Fig. 84. One nurse-and one egg-chamber. From the oöcyte, a process, extends into the nurse chamber. Distal in the oöcyte is the nucleus and near it and on its periphery the small nuclear-like bodies are seen. The proximal end of the oöcyte shows the strand which extends from the oldest oöcyte chamber (PAULCKE), Oöcyte, *oöc*, 0,08  $\times$  15 mm.  $\times 100$ .

Fig. 85. One of the proximally situated nurse-cells from the nurse chamber of last figure. Cell diameter 0,03 mm.  $\times 400$ .

Fig. 86. Nurse chamber and part of an egg chamber, *oöc*. The breaking up of the nurse-cells is noticed in the partial disappearance of their boundaries; the cells no longer fill out the chamber. The opening between the two chambers is seen. Size of oöcyte 18.  $\times 47$  mm.  $\times 100$ .

Fig. 87. Single nurse-cell from nurse chamber of preceding figure. The cell boundary is drawn more regular than it should be.  $\times 400$ .

Fig. 88. Nurse chamber and parts of two egg chambers, *oöc*. The dotted line in the oöcyte (also in figures 84 and 86) shows the cone-shaped mass free from deutoplasm.  $\times 100$ .

Fig. 89. One quarter of a nurse-cell nucleus from one of the nurse-cells of preceding figure.  $\times 400$ .

Fig. 90. Parts of two egg chambers, *oöc*, and between them the nurse chamber, with only three nurse-cells, which belongs to the lower one.  $\times 100$ .

Fig. 91. Part of an egg chamber, *oöc* and two nurse chambers between which a few epithelial cell nuclei can be seen; these represent all that is left of the missing egg chamber.  $\times 100$ .

Fig. 92. An oöcyte nucleus with the surrounding nuclear-like bodies from an oöcyte 0,05  $\times$  0,08 mm. The contents of only a few of the small bodies have been filled in.  $\times 1600$ .

Fig. 93. Nucleus and one of the small bodies from an oöcyte 0,07 mm in diameter.  $\times 850$ .

Fig. 94. Same from an oöcyte, 0,12  $\times$  0,15 mm.  $\times 850$ .

Fig. 95. Same from an oöcyte, 0,14  $\times$  0,24 mm.  $\times 850$ .

Fig. 96. Same from an oöcyte, 0,20  $\times$  0,36 mm.  $\times 850$ .

Fig. 97. Same from an oöcyte, 0,18  $\times$  0,47 mm.  $\times 850$ .

Fig. 98. Same from an oöcyte, 0,29  $\times$  0,47 mm.  $\times 850$ .

Fig. 99. Two dividing cells with the chromatin ring between them.  $\times 1600$ .

Fig. 100. One oöcyte, *a*, and one nurse-cell, *b*, from position just distal to the synapsis nuclei. Oöcyte with two rings.  $\times 1600$ .

Fig. 101. Oöcyte in synapsis showing two of the chromatin rings between it and neighboring cells. The darkened cytoplasm extending through the ring has been drawn.  $\times 1600$ .



Fig. 1.

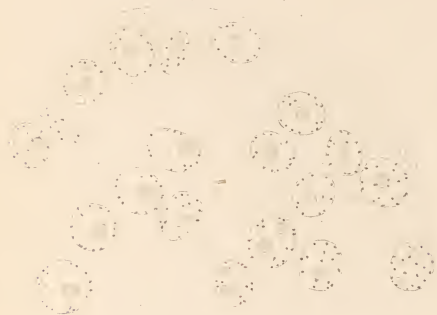


Fig. 2.



Fig. 13.  
Ovary C.

Fig. 17.



Fig. 5.



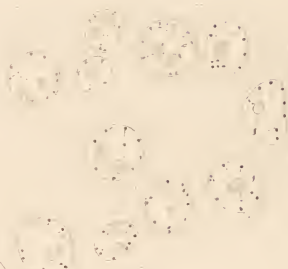
15.  
16.  
17.

odt.

Fig. 4.



Fig. 3.



odt.

Fig. 15.



Fig. 6.  
Ovary A.

Fig. 7.

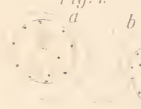


Fig. 19.

20.

21.

22.

Fig. 18.  
Ovary D.

Fig. 8.



Fig. 9.  
Ovary B.

T.

24.

25.

12.

Ov.

Fig. 10.



Fig. 12.



11.

10.

odt.

odt.

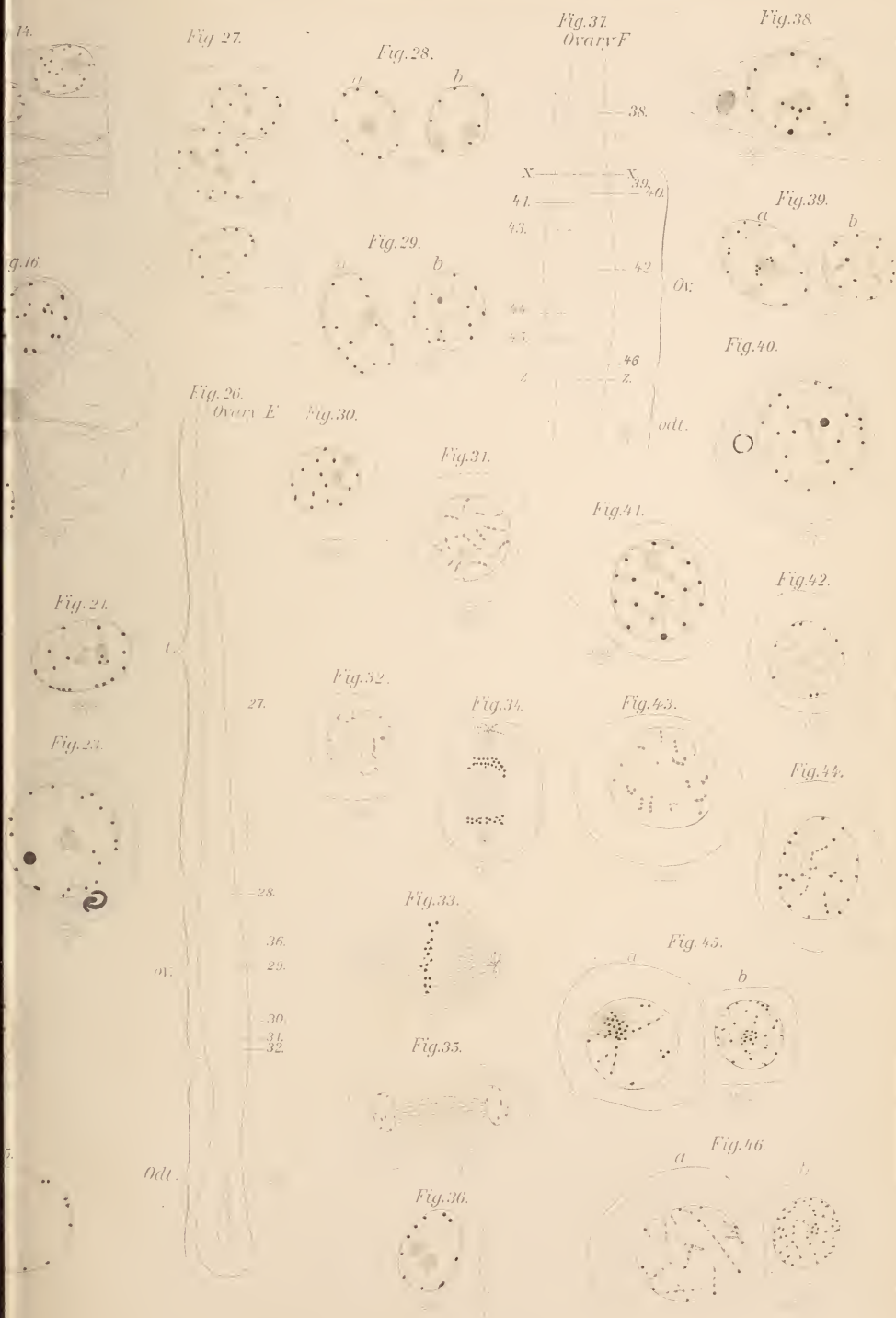
Fig. 11.



odt.

23.





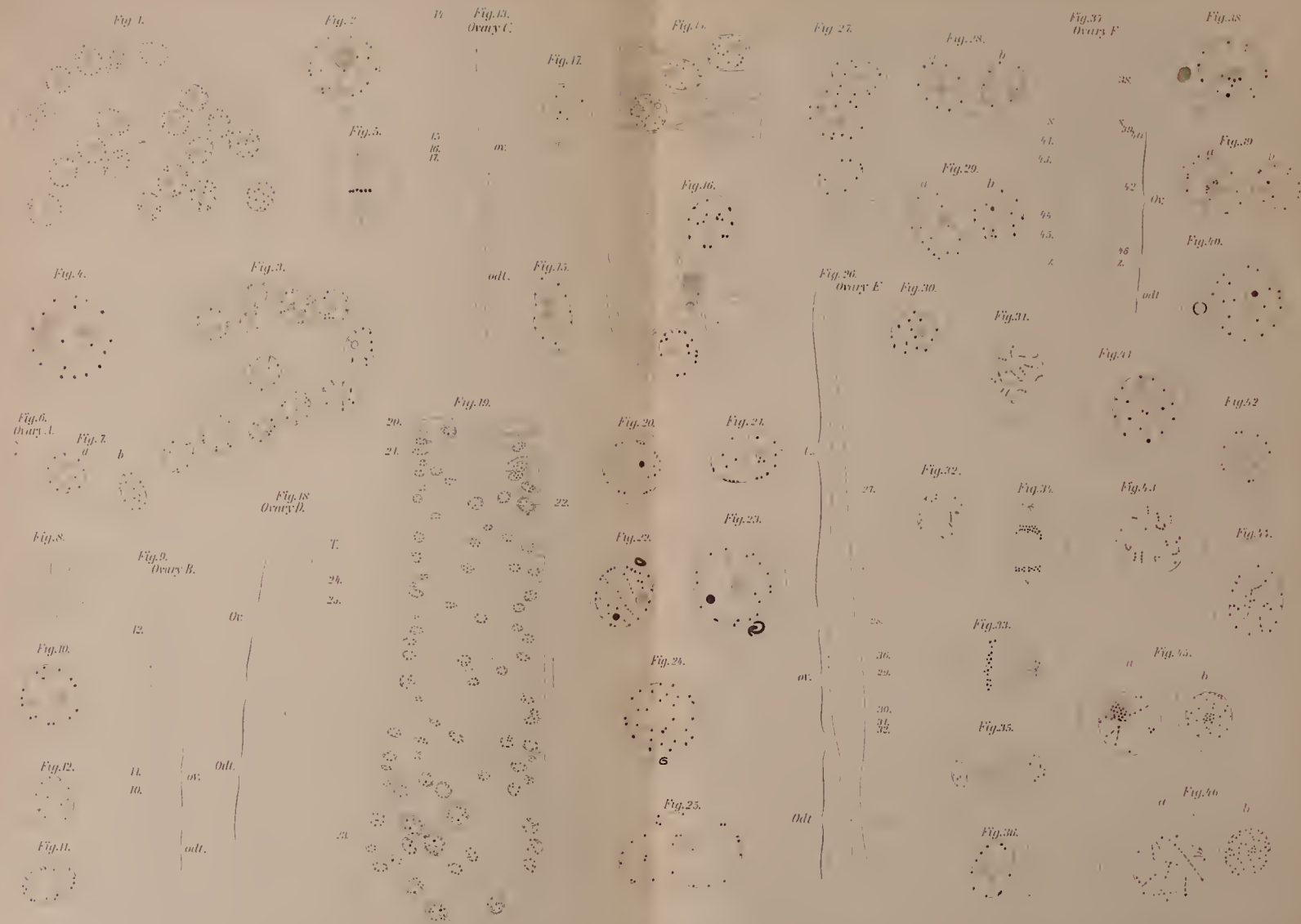




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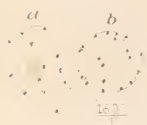


Fig. 49.



Fig. 48.

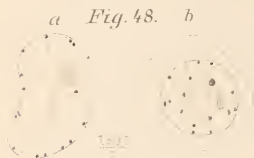


Fig. 50.



Fig. 51.



Fig. 52.

Syn

Fig. 53.



Fig. 55.



Fig. 56.



Fig. 57.



Fig. 58.



Fig. 60.



Fig. 61.



Fig. 63.



Fig. 64.

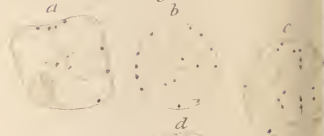


Fig. 65.



Fig. 66.



Fig. 67.



Fig. 68.



Ovary I.

Ovary H.

Fig. 69.

Fig. 70.

Fig. 78.

70

Fig. 71.

Fig. 72.

Fig. 79.

Fig. 74.

Fig. 75.

Fig. 76.

Fig. 77.

Fig. 80.

Fig. 81.

Ovary J

Fig. 73.

a

b

a

Fig. 82.

Fig. 83.

82

81





Fig. 84.



Fig. 86.



Fig. 88.

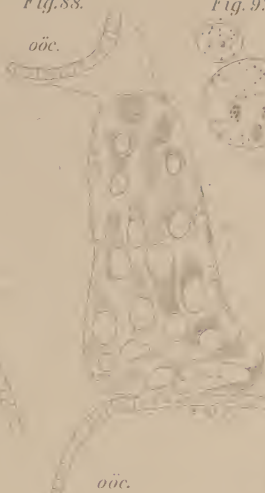


Fig. 93.



Fig. 94.

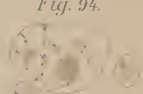


Fig. 95.



Fig. 96.

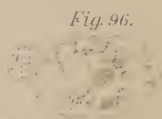


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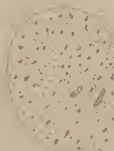


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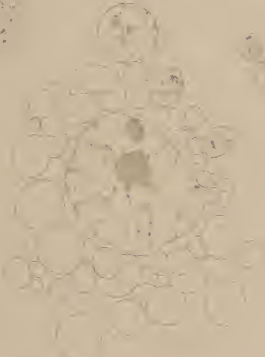


Fig. 97.



Fig. 98.



Fig. 91.



Fig. 85.



Fig. 90.

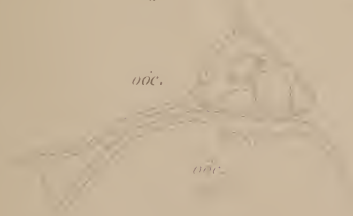


Fig. 89.



Fig. 99.



Fig. 100.



Fig. 101.



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