Nutrition of the Ovum of Scolia Dubia.

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With 2 Plates.

In September, 1906, many larvæ of Allorhina nitida were working in the sandy soil and sparse sod of a certain part of the University campus. These larvæ were being attacked by the very few female Scolia dubia attended by the more numerous males. Specimens of both the larvæ and the wasp were then collected and later sent to Dr. L. O. Howard, Chief of Bureau of Entomology, Washington, D. C., where they were identified by Dr. F. H. Chittenden as "Scolia dubia and its host the 'grub-worm' or white grub, Allorhina nitida, commonly known as the 'green June beetle' or 'fig eater.'"

In September, 1907, the larvæ of Allorhina nitida were very scarce, but in their place an extensive swarm of Scolia dubia appeared. Of these the females, as they crept over the soil or burrowed into it, were caught and various tissues fixed for future study. The material for this work was all taken from adults. The exoskeleton of the adult is so thick as to make it impossible to section the ovaries in place. The ovaries were, therefore, removed from the body cavity with care and fixed. Certain ovaries were fixed in aceto-sublimate two hours, others in chrom-aceto-formalin one hour, and a third supply in Flemming's stronger fluid five hours. All sectioning was done in paraffin. The sections were made 5, 10 and 15 microns thick and mounted in series. All staining was done on the slide. Borax carmine, iron hæmatoxylin, safranin, thionin and methylen green were used in staining. Tissues fixed in chrom-aceto-formalin or in Flemming's stronger fluid and stained with iron hæmatoxylin gave the best results.

The ovary of Scolia dubia is a paired structure. Each member of the pair consists of four ovary tubules which lead into a common oviduct. The histological structure of these tubules is characteristic

GIFT

of the ovary tubules of many insects. Each tubule is anchored with a terminal filament to the peritoneal wall. This filament abuts against a region filled with certain primordial cells. This region of the tubule is called the terminal chamber (Fig. 1). Leading from the terminal chamber is a chain of follicles composed of nurse-cell follicles alternating with egg follicles. In the adult this chain of follicles extends from terminal chamber to the common oviduct.

The terminal filament is composed of spindle-shaped cells which tend to lie parallel to the axis of the filament. Their outline is not well defined. The cytoplasm is densely granular, which makes them conspicuously different from the cells of the terminal chamber (Figs. 1, t. f., and 7, t. f.). The nuclei are oval and have an evident reticular structure; they measure 6 to 8 microns long.

While it is the writer's opinion that the terminal filament is but a peritoneal process serving as an anchorage to the ovary tubule, and having nothing to do with either the origin or the nutrition of the oöcytes, certain early writers have held it to be a more important structure and that by a repeated division of its cellular elements it gives rise to groups of cells which form the primitive elements from which the cells of follicle epithelium, nurse cells and ova were differentiated. The results of more recent investigators point decidedly away from this view. Köhler, '07, deals with this particular feature of the insect ovary and claims that "Bei den Hemipteren ist der Endfaden meist von der Endkammer durch die Tunica propria getrennt. Auch dort, wo dies nicht dar Fall ist, wo sich der Endfaden als Fortsetzung der die Endkammer ausscheidenden Epithelzellen zeigt, besteht eine sharfe Abgrenzung der Epithelzellen des Endfadens gegen die Geschlechtszellen der Endkammer. Der dient ausschliesslich als Aufhängeband."

In Scolia dubia the tunica propria does not completely separate the terminal filament from the cells of the terminal chamber. There is, however, a rather distinct differentiation between the two regions. The cells of the terminal filament stain more deeply than those of the terminal chamber. Between the two regions there can be found no intermediate zone marked by mitotic divisions or other transitional features. (Fig. 7.)

A marked feature of the region in the vicinity of the terminal filament is that peculiar structures appear within the terminal chamber. There are here to be seen oval cells, deeply staining, measuring 10 by 15 microns. Their nuclei are oval and their cytoplasm dense and finely granular. Except for rounded bodies within it the cytoplasm appears homogeneous (Fig. 7 a). At b, Fig. 7, is seen an irregular space containing what may be the remains of one of these peculiar cells broken down. At places smaller cells with what appear to be two, three or more nuclei within each may be found. These figures suggest quite strongly the series shown in Figs. 3 to 19, Taf. I of Will, '86, which this author has interpreted as phases in the development of nurse cells, ova and follicle cells from "oöblasts." The phenomena pictured by Will were at once taken up in dispute by Korschelt, '87. Stuhlmann, Blochmann and Schneider also denounced Will's theory. Among the later writers to dispute Will's interpretation was De Bruyne, '98, who records that some of the cells of the "germigène" undergo a histological transformation characterized by the appearance in the protoplasm of spheres of compact structure serving to support chromophilous fragments more or less numerous. These transformed cells lose their boundaries and their products of degeneration scatter in the cavity of the terminal chamber between the cells which have preserved their general aspect. "Ces produits serrés entre les cellules restées intactes, penetrent ou arrivent par englobement jusque dans celles que l'on reconnaît déjà comme étant les futurs éléments ovulaires et vont y contributer à leur accroissement: il s'agit d'une degenerescence spontanée, debutant et s'achevant sans l'intervention de cellules sanguines et d'une disparition subsequent par englobenment de la part de la cellule-œuf, qui joue ainsi le rôle de phagocyte. Les boules du protoplasme nées dans les cellules nutritives correspondent donc aux produits des oöblasts (Will) et leur partie chromatique est le noyau né, d'après cet auteur, par bourgeonnement de ces mêmes oöblasts. Non seulement des cellules nutritives peuvent, en dégenérant, donner lieu à des substances d'accroissement pour l'ovule, mais l'èpithelium aussi peut secreter de ces boules."

Giardina, '01, shows convincingly that the origin of the nurse cells and oöcytes are quite unlike what Will believed it to be. Giardina describes and figures clearly that an oöcyte and its attending group of nurse cells arise by a series of differential mitoses from a primordial reproductive element of the terminal chamber.

The peculiar features shown in Fig. 7 are found only at the distal end of the terminal chamber. They suggest different stages in the degeneration of certain cells (Fig. 7, a and b). The oöcytes in this region tend to be vacuolated about the periphery and are unusually large. (Compare Figs. 2 and 7.)

The specimens of Scolia dubia were taken very late in their breeding season. It is not probable that all the contents of the terminal chamber would be demanded by the rapidly closing season. This would lead us to expect degeneration phenomena within the distal end of the terminal chamber.

Fig. 7 shows an apparent relation of position existing between one of the unusually large oöcytes and a mass of degeneration products. This is but accidental; for many such bodies are found lying remote from any oöcyte. These products in Scolia dubia, therefore, are considered to have nothing to do with the nutrition of the oöcyte as De Bruyne in the above quotation suggests, but to be mere degeneration products concomitant with the close of the season.

Besides the degeneration cells the terminal chamber contains the primordial elements of future follicle epithelium, young nurse cells and young ova or oöcytes.

Throughout the history of the ovum within the ovary tubule its size increases greatly. The smallest and youngest egg cells within the terminal chamber are about 25 microns long and 20 microns thick. They rapidly grow during their passage down the tubule until they become 1,000 microns long by 350 microns wide. They are throughout this growth highly plastic and readily conform to any irregularities of surface.

During this remarkable growth of the cell body the nucleus remains, so far as comparative measurements of different nuclei show, constant in size. The nuclear contents are highly achromatic except for an irregular mass of chromatin which is always eccentrically situated. This mass is least assembled in the youngest nuclei.

It is interesting to note that the nuclear pattern shows little or no change throughout the entire nutrition of the egg cell.

The terminal chamber of Scolia dubia is limited by a well defined tunica propria, which is composed of a layer of flattened cells similar to the cells of the terminal filament except that they stain less. A similar tunica propria extends throughout the extent of the ovary tubule.

Beneath this layer within the terminal chamber are found the irregularly disposed primordial follicle cells. These cells are crowded within the interstices between the other elements of the terminal chamber. They are the smallest elements within the terminal chamber, their oval nuclei measure about 7 to 9 microns in length. These cells are more or less polygonal in outline and not clearly defined. They are rapidly proliferated by mitoses and as they thus become crowded toward the proximal end of the chamber they assume a spindle shape and lie at right angles to the length of the terminal chamber (Fig. 1, f. c.).

Here they are assembled about an ovum or its attending group of nurse cells to form the follicle epithelium of an egg follicle or the epithelium and scaffolding of a nurse follicle. When entering the formation of the scaffolding of a nurse follicle they have irregular shapes; but in all follicle epithelia the cells are columnar and stand at right angles to the surface of the ovum or nurse cell mass.

Distal to each nurse follicle, follicle cells assemble to separate the newly formed nurse follicle from the egg follicle about to form. In this manner the follicle epithelium develops as a continuous epithelium from the terminal chamber to the end of the ovary tubule; at this latter region it abruptly becomes a much taller columnar epithelium that is more or less convoluted to form the thickened wall of the proximal end of the tubule just as it passes into the structure of the common oviduct. To this thickened region the French give the name "calycule" and the Germans "Wandverdickung."

In the growing follicle cell proliferation continues by mitosis. The nuclei of such follicle cells are oval to spherical and do not stain deeply. About the follicles they elaborate a homogeneous substance called the chorion. Within the folds between the follicles the chorion forms a partition (Fig. 5). As the egg follicle nears its ultimate size, the entire epithelium elaborates a clearly defined cuticle. The cells now become much shorter. The chromatin of each nucleus becomes assembled into four or six irregular masses which stain very deeply. The process continues until the epithelium is reduced to a very thin cytoplasmic layer with greatly flattened deeply staining nuclei.

In Scolia dubia the follicle epithelium is concerned chiefly in the production of the chorion and does not secrete food products for the egg cell; though in the ultimate breaking down of the nurse follicle their disintegration products are most probably taken into the egg cytoplasm for food. Its chief function, therefore, appears to be the formation of the chorion.

The follicle epithelium is, however, in a secondary manner concerned with the nutrition of the egg cell. Bambeke, '97, found that the egg nucleus of Pholcus, at the time yolk is being elaborated out of the material entering the egg cytoplasm from the nurse follicle becomes irregular in contour and at the side nearest the deposit of yolk gives out many slender pseudopods, as if to increase the nuclear surface that many take part in the elaboration of deutoplasm. De Bruyne, '98, quotes Korschelt as saying that the products secreted by the nurse cells are carried to the germinal vesicle and completely transformed by it. Rabes, '00, in speaking of the egg of Rhizetragus solstitialis L., says: "Jedenfalls unterliegt es keinem Zweifel, dass in der Eizelle besonders in der Zeit ihres Wachstums, eine ungemein innige Wechselbeziehung zwischen Kern und Zellplasma besteht, die sich am auffallendsten in den Form- und Lageveränderungen des ersteren zu erkennen giebt."

As over against the apparent remarkable nuclear activity observed in the forms studied by the above investigators, the egg nucleus of Scolia dubia shows no change of contour; its chromatin pattern is comparatively constant throughout the complete nutrition series, and except for its position there is indicated no relation between the egg nucleus and the handling of the food substances from the nurse cells.

In this connection it is interesting to note that there are present at the distal pole of the egg cell a number of smaller nuclei than the egg nucleus, which have a conspicuous nuclear net-work and nucleolus. Their position is a strong indication that they have a rôle to play in the elaboration of deutoplasm or yolk as the secretion of the nurse cells is passed into the egg cell.

Similar nuclei are described by Gross, '01, for the ovum of Vespa vulgaris. The following is quoted from this paper: "In alten Eiern findet sich ausser dem Keimbläschen constant eine Anzahl kleiner Kerne. Dieselben sind zuerst von Blochmann (1886) bei Ameisen und Wespen beobachtet worden. Sie liegen Anfangs in der Nähe des Keimbläschens, entfernen sich aber bald von ihm, rücken an die Peripherie des Eies und bilden hier eine Lage um den grössern Theil des Dotters (Fig. 186). Blochmann nahm an, dass diese Kerne von Keimbläschen abstammen. Korschelt (1886) der ähnliche Gebilde von Musca beobachtete, lässt die Frage nach ihrer Herkunft offen. Bei Hymenopteren, die ich untersuchen konnte, ist die Abstammung der genannten Kerne eine andere und weniger auffallende als die von Blochmann augenommene." * * "Beginnen nun die Nährzellen ihren Inhalt in das Ei zu entleeren, so gelangen auch die Epithelkerne in den Dotter und bleiben hier, nachdem sie die eben erwähnten Ortsveränderungen durchgemacht haben, noch lange erkennbar. Korschelt (1886) meint, dass diese Vorgänge mit der Dotterbildung zusammenhängen. Auch mir scheint dies sehr warscheinlich zu sein. Die Keimbläschen der Hymenopteren sind auffallend klein. Da nun aber, wie Korschelt (1891) gezeigt hat, dem Eikern der Insecten eine wichtige Rolle bei der Umwandlung des dem Ei zuströmenden Nährmaterials im Dotter zugeschrieben werden muss, so könnten die ins Ei gelangten Epithelkerne dem Keimbläschen zu Hülfe kommen und sich mit ihm in die erwähnte Function theilen."

By a careful study including many measurements of egg nuclei no evidence was obtained in support of the above view of Blochmann that these nuclei were given off by the egg nucleus. On the other hand several cases have been found, in which certain follicle nuclei had assumed the appearance of these nuclei and appeared to have been just about to enter the egg cytoplasm. It appears quite probable, therefore, that these migrated nuclei, or as Gross, '01, calls them, "einwandernde Epithelkerne," have been furnished by the follicle epithelium.

The migration suggests the observations made by Metcalf on the ova of Salpa; but the two phenomena differ functionally. In Salpa the nuclei of the follicle epithelium are taken into the egg cytoplasm primarily as a supply of food for the ovum. In Scolia dubia, on the other hand, they have migrated primarily to become the handlers of food material and only when this primary function is terminated do they function in the same manner as the migrated nuclei of Salpa.

At the beginning of the second phase of nutrition when the nurse cells are about to send food material into the egg cell, but one or two of these migrated nuclei are present (Fig. 6). As the egg cell grows and the elaboration of yolk is begun, these greatly increase in number. Along the distal periphery of the egg cell they then form a closely packed layer of clearly defined, rounded to oval nuclei, each of which has a definite nuclear reticulum and a conspicuous nucleolus. They lie in less numbers about the entire periphery of the egg cytoplasm. Those found below the distal fourth of the egg cell are smaller and stain more readily. Have all of these many nuclei come from the follicle epithelium?

In Scolia dubia during the early stages and in favorable places within older egg cells, groups of ova are met with in which one or two large migrated nuclei are found surrounded by smaller ones (Fig. 8). In many other cases large nuclei are found, on which there is a partial constriction which divides the nucleus into a large and a small lobe. In all such cases *two* nucleoli are present; while only few nuclei with two nucleoli and with no constriction were observed (Fig. 9.) These observations have led to the interpretation that the migrated follicle nuclei propagate within the egg cytoplasm by means of amitosis, which results in each case in daughter nuclei of unequal size. In any cases where the egg nucleus is unaided by other nuclei Dotterkerne or yolk nuclei are found. These are so frequent that to give examples in this connection is uncalled for. In the ovum of Vespa vulgaris described by Gross, '01, as having the migrated nuclei, no yolk nucleus is described. Similar conditions are met with the ova of ants and wasps described by Blochmann, '86, and in the ova of Musca described by Korschelt, '86. That ova with these migrated nuclei to aid them in the handling of food material entering them from the nurse cells have in no case a yolk nucleus is significant. It indirectly suggests that the yolk nuclei of other ova are but accumulations of food material that has entered the ovum more rapidly than the cytoplasm with a single nucleus was able to transform it into yolk.

These nuclei are the preparers of the food and are concerned in but a secondary manner with the nutrition of the ovum in Scolia dubia.

We believe that we are warranted in recognizing two phases in the nutrition of the ova of this wasp. The first phase runs its course within the terminal chamber. The second involves the complete history of the nurse follicle after it leaves the terminal chamber. The second phase, therefore, lies entirely outside the terminal chamber.

The follicle cells and their derivatives are now considered somatic cells. Köhler, '07, writes: "Die Zusammengehörigkeit der einzelnen Zellen regelt sich folgendermassen: Als gemeinsamen Ursprungs sind anzusehen: die Zellen des Peritonealepitheles, des Endfadens, des Eiröhrenstieles, des Endkammer- und Follikelepithels. Diesen somatischen Zellen stehen gegenüber die Geschlechtszellen, d. h., die Nährzellen und Keimzellen."

Nurse cells and egg cells have long been known to be the chief cells of insect ovaries. Stein, '47, first recognized them as two distinct elements; but considered them to be masses of homogeneous protoplasm. Meyer, '49, recognized the cellular nature of nurse cells and egg cells.

Folsom, '06, uses a diagram from Lang's Lehrbuch to illustrate his description of three types of ovaries of insects. The first type is represented by ovary tubules composed of a chain of egg follicles without nurse cells. The second type includes the tubules composed of alternating egg and nurse follicles which arise out of a terminal chamber. To the third type belong such ovary tubules as are composed of a series of egg follicles remaining in connection with certain nurse cells, that do not leave the terminal chamber, by means of a protoplasmic strand, which the Germans have called Dotterstränge or Dottergänge and which Lubbock has named yolk ducts. The third type involves, therefore, only the nurse cells as they lie within the terminal chamber. Later it will be shown that Scolia dubia in its nutrition combines the second and third types.

Leuckart, '53, and Lubbock, '60, were the earliest writers to describe the nutrition of the ovum by means of yolk ducts connecting the ovum with the nurse cells of the terminal chamber. Claus, C., '64, describes for Apis platanoides a very characteristic "Dotterstrang" leading from groups of three to six nurse cells to the ovum. Gross, '01, describes for Asopus bidens yolk ducts between the follicle ova and nurse cells within the terminal chamber. Wielowieyski, '06, in describing the ovary of the hemipter Pyrrhocoris apteris says: "Die Dotterzellen sind in einer Endkammer vereinigt und werden mittelst feiner, im Karkraume derselben verlaufender plasmatischer Ausläufer mit ebensolchen plasmatischen Ausläufern der Eizellen verbunden, so dass ein Ernährungssystem entsteht, in welchem dei einzelnen Dotterzellen mit den Eizellen direkt kommuniziren." Kohler, '07, in the ovary of Nepa cinerea describes large yolk ducts which lead from the terminal chamber and give off lateral branches to individual ova.

All the above forms show a nutrition involving nothing but yolk ducts and occurring only through the yolk duct. In Scolia dubia there are two phases of nutrition. The first phase is accomplished within the terminal chamber through short yolk ducts of a peculiar type. Not all the nurse cells take part in this first nutrition phase. The second phase takes place from the nurse follicles and involves all the nurse cells.

The presence of extensive yolk ducts between nurse cells and ova have in the past been taken as sufficient evidence that this was a feature pertaining to the nutrition of the ova. The inference that a nutrition phase of the ovum of Scolia dubia ensues within the terminal chamber is based upon (*a*) the presence of short yolk ducts, and (*b*) the condition of the nuclei of such nurse cells as are attached by these ducts to the ova.

Throughout the extent of the terminal chamber ova are distributed. Except for the one or two at the distal end of the terminal chamber, which are surrounded by apparent degeneration products, all the ova show extremely short yolk ducts which connect them with certain of their attending nurse cells. These yolk ducts have a wall, which appears as a ring formed by the blending or coalescing of a region of the cell membranes of nurse and egg cells, and a central core of cytoplasm. The wall when seen in profile is extremely short, measuring in the youngest stages .25 micron and at its maximum size 1 micron (Figs. 2 to 5). When seen in transverse section it appears as a ring with a comparatively wide body and a diameter of from 2.5 to 4 microns. The wall of the duct stains intensely with all the stains employed as mentioned above. In all the preparations it reveals a homogeneous structure and shows no constituent granules such as Giardina, '01, describes. The cytoplasmic core is not conspicuous except in the late phases of its duration when it becomes greatly elongated (Fig. 5). Unlike the similar structure described by Giardina for Dytiscus this core showed no affinity for particular stains which would give a differential stain.

As the ovum develops and passes proximally through the terminal chamber, the ring-like yolk ducts become more prominent and their core of connecting cytoplasm may be seen. The maximum development is reached before the nurse follicle is formed.

The yolk ducts appear first on all sides of the ovum. After their maximum development is attained, the proximal ones separate from the ovum, and the nurse cells thus freed take a position distal to the ovum which they attend, and enter the cell group of the developing nurse follicle. Thus with the near approach of the complete formation of the nurse follicle, only a few of the nurse cells—the distal ones—retain their yolk ducts (Fig. 5). These few ducts are eventually severed by the crowding of all the nurse cells away from the ovum into the completed nurse follicle (Fig. 6).

The nurse cells may be considered gland cells which secrete material for the ovum. The nuclear pattern of the cells having yolk ducts differs strikingly from that of the other nurse cells. These chromatin differences furnish additional evidence that the yolk ducts above described have to do with a nutrition phase.

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Korschelt, '91, indicated a difference between the nuclei of gland cells that were actually secreting and those that were at rest. He says: "Nach seiner Darstellung enthalten die Kerne secretgefüllter Drüsenzellen ziemlich grobe Chromatinkörner, welche durch Fäden unter einander verbunden sind. Es ist ein derbes Chromatinnetz vorhanden, wie Hermann es bezeichnet. Mit der Entleerung des Secrets findet eine Aenderung der Structurverhältnisse des Kernes insofern statt, als die derben Chromatinbrocken aufgelöst werden und an ihrer Stelle ein feines zierliches Chromatinnetz tritt, das je nach dem Stadium der Secretausstossung noch eine geringe Menge verkleinerter Chromatinbrocken beherberge, bis dieselben in der volkommen secretleeren Zelle gänzlich verschwunden sind. Diese Beobachtungen lassen auf die anschaulichste Weise eine Beziehung der Kerne zu der Thätigkeit der Zelle erkennen." Woltereck, '98, De Bruyne, '99, Rabes, '00, Gross, '01, and others have followed Korschelt in this interpretation. Gross says: "Korschelt (1891) hat entschieden Recht, wenn er diese Erscheinungen als Anzeichen einer starken Betheiligung des Kernes an der secretionschen Thätigkeit der Zelle betrachtet."

In this connection, therefore, it is of interest to note that the nuclei of those nurse cells not attached to the ovum by means of yolk ducts have their chromatin concentrated (Figs. 3, 4, 5). The nurse cells in the distal region of the terminal chamber which have yolk ducts have their chromatin distributed upon a more or less definite, open, reticular net-work (Fig. 2). As the ovum passes down the terminal chamber carrying with it the nurse cells the chromatin in the attached nurse cells becomes finely granular and evenly distributed throughout the nuclear cavity (Figs. 3, 4). In this way the chromatin of these nurse cells behaves in a manner characteristic of many secreting gland cells.

During this phase of nutrition vacuoles appear within the cytoplasm of the ovum together with deeply staining granules. These are held to be nutrition products.

The first phase of nutrition ends with the breaking of the last yolk ducts and the formation of the complete egg and nurse follicles.

The nurse cells of the completed follicles, except for an occasional follicle cell, lie against each other in a compact mass. At the proximal base of the follicle is found a core of follicle epithelial cells. Into this cellular core the ovum sends a cytoplasmic process. The nurse cells have in the meantime undergone an intermediate period of rest and growth (Fig. 6).

With the approach of the second phase of functional or secreting activity the migrated follicle nuclei (described at p. 132) appear within the cytoplasm of the ovum (Fig. 6). The chromatin of the nuclei of the nurse cells becomes evenly distributed. This chromatin feature remains little changed in those cells that have functioned through yolk ducts. In the others the charge of chromatin distribution travels as a wave from the proximal region distally throughout the follicle. The distal nuclei, therefore, are the last to show this chromatin feature.

The nurse cells continue to grow. Their nuclei become more or less irregular in contour. Vacuoles appear in their cytoplasm. Within the ovum the migrated nuclei become abundant and lie at the pole next to the nurse follicle. A large irregular vacuole appears within the egg cytoplasm as the evident recipient of the secretion of the nurse cells (Fig. 10). With these appearances the second phase of nutrition may be considered well under way.

The secretion continues at the expense of the nurse cells. The cytoplasm of some of the proximal cells breaks down. The remaining cells become loosely disposed within the follicle. As the process continues, a wave of cytoplasmic disintegration passes more or less regularly distally through the nurse follicle which is closely followed by the disintegration of the nuclei. In the meantime the nurse follicle collapses (Fig. 10).

The follicle epithelium cells between ovum and nurse cells become loosely arranged so that the secretions of the nurse cells pass freely into the greatly enlarged ovum. These secretions continue to form a vacuole in the multinuclear cytoplasm at the distal pole of the egg cell. By the interaction of the contained nuclei and cytoplasm the material thus taken up is transformed into yolk spherules which are deposited in the proximal half at the periphery of the ovum (Fig. 10, *y. g.*). The deposition of yolk continues until all the cytoplasm has become a reticular meshwork supporting many yolk spherules and the egg nucleus.

The migrated nuclei finally disintegrate to form part of the food supply of the ovum. About the ovum the follicle epithelium builds a complete chorion. These phenomena mark the end of the second phase of nutrition.

The final disintegration of the nurse cells takes place wholly within the nurse follicle. De Bruyne, '98, observed in Dytiscus that after the cytoplasm of the nurse cells had disintegrated the nuclei of these same cells were taken *in toto* into the cytoplasm of the ovum to be consumed by it. Paulcke, '01, discovered a like fate of the nurse cell nuclei in the ovaries of Apis mellifica. In these cases the disintegration of the nurse cell nuclei is similar to that of the migrated follicle nuclei of Scolia dubia.

With this the second phase of nutrition is completed and the ovum with its cytoplasm completely charged with yolk is delivered into the oviduct.

The insect ovary has been a subject of so much scientific investigation during recent years, that except for his observations confirming Giardina's interesting observation, the writer would not be justified in adding to the extensive bibliography. Giardina, '01, was the first to clearly define two phases of nutrition in the ovum of an insect. His observations on Dytiscus has hitherto stood without confirmation. Concerning his priority in this observation he says: "E giustizia notare che, gia nel 1880, Tichomiroff descrisse nel-Bombyx mori un' aperture centrale di communicazione nella parete divisoria tra l'uova e la camera nutrice, e che da questa apertura vedeva penetrare nell' uova sostanza granulosa, simile alla sostanza delle cellule vitellogene. Quantunque la descrizione non sia perfettamente corrispondente alla realta, pure non vi puo esserdubbio che essa si riferisca alle communicazioni protoplasmatiche ova descritte. Anche il Korschelt (1889) non dubitava che delle communicazione tra l'oöcite e le cellule nutrici dovessero esistere, ma gli argomenti da lui adotti non erano molto convincenti."

"Simili connessione attivamente alla nutrizione dell' uovo, e rendono poco verosimile l' opinione del De Bruyne (1898), che esse vi partecipino solo passivamente, lasciandosi divorare dell' oöcyte per via di fagocitosa."

Fundamentally the writer's observations on the ovary of Scolia dubia during the first phase of nutrition are in accord with the findings of Giardina in the ovary of Dytiscus. The heavy walls of the yolk ducts which Giardina describes as being composed of a series of granules were homogeneous structures in Scolia dubia. Within the cytoplasmic core there were no traces of fibrillation, nor could any differential stain be made of this part of the cytoplasm. In these structural details Giardina's Dytiscus material differs from the specimens of Scolia dubia.

Giardina is content to establish the presence of a first phase of nutrition. In Scolia dubia, as shown above, the two phases are distinct; one occurs within the terminal chamber, the other involves only the follicles.

Giardina says that the origin of the yolk ducts is in the unsevered protoplasm and cell membranes of the last differential cell-divisions which result in the formation of a "rosette" of nurse cells attached to an ovum or oöcyte. "L' origine delle connessioni tra l' oöcyte e le cellule nutrici e da ricercasi nella gia noto origine del rosette." Conditions in Scolia dubia indicate that this is a correct interpretation.

Conclusions.

1. The terminal filament does not take part in the supply of the primordial cells from which oöcytes and nurse cells are differentiated.
2. The follicle epithelium is not directly concerned with the nourishment of the ovum.
3. Nuclei within a cytoplasm to which they are exotic may divide amitotically.
4. There are two clearly defined stages of nutrition of the egg.

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Plate I.

Fig. 1. Terminal chamber. t. f., sections of terminal filament; a., degeneration space; e., egg cell; n., nurse cell; y. d., yolk duct; f. c., follicle cells. × 500.

Fig. 2. Two egg cells from distal end of terminal chamber. The lower one shows two nurse cells in a chain of yolk duct attachment. The nurse cells so attached to the egg cell do not indicate secretion activity. y. d., yolk ducts. × 1000.

Fig. 3. An older egg cell. The chromatin of the nurse cell having a yolk duct now shows a marked contrast with other nurse cells. v., vacuole. × 1,000.

Fig. 4. Later stage than preceding. × 1,000.

Fig. 5. The yolk duct about to break permitting the final nurse cell to enter the nurse follicle. ch., chorion. × 1,000.

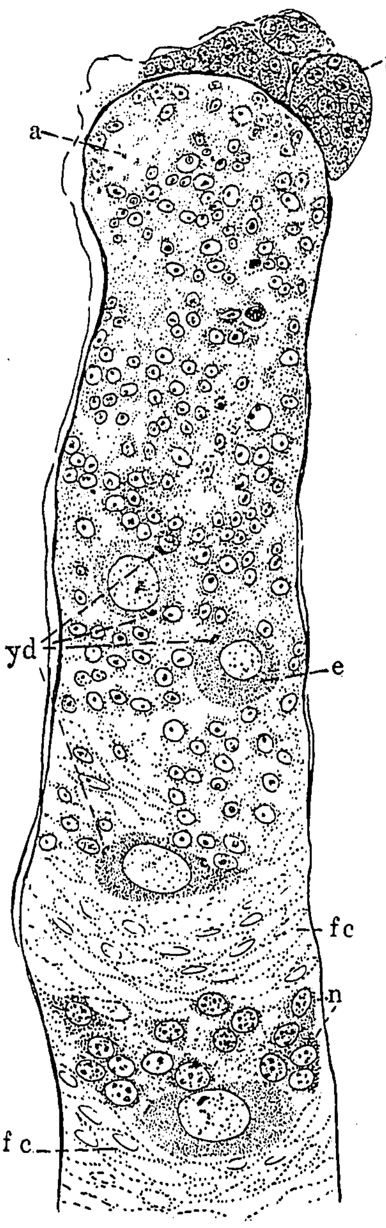
Fig. 6. Completed nurse and egg follicles. e. n., egg nucleus; m. n., migrated nuclei. × 500.

Fig. 7. Distal end of terminal chamber showing degeneration features, a. and b.; t. f., cells of terminal filament. × 1,000.

Fig. 8. Group of migrated nuclei. × 1,000.

Fig. 9. Amitoses of migrated nuclei. × 1,000.

Note.—Figs. 2-9 drawn with Zeiss camera lucida, Zeiss comp, eye-piece No. 6, Zeiss apochrom. 1.5. Figs. 1 and 10 were drawn with Bausch & Lomb camera lucida; B. & L. eye-piece No. 1 and 1-6 and 2-3 objectives.



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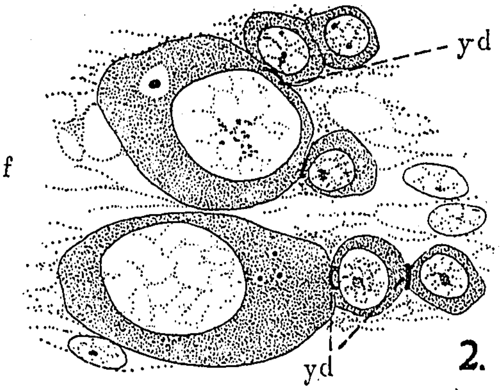

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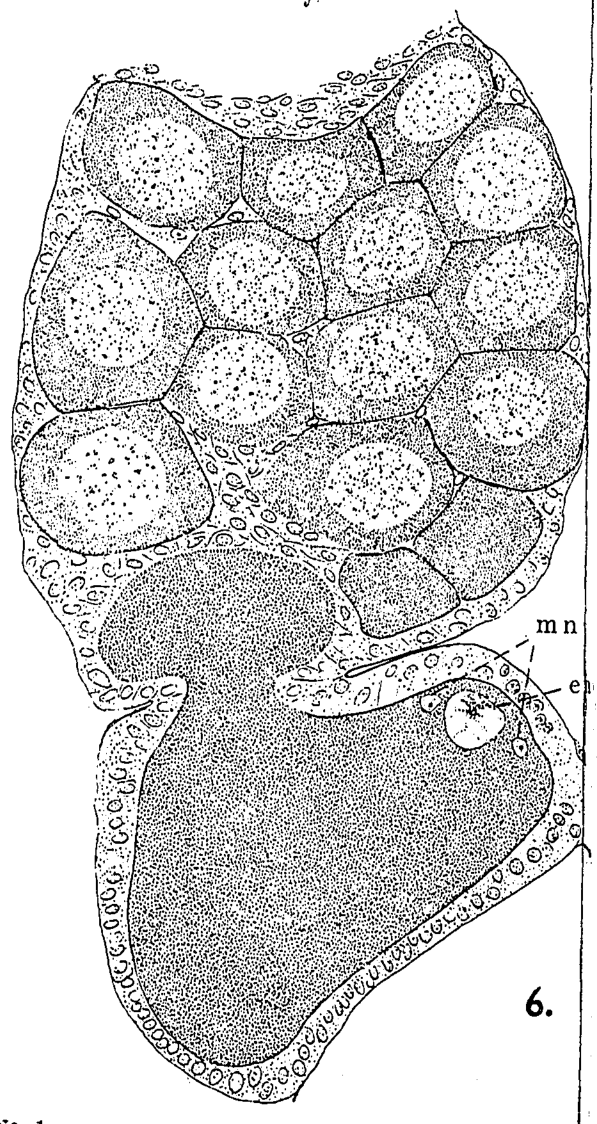

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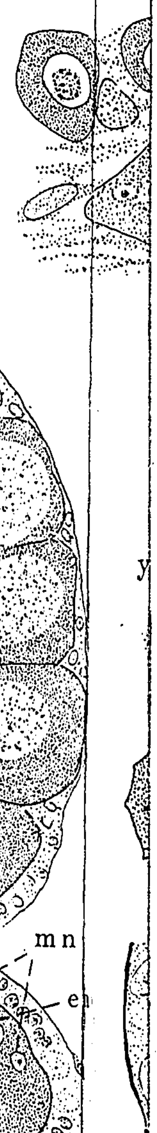

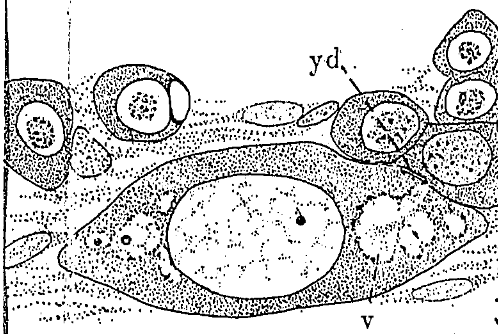

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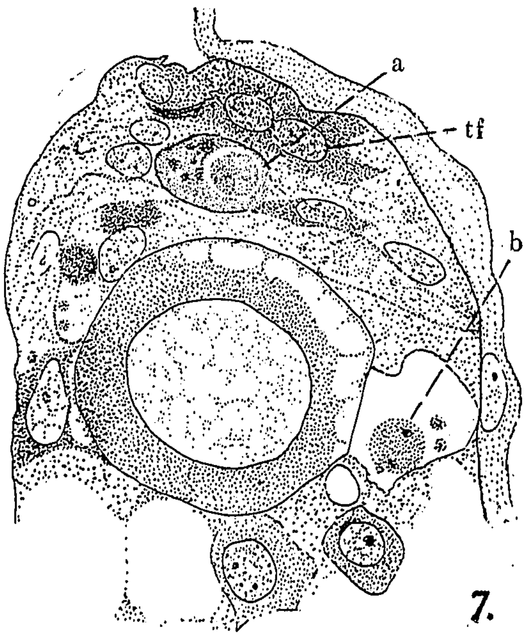

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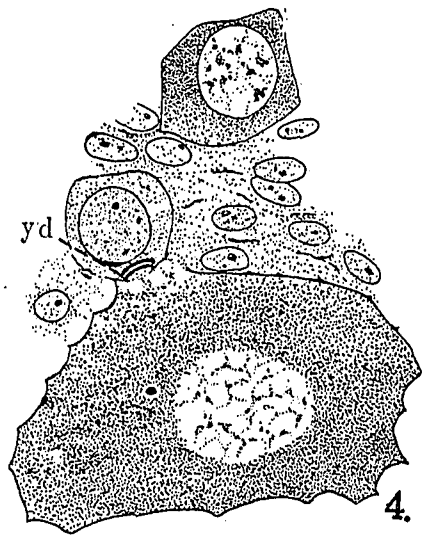

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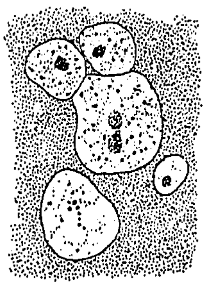
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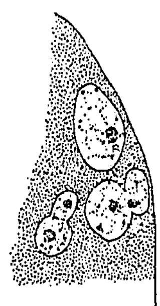
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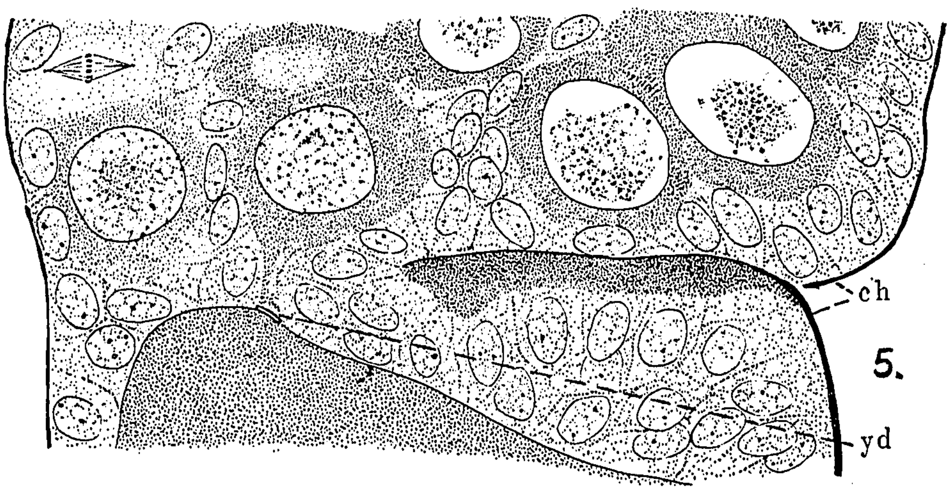
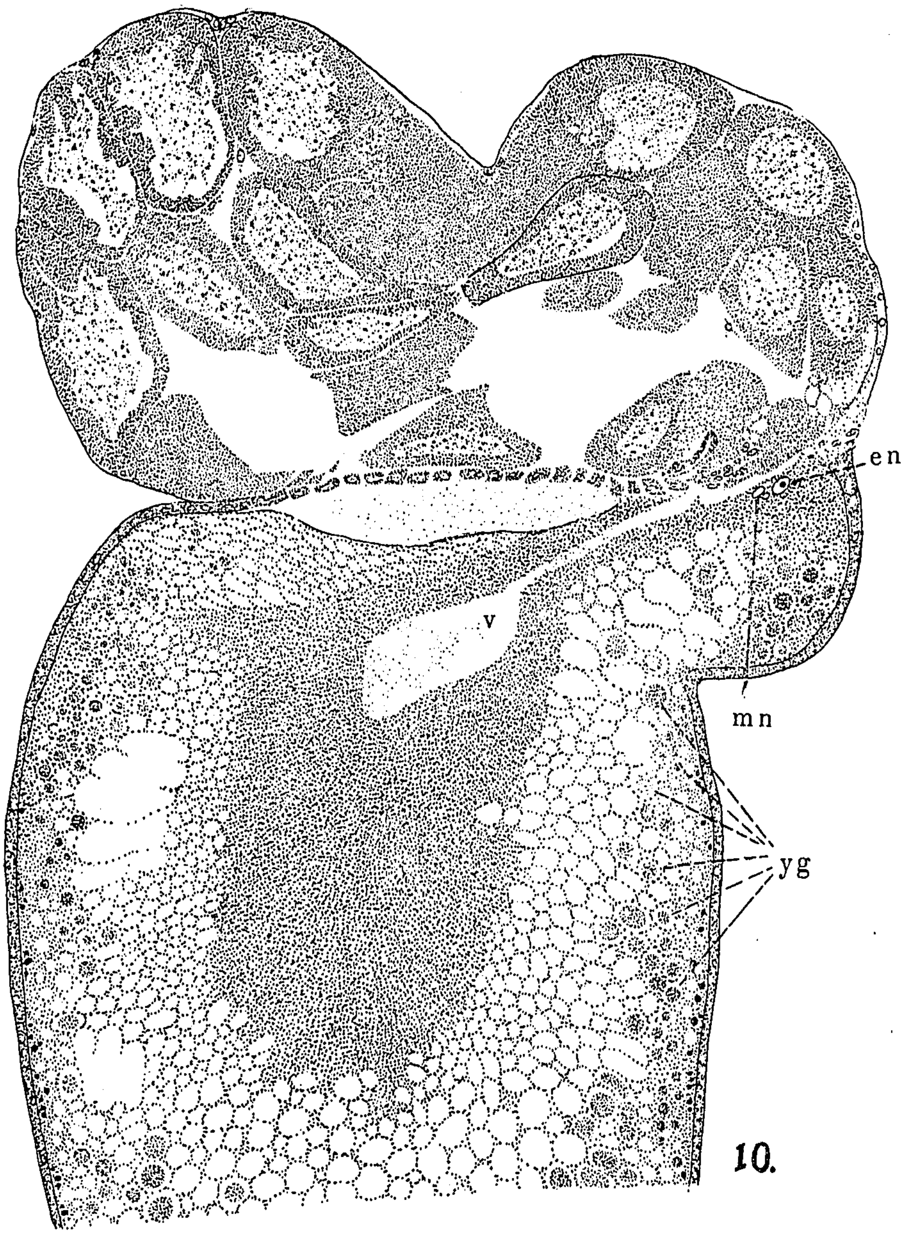


5.

PLATE II.

FIG. 10. Late stage in the second nutrition phase of ovum. e. n., egg nucleus; m. n., migrated nucleus; y. g., yolk granules; v., vacuole. × 166.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