A STUDY OF THE EMBRYONIC DEVELOPMENT OF THE ROCKY MOUNTAIN SPOTTED FEVER TICK

Dermacentor andersoni (Stiles)

(Acarina, Ixodidae)

A Thesis submitted to the Faculty of Graduate Studies in partial fulfilment of the requirements for the degree of Master of Arts in the Department of Biology,

University of Saskatchewan.

by

Martha Pressesky

Saskatoon, Saskatchewan

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INTRODUCTION

The following work describes some phases of the embryonic development of the Rocky Mountain spotted fever tick, Dermacentor andersoni (Stiles), from the time of oviposition until the embryonic development is completed and the larval tick emerges from the chorion.

Research into the embryonic development of Dermacentor andersoni was undertaken for several reasons. The problem was of interest to the writer, since as research into the broad field of arthropod embryology, it was felt to be closely related to the writer's previous study and research in the field of insect embryology. It was thought that elucidation of the various phases of tick embryology might later enable comparison with the comparatively well established concepts concerning embryonic development in insects.

An investigation into the literature dealing with the embryology of arthropods shows that considerable information has accumulated concerning the embryology of the Acarina. Most of this, however, deals with the embryonic development of the mites, and very little describes embryology as found in the ticks, specifically. In addition to the scarcity of information on this subject, almost all the investigations were done in the latter part of the nineteenth century, and are not readily available. No descriptions of the embryonic development of any member of the genus

Dermacentor has been found. The limited information as well as the fact that it is not available without difficulty seems to warrant the present effort.

Many members of the order Acarina are of medical and economic importance. Most ticks are vectors of disease and this is true of Dermacentor andersoni. This species of tick is a vector of Rocky Mountain spotted fever, tularemia, Colorado tick fever, and "Q" fever, and is the cause, in human beings and some domestic animals of the little understood condition, tick paralysis. It is known that in three of the diseases named, the causative organism is transmitted hereditarily from the adult to the larva through the egg. The causative organisms thus transmitted are Dermacentroxenus rickettsi, causing Rocky Mountain spotted fever; Pasteurella tularensis, causing tularemia; and Rickettsia burneti, causing "Q" fever. In order to determine the relationship of the causative organism to the embryonic tissues, knowledge of the embryonic development is required.

The Rocky Mountain spotted fever tick is of particular importance in Saskatchewan since the southwestern part of the province lies within its area of distribution. The Tick and Bubonic Plague Survey of the Saskatchewan Department of Public Health reports finding Dermacentor andersoni infected with a highly virulent strain of tularemia in a number of localities. This was found at Govenlock, Maple Creek, Eastend, Shaunavon, and Val Marie in 1950, and at Eastend in 1951 (1). Medical reports also indicate

the presence of some Rocky Mountain spotted fever infection in these areas. Since Dermacentor andersoni plays such an important role in the transmission of several serious diseases, and since the causative organisms in several instances is passed on from the adult to the larva through the egg stage, it is of importance that the complete development of the tick Dermacentor andersoni be understood.

Dermacentor andersoni requires two full years to complete its life cycle. It passes through three morphological stages--the hexapod larva, the eight-legged nymph, and the sexually mature adult. Each stage requires a mammalian blood meal before moulting. The rather lengthy period required for complete development in the post-embryonic life of this tick is in accord with the long embryonic period of six weeks.

REVIEW OF LITERATURE

Only a few references dealing with tick embryology are available.

A small section of Volume Three of Korschelt and Heider's Textbook of Embryology, published in 1899, is devoted to a survey of embryonic development in the Acarina. This section endeavors to summarize the chief investigations conducted in this field by using them to give a composite picture of embryonic development, and points significant variations from what appears to be the general scheme of development. At no point does the description enter into detail, and is in many parts completely lacking in information. Judging from the investigations reported by Korschelt and Heider most of the work in embryology of the Acarina up to 1900 was concerned only with the mites. Considerable space is devoted to a discussion on the formation and significance of larval integuments during embryonic development. Another lengthy discussion concerns the relationships of the Acarina within the phylum Arthropoda based on embryological evidence.

The only complete account available of embryomic development in ticks is a resume of a very detailed investigation made by Julius Wagner. His complete work was published in 1894 in Russian, and is of a high degree of excellence. The subject of his investigation was the tick Ixodes calacratus,

which belongs to the same family (Ixodidae) as Dermacentor andersoni. The embryonic development of each is similar in most respects. Wagner, like Korschelt and Heider, has given a thorough account of the embryonic integuments of the Acarina, and his opinion of their significance. Wagner does not include any details of his methods, and this is very unfortunate, since the preparation of the material for study involves a number of serious difficulties.

The only other reference available was a small portion of Nordenskiolds brief account "Sur Ovogenese und Entwicklungsgeschichte von Ixodes reduvius" published in Helsingfors in 1910. This research is of little value to the present study since Nordenskiold was concerned mainly with post-embryonic development.

The foregoing again emphasizes the small amount of work done in tick embryology.

MATERIALS AND METHODS

Engorged, fertilized female ticks wre placed in small, round, flat , tin containers, kept under glass. Protection from direct daylight was provided by a sheet of heavy brown paper placed over the glass. It was not necessary to supply hosts for further feeding, since only one complete blood meal is required by the adult.

The flat unfed female tick averages 4 mm. in length. The gravid female onthe other hand measures 15 mm. in length, and its body is very cumbersome. Consequently, the gravid females scarcely move about unless disturbed. Thus two or three ovipositing females may be placed in the same container and their egg masses will remain separate.

Each female may produce several thousand eggs during the period of oviposition. The eggs measure on the average 0.54 mm. in length and 0.35 mm. in width. Oviposition seems to be a continuous process and lasts approximately thirty days. Several hundred eggs per day are produced in the first ten days of oviposition, the number becoming smaller thereafter, and gradually falling off to between twenty and thirty eggs per twenty-four hour period. As oviposition proceeds, the turgid body of the gravid female slowly shrinks and shrivels, becoming flat, and the color changes from greyish to yellowish orange. Soon after oviposition has been completed, the female tick dies.

The eggs are laid in a loose but neat mass. As each egg leaves the genital opening which is located anteriorly, it is pushed forward in front of the capitulum so that the egg mass surrounds the anterior part of the tick. A thin coating of an adhesive substance covers the egg, quite likely the means by which the eggs are held together. Later this adhesive substance hardens, and it is simple to remove a number of eggs from the egg mass with needles.

In order to secure a series of developmental stages over the entire period of embryonic development, egg masses were timed and removed from the tick within an hour of being deposited, and allowed to develop until eggs of the required age wre obtained. Both ticks and eggs were kept at room temperature varying from 68°F to 72°F. The eggs wre placed in small shell vials, covered with distilled water and placed in a water bath which was heated to boiling. This process fixed the tissues. The vials were removed as soon as the boiling point was reached, and when they were cooled, the water was drawn off the eggs and replaced with the preserving fluid, 4 per cent formalin. Bouin's Fluid was used as an alternative fixer with preservation in 70 per cent tertiary butyl alcohol. The results achieved were approximately the same as with boiling water and formalin.

Several difficulties were encountered in preparation of the material for study. Each egg is completely

covered by a tough chorion through which fluids penetrate only with difficulty. The first slides completed showed that infiltration of the paraffin was insufficient. The sections appeared to be torn and collapsed. In order to insure a more thorough infiltration some means of exposing the internal tissues is necessary. This was accomplished by placing the egg in a minute depression in the surface of a lucite block, and slitting the chorion with a fine sharp needle under a binocular microscope. This method was successful in more advanced stages of development, but in early stages the puncturing of the chorion was sufficient to disintegrate the egg contents. Sometimes, particularly in advanced stages, the chorion split and of its own accord came away from the rest of the egg, the contents apparently retained by the peripheral development of the embryo. Such eggs sectioned fairly well. Another method of removing the chorion to obtain thorough infiltration was to place the eggs in a solution of sodium hypochlorite, a method used by Slifer(11). This solution seems to soften and dissolve the chorion. It was found that a three percent solution of sodium hypochlorite affected the chorion of different eggs in varying degrees—from partial to complete dissolution. The ease with which infiltration will occur in an egg thus prepared can be determined by placing the egg in a solution of Borax carmine. Eggs from which the chorion is removed stain a deep red quickly. Those which have an

opened chorion gradually acquire a red color. If the chorion is intact, the stain does not even adhere to the external covering of the egg.

All eggs were stained *in toto* in Borax carmine before further processing. Then they were dehydrated in tertiary butyl alcohol, and embedded in the flat surface of a prepared paraffin block. The paraffin used was Parlax. Parlax has less tendency toward crystallization than ordinary paraffin, and has a somewhat rubbery consistency, which is an advantage in sectioning. Its melting point is 54°C.

In addition to the difficulty encountered in infiltrating the eggs, a second problem arose in attempting to orient the eggs for serial sectioning. Unlike many arthropod eggs, these possess no characteristic pigmentation or reticulation which may be used as a guide in orientation. The ovoid-oblong shape of the egg permits orientation in relation to either the long or short dimension but the dorsal, ventral and lateral aspects remain unknown until the material is studied. In the very early stages no orientation is possible.

Serial sections were made in thicknesses varying from seven to ten microns, depending on the degree of difficulty encountered in sectioning a particular stage. In staining, Delafield's haematoxylin with eosin counterstain was used for the most part and was found to give satisfactory results. Other stains tested experimentally

and found useful were Cresyl Violet, Mallory's Acid Eosin, and Bodian's Protargol.

The illustrations were prepared by projecting the sections on a screen using the Bausch and Lomb triple purpose microprojector, in addition to study by means of a Spencer oil immersion monocular microscope. The drawings were made on ordinary bond paper and then transferred to bristol board. All drawings are somewhat diagrammatic.

EMBRYOLOGY

The embryo of Dermacentor andersoni requires between five and six weeks to complete its development at 70°F. Development seems to proceed quite slowly for the first third of the embryonic period, and there appears to be a marked acceleration of development in the last third.

1. The Egg

The eggs of Dermacentor andersoni resemble those described for other Ixodid ticks. They are oblong-ovate in shape and measure on the average 0.54 mm. in length and 0.35 mm. in width. The surface of the egg is smooth and glossy, lacking the sculpture or reticulation characteristic of many arthropod eggs. The chorion is a structureless membrane, transparent and colorless, and has a tough, somewhat brittle texture. No micropylar opening could be found.

In the egg mass the eggs adhere lightly one to another but there are spaces between the eggs so that even those in the center of the mass are exposed to the air.

At first the eggs are a translucent light yellowish brown in color. As embryonic development proceeds the color changes to a deep rusty brown. The growth of the Malpighian tubules may be observed externally in the developing eggs. At first these appear as a pair of elongate white marks close together on the ventral surface. Much later the Malpighian tubules appear as curvingwhite lines

growing cephalad on either side of the embryonic central nervous system.

The chorion appears to be the only membrane covering the contents of the egg. There is no vitelline membrane as is found in insect eggs. The yolk is found directly beneath the chorion. In fresh unpreserved eggs the yolk granules seem to be spherical. The yolk spheres are fairly large and are of a homogeneous texture. When fixed and preserved, the yolk granules shrivel and assume angular and irregular shapes. In early embryonic development these large angular yolk granules fill the entire egg. As the development of the embryo advances, the particles of yolk break down in size and the amount present becomes progressively less. However, some yolk is present in the mesenteron of the larva as late as two weeks after hatching.

Eggs which are in a very early stage of development do not show the network of protoplasmic strands generally evident in early stages in insect eggs. The only cytoplasm visible at this time is in a small area surrounding the nuclei.

In eggs which are less than one hour old the female pronucleus may be observed in the process of the first maturation division at the periphery of the egg (Fig. 1).

2. Fertilization and Cleavage

In Dermacentor andersoni, it seems most likely that the union of the egg nucleus and sperm takes place following oviposition. This is in agreement with Wagner's observation in Ixodes calcaratus(12) but differs greatly from the condition found in Dermacentor variabilis by Zebrowski (14). The latter's investigation of the reproductive system of the engorged female of Dermacentor variabilis shows that eggs in the uterus and oviducts pass through the stages from zygote to blastoderm formation before oviposition occurs. In the gravid female the entire reproductive tract is filled with eggs in the stage of complete blastoderm formation. In the non-gravid female Zebrowski reported finding spermatozoa all along the course of the oviducts, which led him to believe that the developed intrauterine eggs must have been fertilized. The extent to which embryonic development has proceeded before oviposition, is in keeping with the comparatively short period required for the complete life cycle of Dermacentor variabilis. According to Matheson Dermacentor variabilis requires as little as fifty-four days for the entire cycle from egg to adult, providing there are favourable food and climatic conditions (7).

In the study of the eggs of Dermacentor andersoni which were fixed within one hour after oviposition, the first stage observed is that of the first maturation division

of the female pronucleus. The dividing female pronucleus is located near the periphery (Fig. 1). It was not possible in this division to count the number of chromosomes, but it is evident that the number is small.

In eggs four hours old what is believed to be the synnucleus has appeared, and it is possible to see the three polar bodies which are the products of the reduction division of the female pronucleus(Fig. 2). The polar bodies are found at the periphery,just under the chorion, and the synnucleus, a large nucleus in comparison to the polar nuclei, is found in the same region but deeper down among the yolk particles. The synnucleus is apparently in the process of movement to the central part of the egg, since in other eggs it has been observed in that location, and from which point subsequent divisions seem to take place. Although fertilization has not been observed by the writer, it is believed to occur within a short time after oviposition. It is possible that it occurs during the inward migration of the female nucleus. The polar bodies have not been observed in subsequent stages so it is possible that they undergo a rapid disintegration.

The synnucleus moves toward the centre of the egg. Here the first division takes place perpendicular to the long axis of the egg, approximately twelve hours following oviposition.

The products of this division migrate to opposite poles of the egg. Each nucleus is surrounded by a small mass of

cytoplasm (Fig. 3). When these first cleavage nuclei have moved approximately half the distance to the pole, each nucleus divides again, this time in a plane perpendicular to the plane of the first division (Fig. 4). By the time the egg is twenty-four hours old another division has occurred, this division being in a plane perpendicular to the plane of each of the preceding divisions. The nuclei continue dividing, at the same time moving toward the periphery of the egg. They are uniform in appearance, and by the fourth day of development the eggs have a layer of cleavage cells slightly below the surface. The cytoplasm surrounding each nucleus in the layer seems to be attenuated in a plane parallel to the surface of the egg, (Fig. 5), and is in contact with the cytoplasm of the adjacent nuclei.

Nuclear division takes place as outward migration continues, until, when the cleavage nuclei reach the surface of the yolk just under the chorion, the yolk is completely covered with a single layer of cells, uniformly spaced (Fig. 6). Nuclear divisions are only rarely observed, but in the few cases where they are seen, they occur in a large proportion of the cells simultaneously. This indicates synchronous cell division. When the serial sections show the cleavage cells in the process of mitotic division, those cells dividing which give a polar view of the metaphase plate show the apparent number of chromosomes to be eight (Fig. 7). Wagner(12) was able to observe some mitotic divisions in a polar plane in the metaphase stage, and his

conclusions were that the chromosome number for Ixodes calcaratus was either eight or ten.

Considerable interest has centered around the nature and mode of the outward movement of the cleavage cells in arthropod eggs. Gross and Howland (4), in their study of Prodenia, assume that the outward bound cells move through the yolk by means of a digestive action. Eastham's (3) work on Pieris agrees with this on the basis that during the outward migration he has observed a change in the yolk that has been traversed by the outward migrating cells. The yolk through which the nuclei have passed appears to be clearer than that not yet invaded. In Dermacentor andersoni also, the cleavage nuclei appear to move through the yolk particles by a digestive action (Fig. 5), although there is no apparent change in the appearance of the yolk through which cleavage nuclei have already passed. These observations are at variance with the condition observed in Mamestra configurata by Rempel(10). The latter found that there was an attenuation of the yolk in the region of migration, which he believes is not due to any digestive action on the part of the cleavage cells, but to mechanical displacement of the yolk globules by the outward migrating cleavage cells.

The possible cause underlying the peripheral migration of the cleavage nuclei is also suggested by the above workers. Eastham (3) is of the opinion that a

centrifugal streaming of the reticular cytoplasm draws the nuclei through the yolk to the periphery. In Mamestra, Rempel(10) describes the cytoplasm surrounding the migrating cleavage nuclei as "comet shaped" with the nucleus located in the head of the comet. He feels that peripheral migration is the result of the independent outward movement of the cleavage cells, the movement plausibly under the "guiding influence" of the nuclear material.

In Dermacentor andersoni, neither of the above assumptions are particularly applicable since, in the case of Eastham's theory, there is no reticular cytoplasm visible in the egg, and in the case of the second theory, there is no indication of nuclear control suggested by the position of the nucleus in a comet-shaped mass of cytoplasm. However, nuclear material may be responsible for the peripheral migration, and the difference in shape of surrounding cytoplasm may be due to the difference in the length of time which is required for the completion of outward migration. In Mamestra the cleavage cells reach the periphery in six hours, whereas in Dermacentor andersoni the same stage requires four days, although the eggs are almost the same size.

3. Formation of the Yolk Cells

As soon as the yolk has been covered by a layer of uniform evenly-spaced cells, the blastoderm is complete and the formation of the yolk cells commences. The first indication of their development is the appearance of a new type of cell (Fig. 8), which seems to be distributed evenly throughout the entire blastoderm. The nuclei of these cells are much larger than those heretofore observed in blastoderm cells, and are of a coarse granular texture. They do not stain deeply, and one or two prominent nucleoli may be observed in them. Yolk cells may be observed in various stages of their development from the time they first appear in the blastoderm until they sink down among the yolk granules, and the blastoderm closes over them. At first the yolk cells sink just below the blastoderm, still adhering to it, (Fig. 9), then they separate from it, and gradually sink down into the yolk (Fig. 10). They seem to be evenly distributed throughout the yolk. Wagner claims that in Ixodes calcaratus the yolk undergoes a process of segmentation into large spherical masses, each one including a yolk cell(12). Such a condition may exist in Dermacentor andersoni also, since the evenness of distribution of yolk cells might indicate that one yolk cell exerts its effect upon a particular quantity of yolk. However, there is little indication of a secondary segmentation of the yolk particles into spherical masses in

Dermacentor andersoni. Wagner does not make it clear whether or not the secondary segmentation of the yolk in Ixodes calcaratus persists.

By the end of the eighth day the process of yolk cell formation is completed and there are approximately thirty yolk cells distributed throughout the yolk. As embryonic development progresses, the yolk cells and the yolk undergo a number of changes. Gradually, the yolk particles in close proximity to the yolk cells are broken down and appear as much smaller particles, and in general, the quantity of yolk diminishes so that the partially developed egg does not appear to be so densely filled with yolk. The yolk cells tend to lose their enveloping cytoplasm as development of the egg proceeds; the nuclei lose their coarse granular appearance and nucleoli. Some yolk is present in the mesenteron of the tick larva even after it emerges from the chorion, and is apparently utilized in the first weeks of larval life prior to the first blood meal. In tick embryos which are on the point of hatching, a few pale yolk cell nuclei may be observed.

In certain orders of the Insecta the formation of yolk cells takes place in a different manner. In Collembola, Orthoptera, Strepsiptra, and Lepidoptera the yolk cells are formed when some of the cleavage nuclei lag behind the nuclei moving outward to form the blastoderm. This is quite different from the method of formation in Dermacentor andersoni. Although the process of having

the yolk cells differentiate from the blastoderm has been observed in some orders of Arachnida, it has not been demonstrated to be the general rule (6).

4. Formation of the Endoderm

When the egg is in its eighth day of development the first cells of the endoderm form. Three or four cells, which in appearance resemble the yolk cells, differentiate simultaneously on the dorsal side of the egg (Fig. 11), in the region of the future caudal lobe of the germ band. The nuclei are large and of a granular texture. They stain lightly, as do the yolk cell nuclei.

The endoderm cells appear in only a small area of the caudal region, and at first the cells formed lie close to the cells of the blastoderm. As more such cells appear those first formed are pushed into the yolk. In sections through this part of the caudal lobe, either transverse or longitudinal, the endoderm mass gives the impression of being pyramid-shaped, the base of the mass being at the periphery of the egg. Those cells which have been pushed furthest into the yolk show distinct cell walls (Fig. 15) which are not apparent in the newly formed cells. In this case, the cell walls may be more in evidence due to pressure on the membrane from the cells formed later resulting in concentration of granular cytoplasm at the cell boundary.

Wagner (12) considers the stage of immigration of endoderm cells to be the gastrula phase.

5. Formation of the Mesoderm

Soon after the first endoderm cells have been formed, mesoderm cells begin to appear. They begin as a disturbance in the evenly distributed blastoderm cells in the region of the endoderm. The blastoderm cells covering the endoderm mass proliferate and also concentrate in this area, becoming tightly packed (Fig. 12). Then, on either side of the endoderm mass a small pouch appears into which mesoderm cells rapidly migrate from the blastoderm. In this manner, two strips of mesoderm are formed, extending forward from the caudal lobe along the germ band, toward the caudal pole of the egg. The two pouches in being filled with mesoderm cells form a pair of caudal protuberances externally, separated by the caudal groove (Fig. 13). The caudal groove at the dorsal part of the egg, just at the point of endoderm formation, is deep, and constitutes the beginning of the proctodaeal invagination. The caudal groove becomes less distinct toward the caudal pole of the egg (Fig. 14).

The forming mesoderm cells are easily recognized since they differ greatly in appearance from the endoderm cells. Mesoderm nuclei are small, of an even texture, and stain deeply. They are surrounded by a fine layer of cytoplasm, and cell walls are not discernible.

When the mesoderm cells migrate into the pouch-like formation on either side of the caudal groove, they

at first tend to line the pouch, so that there is a longitudinal cavity within the strip of mesoderm cells. Later the cavity is obliterated and an inner and outer layer of mesoderm cells is formed.

6. Formation of the Body of the Embryo

(a) The Embryonic Membrane

When the blastoderm has just been completed, the cells forming it are uniformly distributed. Simultaneously with the development of the endoderm mass, however, the blastoderm cells begin to concentrate toward the posterior end of the of the egg's surface to form the germ band. Due to continuing cell division and cell concentration, the cells in this area become tightly packed and columnar in shape, whereas the cells in the remainder of the blastoderm become squamous and form a membranous layer(Figs. 15, 16). At this stage the amount of development in the tick egg closely resembles that seen in insect eggs by the time the serosa has been formed. In Dermacentor andersoni that is the extent of formation of embryonic membrane, whereas in the insects it is more complex, with further development of an inner membrane known as the amnion. The condition in Dermacentor andersoni is in agreement with that found in Ixodes calcaratus by Wagner(12) and also with other Acarina and Araneae (6). There are other examples in the Arthropoda whose development in this respect more complex and like

the insects in the development of both a serosa and amnion. This is true of the Scorpiones (6). Regardless of the extent of formation of embryonic membranes, in arthropod eggs which possess a relatively large amount of yolk, the same purpose is served by the membrane, that is, to direct the nutritive material toward the developing part of the embryo. In this way the large quantity of yolk is kept in close proximity to the developing germ band. In Insecta the presence of an inner membrane, the amnion, may be considered to be more specialized, affording the embryo protection from mechanical injury.

In the latter part of embryonic development in Dermacentor andersoni as in other Acarina and Araneae , the squamous portion of the blastoderm forms the external part of the dorsal and lateral body walls. In Insecta the embryonic membranes are usually drawn into the embryo just prior to the dorsal closure of the body wall, so that the protective function of the membranes persists until the insect larva is almost ready to emerge.

The elaborate, persistant double membrane of the Insecta is a protective device apparently not required in the development of the Acarina.

(b) Formation of the Embryo

At first the germ band extends from the point of endoderm formation caudad to a point slightly beyond the caudal pole of the egg (Figs. 16, 17). In this position that part of the germ band which will eventually give rise to the head end of the tick lies at what is later found to be the caudal pole of the egg. At this stage the germ band is quite wide, but as longitudinal growth proceeds it becomes somewhat narrower. The germ band grows rapidly in length, the head end pushing cephalad along the ventral surface of the egg, and eventually passing over the cephalic pole to the dorsal side of the egg to form the cephalic lobe. The germ band at this stage is extremely long (Fig. 18). Wagner(12) found a similar type of germ band development in Ixodes calcaratus, and reports that Salensky(12) describes the same for the water mite, Hydrachna.

It is while the germ band is in this elongate state that the external features appear. Formation of a pair of protuberances in the caudal lobe is observed, and the truncated rudiments of the limbs and mouth parts appear along the ventral surface of the egg (Fig. 18).

Coincident with the development of the mesoderm cells in the caudal portion of the embryo, the caudal groove appears. The groove deepens toward the posterior end of the germ band, and at the posterior end forms the invagination which is the origin of the proctodaeum. The caudal lobe at this stage appears to be segmented, but

this segmentation is of a temporary nature and disappears with the cephalization of the germ band (Fig. 19).

At the same time, along the whole ventral surface of the egg, the appendages appear as six pairs of small protuberances. While the appendages are still in this state of development, the germ band contracts anteriorly. When the caudal lobe has reached the ventral part of the egg its cone-like shape (Fig. 19) disappears and it becomes indistinguishable from the rest of the germ band (Fig. 20). As the growth cephalad of the embryo continues, the yolk is pushed into the posterior part of the egg by the rapid development of the nervous system in the anterior region.

The concentration of the embryo toward the anterior end is demonstrated also in the cephalad migration of the appendages. The anterior two appendage rudiments are destined to form the mouthparts, and become located in the cephalic lobe. The remaining four pairs of limb rudiments elongate, forming the four pairs of legs. This condition is only temporary, the late embryo and larva being hexapod. About the twentieth day, evidence of an embryonic exuviation is present in the form of a granular layer surrounding the embryo (Fig. 29) just below the chorion. Since the embryo hatches as a hexapod larva (Fig. 21), it is possible that the regression of the fourth pair of legs results in this

embryonic exuviation. Wagner(12) reports finding four pairs of legs in the embryo, during part of its development. He too, associates the formation of the larval exuviation (sheath, integument) with the disappearance of the fourth pair of legs. Wagner(12) is of the opinion that all hexapod larvae of ticks pass through an eight-legged stage prior to the hexapod condition, and that the hexapod larva retains under the ectoderm a "more or less developed starting point of the fourth pair of legs".

From their survey of the literature available concerning embryonic development in the Acarina, Korschelt and Heider (6) state that the hexapod larva occurs in most families, but is not universal. It is pointed out that in Phytopta the larvae have two pairs of legs. The adult of this form has two pairs of legs also, and Korschelt and Heider believe that this is a secondary condition in both the larva and adult, and is in keeping with other specialized characteristics of Phytopta.

Korschelt and Heider (6) propose a theory regarding larvae with two pairs of legs, in which this form is considered primitive, and from which has evolved the hexapod form. This idea was held feasible, since the hexapod larva later changes into the eight-legged form (the nymph). However, the records of several workers discredit this theory, by demonstrating that in the development of the hexapod larva, the embryo passes

thruugh a period in which four pairs of legs may be observed. Winkler(13) in his account of the development of Gamasus grassipes clearly observed the formation of four pairs of legs, although the larva is hexapod upon emergence. Wagner's(12) report of an eight-legged condition in the embryo of Ixodes calcaratus and the subsequent appearance of the hexapod larva again confirms the observation that although the larvae of Acarina are hexapod, they first pass through an eight-legged stage. The eight-legged condition in Gamasus grassipes was observed by Winkler only during early development. Nitzsch (8) found that the embryo of Pteroptus vespertilionis commences free life with four pairs of legs, that is, in the nymphal stage. Nitzsch observed however, that the embryo passes through a six-legged stage while the egg is still within the mother. The condition of eight legs during early embryonic development and six legs during late embryonic development and larval stages demonstrated bt Dermacentor andersoni, is thus seen to be fairly general in the Acarina.

(c) The Mouthparts

The anterior two pairs of appendages will form the capitulum. When the stomadaeum begins to invaginate, both pairs are in a ventral and post-oral position, quite widely separated along the ventral mid-line of the embryo. With further development, the first pair migrates antero-

dorsally and eventually come to lie in a position just above the stomadaeum, thus forming the paired piercing and cutting organs, the chelicerae. The second pair of appendages remains in a ventral post-oral position, widely separated, but eventually each appendage grows medially, and they fuse, forming the basis capituli which surrounds the chelicerae and the stomadaeal invagination. Figure 23 shows the anterior two pairs of appendages in an early stage. The anterior pair which will form the chelicerae, are lateral to the oral opening in the process of moving to a position above it.

The second pair of appendages elongates, lying in line with the four pairs of developing legs, and parallel to the long axis of the egg(Fig. 20). Later, however, the position of the second pair of appendages shifts and they come to lie in a position which is at right angles to the long axis of the egg. At this stage a small protuberance forms at the base of each appendage (Fig. 20). The pair of protuberances grows medially and elongates to form the hypostome, a median ventral prolongation of the capitulum, seen in Figure 25. The remaining parts of the second appendages form the basis capituli and the four-segmented appendage, the pedipalpi. The basis capituli is formed by the enlargement and growth together of the bases of second appendages, and their growth around the base of the chelicerae (Fig. 24). The remaining part of the second appendage becomes the four-segmented pedipalp. The pedipalpi, which were previously at right angles to the long axis of the egg, again come to lie in a longitudinal position just lateral to the mouth opening, and tend to enclose the chelicerae and hypostome.

7. Internal Development

(a) The Nervous System

The first evidence of the development of the

nervous system is a slight invagination of the ectodermal cells along the mid-line of the ventral part of the germ band (Fig. 26). This invagination commences soon after the first appearance of the appendage rudiments. The strip of ectodermal cells two or three cells wide which invaginates, separates the ectoderm of the germ band into two longitudinal strips separated by a gradually deepending neural groove. The neural groove appears externally as a continuation of the caudal groove which was observed in the development of the caudal lobe. The ectodermal strips on either side of the neural groove proliferate rapidly so that the nervous system at this stage looks like a pair of elongate masses, several cells in thickness, separated by a constantly deepening neural groove (Fig. 27).

At first the nervous system, like the germ band, is elongate, extending nearly from the anterior to the posterior end of the egg. During this stage the "punctal substance" appears. The term "punctal substance" is the name given by Wagner(12) to a homogeneous granular substance that appears in the nervous system. It tends to stain lightly. The punctal substance is observed first in separate areas along the length of each half of the nervous system. Later, the separate areas of punctal substance fuse transversely (Fig. 28) and then longitudinally. Figure 29 shows how it appears as a longitudinal strip in a sagittal section through one half of the nervous system.

As development of the embryo proceeds, the shortening of the germ band that is noticed in the external development of the embryo is apparent in the progressive concentration cephalad of the nervous system. At the same time, the neural groove grows deeper and narrower (Fig. 30), and is finally obliterated. The cells of the early neural groove may be seen in the interior of the nervous system, forming a compact mass seen just ventral to the stomodaeum in Figure 30. While the nervous system is concentrating anteriorly the stomodaeum invaginates in the direction of the nervous system and grows through it (Fig. 31). Thus the nervous system has the appearance of a large oval mass divided by the stomodaeum into a supraoesophageal portion and a suboesophageal portion (Fig. 32).

Henking (6) speaks of the nervous system of the mature embryo of Trombidium as a large ventral ganglionic mass and a pair of smaller supraoesophageal ganglia. The formation of the nervous system in Dermacentor andersoni is similar to that described by Wagner (12) for Ixodes calcaratus. The outstanding feature of the formation of the nervous system in the Acarina seems to be the marked cephalization which takes place following the development of the elongate form (Fig. 32).

(b) The Digestive Tract and the Excretory System

The endoderm mass which forms on the dorsal side

of the egg at the caudal end of the germ band has already been described. Although the number of endoderm cells increases, the endoderm mass retains its original appearance, (Fig. 33) that of a pyramid of cells, the apex directed toward the yolk, until the shifting forward of the caudal part of the germ band has commenced.

The first change noticeable in this region, is the separation of the endoderm mass into two layers (Fig. 34), the layers being in the same plane as the surface of the egg. The outermost layer divides medially into two equal parts each of which eventually gives rise to a Malpighian tubule. Figure 34 is a sagittal section cut lateral to the anal invagination, and shows the origin of one of the Malpighian tubules.

The growth of the Malpighian tubules may be studied by observing through the surface of the egg their progressive increase in length as embryonic development proceeds. The tubules first appear under the ventral surface of the egg, toward the posterior end, about the twelfth day of development. They are milky white in color and stand out sharply against the rusty brown of the egg contents. Each tubule grows forward gradually, one on each side of the nervous system. When the latter concentrates anteriorly, thus increasing in width as its length decreases, the developing Malpighian tubules are correspondingly pushed out in a lateral direction. Growth of the

tubules takes place in a posterior direction also, toward the rectal vesicle which, like the tubules, can be observed through the ventral surface of the egg as a white bilobed structure. The rectal vesicle is well developed by the twenty-fifth day. By the thirtieth day of development the Malpighian tubes have reached and joined with the rectal vesicle.

Although the Malpighian tubules are endodermal in origin, the rectal vesicle into which they empty appears to be of ectodermal origin (Fig. 35). It is formed as an outgrowth of the dorsal part of the proctodaeal invagination which was observed very early in embryonic development, and which was described in a previous section.

The formation of loops in the Malpighian tubes (Fig. 36) takes place during the last week of embryonic development. They are located laterally and posteriorly to the rectal vesicle, and seem to be the result of growth to accommodate the dorso-ventral mesodermal folds which at this time grow through the yolk.

Korschelt and Heider (6) report that in Henking's study of the mite Gamasus, the conclusion is drawn that the Malpighian tubules are outgrowths of the proctodaeum. They report further that in other orders within the class Arachnida, namely the Scorpiones and Araneae, several workers claim that the Malpighian

tubules are diverticula of the enteron. From their review of the literature Korschelt and Heider (6) conclude that the question of the origin of the Malpighian tubes in the Arachnida is not settled beyond doubt.

The inner layer of endoderm (Fig. 34) grows anteriorly between the great quantity of yolk that is present and the nervous system, to form at first the basal part of the large, branched mesenteron. For the major part of embryonic development, the large volume of yolk is enclosed only by the ectoderm. In the last seven or eight days of development, the yolk mass is broken down into smaller sections by the growth through the yolk of mesodermal folds. These arise ventrally and grow dorsad. As the mesodermal folds are formed, the basal plate of endoderm grows upward around the yolk, enclosing it. All branches of the mesenteron contain yolk particles (which may be observed even in hatched larvae two weeks old). The only exception is the ventral posterior outgrowth which projects posteriorly as far as the proctodaeum (Fig. 32). This outgrowth is located posterior to the concentrated nervous system and anterior to the rectal vesicle. This posterior outgrowth of the mesenteron does not have any direct connection with the rectal vesicle but is closely applied to the proctodaeum just below the origin of the rectal vesicle (Fig. 32).

Wagner (12) is of the opinion that the embryo

of Ixodes calcaratus there is no actual opening between the posterior outgrowth of the mesenteron and the proctodaeum. He has demonstrated to his own satisfaction that in hatched larvae the posterior outgrowth of the mesenteron is blind. His conclusion is that in the embryo all excretory function is performed by the Malpighian tubules. In examining the connection between the posterior outgrowth of the mesenteron and the proctodaeum in Dermacentor andersoni no clear passage from one to the other has been observed, and this writer is therefore inclined to agree with Wagner that excretory function is carried on, in the embryo at least, by the Malpighian tubes.

(c) The Stomodaeum and Salivary Glands

The stomodaeal invagination begins later than the proctodaeal invagination, after the appearance of the appendage rudiments on the ventral surface of the egg (Fig. 22). With the development and contraction and subsequent thickening of the nervous system the mesenteron becomes pushed farther from the oral opening and consequently the stomodaeum increases in length. It joins the mesenteron at the point where it emerges from the surrounding nervous tissue. With the translocation of the chelicerae to a pre-oral position and the growth of the base of the pedipalpi to form the ring-like basis capituli, the distal portion of the stomodaeum compresses dorsoventrally and assumes an arched appearance (Fig. 32). The

segmental mesoderm of the oral segments forms the muscles which operate the blood sucking apparatus. The large muscles which activate the chelicerae begin to develop about the twenty-seventh day, and when fully formed extend posteriorly from the base of the chelicerae and are inserted in the hypodermis of the anterior part of the dorsal body wall. After the formation of the stomodaeal muscles, the chitinous lining of the anterior part of the stomodaeum becomes evident.

The paired salivary glands of Dermacentor andersoni develop as ectodermal invaginations from the buccal cavity of the basis capituli into the haemocoele. Theinvaginations deepen, growing back into the body cavity (Fig. 24), and form the main ducts of the salivary glands. The salivary ducts are located laterally, one on each side of the central nervous system, and above the anterior prolongation of the Malpighian tubules (Fig. 36).

The main duct gives off short lateral branches, which terminate in glandular cells. The glandular cells form first in the anterior part of the body. These may be first seen about the thirtieth day of embryonic development. Toward the end of the embryonic period fully developed salivary gland cells may be observed as far back in the body cavity as the posterior outgrowth of the mesenteron. The cells may be seen in various stages of transformation into glandular cells. The terminal

cell (Fig. 31) of a lateral duct enlarges, the cytoplasm becomes coarse in texture and appears to be somewhat vesicular. The nucleus becomes large and prominent.

The main duct of the salivary gland appears to be chitinized in embryos which are thirty-four days old. The chitinous portion extends only a short distance from the point of origin, but may be clearly seen in cross-sections through the anterior part of the body (Fig. 24). Wagner(12) finds that in Ixodes calcaratus, the internal spiral thickening of the discharge duct does not occur until the post-embryonic period.

(d) The Reproductive System

The development of the reproductive system in the embryo of Dermacentor andersoni has not been fully studied, but judging from the isolated instances in which parts of the reproductive organs have been observed, their formation in this tick resembles the development which is generally found in the Acarina. Korschelt and Heider (6) briefly describe the development of the genital glands as paired "bean-shaped bodies" in the beginning, and which "only in the further course of development fuse to form the unpaired genital gland known in the adult".

In Dermacentor andersoni the genital glands are first noted as paired cell masses which lie on either side of the rectal vesicle just anterior to and above it. These

are seen by the twenty-eighth day of development. As in Ixodes reduvius, reported by Nordenskiold (9), the cells in the paired masses are of two types, one kind containing large nuclei, and the other containing small narrow nuclei. The former type of cell grows around and envelopes the latter. Wagner identifies the cells with large nuclei as the primary sex cells. The second type, he claims, are destined to form the genital ducts.

The writer has not as yet been able to prepare material from which a complete study of the reproductive system can be made.

8. The Embryonic Integuments

In the study of the embryology of various Acarina, many workers have recorded the formation of one or more delicate structureless membranes just below the chorion of eggs containing partially developed embryos. The origin and significance of this peculiar formation has been the subject of much speculation by investigators in the field of arthropod embryology.

The membranes observed have been identified in various ways by different workers, yet from their descriptions we may assume that the different terms are synonymous. Thus, we have the deutovum and tritovum membranes of Claparède (2); the embryonal sheath of Henking and Salensky (5); the cuticula blastodermica

mentioned by Wagner(12); the term larval integument employed by Korschelt and Heider (6).

A membrane which in some respects appears to be analogous with these has been observed in Dermacentor andersoni. As early as the eighteenth day in some embryos, the membrane appears surrounding the embryo just under the chorion (Fig. 29) and seems to be composed of minute chitinous granules. It follows the external contours of the body. The membrane is observed in all stages from the time of its initial appearance until the hatching of the larva.

Wagner(12) describes a membrane in the development of Ixodes calcaratus as follows: "After the formation of the mesodermic cells a very thin sheath makes its appearance under the chorion. This individual embryonal sheath is visible in sections as a thin granular line, and the individual parts look from above as if they were covered with very small grains".

The membrane observed by the writer in the study of Dermacentor andersoni resembles in appearance that described by Wagner, but is formed much later in the embryonic development. The membrane in Ixodes calcaratus must have appeared as early as the seventh or eighth day.

Korschelt and Heider (6) in their review of the literature give the following description: Even before the truncated limb rudiments have appeared....."a delicate structureless integument separates, in Atax, from the

embryo, and surrounds it, like a second egg integument, in the form of a closed envelope". In other Acarina, this process takes place later, only when the legs are already present, so that they each project into a sheath formed by the envelope.

Claparède (2), in Myobia, describes two successive cuticular integuments which he terms the deutovum and the tritovum membranes. In some Acarina where the embryo is enveloped by two integuments, the embryo may actually leave the egg shell surrounded by them. In Myobia a modification of this takes place. The egg shell splits, and this allows only part of the deutovum to emerge. In many other Acarina, including Atax and Trombidium the egg shell is entirely cast off and the embryo continues to develop surrounded only by the cuticular deutovum. This situation is not confined to members of the Acarina but occurs also in some Crustacea (Apus), Korschelt and Heider report(6).

Wagner(12) describes the formation of only one integument in Ixodes calcaratus. This appears at an early stage soon after the development of the mesoderm cells. However, in a later section of his work dealing with the development of the hexapod condition in Ixodes larvae, Wagner says.."Ixodes embryos have four pairs of legs. The last pair has a fully developed cavity, and is provided with an articulation. It becomes stunted when the final larval sheath is formed[x]". From this, the writer assumes that

[x] Underlining by writer.

Wagner has observed the formation of more than one membrane in Ixodes . Wagner (12) in a brief review of the literature presents the views of several workers with regard to the origin and significance of the membranes. Henking, Wagner says, is of the opinion that both the embryonic membrane and the ecdysis between the different post-embryonic stages are of the same significance and calls both "apoderma". He believes that both are formed by free amoeboid cells which arise from the body of the embryo. He reports having observed amoeboid cells in both the embryo and during nymphal moults. Such amoeboid cells have been observed in several Acarina. Bourgignon, according to Wagner, describes gramular corpuscles surrounding the embryo of Sarcoptes visible on the sixth day of development but could not explain their origin or function. Wagner mentions that Claparède described such cells as blood corpuscles but was unable to trace their development. Wagner reports that Salensky, working independently, observed granular protoplasmic cells forming after the appearance of embryonic sheath in Hydrachna cruenta. Salensky made a very thorough examination of tha amoeboid cells, finding the embryo almost covered with a layer of them, moving by means of pseudopod formation. Salensky believed that they served as a nutrient for the developed embryo, but that not all were consumed and quite a number remained at the completion of embryonic development. Wagner(12) draws the following conclusions regarding the amoeboid cells described in the

work of Clarapède, Bourgignon and Salensky: a) these cells are formed very early in embryonic development; b) the number increases by cell division, and they feed and grow outside the body of the embryo; c) the cells move in an amoeboid manner; d) they do not participate in the formation of the embryonic body nor in the elimination of the deutovum membrane. Henking thought that the cells formed the embryonic membrane, but this cannot be true since the membrane appears in forms such as Wagner's Ixodes and Claparède's Myobia which lack the amoeboid cells. In the present investigation of Dermacentor andersoni, it is found that although the membrane is formed, there is no evidence of any amoeboid cells, so that this writer must agree with Wagner's fourth conclusion. In the three species mentioned above, at least, the embryonic membrane would be lacking if its formation was according to Henking's view.

Wagner believes that the amoeboid cells are corpuscles which appear in embryonic development in some cases, as a carry-over from an ancestral form in which they had a protective function. It is Wagner's opinion that the formation of the embryonic membrane, and the moults which take place in post-embryonic metamorphosis are two different types of phenomena. He tries to validate this by theorizing that the ticks' ancestral form passed through a number of larval phases which were different from the present post-embryonic metamorphosis from larva to nymph and from nymph

to adult. In the passage from one larval stage to the next the larval skin became detached at certain points from the body surface, even before the formation of the next larval integument. In case of injury the larval body discharged leucocytes to the injured part. As different species of ticks evolved, the embryonic period lengthened so that the exuviation of the first larval phase took place in the egg. Also, with some species the formation of leucocytes became a permanent rather than an incidental phenomenon, while in other species leucocytes continued to be produced only after injury. Thus in species which form leucocytes at the time of the embryonal exuviation, and in species which form only the deutovum membrane, the phenomena are of the same significance namely, the exuviation at the end of a larval phase.

Wagner holds that there is only one true embryonic exuviation and that it is the deutovum membrane discussed above. The tritovum membrane which occurs in Myobia is characterized by a histolysis similar to those which are found when the larva becomes a nymph, and the nymph an adult. It is therefore, not a second larval sheath, Wagner claims, but a different type of formation resembling that found in the metamorphic moults.

In Dermacentor andersoni the membrane observed by the writer does not appear before the eighteenth day of the development. It resembles the deutovum membrane described by Wagner, but appears much later than the former. Further,

only one membrane appears in Dermacentor andersoni, and Wagner does not clearly state if a second membrane appears in Ixodes calcaratus, but implies that a "final larval sheath" is formed in connection with the regression of the fourth leg as seems to be the case in Dermacentor andersoni.

If the membrane observed in Dermacentor andersoni is homologous to the "final larval sheath" of Ixodes calcaratus then it appears that the deutovum membrane of early stages is omitted. If such is the case, this type of development could be considered as a simplification from that of Ixodes calcaratus, an apparently unnecessary step having been omitted.

Embryonic development is completed in approximately thirty-five days. The larval tick emerges through a dorsal longitudinal slit in the chorion. Upon emergence the globular form of the embryonic tick which was due to the enclosing chorion, is lost, and the typical dorso-ventrally flattened appearance of the post-embryonic tick is assumed.

SUMMARY

The egg is oblong-ovate in form, averaging 0.53 mm. in length and 0.35 mm. in width. The micropyle is not visible. The eggs are laid in a loose irregular mass. Fertilization occurs within one hour after oviposition.

The cleavage nuclei seem to digest their way through the yolk. Upon completion of the blastoderm, cells differentiate from the ordinary blastoderm cells and migrate back into the yolk to form the yolk cells.

The germ band appears first as a short broad area in the posterior dorsal region. The part of the germ band which is the caudal pole of the egg grows in a ventral anterior direction passing over the cephalic pole to the dorsal side of the egg. The germ band undergoes a gradual shortening process cephalad until the posterior end no longer extends further along the ventral part of the egg than the caudal pole.

The endoderm differentiates from the ectoderm at the caudal end of the elongate germ band, and remains in the form of a pyramidal mass until the caudal end of the germ band has reached the ventral side of the egg.

The mesoderm cells migrate inwards from the ectoderm in the region of the endoderm, forming paired lateral strips that extend forward to the anterior part of the germ band.

The germ band in the elongate state shows distinct segmentation in the abdominal region.

Proliferation on both sides of the ventral neural groove gives rise to the nervous system, which is at first paired, and later fuses and shortens to form a single compact mass about the stomodaeum.

The endoderm mass divides, one part being the primordium of the paired Malpighian tubules. In the last part of embryonic development the Malpighian tubules grow in a posterior direction and join the rectal vesicle, an outgrowth of the anal invagination. The remaining endoderm tissue grows anteriorly beneath the yolk forming at first the ventral part of the mesenteron. After the yolk has been divided by the growth through it of the mesodermal dorso-ventral folds, the endoderm grows dorsad to enclose the yolk. In this manner the branched mesenteron is formed.

Six pairs of appendage rudiments appear ventrally while the germ band is in the elongate state. These move cephalad with the shortening of the germ band. The anterior two pairs form the capitulum. The remaining four pairs become the legs. In the latter part of development only three pairs of legs are present, the fourth pair having regressed and withdrawn beneath the embryonic ectoderm. The withdrawal of the fourth pair of legs may be associated with the embryonic exuviation which occurs at this time.

Embryonic development is completed in approximately thirty-five days.

RECOMMENDATIONS

This work has been an attempt to describe the gross morphological changes occurring during the embryonic development of Dermacentor andersoni. As a result, the general scheme of development is demonstrated but several important questions still remain unanswered. For instance, it is still not clear how the development of the reproductive system occurs, nor has any study been attempted of the circulatory and muscular systems.

The nature of the transmission of infective organisms from adult through egg to larva is not yet understood. The writer feels that only through a detailed study of the more minute phases of development of infected eggs, can the true relationship of the organism to the vector be established.

It would also be of interest to trace the development of the post-embryonic period up to the time of the first blood meal, at least.

It is hoped that the present work might serve as a basis for the researches suggested above.

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Abbreviations

ab seg	—	abdominal segments
bl	—	blastoderm
cap	—	capitulum
c c	—	cleavage cells
ce p	—	cephalic pole
c g	—	caudal groove
chel	—	chelicera
chel m	—	cheliceral muscle
cho	—	chorion
c l	—	caudal lobe
c p	—	caudal pole
deut m	—	deutovum membrane
dr	—	dorsal
ect	—	ectoderm
end	—	endoderm
e n g	—	cells of the early neural groove
g b	—	germ band
hyp	—	hypostome
L	—	leg
malp	—	Malpighian tubule
mes	—	mesoderm
mesent	—	mesenteron
mes f	—	mesodermal fold
n c	—	nerve cells
n g	—	neural groove
p b	—	polar body
pdp	—	pedipalp
proct	—	proctodaeum
p s	—	punctal substance
r v	—	rectal vesicle
s d	—	salivary duct
s g	—	salivary gland
sh	—	cheliceral sheath
stom	—	stomodaeum
vn	—	ventral
y	—	yolk
yc	—	yolk cell
zyg	—	zygote

Explanation of Figures

1. Egg with female pronucleus in first maturation division.

2. Cross section of egg one hour old. Fertilization has taken place. Zygote and three polar bodies are present.

3. Egg after first cleavage division. Median vertical section.

4. Egg after second cleavage division. Vertical section.

5. Egg four days old. The cleavage cells have formed a complete layer below the surface of the egg.

6. Blastoderm complete. Transverse section.

7. Nucleus of cleavage cell in mitotic division.

8. Egg six days old. First appearance of yolk cell in blastoderm.

9. Yolk cell has sunk down below blastoderm. Blastoderm cells have closed over it.

10. Yolk cell has sunk down among the yolk particles.

11. Egg eight days old. Beginning of formation of mass of endoderm cells. Transverse section showing endoderm mass on dorsal side of egg.

12. Vertical section through mass of endoderm cells showing formation of first mesoderm cells.

13. Transverse section through the paired caudal protuberances at point of endoderm formation. Note the deep caudal groove.

14. Transverse section through paired caudal protuberances at posterior part of endoderm mass, where caudal groove is less marked. At a later stage than Fig. 13.

15. Transverse section through endoderm mass showing width of germ band.

16. Egg ten days old. Longitudinal section through the endoderm mass showing the length of the germ band at this stage of development.

17. External view of egg showing extent of germ band development in embryo nine days old.

18. Egg eleven days old. External view, germ band at maximum length, appendage rudiments present.

19. Sagittal section through a caudal protuberance. Same stage of development as Fig. 18.

20. Eighteen day embryo. External view of embryo in eight-legged stage.

21. Thirty-three day embryo. External view of embryo in hexapod form.

22. Egg twelve days old. Sagittal section through the stomodaeal invagination.

23. Transverse section through stomodaeal region. Note lateral position of chelicerae.

24. Transverse section through the capitulum showing the salivary ducts.

25. Coronal section through the capitulum.

26. Egg nine days old. Ventral part of transverse section showing mid-ventral strip of cells which invaginates to form the neural groove.

27. Egg eleven days old. Ventral part of transverse section showing proliferation of nerve cells on each side of neural groove.

28. Transverse section through posterior part of embryo showing relationship between nervous system and Malpighian tubules.

29. Egg twenty days old. Sagittal section through the nervous system. Areas of punctal substance visible.

30. Transverse section through nervous system in region through which stomodaeum passes.

31. Transverse section through nervous system anterior to section shown in Fig. 30.

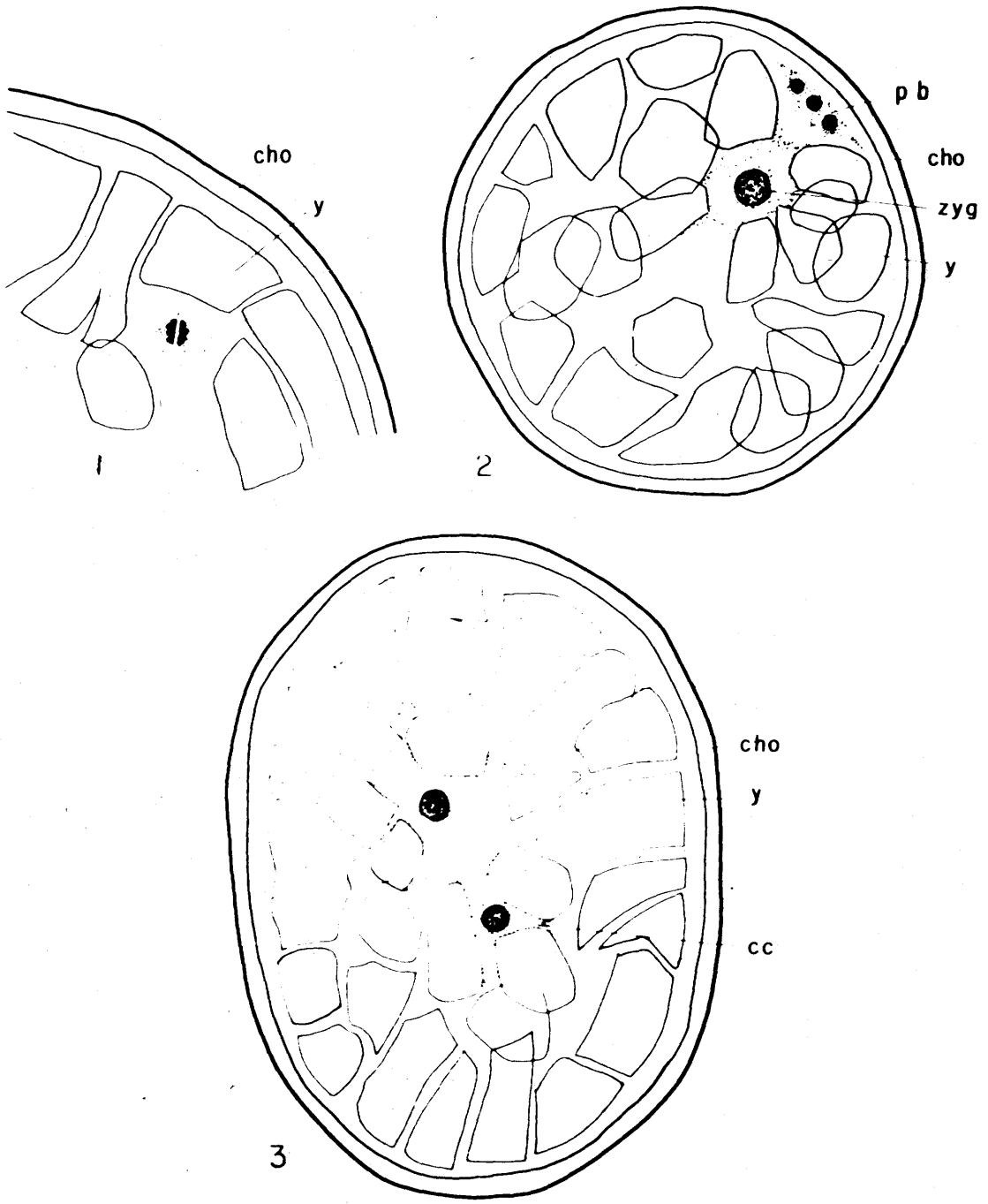
32. Sagittal section showing relationship of nervous system and digestive tract. Embryo thirty-three days old.

33. Sagittal section through the endoderm mass early in embryonic development.

34. Similar section to that in Fig. 33, but in a further developed stage, the endoderm mass being divided into an inner and outer layer.

35. Transverse section through posterior part of the egg, the caudal end of the germ band located at the caudal pole of the egg.

36. Three dimensional view of embryonic nervous system and digestive tract. Embryo thirty-three days old.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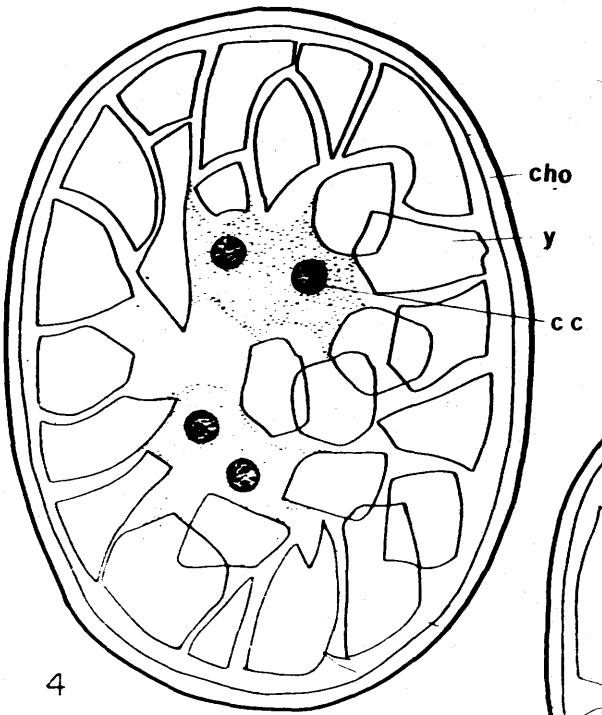

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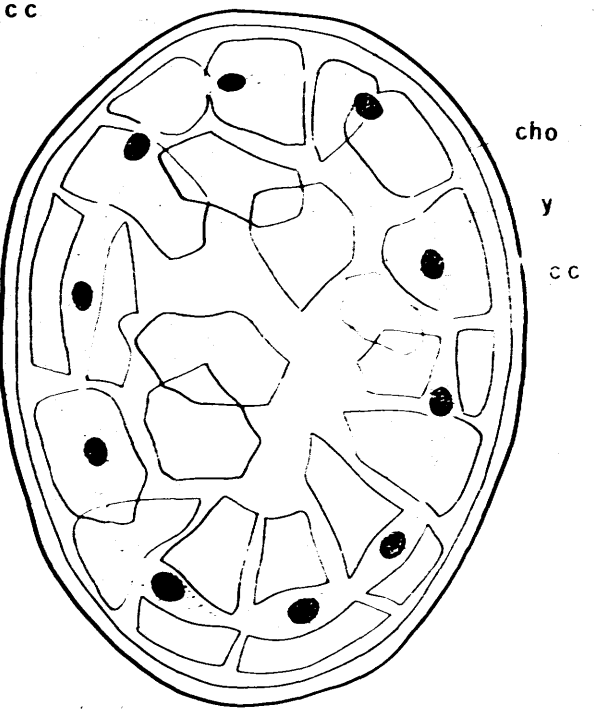

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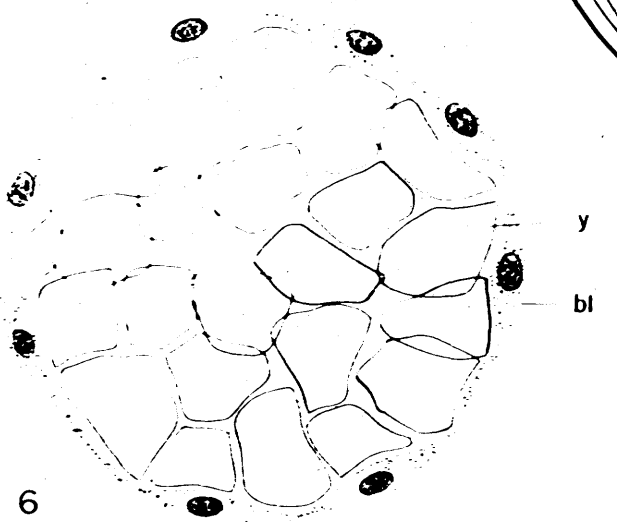

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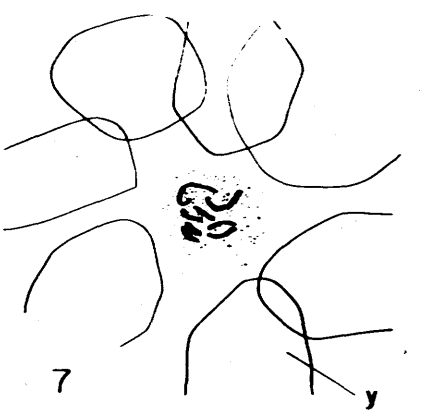

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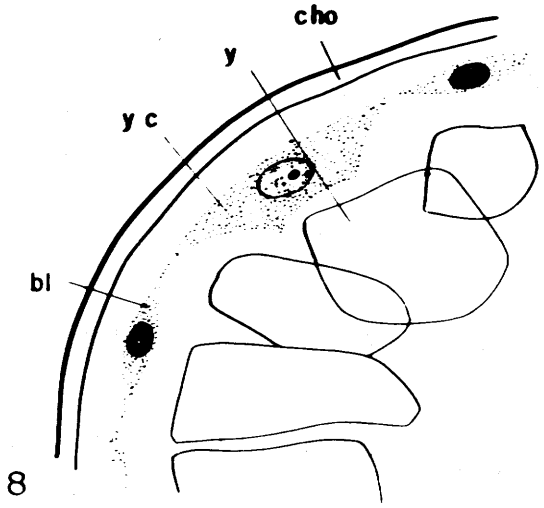

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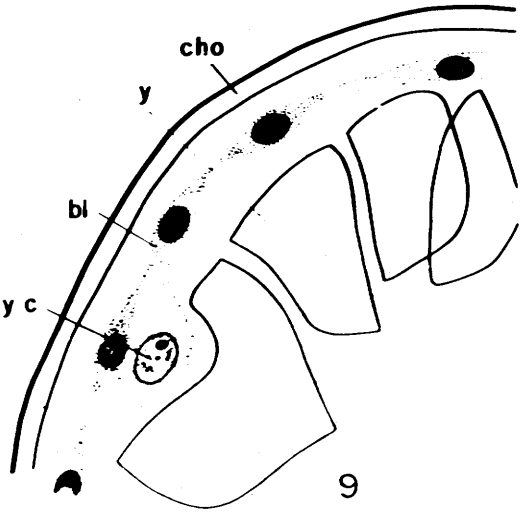

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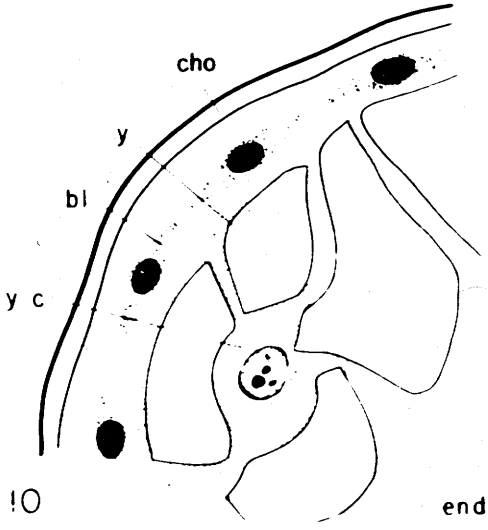

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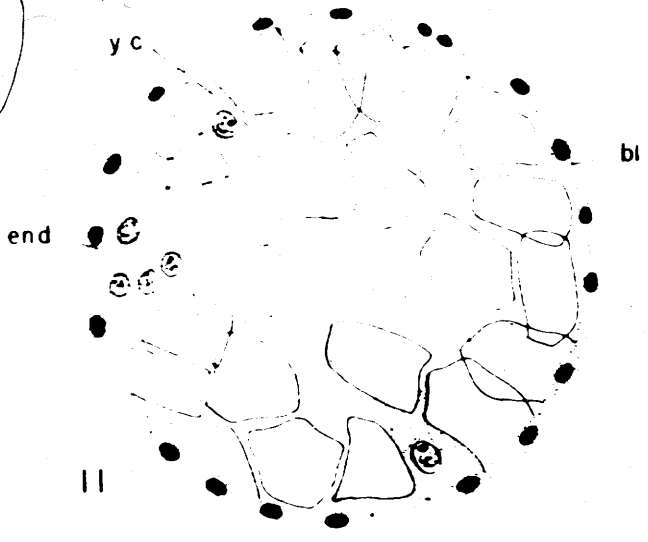

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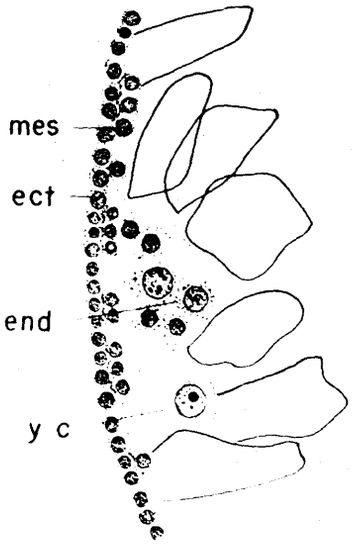

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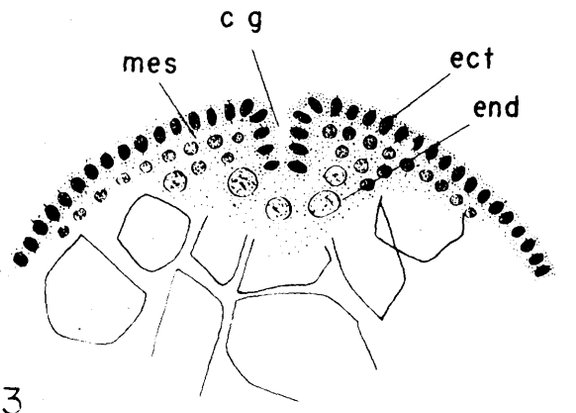

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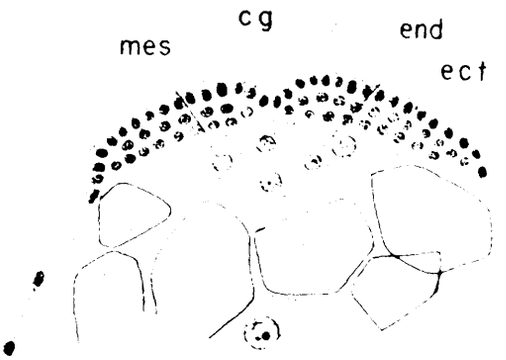

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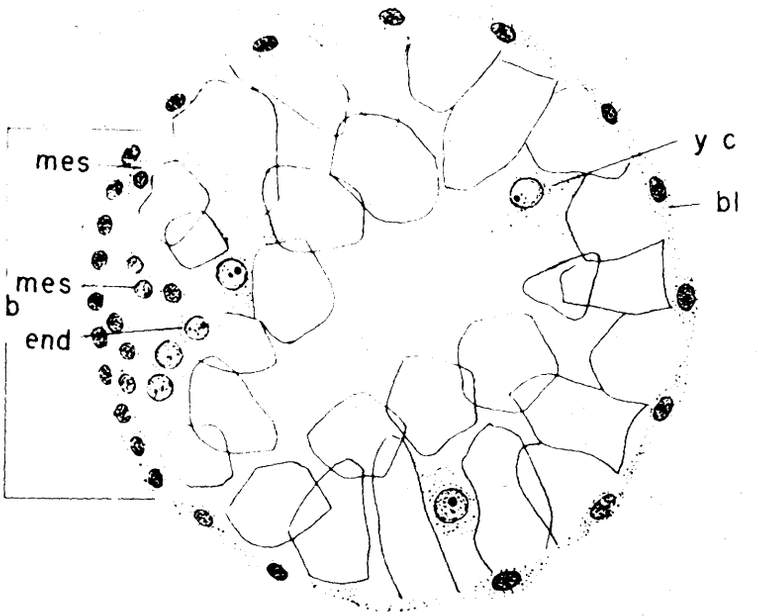

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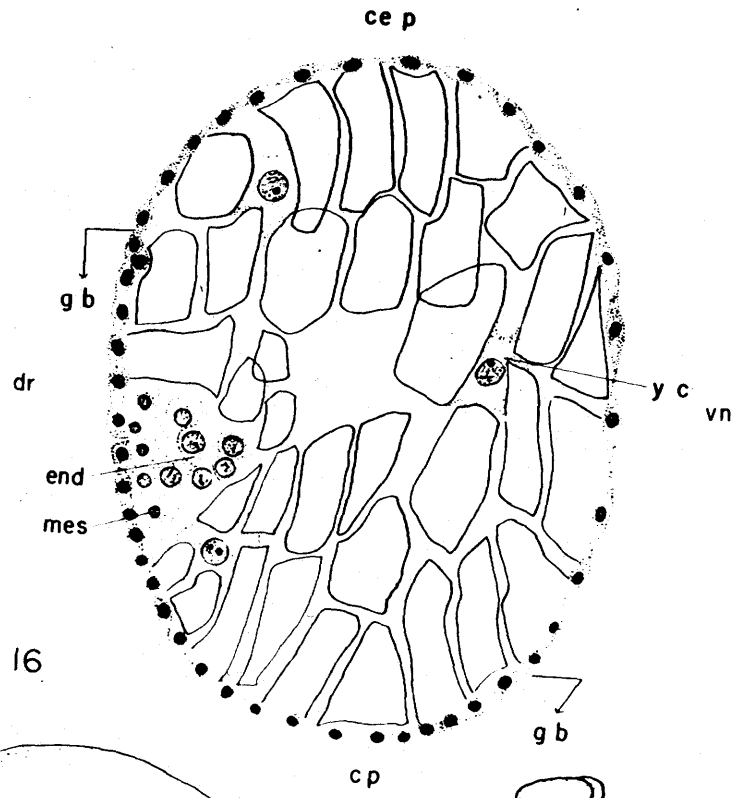

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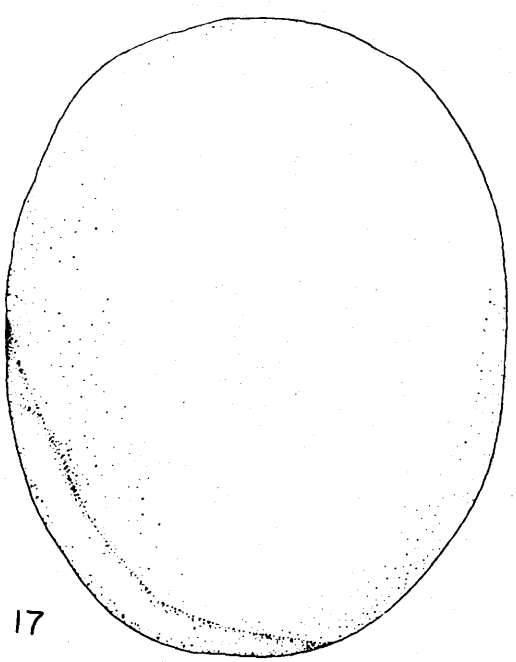

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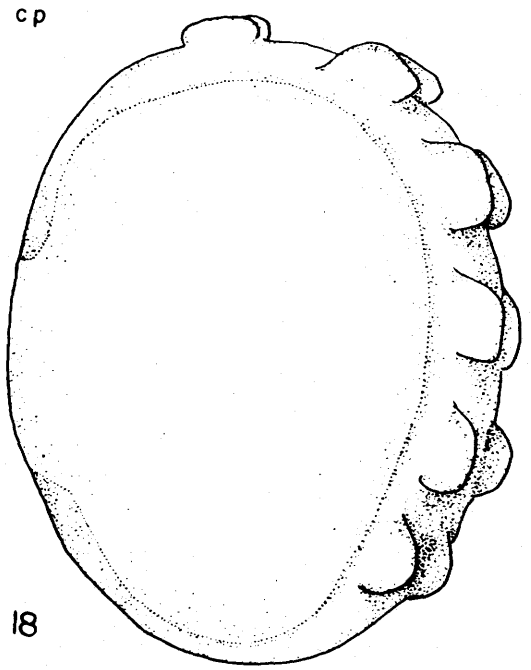

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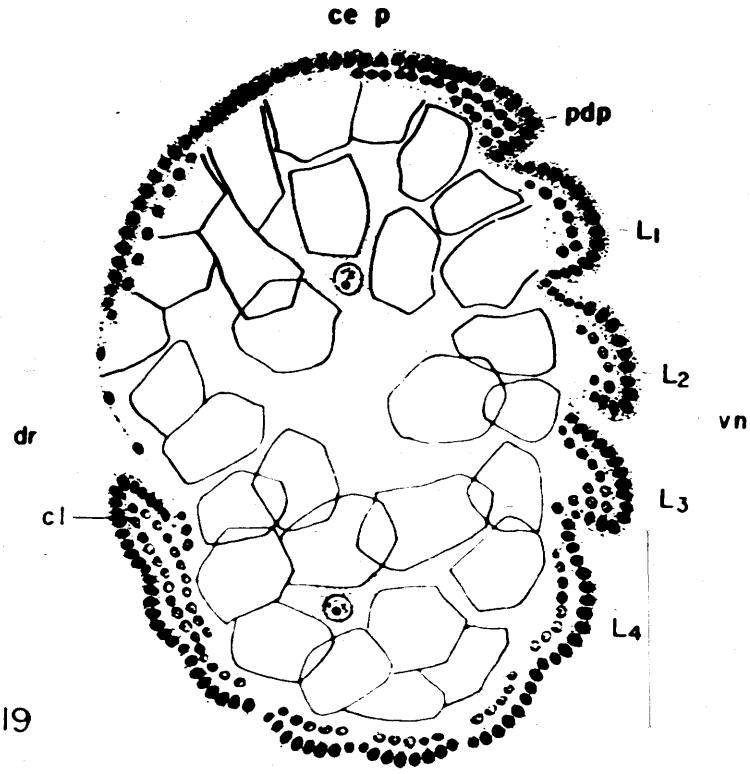

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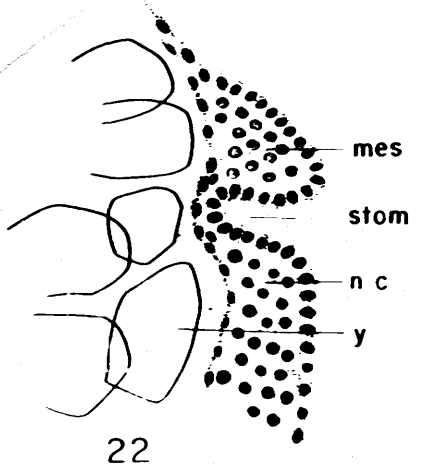

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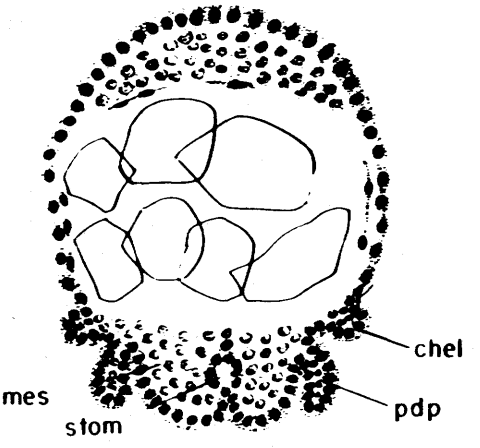

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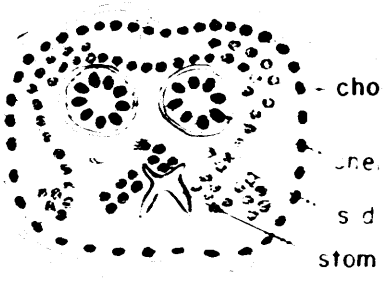

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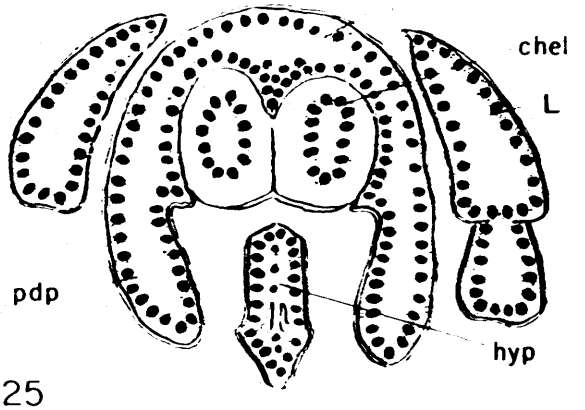

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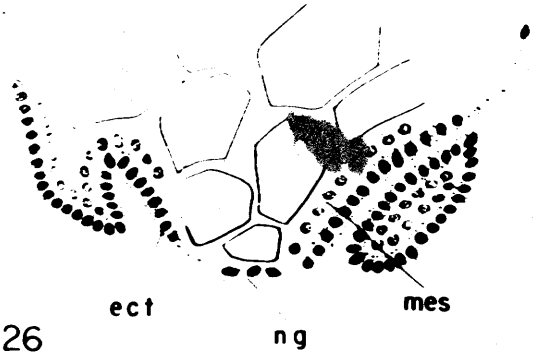

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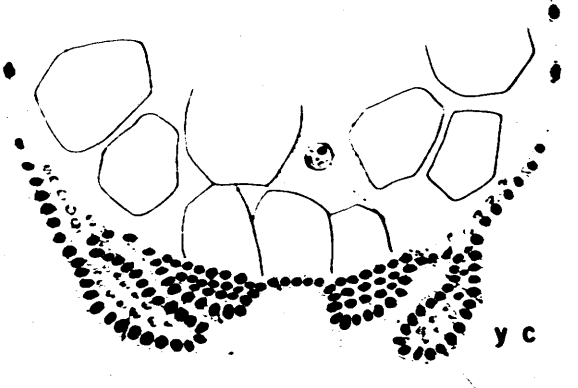

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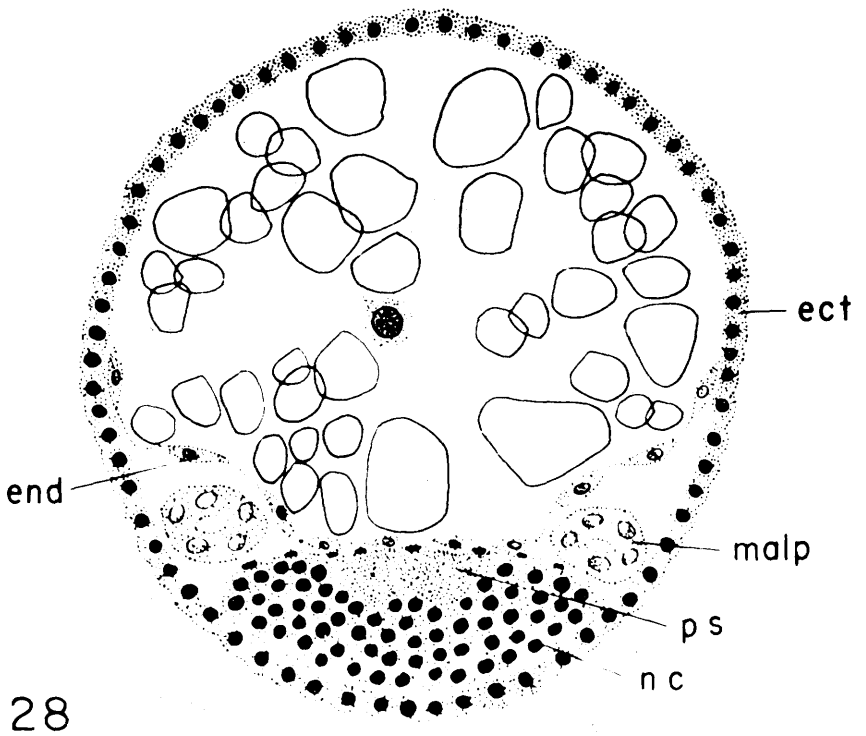

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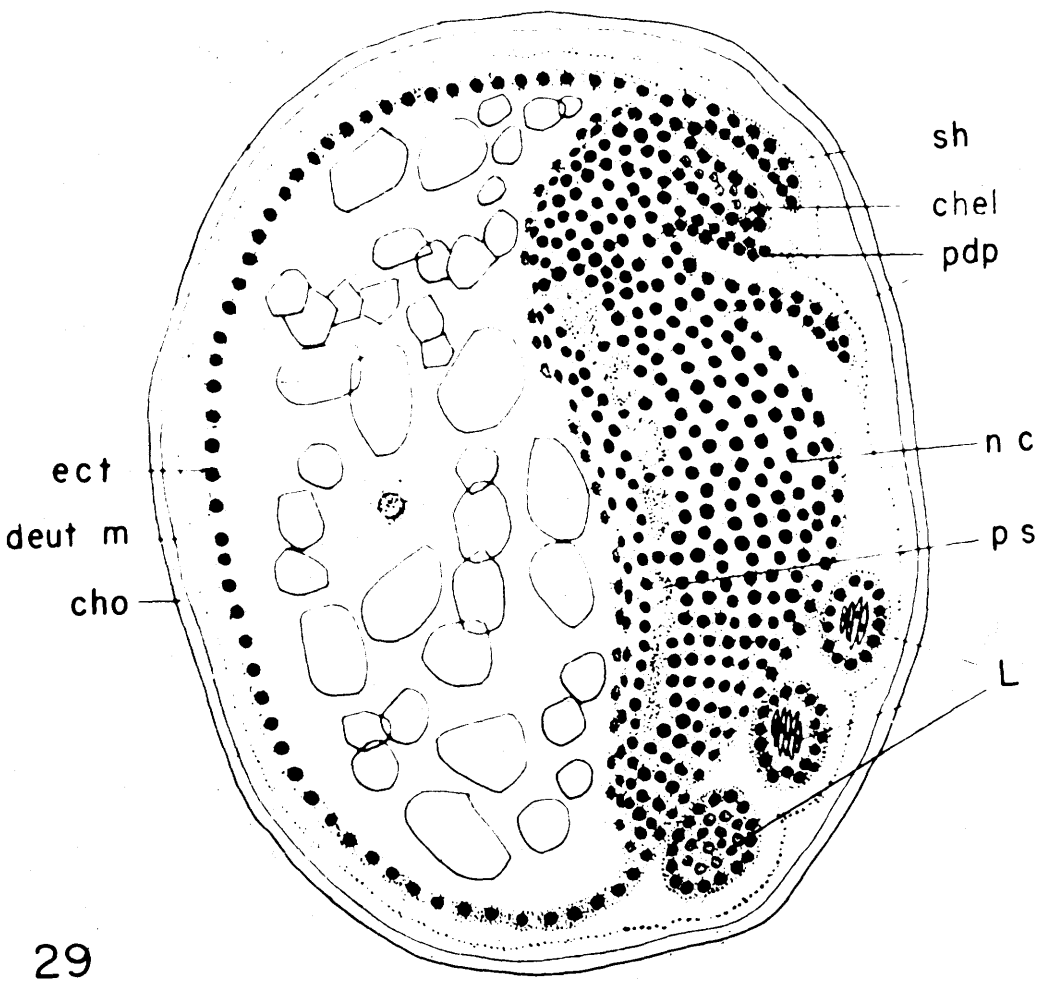

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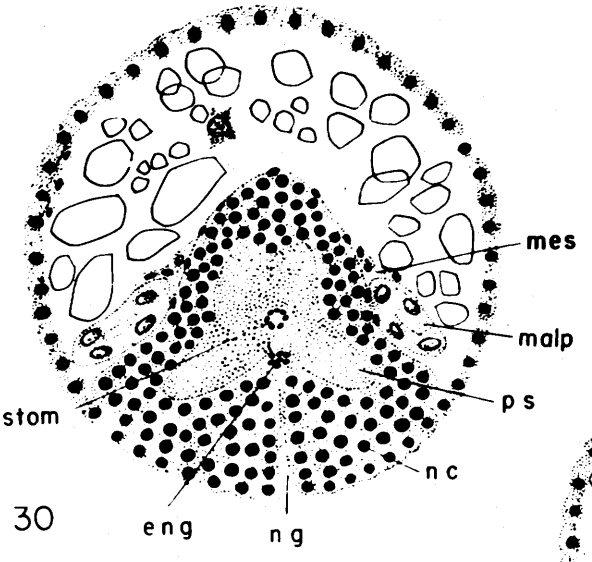
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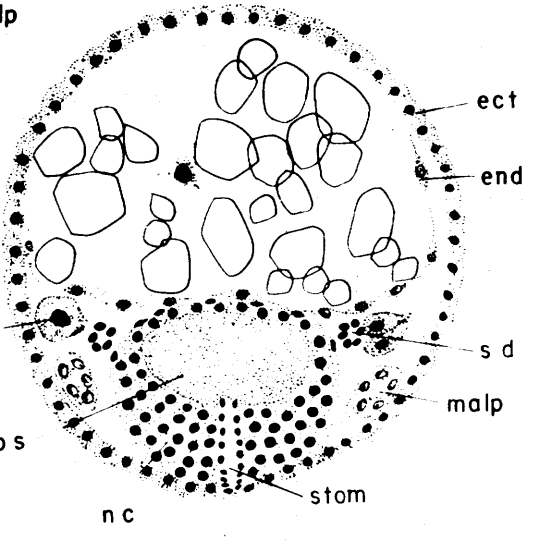
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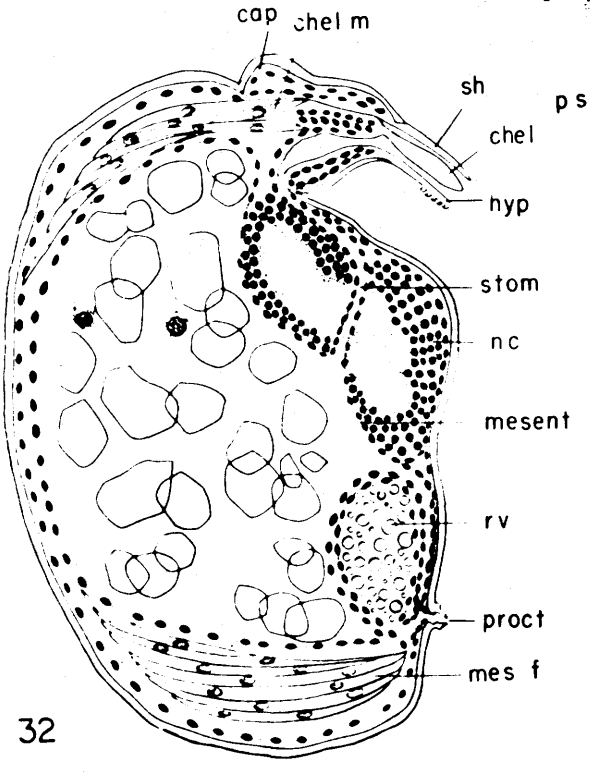
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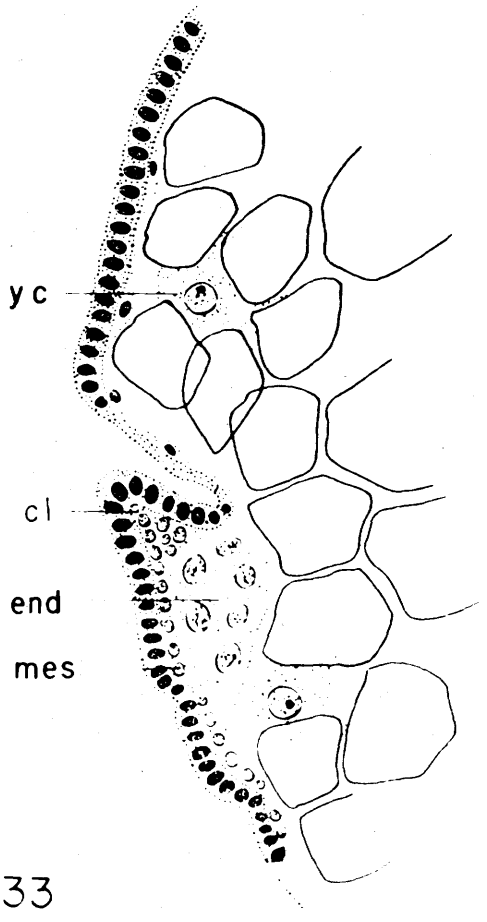

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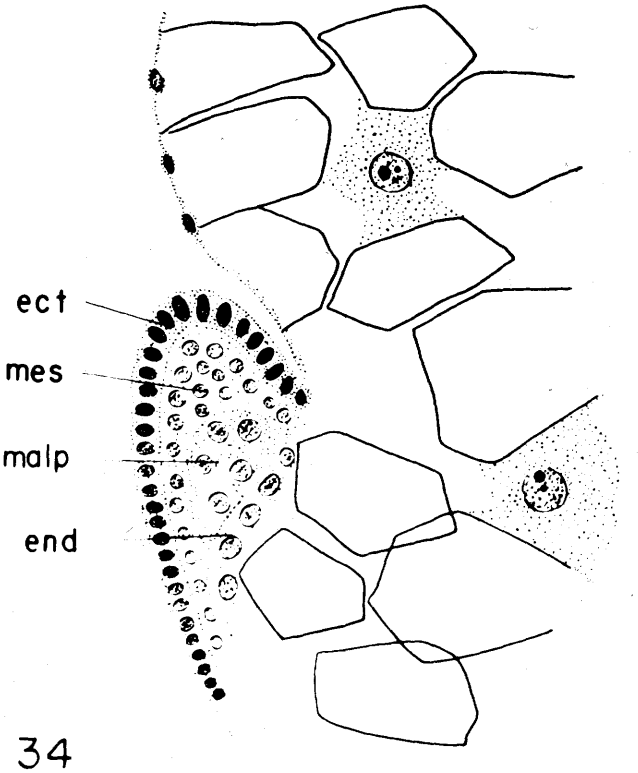

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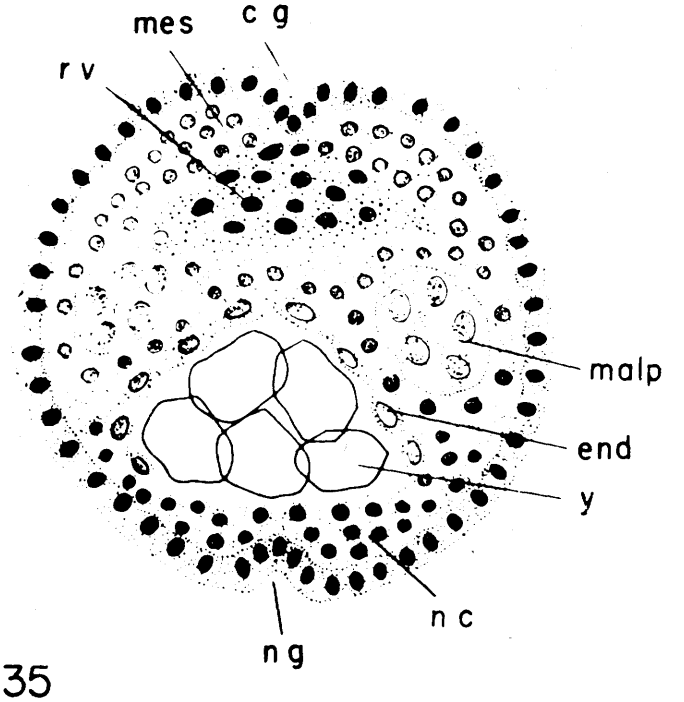

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