AN EMBRYOLOGICAL STUDY OF THE SPRUCE BUDWORM, *Choristoneura fumiferana* (Clements), (Lep., Tortricidae)

By

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A THESIS

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CONTENTS

I. INTRODUCTION

The most complete accounts of Lepidopteran embryology are by Toyama (1902), Eastham (1927), Johannsen (1929), Gross and Howland (1940), Rempel (1951) and Presser and Rutschky (1957). There are many other papers that either deal with the development of only one or a few structures or review knowledge of a specific subject. In this group are the reviews by Eastham (1930) on the formation of germ layers in insects, Richards (1932) on the origin of the midgut in insects, Richards and Miller (1937) on experimental embryology of insects, Roonwal (1936) on gastrulation in insects and Tiegs and Murray (1938) on many phases of insect development, particularly muscle formation. Snodgrass (1935), Imms (1941) and Hirschler (1924) have included in their handbooks accounts of various phases of insect development but the book by Johannsen and Butt (1941) is the only one devoted entirely to the embryology of insects and myriapods.

The only paper available on the embryology of a tortricid is an excellent account of early development of the embryonic rudiment of the holly tortrix moth, Eudemis naevana (Hb.) (Huie, 1918). Another study of tortricid embryology was made by Dr. C. W. Rutschky on the oriental fruit moth Grapholitha molesta Busck. He presented the results in a doctoral thesis at Cornell University in 1949. I was unable to obtain a copy of this thesis but Dr. Rutschky informs me (personal communication, 1957) that it is being prepared for publication.

Very little has been published on the internal morphology of C. fumiferana and this study is confined to some features of

oogenesis, fertilization, maturation, and development from cleavage to the first instar.

II. METHODS

The insects used in this study were collected in the Lake Nipigon region of northwestern Ontario. Larvae and pupae were reared in the laboratory under nearly natural conditions and adults were allowed to mate in eight-ounce glass rearing jars. After mating the males were removed so that the females could oviposit unmolested on the needles of two-year old balsam fir twigs.

Moths oviposited throughout the day, but most intensively between 4 p.m. and 9 p.m. Since a moth required about 15 minutes to lay an average mass of 30 eggs the rearing jars were examined and egg masses were removed every half hour in order to obtain a good series of the early developmental stages (zero to eight hours). Timing for the later stages was not as important so jars were examined only every three or four hours. The egg masses, and the needles on which they were laid, were transferred to an incubator and reared at 25 degrees centigrade. Since the eggs are very delicate they were not removed from the needles until after they had been fixed and preserved.

Cold Kahle's modified formo-acetic-alcohol was used exclusively as a fixing solution (95 per cent alcohol-15 parts, formalin-six parts, glacial acetic acid-one part) (Smith, 1943). Eggs were left in this for 24 hours. The chorion is very thin and relatively fragile, thus it was not necessary to remove or pierce it before fixation in order to obtain good penetration. After the material was removed from the fixing fluid it was washed for a few minutes in distilled water and then preserved in 70 per cent alcohol. Most of the chorions could be removed without damaging the eggs after

storage in alcohol for more than a day. However, if the serosal envelope was not formed it was difficult to remove the chorion without damaging the tissue inside it, nevertheless, in a few instances perfect removal was accomplished.

Both whole mounts and sections were prepared from material handled as above. Whole eggs, without their chorions, were stained overnight in Grenacher's alum carmine solution in order to study their internal macroscopic organization. They were usually over-stained and then immersed in acidulated alcohol for an half hour to remove the stain from the yolk and leave the embryo bright red. The eggs were then washed in 95 per cent alcohol for five to ten minutes and cleared in cedar wood oil or xylol. Cedar wood oil gave excellent results while xylol caused considerable distortion, therefore, the former was used exclusively. Cleared eggs were then mounted in thick Canada balsam on standard microscope slides. By using this technique it was possible to follow the external development and blastokinetic movements of the embryo.

For the preparation of sections, fixed preserved material was embedded in 56-58 degrees C. paraffin according to two different schedules. In the first, entire egg masses with their chorions intact were dehydrated, infiltrated and embedded using essentially the same method as described by Smith (1943). In the second schedule masses were dehydrated in increasing concentrations of ethyl alcohol, cleared in cedar wood oil and infiltrated with and embedded in a rubber-base paraffin (Fisher Tissuemat). The procedure for each method is shown in Table I. Sections of egg masses embedded according to procedure A were very good, and this method was used almost

exclusively.

Entire egg masses were embedded in blocks of paraffin; blocks containing egg masses of a similar age were stored together. Before sectioning, each egg mass was reembedded in a watch glass smeared with glycerin and accurately orientated with warm dissecting needles. Two or more eggs were usually separated and orientated to provide sections through a number of desired planes in one cut, thus facilitating examination.

Table I. Schedules for embedding material fixed in Kahle's (modified) solution. A. Modification of Smith's method (1943). B. Alcohol-cedar wood oil method.

A.

Step	1	2	3	4	5	6	7	8	9	10	11
Time (hours)	1	1	1	1	1	2	2	2	2	8	
					Per cent						
Water	25	10	1	-	-	-	-	-	-	-	
Ethyl alcohol	50	40	24	5	-	-	-	-	-	-	
N-Butyl alcohol	25	50	75	95	100	75	50	25	-	-	
Paraffin (56-58C)	-	-	-	-	-	25	50	75	100	100	

B.

Step	1	2	3	4	5	6	7
Time (hours)	1	1	1	8	1	8	
				Per cent			
Water	20	5	-	-	-	-	
Ethyl alcohol	80	95	-	-	-	-	
Cedar Wood Oil	-	-	100	100	-	-	
Paraffin (56-58C)	-	-	-	-	100	100	

Sagittal sections of entire egg masses (with chorions intact)

were cut as thin as four micra. These and transverse sections were superior to those cut through any other plane and most of the illustrations are of eggs sectioned along these two planes. A rotary microtome was used to cut ribbons of serial sections six to eight micra thick. Five or six standard microscope slides usually accommodated an entire egg mass sectioned sagittally.

For staining sections the following nuclear stains were used: Heidenhain's iron hematoxylin, Delafield's hematoxylin, crystal violet and toluidine blue. Best results were obtained with Heidenhain's method and it was employed more often than any of the others. Several schedules were followed in an attempt to find one which gave the desired detail most frequently with the least amount of time. Sonnerblick (1950) employed the following schedule for staining Drosophila embryos: "Mordant with four per cent iron alum for one hour, wash for several minutes, and then stain for 45 to 90 minutes and destain in two or four per cent iron alum." This, however, was applied unsuccessfully to sections of budworm eggs, chiefly because destaining or differentiation was very rapid and difficult to control, thus results were extremely variable. Best results were obtained using the following method: mordant 12 hours in two per cent iron alum, stain for 18 to 24 hours, rinse in distilled water to remove excess stain, destain in two per cent iron alum until sections have a dark straw colour, and wash in tap water to blue the stain. A combination between this method and that of Sonnerblick was used to obtain satisfactory results in a minimum of time as follows: mordant in two per cent iron alum for three hours, stain for three to four hours, rinse in

distilled water to remove excess stain, differentiate in two per cent iron alum, and wash in tap water to make the stain blue. Occasionally sections were counterstained with light green.

For the study of ovarioles, adults were treated the same as the eggs except that they were left in the solutions longer and embedded following procedure A (Table I) only.

III. OOCYTE DEVELOPMENT

A. STRUCTURE OF OVARIES

In adult females of C. fumiferana there are two ovaries, each with four ovarioles, filling the adult abdomen. Each ovariole is attached to the mid-dorsal body wall near the anterior end of the abdomen by means of a short mesodermal filament that extends posteriorly over the ovariole forming a sheath. The anterodorsal region of each ovariole is known as the germarium which contains both germ cells, or oogania, and mesoderm cells in an undeveloped state. As they pass posteriorly into the vitellarium or region of growth the cells become differentiated; the oogonia divide to form oocytes and nurse cells while the mesoderm cells divide and form follicular epithelium around the oocytes (Fig. 1). Each egg tube or ovariole increases in diameter posteriorly to accommodate the rapidly developing oocytes.

Ovarioles in insects may be divided into two groups according to the method used to nourish their oocytes. If oocytes are nourished by their follicular epithelium the ovarioles are panoistic but if they are nourished by special nurse cells they are meroistic. Futhermore, if all the oocytes of a meroistic egg tube are nourished by a single group of nurse cells it is acrotrophic, but if there is a group of nurse cells associated with each oocyte it is polytrophic (Snodgrass, 1935; Johannsen and Butt, 1941). In C. fumiferana, as in Lepidoptera in general (Johannsen and Butt, 1941), the ovarioles are polytrophic.

B. STRUCTURE OF NURSE CELLS, OOCYTES AND FOLLICLES.

1. Nurse Cells

Hirschler (1929) has described the formation of nurse cells in Lepidoptera as follows; An oogonium divides while passing from the germarium into the vitellarium giving rise to two daughter cells. One daughter cell divides twice to form four nurse cells and the other divides once, giving rise to the oocyte and another nurse cell (Fig. 2). Although this sequence was not observed in C. fumiferana the five nurse cells for each oocyte probably arise in the same manner.

In C. fumiferana the cytoplasm of each oocyte and its sister nurse cell is continuous and nutrient material is probably passed through this cytoplasm to the developing oocyte (Snodgrass, 1935). Each oocyte becomes surrounded by a follicle of mesodermal epithelium except where the cytoplasm of the oocyte is continuous with that of the sister nurse cell (Fig. 3).

The nurse cells, except for a membranous sheath, are in contact with the blood and form a semi-spherical dome at the anterior end of the oocyte, the sister nurse cell of the oocyte being surrounded by the other four (Fig. 3). The cytoplasm of a nurse cell is confined to the peripheral region while the nucleus occupies the central portion. A nurse cell with a diameter of 80 micra has a nucleus 40 micra in diameter and its cytoplasm is about 20 micra thick. As the oocyte grows it passes towards the distal end of the ovariole where its nurse cells disintegrate (Fig. 4).

2. Oocytes

An oocyte at the anterior end of the vitellarium is about the same size as one of its nurse cells but, as it passes posteriorly it grows very rapidly and is soon many times its original size.

Its nucleus, however, does not increase in size and seems to undergo a minimum of change. In an immature oocyte it is about 50 micra in diameter with a large vacuole and scattered clumps of densely staining chromatin (Fig. 1). In a mature oocyte the nucleus is slightly larger and its chromatin is scattered along its perimeter. Nuclei at all stages have a rod-shaped inclusion that absorbs cytoplasmic stains slightly but does not take nuclear stains (Fig. 5).

An immature oocyte has little yolk, and as development progresses, yolk material is absorbed and stored in its center between strands of cytoplasm. The ratio of the amount of yolk to that of cytoplasm increases and when the oocyte is mature most of the cytoplasm is concentrated in the peripheral region where it becomes known as periplasm (Snodgrass, 1935). A cytoplasmic reticulum persists between the yolk globules and the nucleus is connected to the periplasm by this reticulum. Throughout oocyte development the yolk undergoes few visible morphological changes. In immature oocytes it is relatively homogenous with no affinity for nuclear stains.

3. Follicles

A follicle of epithelium surrounds each oocyte while it passes down the length of the ovariole and the cells of this epithelium are continually dividing and growing to accommodate the growing oocyte (Fig. 1). The cells of the follicle are square in transverse section but hexagonal in tangential section and when the oocyte is mature they secrete the chorion or egg shell. Apparently this is their most important function in C. fumiferana

except, perhaps that of protecting the oocyte during its development. Anatomical observations indicate that they do not have a nutritive function. They are separated from the oocyte by their own cell walls and by the vitelline membrane or cell wall of the oocyte. Just before the oocyte is laid they disintegrate in the same manner as the nurse cells.

C. STRUCTURE OF THE EGG AT OVIPOSITION

In C. fumiferana, as in other Lepidoptera, a mature oocyte is laid (Johannsen and Butt, 1941). The following is a description of an oocyte immediately after oviposition and before the spermatozoon has affected it.

The chorion has not hardened completely because the shape of the egg depends on its association with others. It is convex if deposited separately (Fig. 6b) but in close contact with others it becomes almost rhomboid in lateral view (Fig. 6d). It is about 700 micra long, 500 micra wide and 200 micra deep.

Soon after oviposition the chorion (approximately three micra thick) hardens to give the developing tissues protection. The only opening in the entire chorion is a minute hole, five to seven micra in diameter at the anterior end of the egg. This is the micropyle through which male spermatozoon has entered (Fig. 7). Ridges of chorionic material, formed when the follicular cells secrete the chorion, radiate from the micropyle. Thus, the external surface appears hexagonally sculptured (Fig. 7).

The cell wall or vitelline membrane of the oocyte, a true plasma membrane less than one micron thick, lies between the periplasm and the chorion (Fig. 8). The periplasm is slightly

basophilic and varies in thickness from six micra on the dorsal anterior side to 15 micra on the ventral posterior side. It is in this latter region that the germ rudiment will form. At this stage the polar plasm and the "Richtungsplasma" (Huie, 1918) are not differentiated or, at least, not made visible by iron hematoxylin.

Most of the egg is yolk (60-80 per cent by volume). The spherical particles of the yolk are of various sizes and none have an intense affinity for nuclear stains. A cytoplasmic network is distributed between the yolk globules and occasionally small masses of this network appear in sections of almost any part of the egg (Fig. 8).

Presser and Rutschky (1957), Rempel (1951) and Gross and Howland (1940) report that the yolk of a mature oocyte contains many basophilic particles concentrated in a region midway between the center of the egg and its periplasm. Huie (1918) observed a similar concentration in E. naevana. In C. fumiferana some particles retained iron hematoxylin more than others but they were very easily destained in iron alum.

At oviposition the oocyte nucleus stains darkly and definite chromatic details are difficult to see but observations suggest it is near the dorsal side of the egg, midway between the anterior and posterior poles. At the same time the sperm is in an island of cytoplasm immediately beneath the micropyle (Fig. 9).

IV. MATURATION TO GERMBAND FORMATION

A. MATURATION

During the first hour after oviposition the oocyte nucleus enters the periplasm to begin maturation division. This area of periplasm, which is now more finely granular than that surrounding it, is known as the "Richtungsplasma" (Huie, 1918). Actual division was not observed in the budworm but Huie (1918) has described it in detail for Eudemis as follows: The oocyte nucleus enters the "Richtungsplasma" during the first hour after oviposition. It divides once giving rise to two daughter nuclei. Each of these daughter nuclei divides again, one giving rise to two polar bodies and the other giving rise to one polar body and the female pronucleus. The sister body of the pronucleus is larger than the other two polar bodies. In C. fumiferana, however, I have found only two polar bodies, one of which is much larger than the other. The large one appears to be the first undivided polar nucleus and the small one the sister nucleus of the female pronucleus.

The female pronucleus migrates to the center of the egg where it fuses with the male pronucleus to form the zygote nucleus (Fig. 10). The polar bodies are present in three-hour, but not in four hour eggs. Apparently they disintegrate as described by Huie (1918) and do not contribute further to development.

The following observations suggest that additional work is necessary to establish whether or not the first polar nucleus divides. Firstly, since the "Richtungsplasma" is very delicate it is difficult to obtain perfect sections of it. Secondly, even when good sections are obtained the polar nuclei often adsorb stains to

the same degree as the surrounding cytoplasm and are, therefore, almost invisible. A more refined treatment of this area of periplasm is necessary. The chorion perhaps resists penetration by the fixing, dehydrating and embedding materials. If this is so the chorion should be punctured or removed. Furthermore, a more highly selective stain (e.g. Feulgen reagent) might give better differentiation between the polar bodies and cytoplasm.

B. CLEAVAGE

During the third hour, following the fusion of male and female pronuclei, the egg is a mature zygote, the one-cell stage of the future many-celled individual (Fig. 10). This stage is essentially the same in all bisexual organisms. That is, the egg has a periplasm (with its reticulum), a yolk, and a nucleus containing gene complements of both sexes. It is now that cleavage begins. In many animals the entire cell divides, giving rise to two identical daughter cells, while in many others only the cell nuclei divide. The former is known as holoblastic or total cleavage while the latter is meroblastic or superficial cleavage (Snodgrass, 1935). In insects in general and Lepidoptera in particular cleavage is typically meroblastic (Johannsen and Butt, 1941).

In C. fumiferana the zygote nucleus stains darkly, its chromatic material is not obvious and its nuclear membrane is obscure (Fig. 10). However, when cleavage begins the nucleus increases in diameter and structural details become more obvious. By the completion the fifth synchronous cleavage the nuclear membrane, nucleolus and vacuoles are evident. Immediately before the eighth cleavage, nuclei have maximum diameters of 30 to 40 micra and immediately following they are 15 to 20 micra in diameter (Fig. 11).

These were the largest nuclei observed during embryonic development.

Although patterns of cleavage were not traced, by the fifth hour cleavage nuclei, with their surrounding cytoplasm, are arranged in a solid sphere near the center of the egg. This sphere of nuclei is approximately 200 micra in diameter and the 32 nuclei forming it are distributed uniformly. Nuclei on the perimeter appear the same as interior ones and do not seem to move towards the periplasm in an orderly manner. There are no cytoplasmic "tails", such as described by Eastham (1927), Gross and Howland (1940), and Presser and Rutschky (1957). In fact, the daughter nuclei seem to occupy the most easily filled spaces at each cleavage; at least, mitotic spindles are not regularly orientated in relation to the surface of the egg.

These observations suggest that migration of cleavage nuclei in C. fumiferana is controlled, not by the streaming of cytoplasm (Eastham, 1927), but to some extent by variation in viscosity of the yolk and by the continuance of mitotic forces immediately following cleavage.

C. FORMATION OF THE GERMBAND AND ITS ENVELOPE

Nuclei first reach the periplasm in the posterior region of the egg during the seventh hour and by the eighth hour a somewhat superficial blastoderm is formed. The outer cell walls and part of the intercellular walls are evident but the inner margin of the periplasm is still continuous with the cytoplasmic reticulum (Fig. 12). Completion of inner cell walls and differentiation of the blastoderm into serosal and germ rudiment cells occur concurrently during the ninth hour (Fig. 13). Germband cells are mononuclear with small

vacuoles while serosal cells are multinuclear (three or four nuclei, Fig. 14) with large areas (probably vacuoles) along their inner margins which do not adsorb stains (Fig. 13). Blastoderm differentiation is complete by the tenth hour and the germband or embryonic rudiment occupies most of the ventral half of the peripheral region of the egg, while the serosal cells occupy the remaining peripheral portion (Fig. 16a). Yolk and yolk nuclei (vitellophags) fill the entire egg internal to the blastoderm (Fig. 13).

Immediately following blastoderm differentiation the serosal cells spread over the germband forming a cellular membrane or envelope surrounding the entire egg just beneath the vitelline membrane. Apparently, the cells merely flatten slightly and spread laterally until the germband is enclosed. Some cells in the anterior region of the egg retain their form and appear to assist in the digestion of yolk. The serosa persists until the embryo is fully developed (150 hours) at which time it is consumed by the embryo.

During the eleventh hour, the second embryonic envelope, known as the amnion, begins to form from the edges of the germband (Fig. 15). Amnionic cells are mononuclear and they spread very thinly over the ventral side of the band, separating this side from the surrounding yolk. Thus, yolk contact with the embryo is limited to the dorsal side. After the embryo closes dorsally (80 hours) the amnion completely separates it from the yolk for the next 40 hours. (Here, "ventral side" refers to the side in which the neural groove will appear and from which the appendages will be evaginated.) During this 40 hours, however, the embryo continues to develop and at about

120 hours it breaks through the anterior end of the amnion and begins to "ingest" the yolk cells. Eventually, the amnion is consumed along with the yolk.

V. MACROSCOPIC CHANGES IN THE EMBRYO

At the time of serosal formation the germband occupies most of the posterior part of the egg and can be seen immediately beneath the transparent chorion and vitelline membrane (Fig. 16a). However, during the thirteenth hour the band begins to sink into the yolk and soon is not visible externally. Its edges fold inwards while the amnion is forming and it has the appearance of a hollow pouch (Fig. 16b). At this time it has exactly the same orientation or polarity as the adult, thus conforming to Hallez's (1886) theory that the polarity of the embryo at oviposition is the same as that of the adult.

During the next three hours the embryo elongates slightly and turns either to the right or left along its longitudinal axis (Fig. 16c). It is not possible to predict which way it will turn and an examination of a number of eggs revealed that equal numbers turn in either direction (the illustrations are of an embryo which turned left). Huie (1918) reported a similar movement in Eudemis and he believed that the embryo made such a move in order to find space in which to develop because of the almost two-dimensional structure of the egg (Fig. 6). This may be the reason for the rotation in C. fumiferana.

Although most authors apply the term "blastokinesis" to the final revolution of the lepidopteron embryo, I believe the first movement in tortricids should also be regarded as blastokinetic (anatrepsis). Katetrepsis in C. fumiferana will be described later.

By the nineteenth hour the embryo has completed its anatreptic

movements and now lies with its head lobes and caudal pouch directed towards one side of the egg, having turned through 90 degrees around its longitudinal axis (Fig. 16d). It is shaped like a hollow, U-shaped cylinder, 300 micra wide and 450 micra long. The hollow is filled with yolk which is connected to the main yolk mass through the dorsal opening of the embryo (Fig. 16d).

During the twenty-first hour the embryo still has the appearance of a hollow, U-shaped cylinder but is about 100 micra wide and 500 long. The head lobes are more pronounced and the stomodaeum is beginning to invaginate (Fig. 17a). Five hours later (26 hours) the neural groove appears on the ventral side of the embryo in the portion destined to form the gnathal and thoracic segments (Fig. 17b). No further differentiation is evident externally except for slight changes in shape. The lobes of the head have developed laterally and rotated slightly owing to the lack of space (Fig. 17b). The blastocorm is now nearly 600 micra long and 70 micra wide.

It is obvious that as the length of the embryo increases its diameter decreases. However, when it reaches a length of 850 micra (40 hours) and segmentation is complete it grows shorter and wider until it is about 600 micra long (96 hours, Fig. 18b). From this time it increases in both length and diameter and when mature is 1400 micra long and 300 micra wide (Fig. 18c).

Segmentation begins during the twenty-eighth hour in the anterior region (gnathal and thoracic), proceeds posteriorly, and is complete by 40 hours. At this time there are three gnathal, three thoracic and ten abdominal segments in addition to the undefined head segments and caudal pouch. Appendages have begun to

develop on the head, gnathal and thoracic segments. Labral and antennal lobes are evident on the head. The two labral lobes approach the mesal line and lie just anterior to the mouth. The antennal lobes, on the other hand, project from the posterolateral edges of the head. At this time the gnathal and thoracic appendages are very similar, appearing merely as paired outgrowths (Fig. 17c). As development progresses the gnathal appendages shift anteriorly and become grouped around the mouth, thus, any evidence of previous segmentation is lost (Fig. 18a). The labral lobes fuse and lie anterior to the mouth while the maxillary and labial lobes form the spinneret posterior to it. The mandibles are laterad the mouth and the antennae lie just dorsad the bases of the mandibles. The thoracic legs become segmented and paired appendages are evident on abdominal segments, three, four, five, six and ten (Fig. 18a). By 72 hours the intersegmental lines are quite definite on the ventral and lateral sides, and the lateral walls of the embryo have grown dorsally, nearly closing along the middorsal line.

Within the next eight hours (72-80) the lateral walls close dorsally. The embryo rotates through 180 degrees around its longitudinal axis (the axis at this time is an oblique U-shape) and assumes the position shown in Fig. 18b. Many authors contend that this constitutes blastokinesis in Lepidoptera but here there is justification for calling it merely the katetreptic phase, the anatreptic phase occurring earlier as described. Further slight changes in orientation follow and the mature embryo lies in a position illustrated in Fig. 18c. The head has turned through 45 degrees and, although it is not as obvious in the diagram, the

entire body has turned through the same angle around its longitudinal axis.

Following dorsal closure and blastokinesis the embryo no longer adsorbs Grenacher's alum carmine staining solution because the ectoderm has begun to secrete the cuticle. Since the embryo is not able to absorb nutrient material through the cuticle, any further development must depend on yolk that is trapped in the midgut during closure. Sections show that a small amount of yolk is enclosed in the midgut in comparison with the volume still unused in the egg. This small amount must be insufficient for nourishing the embryo during its growth from 120 hours (Fig. 18b) to 144 hours (Fig. 18c). Therefore the excess yolk must pass into the alimentary canal through the mouth of the embryo.

The question now arises; is the embryo developed sufficiently to feed at 120 hours? At 96 hours the mandibles are the only sclerotized structures and by 100 hours the body muscles, stomodaeum and mesenteron appear functional (Figs. 19, 20, 21 and 22). It seems, therefore, that the embryo is able to ingest yolk at this time and that the yolk enclosed in the midgut during closure does nourish cellular differentiation during the period from 80 to 120 hours. Further growth then is probably nourished by ingested yolk. By the time the embryo is mature it has ingested all the yolk and embryonic membranes leaving only the chorion. The foregut is definitely functional and the midgut is filled to capacity with yolk (Fig. 23).

Typically, in Lepidoptera most of the yolk is surrounded by the lateral walls of the embryo as it closes dorsally (Johannsen and Butt, 1941). Thus, a large amount of yolk is trapped in the

midgut and a relatively small amount is left to be eaten by the mature embryo. Since yolk consumption in C. fumiferana is not typical it should be investigated in other Tortricidae and related groups.

VI. MICROSCOPIC CHANGES IN THE EMBRYO

A. CELLULAR STRUCTURE OF THE GERMBAND

The embryonic rudiment remains a one-celled layer until its cells are uniformly 15 by 15 micra with nuclei eight to ten micra in diameter (Fig. 13). During the next two hours the germband cells become columnar and not nearly so uniform (Fig. 15). As the embryo elongates division is most active in the blastocorm and although the cells become quite variable in shape and size they retain their compact association (Fig. 24).

B. FORMATION OF MESODERM

At about 23 hours the cells that are destined to form the mesoderm invaginate along the mid-ventral side of the blastocorm, beginning in the future labial and prothoracic segments (Fig. 24). The entire plate invaginates and the lateral sides grow mesally and nearly meet beneath it (Fig. 25). This process is continued anteriorly and posteriorly forming a second layer of cells and a mid-ventral (neural) groove throughout the length of the embryo. The groove, however, is less evident in the posterior segments because overgrowth of the lateral plates is more complete here.

The cells of the second layer divide and spread laterally to the dorsal edges of the embryo where they form coelomic sacs (Fig. 26). A pair of these sacs is formed in each segment and the muscles, heart, blood cells, fat body and gonadal sheath are formed from them (Snodgrass, 1935). In C. fumiferana the ventral somatic mesoderm probably differentiates to form the ventral intersegmental muscles, whereas the lateral mesoderm forms the remaining segmental muscles. The inner wall of the coelomic sac (splanchnic mesoderm)

probably forms many of the muscles of the alimentary canal while the dorsal cells form the heart and fat body. Blood cells probably arise from the mesoderm immediately dorsad the ganglia. The gonadal sheath is formed in the fifth abdominal segment probably from the dorsal part of the somatic layer. The foregoing statements are probably and further study will be necessary to establish the true relationship of these structures with the primitive mesoderm in C. fumiferana.

C. GERM CELLS

The germ cells are enclosed by the mesoderm of the fifth abdominal segment and at 40 hours they appear as shown in Fig. 27. They are relatively large cells with strongly vacuolated cytoplasm and central nuclei. Although germ cells were not observed earlier they were probably differentiated before 35 hours. The most recent observations of Presser and Rutschky (1957) on Heliothis show that germ cells are invaginated from the undifferentiated germband near the posterior part of the egg. In other insects, notably Diptera and Hymenoptera, they are morphologically differentiated at the time of blastoderm formation (Sonnerblick, 1950). Apparently this is not true in Lepidoptera, although specialized techniques might reveal their presence here also. These limited observations in C. fumiferana merely show the form and location of the germ cells in a 35 hour embryo and it is hoped that this will make a good starting point for further study of their development.

D. CENTRAL NERVOUS SYSTEM

The central nervous system is usually one of the first structures to develop (Snodgrass, 1935). In C. fumiferana neuroblasts

are differentiated from the ectodermal tissue while the coelomic sacs are being formed. By the time the sacs are completed neuroblasts have proliferated daughter cells nearly as large as themselves which seem to become smaller as they are pushed further away from their mother neuroblasts (Fig. 26). The groove that began when the mesoderm invaginated deepens as the neural cells are formed. Two large masses, continuous throughout the length of the embryo, one on each side of the neural groove, will form the segmental ganglia. A central mass between these two and dorsad the central groove appears distinctly in the thorax but its destiny has not been observed (Fig. 28). As segmentation progresses each lateral nerve mass becomes divided into 16 smaller masses connected by strands of neural cells (Fig. 20). The ganglia of the gnathal segments are separate at the beginning of segmentation but as the appendages become grouped around the mouth they unite to form the suboesophageal ganglion immediately beneath the oesophagus. The ganglia of segments eight, nine and ten of the abdomen become united in the eighth segment while the thoracic ganglia unite slightly.

Soon after segmentation is complete a central neuropile forms in each ganglion (Fig. 19). This is the region in which impulses are transferred from one nerve cell to another through the dendrites which comprise it. The neural cells become arranged around the neuropile and send out their axons to the sense cells. These axons also form the connectives between the ganglia (Fig. 20). Finally, a cellular, membranous neurolemma surrounds the entire ganglion and its connectives. Its derivation is a subject of controversy (Snodgrass, 1935). Some authors believe it is

mesodermal, others believe it is delaminated nerve cells and still others believe it is delaminated dermal cells. Presser and Rutschky (1957) are the most recent to describe it as arising from the nerve cells. Detailed observations on its formation in C. fumiferana were not made.

E. FORMATION OF THE ALIMENTARY CANAL

1. Stomodaeum or Foregut.

The ectoderm at the anterior end of the embryo between the headlobes begins to invaginate at 21 hours (Fig. 17a). This invagination eventually forms the foregut or stomodaeum as it appears in a longitudinal section of a mature embryo (Fig. 23). Although it is evident externally at 21 hours it continues to invaginate relatively slowly and at 40 hours has invaginated only slightly (Fig. 29). At this time the internal limiting layer of cells is evident and some of the mesoderm can be seen invaginating with the ectoderm. No ring or section of the stomadaeum exhibits any difference in appearance after treatment with normal stains but in view of Henson's work (1932) midgut cells are probably proliferated from its posterior end.

Most of the muscles originate from the layer of mesodermal cells carried inwards by the ectoderm (Fig. 29). As the foregut differentiates into a buccal cavity, oesophagus and crop the muscles arrange themselves so that the insect may swallow food particles which are pushed into the buccal cavity by the mouthparts. The entire invagination becomes surrounded by an inner longitudinal layer and an outer circular one. However, the layers are not spread evenly over the surface of the stomodaeum but become concentrated

in regions where they are most useful, being fairly well developed around the oesophagus and poorly developed around the crop (Fig. 23). They reach their highest degree of development at the posterior end of the foregut where they form a sphincter valve anterior to the midgut (Fig. 23).

The formation of the stomatogastric nervous system was not followed in Choristoneura but it probably forms in essentially the same manner as in Pieris (Hensen, 1932), that is, by invagination of the dorsal stomodaeal wall. The frontal ganglion, recurrent nerve, hypercerebral ganglion, corpora cardiaca, ventricular ganglion and their connectives constitute this system. It is joined to the central nervous system by commissures running from the frontal ganglion to the brain.

The epithelial cells of the foregut, being ectodermal, secrete a typical cuticle which is shed along with the exoskeleton every time the insect molts, thus, the foregut is easily differentiated from the midgut which does not secrete a cuticle. As the embryo emerges from the amniotic sac (120 hours) the limiting layer of cells at the posterior end of the stomodaeum breaks down allowing the orally ingested yolk to pass into the mesenteron (Fig. 23).

2. Proctodaeum or Hindgut

The hindgut is formed from the posterior end of the embryo by a median invagination that begins in the caudal pouch or telson. As the invagination continues anteriorly the telson and the eleventh abdominal segment are lost, so that in a 72 hour embryo the hindgut opens through the tenth abdominal segment (Fig. 18a). As in the foregut, a layer of mesoderm is carried inwards to form an inner longitudinal, and an outer circular, layer of muscle.

Also, as the hindgut differentiates into an ileum, colon, and rectum the muscles become differentiated; development is greatest at the ileo-colic and rectal valves.

An inner limiting layer of cells, similar to that in the foregut, is present and apparently does not disintegrate until some time after the embryo has hatched. Although none of the cells near the anterior end of the hindgut appear different from one another the posterior part of the midgut is apparently proliferated from this region. Again, midgut cells do not secrete a chitinous intima as do those of the proctodaeum, so the boundary between the two is clearly defined.

The Malpigian tubules are usually described as originating by invagination of the blind ends of the proctodaeum (Snodgrass, 1936). However, Hensen (1932) claims they originate from the posterior end of the mesenteron. Their development in C. fumiferana was not followed in detail but limited observations suggest that they arise from the anterior end of the proctodaeum.

3. Mesenteron or Midgut

The formation of midgut in insects is a subject of controversy, but in Lepidoptera it is generally believed to be proliferated from the blind ends of the stomodaeum and proctodaeum. Detailed studies of its formation in C. fumiferana were not made but limited observations suggest it is formed in the typical lepidopteron manner.

Proliferation from the proctodaeum probably begins at 50 hours and a few hours later from the stomodaeum. These cells spread along the splanchnic walls of the coelomic sacs which break down to form an inner circular and an outer longitudinal

layer of muscles. The two strips of midgut cells meet first in the ventral region and grow dorsally surrounding a small amount of yolk. Just before katetrepsis (80 hours) the dorsal wall closes, trapping some of the yolk.

By 100 hours the midgut cells appear functional (Fig. 30). They now form a columnar epithelium 20 micra thick, and their internal borders, although not striated, have absorbed some of the yolk material (Fig. 30). The embryo soon begins to "feed" on external yolk and as the volume of yolk increases in the midgut its cells become less and less columnar until in a mature embryo, which has ingested all the yolk and embryonic membranes, they are a thin squamous layer (Fig. 23).

VII. YOLK, YOLK NUCLEI AND YOLK CELLS

Immediately following oviposition the yolk granules are spherical and range in diameter from 0.5 micra to 5.0 micra. Parts of the cytoplasmic reticulum appear as darkly staining, irregular masses (Fig. 8). When spermatozoon enters the egg and maturation occurs, there are no obvious changes in the yolk but as soon as cleavage begins many yolk granules acquire an intense affinity for iron hematoxylin (Fig. 31). Furthermore, as cleavage nuclei move towards the periplasm the granules become less basophilic and after most of the nuclei have entered the periplasm no darkly staining granules are evident.

Some of the cleavage nuclei, each surrounded by an island of cytoplasm, remain behind in the yolk and continue to divide synchronously to the tenth cleavage (Fig. 32). During the ninth and tenth cleavages they remain in the same mass of cytoplasm. Eventually, however, each nucleus becomes separated and the yolk becomes divided into cells each with one, two, or three nuclei (Fig. 33). The nuclei of these yolk cells are generally known in the literature as vitellophags. Many believe they assist in the digestion of yolk and their function in C. fumiferana, as suggested by morphological observations, seems to be primarily that. At 120 hours, as the embryo pushes through the amnion to begin ingesting the yolk orally, the granules of yolk are much larger than at any time previously ranging from 1.0 to 12.0 micra in diameter (Fig. 34). This suggests that some coalescence or digestion has occurred. More evidence of coalescence is seen after comparing sections of 40-hour eggs (Fig. 33) with those of 120-hour eggs (Fig. 34).

Yolk cells of 120-hour eggs are mononuclear while those of 40-hour eggs are multi-nuclear.

During closure of the embryo some yolk cells are trapped in the midgut and their walls do not break down until the midgut epithelium becomes functional, about 100 hours. The walls next to the epithelial cells appear to be the first to break down, since the distal portions of the epithelial cells contain material similar to that in the yolk cells (Fig. 30). The lateral yolk cell walls appear to remain intact longer. Meanwhile, the nuclear material becomes more dispersed and the yolk granules coalesce still further until some are nearly 15 micra in diameter (Fig. 30).

This small amount of yolk trapped by the midgut during closure is hardly enough to account for the great increase in the size of the embryo during the last 30 hours of its embryonic life (Compare Figs. 18b and 18c). This growth must be supported by the surrounding yolk. The embryo ingests the yolk and its midgut cells become stretched as the midgut is filled to capacity. The nuclei and walls of the yolk cells disappear and the yolk in the midgut of a mature embryo is quite homogeneous (Fig. 23).

VIII. SUMMARY

This study was undertaken to provide information on the internal development of Choristoneura fumiferana (Clements). The structure of the ovaries and development of oocytes, follicles and nurse cells are described. An oocyte immediately following oviposition is described. Some details of maturation, fertilization and zygote formation are presented. Cleavage, blastoderm and germband formation are followed. The formation and differentiation of many parts of the embryo and its envelopes are described in various detail. Changes in the yolk during development are also described and probably the most important result of the study is the discovery of the mode of consumption of yolk by the embryo. The embryo develops for 120 hours in the posterior half of the egg at the expense of a small percentage of the total volume of yolk. By 130 hours the embryo is orally ingesting the yolk in the anterior region of the egg. It grows very rapidly until it hatches at about 150 hours.

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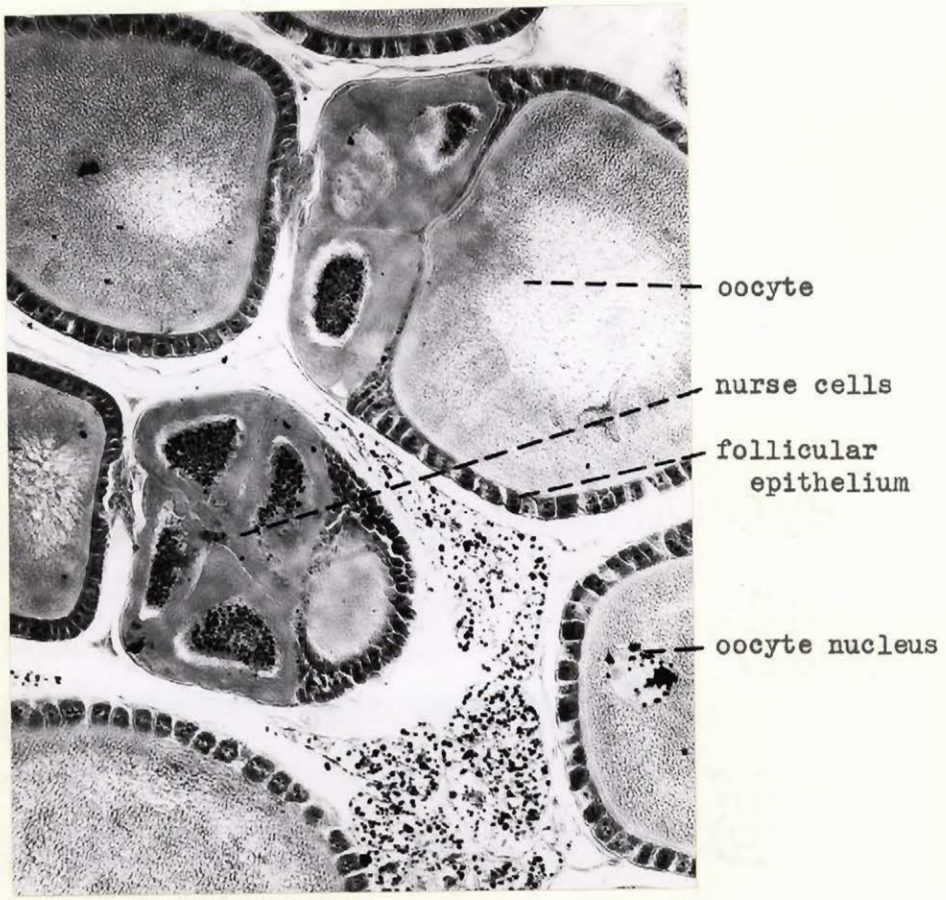


Fig. 1. Longitudinal section of the ovary showing the development of the oocytes. Notice the densely-staining chromatin of the nucleus of the oocyte in the lower right corner. Photomicrograph.

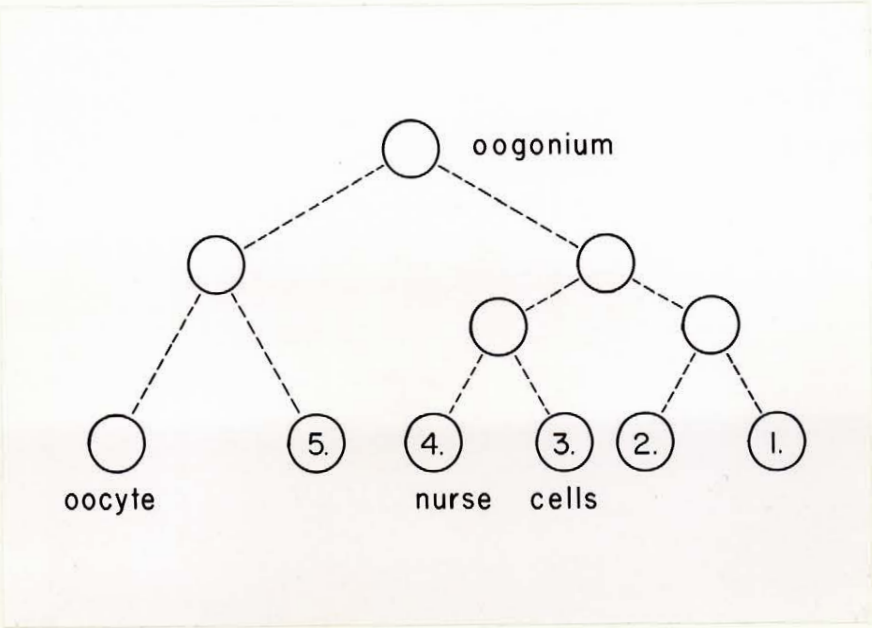


Fig. 2. Diagram showing the formation of an oocyte and its nurse cells from an oogonium. Nurse cell "5" is the sister cell of the oocyte.

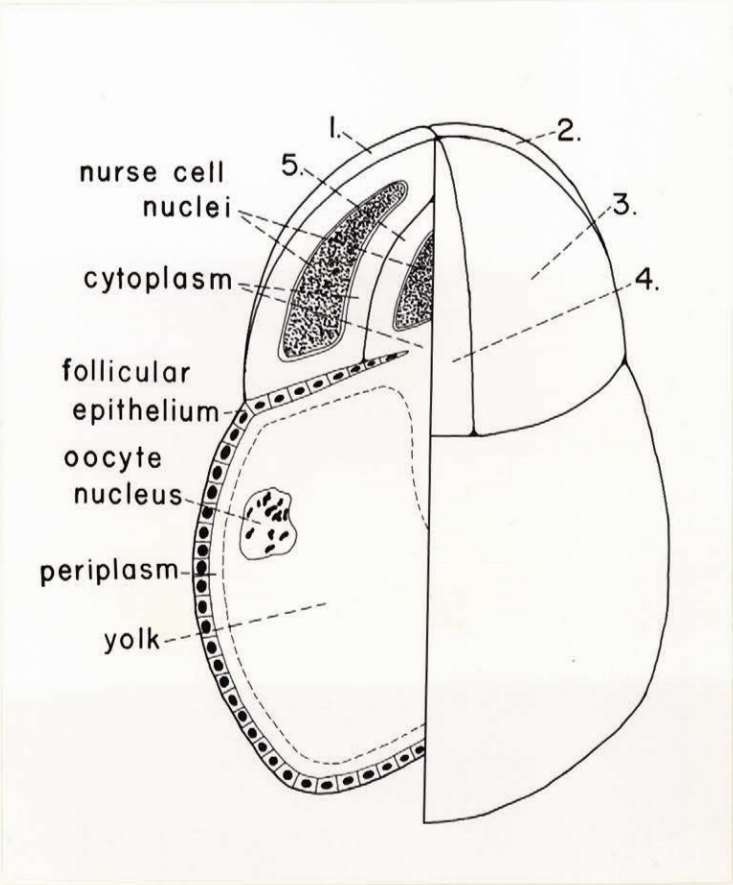


Fig. 3. Diagram of an oocyte in the anterior end of the vitellarium showing the arrangement of its cytoplasm (periplasm), follicular epithelium, and nurse cells. The numbered cells are nurse cells; "5" is the sister cell of the oocyte.

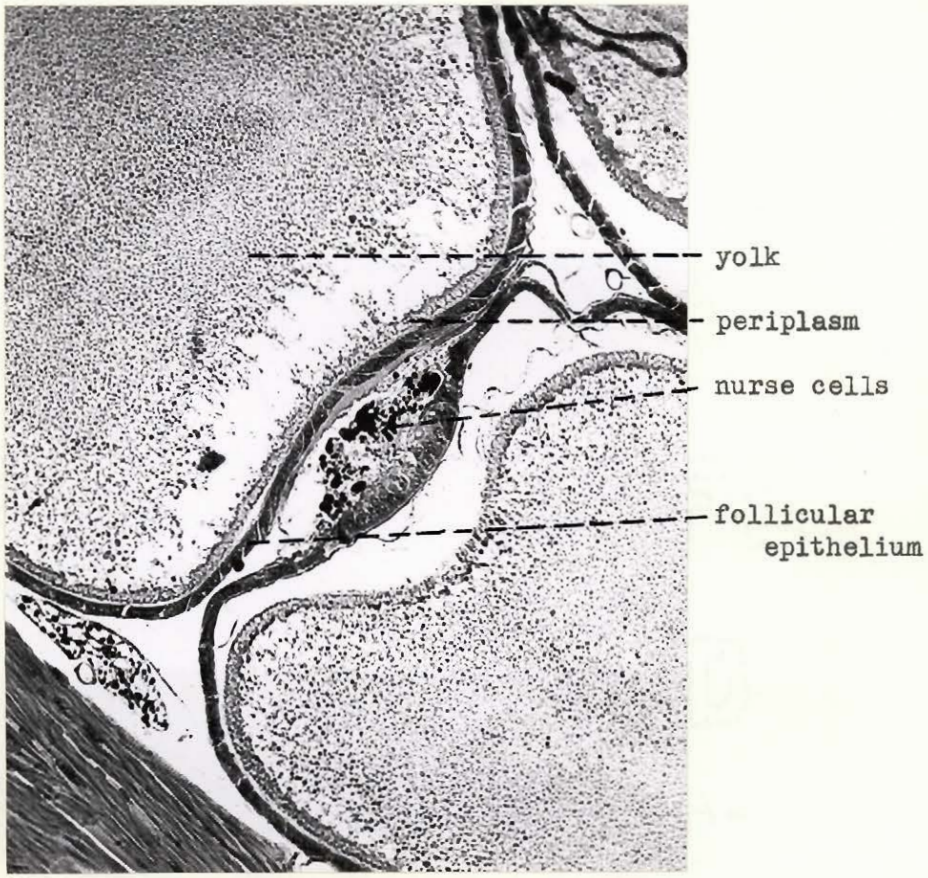


Fig. 4. Longitudinal section showing the disintegrating nurse cells. Photomicrograph.

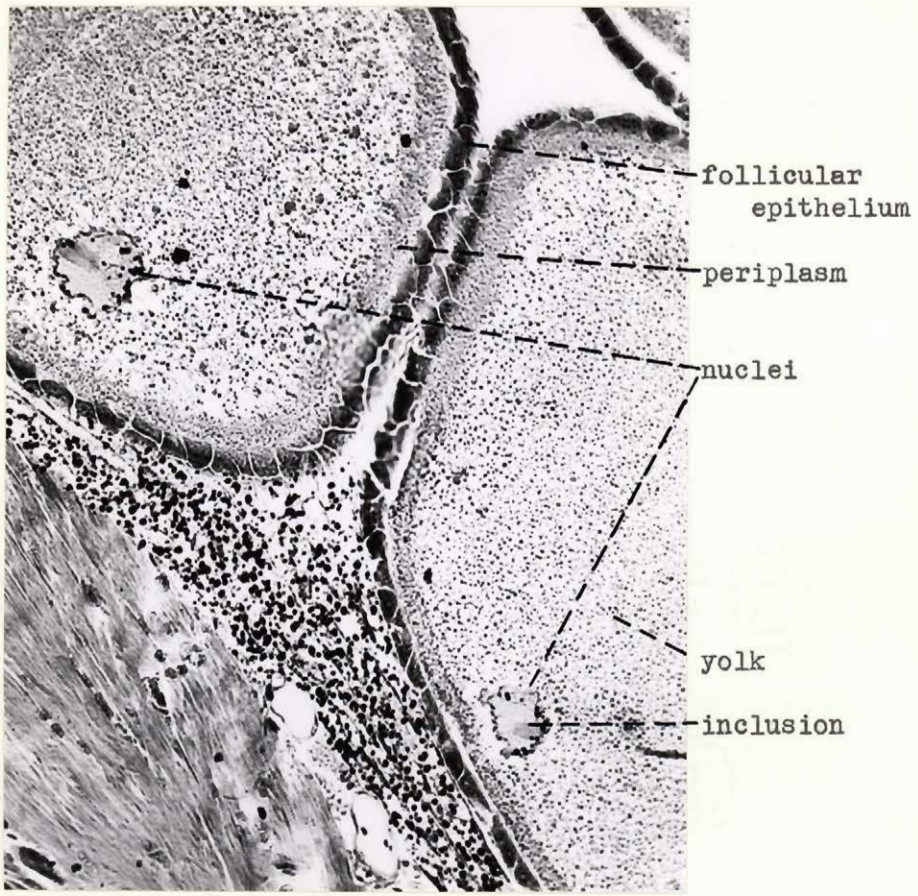


Fig. 5. Longitudinal section the position of nuclei of mature oocytes. Notice the rod-shaped inclusion in each nucleus. Photomicrograph.

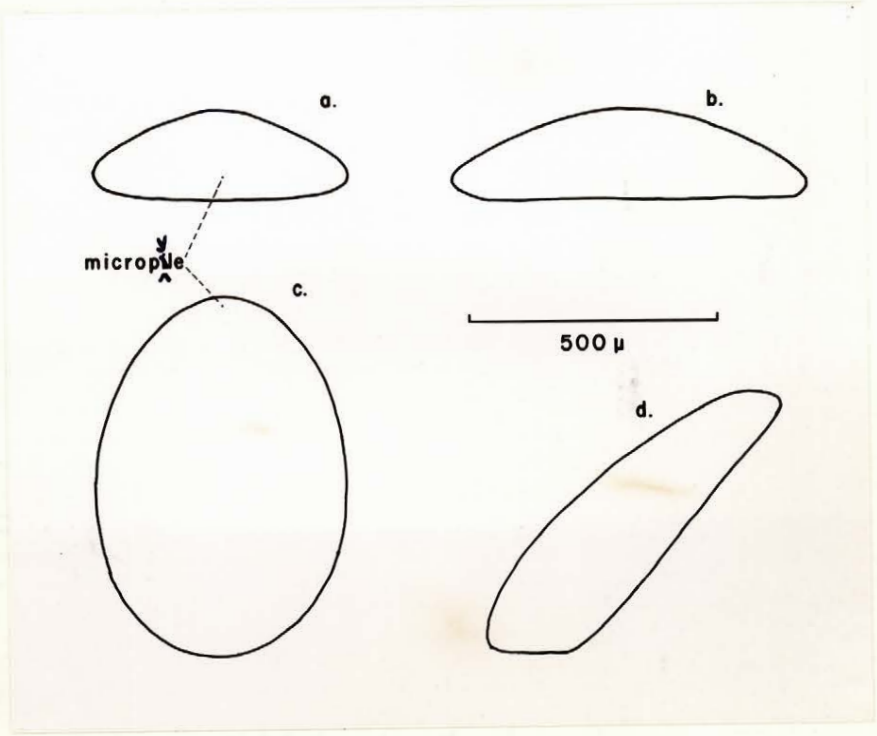


Fig. 6. Diagram showing the dimensions and shape of an egg of C. fumiferana.(a) Anterior view, (b) lateral view, of an egg laid on a flat surface, (c) dorsal view, (d) lateral view of an egg laid in a mass against another egg.

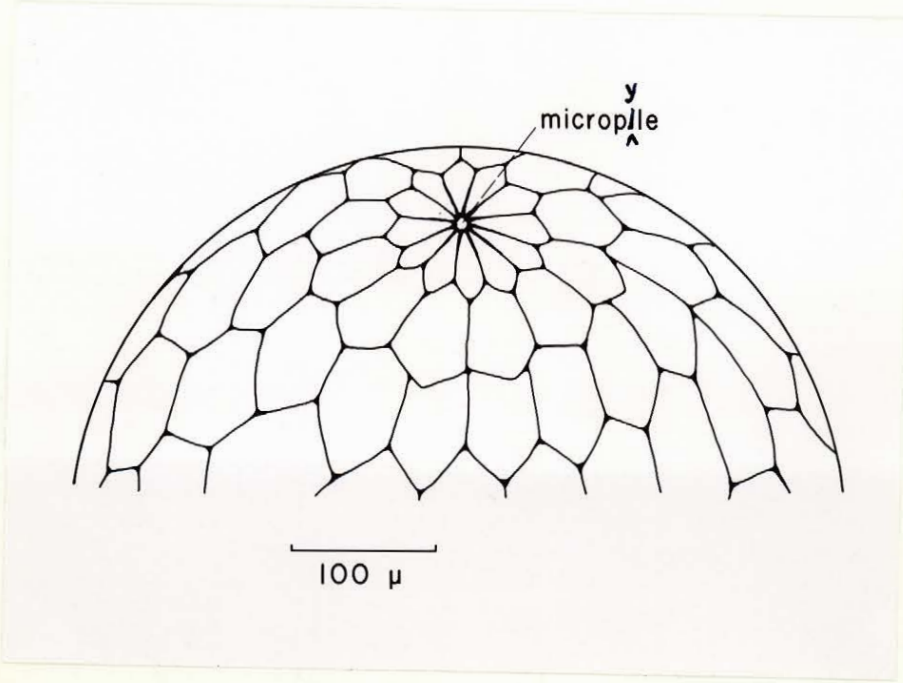


Fig. 7. Diagram of a dorsal view of the egg showing the micropyle and the sculpturing on the chorion.

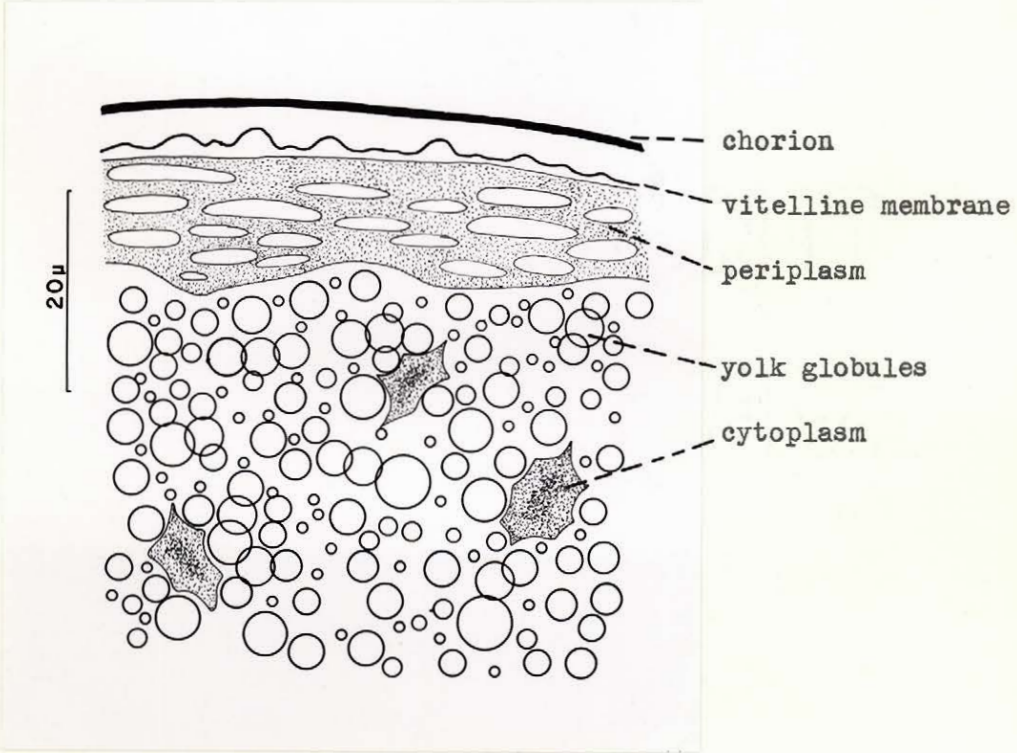


Fig. 8. Diagram of a section of an oocyte immediately after it has been laid. Notice that there are no basophilic yolk globules.

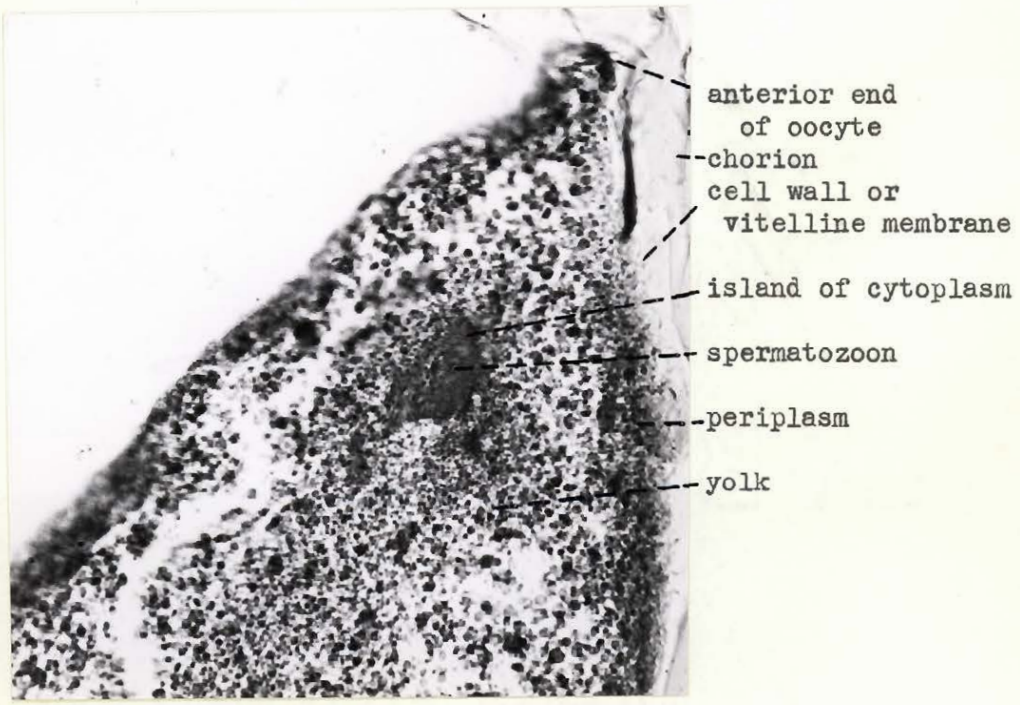


Fig. 9. Longitudinal section of the anterior end of the egg showing the spermatozoon in an island of cytoplasm. Photomicrograph.

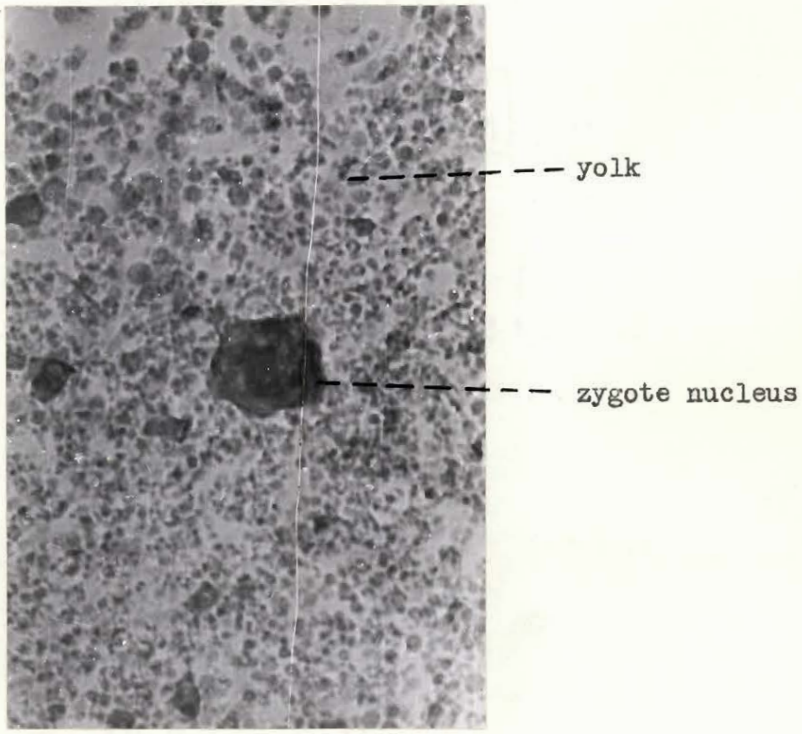


Fig. 10. A section of a three-hour egg showing the zygote nucleus. Photomicrograph.

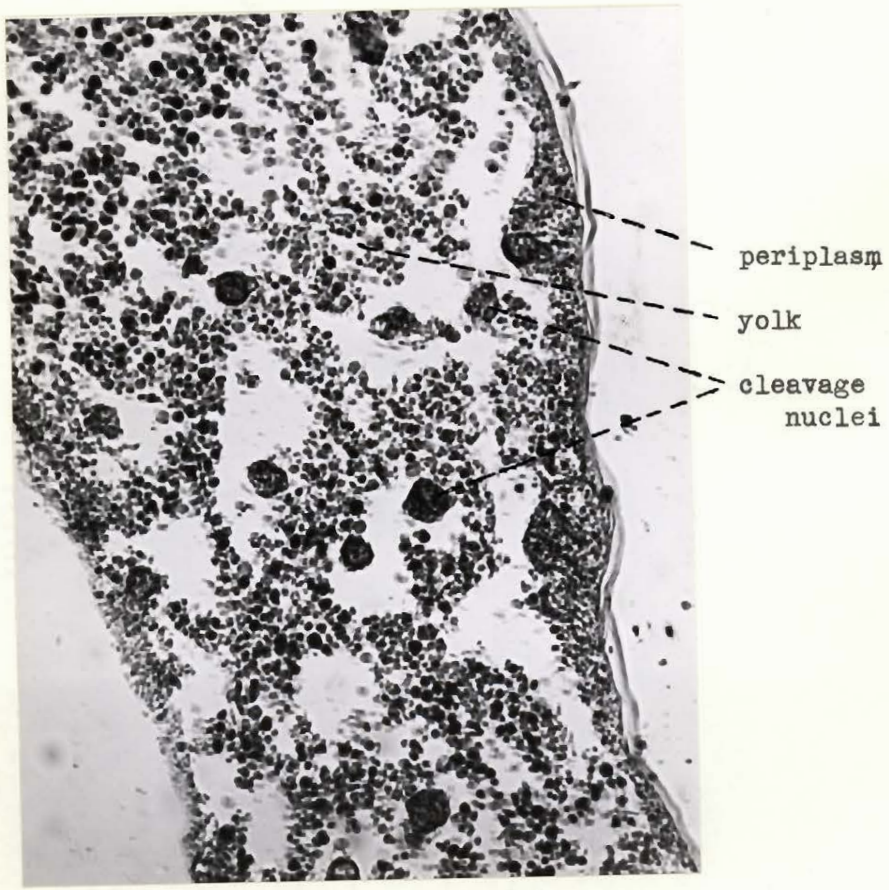


Fig. 11. Sagittal section of an egg immediately following the eighth synchronous cleavage. Photomicrograph.

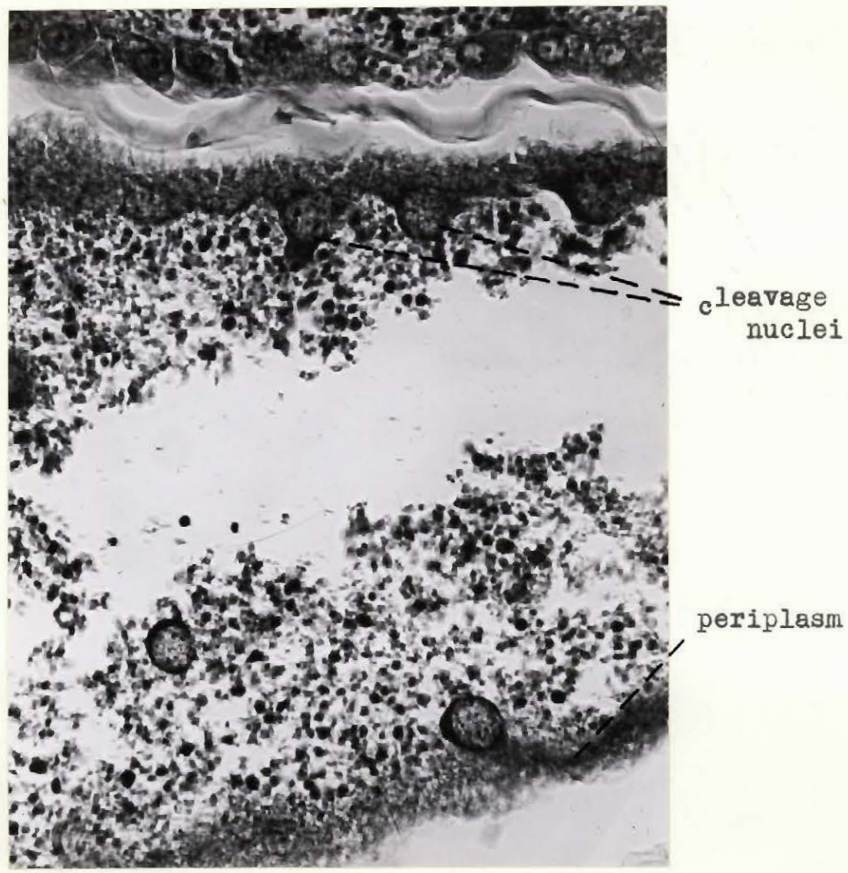


Fig. 12. Sagittal section showing cleavage nuclei entering the periplasm just before the tenth synchronous cleavage. photomicrograph.

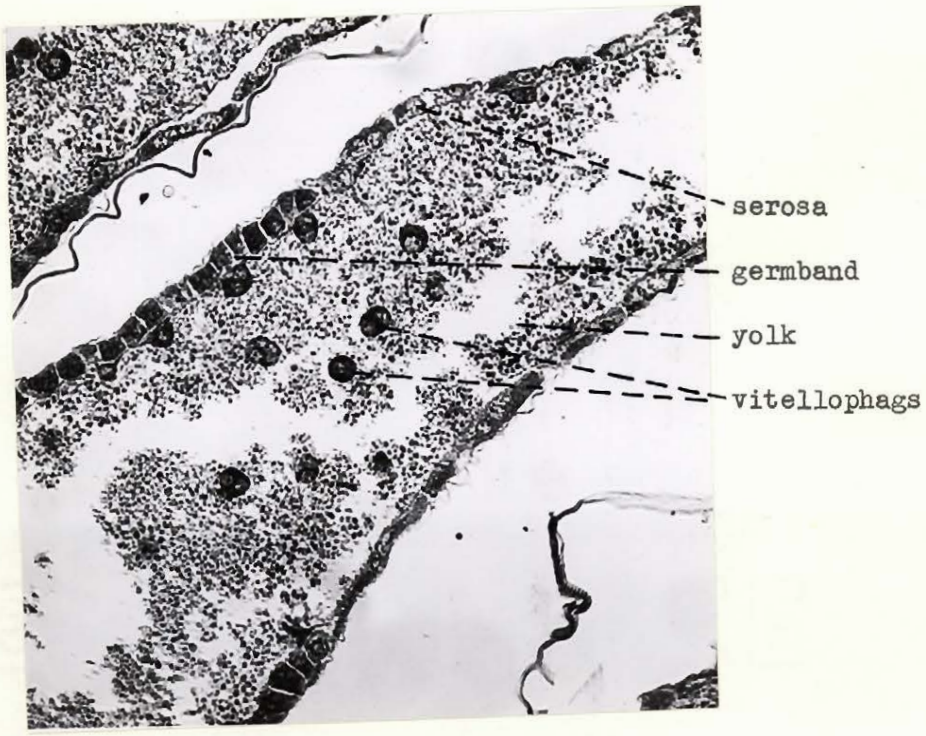


Fig. 13. Sagittal section of an egg at ten hours. Notice that the serosa is beginning to spread over the germband. Photomicrograph.

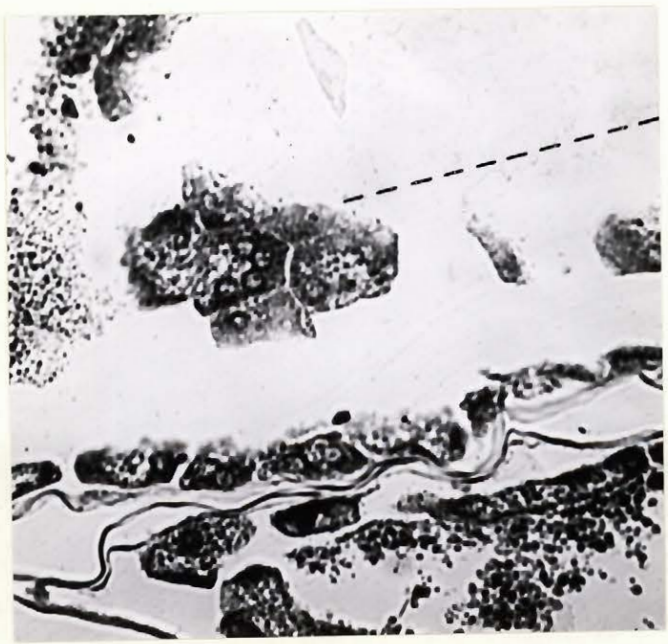


Fig. 14. Tangential section of a few serosal cells immediately following the completion of the serosa. Photomicrograph.

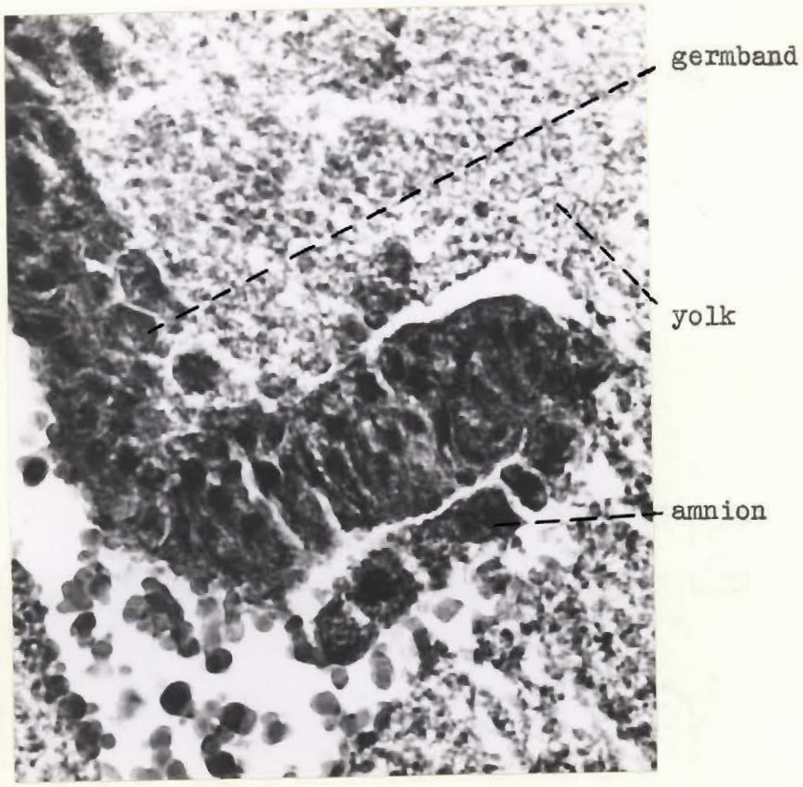


Fig. 15. Transverse section of the germband showing the amnion beginning to form. Notice that the cells are quite compact and columnar. Photomicrograph.

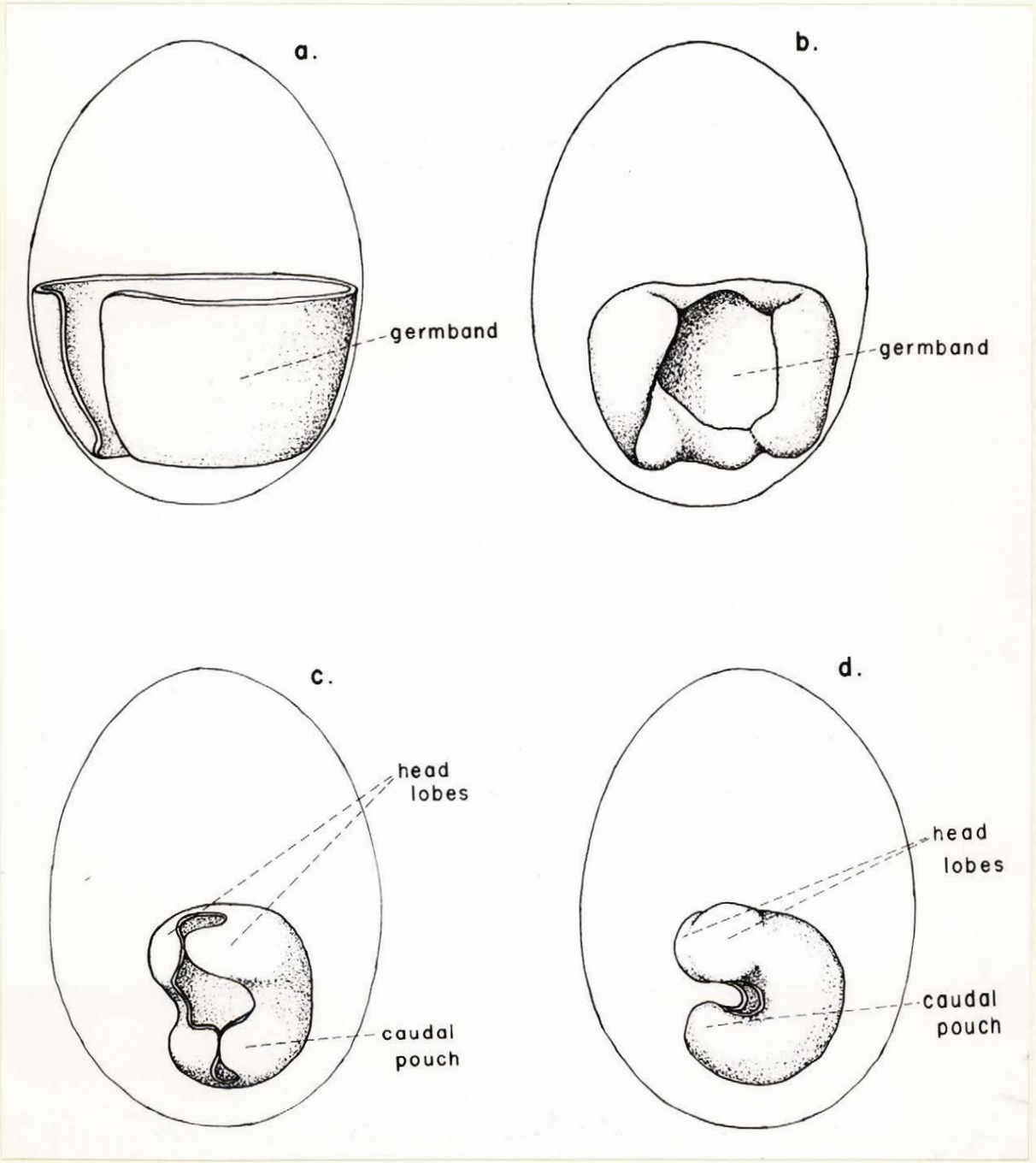


Fig. 16. Diagrams of macroscopic embryonic development. (a) Embryo at 10 hours,(b) at 13 hours,(c) at 16 hours and (d) at 19 hours.

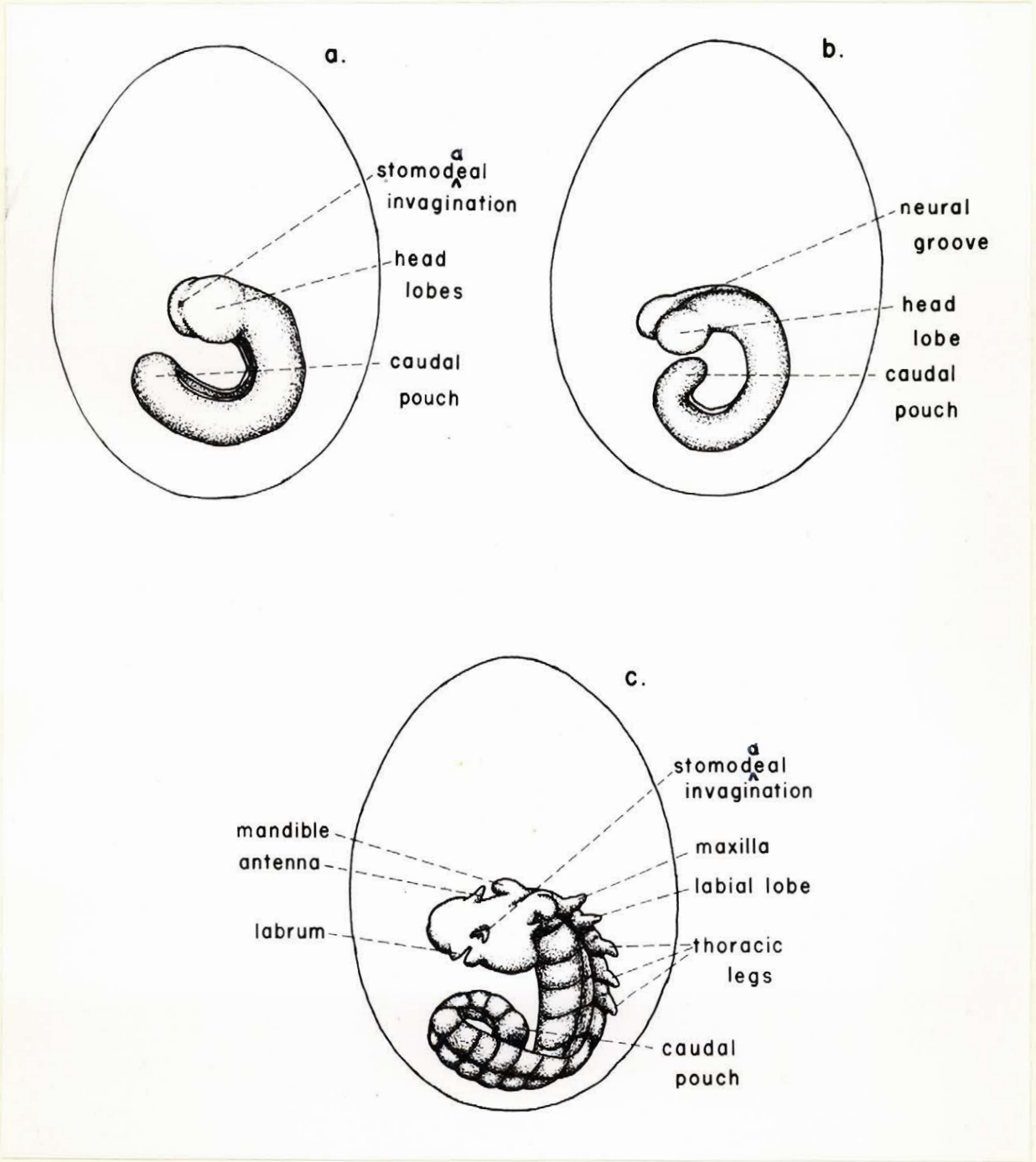


Fig. 17. Diagrams of macroscopic embryonic development. (a) Embryo at 21 hours, (b) at 26 hours and (c) at 40 hours.

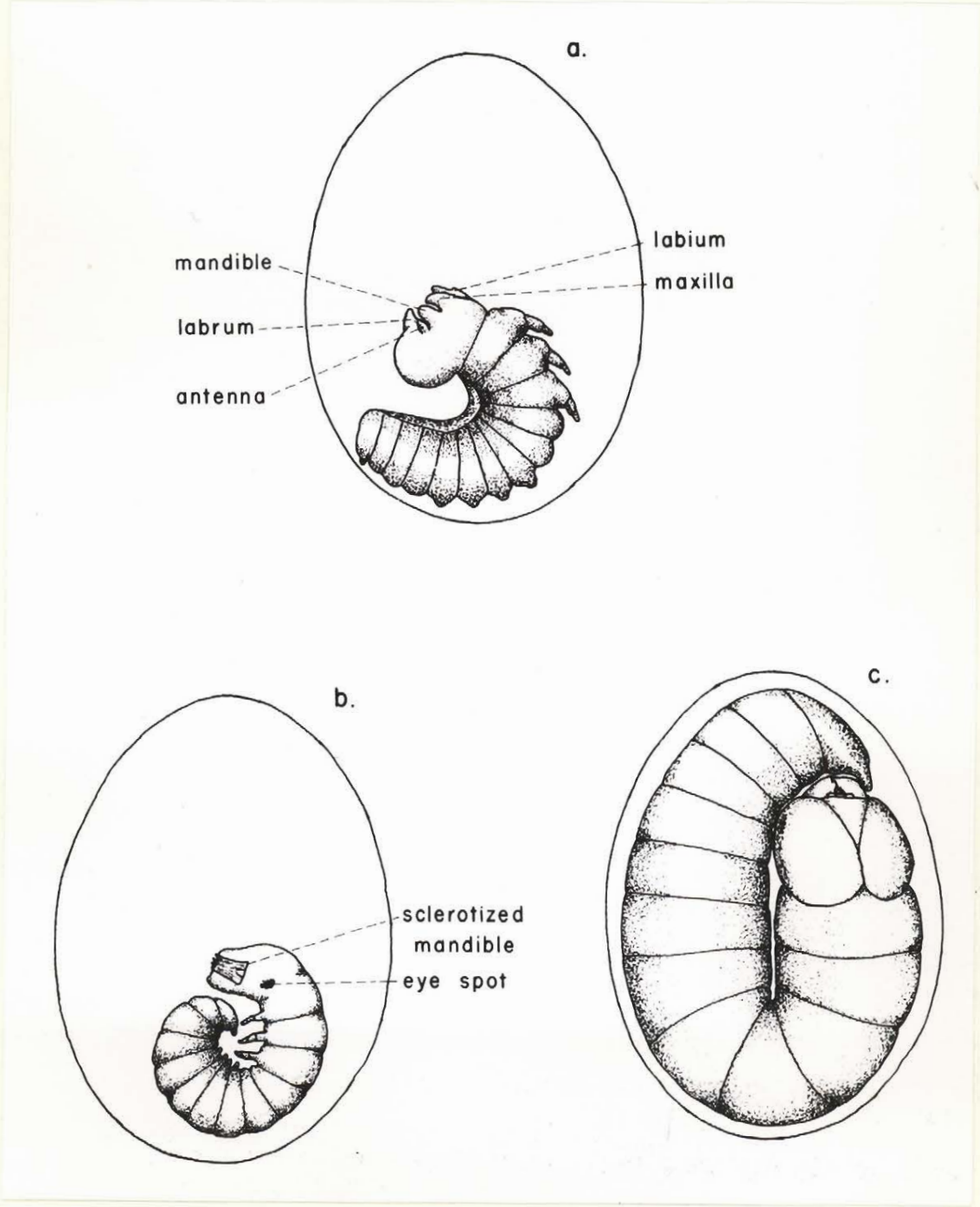


Fig. 18. Diagrams of macroscopic embryonic development. (a) Embryo at 72 hours, (b) at 96 hours and (c) at 144 hours.

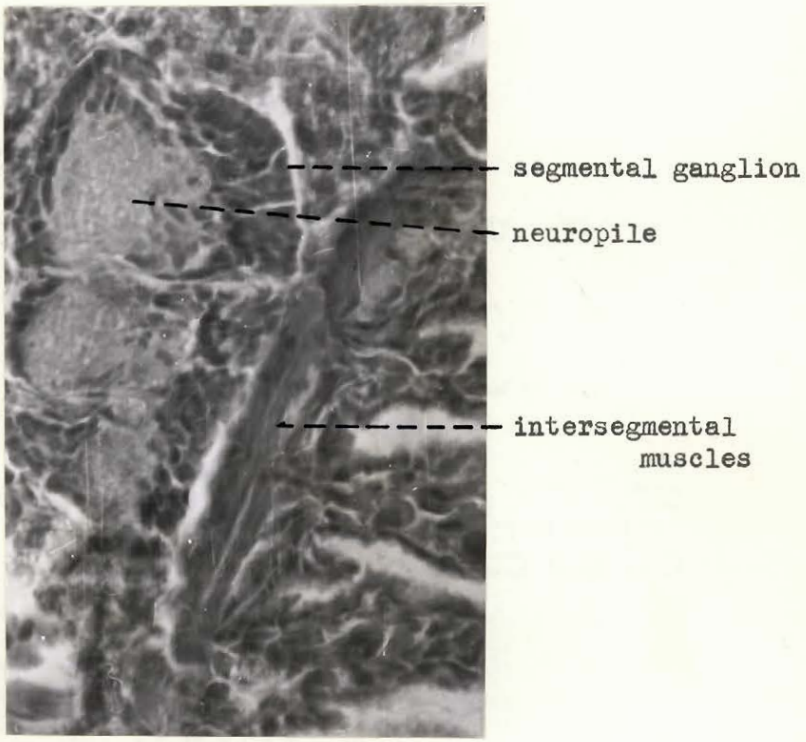


Fig. 19. Longitudinal section of a few abdominal segments of a 100-hour embryo showing the degree of development of ganglia and muscles. Photomicrograph.

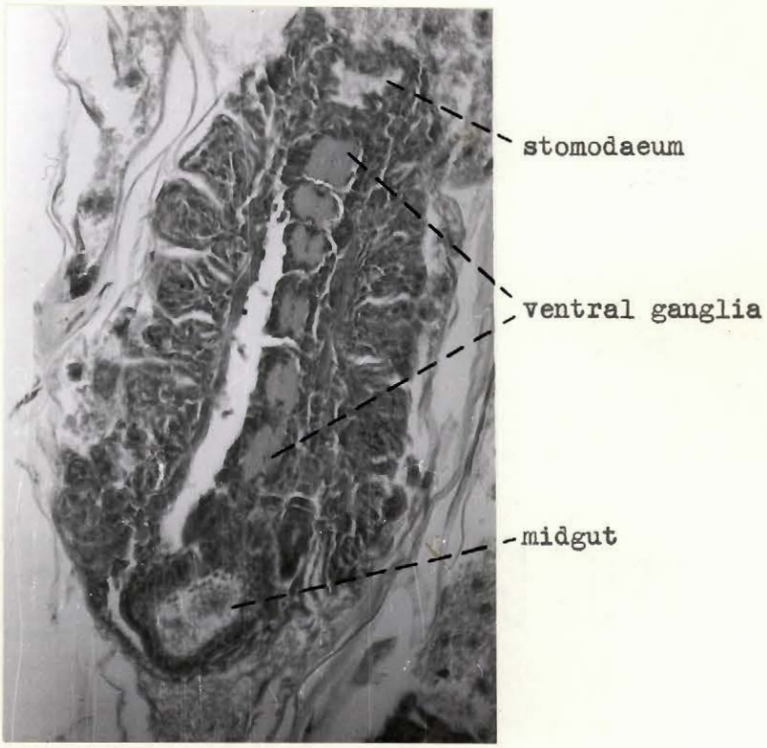


Fig. 20. Longitudinal section of a 100-hour embryo showing segmental ganglia, foregut, and midgut. Photomicrograph.

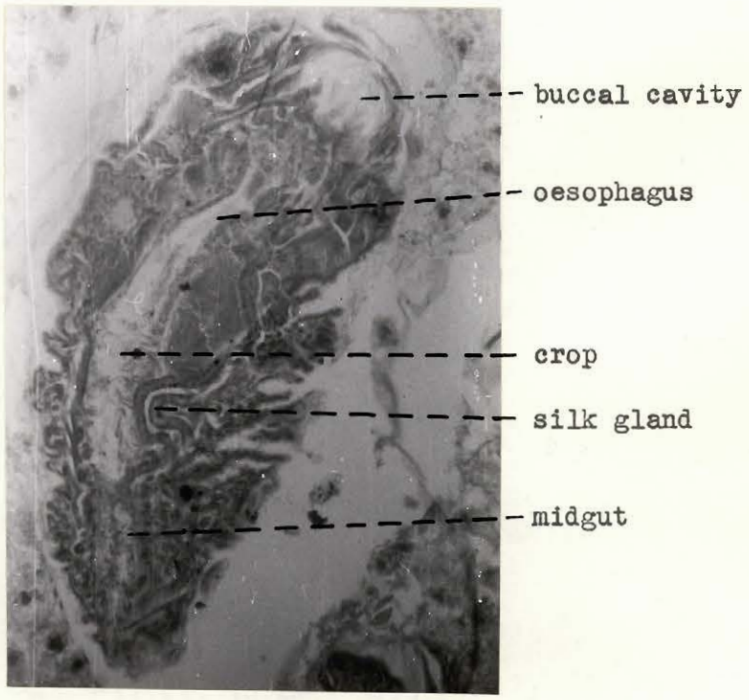


Fig. 21. Longitudinal section of the anterior end of a 100-hour embryo. Photomicrograph.

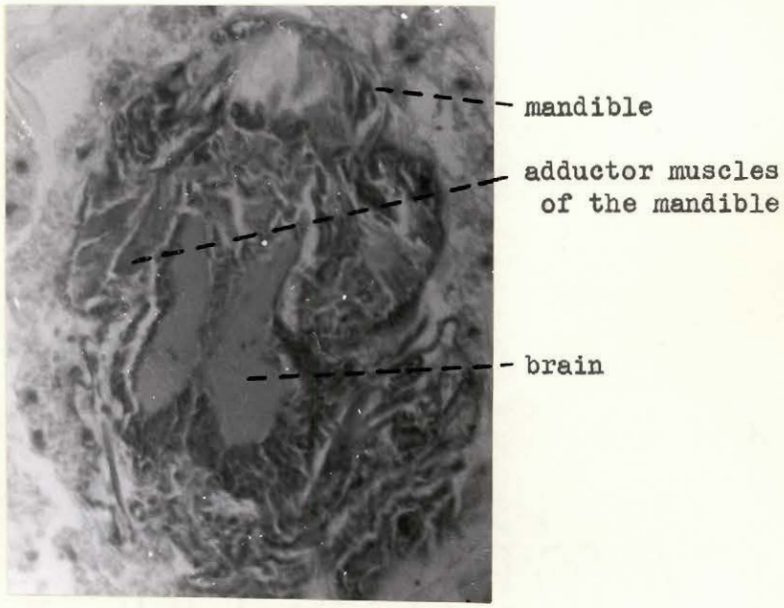
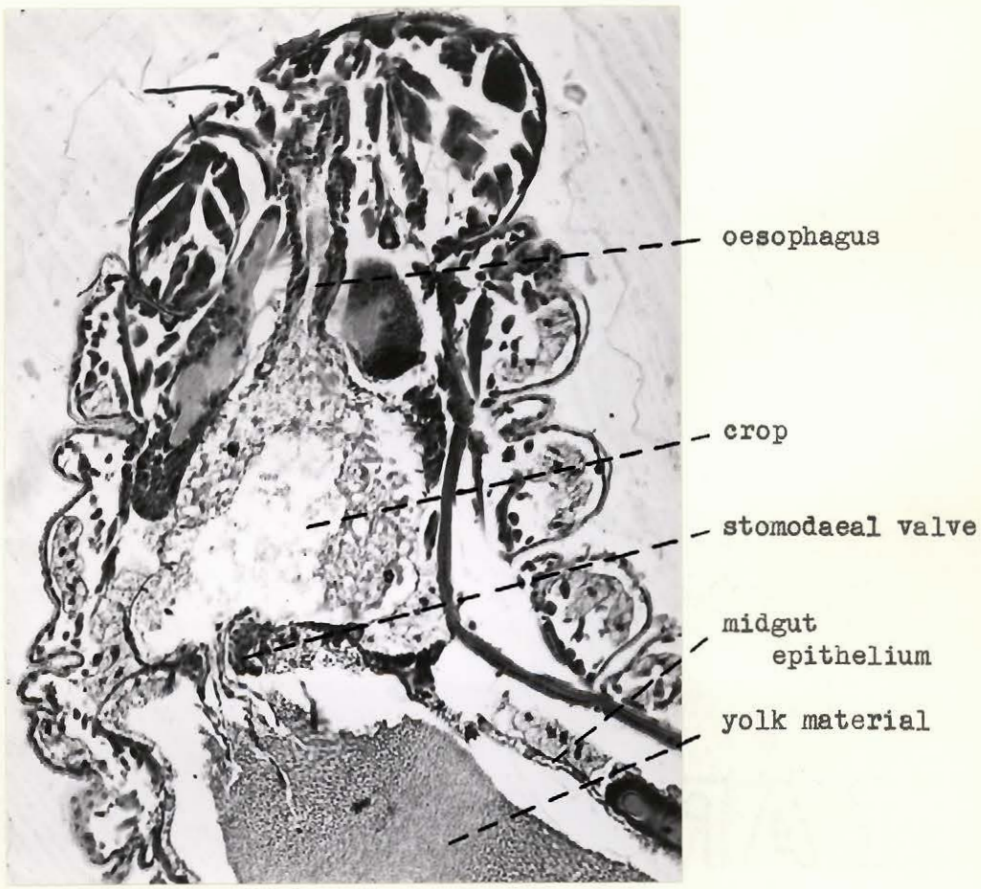


Fig. 22. Longitudinal section of the head of a 100-hour embryo showing the degree of development of the mandibular muscles and the brain. Photomicrograph.



Fig, 23. Longitudinal section of the anterior end of a newly-hatched larva. Photomicrograph.

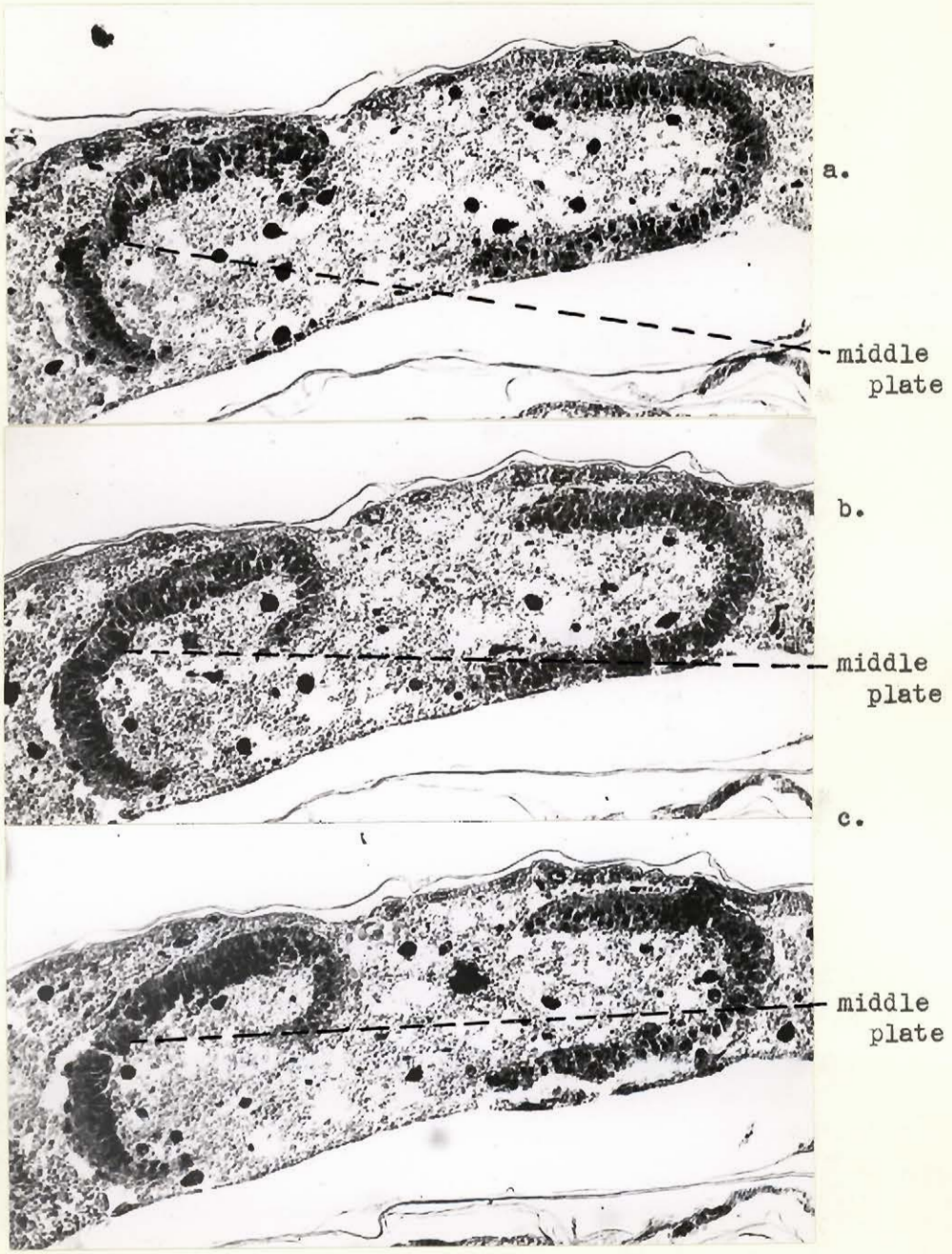


Fig. 24. Transverse sections of a 23-hour embryo showing the mesoderm beginning to invaginate. Photomicrographs.

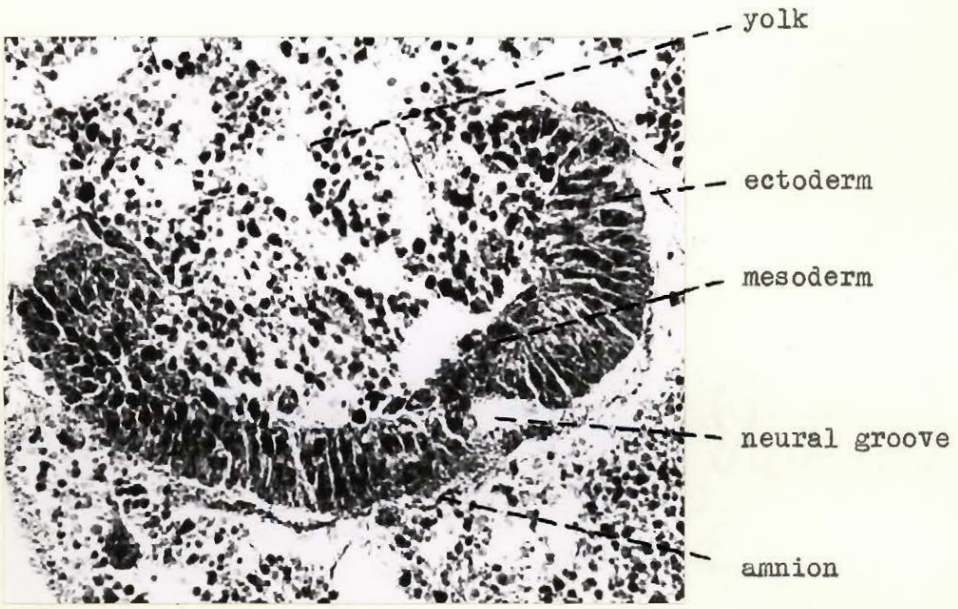


Fig. 25. Transverse section of a 26-hour embryo shortly after the invagination of mesoderm. Photomicrograph.

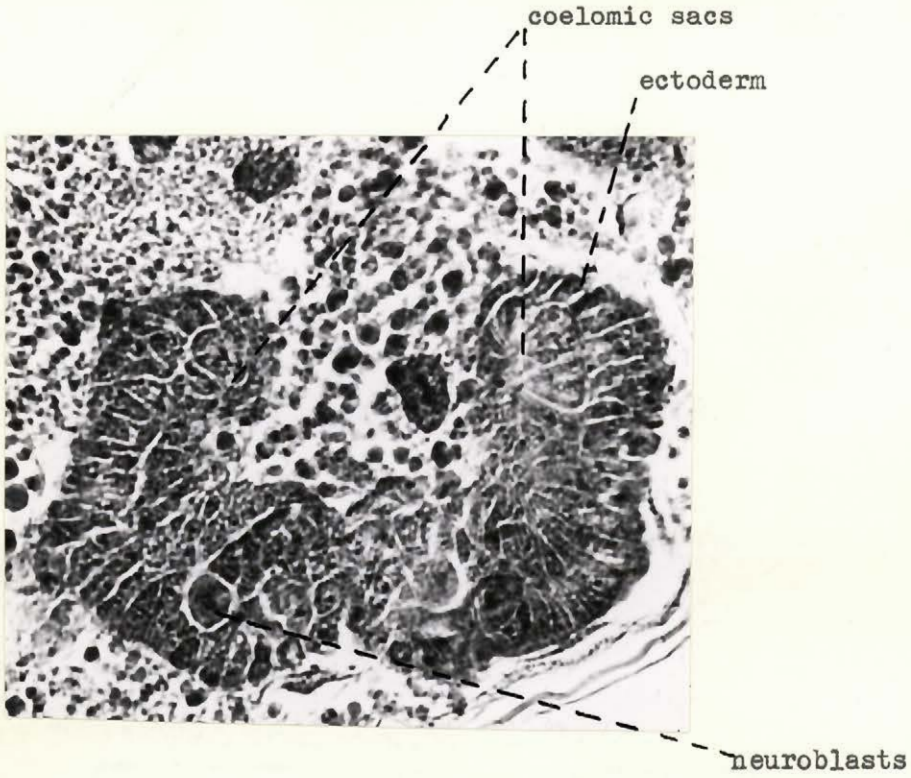


Fig. 26. Transverse section of a body segment of a 40-hour embryo showing the completed coelomic sacs and the beginning of nerve cell proliferation by the neuroblasts. Photomicrograph.

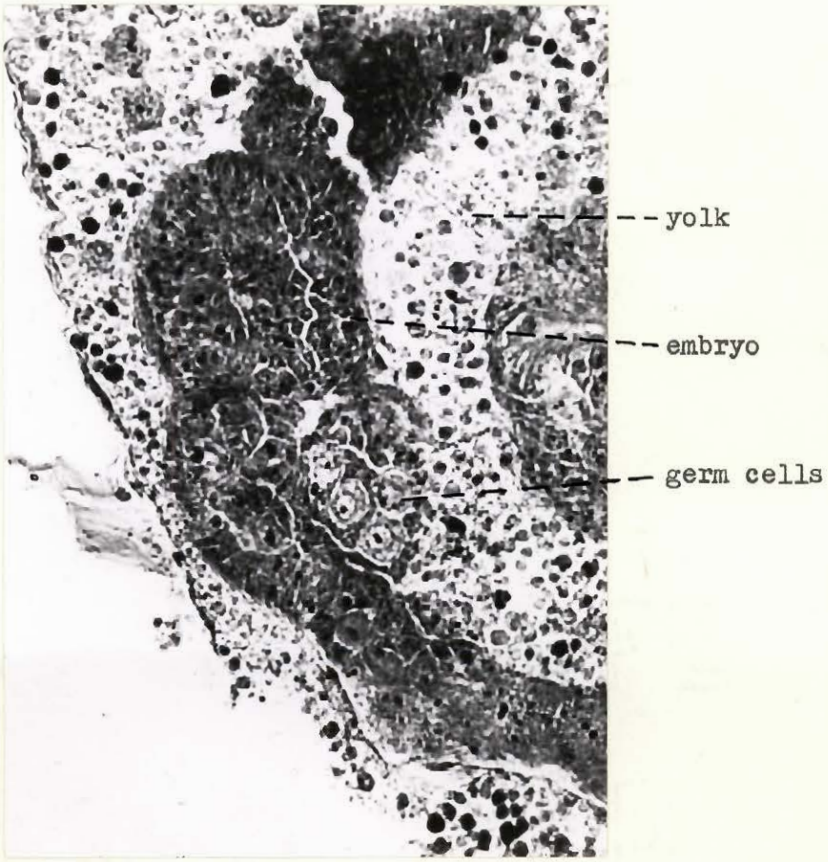


Fig. 27. Longitudinal section of part of a 40-hour embryo showing the germ cells in the mesoderm of the fifth abdominal segment. Photomicrograph.

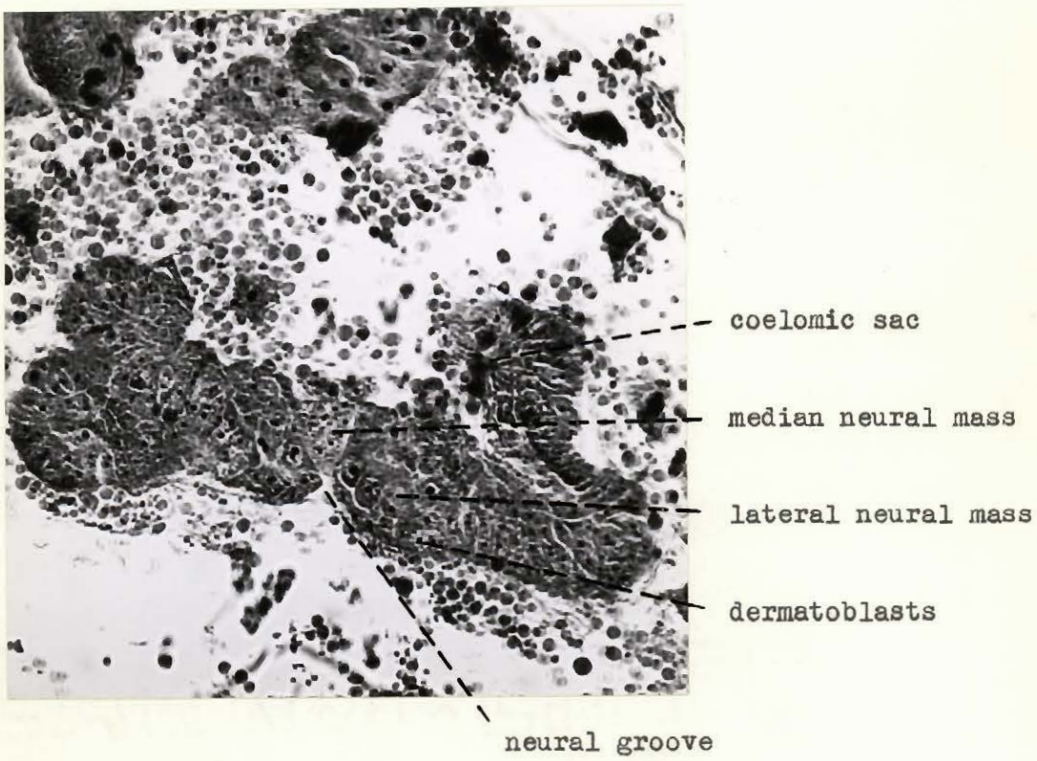


Fig. 28. Transverse section of a thoracic segment of a 40-hour embryo showing the median neural mass. Photomicrograph.

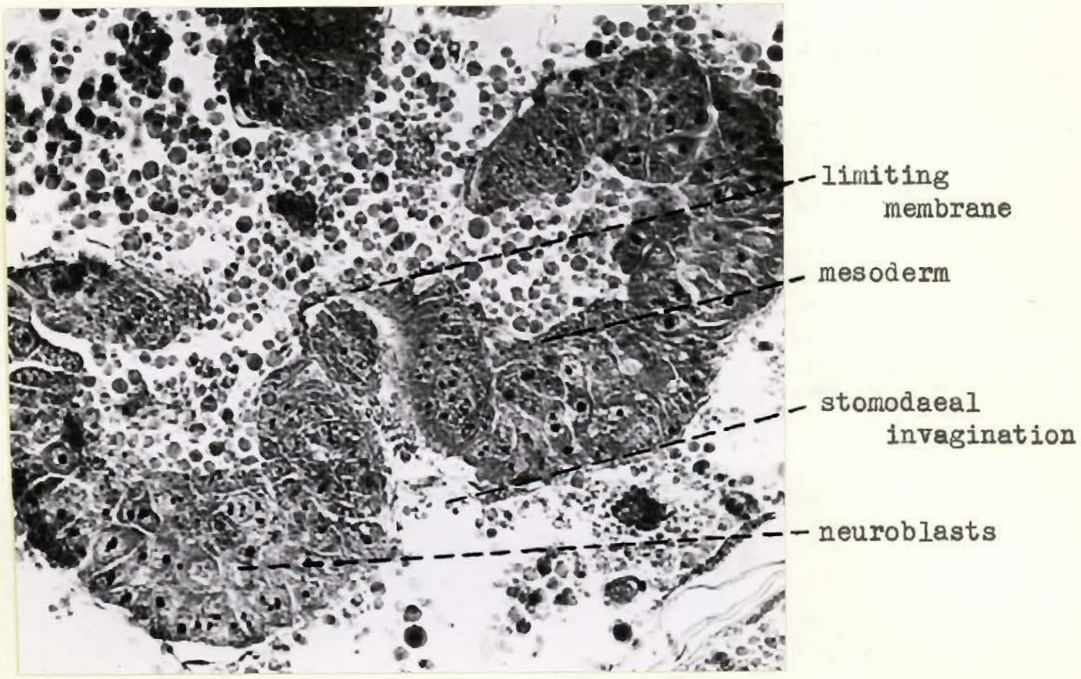


Fig. 29. Transverse section of a 40-hour embryo through the head lobes showing the stomodaeal invagination. Photomicrograph.

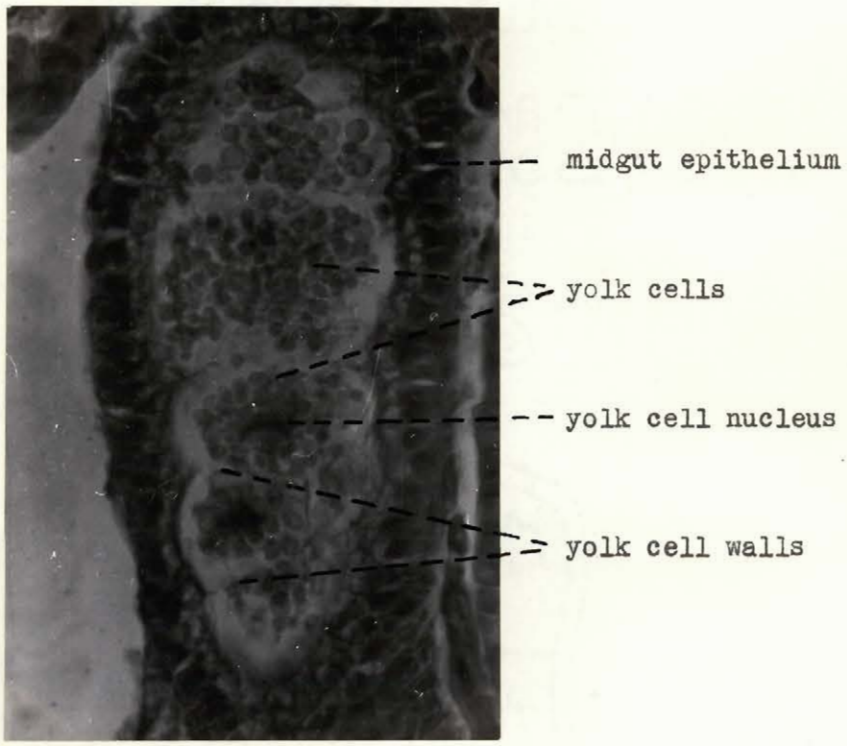


Fig. 30. A section of the midgut epithelium of a 100-hour embryo. Photomicrograph.

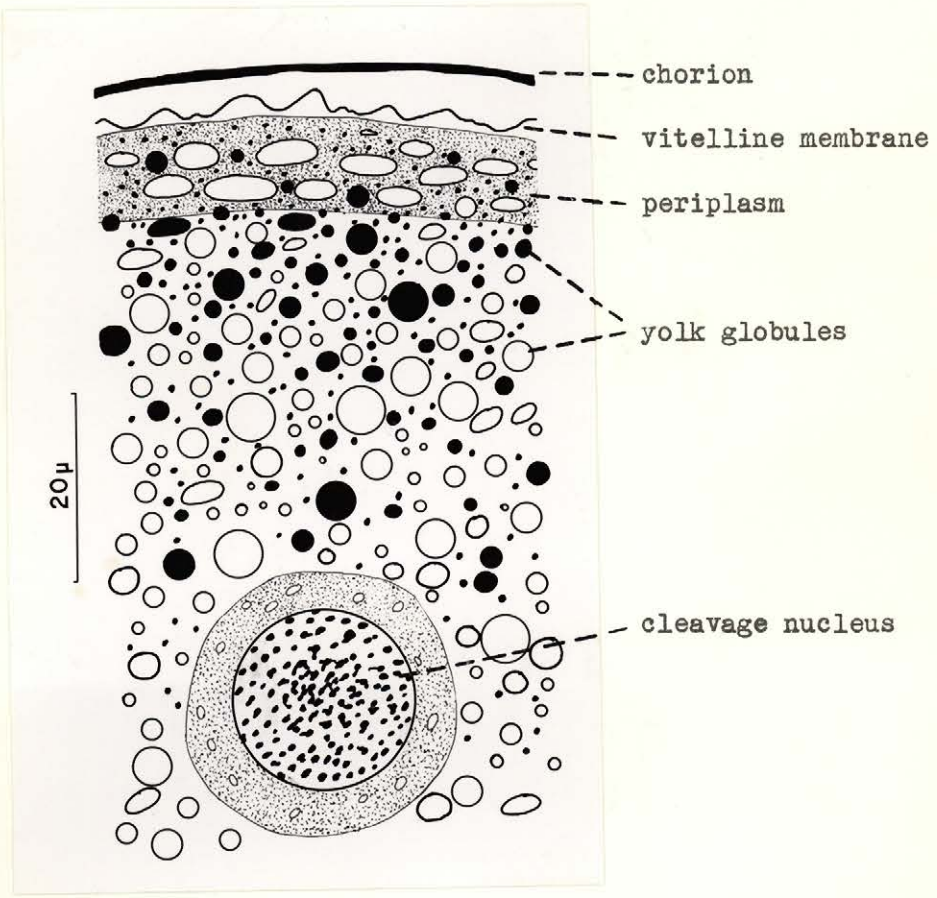


Fig. 31. Transverse section of part of an egg at about seven hours showing basophilic yolk globules external to the cleavage nucleus.

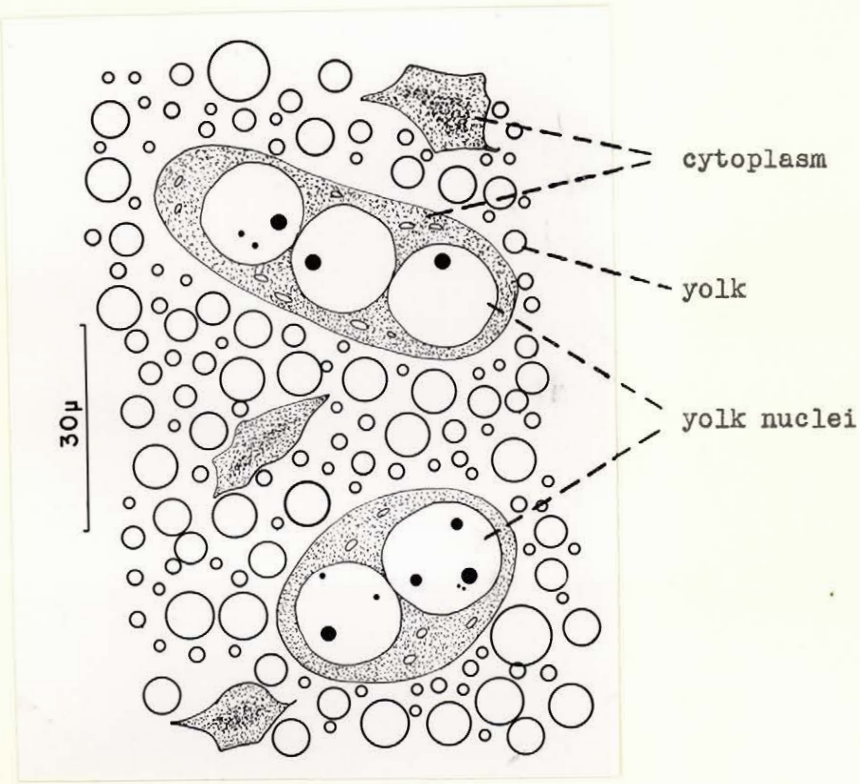


Fig. 32. A drawing of a section of yolk at the time of blastoderm formation.

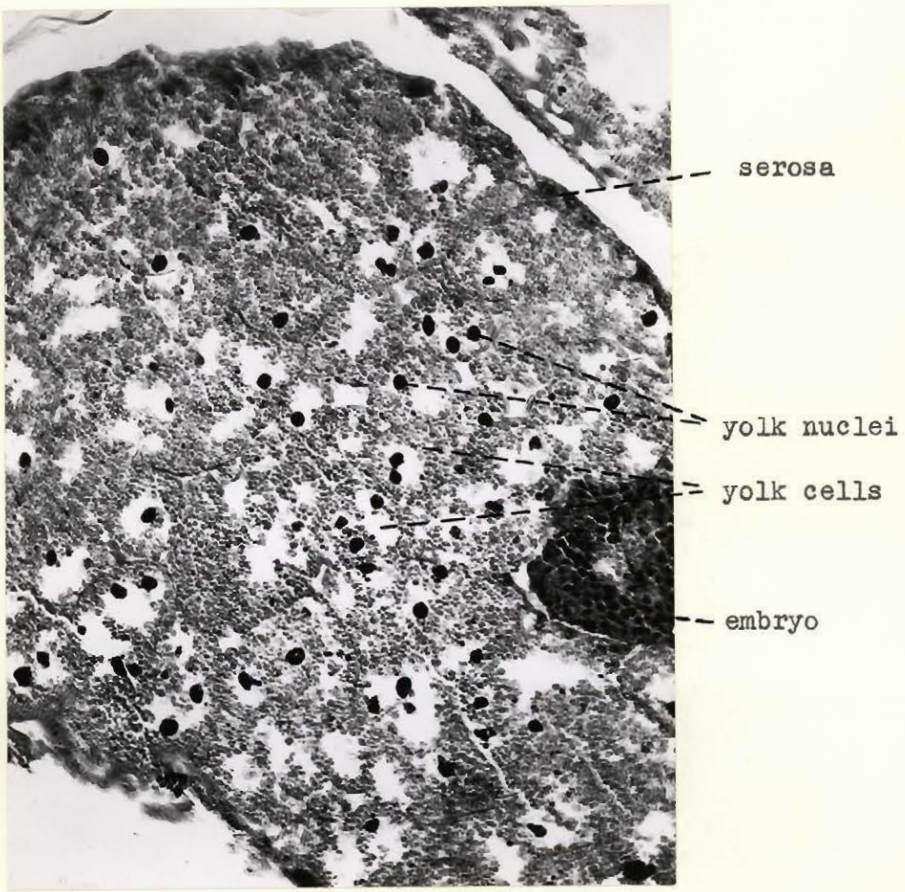


Fig. 33. Sagittal section of the yolk of a 40-hour egg showing the multinucleate yolk cells. Photomicrograph.

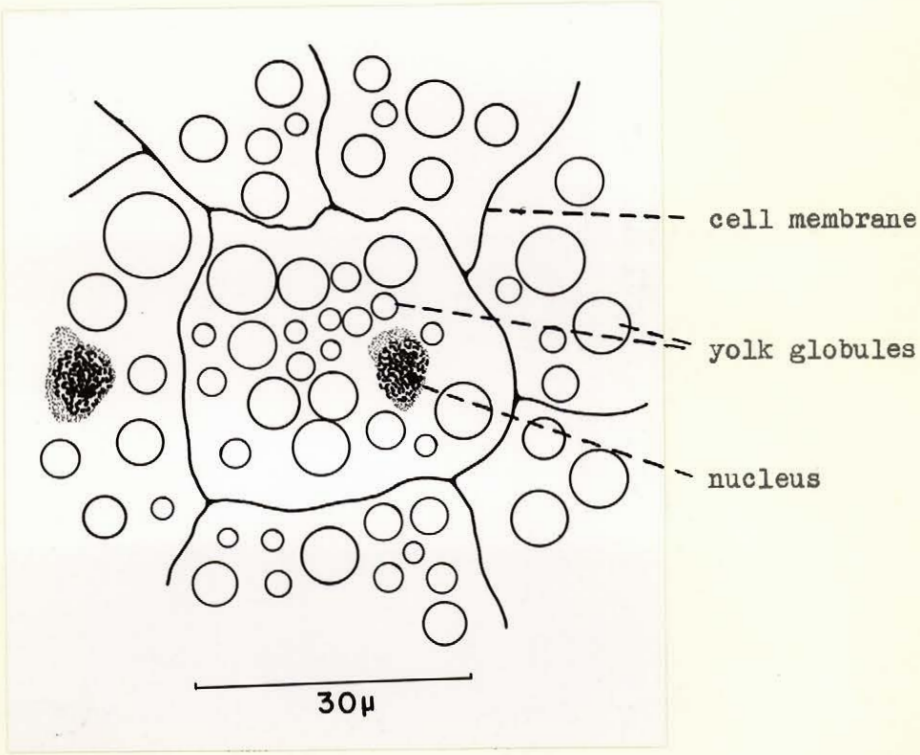


Fig. 34. A drawing of a section of yolk at 120-hours. Notice that the cells have single nuclei.