THE POLYEMBRYONIC DEVELOPMENT OF PLATYGASTER VERNALIS

BY

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(Contribution from North Carolina Agricultural Experiment Station and Bureau of Entomology)

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THE POLYEMBRYONIC DEVELOPMENT OF PLATYGASTER VERNALIS [1]

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INTRODUCTION

In a previous paper the writers (*4*) [3] referred to the desirability of demonstrating insect polyembryony in a species in which but a few individuals are developed from a single egg. The development of such a species was in part described for *Platygaster hiemalis*, a parasite of the Hessian fly.[4] It was found, however, that polyembryony was carried only to the point where twin parasites were produced from a single egg, thus demonstrating the simplest type of polyembryony possible.

A slightly more complex form of polyembryony will be described here for *Platygaster vernalis* (Myers), another parasite of the Hessian fly, in which an average of about eight individuals are developed from a single egg of the parasite. A knowledge of the development of this species will furnish a further clue to the more highly specialized forms of polyembryony in which as many as 150 to 2,000 individuals are produced from a single egg.

Platygaster vernalis develops only in the mid-intestine of the host larva. The development of a closely related species, *Polygnotus minutus* Lindemann, which is also confined to the mid-intestine of the Hessian fly larva in France, has been described previously by Marchal (*5*). Marchal's paper upon this insect is now difficult to obtain. However, his paper does not treat of the precleavage or cleavage stages of development in a sufficiently detailed manner to demonstrate polyembryony, but it does describe quite fully the organogeny of the embryos. There are also some indications that *P. minutus* and *P. vernalis* differ slightly in the details of their development, although it is difficult to determine this definitely, because the development of Marchal's species is not illustrated with sufficient histological preparations. In the present paper, therefore, emphasis has been placed upon a study of microtomic sections, and the paper has been illustrated [5] from this viewpoint rather than upon gross examinations.

RELATION OF PARASITE TO HOST

The biology of *P. vernalis* with particular reference to its economic importance as a parasite of the Hessian fly, which is a serious pest of wheat, has been previously dealt with by the junior writer (*2*). The adults emerge from their

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[2] The writers gladly acknowledge the continued interest shown by Dr. L. O. Howard and W. R. Walton in the studies reported in this paper. To Doctor Howard is due the chief credit for calling the attention of American investigators to insect polyembryony. Our studies on the development of the Platygaster parasites of the Hessian fly were initiated at the direction of Mr. Walton.

[3] Reference is made by number (italic) to "Literature cited," p. 839.

[4] *Phytophaga destructor* Say.

[5] All of the illustrations of microtomic sections except figures C and D on Plate 1 were drawn by the senior writer. The remainder of the drawings were prepared by the junior writer. The photomicrographs were made by the photographer of the Bureau of Entomology.

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cocoons (Pl. 1, E) during April and the early part of May. A group of approximately eight individuals emerge from each host puparium of the Hessian fly when parasitized by this insect. The female parasites, whether fertilized or unfertilized, deposit their eggs in the eggs of the host (Pl. 1, A, B) which are deposited on the wheat plants by the spring generation of flies. The parasite eggs begin development immediately (Pl. 1, C), the embryos being fully formed in the nearly mature host larvæ (Pl. 1, D) by the first of June. During June and July the parasite larvæ feed upon the contents of the host larvæ, each group of parasites devouring all of a host larva (Pl. 8, C) with the exception of its integument. After the parasite larvæ are fully grown (Pl. 8, B) they remain for some time in their cocoons which they have prepared inside the host larval integument. About the latter part of July the larvæ transform to pupæ (Pl. 8, D), these in turn transforming to adult parasites some time during August.

The adult parasites remain in their cocoons (Pl. 1, E) during the winter, being protected by the puparium or toughened integument of the host; and emerge from their cocoons and the host puparia in spring, by gnawing one or more holes through the cuticula and puparium of the host (Pl. 1, F). These adult parasites then search for eggs of the spring generation of the Hessian fly in which to oviposit.

PRECLEAVAGE DEVELOPMENT OF THE EGG

The eggs of *Platygaster vernalis* are always deposited singly, but occasionally a second or third egg may be placed in the same host egg by other females. However, the same female parasite usually avoids ovipositing in any host egg more than once. This parasite differs from *Platygaster hiemalis*, another parasite of the Hessian fly, therefore, in the number of eggs deposited at one time; *P. hiemalis*, as has been shown by the writers (*4*), placing a cluster of four to eight eggs in the host egg at each oviposition.

The egg of *Platygaster vernalis* is always so placed in the host egg that it is eventually found in the mid-intestine of the host embryo or young larva with unfailing regularity (Pl. 1, C). The egg does not and apparently can not undergo development in any other part of the host. The proper placing of the egg is doubtless accomplished by a complete coordination of certain factors, among which are the orientation of the host egg, the manner of striding the egg by the parasite, and the length of the parasite's ovipositor. It is of interest to note, on the contrary, that *P. hiemalis* always deposits its eggs in the host egg, so that they are never lodged in the mid-intestine, where apparently they would fail to develop.

THE NEWLY DEPOSITED EGG

The newly deposited egg is somewhat elongate, but it soon becomes more compact, cylindrical in shape, and rounded at both ends (Pl. 2, A), measuring, according to fixed and sectioned material, approximately 21 μ in length and 8 μ in width. The protoplasm of the egg appears very finely granular and uniform, and contains a spherical and darkly staining concentrated nucleus which measures 3 μ in diameter. Immediately after oviposition the nucleus is found in or near the center of the egg. The long and thread-like sperm (Pl. 2, B) is somewhat difficult to demonstrate in all eggs deposited by fertilized females, probably because of its wavy and spiral position in the protoplasm of the egg. There is also some evidence that not all eggs deposited by impregnated females are inseminated. The nucleolus or germ-cell determinant is wanting, just as it is in *Platygaster hiemalis*, and in *P. dryomyiae* as shown by Silvestri (*8*).

MATURATION AND THE ORIGIN OF THE PARANUCLEAR MASSES

In a general way the maturation of the egg of *Platygaster vernalis* is similar to that described for other species of Platygaster. As far as can be ascertained, it is identical in fertilized and unfertilized eggs. As in previously described polyembryonic and some monembryonic Hymenoptera, the polar bodies are retained in the egg and eventually give rise to the paranuclear masses which have a nutritive function in the course of development of the embryos; while the oöcyte nucleus, whether fertilized or not, gives rise to the embryos.

The first maturation of the oöcyte nucleus is completed during the first eight hours after oviposition. Within the first 30 minutes the nucleus begins to expand, and by the fifth hour it is in the prophase stage of mitosis (Pl. 2, B, C). Thereafter maturation is completed quickly; the chromosomes being found first, grouped together at each end of the spindle but distinct from each other (Pl. 2, D), and then, later, somewhat concentrated into two separate nuclei (Pl. 2, E). The chromosomes of each of the two nuclei then condense further and form irregularly shaped, homogeneous, dark staining nuclei (Pl. 2, F, G), which are always quite conspicuous in the egg in spite of their small size. The first maturation takes place longitudinally in the anterior half of the egg, and results in the production of the first polar body, which passes to the anterior edge of the egg, and the oöcyte nucleus of the second order, which remains near the center of the egg.

Between the eighth and the twelfth hours after oviposition the first polar body and the oöcyte nucleus of the second order remain quiescent, but about the twelfth hour a second maturation of the oöcyte nucleus commences, and the mitotic division results in the production of two distinct groups of chromatin material in the central region of the egg (Pl. 2, H–J). The second maturation spindle is always considerably shorter than the first. Various stages of the division can be observed in eggs that are 12 hours old, indicating that second maturation is completed quickly. The anterior of the two centrally-disposed nuclei is the second polar body, which later migrates toward the polar or anterior region of the egg by the side of the first polar body. The posterior nucleus of the second maturation spindle becomes the female pronucleus of the fertilized egg or the cleavage nucleus of the unfertilized egg. It remains in the posterior half of the egg from the twelfth to the twenty-fourth hour, increasing in size during this interval from a diameter of 2.2 μ just after maturation is completed to 5.4 μ at the time of fusion with the male pronucleus.

About 24 hours after oviposition the two polar bodies begin to expand, becoming at first spherical and then oval (Pl. 2, K, L; Pl. 3, A, B), thus assuming the shape and appearance of typical paranuclear masses, as described for other polyembryonic Hymenoptera. As the polar bodies expand the chromatin breaks up into numerous small granules which are scattered throughout the plasm of each nucleus. As will be shown later, the polar bodies (henceforth known as paranuclear masses) migrate about in the egg and divide amitotically into similar secondary masses. It should be noted that simultaneously with the expansion of the polar bodies the egg increases in size, measuring in its greatest diameter when 1 day old approximately 28 μ in length and 9 μ in width.

FERTILIZATION

During the first six hours after oviposition the sperm transforms into an oval nucleus (Pl. 2, D, E), which is always located in the posterior region of the egg. A stage of the male nucleus is then evident, when the chromatin resembles a tightly coiled thread (Pl. 2, F). The threadlike chromatin next breaks up into numerous pieces (Pl. 2, I), which are then distributed over a matrix (Pl. 2, G, H). Meanwhile, the nucleus has expanded until it measures approximately 5 μ in diameter. These changes undergone by the male nucleus are not always synchronous with the various stages of maturation but they take place between the tenth and twelfth hours after oviposition. From the twelfth to the twenty-fourth hours the male nucleus remains in a quiescent condition.

Fusion of the male and female pronuclei is effected about 24 hours after oviposition, in the posterior half of the egg (Pl. 2, K, L; Pl. 3, A, B). The egg now contains two paranuclear masses in its anterior region, and a cleavage or embryonic nucleus in its posterior region.

DIFFERENTIATION OF TROPHAMNION AND EMBRYONIC REGION

Shortly after the cleavage nucleus is formed, an area is seen to encompass it which is somewhat less dense than the remainder of the egg. This more lightly stained area is the embryonic region (Pl. 3, B), which together with the cleavage nucleus gives rise to the embryos. The remainder of the egg containing the two paranuclear masses constitutes the trophamnion.

The egg is henceforth properly known as the parasite body, for it now begins to increase in size. The active feeding of the host, which is soon to commence, permits the trophamnion to absorb the elements from the chyle in the host which are necessary for further growth. Similarly, the elaboration of the trophamnion and the distribution of the paranuclear masses within it are conducive to cleavage and the development of the embryos.

SUMMARY AND DISCUSSION OF PRECLEAVAGE DEVELOPMENT

In most respects the precleavage development of *Platygaster vernalis* is not unlike that previously described by the writers (*4*) for *P. hiemalis*, nor even greatly different from that described by Silvestri (*8*) for *P. dryomyiae*, which develops monembryonically. In *P. dryomyiae* the first polar body divides during the second maturation of the oöcyte nucleus, and the second polar body, produced at second maturation, unites with the posterior half of the divided first polar body to form one paranuclear mass; while a second paranuclear mass is developed from the anterior half of the divided first polar body. In *P. hiemalis* the writers have shown that the two polar bodies, resulting from the two maturations of the oöcyte nucleus, unite to form one polar nucleus, which later divides to form two subequal paranuclear masses. It has been shown above that in *P. vernalis* the two polar bodies neither divide (as they do in *P. dryomyiae*), nor unite (as in *P. hiemalis*), but that they develop directly by expansion into two subequal paranuclear masses.

If there is any significance in the fact that the first polar body divides before forming a paranuclear mass, or that the two polar bodies coalesce, or that they develop directly into paranuclear masses, it is not yet understood. In any event, the object accomplished is identical: paranuclear masses originate from the polar bodies, and are confined thereafter to a differentiated plasm of the egg known as the trophamnion. The function of the trophamnion is nutritive. It elaborates as the parasite body grows, and portions of it eventually surround each embryo of a polyembryonal mass, thus permitting each embryo to be so nursed that it can develop into a young larva. Henceforth the insect is able to provide for itself by direct feeding upon the host.

In other polyembryonic hymenopters Leiby (*3*) and the writers (*4*) have shown that the egg, before cleavage or very shortly thereafter, must be encompassed by host tissue in order to continue development. Eggs which are not so provided cease development and become aborted. The egg of *Platygaster vernalis* is not encysted by host tissue, but is able to continue its development by reason of its location in the chyle of the host's stomach. The chyle, it should be recalled, is in reality the sap of the wheat plant, although it may be altered chemically by the nuclei of the intestinal lining; so that in one sense the parasite body may be regarded as developing in host tissue.

DEVELOPMENT OF THE PARASITE BODY IN THE HOST LARVA

The development of the embryos of *Platygaster vernalis* covers a period of about thirty days and takes place largely during the month of May, while the host larva is feeding and maturing upon the wheat plant. The presence of the parasite body in the stomach of the host apparently does not affect its growth. Like other species of polyembryonic parasites the *P. vernalis* larvæ are not developed to the point where they begin feeding upon the host until after the host is completely grown. Precocious development of the parasite larvæ, and feeding before the host is fully grown, would result either in their starvation or a reduction in the number produced from a single egg caused by the feeding of some of the parasites upon others of the brood.

The pupal and adult stages of the parasites are developed in individual cocoons (Pl. 1, E) formed by the larvæ in the carcass of the host during late summer. The adults remain in the cocoons during the winter and emerge the following spring.

EARLY CLEAVAGE AND THE FORMATION OF THE POLYGERM

About the second day after oviposition the cleavage nucleus migrates toward the center of the egg, where it divides to form two daughter embryonic nuclei (Pl. 3, C) of equal size. A second, third, and fourth cleavage of the embryonic nuclei take place between the third and sixth days after oviposition. These divisions of the nuclei produce parasite bodies which contain four, eight, and sixteen embryonic nuclei respectively (Pl. 3, D–F; Pl. 4, A). The embryonic nuclei are located in the central part of the parasite body in an enlarged embryonic region, which was differentiated around the cleavage nucleus when the parasite body was one day old. They are all of about the same size and measure approximately 4 μ in diameter.

While the first four cleavages take place, the trophamnion becomes elaborated proportionately. The earliest change is observed at the first cleavage, when one of the paranuclear masses divides to form two masses which immediately proceed to increase in size (Pl. 3, C). At this time the parasite body measures 24.3 μ in length and 10 μ in width. At the second cleavage four paranuclear masses (Pl. 3, D) are observed in the trophamnion. At the third cleavage there may be as many as twelve paranuclear masses. As is shown in Plate 3, E, the masses are distributed uniformly throughout the trophamnion. The parasite body now measures approximately 27.3 μ in length and 14.5 μ in width. Its form is still ovoid, and similar to that of the original egg.

The development of *Platygaster vernalis* reaches that stage which is comparable to the polygerm stage of other polyembryonic hymenopters at the end of the fourth cleavage (Pl. 4, A), at which time the parasite body contains sixteen nuclei in the embryonic region if all nuclei have divided regularly. Each embryonic nucleus then becomes separated from the others, and, surrounded by a bit of the embryonic cytoplasm, the cytoplasm with its nucleus becomes invested by a membrane. A cell is thus formed and recognized as a germ. The germ becomes the progenitor of one or two parasites, depending upon whether or not it divides once in the morula stage. The parasite body now represents a typical polygerm.

If all the nuclei divided regularly at the fourth cleavage the polygerm should contain sixteen germs. If only seven of the eight embryonic nuclei divided at the fourth cleavage, the polygerm would contain only fifteen germs. Difficulties of interpretation as to whether each germ is originally composed of a single cleavage nucleus are often encountered by reason of the fact that the single nucleus of a germ divides to form a binucleated germ before others of the same polygerm divide. Moreover, the nuclei are often so disposed (one above the other) and the germ membrane is sometimes so indistinct, that it appears as if some germs might originally be composed of two embryonic or cleavage nuclei.

The contention that a germ originates from a single embryonic nucleus in this polyembryonic species is based upon a study of such preparations as are illustrated by Plate 4, A and B. Figure A shows that most of the nuclei are each surrounded by a portion of the embryonic cytoplasm, which is in turn encompassed by a membrane. Figure B represents a later stage, and shows two germs containing but a single nucleus each, while two other germs contain two nuclei each as a result of a recent division of the original nucleus of each of the two germs. A fifth germ contains four nuclei, while a sixth has already advanced to the morula stage.

The polygerm is either slightly ovoid or spherical at this time and measures approximately 47 μ in its greatest diameter. The trophamnion now is no longer confined to the periphery of a central embryonic region but penetrates toward the center of the polygerm so that it surrounds each germ.

About the twelfth day the polygerm (Pl. 4, C) measures about 57 μ in diameter. With the increase in size of the polygerm there is also noted an increase in the size of each germ. At this stage certain of the germs divide in toto to form two daughter germs. Not all of the germs divide, and when division takes place the parent germ is usually composed of approximately eight nuclei. A similar division has been recorded by Marchal (*5*) in *Polygnotus minutus* and by the senior writer (*3*) in *Copidosoma gelechiae*. A typical case of germ division is illustrated in Plate 4, C. Here two of the four germs are daughter germs that are still in contact, and separated only by a very thin portion of the trophamnion which filtered between them immediately after division. Henceforth the embryos develop rapidly into the blastula stage.

THE POLYBLASTULA STAGE

About thirteen days after oviposition the parasite body (Pl. 4, E) represents a typical polyblastula stage. The germs have increased both in size and in number of their nuclei. The nuclei become lodged in cells and are arranged regularly in the periphery of the germ, so that a median section through an embryo illustrates a true blastula. At the thirteenth day the polyblastula measures about 55 μ in its greatest length and 45 μ in width.

Between the thirteenth and eighteenth days the polyblastula increases in size until it measures about 75 μ by 68 μ (Pl. 4, F; Pl. 5, A). The oval or spherical shape of the parasite body is maintained. During this interval the blastulas and the cavities in which they are found increase in size proportionately, as do the paranuclear masses of the trophamnion. The trophamnion also appears somewhat vacuolated.

By the twentieth day the polyblastula measures when spherical about 114 μ in diameter. At about this stage one observes definitely that all of the embryos are not approximately of the same size. Some of the embryos are normal blastulas, while others are composed of but four to ten nuclei and are in reality in the germ stage. Instead of the cavities in which the germs are located measuring 40 μ in diameter, as do those of the typical blastulas of this age, they measure only 18 μ in diameter. Two such germs are represented in the polyblastula illustrated in Plate 5, B. These germs have failed to develop, and will degenerate. They are similar to the pseudogerms described by the writers (*3, 4*) in *Copidosoma gelechiae* and in *Platygaster hiemalis*. Pseudogerms apparently do not occur in all *P. vernalis* parasite bodies. The writers believe that they are developed as a result of total division of a daughter germ, and that the resulting components do not contain sufficient potential elements to allow them to mature.

At about the twenty-fourth day the polyblastula is usually elongate. The one represented by Plate 6, A, measures approximately 0.4 mm. in length and 0.07 mm. in width. It contains seven blastulas which are about ready to begin organogeny of the embryo. In this particular parasite body the paranuclear masses are found at each end. In the less elongate but more ovoid parasite bodies the paranuclear masses continue to be distributed regularly throughout the trophamnion.

THE POLYEMBRYONAL MASS

Between the twenty-sixth and thirty-second days after the egg has been deposited the parasite body represents a typical polyembryonal mass. During this period organogeny of the embryos takes place. The parasite body is usually found intact in one end of the mid-intestine of the host at this time (Pl. 7, B), although occasionally it may become split up into two or three secondary polyembryonal masses.

A twenty-seven day old parasite body is illustrated in Plate 6, B. This parasite body measures approximately 0.52 by 0.19 mm. The embryos continue to be held together by the trophamnion, which is less dense than in the preceding stages. The paranuclear masses have increased in size, but these, too, are less concentrated.

The organogeny of the embryos, as far as has been determined in a general way, takes place similarly to that described by Marchal (*5, 6*) for *Polygnotus minutus* and other Platygasters and by Silvestri (*9*) for *Platygaster dryomyiae;* and it will therefore not be referred to in detail. The blastulas become somewhat oval (Pl. 6, A) before the embryonic layers are differentiated. Prior to the formation of the mesenteron the embryos assume the U-shape (Pl. 6, B) described for other polyembryonic Hymenoptera, and then straighten out to form the primary larval stage (Pl. 7, B). During organogeny the embryos increase in size from 0.09 mm. in length, when they are somewhat U-shaped, to approximately 0.14 mm., when they resemble typical primary larvæ.

The final stage of the polyembryonal mass is illustrated in Plate 7, B, which represents a section through a parasite body at about the thirty-second day. The parasite body measures 0.8 by 0.4 mm. The embryos are in reality fully formed primary larvæ which have not yet taken any food, and measure approximately 0.23 by 0.07 mm. The fixed host in which this polyembryonal mass is found measures 3.8 mm. in length, while its mid-intestine measures 2.1 by 0.47 mm.

About the thirty-fourth day the membranelike trophamnion is ruptured by the first feeding of the primary larvæ, whereupon the larvæ are set free in the mid-intestine of the host (Pl. 1, D).

SUMMARY AND DISCUSSION OF CLEAVAGE TO LARVAL STAGES

It has been demonstrated above that the egg of *Platygaster vernalis* is organized into a typical polygerm, and finally into a polyembryonal mass, during the course of its later development, and before the newly formed primary larvæ are set free. This development takes place entirely in the mid-intestine of the host, the parasite body being tossed about within it by peristaltic action, at least until the polygerm stage is formed. Thereafter, the polygerm becomes, as a rule, lodged in one end of the mid-intestine, where the germs develop into true embryos, and finally into the primary larval stage before the parasite body is broken up.

The development of *Platygaster vernalis* is therefore similar, in a general way, to that of *Polygnotus minutus*. In other described polyembryonic species, such as *Copidosoma gelechiae* and *C. truncatellum*, development takes place in the body cavity of the host, the polygerm breaking up into secondary masses which are held together in a group by adipose tissue of the host in the case of *C. gelechiae*, and scattered throughout the body of the host in the case of *C. truncatellum*. Each individual blastula or embryo of *C. gelechiae* and *C. truncatellum* becomes separated from all others and is invested with a portion of the trophamnion and paranucleus, thus completing its development independently of any other individual. In *Platygaster vernalis* and *Polygnotus minutus* the parasites are developed to the primary larval stage in the common original trophamnion, although, as mentioned above, the parasite body may, in a few instances, at least in *P. vernalis*, become accidentally divided into two or three secondary masses. In this event one of these secondary masses may not develop completely.

Before cleavage the egg contains a cleavage or embryonic nucleus in a differentiated embryonic region, the remainder of the egg comprising the trophamnion, which contains two paranuclear masses of polar body origin. Four cleavages of the original embryonic nucleus result in the production of twelve to sixteen daughter embryonic nuclei, each of which lies within a small portion of the embryonic plasm and forms a germ. Some of the germs when composed of eight embryonic nuclei divide to form two daughter germs, but in any event each normal healthy germ finally develops into an embryo. From eight to twelve embryos are thus developed from a single *Platygaster vernalis* egg.

The development of the *Platygaster vernalis* egg therefore represents a simple type of polyembryony; not as simple as that shown by the writers (*4*) in *P. hiemalis*, nor as complex as that demonstrated in other polyembryonic insects by Marchal (*5*), Silvestri (*7*), and Leiby (*3*). In *P. hiemalis* we have shown that immediately after second cleavage some of the eggs divided into two equal parts, each part containing two embryonic nuclei and two paranuclear masses which together form an embryo. Twin embryos are thus developed from some eggs, while other eggs do not become so organized, and develop but a single individual. In *P. vernalis* approximately four cleavages take place before the germs are formed and an average of eight embryos are thus matured from a single egg. In *Copidosoma gelechiae* the senior writer (*3*) has shown that the germs are not organized until after the seventh cleavage, with the result that from 150 to 225 embryos are produced from a single egg. In any event, it appears that the number of embryos produced nearly always approximates the number of parasites that the host larva is able to mature. The size of the host appears to be the governing factor. Where but one egg is normally deposited in the host egg, as in the case of *C. gelechiae* and *P. vernalis*, cleavage continues to the point where the maximum number of parasites is developed that the host can mature. When cleavage extends beyond that number the embryos become aborted in their different stages. In the case of *P. hiemalis* two individuals develop from some of its eggs, and a single individual from others. This parasite therefore deposits from five to eight eggs at a time in one host egg, and in this way are developed the maximum number of individuals of this species that the Hessian fly larva can mature.

The failure of some of the germs to keep pace in their development with other germs in the same host has been briefly referred to above; the fault being ascribed to cleavage of daughter germs. A similar condition obtains for some of the blastulas, but the instances are rare. Occasionally a group of three or four blastulas will fail to continue development. Examination of the parasite body shows in such instances that a portion of it has become separated in the host intestine from the rest of the parasite body, and that the detached portion did not happen to become provided with a sufficient amount of the trophamnion and paranuclear masses. Such blastulas then become aborted.

Occasionally host larvæ are met with which contain more than one parasite body, in spite of the tendency of the parasite to oviposit in an egg only once, and to deposit at that time only a single egg. At such times one of the parasite eggs may fail to continue development. An undeveloped parasite body of this kind is illustrated in Plate 4, D.

Aborted eggs, germs, blastulas, or larvæ in *Platygaster vernalis* are rare in comparison to those which have been observed in *Copidosoma gelechiae* and in *C. truncatellum*, a condition which is to be expected in polyembryonic insects which show a comparatively simple type of polyembryonic development.

THE LARVA

As has been shown by the junior writer (*1*, *2*) the larva passes through two distinct stages in completing its development. When the insect takes its first food it is known as the primary larva (Pl. 8, A). In this stage the larva is elongate oval, bluntly rounded at both ends, and possesses two relatively large mandibles. This stage is further characterized by a lack of distinct body segmentation, and the presence of two very prominent lateral knoblike projections which are located in the head region at the base of the mandibles. The primary larva measures about 0.54 mm. in length and 0.18 mm. in width.

The mature larva (Pl. 8, B, C) is white, ovoid, and measures about 1 mm. in length and 0.5 mm. in width. Spiracles are present in this stage on the second and third thoracic segments, and second abdominal segment only. The mandibles of the mature larva are less than half the length of those of the primary larva. Eleven distinct body segments are defined in the mature larva.

Feeding commences when the primary larva is fully formed. The larvæ of a brood first consume the remnants of the trophamnion, whereupon they become liberated in the mid-intestine of the host (Pl. 1, D) and ingest the chyle. The intestine is next ruptured and the fatty tissues consumed. In the course of feeding, the entire contents of the host larva are consumed, leaving only the outer cuticle (Pl. 8, C) to contain the fully developed parasite larvæ. In the process of feeding, the superior lip is moved toward and away from the inferior lip by radiating muscles which are quite prominent in the head region.

A parasitized larva very seldom succeeds in pupating, but it does form the puparium. Each parasite larva, when fully grown, forms a cocoon, so that a brood of the parasites in the larval or pupal stages is contained within a cluster of cocoons (Pl. 1, E), which is in turn confined in the puparium of the host (Pl. 1, F).

THE PUPA

The pupa (Pl. 8, D) is formed in the cocoon. At the time of transformation it is white in color, but gradually the eyes and body darken until it is a shiny black. The parasites spend two to three weeks in the pupal stage.

THE ADULT

The adult is shiny black and measures from 0.7 to 0.9 mm. in length. An average of about eight individuals, which are nearly always of the same sex, are reared from one host. After emergence in spring they will live in confinement from three to twenty-nine days, the length of time depending upon the food and the humidity of the atmosphere.

Oviposition by fertilized or unfertilized females takes place immediately after emergence if the parasite happens to come in contact with a host egg. Experiments conducted by the junior writer (*2*) show a definite tendency for a female to oviposit only once in a host egg. The preparations, studied by the writers, which resulted from ovipositions controlled in the laboratory, indicate conclusively that a single egg is deposited at each oviposition.

SEX RATIO

A study of the sex of individuals of the broods indicates that usually all of a brood are either males or all females. Of 48 broods, 40 were either pure male or pure female broods, and 8 were mixed. A similar ratio has been shown by Marchal (*5*) for *Polygnotus minutus;* in the article cited he records the sex of 16 broods; eight being pure female broods, six male broods, and two mixed. Marchal believes that a mixed brood originates as a result of a fertilized and an unfertilized egg being deposited in the host. The writers believe that a similar explanation will answer for *Platygaster vernalis.*

SUMMARY

(1) *Platygaster vernalis* develops polyembryonically in the larva of the Hessian fly, one egg giving rise eventually to approximately eight individuals. There is but one generation annually.

(2) The adult parasites emerge from their cocoons in spring and almost immediately oviposit in the eggs of the host. By the first of June the embryos are fully formed in a well-grown host larva. The larvæ feed upon the host during June and July, and then transform to pupæ, which in turn become adults in August.

(3) A single egg is deposited by the parasite at each oviposition, and in such a manner that the egg always becomes lodged in the host's mid-intestine, where development to the larval stage is completed. Development begins immediately, whether the egg is fertilized or unfertilized.

(4) If more than one egg is deposited in the same host by different females one of the eggs may become aborted.

(5) In the course of maturation two polar bodies are formed, which become the two original paranuclear masses. The maturated oöcyte or cleavage nucleus becomes the progenitor of the embryos.

(6) Four divisions of either the conjugated or parthenogenetic cleavage nucleus result in the production of twelve to sixteen embryonic nuclei, each of which apparently gives rise to a germ. The germs develop in the central part of the parasite body and are encompassed by the trophamnion containing paranuclear masses.

(7) Some of the germs divide once, at the time they are composed of eight nuclei, to form two daughter germs. A further division of the daughter germs apparently results in the production of pseudogerms.

(8) The group of germs comprising a parasite body is known as a polygerm. Each normal germ passes through the blastula and late embryonic stages, and finally becomes a primary larva. During the course of this development the parasite body increases in size and remains intact in the mid-intestine of the host.

(9) When the primary larvæ are formed they rupture the thin trophamniotic membrane and begin to feed upon the contents of the host's mid-intestine. Later the mid-intestine is ruptured, whereupon the secondary or mature larvæ devour the entire contents of the host, leaving only the cuticula.

(10) Each larva constructs a cocoon in which it transforms to a pupa and later an adult parasite. The cluster of cocoons is surrounded by the cuticula of the host, and is further protected during the winter by the host's puparium.

(11) The adults of a brood are usually of the same sex. It is believed that the occasional mixed broods originate from a fertilized and an unfertilized egg deposited in the same host egg.

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PLATE 1

Platygaster vernalis

A.—Female parasite poised on a blade of wheat and ovipositing in egg of Hessian fly. ×45.

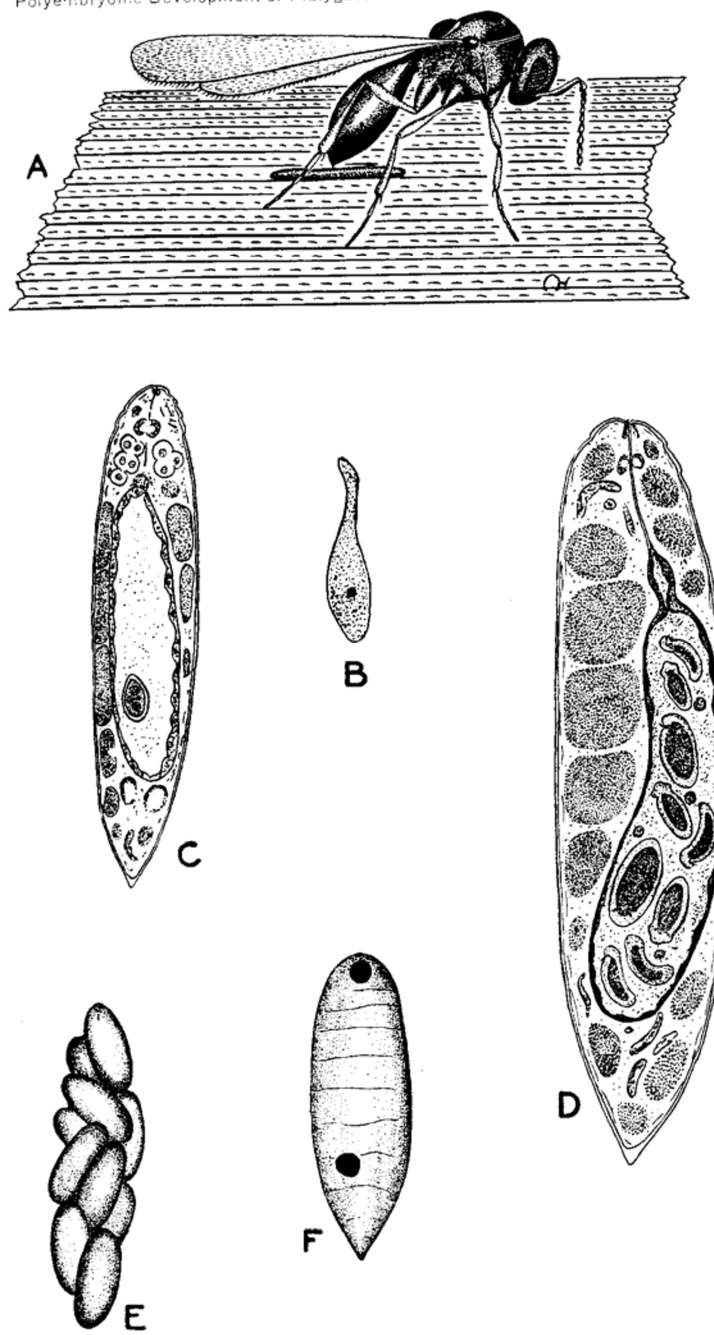
B.—Egg of *P. vernalis* before oviposition. Much enlarged.

C.—Longitudinal section through a young Hessian fly larva, showing a *P. vernalis* parasite body in the mid-intestine. ×55.

D.—Longitudinal section through a well grown Hessian fly larva showing primary stage larvae of *P. vernalis* in the mid-intestine. ×40.

E.—Host larval carcass (cuticula) containing ten *P. vernalis* cocoons. ×15.

F.—Host puparium showing exit holes made by adult parasites. ×14.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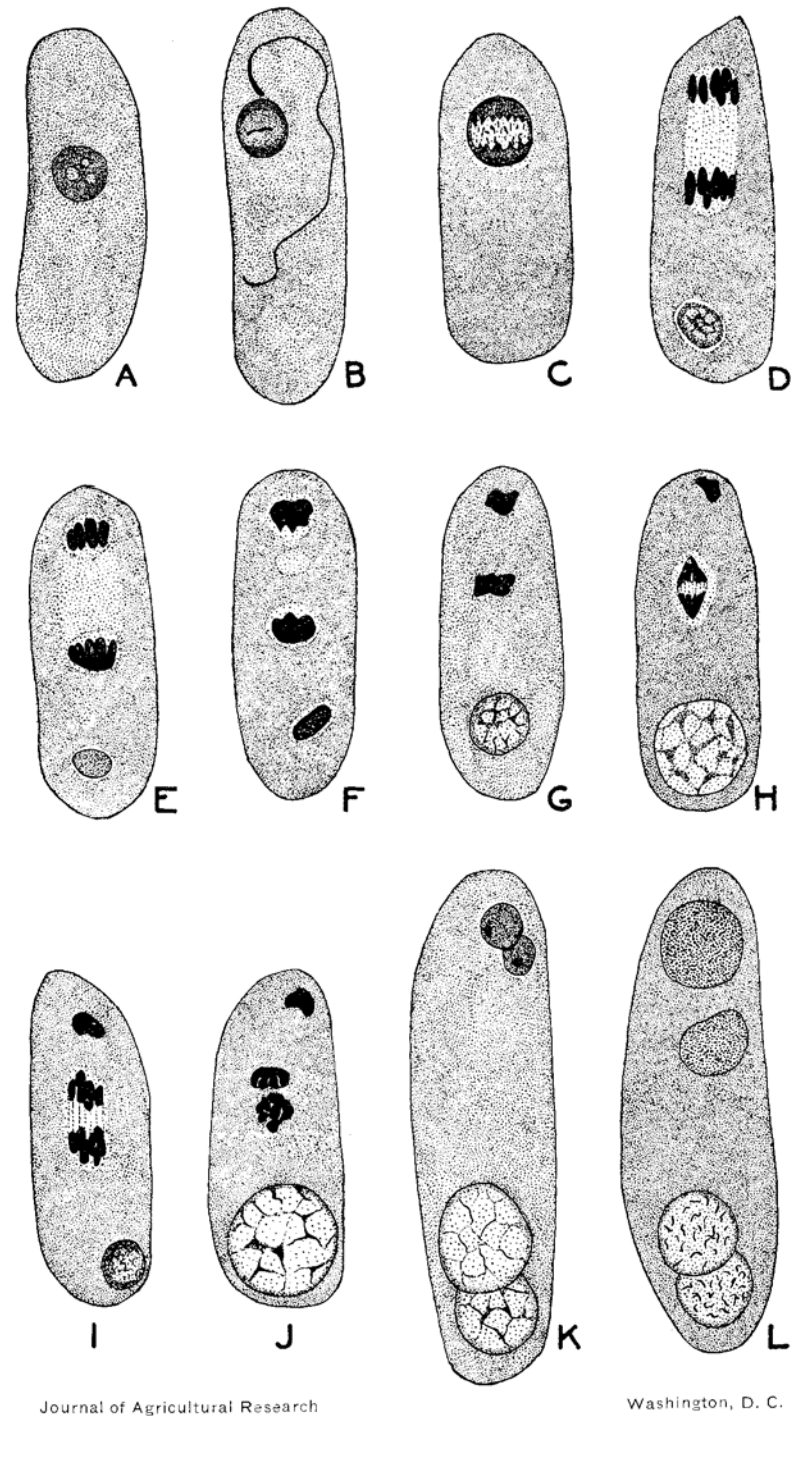
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PLATE 2

Platygaster vernalis

All figures drawn 2,200 times natural size

A.—Unfertilized egg, immediately after oviposition, containing the nucleus.

B.—Fertilized egg 30 minutes after oviposition, with nucleus and vermiform sperm.

C.—Unfertilized egg five hours old, with the nucleus beginning the first maturation.

D.—Fertilized egg six hours old, showing the male nucleus and the first maturation spindle.

E.—Like D, but at slightly later stage. The anterior nucleus becomes the first polar body, the middle nucleus the oöcyte nucleus of the second order.

F.—Like D and E, but slightly further advanced.

G.—Fertilized egg 10 hours after oviposition. The first polar body and the oöcyte nucleus of the second order show their nuclear material much condensed, while the male nucleus has meanwhile expanded and become spherical.

H.—The beginning of second maturation of a fertilized egg, about 12 hours after oviposition.

I.—Like H, but a slightly later stage.

J.—Egg 12 hours old. Second maturation is completed, and a second polar body and the female pronucleus are formed.

K.—Between the twelfth and twenty-fourth hours the egg increases slightly in size. The two polar bodies have migrated to the anterior end of the egg, and begin to increase in size.

L.—Polar bodies become elaborated and are henceforth known as paranuclear masses. Male and female pronuclei about to unite. Egg 24 hours old.

PLATE 3

Platygaster vernalis

All figures drawn 2,200 times natural size

A.—Portion of fertilized egg 24 hours old with male and female pronuclei at prophase.

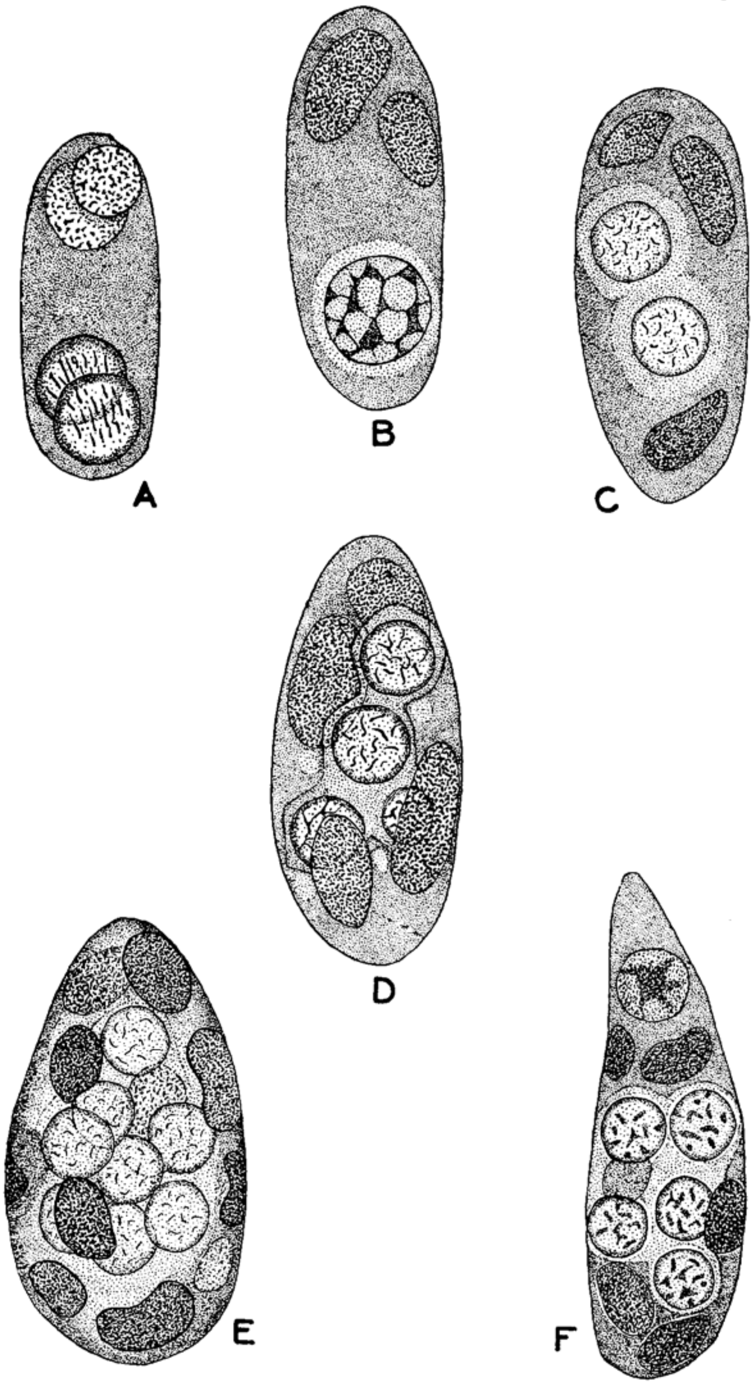
B.—Fertilized egg one to two days after oviposition, with the cleavage nucleus in a differentiated embryonic region. An unfertilized egg of this age has a similar appearance, except that the cleavage nucleus is not quite so large.

C.—Egg (now the parasite body) two to three days old. One of the paranuclear masses has divided amitotically. First division of the cleavage nucleus has produced two cleavage nuclei in a differentiated embryonic region.

D.—Section through a three-day old parasite body, showing four cleavage or embryonic nuclei and four paranuclear masses.

E.—Drawing of entire parasite body four days old. The eight embryonic nuclei are located in a central or embryonic region, which in turn is surrounded by the polar region now known as the trophamnion containing paranuclear masses.

F.—Section of a parasite body about six days old. Five of the eight embryonic nuclei are shown.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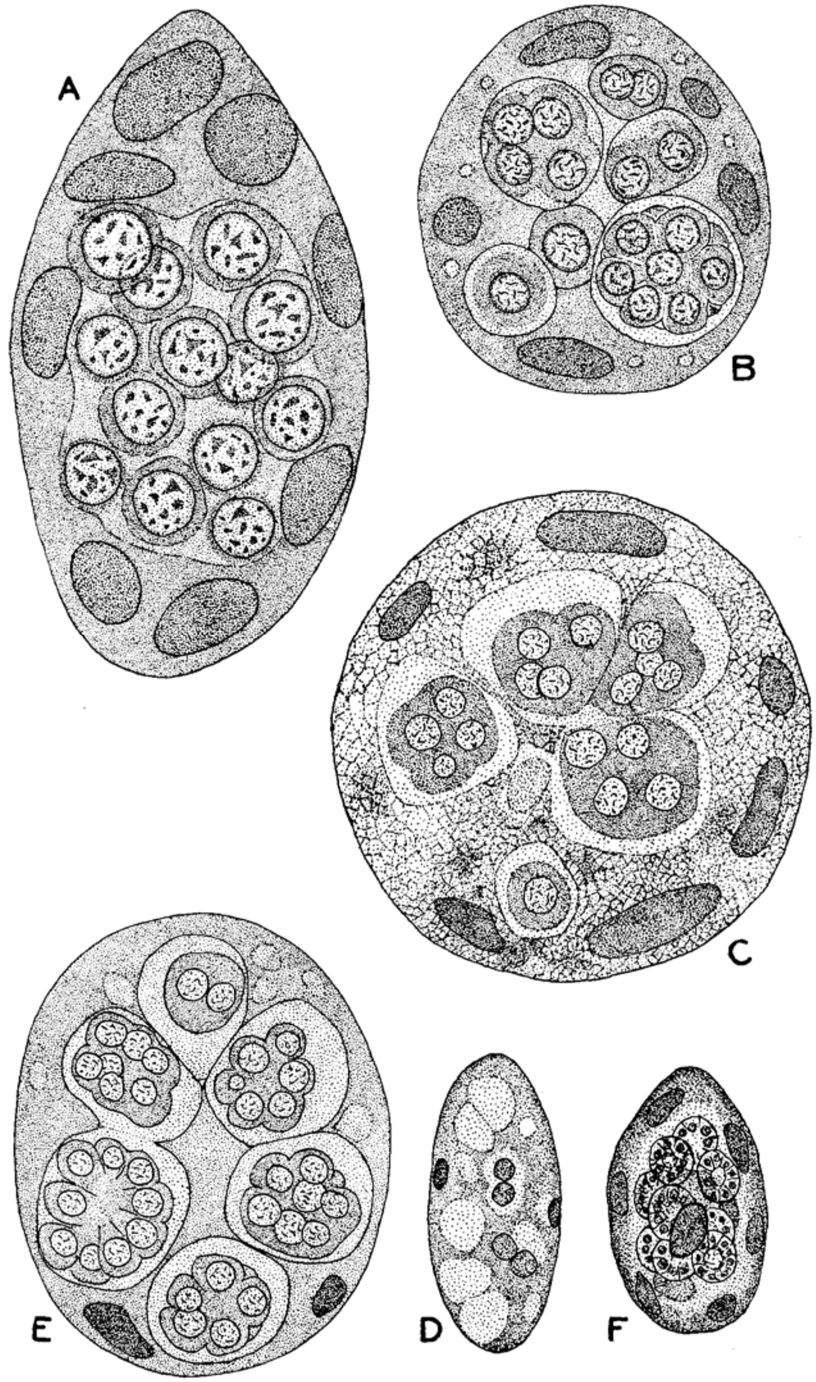


PLATE 4

Platygaster vernalis

A.—Section of parasite body about seven days old, showing thirteen of its sixteen embryonic nuclei forming germs. The parasite body has increased in size considerably. ×2200.

B.—Section through a polygerm about ten days old, showing five germs and one morula. The darker nuclei in the periphery are paranuclear masses. ×1100.

C.—Section through a polygerm about twelve days old, showing portions of five germs. The two upper germs have just been produced by the division of a parent germ. Each germ is lodged in an embryonic cavity which is surrounded by a somewhat vacuolated trophamnion containing paranuclear masses. Since development takes place in the mid-intestine of the host, no cyst of host tissue ever surrounds the parasite body. ×1100.

D.—Aborted or pseudoparasite body, found sometimes in host with a healthy parasite body, in the process of degenerating. Twelve days after oviposition. ×1100.

E.—Section of a polygerm about thirteen days after oviposition, showing portions of six embryos in the germ or early blastula stage, and two paranuclear masses in the trophamnion. ×1100.

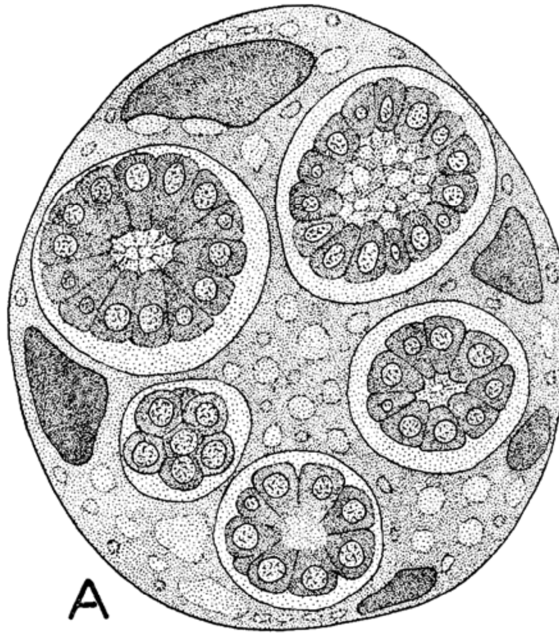
F.—In toto drawing of a polyblastula with nine blastulas in the embryonic region which is surrounded by the trophamnion, containing its dark staining paranuclear masses. ×550.

PLATE 5

Platygaster vernalis

A.—Section through an 18-day-old parasite in the polyblastula stage showing the embryos at the early blastula stage. The trophamnion becomes noticeably vacuolated and surrounds the embryonic cavities on all sides. ×1100.

B.—Section through a 20-day-old parasite body, showing four normal embryos in the blastula stage, two paranuclear masses and two pseudogerms which are apparently degenerating because division of the parent germ was carried too far. ×550.



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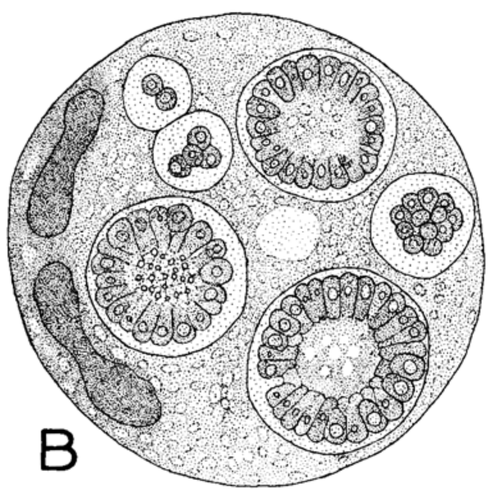

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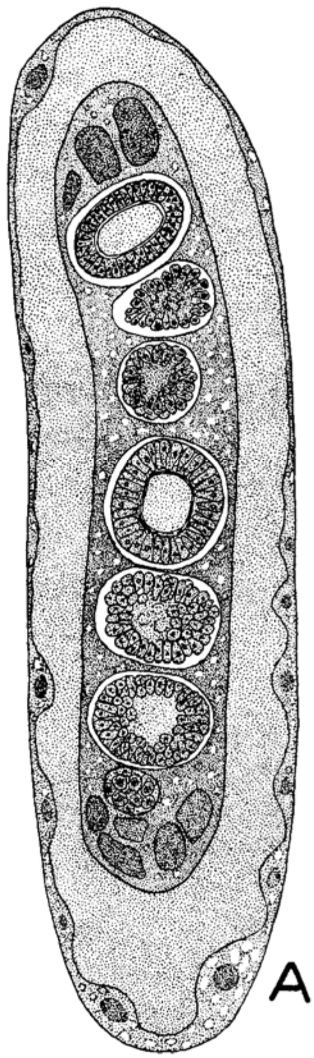


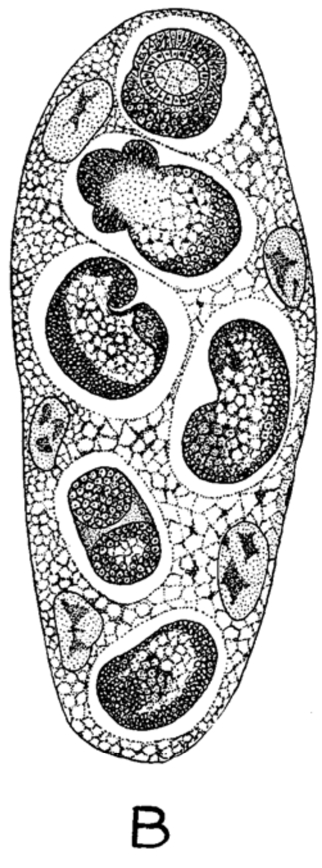


PLATE 6

Platygaster vernalis

A.—Section of a polyblastula 23 days old located in the chyle of the mid-intestine of the host. Portions of seven of the eight blastulas which compose this parasite body are shown. Two of the blastulas are cut through the center. This parasite body has assumed an elongate shape and now measures about one-fourth the length of the mid-intestine of the host. The paranuclear masses are shown at each end. The periphery of the drawing represents the epithelium of the mid-intestine, the lighter stippled area the chyle of the mid-intestine. ×275.

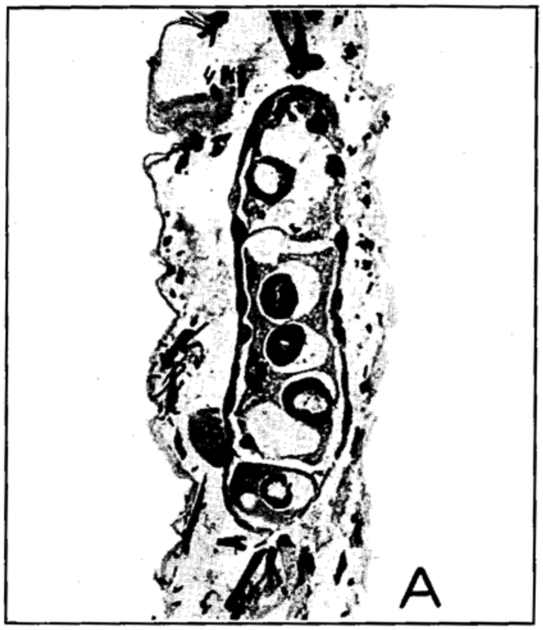
B.—Longitudinal section through a polyembryonal mass about 26 days after the original egg was deposited, showing portions of six embryos. About this time the embryos are forming the germ layers and organs of the young larvae and are somewhat Gothic-like-shaped. Note that the trophamnion is less dense, and that the paranuclear masses are no longer so conspicuous. ×200.

PLATE 7

Platygaster vernalis

A.—Photomicrograph of polyembryonal mass distributed throughout the mid-intestine of the host. Sections of five embryos are shown which have jus differentiated the germ layers. Note that the trophamnion is vacuolated ×93.

B.—Photomicrograph of polyembryonal mass lodged in the anterior end o the host's mid-intestine. The embryos have almost or quite reached the stag of the primary larva. The black area surrounding the embryonal mass rep resents the chyle of the host. The lower primary larva is just beginning t devour the remnants of the trophamnion. ×62.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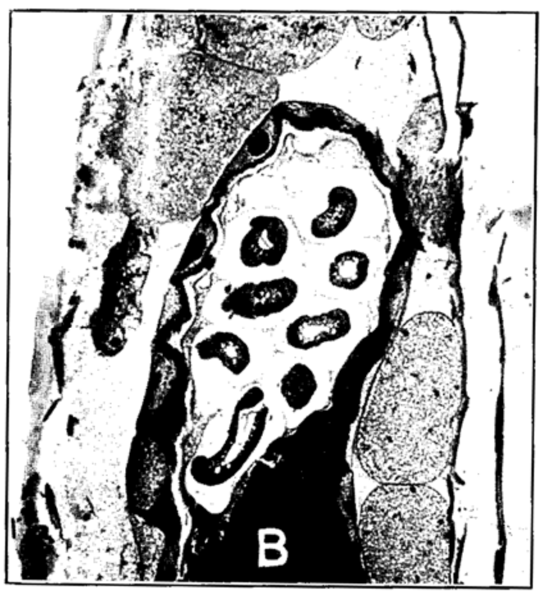
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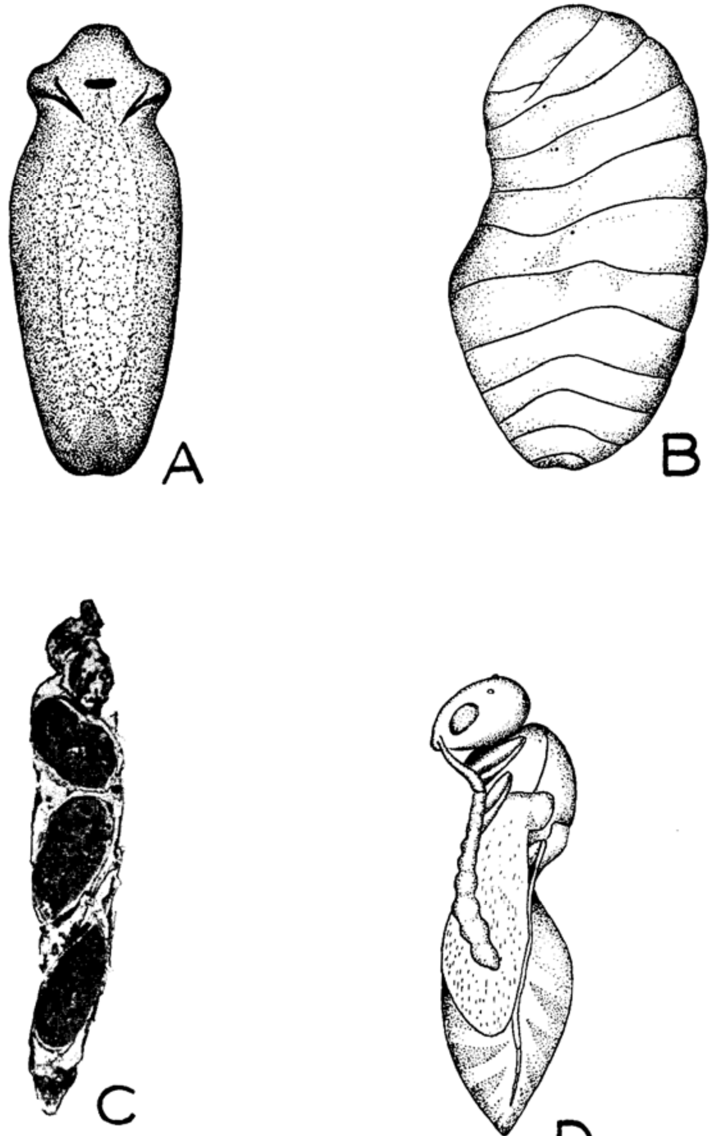
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PLATE 8

Platygaster vernalis

A.—Primary larva. ×111.
B.—Mature larva. ×62.
C.—Photomicrograph of section through a host larva (carcass), showing three *P. vernalis* larvæ that are almost mature. ×25.
D.—Pupa of *P. vernalis*. ×62.

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