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OBSERVATIONS ON THE DEVELOPMENT OF COPIDOSOMA GELECHIÆ.

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I. INTRODUCTION.

The discovery of polyembryonic development among certain of the hymenopterous parasites has opened up an extremely interesting field for investigation. Like most other important biological discoveries, this one was foreshadowed by the observations of several different naturalists. In a paper of this nature it is not necessary to give an extended account of the history of this discovery. We shall therefore be content with a brief

statement on this point, limiting the account almost entirely to the species with which the paper deals.

The general features of polyembryony in insects have been given in the well-known papers of Marchal ('98, '04) and Silverstri ('06, '08), but there are many points concerning the details of this process which have not as yet been worked out. It was with the view of studying certain of these details that led the writer three years ago to seek an American species upon which such studies could be made. Dr. L. O. Howard[1] suggested that *Copidosoma gelechiæ*, which parasitizes the larvæ of the *Solidago* gall moth, *Gnorimoschema gallæsolidaginis*, would be a good form upon which to work, as it seemed to be an undoubted case of polyembryony.

The *Gnorimoschema* moth makes the ellipsoidal galls on the stems of several species of goldenrod. Baron Osten Sacken ('63) seems to have been the first to have published a description of the inflated carcass of the *Gnorimoschema* larva, caused by the chalcis parasite; but apparently he was not acquainted with the maker of the gall. In 1869 in connection with his account on the life history of this moth, Riley states that the caterpillar serves as a host for no less than six different species of hymenopterous parasites. One of these, which is shown in his Fig. 6, Plate 2, is described as a "little fly of a dark metallic green color, with reddish legs." This is clearly *Copidosoma*. Riley states that the larvæ of this species infests the caterpillar in great numbers, more than 150 having been obtained from a single host. He supposed that the "mother fly" gnawed a passage through the gall and desposited her batch of eggs in the inmate. He pointed out that the larval parasites cause the caterpillar to swell to three or four times its natural size, and after having absorbed all the juices of the victim, form very small brownish cocoons, which are so packed together that they give to the worm the puffed-up appearance which is typical of the mummified carcasses of lepidopterous larvæ that have been parasitized by a polyembryonic species. It was this inflated condition of the larval host that led Riley to call the parasite the "Inflating Chalcis

[1] For this as well as for other suggestions received throughout the progress of the work, the writer is greatly indebted to Dr. Howard.

Fly." Howard ('85) later named this species *Copidosoma gelechiæ.*

Upon examining the various goldenrods about Woods Hole, Mass., for galls of *Gnorimoschema*, it was found that *Solidago sempervirens* furnished the best opportunity for obtaining material. However, the common gall maker of this solidago proved not to be *Gnorimoschema gallæsolidaginis* Riley, but a closely related species, *G. salinaris* Busck. The parasites infesting these two moths are varieties of the same species, *Copidosoma gelechiæ.*

The selection of this species has not proved altogether satisfactory, because the gall-making habit of the host complicates the life history and renders the collecting of material for early stages of the parasite somewhat more difficult than from a host which feeds openly. Furthermore, the moth, and likewise the parasite, has but one generation a year. In addition to these objections, there is the further one that the egg of *Copidosoma* gives rise to a relatively large number of individuals (about 191 on the average). In attempting to obtain material for the studies which the writer has in mind, it seems best to seek to find a host which is an open or semi-open feeder, which has two or more generations a year, and which harbors a parasitic egg giving rise to but few individuals. During the past summer at least two species have been found which in the main seem to fulfill these conditions. It therefore seems best to publish the main facts concerning the development of *Copidosoma* before giving it up for more favorable material.

There is one feature in the development of *Copidosoma* which makes further study desirable. We refer to the abortive embryos (presently to be described), which at first were thought to be comparable to the so-called asexual larvæ of *Litomastix truncatellus.* It will be recalled that Silvestri ('06) described in this species the development of both sexual and asexual larvæ from a single egg. In one instance he secured from a caterpillar of *Plusia gamma* 1,700 sexual and 220 asexual larvæ of *Litomastix.* He believes that the asexual larvæ play the rôle of raspers for the normal larvæ, tearing the tissues of the host so that the sexual larvæ may the more easily secure the necessary food. It may

be stated here that the abortive larvæ of *Copidosoma* are in no way comparable to the asexual larvæ of *Litomastix* as described by Silvestri.

II. Note on the Life History of Gnorimoschema.

In order to collect polyembryonic material it is essential to know something about the life history of the host; especially is this true in cases like *Gnorimoschema* in which the larval host is a gall maker. Considerable attention has therefore been given to a study of the life history of *G. salinaris*.

The general habits of the *Solidago* gall moths were first made known by Riley's ('69) studies on *G. gallæsolidaginis*. According to Riley this species winters over in the imago stage and may be seen flying in the month of May. When the young plants (*Solidago nemoralis*) are about six inches high the female moth lays her egg either in the terminal bud or at the side of the stalk immediately below the bud. The young caterpillar upon hatching burrows into the stalk and starts the development of the gall. By the first of June the gall has just begun to form and contains a larva about one-third grown. The larva and its ellipsoidal gall reach their full size by the middle of July. The caterpillar which now measures over half an inch in length prepares for the change into the chrysalis state by first eating a round passage-way through the wall well toward the upper end of the gall. The orifice is then closed by a secretion of liquid silk, which hardens to form a silken plug. After closing the orifice, the caterpillar lines the passage-way and the walls with a delicate silk, and then transforms into a shiny, mahogany-brown pupa, about a half inch long. The moths begin to emerge about the middle of August and continue to appear until the beginning of October.

Many phases of the life history of *G. salinaris* are similar to those of *G. gallaesolidaginis*, but there are some important differences. The earliest date at which galls of the marsh goldenrod have been secured was June 12, 1914, and at that time many of the galls were well started. Between June 12 and 15, 63 galls of various sizes were collected and examined. They varied in size from 8 to 12 mm. in length and from 4 to 17 mm. in transverse section. In shape the galls also vary greatly. Some are distinctly

pear-shaped, while others are fusiform, with various gradations between these two general types. The galls occur at different heights on the stem, but the vast majority of them are located at or near the base of the stalk (Fig. 1). Their position is undoubtedly determined by the location of the point at which the larva penetrates the young shoot. If this point is located toward the base of the young stalk, the gall will naturally appear near the base of the fully grown plant; but if it is located in or near the terminal bud, the gall will appear some little distance up on the stem. Occasionally two galls are found on the same plant (Fig. 8). A few cases have been observed in which the gall was located at the tip of the terminal bud, producing a stunted plant without a central, flower-bearing stalk. With these few exceptions, the gall of *G. salinaris* does not seem materially to affect the growth and vigor of the plant. It is true that many galls are found on plants that are apparently stunted but such dwarfing is to be attributed to the adverse conditions under which the plant sometimes grows. In regions that are very much exposed to the wind, like the banks along the coast, many of the goldenrods are small and clearly dwarfed; but this condition applies as well to the plants that are free from galls as to those that are infected.

The habits of gall making are similar in the three common species of *Gnorimoschema*, although the following differences may be pointed out. *G. gallæasteriella* produces a triangular gall at the top of the dwarfed or stunted stems of *Solidago cæsia*, *S. axillaris*, *S. latifolis*, and *Aster divaricatus*.[1] The form of the gall differs somewhat with the plant. The gall of *G. gallaesolidaginis* may occur toward the top of the stem, but usually it is located just below the middle, especially is this true of the galls on *S. canadensis*. The galls of this moth do not dwarf the plant. The condition of the galls of *G. salinaris* on the marsh goldenrod has already been described. They occur nearer the base of the stem than do those of last species, and like the latter there is little or no tendency to dwarfing the plant.

The larvæ secured from the galls collected between June 12 and 15 varied from 3 to 8 mm. in length. Beginning with the middle of June, the young caterpillars grow rapidly, doubling

[1] Part of these data were kindly furnished the writer by Dr. T. M. Forbes.

their size within a fortnight. By the middle of July they have reached their full growth, and are beginning to show signs of undergoing pupation, which is evidenced by the construction of the passage-way. The passage-way and its orifice differ in two respects from those of *G. gallæsolidaginis* as described by Riley ('69). The silk lining does not extend much beyond the lower limits of the passage-way, and hence does not cover the inner surface of the wall. The second difference is seen in the character of the orifice and its silk plug. The caterpillar of *G. salinaris* does not cut the passage-way quite through the wall, but leaves the very thin epidermis of the stem, which is used as a background for the construction of the plug (Fig. 7).

TABLE I.

TABLE SHOWING DATES OF PUPATION AND EMERGENCE OF COPIDOSOMA AND GNORIMOSCHEMA.

Pupation (Beginning of)	Copidosoma	Aug. 6, 1912.
		Aug. 5, 1913.
		July 31, 1914.
		July 30, 1915.
	Gnorimoschema	Aug. 6, 1912.
		July 23, 1913.
		July 30, 1914.
		July 26, 1915.
Emergence	Copidosoma	Aug. 25 to Sept. 12, 1912.
		Sept. 3 to Sept. 13, 1913.
		Aug. 30 to Sept. 18, 1914.
		Aug. 24 to Sept. 21, 1915.
	Gnorimoschema	Aug. 25 to Sept. 10, 1912.
		Aug. 25 to Sept. 10, 1913.
		Aug. 22 to Sept. 11, 1914.
		Aug. 24 to Sept. 14, 1915.

Pupation occurs during the last week of July and the first week of August (Table I.). The imagines begin to emerge about August 25, and continue to appear until September 10. The moth has been seen flying in the open during this period.

Females kept in captivity often lay eggs. This they do within ten days after emerging, and irrespective of their association with males. As a rule the moths simply drop the eggs on the bottom of the cage, or they may lay them on the leaves and flowers of goldenrods placed in the cage. At first it was thought that *G. salinaris* must differ from *G. gallæsolidaginis* in respect to its egg-laying habits, for Riley states that the latter species although emerging in the fall, hibernates as an imago and lays

its eggs in the following May. It has been discovered, however, that *G. gallæsolidaginis* from the galls of *S. canadensis* in western Ohio likewise drops several eggs soon after emerging from the pupa in September. This raises the question as to whether these fall eggs develop into larvæ, for if so it would be difficult to explain how the young caterpillars could withstand the winter and succeed in the spring in finding a young goldenrod bud or shoot in which to start the new gall.

In reply to an inquiry, Mr. A. Busck of Washington kindly informed the writer that the laying of eggs by *Gnorimoschema* was of no particular significance, as it is not uncommon for certain Lepidoptera to drop their eggs prematurely, especially if kept in captivity. In view of this fact an observation made in the fall of 1913 is of special interest. During the first few days of September of that year a single female, confined in a cage with several males, laid a dozen or more eggs on goldenrod leaves and flowers. On the thirteenth of the month three larvæ hatched from this batch of eggs! There can be no possible doubt as to the correctness of this observation, for the hatching of one of the little caterpillars was actually observed under a hand lens.

It is difficult to explain the development of these larvæ from fall eggs, except on the basis of parthenogenesis. It is true that the female which laid the eggs from which the larvæ developed had been confined with males; but although males and females have been kept together for several weeks during each of the last three seasons, yet mating has never been observed. The supposition that the fall eggs of *G. salinaris* may develop by parthenogenesis receives strong support from a study of sections of eggs laid by a female not associated with males. In Fig. 20 is shown a transverse section of one of her eggs and it can clearly be seen that development is well started. Twelve eggs out of the batch were sectioned, and it was found that eleven had started to develop, although apparently not in a normal manner. It is not improbable that some few eggs may develop normally and eventually produce larvæ. The question of parthenogenesis in the *Solidago* moths is one needing further study.

It might be worth while to add that parthenogenetic development among Lepidoptera is by no means unknown. DeGeer is

given credit for having first discovered long ago that certain butterflies belonging to the genus *Solenobia* lay unfertilized eggs which develop into normal imagines, and later von Siebold not only confirmed this observation, but also discovered that *Psyche helix* reproduced parthenogenetically. It has since been shown by several workers that the silk moth, *Bombyx mori*, may under certain conditions reproduce by parthenogenesis.

III. Parasites of Gnorimoschema salinaris.

Riley reports six hymenopterous parasites for *Gnorimoschema gallæsolidaginis*, and in addition to these he found a beetle larva and another lepidopterous larva which intrude as inquilines within the cavity of the gall made by this species. At least five hymenopterous parasites have been found associated with *G. salinaris*. The most important of these is *Copidosoma gelechiæ*, which is by far the most common parasite attacking the moth. The other four species are *Calliephialtes notanda* Cress, *Epiurus* sp., *Eurytoma* sp. (pupa), and *Pseudacrias sexdentatus* Girault. The first of these four occurs most frequently, while the last has been observed but a few times. However, it is of special interest, inasmuch as it is the only observed case of a second parasite emerging along with *Copidosoma*, although the larvæ of other species have been found associated with the larvæ of *Copidosoma*. On September 3, 1914, six individuals, all females, emerged together with a brood of about one hundred Copidosomas from a single carcass. The small pupæ of *Pseudacrias* lying among those of *Copidosoma* were observed through the transparent chitin of the carcass of the host some days prior to their emergence. They were not grouped together but scattered about in different parts of the carcass. Each pupa was inclosed in a chamber very much smaller than, but exactly similar to that containing a *Copidosoma* pupa.

Usually *Pseudacrias* larvæ do not pupate until after the larval host has undergone this process. About a dozen *Gnorimoschema* pupæ have been found containing *Pseudacrias* pupæ, which later emerged. It is not probable that *Pseudacrias* is polyembryonic. First, because both male and female individuals usually emerge from the same pupal host; and second, because the individuals

do not come out at the same time. The single instance of six females ssuing simultaneously with the brood of *Copidosoma* can be explained by assuming that a single female deposited six fertilized eggs in the host at the same time. However, this case is of special interest as it demonstrates the synchronous development in a single host of the broods of two distinct parasites, and thus supports Wheeler's ('10) suggested explanation of Silvestri's so-called asexual larvæ in *Litomastix*.

In addition to the five hymenopterous parasites, there are two insect larvæ associated with the larva of *G. salinaris*. They are undoubtedly inquilines. One of these is a beetle and the other a lepidopterous larva (Fig. 5). Judging from Riley's account, these two species are very similar to if not identical with the corresponding inquilines reported by him for the galls of *G. gallæsolidaginis*.

IV. Development of Copidosoma gelechiæ.

1. *The Polygerm Stages.*

(*a*) *Youngest Stages.*—We have not secured the cleavage stages of *Copidosoma*, owing to the fact that they occur earlier in the year than we have been able to reach Woods Hole. Therefore, in describing the developmental processes which have their inception in the cleavage stages, we must rely upon the work of other investigators in this field for our interpretation of the significance of these processes.

The youngest stages secured were found in a small larva of *Gnorimoschema*, taken June 21, 1913. The series of sections of this small caterpillar contains three young polygerms of *Copidosoma*. Evidently the egg from which the caterpillar developed had had three parasitic eggs deposited in it. Two of the polygerms, which lie close together, are situated in the first and second body segments of the larva, just beneath the hypodermis; while the third is found in sections 5 to 14 posterior to these, and is also situated just beneath the hypodermis of the host.

The three polygerms are not of the same size, as is indicated by the following measurements: Of the two specimens lying close together, the larger measures 150 μ by 82 μ and runs through 15 sections (150 μ), the smaller measures 103 μ by 71 μ, and is

found in 12 sections; the single specimen measures 179 μ by 95 μ and occupies 8 sections only.

In structure the three polygerms are practically identical. Each consists of two distinct zones: (1) An outer relatively thick zone containing a large number of nuclei irregularly placed, and (2) a central region containing the embryonic nuclei (Fig. 19). In the absence of the earlier stages, it is not an easy matter to interpret the conditions seen in these polygerms. In the main they correspond most nearly to the conditions in the egg of *Litomastix* (*Copidosoma*) *truncatellus*, as described by Silvestri ('06). I therefore interpret the outer zone to be the product of the "polar oöplasm" plus the "polar nuclei," while the central region contains the true embryonic nuclei, derived from the fertilized nucleus, or in the case of parthenogenetic development, from the matured egg nucleus.

There is of course one essential difference in the corresponding stages of *Litomastix* and *Copidosoma*. In the polygerm of the former the central region is composed of a solid mass consisting of distinct cells, while in the latter this region is on the point of being broken into multi-nucleated masses, which form the primordia of the embryos (cf. Fig. 19 *A* with Silvestri's Fig. 33, Pl. III.). It may be that the embryonic nuclei are delimited by cell walls in *Copidosoma*, although one can not make them out with certainty, even under the highest powers of the microscope. Judging from the work of other investigators, one would expect to find the embryonic nuclei surrounded by cell walls. In *Ageniaspis*, Marchal ('04) first reported that the early embryonal masses were pluri-nuclear in character, but Silvestri ('08) and Martin ('14) have later demonstrated that the nuclei are surrounded by cell walls. In *Copidosoma* the embryonic nuclei are often so closely packed together that the demonstration of cell walls would be extremely difficult.

The three polygerms mentioned above are of particular interest, in that they show very clearly the manner in which the central mass with its nuclei breaks up to form the primordia of the multiple embryos. The central region itself consists of two distinct substances. (1) A granular protoplasm in which the embryonic nuclei lie, and (2) a more fluid-like material which

becomes greatly shrunken during the process of fixation, and which in sections appears as a precipitated substance (Fig. 19 *A*, *P.M.*). As to the origin of these different substances we know nothing, but their subsequent history is clear. For the sake of clearness in description we shall use the following terms: (1) *Nucleated Membrane* for the outer zone; (2) *Granular Layer* for the protoplasm containing the embryonic nuclei; and (3) *Precipitated Material* for the shrunken fluid-like substance.

(*b*) *The Nucleated Membrane.*—In these young polygerms the outer zone stains more deeply than the central mass. The "polar nuclei" have no definite arrangement, but are irregularly scattered throughout the protoplasm. The entire zone therefore is in every sense of the word a syncytium. As the polygerm grows in size the nuclei become arranged into a single layer, and the protoplasm thins out, thus forming a true nucleated membrane about the central or embryonic portion of the egg (Fig. 21, *N.M.*). In the later history of the polygerm some of the nuclei are clearly surrounded by cell walls, that is, there is a tendency for the membrane to become cellular.

At first the young polygerms are naked, that is there are no elements from the host tissue laid down on the outer surface of the nucleated membrane. Later a few mesenchyme cells are found on the surface of the membrane, and still later these cells give rise to the adipose tissue (Fig. 22, *A.T.*), which may completely surround the polygerm.

(*c*) *Precipitated Material.*—This material occupies the central portion of the polygerm. Apparently it is formed through the action of the fixing reagent upon the fluid-like protoplasm. In sections it is very much shrunken, thus leaving an irregular clear space (Fig. 21, *C*). As we shall see later, it persists throughout the entire polygerm phase of development.

(*d*) *The Granular Protoplasm and the Embryonic Nuclei.*—In Fig. 19 the condition of the embryonic nuclei and their surrounding granular protoplasm is especially clear. Most of the nuclei are indifferently scattered in the protoplasm, but some of them are collecting into groups. The number of nuclei in each group is variable; some groups contain only two or three nuclei, while others may have as many as ten or twelve. The granular pro-

toplasm surrounding a group of nuclei soon rounds off and the primordial embryo with its surrounding layer lies free within the more fluid contents of the central region of the egg (Fig. 19 *A*). The more usual condition is for the spherical mass to remain attached at one side to the peripheral layer of the granular protoplasm (Fig. 19 *B*, *P.E.*). Eventually all of the embryonic nuclei thus become included in these spherical masses of protoplasm, and thus become isolated as primordia of the embryos.

The condition at the close of the formation of the primordia is shown in Fig. 21. This specimen was found in a series of sections of a 3 mm. caterpillar, taken June 15, 1914. In the median section it measures 113 μ by 203 μ, and runs through 40 sections (280 μ). It lies in the middle portion of the body cavity, to one side of the intestine, which on account of the size of the polygerm is pushed out of place. As compared with the preceding polygerms this one is very much larger and shows a number of important changes. The nucleated membrane has become much thinner and its nuclei are arranged more or less into a single layer. The adipose tissue is being laid down on the outer surface of the membrane. The most important change, however, has occurred in the embryonic masses themselves. The protoplasm which surrounds a group of nuclei is differentiated into two distinct regions. The central part, crowded with nuclei, stains somewhat lighter than the peripheral zone, which forms a relatively dense layer about the central core (Fig. 21, *P.E.*). There are still a few nuclei which have not as yet been surrounded by the dense layer, but this stage marks approximately the end of the division of the germ into separate embryos.

(*e*) *Growth of the Polygerm and the Formation of the Primary Divisions or Masses.*—Upon the completion of the primitive embryos, the polygerm grows very rapidly. It first extends in the direction of its long axis, soon transforming into an elongated cylindrical structure. One specimen showing this condition measures in section 148 μ by 430 μ. It never becomes an elongated tube as does the polygerm of *Ageniaspis*. During this growth the adipose tissue is laid down in the form of a thick layer about the polygerm. One of the easiest ways in which to find a polygerm of this and later stages is to examine the large

fat bodies lying in the middle region of the body cavity of the larval host. If the caterpillar is parasitized one of these bodies is almost certain to contain the polygerm.

After the elongated condition is attained, the further growth of the polygerm may take place in any direction. In some cases the extension is mainly in one plane, thus transforming the polygerm into a flat, plate-like structure (Fig. 13). In other cases it forms a thick irregular mass (Fig. 11), and when viewed as a whole mount shows many elevations on its surface, due to the breaking up of the entire polygerm into single masses, each of which contains an embryo.

During the rapid expansion of the polygerm a very important change takes place in its structure, whereby each embryo become enclosed in a double involucre. The first step in this process begins just prior to that period of development in which the polygerm attains its elongated, cylindrical shape. It consists in the formation of constrictions in the nucleated membrane which break up the single polygerm into a series of primary divisions or masses (Fig. 15). In the specimen shown in this figure there are about twelve of these masses. Each primary mass has the same general structure as the original single polygerm. It is surrounded by a portion of the nucleated membrane, contains precipitated material, and has a variable number of embryos, from five to fifteen or more.

In Fig. 22 one end of a longitudinal section of a polygerm is shown with the completed primary masses. Three of these masses are seen in the figure, together with a portion of a fourth. Attention should be called to the fact that the adipose tissue, although in contact with the polygerm, is still a distinct structure. In the process of forming the primary masses not all of the elements of the nucleated membrane are taken into these structures. Some of them are left behind and later lie in the inter-embryonal spaces or interstices. In Fig. 22 a number of these elements (cells and nuclei) are shown at the point marked "*N*," lying between the primary masses and the adipose layer.

In another portion of the same polygerm a single primary mass is being constricted off laterally. It appears as a bud extending from the main body of the polygerm. It is such cases

as this which give rise to the condition frequently seen in whole mounts, in which the surface of the polygerm displays many protuberances.

(*f*) *Formation of the Secondary Masses.*—The primary masses soon become broken up into secondary masses. This is also brought about by constrictions of the nucleated membrane (Fig. 23). These secondary masses may contain more than one embryo, in which case they immediately form constrictions which result in producing still smaller masses, each of which contains a single embryo.

In the constrictions which lead to the cutting off of a single embryo with its involucres, some of the precipitated material is enclosed between that portion of the granular layer which is in contact with the embryo and that part lying adjacent to the inner surface of the nucleated membrane. These two parts of the granular layer then fuse, forming a single involucre in which are the spaces containing the precipitated material (Fig. 24). The embryo is thus surrounded by two involucres, a granular layer, and a nucleated membrane (Fig. 26). In some cases the precipitated material may be so abundant as to form a solid zone between the inner and outer parts of the granular layer; in others it is small in amount and gives the appearance of much flattened nuclei lying within this layer (Fig. 26, *P.M.*).

(*g*) *The Inter-embryonal Substance.*—At the close of the formation of the single embryonic masses and their involucres the inter-embryonal interstices are already filled with a substance derived from several different sources. It consists of a plasma-like matrix in which are embedded cells and nuclei. We have already noted that during the formation of the primary and secondary masses some of the elements from the nucleated membrane are not included in the outer involucre, but are left in the inter-embryonal spaces. During the early history of the inter-embryonal substance, it consists mainly of product from this membrane. Later cells from two other sources enter into its formation. First, leucocytes from the host are found embedded in the matrix. They are especially abundant in those regions of the polygerm exposed directly to the body cavity, that is near a surface barren of adipose tissue. Second, fat cells

from the adipose layer invade the inter-embryonal spaces. The fat cells are the last elements to enter the inter-embryonal substance. In Fig. 13 a wedge-shaped mass of fat tissue is seen lying between the embryos in the middle region of the polygerm, on the upper side. Perhaps it would be more correct to say that the embryos bud out into the adipose tissue. Thus in Fig. 24 a single primary mass has been budded off into the adipose tissue.

The final condition of the polygerm at the end of the formation of the inter-embryonal substance is shown in Fig. 16. The adipose tissue has invaded the inter-embryonal substance from all sides of the polygerm and has become an organic part of this substance. The fat body and the included polygerm become an extremely complex structure, which may be called the *polygermal mass.*

2. *Dissociation of the Polygermal Mass.*

The setting free of the larval parasites into the body cavity of the host is brought about through the dissociation or disintegration of the inter-embryonal substance. The fat brought into close contact with the embryos by the invasion of the adipose tissue is digested and absorbed by them. It is therefore the first component of the inter-embryonal substance to disappear. That the fat is digested and consumed by the embryos is evidenced by the fact that the numerous other fat bodies remain intact during this period. The disappearance of the fat leaves the embryos loosely held together by the plasmalike matrix, which in turn soon disintegrates, freeing the larvæ.

The first larvæ to be set free are those situated at the periphery of the polygermal mass. Such larvæ are usually the largest present in the mass. As the inter-embryonal substance slowly disintegrates the remainder of the larvæ are gradually set free (Fig. 17). The earliest date at which free larvæ have been found was July 19; the latest, July 31. In the vast majority of cases the mass dissociates during the last week of July.

The larvæ retain the involucres for some time after being set free (Fig. 18). Once free in the body cavity they proceed to devour the contents of the host, first consuming the fat tissue, and finally the various organs. The last internal organ to disappear is the intestine.

3. *Pupation, and the Emergence of the Imagines.*

Pupation in *Copidosoma* occurs during the first ten days of August. The pupa stage lasts twenty-eight days. As stated above, the larvæ destroy all of the internal organs of the host, and consume such portions as are dissolved by the action of their salivary secretions. The undissolved portion consists largely of the chitinous parts of the tracheæ. The larvæ also destroy all of the body wall except the superficial layer of chitin. During the process of pupation the non-digested content of the caterpillar hardens and forms the thin-walled, oval chambers in which the parasitic larvæ lie and in which they undergo their transformation into pupæ. The superficial layer is perfectly transparent, and at first is very flexible. Later, as drying occurs, it shrinks in on the walls of chambers and becomes hard and rigid, the whole forming the typical mummified carcass (Figs. 2, 4, 6). Practically all of the pupæ are oriented in a definite fashion in the carcass. Their heads are directed toward the anterior end of the carcass. Just before becoming immobile, the *Gnorimoschema* larva almost invariably turns the head upward in the gall chamber; likewise, the parasitic larvæ, just before pupating, orient themselves so that their heads are directed upward, in the direction of the anterior end of the carcass.

The imagines come out during the last week of August and the first week of September (Table I.). They escape by gnawing holes through the walls of the chambers and the superficial chitinous layer, both of which become very fragile. As a rule they all emerge practically at the same time. Several cases have been observed in which the entire brood has escaped within a period of ten minutes.

Once free from the carcass, they immediately gnaw a hole through the wall of the gall. Their escape is greatly facilitated by the habit of the caterpillar, just before becoming immobile, of eating out a passage-way to, or nearly to the epidermis of the plant. But in no case does the parasitized caterpillar secrete a silken plug. Hence, in order to escape to the exterior, the parasites have only to cut through the remaining thin portion of the wall.

The parasites must winter over in the imago state; otherwise

they would not be able to parasitize the normal or spring eggs of *Gnorimoschema*. Copulation, however, takes place immediately after the adults emerge, but the females do not parasitize the

TABLE II.

TABLE SHOWING VARIATION IN LENGTH OF LARVÆ IN THREE LOTS OF COPIDOSOMA.

Length in Lines.	Lots.			Length in Lines.	Lots.		
	I	II	III		I	II	III
1				29		3	3
2				30		10	7
3			2	31		3	1
4			3	32	1	5	
5			10	33		1	1
6		3	14	34		2	3
7			13	35		4	3
8		1	12	36		2	1
9			4	37		8	5
10		1	3	38		1	4
11		1	4	39	1	2	2
12			2	40	3	3	9
13			2	41	4	4	4
14			2	42	2	7	5
15	1		6	43	2	3	7
16	1		3	44	1	3	7
17	4	4	12	45	3	5	2
18		1	7	46	1	8	4
19	1	6	16	47	1	5	3
20		4	17	48	1	10	1
21			9	49	2	13	2
22		9	7	50		10	2
23		5	6	51		4	
24		3	4	52	2	8	
25		3	13	53		1	
26		3	5	54			
27		1	4	55	1	2	
28		4	1	56			

fall eggs of this moth. Only on one occasion has an attempt to ovipost in such eggs been observed. In this instance the few females which made the attempt were not able to penetrate the shell of the egg with the ovipositer.

4. *The Abortive Embryos.*

One of the most interesting discoveries made in connection with the study of *Copidosoma* is what we shall call the abortive embryos or larvæ, to which brief reference has already been made. Abortive embryos occur in the development of many different species of both invertebrates and vertebrates. They

are especially common in mammals. For example, my colleague, Dr. C. G. Hartman, has found a great mortality of embryos in the development of the opossum. Degenerating embryos are found throughout the brief but entire period of gestation. Abortive embryos have been found in at least three other species which have a polyembryonic type of development. One of the two embryos which develop from a single egg of the earthworm, *Lumbricus trapezoides*, sometimes degenerates. Fernandez ('09) has observed rudimentary embryos in the South American armadillo, *Tatusia hybrida*, and I have on several occasions seen them in the blastocyst of *Tatusia novemcincta*. But in no case with which we are acquainted is their number and constancy of occurrence so striking as in *Copidosoma*.

Our attention was first attracted to these abortive embryos while dissecting out a batch of larvæ from a large caterpillar. Most of the larvæ in the lot were large and about on the point of undergoing pupation, but in addition to these large individuals, there were a number of smaller ones. At first it was supposed that two distinct species of parasitic larvæ were present, or that we had a condition similar to that described by Silvestri for *Litomastix*, of sexual and asexual larvæ. It was noted, however, that the small larvæ had the same general structure as the larger individuals, except that they still possessed the two involucres typical of all of the younger larvæ of this species.

A study of serial sections of more than a hundred polygerms has completely demonstrated beyond any possibility of doubt that the small rudimentary embryos are derived from the same egg as larger normal larvæ, and consequently do not belong to a different species. The sections show that degenerating embryos are to be found in every stage of development of the polygerm, from the time of the formation of single embryos until the larvæ are set free into the body cavity of the host. In Fig. 24 is shown a degenerating embryo which has not yet been completely cut off from its fellow by the constriction of the nucleated membrane. Its nuclei have already completely disintegrated. In Fig. 26 is another embryo well on the way to complete disintegration. Finally Fig. 17, which is a portion of a polygermal mass about at the close of dissociation, contains at least four or five rudimentary embryos. They stain darker than the normal individuals.

The degeneration of embryos or larvæ does not cease immediately after the dissociation of the polygermal mass, but such embryos are found up until the beginning of pupation. About fifty lots of free larvæ have been dissected out of caterpillars, and

TABLE III.

TABLE SHOWING THE NUMBER OF PARASITES IN FEMALE BROODS.

Brood.	No. of Individuals.	Brood.	No, of Individuals.
1	25	46	200
2	42	47	201
3	49	48	207
4	52	49	210
5	73	50	210
6	89	51	212
7	91	52	213
8	95	53	213
9	100	54	214
10	100	55	215
11	106	56	215
12	108	57	216
13	115	58	216
14	119	59	217
15	120	60	229
16	121	61	234
17	122	62	236
18	124	63	236
19	124	64	237
20	125	65	245
21	131	66	248
22	137	67	250
23	142	68	251
24	145	69	254
25	146	70	256
26	150	71	257
27	151	72	260
28	153	73	261
29	154	74	264
30	156	75	272
31	161	76	275
32	163	77	280
33	164	78	284
34	167	79	286
35	174	80	292
36	174	81	301
37	178	82	314
38	178	83	328
39	179	84	335
40	181	85	338
41	183	86	340
42	189	87	347
43	192	88	378
44	194	89	385
45	195	90	395

Total = 17,864.
Average = 198.48.

almost without exception degenerating individuals were found. During the early period of the free larval stage, any given lot will show great variation in the size of the larvæ. To show this, all of the individuals of three lots have been measured in the terms of lines on the eye-piece micrometer scale (Table II.). In Lot I. there were only thirty-two larvæ. All but six of these would have reached maturity. Lot II. contained 176 larvæ, but at least twenty of these were degenerating. Lot III. contained 257 larvæ, and probably more than a hundred of them would have degenerated eventually.

A series of sketches of these larvæ is shown in Fig. 25, *A* to *H*. The first four or five of these types would have developed to maturity, but such larvæ as those illustrated in *F* to *H* degenerate. The most common types of degenerating embryos are the small spherical or oval-shaped masses (*G*, *H*). In one extreme case the lot of embryos consisted of about thirty of these masses, together with only a single normal larva. Doubtless many other similar masses had already degenerated.

It is difficult to assign any definite cause to the degeneration of these embryos, although it probably has something to do with nutrition. In some cases it seems to be due to the fact that the division of the egg has been carried too far. Some of the primordia receive but few embryonic nuclei, and these are invariably the first to degenerate in the polygerm. In other cases the degeneration is apparently due to the lack of proper nutrition. Most of the polygerms are early surrounded by the thick layer of adipose tissue, upon which the early development of the embryos depends. But other polygerms are almost if not entirely barren of adipose cells, and it is an observed fact that the mortality of embryos in such cases is exceedingly high. In Fig. 14 one of these cases is shown. This polygerm, which is devoid of fat tissue, contains more than a hundred embryos, not more than thirty or thirty-five of which have developed normally.

V. Number and Sex of Copidosoma Parasites Found in Gnorimoschema.

The number of matured parasites developing in the *Gnorimoschema* larva has been determined in 162 cases. This has been

done by removing the carcass from the gall chamber a short time before the emergence of the parasites, and enclosing it in a small vial. After all of the parasites have emerged they are killed by filling the vial with 80 per cent. alcohol, and then counted under a binocular microscope. This procedure has the advantage of eliminating the possibility of contamination from other polyembryonic broods. Furthermore, the use of the binocular in counting enables one to distinguish readily the two sexes. The strong sexual dimorphism in *Copidosoma* makes this task rather easy. The females have the enlarged club-shaped,

TABLE IV.

TABLE SHOWING THE NUMBER OF PARASITES IN MALE BROODS.

Brood.	No. of Individuals.	Brood.	No. of Individuals.
1	41	32	178
2	53	33	179
3	61	34	180
4	67	35	180
5	90	36	180
6	93	37	182
7	96	38	190
8	100	39	190
9	101	40	192
10	106	41	199
11	107	42	199
12	113	43	202
13	118	44	204
14	119	45	214
15	124	46	215
16	124	47	218
17	124	48	223
18	127	49	225
19	128	50	232
20	136	51	233
21	137	52	236
22	138	53	236
23	139	54	245
24	142	55	247
25	147	56	272
26	152	57	277
27	168	58	278
28	171	59	323
29	172	60	324
30	177	61	328
31	177	62	345

Total = 19,874.
Average = 175.32.

terminal segment of the antenna, and bright yellow legs, while the males do not have the enlarged segment and the legs are of a

dark, more or less mottled color. One can therefore readily detect a mixed brood under the microscope.

The 162 broods studied were taken at random from the field, and therefore in all probability the data on numbers and sex yielded by them represent the approximate sex ratio for the species. These 162 broods contained a total of 31,001 individuals, or an average of over 191 to the brood. Ninety of these, or 55.56 per cent., contained only female parasites, 62, or 38.27 per cent., contained only male parasites, and 10, or 6.17 per cent., contained mixed broods of males and females.

There are therefore not only a larger number of female broods than male, but the average number of individuals in the former exceed that of the latter. Female broods average a little over 198 individuals to the brood (Table III.), while male broods average only about 175 (Table IV.). The range in the number of individuals in these broods (from 25 to 395 in the female, and from 41 to 345 in the male) makes it evident that these averages are of little significance, except, perhaps, to show that the fertilized egg is slightly more prolific than the unfertilized egg.

Of the total number of individuals (31,001), 63.41 per cent. are females and 36.59 per cent. males; but obviously the true sex ratio can not be based on these figures. It must be determined from the number of male and female broods. It would not be a difficult matter to determine this ratio were it not for the uncertainty of the origin of some broods. There is always the possibility in these insects that more than one parasitic egg has been laid in the egg of the host, and hence the parasites which later emerge may not constitute a true polyembryonic brood, but in fact represent two or even more such broods. Under the circumstances, the best that one can do is to determine approximately the sex ratio for the species. This can be done in the following manner. If we assume, as all previous workers have done, that each of the mixed broods is the product of at least two eggs, then, in accordance with the law of probability, we can determine the number of unmixed male and female broods, each of which must also have been produced from two eggs. Worked out on this basis, it is found that the ratio of females to males is 106/76 or a sex ratio of approximately 3 : 2

This leads to a discussion of mixed broods, and especially to a consideration of the question as to how such broods have come into existence. The obvious explanation of their origin is the one given above, viz., that they arise from two eggs. Marchal and Silvestri, who have studied the development of polyembryonic insects, both offer this explanation. They support the conclusion by citing the fact that two (or more) parasitic eggs are sometimes laid in the egg of the host. According to Marchal, such eggs develop independently, each producing a distinct polygerm and consequently a distinct brood. If the two eggs are of the same sex potentiality, the individuals developing from them will be either all females or all males, according to whether or not the eggs are fertilized or unfertilized. The dual origin of these double broods naturally elude detection in lots that have emerged. But if one of the two eggs is unfertilized and the other fertilized, the result will be a mixed brood, consisting of males and females. This conclusion of Marchal and Silvestri is strongly supported by the facts of polyembryonic development in the armadillos, in which it has been conclusively demonstrated (Fernandez, '09, Patterson, '13) that all of the embryos of a given pregnancy are the product of a single egg. As a result, mixed litters are never found in these mammals.

That mixed broods may arise from two eggs in *Copidosoma* is supported by the fact that two polygerms are sometimes found in a single *Gnorimoschema* larva. However, certain facts concerning the condition of mixed broods in this species, make it doubtful whether the origin of all such broods can be explained in this obvious way. Careful dissections of something over a hundred parasitized *Gnorimoschema* larvæ have revealed only two cases in which a single larva contained more than one polygerm. Since 6.17 per cent. of all broods are mixed, and since a similar number of unmixed broods would have a dual origin, we should expect to find over 12 per cent. of all parasitized larvæ containing two polygerms, but instead, less than 2 per cent. are found.

Another line of evidence which is not in harmony with the view that mixed broods are always the product of two or more eggs, is the great preponderance of females in certain lots. Of

the nine complete lots (Broods 2 to 10) listed in Table V., the number of females in each case is greater than the number of males. In some cases (Broods 3, 4, 5, 7, 8), this difference is not so great but that the origin of each lot can be explained on the assumption that two eggs have been deposited in the egg of the host. But in Broods 2, 6, 9, and 10 the number of females in excess of males is indeed striking, making it difficult to explain the origin of such broods on the basis of two eggs.

In view of these facts, the writer is convinced that some other explanation must be offered for the origin of certain mixed broods; in fact, one involving the idea that a single fertilized egg may give rise to a few males as well as a relatively large number of females. This would be possible on the basis of the following assumption.

TABLE V.

TABLE SHOWING THE NUMBER OF PARASITES IN MIXED BROODS.

Brood.	No. of Individuals.	Females.	Males.
1*	89	20	69
2	162	153	9
3	172	92	80
4	207	126	81
5	216	176	40
6	235	223	12
7	241	161	80
8	300	235	65
9	304	292	12
10	337	316	21
Totals.....	2,263	1,794	469
Average.....	226.3	179.4	46.9

* This brood is not complete, owing to the fact that some of the larvæ and pupæ had been destroyed by a large dipterous larva.

If *Copidosoma* conforms to the general scheme for sex determination in insects, the females must have the 2 X chromosomes, and males the single X chromosome. Ordinarily, during the process of cleavage, all of the chromosomes in the fertilized egg divide equally, so that all of the nuclei entering into the formation of the embryos will carry the XX chromosomes, thus producing a brood of females. But if during the early development of the egg it should happen that the two X chromosomes in one or more cleavages should not divide but separate, one going to each pole of the spindle, each daughter nucleus would then receive a single

X chromosome. If such nuclei later divided in the typical manner and gave rise to embryos, such embryos would be males. One is encouraged to make this suggested explanation in the light of Bridges' ('13) discovery of the non-disjunction of the sex chromosomes in *Drosophila*. In *Copidosoma* the separation of the sex chromosomes during cleavage would be a case of "somatic" or "cleavage disjunction," while in *Drosophila* these chromosomes fail to separate or "disjoin" in the reduction division of the egg.

In conclusion attention should be directed to the frequency of *Copidosoma* in nature. At Woods Hole about twenty per cent. of all *Gnorimoschema* larvæ are infected with this parasite

TABLE VI.

TABLE SHOWING PERCENTAGE OF PARASITIZED CATERPILLARS IN THE GALLS OF SOLIDAGO SEMPERVIRENS.

Number of Galls.	Date.	Parasitized by *Copidosoma*.	Normal Galls	Empty.	Parasitized by Other Parasites.
9	7–29–12	7	2	0	0
33	8– 5–12	5	15	0	13
33	8–17–12	9	16	5	3
56	8– 8–12	7	26	10	13
29	8–12–12	8	16	0	0
141	8–25–12	20	56	33	32
14	7– 7–13	1	13	0	0
16	7–14–13	0	13	0	0
39	7–15–13	8	31	0	0
38	7–19–13	4	*	*	*
23	7–23–13	2	20	0	1
38	7–26–13	6	*	*	*
27	8– 5–13	4	*	*	*
24	8–25–13	4	17	3	0
18	6–15–14	2	14	2	0
19	6–18–14	3	16	0	0
43	6–22–14	19	19	3	2
40	6–24–14	9	20	10	1
20	7–16–14	1	19	0	0
24	7–30–14	0	21	0	3
25	7–23–15	3	12	7	3
18	7–26–15	5	11	0	2
66	7–30–15	25	37	2	2
35	8– 4–15	14	35	1	6
Totals. . 828		166			

* Record incomplete. About 20 per cent. of the caterpillars are parasitized by *Copidosoma*.

(Table VI.). As may be seen from the table, the extent of infection varies greatly in the lots of galls taken from different regions (those collected on a given date are all from a single locality). Plants which grow in exposed places, as along the

roadside or barren spots, carry a higher percentage of galls than do those which are located in protected regions. Likewise, the moth larvæ from the galls of the former are more highly parasitized.

Summary.

1. *Copidosoma gelechiae*, which is a parasite in the Solidago Gall Moth, *Gnorimoschema salinaris*, has but one generation a year.

2. The egg of this parasite is probably laid during the month of May.

3. The type of development in *Copidosoma* is polyembryonic. The number of individuals average about 191 per brood.

4. In the youngest stages secured the process of division of the egg into embryonic primordia is already in progress. The young polygerm consists of two distinct regions: (1) An outer zone, or nucleated membrane, containing the free polar nuclei; (2) a central region, containing the true embryonic nuclei.

5. The embryonic nuclei segregate into groups, which become surrounded by a dense layer of granular protoplasm and form the primordia of the multiple embryos.

6. During early growth the polygerm elongates into a cylindrical-shaped structure, which becomes broken up into several spherical, primary masses by the formation of constrictions in the nucleated membrane. Each primary mass receives several of the primitive embryos.

7. The primary masses become broken up into secondary masses by further constrictions of the nucleated membrane. At the end of these divisions, each embryo is separated from the others and is surrounded by an inner and an outer involucre—the former derived from the granular protoplasm and the latter from a portion of the nucleated membrane.

8. The interstices between these masses become filled with an inter-embryonal substance derived from at least three sources: elements from the nucleated membrane, leucocytes, and cells from the adipose tissue, which usually is laid down in the form of a thick layer on the outer surface of the polygerm. The entire structure thus becomes a complex, which may be called the polygermal mass.

9. The dissociation of the inter-embryonal substance sets the larvæ free in the body cavity of the host. This occurs during the last week of July.

10. Abortive or degenerating embryos are found throughout the entire period covered by the polygerm and free larval stages.

11. The free larvæ destroy the entire contents of the caterpillar, except the chitinous parts of the trachae, and leave only the superficial layer of chitin of the body wall intact. The detritus left in the larval chitin hardens to form thin-walled, oval chambers in which the larvæ lie and undergo pupation The superficial layer of chitin also hardens, and the larval skin thus becomes transformed into the typical mummified carcass, filled with the parasitic pupae.

13. Pupation takes place during the first ten days of August and lasts about a month.

14. The number of adult parasites emerging from the carcasses varies from 25 to 395. There is a preponderance of females, about 55 per cent. of all broods being females. Furthermore, the average number of females emerging from a single carcass is 198 as compared with 175 for the males. Ten mixed broods of males and females have been obtained. Some of these have doubtless arisen from two or more eggs; but it is suggested that such broods may also arise from a single fertilized egg, by a process of disjunction of the sex chromosomes during the early cleavage stages.

Woods Hole, Mass.,
August 12, 1915.

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DESCRIPTION OF PLATES.

PLATE I.

FIG. 1. A typical gall of *Gnorimoschema salinaris*, Busck, situated at the base of the stalk of the swamp goldenrod, *Solidago sempervirens*. × ¼.

FIG. 2. Gall cut open to show the position of the mummified carcass of *Gnorimoschema*. Natural size.

FIG. 3. Gall cut open and carcass removed to show the shape of cavity. Note that the walls of the cavity are smooth and that the excrement from the caterpillar is packed in the bottom of the cavity. Natural size.

FIG. 4. Mummified carcass from gall shown in Fig. 3. Natural size.

FIG. 5. Lepidopterous larva which is an inquiline in the gall of *Gnorimoschema*. Note the irregular shape of the cavity which contains scattered trash and excrement. Natural size.

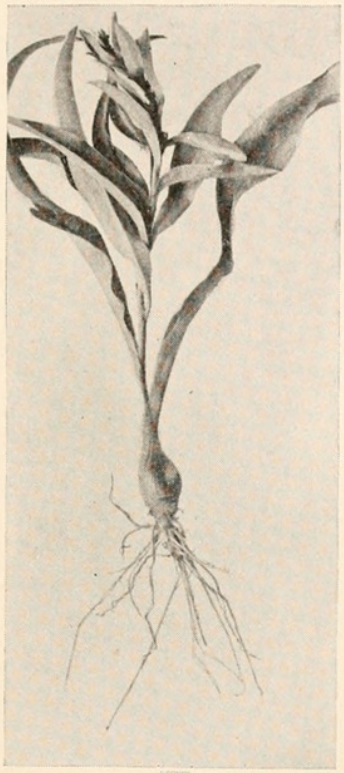
FIG. 6. This gall shows an incomplete passage-way, lying just above the head of the carcass. Normal size.

FIG. 7. Side view of a gall showing the orifice of the passage-way, closed by silken plug. Natural size.

FIG. 8. Stalk of swamp goldenrod containing two galls. × ¼.

FIG. 9. Gall containing a non-parasitized caterpillar. Natural size.

FIG. 10. Gall containing a parasitized caterpillar. Natural size.



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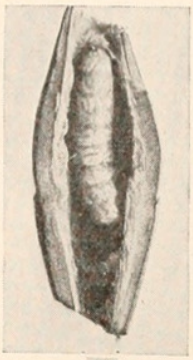

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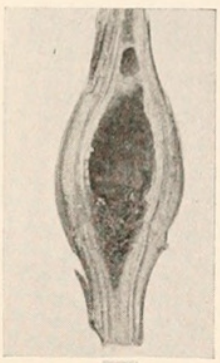

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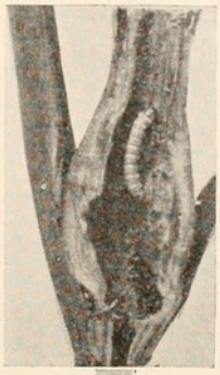

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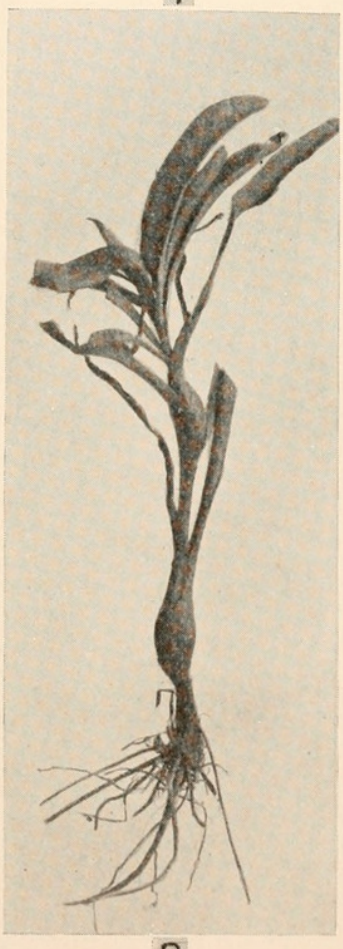

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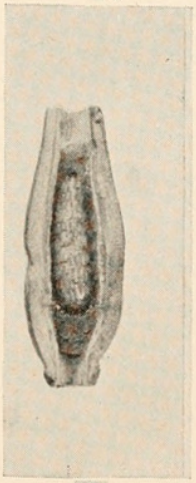

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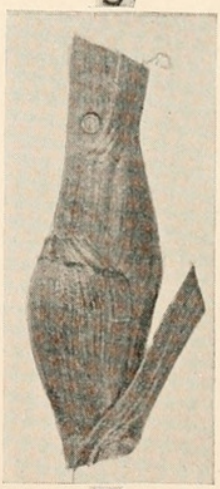

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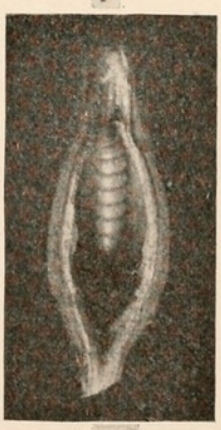

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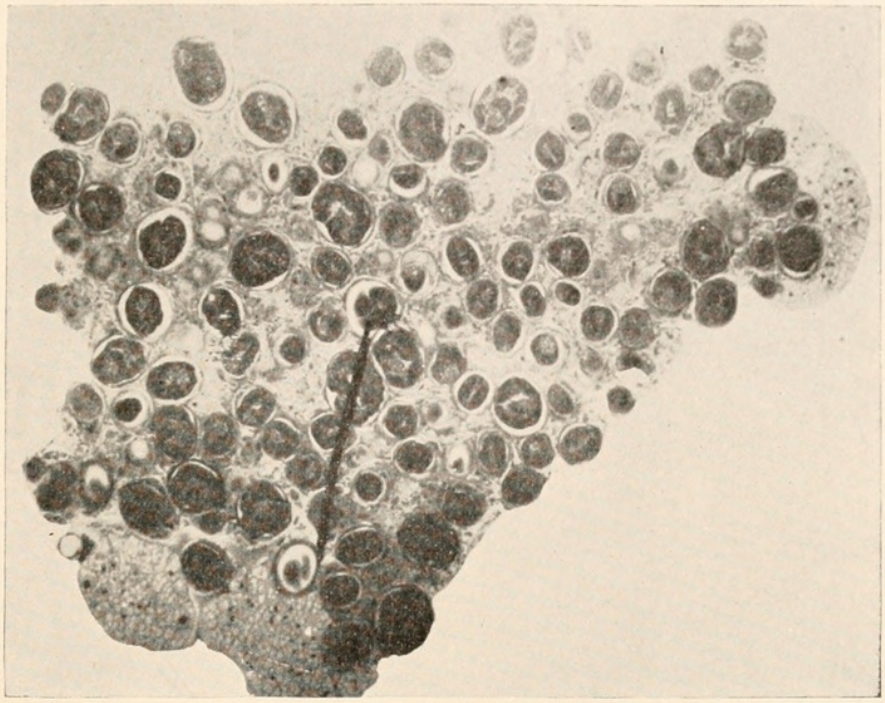
PLATE II.

FIG. 11. Photomicrograph of a section of an irregular polygermal mass. ×40.

FIG. 12. Photomicrograph of a single embryo from mass shown in next figure. ×180.

FIG. 13. Photomicrograph of a longitudinal section of a flat, plate-like polygermal mass. × 40.

FIG. 14. Photomicrograph of a spherical polygermal mass which is barren of adipose tissue. × 40.



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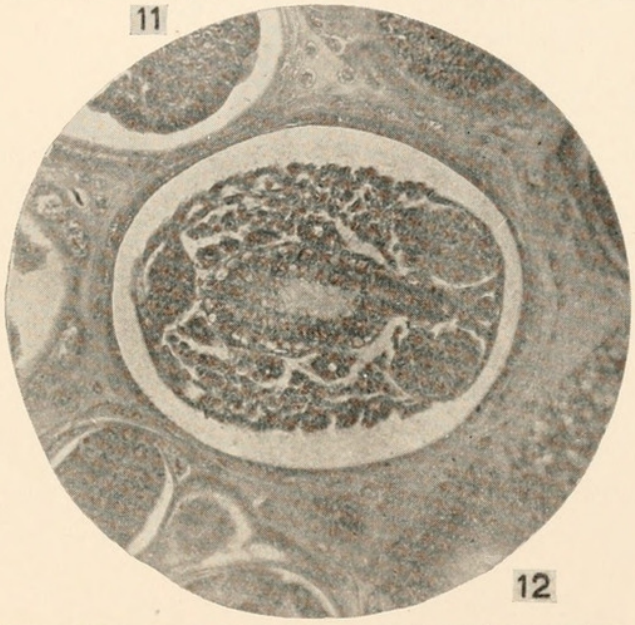

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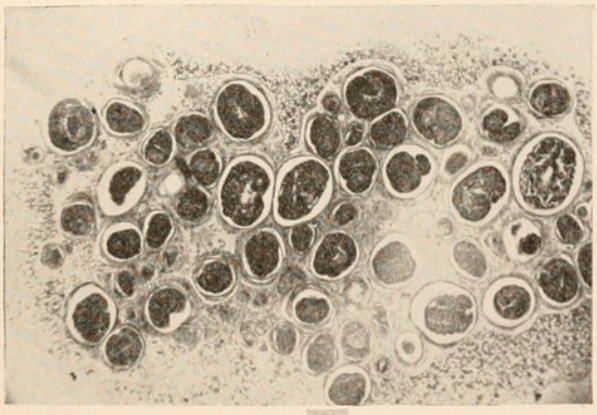

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PLATE III.

FIG. 15. Photomicrograph of the middle portion of longitudinal section of a small caterpillar. A fat body containing a polygerm lies just below the intestine. × 44.

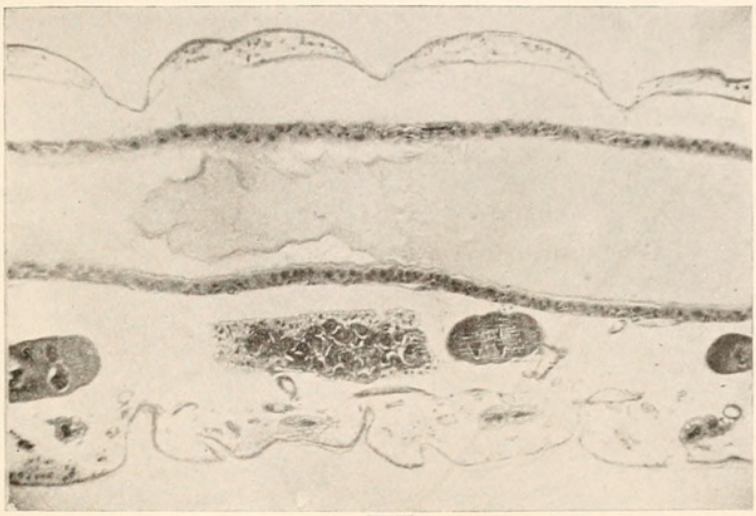
FIG. 16. Photomicrograph of a portion of a section of a polygermal mass which was about to begin disintegration. × 44.

FIG. 17. Photomicrograph of a section of a polygermal mass undergoing dissociation. × 44.

FIG. 18. Photomicrograph of a mass of free larvae from the body cavity of the caterpillar. Note that each embryo is still surrounded by the involucres. × 44.

Reference Letters Used in Plates IV.–VI.

A.E., Abortive Embryo.
A.T., Adipose Tissue.
C., Clear space left by contraction of Precipitated Material.
E.N., Embryonic Nuclei.
G.L., Granular Layer.
I.I., Inner Involucre.
I.S., Inter-embryonal Substance.
N.M., Nucleated Membrane.
O.I., Outer Involucre.
P.D., Primary Division of polygerm.
P.E., Primitive Embryo.
P.M., Precipitated Material.



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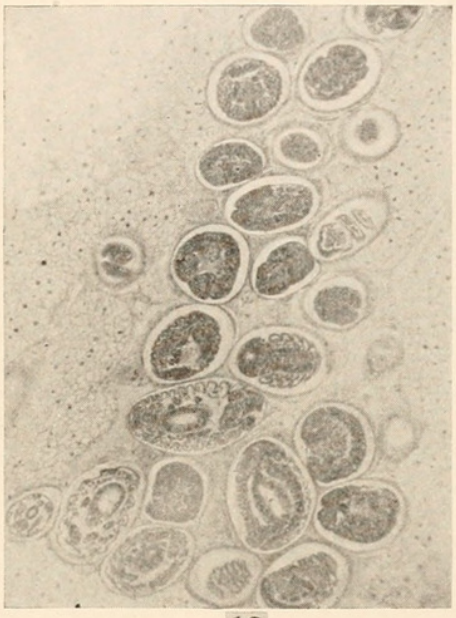

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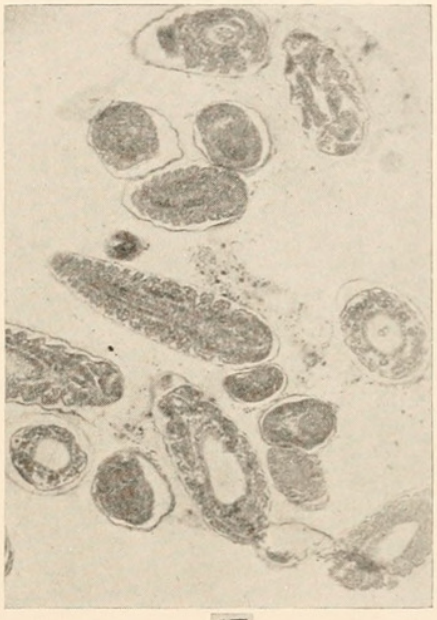

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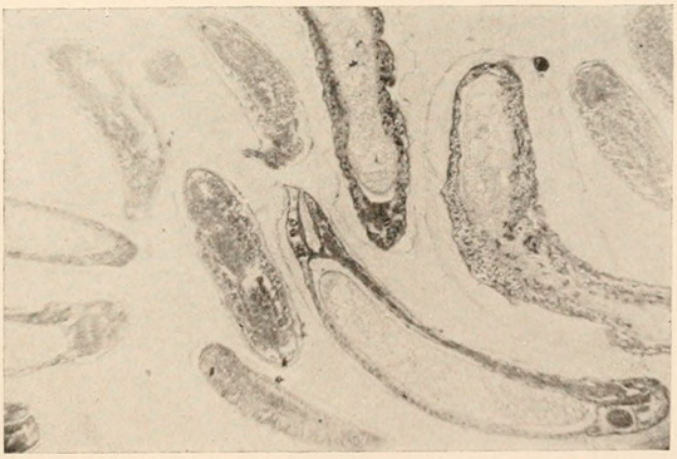

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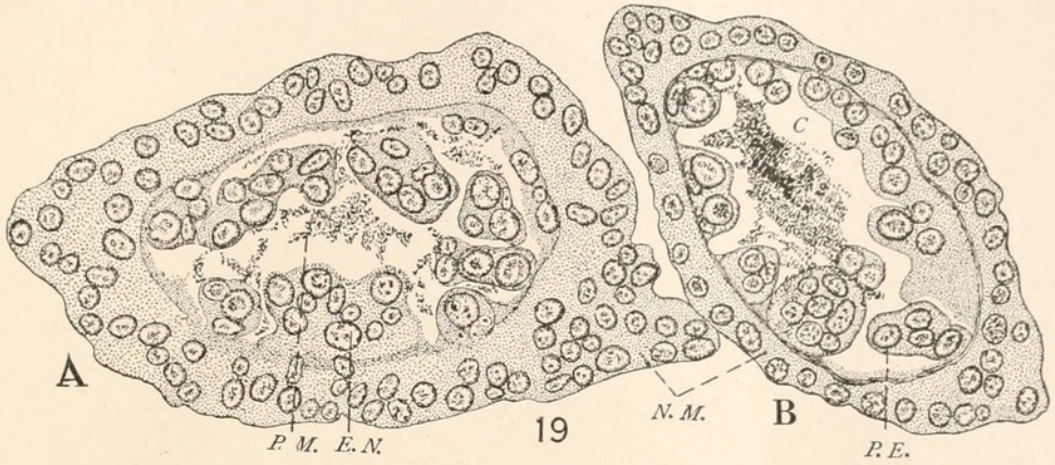
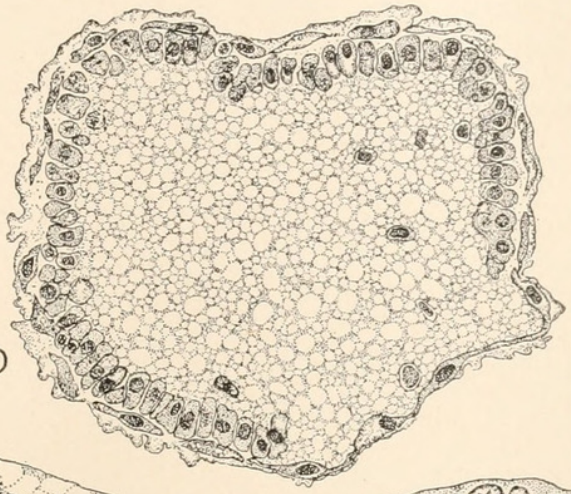
PLATE IV.

FIG. 19. *A* and *B* longitudinal sections of two polygerms lying close together in the same caterpillar. These polygerms show an early phase of the segregation of the embryonic nuclei to form the separate embryos. × 489.

FIG. 20. Section of an egg of *Gnorimoschema* which has started to develop parthenogenetically. × 173.

FIG. 21. Longitudinal section of a polygerm showing the end phase of embryo formation. × 480.





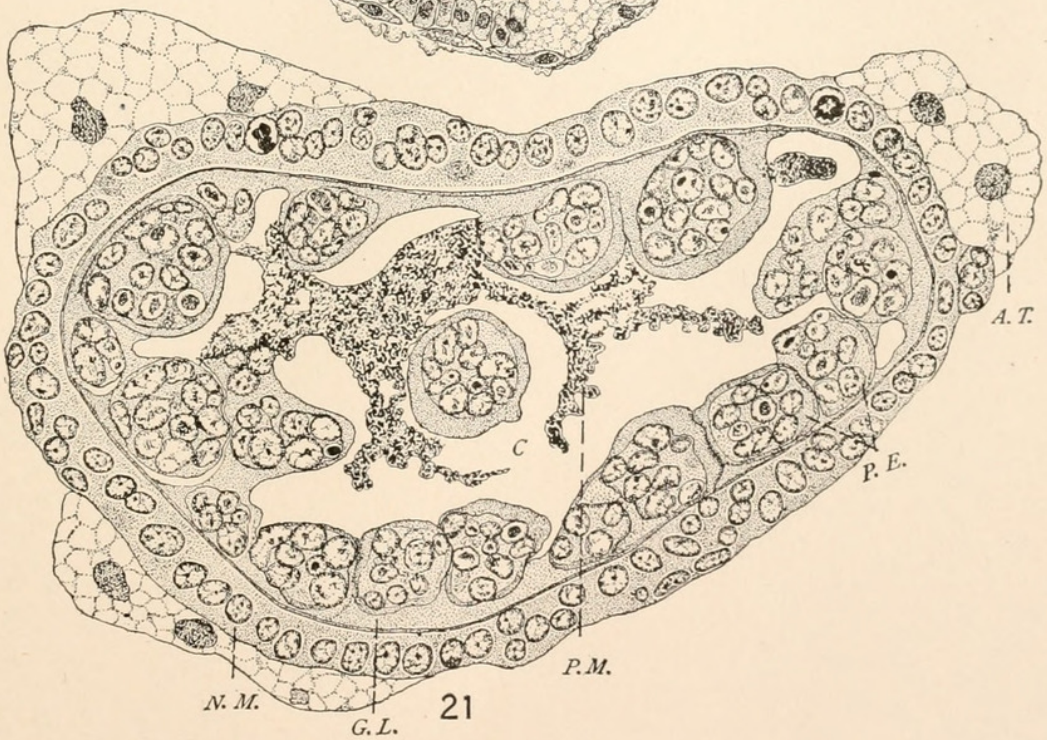


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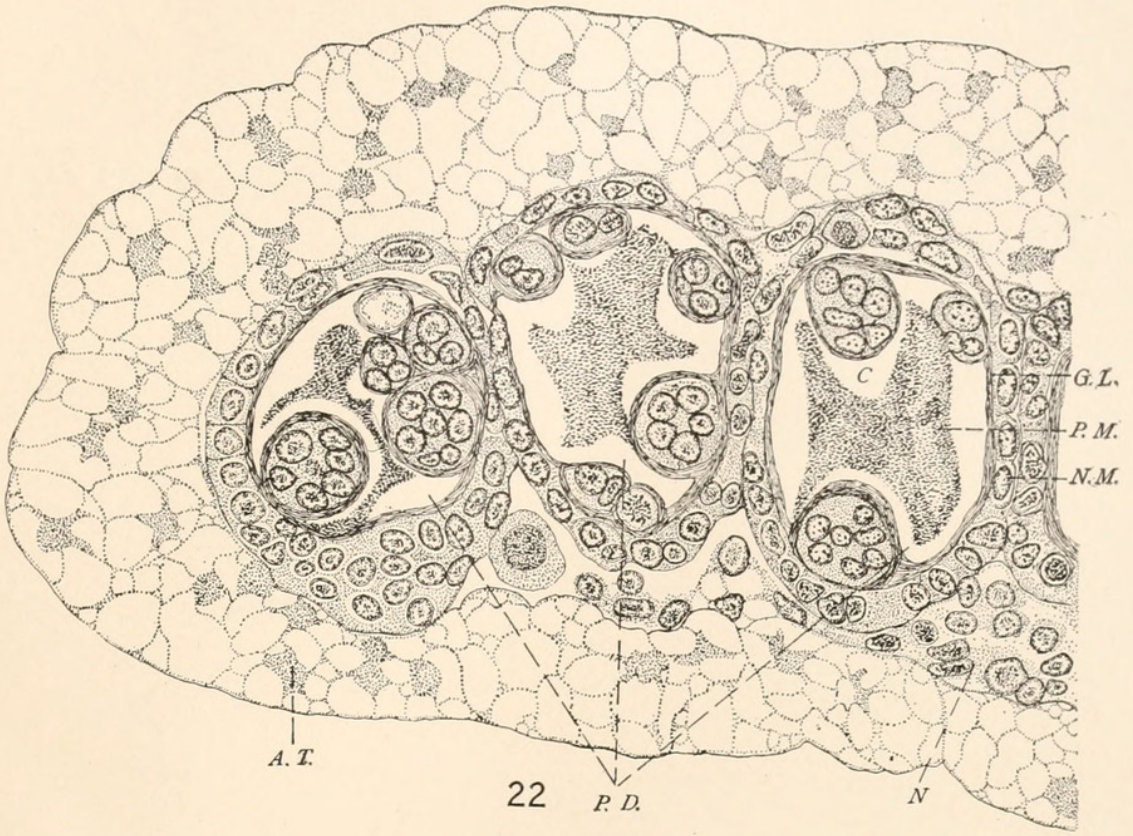
PLATE V.

FIG. 22. One end of a longitudinal section of a polygerm showing three of the twelve primary divisions into which it has been divided by constrictions of the nucleated membrane. × 373.

FIG. 23. Section of a primary mass showing the process by which it is further divided up into secondary masses by constrictions of the nucleated membrane. × 508.

FIG. 24. Section of a single isolated, primary mass about at the close of its division into single embryonic masses. × 257.



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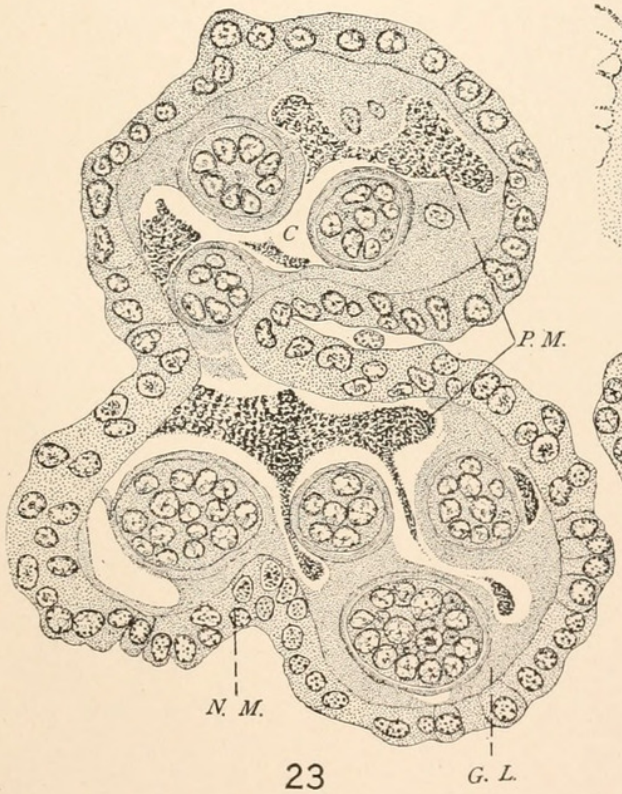

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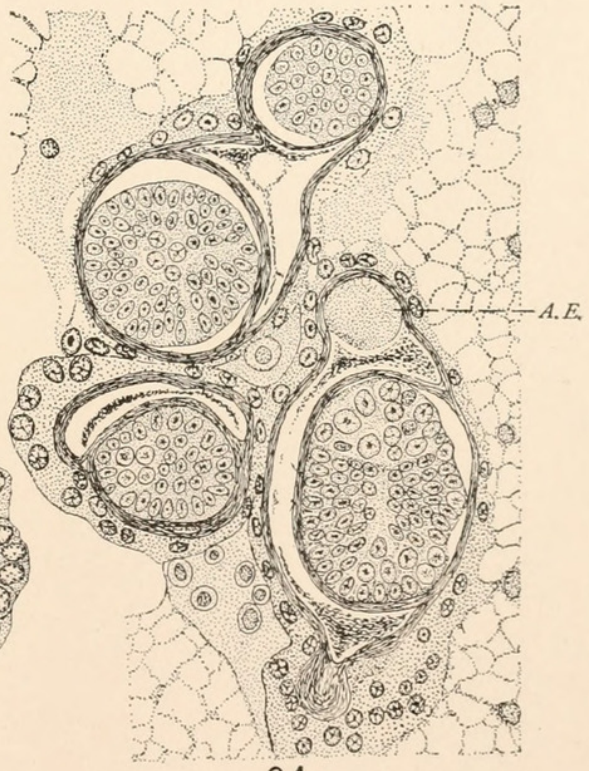

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PLATE VI.

FIG. 25. *A* to *H*, Series of sketches from Lot III of the free larvae listed in Table III. This figure shows the great variation in size of the larvae from a single caterpillar. They are all drawn to the same scale.

FIG. 26. Detailed drawing of a section of one of the embryos seen in Fig. 13. It shows the relation of the inter-embryonal substance and involucres to the embryo. × 187.

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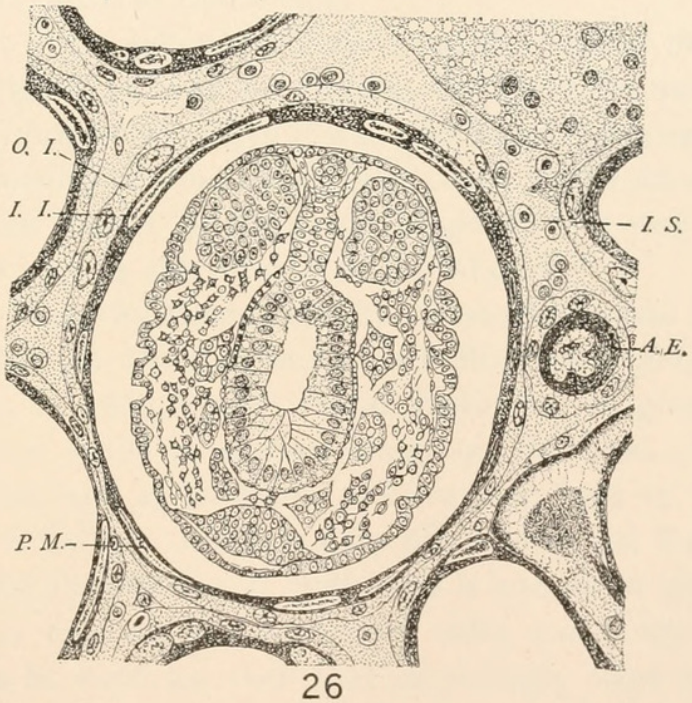

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DISTRIBUTION OF FOLLICULINA IN 1914.

E. A. ANDREWS.

The finding of vast hordes of the *Stentor*-like infusorian *Folliculina* both in 1912 and 1913 throughout the whole extent of the Severn River which is a brackish side branch of the Chesapeake Bay, led to further examination in 1914 to see if this were a phenomenon to be repeated annually or only a rare inroad of an outside fauna into new territory.

In 1913[1] *Folliculina* was found in inconceivable numbers living upon the leaves of the fresh water plants *Elodea* and *Potamogeton*, which have taken possession of definite zones of shallow brackish water along some fifty and more miles of extent of the river and its side creeks. It was also found on *Elodea* in Whitehall River, just to the north of the Severn.

In 1914 it was taken on *Elodea* from the head of the Magothy River, August 13, and on floating *Elodea* in the mouth of the Magothy, August 23, when it was also found living upon stunted *Elodea* growing in the narrow inlet canal to the nearly shut off side branch known as the Little Magothy. It was taken also at Deep Creek, a side branch of the Magothy.

As the Magothy opens into the Chesapeake some seven miles from the Severn, the distribution of Folliculina is quite extensive. Moreover, in 1880 Ryder[2] found *Folliculina* in great numbers upon oyster shells in shallow water on the west coast of the Chesapeake, and as he seems to have then been at St. Jerome, St. Mary's County, which is sixty miles down the Bay from the Severn, the distribution of *Folliculina* is known for side branches of the Bay opening into it seventy miles apart, approximately.

It is to be expected then that exceedingly large areas of the side waters of the Chesapeake may be inhabited by this little-known protozoan, which in the mid-summer season adds greatly

[1] See BIOL. BULL., XXVI., No. 4, April, 1914.

[2] *Am. Nat.*, 14, 1880.

to the plankton, or swimming fauna, as well as to the microscopic life attached to the summer vegetation of these waters.

Its advent and departure in Chase's Creek, a branch of the Severn, showed in 1914 even more suddenness than in 1913, while its time of abundance was noticeably less though actual numbers present were even more vast.

Though searched for from the middle of June, every few days, *Folliculina* was found first on July 19, 1914. It then appeared only here and there, not on every plant of *Elodea* and on very few plants of *Potamogeton*. On the sprays of *Elodea* the *Folliculina* showed on comparatively few leaves, like black soot stuck on the leaves; both isolated individuals and aggregates occurred

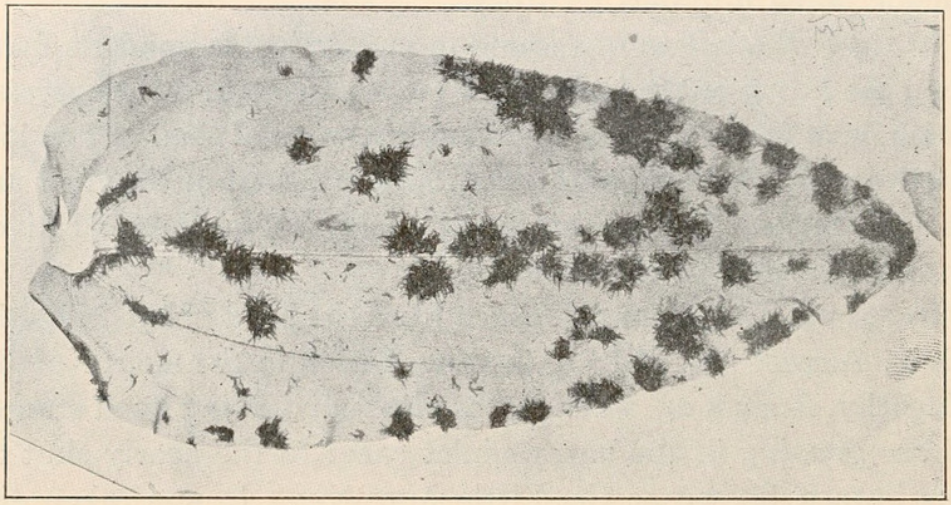

FIG. 1. Leaf of *Potamogeton* showing scattered colonies of *Folliculina*. ×3 diam. Photograph of preserved specimen.

but there were very few large aggregates covering half the surface of a single leaf. Most leaves had none, some leaves had many scattered individuals. On the stems there were noticeable numbers of the small form of sac. The occurrence on leaves seemed entirely arbitrary as if from settlements of swimmers: the *Folliculina* was not now crowded toward the tips of the sprays but scattered along many inches of the spray.

At the date of this first appearance, jellyfish had been common for two weeks but the other conspicuous summer visitor to these waters, the young menhaden now for the first time came along the shores over the *Elodea*, which may be correlated with the

feeding of the menhaden upon plankton in which the free swimming *Folliculina* may be included as possible food for the menhaden.

At this date the *Elodea* had grown up to a height of twenty inches and formed some flower stalks and buds at the surface, so that there had been a long period in which suitable attachment base for *Folliculina* was present but the *Folliculina* had been absent.

July 21 the water after long drought was turbid from the presence of plankton and the *Folliculina* had increased but little, appearing as black spots on one out of several hundred sprays of *Elodea* and one out of many thousands of *Potamogeton* sprays. Only a few of the leaves on each inhabited spray had dense aggregates, so that the question arises: why do the *Folliculina*

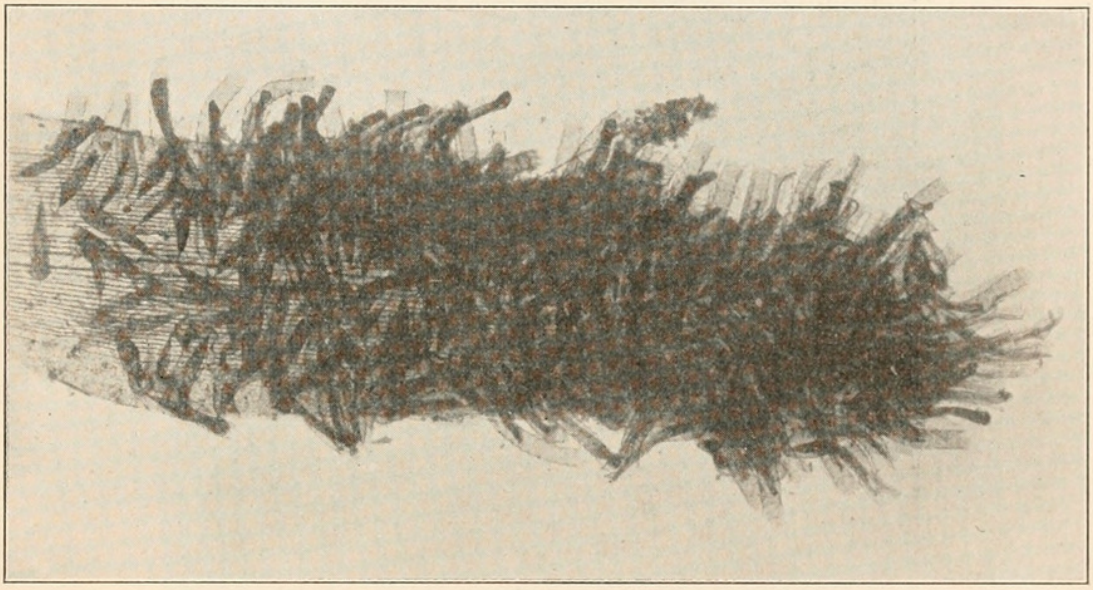

FIG. 2. Tip of leaf of *Elodea* covered with a colony of *Folliculina*. × 15 diam. Photograph of preserved specimen.

crowd together in these rare, isolated aggregates? When sprays of these dates were put into aquaria they gave rise to free swimming forms, thus showing that these early settlers need not remain fixed but might contribute to additional distributions.

On July 27 *Folliculina* had become much more abundant upon sprays of *Elodea* and *Potamogeton;* some of the free-floating fragments on the surface appeared black with the accumulated

Folliculina. In the water also some free-swimming *Folliculina* could be seen near the surface swimming all through the water as well as close to floating plants.

Out in the Severn River a two-quart jar of water taken up at random at the surface showed several free-swimming *Folliculina;*

FIG. 3. Photograph of a preserved colony that had been formed on surface of the water in aquarium; showing form of case and tube spirals as well as animal rectracted within case. Enlarged 30 diameters.

three days later these had settled down on the side of the jar and were in two groups, two individuals in one and five in the other, so that at least seven were in the two quarts of surface water, which would make an immense number for the entire river.

By August 1 much of the *Elodea* growing in the *Elodea* zone along shore was black with aggregates of *Folliculina*. Free swimmers were in the water of the creek in vast numbers: a quart dipped from the surface at random showed in a white bowl from fourteen to one hundred, by actual count, for each quart of water from the surface. By drawing the bowl along the surface, the *Folliculina* swimming free were concentrated till thousands in a quart made it dark as if sprinkled with black pepper. Though these free-swimming Folliculinas easily escape notice in the greenish water turbid with plankton and sediment, they are readily observed in calm water by an eye near the surface; and standing in water five feet deep one may see them swimming

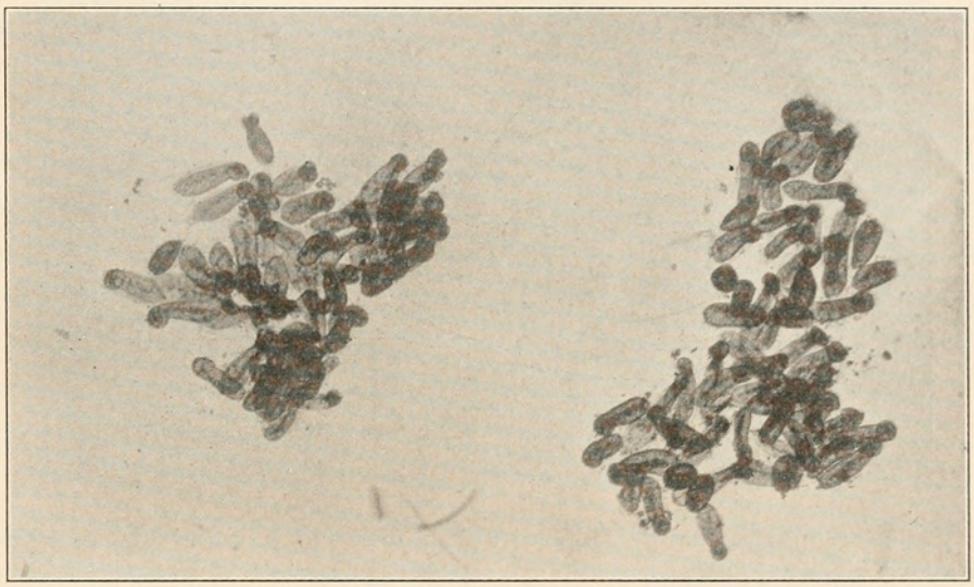

FIG. 4. Photograph of two young colonies of free swimmers that have just settled on surface of water in aquarium and formed sacs but no tubes: one individual on extreme left is still in motile form. Preserved specimen, × 20 diam.

rapidly in all directions, individually in straight and in curved paths. Many deep down in the water were seen best by holding a white object below them, but most of them were near the surface where they congregated especially about any floating object as fallen leaf or floating chip, seemingly influenced by its presence so that they swam toward it.

While at this time the *Folliculina* continued to colonize the new growths at tip of the *Elodea* as fast as it grew so that the

black aggregates crowded on the young leaves nearly to the tip where only the newest leaves were as yet unoccupied; by August 18 the extension of the *Folliculina* hosts had ceased. The tips of the growing *Elodea* were now bare or free from *Folliculina* back some twenty leaves from the tip and many of the old dwellings on the lower leaves were deserted. These dense black colonies on old leaves contained in fact but few living Folliculinas.

FIG. 5. Photograph of natural size sprays of *Elodea* preserved to show successive phases of colonization in 1914. Spray on left has grown enough to form flower but as yet but a very few isolated individual *Folliculina* have settled upon it. The next spray shows scattered tubes all along its length. The third spray shows dense aggregations of colonies even up to the tips of the rapidly unfolding new leaves. The fourth spray illustrates the subsidence in colonization: the new colonies no longer cover the leaves at the tip of the spray but these grow more rapidly than the new colonists occupy them and are left more nearly free from any Folliculinas.

By August 26 this falling off in the colonization and rapid disappearance of *Folliculina* was most pronounced: the *Elodea* sprays showed an abrupt transition from the lower leaves black from dense population of tubes, for the most part empty, to the upper leaves only sparsely inhabited with scattered individuals. Evidently some sudden change had operated not only to check the previously rapid spread of the Folliculinas onto new leaves but to

almost exterminate them. Yet many remained alive here and there so that when large quantities of the *Elodea* were put into aquaria many free swimmers escaped. Yet these after forming new tubes on the surface of the water did not remain alive but had all vanished September 5, though in such apparently normal environment others had been kept two weeks in captivity earlier in the season.

Thus while appearing after the middle of July and being extraordinarily abundant in August, the *Folliculina* were all gone about the end of August and no way was found of keeping them longer. Their period of existence in accessible regions of the river was scarcely six weeks.

In 1913 they appeared before the end of June and a few lingered on to the first of September in nature and were kept in aquaria in a warm room till the 27th and a few till November 11.

In 1912 no live ones were found after September 8. This enormous crowding of the waters with free-swimming *Folliculina* and dense settlements of the case-making Folliculinas during about a month, the last weeks of July and the first of August, coincides with very high temperatures and abundance of microscopic plankton in these waters but it is not at all evident either why the Folliculinas should not come earlier, as they did in 1913, or remain later as they did in 1913 and 1912.

The great rapidity of their colonization of large areas suggests either very great immigration or else very rapid multiplication, or combination of both. As all material searched in the daytime in 1913 failed to show more than a few cases of multiplication, most all the free-swimming forms being merely the case-making forms again freed, material was collected at all times of the night in 1914, but here again but few cases of division were observed.

Hence it seems unlikely that fission of a few immigrants actually produced the vast numbers found on the leaves of plants, and it is probable that very large numbers came into the river suddenly from some outside source and these settling down, migrating out again, and in some cases increasing by fission, gave rise to the succession of dwellings covering the leaves for some two months.

The causes leading to the immigration as well as the causes of rather sudden diminution of numbers and utter disappearance remain entirely unknown.

The food of the case-inhabiting *Folliculina* being bacteria and some larger forms of plankton, the disappearance of *Folliculina* may well be associated with changes in food supply, in turn brought about in connection with such changes as those of temperature and salinity.

The motile forms take no food and may be enabled to settle and to continue migration and multiplication only when feeding conditions allow the sessile form to accumulate enough energy.

Summary.

1. The vast swarms of swimming protozoans of the genus *Folliculina* that were found to settle down over the aquatic plants along the shores of side branches of the Chesapeake Bay in 1912 and 1913, came in even greater numbers in 1914, and it is therefore probable that this immigration and colonization is a regular annual phenomenon.

2. The incursions of swimming *Folliculina* do not take place as soon as the plants have grown enough to supply places for attachment, and the departure or disappearance of the living Folliculinas antedates the cessation of growth and final dying down of the plants upon which they settle.

3. As far as evidence is available the numbers that crowd the leaves arise more from immigration from without the area than from division of animals that have already settled in the area.

4. The times of appearance and disappearance differ in successive years.

5. It is suggested that conditions of food possibilities are determining factors in these inroads into the brackish fauna.

6. The great number of free swimming forms makes them, for the time being, an important factor in the plankton.

7. The crowding of the dwellings or cases on the leaves all along the shores is a considerable element in the transformation of matter which, arising from decay of organic materials, is transformed into bacteria and other plankton organisms, which in turn are eaten by *Folliculina* and enable them to secrete resisting tubes and sacs which finally settle to the bottom of the river.

PHENOMENA OF ORIENTATION EXHIBITED BY EPHEMERIDÆ.[1]

F. H. KRECKER.

It is a well-known fact that in alighting Ephemeridæ orient positively to a breeze. I became interested in this reaction and the observations made naturally lead to others on reactions to gravity and to light, and to the results of a conflict between any of these three stimuli.

The observations were made during the summer of 1915 at the Lake Laboratory of Ohio State University at Cedar Point on Lake Erie. Ephemeridæ appear here in almost incredible numbers. When a brood is at its height it is a very common occurrence to find piles of the insects three or four feet square and six to eight inches deep under electric lights. At a neighboring amusement resort several carts were required each morning to haul away the dead insects. The species with which the following observations are especially concerned is *Hexagenia variabilis*. The number, variety and arrangement of lights at the resort presented favorably conditions for observing the reactions to light of great numbers of individuals in what may be termed natural surroundings. The equipment used for experiments with air currents and gravity was simple and largely improvised. Nevertheless, since it is not primarily my purpose to measure intensity of stimuli or rapidity of reaction, I believe the results obtained have some interest and value.

Reactions to a Current of Air.

There was a question in my mind as to whether the positive orientation of the Ephemeridæ to a breeze is a response to the breeze per se or whether other factors are concerned. In order to test this I took a piece of glass tubing several inches long and sent through it a weak but steady current of air so directed

[1] Contribution from the Department of Zoology and Entomology, Ohio State University, No. 43.

as to strike the insects on the side of the body. They were resting on boards placed horizontally. A few of them flew away but most of them eventually faced the current. Individuals placed on a rough surface, such as a wire screen, which afforded a better foothold frequently tried to walk away. When facing the current of air an individual would raise its long, slender front pair of legs and extend them forward and upward at an angle of about 40 degrees. When held in this way the legs resemble antennae and it is possible they have a sensory function. However, cutting them off had no apparent effect on the reactions here in question. The time required for the turning reaction varied from an almost instantaneous response to two minutes. In the majority of cases the response was gradual and occupied from 30 seconds to one minute. The rapidity of reaction depended upon a correlation between the strength of the breeze and the part of the body it struck.

The influence of the area stimulated is shown in experiments with the wings. The latter are large in proportion to the body and meet over the back in a perpendicular position. They, therefore, present quite a broad surface. When a current of air of an intensity sufficient to blow the wings slightly to one side was directed against them individuals would react in fifteen to thirty seconds, whereas when this current was directed against the thorax or the abdomen the response was slower, if indeed any occurred. A stronger current directed against any of these parts brought about a correspondingly more rapid reaction.

In another series of experiments a current of air was directed from the posterior lengthwise of the body along the dorsal surface of a number of individuals. The response in these circumstances was also an eventual facing about to the current. A current of air striking an individual longitudinally along the mid-dorsal surface is neutral so far as lateral directions are concerned. In the cases here in question the current blew the wings to one side or the other and then as before the insects turned around toward the side on which the strain was exerted.

The experiments were repeated on a group of individuals from which the wings had been removed. The results from a current of air striking the insects on the side of the body were the same

as before; the insects faced the current. However, when a current was directed from the rear longitudinally along the dorsal surface of the body the previous results were not repeated. In some cases the insects crawled with the current and away from the point of origin. In other cases they remained stationary and took an attitude similar to that assumed when facing the current. If the current became very strong they either attempted to crawl away or they retained the attitude until blown off their feet. When the current veered sufficiently to strike them on the side they began to turn toward it.

In these experiments with air currents the first noticeable response from the insects was an attempt to hold on to the surface upon which they were resting. This they did by fastening their claws firmly and even changing the position of the legs. When the current became so strong as to make it difficult to remain attached and especially when the body was blown over to one side the insects began to change position, rather hesitatingly it appeared, and to face about toward the direction from which the current came. When an insect reached a position where it did not seem to have difficulty in maintaining its hold it came to rest. This usually meant that it was directly facing the current, although sometimes it stopped at a point between a half and a complete about face. A half about face could generally be made complete by increasing the strength of the current.

When directly facing a current of air an individual is in the optimum position for resistance; it presents the least surface and the claws because of their backward curve have the maximum effect in holding the body. On the other hand when an individual stands sidewise to the current a greater surface is presented, the claws are not in a relatively favorable position and attachment is clearly more difficult. With regard to the more rapid reactions which result when the current strikes the wings it may be said that the proportionately great expanse of the wings above the body's center of gravity gives them such a leverage that the body is more easily tipped over, a strain is more quickly felt and attachment more quickly made difficult. In those cases in which a current struck wingless individuals from the posterior there was practically no obstruction to the current

and it consequently did not so easily cause strain or seriously disturb the attachment and there was therefore no turning reaction.

It would appear from the foregoing experiments that the Ephemeridæ do not change position under the stimulus of a breeze until a strain is exerted on the organs of attachment. That this does not merely mean that the response was delayed, until a breeze of a given intensity developed is shown by the fact that a comparatively weak breeze directed against the wings alone had the same effect as was caused by a somewhat stronger breeze against the thorax. There is, therefore, evidence, I believe, for concluding that Ephemeridæ do not orient positively to a breeze because of sensations derived from the breeze per se but that they react positively to tension exerted on the muscles of attachment.

Reactions to Gravity.

The position of Ephemeridæ when resting upon a perpendicular surface is negative with regard to the earth's surface and usually approximately vertical to it, although variations as great as 45 degrees occur. On comparatively smooth surfaces the orientation is more generally an approximation to the vertical, whereas on surfaces such as a wire screen, which affords a good foothold at any angle, variations from the vertical may occur in 50 per cent. of the individuals concerned. Individuals picked up by the wings and replaced head downward, if they are not so disturbed as to fly away, will struggle to gain a foothold. The position of the claws, which are adapted to a vertical position, make attachment rather difficult. This difficulty is increased by the fact that the long abdomen is thrown forward and downward and thus tends to destroy equilibrium. On comparatively smooth surfaces such as a planed board the insects rarely succeeded in maintaining their equilibrium long enough to gain a footing. On a wire screen they were more often successful and once they gained a footing and their equilibrium they retained the new position. The picking up process caused so many of the insects to fly away that other methods were tried. Several individuals were placed in a vertical position on a straw hat held perpendicularly and then the hat was slowly revolved until the

insects were upside down. The overhanging abdomen disturbed the equilibrium of some of them sufficiently to cause them to lose their hold and fly off. The others retained their footing, in some cases by changing the position of the legs, and remained in the inverted position for ten to fifteen minutes which was as long as they were watched.

In explanation of the position normally assumed on an upright surface the evidence derived from the experiments seems to indicate that the position taken is not a negative reaction to gravity per se but that it is largely, if not entirely, due to the character of the insect's means of attachment.

Results obtained from experiments performed to test the influence of a breeze upon the position of the insects on a perpendicular surface support this view. A current of air was directed against the side of individuals resting in the normal upright position on a perpendicular surface. As they turned the current was so directed as to bring them still further around. During the process some of them could not retain their foothold and flew off. The others turned completely around and faced directly downward. They maintained the inverted position at least as long as they were under observation, ten to fifteen minutes, which length of time, in view of a constant coming and going among those normally situated, seemed sufficient.

Reaction to Light.

The conclusions with regard to reactions of the Ephemeridæ to light are largely the result of observations made in the amusement resort already mentioned. The observations have to do mostly with artificial light. The insects react negatively to bright sunlight and seek the shade. They are strongly attracted to the lighter colors of artificial light. In the resort there are a great many electric lights of sixteen candle power intensity with colorless glass bulbs. Many of them are attached in a horizontal position to the sides of buildings in such a way that there is a perpendicular surface either above or below them and frequently on all sides.

The reaction to these lights seems to be satisfied if the insects can come to rest within a zone which begins approximately six inches from the light and covers a radius extending outward for

about twenty-four to thirty inches. When individuals enter this optimum zone they alight, if a surface is available, and orient themselves in such a way that the body is parallel with a radius projecting from the light. After alighting the insects usually remain at rest, although there may be a certain amount of crawling toward a position nearer the center. This is more often done by those nearer the outer limits of the zone. When the insects are numerous they become arranged in rows consisting of individuals either directly behind one another or slightly to one side and they thus form a striking pattern of radiating lines.

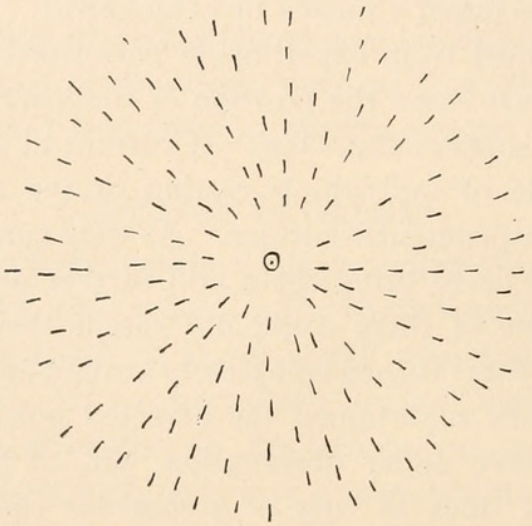

FIG. 1.

The accompanying figures illustrate the positions assumed with regard to lights in different positions and combinations.

The first figure illustrates the position assumed when the surface extends about a light in all directions whether the plane be horizontal or vertical. When any portion of the surface is absent the pattern is of course interrupted to a corresponding extent. The clear zone immediately surrounding the light was approximately six inches wide. I shall call it the excitement zone. Individuals that entered this zone became greatly excited and fluttered about the light in a confused state. There was no evidence to show that individuals at rest deliberately entered the excitement zone. Those immediately bordering on it were rather restless and occasionally in crawling about some were pushed into it and others on taking wing came within the influence of the light.

The second figure shows lights arranged along the lower edge of a perpendicular surface at intervals of twelve to fifteen inches. About each light was the usual excitement zone and upward from this extended the radiating lines of insects in the optimum zone. As shown in the diagram these lines were rarely at an angle of less than 35 degrees. This was due to the fact that below this point the lines from neighboring lights conflicted and caused such confusion among the insects as to obliterate regular alignment. The greatest confusion occurred in the comparatively short space

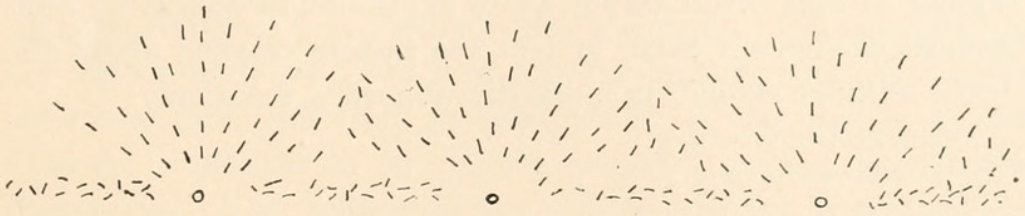

FIG. 2.

between the lights where insects attempting to arrange themselves about one light constantly came into conflict with others attracted to the neighboring light.

When the insects rested on a horizontal plane about a light they faced it. The most striking feature connected with the arrangement of the insects on a perpendicular surface was that the individuals on opposite sides of a horizontal plane passing through the center of a light had opposite ends of the body directed toward the light. The insects below the plane or parallel with it faced the light, whereas those that were above the plane were turned away from the light. In other words all the insects, except those parallel with the horizontal plane, approximated a vertical position with the anterior end uppermost. Those above the plane and with the posterior end directed toward the light were apparently as well content as those below the plane and facing the light.

The position of the insects on a horizontal surface shows that other things being equal they face the light. It is reasonable to conclude that their normal reaction to light is positive. The negative position assumed on a perpendicular surface above a light can be explained, in view of the air current and the inversion experiments, as being due to the difficulty experienced in maintaining a foothold in the inverted position.

Some observations were also made on the relative influence of white and colored lights. On the sides of one of the buildings in the resort there was a succession of alternating white, red and blue lights. The slightly yellowish white bulb attracted the insects in greatest numbers. There was the usual excitement zone and the regular alignment of those at rest. The number of insects about the red and the blue bulbs was decidedly small and as between the two lights about the same. These lights appeared to have a quieting effect on the insects. The alignment was similar to that described for white lights but there was no well-defined excitement zone, in fact the insects crawled about the bulbs without exhibiting markedly abnormal reactions.

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CELL MULTIPLICATION IN THE SUB-CUTICULA OF DILEPIS SCOLECINA.[1]

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Introduction.

The process of cell division in cestodes as compared with that in other Metazoa is apparently quite abnormal. An examination of cestode material at once reveals the fact that mitotic figures are very rare, and that an explanation of the process of cell division analogous to any of the common types is apparently impossible. The opinion of the various workers in cestode cytology, as to how cell division is taking place, varies greatly. Some state that it occurs by mitosis, others by amitosis, while it has been asserted that nuclei arise 'de novo' from the cytoplasm.

Child ('07) noted the apparent infrequency or total absence of any evidence of mitosis in *Moniezia*, even in regions where rapid growth was taking place. He says, "If my observations are correct, amitosis is the more common method of division in the generative cycle, except during the period of maturation and early cleavage. And in the somatic cells of the adult body it appears to be the usual method at all times."

Young ('08), working with *Cysticercus pisiformis* describes what he calls the "de novo" formation of cells. He observed irregular masses of coarsely granular cytoplasm lying in the meshes of the parenchyma network. These masses contain numerous small deep staining granules scattered haphazard through the mass. Shortly succeeding the formation of these granules, a nuclear membrane is formed around them; the newly formed nucleus, together with a small mass of cytoplasm, becomes partly constricted from the parent mass; and the daughter cell has been formed."

Further, he says: "I believe that the nucleus in these forms is not a morphological, but a physiological entity; that the

[1] A thesis presented to the graduate faculty of the University of North Dakota in partial fulfilment of the requirements for a master's degree.

nuclear granules are fundamentally the same as the remaining protoplasm of the cell, but are differentiated therefrom under physiological conditions which we do not at present understand; that the granules are perhaps reserve material stored up in the nucleus for future use, the entire cell body being thus occasionally converted into a nucleus; and the nucleus varies in structure from time to time in response to the varing physiological demands made upon it. . . . Further if my interpretation of my observations be correct, then distinction between germ and somatic plasm is obviously impossible, a special vehicle for the transference of hereditary qualities is entirely wanting; such qualities must be transmitted by the undifferentiated protoplasm; cell lineage is manifestly lacking; a mosaic theory is plainly untenable; and the fate of any given embryonic element—whether it shall form parenchyma, muscle, nerve, etc.—must be determined by physiological causes alone."

Richards (1911), working with *Moniezia*, does not agree with Child. He says (p. 158): "I have after diligent search upon carefully prepared material been unable to establish a series of stages in the autoconstriction and subsequent division of the nucleus and cell body by amitosis. Considering the evidence as set forth, it seems to the writer that one is forced to the conclusion that mitosis is the method by which pre-oögonia and cleavage divisions are accomplished."

Mary T. Harman ('13, p. 223) states: "My observations have not shown that amitosis does not take place in *Taenia* or *Moniezia*, but they have shown no condition which cannot be as readily explained as the result of mitotic as of amitotic division."

Materials and Procedure.

The form I worked with was *Dilepis scolecina* parasitic in the small intestine of the double-crested cormorant (*Phalocrocorax dilophus*). These birds are found abundantly near the shores and on the islands of Devils Lake, North Dakota.

Immediately after the bird was killed, the cestodes were removed from the intestine and placed in fixing solution. Flemming's solution and cestode mixture were the fixatives used. Flemming's solution blackened the tissue so that the results

from it were not satisfactory. The cestode mixture, however, gave excellent results.

The stains used were the following: Heidenhain's iron-alum-hæmatoxylin without counterstain; safranin counterstained with light green; thionin counterstained with acid fuchsin; methyl green counterstained with acid fuchsin; and safranin counterstained with water blue.

Observations.

I began my study of cell multiplication in cestodes without any previous knowledge of what had been done in the field of cestode cytology. Moreover, I completed the study of my material and drew my conclusions before I read any of the literature on the subject.

I have confined my study of cell multiplication in Dilepis to the sub-cuticula. In this tissue I have searched in vain for a single clear case of mitosis or amitosis. Moreover, in order to be certain I had not overlooked any, I counted 10,000 resting nuclei in the sub-cuticula of the neck regions of ten worms with the same result. Certainly active growth must have been taking place in this region, but it could not be accounted for by mitotic or amitotic division.

I have, however, observed numerous places in this region in which active cell multiplication was apparently taking place. Here multinucleate cells, such as shown in Fig. 1, have been observed. In addition to these, large protoplasmic masses were present, which varied in size from that of a single cell to that of perhaps fifty cells massed together. Fig. 2 shows a typical mass. These masses stain rather deeply with nuclear stains, and contain from one to five nuclei.

These masses are found abundantly in the neck region of every worm I examined, and occur, although less frequently, in the body region.

By reference to any of these figures it is seen at once that the mass of cytoplasm is out of proportion to the mass of the nuclei. Moreover, I have observed numerous lobes and occasionally even entire masses in which I was unable to find any trace of a distinct nucleus. Fig. 7 shows a lobe,[1] *i*, and Fig. 6 a mass of

[1] At focal levels other than that shown in the figure the lobe was seen to be continuous with nucleate masses.

protoplasm, *h*, in which no well-defined nucleus is present. However, in this latter case the mass is so close to a nucleate mass that I cannot say positively that it is not continuous with it.

By closely examining the nuclei present in these masses, I find that the nuclear membranes are very indistinct in many cases. Fig. 2 shows a mass in which the nuclei have indistinct membranes. Also one of the nuclei, *c*, has a somewhat less distinct membrane than the other, *b*. And this latter membrane is in turn less distinct than the membranes of the nuclei in the cell syncytium above it.

Moreover, a large number of nuclei have been seen which lack membranes completely. The nucleus consisted of a "nucleolus" or "karyosome" surrounded by a clear zone. Figs. 3, 4, and 5 show "karyosomes" which lack membranes. As Child and Young have already suggested, I believe this "nucleolus" represents the chromatin material of the nucleus.

By observing the protoplasm under high magnification (2,000 diameters) it is seen that the protoplasmic strands contain many dark staining granules of various sizes and shapes. Some of these granules were as large as the "nucleoli" of the complete nuclei; others, however, were so small as to be scarcely discernible. Fig. 4 shows a mass which contains a number of varying-sized granules. Fig. 5 shows a mass which contains a number of varying-sized granules one of which, *g*, is becoming surrounded by a clear zone.

The protoplasmic masses apparently arise by the outgrowth of protoplasm from certain cells of the syncytium. Figs. 2, 3, 4, and 6, show masses of protoplasm continuous with the syncytial cells around them. In Fig. 6, the developing mass is very small and contains no definite nucleus. In Figs. 2, 3, and 4, the masses are very large and contain from one to five complete nuclei. A large number of masses have been observed varying in size between these extremes. The nuclear membranes of the nuclei in the cells from which these masses are developing, contain very small, irregular granules which stain darkly like the granules in the cytoplasm. I have insufficient evidence for or against Young's view of the "de novo" origin of these granules. The chromatin granules may arise "de novo" in the cytoplasm and

develop to complete nuclei in situ. Young bases his theory of the independent origin of granules from a cytogenic protoplasmic mass upon the following facts:

1. The occurrence of masses of granular protoplasm lacking any evident nuclei.

2. The occurrence of isolated " nucleoli" of varying size from ¼ to 1 micron in diameter, which are usually found in the above mentioned masses of protoplasm but occasionally lie free in the parenchyma strands.

I believe, however, that these facts may be equally well accounted for by assuming the extrusion of chromidia from a mother nucleus. Masses of granular protoplasm without any evident nuclei, which occur but rarely may be explained as having been severed from parent masses after impregnation with chromidia. The occurrence of isolated "nucleoli" can be accounted for just as well by assuming the migration of chromidia from the nuclei along the strands of the cytoplasmic network, as by the assumption of their development from the protoplasm in situ.

Young, in a later paper ('13) dealing with gametogenesis, in *Tænia pisiformis* says (p. 375): "I believe that new nuclei arise either from chromidial extrusions from old nuclei, or 'de novo' in the cytoplasm. . . . The structure of the nucleus—a loose collection of chromatin bodies without a membrane—renders the extrusion of chromidia an easy matter. After their extrusion new chromatin is added and that part of the cell containing them is constricted off, to give rise in its turn to other cells. . . . It is obviously impossible to say, however, whether any chromatin granule in the cytoplasm is a chromidial extrusion or a 'de novo' formation."

Since I have seen these very small granules, all of about the same size, present in the nuclear membrane as though impeded by it in their exit, along the strands of the protoplasmic network, from the nucleus to the cytoplasm, I believe that these granules are extruded from the mother nucleus. Moreover, since I have observed granules of various shapes and sizes, many of the larger ones appearing to be composed of three or four smaller ones partly united, and since I have often seen a number of

granules clustered together, I believe that the larger granules are the result of the union of many smaller ones. Thus, I believe that the small particles of chromatin or "chromidia" are extruded from the mother nucleus. Then these "chromidia" unite here and there throughout the protoplasm to form larger granules or "karyosomes" which become surrounded by a clear zone. Finally the nuclear membrane is formed, producing a daughter nucleus. When a number of nuclei have been formed multinucleate cells are the result. Since the tissue is always a cell syncytium, constrictions of the cytoplasm around a nucleus finish the production of a daughter cell. Thus one mother cell may produce a large number of daughter cells.

Comparison with Tænia pisiformis.

In order to compare the process of cell multiplication in *Dilepis* with that in other cestodes, Dr. Young has permitted me to examine his slides of *Tænia pisiformis*, and *Cysticercus pisiformis*. Here I have identified the protoplasmic masses in both the adult and the larva. These also contain nuclei in the various stages of formation from chromidia to complete nuclei. The young larvæ show large numbers of protoplasmic masses developing in the cell syncytium. In the older larvæ the masses often show four or five nuclei developing membranes at the same time.

Discussion.

Cell multiplication by means of protoplasmic masses and the development of nuclei from chromidia, has, so far as I am aware, never been observed heretofore in Metazoa by anyone except Young. He has described the process as it occurs in *Cysticercus pisiformis* (Young, '08) and has noted it in some other cestodes (Young, '10) although his interpretation varies slightly from my own. I have, in the present paper given an account of it as it occurs in the sub-cuticula of *Dilepis scolecina*. It is true that chromidia have been observed in certain Metazoa, but no account of their functioning in the reproduction of the cell has ever been given previous to Young's paper on the "Histogenesis of *Cysticercus pisiformis*."

If cells are actually developing from protoplasmic masses in

the manner described, we have here an exceptional method of cell multiplication, unlike anything previously described in Metazoa.[1] Moreover, if future research supports this view, the present theories of the role of the nucleus in heredity will have to be greatly modified at least with respect to cestodes.

As Young has previously suggested, the explanation of such a method of cell multiplication as this may rest on the fact that the cestode is highly degenerate in most characteristics due to its long period of parasitism. In the development of cells from protoplasmic masses the nucleus passes through a cycle in which occur stages resembling nuclei of lower forms. The protoplasmic mass with its diffused nuclei in the form of chromidia is comparable to a cell of the Bacteria or of the Myxophyceæ. In certain Protozoa also, as noted by many observers, the nuclear material at certain periods diffuses throughout the cytoplasm in the form of chromidia which may give origin to secondary nuclei, and these in turn to gametes. It is possible that the cestode nucleus has lost the power of mitotic division, accompanying the somatic degeneration of the worm due to parasitism. Richards, Harman, and others have shown, however, that we still find cell division taking place by mitosis in the sex cells and developing embryos.

Conclusions.

I have made the following conclusions in regard to cell multiplication in the sub-cuticula of *Dilepis scolecina*.

1. After a careful examination, and after counting 10,000 of the nuclei in this region, I conclude that the growth of the sub-cuticula cannot be accounted for by mitotic or amitotic division.

2. Tissue growth is taking place rapidly in this region by the development of protoplasmic masses. My reasons for believing this are the following:

A. The nuclei in the multinucleate cells are frequently seen crowded together as if they had developed in protoplasmic masses.

B. In the protoplasmic masses the quantity of cytoplasm is out of proportion to the number of complete nuclei present.

C. Developing nuclei have been actually observed in the cytoplasm. The different stages of nuclear formation are shown by the following:

[1] A similar process was suggested long ago by Schleiden and Schwann.

(*a*) The chromidia, or diffused nucleus.

(*b*) The irregular chromatin granules formed by the union of numerous chromidia and surrounded by a clear zone.

(*c*) The nuclear membranes of the nuclei in the masses vary considerably from delicate, scarcely discernible membranes to heavy, well developed ones.

D. These masses appear to arise by the simultaneous growth of cytoplasm and chromidial extrusions from the nuclei of certain cells.

3. The degenerate character of the nucleus is perhaps the result of the parasitic habit of the cestode.

I wish here to express my sincere thanks to Dr. R. T. Young, whose valuable criticisms and suggestions made this work possible. I also wish to express my indebtedness to Dr. B. H. Ransom for identifying my material.

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Explanation of Plate.

Fig. 1. Multinucleate cell, *a*.

Fig. 2. Nuclei with indistinct membranes, *b* and *c*.

Fig. 3. Nuclei, *d* and *e*, lacking nuclear membranes.

Fig. 4. Chromatin granules, *f*, in the cytoplasm.

Fig. 5. Large chromatin granule, *g*, in cytoplasm.

Fig. 6. A developing protoplasmic mass, *h*, in which no definite nucleus is present.

Fig. 7. A lobe, *i*, of a protoplasmic mass in which no definite nucleus is present.

Fig. 8. A large protoplasmic mass in the body region which contains only one nucleus, *j*.

Fig. 9. Protoplasmic masses, *k*, developing in the body region.

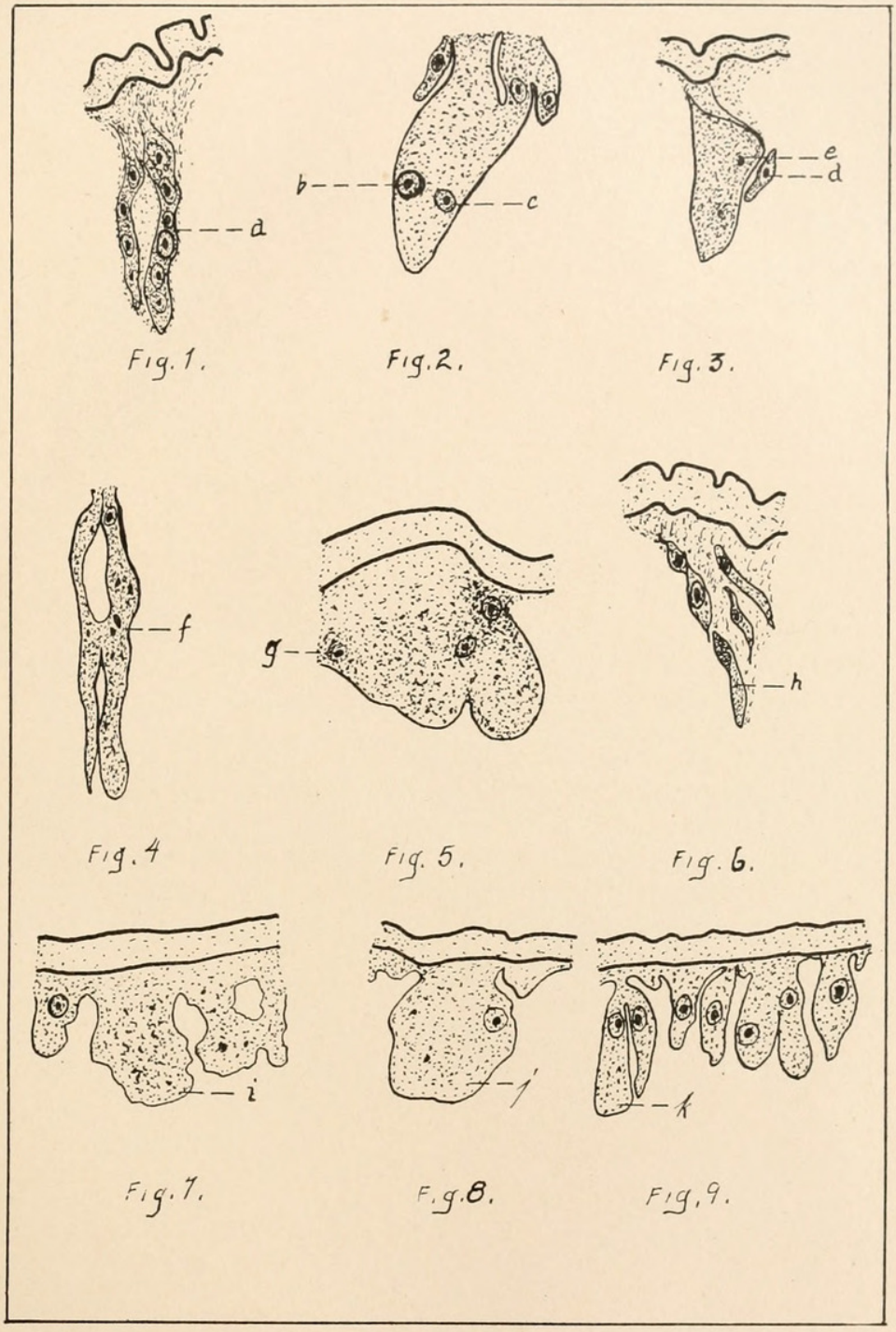

Fig. 1. Fig. 2. Fig. 3.

Fig. 4 Fig. 5. Fig. 6.

Fig. 7. Fig. 8. Fig. 9.