SIGNIFICANCE OF LARVAL MANTLE OF FRESH-WATER MUSSELS DURING PARASITISM, WITH NOTES ON A NEW MANTLE CONDITION EXHIBITED BY LAMPSILIS LUTEOLA.

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CONTENTS.

INTRODUCTION.

The developmental stages of fresh-water mussels while parasitic on fishes have received considerable attention from German workers, different species of Anodonta having been used as subjects for classical studies. These mussels have no commercial value, and there has been no artificial propagation in Germany that might have led to studies of other unrelated forms. In this country, however, we have the artificial propagation by the U. S. Bureau of Fisheries[1] of several species of mussels, particularly of *Lampsilis luteola*, which is of a different genus from those studied abroad and one in which, therefore, the developmental stages might be dissimilar to those described for Anodontas. In this connection the examination of larval mussels of this species has revealed a condition of the larval mantle cells considerably different from that reported by German writers.

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[1] The artificial propagation here mentioned has been carried on under the direction of the Fairport Biological Laboratory. The author is particularly indebted to H. W. Clark, scientific assistant, of the Fairport staff, for criticism in reviewing the manuscript and for suggestions during the course of the work.

The rôle played by the mantle cells during parasitism and the changes that occur during transformation are alike most interesting, and a continuance of these studies (which the writer intends to carry out) will probably result in the finding of still further adaptations that will make this an attractive and valuable field for study. These changes are correlated with embryological development as a whole; but since the development of organs of mesodermal or endodermal origin are very similar with different mussel species and differ only in the extent of development prior to and at the completion of the encystment stage, they are referred to only in so far as they have any relation to mantle cell conditions. Whenever citations are made in this paper to mussel literature they are so stated, with the author referred to; otherwise all statements herein are the result of the worker's observations.

MANTLE CELLS OF GLOCHIDIUM.

In order to understand the references that follow, it is necessary to have a slight knowledge of the histology of the glochidium, and this is briefly described from the standpoint of the mantle cells, since a more detailed treatment is not necessary in a study of this nature.

The mantle consists of two layers. Immediately underlying the internal surface of each valve of the shell is a very thin membrane, which represents the inner mantle (fig. 1). This lies between the valves and the remainder of the glochidial tissue, with the exception of the areas of attachment of the adductor muscle. It is sometimes difficult to distinguish in the glochidium, but less so during later development, when the cells increase in size and become somewhat conspicuous.

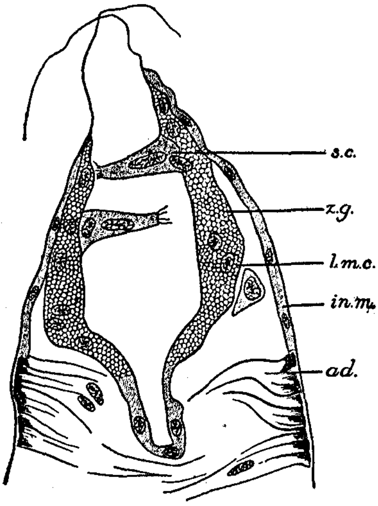

FIG. 1.—Section through adductor muscle of glochidium of *luteola*. *s.c.*, sensory cells; *l.m.c.*, large mantle cells; *in. m.*, inner mantle; *ad.*, adductor muscle; *z. g.*, zymogen granules.

Covering this layer, in turn, are the large cells giving rise to the outer mantle, which overlies the greater portion of the interior of the glochidium. This mantle is in the form of two folds—one for each valve—which are continuous with each other with the exception of a small area ventral to what Lefevre and Curtis have termed "the area of flexure." On each lateral portion of the mantle these cells are large, flat, and deeply granulated, with large nuclei (fig. 1). Ventral to the adductor muscle, however, the mantle cells are extremely flat, with smaller nuclei, and the granulations are absent (fig. 1).

On the posterior side of the adductor muscle is a group of crowded cells with large nuclei. Lateral extensions of this tissue, which is of mesodermal origin, give rise to the heart, pericardium, and kidney, and extensions backward, somewhat under the mantle (fig. 2, *l. p.*), represent lateral pits from which the gills originate. These lateral pits enter largely into the mantle cell transformation of the Anodontas. Of the median group of cells the upper or ectoderm cells give rise to the covering of the foot fold; the deeper cells are the endoderm. This area is the only portion not covered by the mantle cells. The groups of sensory cells, although of ectodermal derivation, consisting as they do of modified mantle cells, nevertheless atrophy soon after encystment takes place and have no further interest.

Thus, the mantle cells constitute the greater part of the cellular structure of the glochidium and, with the exception of the adductor muscle and the small group of cells of the endoderm, foot fold, and lateral mesoderm, they represent the main organogeny of the glochidium, so that the first encystment stage might well be called the mantle cell stage, since the mantle is the dominating structure during this period.

A characteristic feature of the large mantle cells, as has already been mentioned, is their heavy granulation. These granules, which give a firm, dense texture to the cells, are so large as to be conspicuous elements, and in their staining reactions they have the same relations as have the zymogen granules of the liver cells of the more developed larva. They are thus probably cells with a secretory function and may be capable of forming a digestive ferment that would make the larval mantle of the glochidium a digestive organ, constituting an anomalous condition in view of its ectodermal nature.

This granular feature is readily seen through the thin shell of the glochidium when viewed alive, and it is especially noticeable with such forms as Anodonta, where the mantle cells have a brownish tinge, which gives a dark brown coloration to the glochidium. This granulation is a temporary character, however, of the glochidium only, since it disappears soon after encystment takes place, as will be explained later.

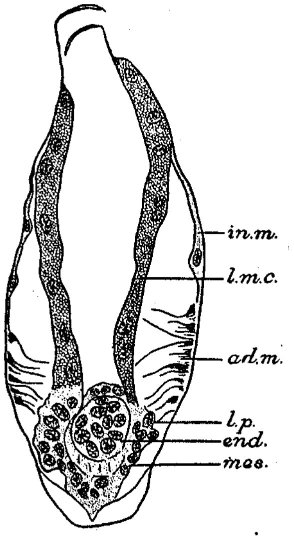

FIG. 2.—Section through glochidium of *luteola*, posterior to adductor, showing area not covered by large mantle cells. *l.m.c.*, large mantle cells; *l. p.*, lateral pits; *end.*, endoderm; *mes.*, mesoderm; *in. m.*, inner mantle.

PARASITISM OF SPECIES OF ANODONTA.

Since the hooked glochidia are fin parasites and the hookless gill forms, the conditions underlying their parasitism are not of the same fundamental nature. Faussek, Harms, and others have found certain anatomical and functional characteristics of the developing glochidia typical of European Anodontas. Herbers (1913), corroborating previous work on Anodonta, says that its developmental and anatomical distinctions are none the less applicable to certain Unio and Margaritana glochidia. Although the first accounts of parasitical conditions were very general, yet the subsequent studies have been all that could be desired, and at present we have a knowledge of the details of encystment stages of development that allows a full comparison in additional studies.

Since previous work has been confined chiefly to the hooked glochidium and since most of the deductions have been drawn from the studies on the development of these forms, a discussion of our present knowledge is in reality a discussion of the hooked type of glochidium and will be referred to as such.

The early encystment stage is one characterized by a marked transformation and subsequent degeneration in character, function, and appearance of the mantle cells.

By virtue of the large hooks the glochidia have little difficulty in securing attachment to the fin or body proper, inclosing in this way a considerable portion of host tissue, the fold extending into the mantle space consisting of epidermis, dermis, and connective tissue; and it is from this inclosed tissue that the newly attached larva gets its first nourishment. This tissue breaks down into its constituent elements by a combined process of disintegration and possible digestion, the latter by virtue of the supposed digestive action of the secretions of the mantle cells. These mantle cells as seen in preparations closely envelop the host tissue and would thus be enabled to pour the digestive juices directly upon the epidermis cells. Examinations of early stages of encystment of *Anodonta corpulenta* have shown that the so-called zymogen granules are lost shortly after encystment begins, which indicates that the secretory action would begin as soon as attachment is made and last but a short time.

Coincident with attachment, the process of cell disintergration of inclosed fish tissue takes place very rapidly. Apparently the secretions of the mantle cells are sufficient to start the breaking down of the intercellular elements, with the results that the mantle cavity soon contains a quantity of broken-down cells, epidermis, connective tissue, red blood cells, leucocytes, and cellular detritus. Even though there at times is a noticeable hyperplasia of the tissue at the base of the cyst during early formation, red blood cells being especially noticeable, it is doubtful if there is ever an appreciable migration of leucocytes between the valves and, whenever it does occur, it probably represents a pathological condition detrimental to the mussel. Faussek records a phagocytic reaction of the leucocytes against encysted mussels that is a specific cause for death, and the author has evidence of a similar mortality of *Lampsilis luteola* occasioned in the same way. However, the author is of the opinion that the larval mussel is normally resistant to leucocytes, and, when phagocytosis occurs, it represents a condition of susceptibility of the mussel occasioned in some unexplainable manner, possibly associated with mantle cell reactions, or perhaps in a larger sense with possible immunity reactions between host fish and its mussel parasite.

INGESTION OF FOOD.

The mantle cells in the meantime have lost their original character and appearance. The so-called zymogen granules, which were such conspicuous elements of the glochidial mantle cells in stained preparations, are no longer in evidence, and there is a probable transformation from a secretory to an ingestive function, the mantle cells becoming looser in texture, somewhat vacuolated, and characterized by a tendency toward pseudopodial formation by which the broken-down cell matter is ingested and taken to the interior (fig. 3), so that nutrition becomes an intracellular process. Stained material at this stage shows the mantle cells containing an abundance of disintegrated cells, the nuclei of which are readily distinguished from those of the mantle cells, since the nuclei of the latter, along with the transition to a nutritional character, have become greatly enlarged, the micronuclei also becoming large and distinct. Faussek speaks of these ingested cell elements while being consumed lying in special vacuoles, which at times take up the greater part of the cells, these vacuoles being similar to the food vacuoles of Protozoa. Although such a condition undoubtedly occurs, it is probably not as conspicuous as Faussek's words might infer, and the writer has not been able to determine this condition as at all prominent. Vacuoles have been seen inclosing food matter, but in other cases they were observed having no connection with ingested material, and they may represent merely a vacuolated condition not necessarily of a digestive nature.

It was found with *Anodonta corpulenta* that the disintegration of the inclosed tissue progressed slowly for at least two days at a temperature of 18° C. before the epithelial cells of the host tissue were completely utilized, and it required at least another day before the ingested cell matter in the mantle cells disappeared. During all this period the mantle cells became larger and the vacuolated condition more pronounced, so that it is hard to escape the conclusion that there is an actual increase in size as a result of the ingestion of the not inconsiderable amount of food, and this is supported by the fact that during this short period of three days the development of the larva progressed very slowly, the lateral pits became more pronounced, and the endoderm sac conspicuous; yet there was no growth of the remaining organs commensurate with the large amount of food ingested.

In the glochidium the cell boundaries of the larval mantle are indistinct, and as parasitism progresses a fusion of the cells takes place, so that the mantle at the end of the first three days appears only as an enlarged, clear, vacuolated mass, with the outer edge or surface very irregular, characterized by the large nuclei with conspicuous nucleoli, so different from the nuclear structures of the remainder of the organs of the developing larva.

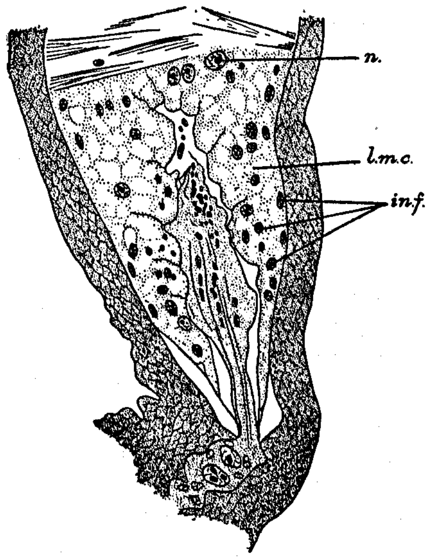

FIG. 3.—Section through encysted glochidium of *A. corpulenta*, one day after attachment, showing fusion of mantle cells, with pseudopodial processes noticeable. Ingested food material visible. *n.*, nucleus of larval mantle; *in.f.*, nuclei of ingested cellular detritus.

Up to this time the mussel has passed through what might be termed the first encystment stage. With the examples of *corpulenta* that have been studied this covered the first three days of parasitism. During this period the mantle underwent a remarkable histological metamorphosis, and nutrition by reason of these mantle cells occurred in an intracellular manner. This method of intracellular nourishment, as Faussek points out, is present with the embryos of mammals. The cells of the chorion villi and the chorion epithelium, especially, possess this phagocytic ability. With the ruminants they take in the elements of the uterine milk, with the carnivores the constitutents of the blood, and in all these cases the embryos have a like parasitical relation to the mother that the developing glochidium has to its fish host.

During this first encystment stage, as observed with *Anodonta corpulenta*, there has not been a very extensive development of the definitive organs. The lateral pits have increased in size and are beginning to show the ridges that give rise to the first pair of gill buds. The endoderm sac has developed lateral evaginations which represent the liver sacks. The foot fold has been given prominence, and the mantle cells, although still having an intracellular nutritional function, have fused into a solid layer with no discernible cell boundaries. The larval adductor muscle has remained unchanged.

FORMATION OF "MUSHROOM BODY."

Following this there is the condition which Herbers calls "the stage with the ring of mantle cells," which is conceded to be brought about by a mechanical process. The gill buds, which develop at this time from the lateral pits, cause the mantle cells to recede from the region of the body proper, so that they are confined more to the edges of the valves, which gives rise to Herbers designation. With the beginning of this stage, the rôle of the larval mantle as a nutritional organ is supposedly ended, the intracellular method of nutrition which it has been exhibiting disappearing as a result. Due to the formation of gill buds, the mantle cells are pushed away from the neighborhood of the foot fold, and coincident with this is the appearance from the edges of the valves of the cells of the definitive mantle, which ultimately supplants the larval mantle. The cells of the definitive mantle are short, cylindrical, and with smaller, more deeply stained nuclei than the larval mantle cells, and the conspicuous nucleoli are absent. They are thus readily distinguished from the larval mantle, for whose destruction they are held so largely responsible. Herbers also records for *Anodonta cellensis* the formation of definitive mantle cells from the body proper, so that the larval mantle folds of the two valves become separated and are confined to the central portion of each valve (figs. 4 and 9), where they project into the mantle cavity and give rise to what Braun, who first described it, called the "Pilzformiger Korper" or "mushroom body," naming it for its peculiar mushroom resemblance.

According to Faussek, Braun held this body to be a provisional nutritional organ of the larva, giving it the function of dissolving and absorbing the lime salts of the bony skin fold of the fin of the fish. Schmidt at a later date agreed with Braun, saying, quoting from Faussek, "die von Parasiten erfassten Teile des Flossenskelets stets ganzlich zerfallen und dass im Protoplasma der Zellen des 'Pilzformigen Korpers' verschiedener grosse Korperchen, die vollstandig des Zerfallsprodukten gleichen, nachweisbar sind." Faussek, as a result of his own work, concluded that both Braun and Schmidt had only partially solved the question and considered the mushroom body to be merely the atrophied remains of the larval mantle, and as the real nutritional organ he held the layer of definitive mantle cells arising principally at the edges of the valves, giving these cells the ability to absorb and transmit nutriment from the lymph fluids of the cyst cavity.

Herbers accords with Faussek in considering the mushroom body to be a functionless organ, representing merely the remains of the larval mantle. He differs somewhat, however, in not attributing a functionless condition until a later period than Faussek, who considers that the nutritional function is lost with the formation of the mushroom body. Herbers's view apparently seems the more rational. Undoubtedly the mushroom body plays a part in the destruction and absorption of the larval adductor muscle, for in preparations of *Anodonta corpulenta* it was not uncommon to find abundant remains of the disintegrated adductor fibers within the mushroom body, apparently being transformed into food. Without the intermediation of some such organ it would be difficult to understand how so large a group of tissue cells as make up the larval adductor could disintegrate without causing some fatal cytolytic response with the developing larva.

It is only on the complete destruction of the larval adductor, which occurs about the middle of the parasitic period and when an advanced development of the definitive mantle has taken place, completely encircling the transformed larval mantle, that the pronounced "mushroom stage" of the latter is reached. From each side the degenerated larval mantle, very much vacuolated and containing only a small amount of cell detritus, which probably represents the remains of the larval adductor, flows into and practically fills the mantle space, touching from each side and enveloping the foot. One might conclude that at about this time the functional character of the larval mantle is ended, and that henceforth the mushroom body is in reality purely an atrophied organ, serving no well-defined purpose, with the nutritional function being taken over by the definitive mantle. While during its early development the cells of the definitive mantle are formed in a single layer, yet during the close of the parasitic period there is a bunching of these cells at the edge of the valves and the formation of a noticeable mantle sinus, which would offer a ready explanation for transmission of nutriment from the mantle to the remainder of the definitive organs.

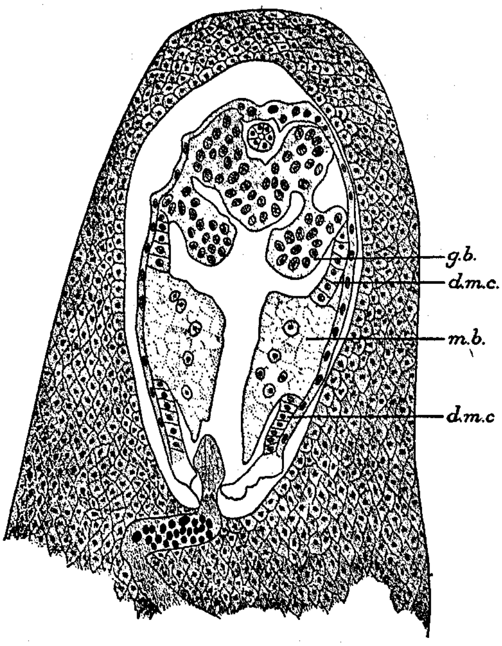

FIG. 4.—Mushroom stage of encysted *A. corpulenta*, showing progress of definitive mantle cells (*d. m. c.*) from edge of valve (below) and also from region of gill buds (above). *m. b.*, mushroom body; *g. b.*, gill bud.

Faussek, while attempting to explain the nutrition during the latter stages of parasitism, seemed inclined to the view that the enteric canal came forward as a nutritional organ, but was never able to convince himself of the validity of this interpretation.

Possibly some deductions might be drawn from the experiments of Churchill (1916) on the ability of fresh-water mussels to absorb nutriment from solution. Working on adults he came to a number of conclusions that are of interest in connection with the nutritional phases of the larva, since the correlations of tissues and functions must be very similar. With fats as emulsions, dissolved proteins, and starch in solution he came to the conclusion that the adult mussels are able to take up food directly from solution; that the outer epithelial cells of the body possess this ability to some extent, and in this connection he makes the following observation:

> As these animals are provided with a well-developed digestive apparatus, we may suppose that the absorbing power of these epithelial cells is a property that has been retained from a more primitive state in which the cell was less highly specialized, and that this property is not a special adaptation correlated with the lack of a functional digestive system.

Nutriment from solution could also be taken up by the alimentary canal, which, of course, is highly specialized for this purpose. Although uncertain as to the nature of the absorption of the foods by the epithelial cells he concluded that this may have been effected by phagocytic or amoeboid action of the cells or by solution in the plasma membrane and reprecipitation within the cells. Granules of albumen were carried entire to the interior of the cells, supposedly by phagamoeboid or phagocytic action. Possibly in referring to the "primitive" origin of the nutritional capacity of the epithelial cells, Churchill may have had reference to the encysted larvæ, and even though this is not the case, it is interesting to note how applicable his words are in connection with the nutrition of the developing larva, where all the epithelium is bathed in body fluids of the host and where an absorbing power by the cells is evident. This would seem to bear out the belief that during the latter stage of development of the larva the definitive mantle is a nutritional organ and also seems to give some reason for considering that the developed alimentary canal also may function in this manner to a limited extent.

COMPLETION OF DEFINITIVE MANTLE.

During the latter third of the parasitic period the mushroom body is pushed more to the center of the valve, due, apparently, to the development of the definitive mantle, so that the base of this body becomes smaller, with the overflowing appearance more pronounced. However, a gradual reduction in size of the mushroom body takes place, since its substance is absorbed and utilized as food, and finally toward the end of the parasitic period it disappears altogether, with the definitive mantle becoming complete at the same time. This marks the end of the regeneration process of tissue formation of the developing larva, in which the larval adductor and the larval mantle were so largely concerned. From now on development proceeds in a normal manner. Both anterior and posterior adductor muscles appear, the alimentary canal becomes a completely connected organ with large liver sacks, the lateral pairs of gill buds become conspicuous, with complete ciliation, the foot projects widely into the mantle cavity, a lymphatic system is developed, leucocytes make their appearance, with the heart and kidneys becoming noticeable, and the ganglia take on an extensive development, so that by the time the young mussel is liberated it has become a miniature of the adult, with practically all the adult organs in evidence.

With the larvæ of the Anodontas, as brought out by the foregoing discussion, development during parasitism is characterized by the following features, to which attention is directed.

There occurs, first, a complete destruction of host tissue inclosed within the valves, with the initiation of an intracellular nutrition by means of the pseudopodial ability of the transformed mantle, which very early tends to lose its cell distinctions and fuse together. Following this there occurs the appearance of the definitive mantle through whose development the mushroom stage of the larval mantle arises, which is considered to be a relatively functionless organ, with endosmotic nutrition apparently centered on the cells of the definitive mantle; and, finally, there is the complete absorption of the mushroom body, coincident with the completion of the definitive mantle, which marks the end of the parasitic period.

Of all the many unionids the development of the hooked larva of Anodonta alone has been studied closely, yet when one considers the great number of forms of naiads, the diversity in their structure, and the lack of complete uniformity in manner of metamorphosis from the glochidium to the juvenile stage—some species developing without parasitism—it is perhaps not surprising to find a digression from the Anodonta type in the larval development of *Lampsilis luteola.*

METAMORPHOSIS OF LAMPSILIS LUTEOLA.

In the course of experimental field propagation of this mussel during the summer of 1921 at Lake Pepin, Minn., larval mussels were collected from experimental pen bottoms shortly after they were shed from their host fish. The examination of the material thus collected brought to light the unexpected presence of primitive mantle cells. In addition these cells exhibited differences in structure and function from the mantle cell conditions of Anodonta. This, prompting a study of the earlier parasitic development, led to the discovery of the retention of the individual larval mantle cells without fusion until the end of parasitism, they having apparently a nutritional function throughout. This has been determined to be a normal condition by series of sections at different stages of development. The author has sectioned about 100 encysted individuals[2] of parasitic larvæ and about 50 of free larvæ after the completion of metamorphosis, so that the different stages of development have been determined with certainty.

The only similar studies having been on several European and a single American species of Anodonta, it was considered desirable to obtain further data on another American Anodonta, both for comparison with former observations and for the purpose of thoroughly familiarizing the author with the material published. With this in mind preparations of the larvæ of *Anodonta corpulenta* on *Lepomis humilis* were made and studied.

The sections of glochidia of *Lampsilis luteola* were used as type forms in the illustrations in view of the gross similarity in cell structure of both the hooked and hookless glochidia. The only differences that do occur are concerned with the presence of the larval thread in the former and a lack of similarity in sensory cell conditions; otherwise the embryonic structures are of the same order, although the

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[2] Part of this material was obtained from H. W. Clark.

degree of development is usually less with the hookless glochidium. The endoderm has the same prominence, as have the lateral pits of the mesoderm. The larval mantle cells have the same relation to the underlying tissues, with a break in continuity in the region just posterior to the adductor, leaving this area uncovered. One would expect, then, to find a similar development with such relatively close histological uniformity.

FIRST ENCYSTMENT STAGE OF LAMPSILIS LUTEOLA.

On implantation one observes the same coordination of functions as have been discussed with the hooked type. For a short time after attachment, for a period of a couple of hours at least, the supposed secretory function predominates and sections, as Figure 5, show the mantle cells still retaining their original appearance. They are remarkable for their compactness; they have a very firm, almost a hard texture in view of the excessive refractive granulation; the nuclei are small, the nucleoli are not prominent, and the individual mantle cells are flat, rather than cylindrical. Cell boundaries, as with the Anodontas, are very indistinct, so that the mantle appears to be a homogenous nucleated structure. Disintegration of the inclosed tissue is observable at this time, so that the mantle space contains a quantity of cellular débris, although there is no evidence of mantle cell transformation at this time. With the close of the supposed secretory function of the Anodonta glochidium there is initiated the series of degenerative changes that result in the formation of the "mushroom body." With the loss of granulation the cells of the mantle of *Lampsilis luteola*, instead of fusing, merely lose their granulation, and the cell boundaries, instead of becoming obscured, become more distinct. The free inner surfaces project into the mantle cavity as individual cell areas; there is the appearance of the pseudopodial character and the intracellular ingestion of the disintegrated host cells. Because of the retention of individualism the mantle cells project more into the mantle cavity and envelop the fold of fish tissue more closely than does the fused mantle of the Anodontas, which takes on somewhat of a blanket structure, with short pseudopodial processes, so that there can be no individual extension of whole cells as with *luteola*.

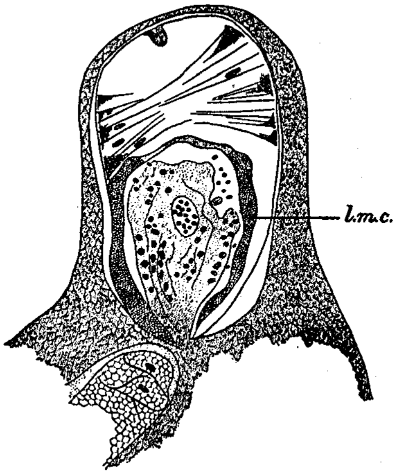

FIG. 5.—Encysted glochidium of *L. luteola* 1½ hours after attachment, showing retention of original granular nature of larval mantle cells. The pseudopodial nature has not become evident.

As ingestion of cell material is initiated the large mantle cells undergo a marked increase in size, and there is undoubtedly a storing up of food matter through the conversion of the ingested food cells. In the Anodontas the mantle early takes on a vacuolate structure, which becomes pronounced as degeneration progresses, and there is the rapid transformation in the character of the nuclei, which become distended, with the nucleoli conspicuous. Relative to intracellular nutrition of *luteola*, however, there is no indication of any vacuolated nature that in the Anodontas represents an excessive hypertrophy of the structure. This first increase in size probably represents the transformation from a secretory cell to a nonsecretory ingestive cell, characterized by a protoplasm of a more watery consistency. There is not the rapid change in the appearance of the nuclei, which retain their dark compactness into an advanced stage, so that they are not as readily distinguished from ingested cell nuclei as is true with the Anodontas. The nucleoli are only rarely prominent at this time.

These individual mantle cells, although separated at their free ends, are closely attached along their bases and undoubtedly have the same function as has the larval mantle of Anodonta, although with the latter the transmission of food is apparently of a simpler nature in view of its homogeneity. With the individual cells of *luteola* there must occur a transfusion from cell to cell, so that the matter of functioning presents this slight difference.

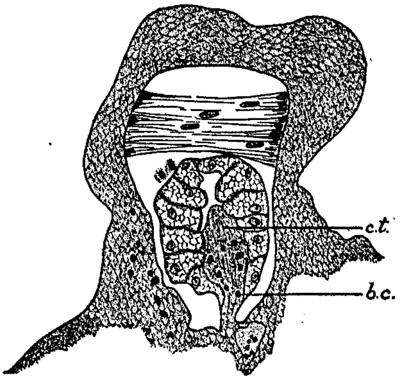

FIG. 6.—Encysted glochidium of *L. luteola* three days after encystment. The epithelium of the fold of host tissue has become completely disintegrated, leaving only the connective tissue (*c. t.*) in which are visible the red blood cells (*b. c.*). All the ingested food of the mantle cells has been utilized, no nuclei or cell detritus remaining.

At the end of the third day we have with *luteola* a stage represented by Figure 6, in which all cellular detritus has been utilized, the individual mantle cells standing out clear and distinct, which is a contrast at this stage with the agglutinated appearance of the Anodontas, in which the boundaries of the mantle are so poorly indicated.

There is a similarity at this time in both *corpulenta* and *luteola* in connection with the development of the lateral pits, which form inward involutions, later giving rise to the gill buds and having the effect of pushing the mantle cells from the region of the body proper. As a result of confining the mantle cells to a smaller area they become more elongate, which condition is retained from this time to the end of parasitism. At no time during subsequent stages is there any sign of degeneration.

ABSENCE OF "MUSHROOM BODY."

In the Anodontas the evidence of degeneration supposedly lies in the transformation of the mantle into the mushroom body, which marks the end of the intracellular nutritional function of the larval mantle, after which nutrition is taken care of by the definitive mantle. Thus, in the absence of any mantle transformation in *Lampsilis luteola*, there is this important difference, that the larval mantle functions as a nutritional organ during its whole existence, showing a more adaptive relationship than does that of the hooked glochidial type.

FORMATION OF DEFINITIVE MANTLE.

Herbers has emphasized the fact that the Anodontas are characterized by the formation of the definitive mantle from two directions, first from the edges of the valves and secondly from the body proper, as indicated by Figure 4, which shows the definitive mantle cells springing from the regions of the gill buds. This brought about the isolation of the larval mantle to the two concentric areas at the center of each valve.

With *Lampsilis luteola*, even with advanced cases, the author has never been able to find any trace of the appearance of the definitive mantle cells from other than the edges of the valves, with the result that the larval mantle cells as development progresses recede only from the edges of the valves, never from the body proper, to which they usually extend.

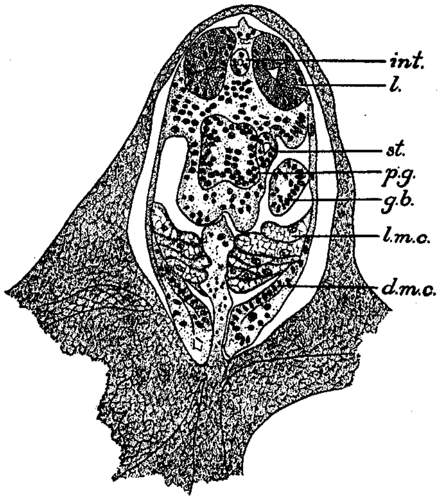

FIG. 7.—Encysted *L. luteola* shortly before end of parasitism, showing placental-like relationship existing between larval mantle cells and involution of host tissue. *int.*, intestine; *l.*, liver cells; *p. g.*, pedal ganglion; *st.*, statocyst; *g. b.*, gill bud; *l. m. c.*, larval mantle cells; *d. m. c.*, definitive mantle cells. The nuclei of red blood cells are pictured in the involution of host tissue.

It was often observed with *luteola* that there was a marked tendency for the larval mantle cells during the late stages of development to migrate toward the lower portions of the valves, such as is seen in Figure 7, which shows the bare areas on the valves just below the bases of the gill buds. This seemingly contradicts the reason usually advanced with Anodonta for the destruction of the larval mantle, which is said to be brought about in a mechanical way through the advancement of the definitive mantle cells from all directions. Cause, then, may have been mistaken for effect, and the advance of the definitive mantle may merely be the result of a contraction of the larval mantle, so that the formation of the mushroom body is a natural process.

MIDDLE PARASITIC STAGE.

In the Anodontas this period is occupied with the destruction and absorption of the larval adductor muscle, in which the larval mantle is concerned, and without the aid of which this change could possibly not be accomplished. The studies of *luteola* were made from collected material of various stages, but none were at hand showing the atrophy of the larval adductor, so, unfortunately, the part played by the larval mantle cells in this process was not observed. It is hoped, however, to cover this stage with further work when the several developmental stages of *luteola* will be taken up more in detail. It is presumed, however, that the disintegration and fusion of the muscle filaments occur as with *Anodonta corpulenta*, with ingestion by the individual cells of the larval mantle.

FINAL PERIOD OF PARASITISM.

During the last stage of development the foot of the larval mussel is largely developed and almost fills the mantle cavity. The definitive mantle has undergone considerable development and is readily recognizable, standing out very sharply from the rest of the tissue because of the uniformity of its short cylindrical cells. These definitive mantle cells take the form of a pyramid structure, giving rise to the internal mantle sinus, which is an important part of the lymphatic system.

It is difficult to know what interpretation to place on obvious nutritional organs. With the Anodontas it is assumed that the nutritional function is carried by the definitive mantle, since the larval mantle is being absorbed at this time. With *luteola*, of which the larval mantle cells retain a uniform condition and appearance throughout the entire period of parasitism, it seems only fair to assume that as they have a nutritional function during the beginning of the parasitic period they have the same rôle at the close of parasitism, their appearance remaining the same. With this the case, we have nutrition being taken care of by both the larval and the definitive mantle cells, and this at the present time seems to be the only interpretation that can be given.

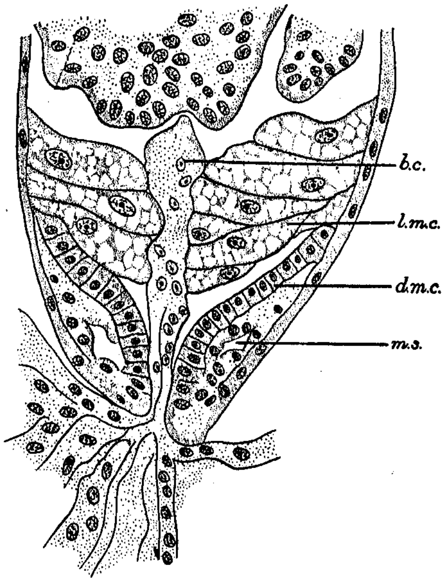

FIG. 8.—View similar to Fig. 2, but more enlarged. *m. s.*, mantle sinus. Other symbols same as in Figs. 6 and 7. The red blood cells in host tissue clearly shown.

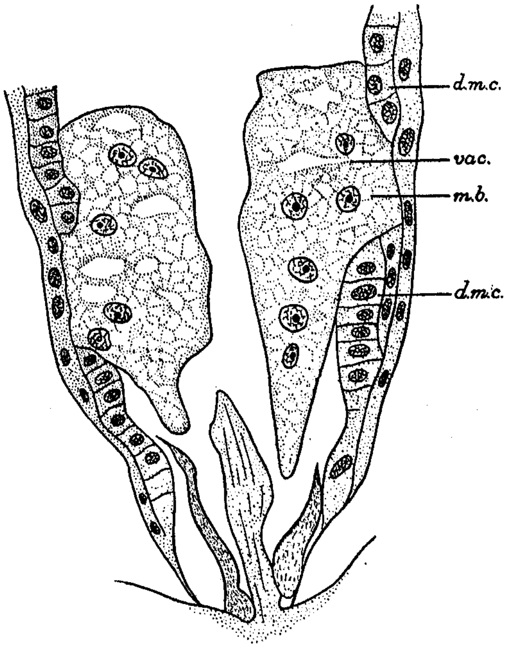

FIG. 9.—Enlarged view of mushroom body of *A. corpulenta*, with the vacuolate character in evidence. The high cylindrical cells of the definitive mantle are well shown.

One noticeable characteristic of the larval mantles of both *Anodonta corpulenta* and *Lampsilis luteola* is their almost negative staining reaction to eosine. Even heavy exposures to this stain during the end of the parasitic period fail to take, and the mushroom body of *corpulenta* and the individual mantle cells of *luteola* are always remarkable for their transparency in stained preparations. Churchill (1916) alludes in his work to the lack of staining capacity of fasting or starved cells, using this as a basis for determining the presence of epithelial food absorption. He found that starved cells took a very much lighter eosine stain than normal tissue. With the mushroom body of Anodonta there is a constant utilization and resorption, being a drain on its protoplasmic resources by the remainder of the organism, giving it its analogy to a fasting or starved cell, especially so during the latter stages of encystment, so that the lack of staining ability is readily understood. So, in this case, with the mushroom body, we have lack of staining associated with degenerative changes.

With Anodonta, regeneration of the mantle is complete by the end of parasitism, the mushroom body disappearing and the definitive mantle attaining its complete development, so that when liberation occurs the tissue transformation has been complete.

The development of the definitive mantle progresses more slowly in *luteola*, the larval mantle cells being in evidence at the time the mussel is shed by the fish. The final completion of the definitive mantle takes place very quickly, however, and many specimens collected two days after shedding showed the definitive mantle in an almost complete condition (fig. 11). Many of these individuals still possessed the larval mantle cells, which were interesting in that they were not extended, as during encystment (fig. 8), but were contracted, with the pseudopodial processes very evident. Whether or not there could have been any ingestion of food by these mantle cells by virtue of their pseudopodia is a matter of question. Granules were seen in many of these cells, but their nature could not be determined.

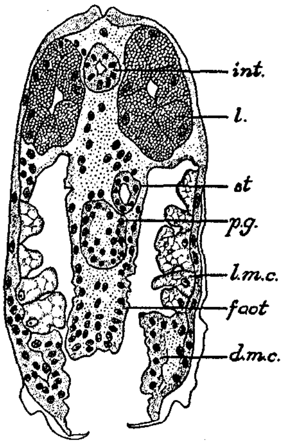

FIG. 10.—Developed free larval mussel of *L. luteola*, but with larval mantle cells still present. Symbols same as Fig. 7.

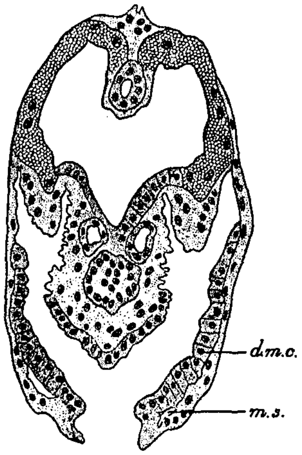

FIG. 11.—Developed free larval mussel of *L. luteola*, with larval mantle cells replaced by definitive mantle. *d. m. c.*, definitive mantle cells; *m. s.*, mantle sinus.

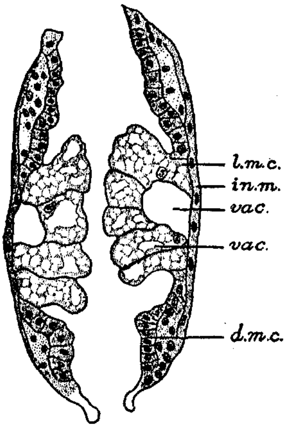

FIG. 12.—Transverse horizontal section through mantle cells of developed free larval mussels of *L. luteola*, showing nature of shedding of larval mantle cells. *d. m. c.*, definitive mantle cells; *l. m. c.*, larval mantle cells; *vac.*, vacuoles.

In view of the rapidity with which the completion of the definitive mantle takes place, it is only to be expected that whenever retention on the fish is prolonged the final transformation of the mantle takes place during parasitism instead of following it.

No indication has been seen so far of atrophy of these larval mantle cells as the means whereby their final disposal takes place. With free mussels the throwing off of these cells is shown in Figure 12. Here there is a very obvious disintegration, the cells becoming vacuolated and large spaces appearing underneath them, so that they apparently were thrown off in a mass. During encystment this might probably be replaced by resorption by the remainder of the larval tissues.

OCCURRENCE OF A PLACENTAL-LIKE RELATIONSHIP BETWEEN LAMPSILIS LUTEOLA AND HOST.

In Figures 7 and 8 there is pictured a very peculiar condition observed with encystments of *Lampsilis luteola* at nearly the close of the parasitic period. This illustrates very well the presence of a fold of host tissue within the valves, with the larval mantle cells grouped together near the tips of the valves, greatly extended and in contact with the host tissue. The involution also shows very well the red blood cells within its interior. With this doubtful arrangement there is a possible direct passage of the lymph from the involution to the mantle cells, which would establish a placental connection between mussel and host. To what extent the retention of the fish fold occurs has not been determined at the present time. In one lot of encysted mussels involutions were determined in 47 cases out of a possible 51, 4 being met with in which its occurrence was doubtful.

This condition has no precedent in previous conceptions, since it has been conceded that all inclosed tissue lying within the valves undergoes a complete disintegration. A placental-like relationship is made possible, however, in this case, by the nature of the larval mantle cells, which exhibit a pseudopodial capacity at this late stage. Reverting back to a comparison between the hooked and hookless glochidia it is seen that the structural differences modify the character of the attachment of the host, so that fin infections vary to some extent from gill infections. With the former the large hooks bring about an extensive laceration of the entire tissue within the glochidial valves, and the "bite" is usually hard enough to almost sever the involution from the rest of the tissue. Very little connective tissue as a rule is inclosed on account of the extreme thickness of the epidermis, and after the first two days of encystment all that remain are a few strands of connective tissue or cartilaginous or bony material.

The hookless form of glochidium as represented by *Lampsilis luteola* makes a clean perforation; there is no tearing of the tissue or any laceration, as is characteristic of the hooked form. As far as the author could determine the initial bite cuts down only through the layer of epidermal cells and does not extend through the connective-tissue dermis, although this is very much constricted at the junction of the valves. One can readily determine the relation of this connective tissue to the remainder of the gill filaments. This represents a noticeable variation exhibited by the hookless glochidium and undoubtedly is the result of more specialization, dealing, as we are, with a more degenerate form.

The disintegration and ingestion of inclosed tissue is concerned largely with the cells of the epidermis, the connective tissue not having been perforated, giving rise to the tendency that has already been mentioned for this portion of the host tissue to persist within the valves. Figure 6 shows this condition very clearly at the end of the third day of parasitism. No cellular structure can be made out, so that it consists of connective tissue alone, with the epidermis completely removed.

During the early encystment stage this condition is constant. There is a uniform retention of the connective tissue involution, and as the mantle envelops it very closely in view of the pseudopodial nature of these cells this at once establishes a placental-like relationship. There seems to be, however, a certain shrinking action on the part of the involution, and in the late stages of development the foot fills up the mantle cavity so largely that the involution is necessarily pushed outward, as we have in Figures 7 and 8.

SPECIALIZATION AS INDICATED BY MANTLE CELL CONDITIONS.

In their relation to fish hosts it has been recognized that some mussels exhibit a lesser degree of dependence, which is related to the extent of specialization these species possess. With such species as *Strophitus edentulus* and *Anodonta imbecillis* this has been carried to the point where metamorphosis may take place in the absence of parasitism. These two forms would, therefore, represent the lowest degree of adaptation to parasitic life—that is, the least degree of degeneration—and this is indicated most clearly by the character of the mantle cells, so that a study of the mantle cell transformation of these two species will very likely reveal adaptations associated with a lack of parasitism. In the Anodontas there are forms that are said to occupy the middle ground of limited dependence upon fish; they must live on fish, but require little from them (Coker et al., 1921). With the mantle cells we observe a functional degeneration during parasitism that apparently is the result of lessened dependence, the mantle cells ceasing to function at an early period. This evidently is characteristic of fin attachments, since with these forms there is less diffusion of lymph into the cystic cavity, and consequently there is less need for any specialized structures for nutritional purposes.

With the hookless glochidium, as typified by *Lampsilis luteola*, the closest relation exists in its connection with the fish host. Being a gill parasite, this attachment affords it a more direct connection with the lymph stream, so that lymph diffusion takes place freely. Its very evident dependence on the fish host is reflected in the retention of the larval mantle as a nutritional organ, which is evidence that the nutritive process is in active operation and is a dominant factor in its larval development. This represents the more degenerate condition exhibited during metamorphosis, since the first evidence of specialization leading to greater dependence takes place with the larval mantle cells. The value of mantle cell studies is thus emphasized as a necessary preliminary to any study of larval relationships.

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