THE NUTRITION OF GLOCHIDIA DURING METAMORPHOSIS

A MICROSCOPICAL STUDY OF THE SOURCES AND MANNER OF UTILIZATION OF NUTRITIVE SUBSTANCES[1]

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ONE PLATE (ELEVEN FIGURES)

AUTHOR'S ABSTRACT

Part of the nutriment of metamorphosing glochidia is supplied by the cellular host tissue, bitten by the larvae during attachment. Some of this is taken up piecemeal by the mantle cells and digested intracellularly. The coarse granules that first pack the mantle cells are apparently the precursors of a digestive secretion, some of which escapes into the mantle cavity. Here it also causes the *prompt* dissolution of additional utilizable host tissue.

Another source of nutriment is furnished by the provisional larval adductor muscle which undergoes degenerative changes in situ, then fragments, and finally is carried away bit by bit by amoeboid cells. These turn over their muscle content to the larval mantle, where the particles are further reduced beyond recognition. The mantle remnant itself is finally *sacrificed and doubtless becomes* an additional source of nutriment.

The gut serves as an organ of nutrition throughout the last two-thirds of the parasitic period. It appears to admit and digest pieces of the adductor muscle and certain unidentified particulate matter. In addition, the gut, like the definitive mantle and other organs, doubtless absorbs tissue transudate from the host.

Special vascularization of the host tissue to facilitate the passage of nutriment from host to parasite does not occur, yet there is no reason to doubt that an appreciable part of the larval nutrition results from transuding tissue juices.

CONTENTS

INTRODUCTORY

It is well known that the larvae of fresh-water mussels cannot metamorphose without passing a semiparasitic period,

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buried in the superficial tissues of appropriate fish hosts.[2] This relationship between host and parasite is quite specific and each mussel has a particular host (or hosts) on which its development is restricted.

When the glochidium attaches to a gill or fin it is a tiny bivalve about one-fourth of a millimeter in diameter; the shells are lined by a simple mantle, a provisional adductor muscle closes the valves, and a small, undifferentiated mass of cells comprises the building material for the future organs (fig. 1). At the end of parasitism the juvenile mussel has not increased in gross size,[3] but the internal transformation is marked. The larval mantle is replaced by a new one; the adductor undergoes destruction and two others arise de novo; in addition, there are representatives of the foot, gut, liver, heart, kidney, gills, and ganglia.

It is obvious that considerable nutriment is necessary to condition such growth. The purpose of the present paper is to inquire what the sources of the requisite food may be. Besides the academic interest in the food requirement and its supply, there is a practical bearing. The Bureau of Fisheries is attempting the rearing of mussels without parasitism; information of the sort here attempted forms a scientific basis for the intelligent conduct of such experimentation.

There are three morphological types of glochidium. The most numerous is the 'hookless' group, shaped like a boot heel or the bowl of a spoon; these are gill parasites. Next abundant are the triangular 'hooked' forms which attach to fins as well. Least common is the 'axe-head' group, gill parasites with a contour suggesting their appellation. These groups demand separate consideration.

The material studied was obtained mostly from artificial, controlled infections, samples of host tissue being removed as wanted and fixed immediately either in Zenker's or Bouin's

[2] In Anodonta imbecillis parasitism is facultative. Independent development has also been reported for Strophitus, but attempts to confirm this have failed repeatedly.

[3] Exceptions occur in the genus Proptera.

fluid. Paraffin sections, cut serially at 6 μ and stained with hematoxylin and eosin, completed the technical procedure.

OBSERVATIONS

A. Hookless glochidia

Lampilis luteola may be chosen as a representative of the hookless glochidia. Its history throughout the period of parasitism will answer in the main for the entire group. The detailed statements that follow refer to encysted series which completed parasitism in thirteen to fourteen days on a natural host, the large-mouth black bass (Micropterus salmoides); this is about the average period during warm summer weather. Cysts to the number of 132 have been studied in serial section.

An account of nutrition during metamorphosis centers largely around the provisional mantle which appears to exist chiefly for this purpose. There are two mantle layers, an outer one next the shell, and an inner, thicker layer bordering the mantle cavity (fig. 1, *i.m.*, *o.m.*). The outer mantle layer is thin and inconspicuous (figs. 8 and 10, *o.m.*). It is composed of highly squamous cells, with flattened nuclei; as metamorphosis progresses, these cells become somewhat thicker and more prominent. Since they have no demonstrable connection with the problem at hand, they may be thus summarily dismissed. The inner mantle cells are of low cuboidal shape, 5 to 9 μ high (figs. 1 and 8, *i.m.*). When the glochidium is attached to a gill filament, the pressure of the material bitten flattens these cells to 3 to 5 μ, and their nuclei are correspondingly compressed. The nuclei are normally rounded and chromatic, with a nucleolus which does not always show prominently. Cell boundaries are not easily distinguishable, due to the granular content of the cytoplasm. These acidophilic granules, which may be observed in the living glochidium, are spherical globules between 2.0 and 3.5 μ in diameter (compare fig. 3, *z.g.*). On account of their large size a few suffice to pack the cell; more will be said of them in another paragraph (p. 205). A delicate surface membrane

—unlike the striking cuticula in the hooked forms—caps the mantle cells and is continuous with the external cuticular covering of the shell (compare figs. 3 and 4, *m.c.*); thus a single cuticula bounds the glochidium inside and out (Arey, '24).

Fate of the host tissue. The amount of gill tissue bitten at the time of attachment is relatively great. Usually the glochidium's interior is filled from mantle to mantle (fig. 1, *h.t.*). In sections cut vertically through both valves, this mass measures to a maximum of 70 × 120 μ. It bulks about a quarter of the cubical contents of the entire glochidium. The material bitten consists usually of epithelium, much cellular connective tissue, and an internal core of blood vessels and fibrous connective tissue. Those larvae which attach to the gill lamellae tend to be stuffed with the compressed gill layers.

Five hours after a glochidium attaches there is little or no apparent change in the mantle cells, but the cellular peripheral tissue of the bitten gill filament is variously affected. Some specimens show practically no change; in others this epithelial and cellular connective tissue loosens, the cells losing their mutual connections and becoming separate; in still others there is evidence both of tissue disorganization and disintegration.

By nine hours the soft constituents of the bitten gill may be reduced to a formless mass of débris with a pulpy, necrotic look. In some cases the dissolution is but partial, and naked nuclei are seen (compare fig. 2, *a*). The mantle cells contain pyknotic nuclei and cellular fragments; hence phagocytosis has been operative (compare figs. 2, 3, and 4). As a result of ingestion these cells increase in height, and the tallest reach 15 μ. The sensory cells, having fulfilled their function when attachment occurs (Arey, '21), disappear early.

At the end of the first day phagocytosis is active. The granules of the mantle cells are plentiful and prominent, though somewhat diminished in number. Those remaining retain their original size, but stain less brilliantly than at first—a condition detectable as early as nine hours.

After two days the granules are practically gone (compare fig. 3, *z.g.*) and the nucleoli become prominent. The general cytoplasm is coarsely granular and contains miscellaneous ingested cell fragments and nuclei. At this time, and in some instances earlier, the chief cellular tissues of the bitten gill have entirely disappeared.[4] This leaves exposed the central core of fibrous tissue and blood vessels (fig. 11, *h.t.*). In the numerous cases where the glochidium attaches to an edge of the blade-like gill filament the core is ensheathed by a dense lamina of connective tissue (figs. 10 and 11).

At five days there is evidence of the retrogression of the tougher remnant of the enclosed gill (figs. 10 and 11). By the ninth day it is shriveled, and at eleven days it is a mere fibrous strand with a few pyknotic nuclei. During the last days of encystment the persisting host tissue becomes still less conspicuous.

How are these several facts, concerning which observations have also been recorded by Faussek ('95) and others, to be interpreted? The dissolution of the cellular inclusion at five hours and its disintegration by nine seem to imply an early enzymic digestion. The granules of the mantle cells exactly resemble those which disappear from vertebrate glands during extracellular secretion. Since, moreover, the gradual loss of these glochidial granules coincides precisely with the period of reduction of the bitten host tissue, it is only logical to infer that the mantle cells also have an external digestive secretion of which the granules are the visible presecretory index. Otherwise the bitten tissue should not disintegrate so promptly, for it is easily demonstrable that excised gill filaments not only live for hours in a watch-glass, but even complete cysts of extra size about attaching glochidia (Arey, '21). Hence the bitten gill tissues, protected by an enclosing gill cyst, break down entirely too readily for the process to be

[4] In a series of Lampsilis anodontoides, which completed metamorphosis on the gar-pike in six days, the bitten gill tissues were not digested until the end of the third day.

purely spontaneous. Furthermore, although phagocytosis is undoubtedly active, and cellular fragments in the cytoplasm are relatively abundant, yet the visible evidence of such ingestion is not sufficient to permit the belief that all the disappearing gill tissue is taken up piecemeal and disposed of by intracellular digestion. On the contrary, it is reasonable to conclude that part of the bitten gill tissue undergoes digestive liquefaction in situ,[5] whereupon it is absorbed; in addition, many nuclei, cellular fragments, and miscellaneous débris are first ingested and then digested by the mantle cells.

There is a suggestive correlation between the tardy onset of metamorphosis and the time consumed in receiving and digesting the bitten host tissue. Structural changes in the transforming glochidium first become noticeable after the second day of encystment; during this same period the mantle is occupied with reducing the cellular tissue of the host. Does the start of metamorphic development wait upon the conversion of this material and its reception as available food? Is the initial stimulus bound up in this phenomenon? Such questions can be raised, but not answered except by inference.

Mention has been made repeatedly of the blood vessels in the center of the bitten mass. These are invariably found in well-encysted specimens whether the attachment is to the gill lamellae (capillaries) or to the blade-like edges of the filament (larger vessels). It might be thought that the blood continues to circulate in such vessels, and hence the glochidium by diffusion is supplied with a constant source of nutriment throughout its parasitic period.[6] This possibility is particularly appealing, inasmuch as the host makes no attempt whatever to vascularize the tissues adjoining its guests (Arey, '32 a).[7] Yet such an hypothesis is clearly untenable, for it is not supported by fact. In the first place,

[5] Corroborative are the bare nuclei often encountered (compare fig. 2, *a*).

[6] Blystad ('23–'24) has recently advanced this theory. He holds for a placental-like relation between host and parasite.

[7] A few mussels, including the axe-head genus Proptera, not only metamorphose internally, but increase greatly in general body size while parasitic. I have

the adductor muscle brings the valves so nearly into apposition that the tissue included is constricted in a highly compressed fashion (fig. 11, *f*). The lumina of the vessels are thereby obliterated at this level, and the condition persists until the end of metamorphosis (Arey, '24). To state dogmatically that there can be no seepage of plasma through this isthmus is unwarranted; it conceivably may occur. But to dignify it by the term 'circulation' is to ignore the evidence of well-preserved specimens which have not been subjected to distortional violence. In any event, there is not enough passage of plasma to keep alive the host tissue beyond. The progressive involution of the fibrous core (after the digestive phase is finished) is eloquent testimony to the limited extent of such possible intravascular flow (figs. 10 and 11, *h.t.*). Still, there doubtless is fluid interchange between host and parasite, although the tissue juices which bathe the glochidium are more probably utilized for this purpose; (the gill glochidial parasite usually lies embedded in cellular connective tissue, roofed over by epithelium; Arey, '32 a). Conversely, during the extensive internal organ building of metamorphosis, as well as in the digestion of bitten host tissue and the sacrificed larval adductor, there arise katabolic wastes which demand removal; these undoubtedly follow the same diffuse path.

Involution of the larval adductor. A new and most remarkable process begins by the third or fourth day. At this time the mantle cells have lost their zymogen granules and the previously phagocytosed bits of gill tissue are digested. Now the cytoplasm again becomes coarsely and irregularly granular by the incorporation of many cellular fragments (figs. 8 and 11, *m.f.'*) It is easy to overlook the discontinuity of these two ingestive and digestive periods, and fall into the error of believing that they constitute one protracted process.

observed encysted P. laevissima which had increased their volume some forty times. This necessitates a considerable supply of nourishment from the host. In fact, the fleshy cyst is vascularized to a slight extent, and it may be further significant that these forms alone have their valves bowed so they meet only along their ventral edges (Arey, '32 a).

What is the source of these new inclusions? In brief, the larval adductor breaks down (figs. 5, 6, and 7) and is transported bit by bit by amoeboid cells (figs. 5 to 8, *t.c.*) to the larval mantle (figs. 9 and 10, *t.c.*) which receives the fragments (figs. 7 and 10, *m.f.'*) and reduces them to finer particles (figs. 8 and 11, *m.f.'*) that are utilized as food for the growing organism. With the perspective gained by this general statement, the detailed history of the adductor muscle and inner mantle may now be followed.

On the third or fourth day there are indications of the future decline of the larval adductor. Some of its muscle fibers begin to swell, become somewhat opaque, and stain less brilliantly (fig. 5, *a.m.'*). At the same time amoeboid cells appear among these fibers (figs. 5 to 7, *t.c.*). They have basophilic cytoplasm and vesicular nuclei; the cytoplasmic contour is irregular and often drawn out into processes. Passing alongside the swollen and fragmented muscle fibers, the wandering cells ingest irregular or rounded pieces of muscle (figs. 6 and 7, *m.f.*). Often a nest of such cells is seen in a restricted area which not always is at the periphery of the muscle as a whole (fig. 7, *t.c.*). The fragment taken in is frequently larger than the transportive cell itself (fig. 8, *m.f.*); the largest ingested piece observed in my series measured $12 \times 20\,\mu$. On the other hand, a cell may contain several fragments. In such instances of marked distention it is sometimes difficult to identify a continuous cytoplasmic rim (fig. 8, *t.c.*). At other times, large fragments are surrounded by the joint effort of several fused cells. These transportive cells then move ventrad between the two mantle layers (figs. 8, 10, and 11, *t.c.*) and come to lie just outside the base of the inner mantle and in close apposition with it. Some are found pressing and indenting the mantle cell layer (fig. 9, *t.c.*). The load is then delivered to the mantle cell, but the carrier is not incorporated as well; its nucleus and cytoplasm are distinctive and do not appear within. Furthermore, unladen cells of the same type are found near the mantle and these apparently are the former transportive elements, now empty.[8]

The grosser portions of muscle become reduced promptly to smaller bits (figs. 7, 8, and 11, *m.f.'*). It is possible that this may be begun in part by the amoeboid carriers, but, even so, it is certain that many transportive cells reach the mantle with large pieces of muscle still intact (figs. 8 and 9, *m.f.*); in transit, fragments as large as $12 \times 20\ \mu$ have been observed. There is some indication of a breaking down while the transportive cells lie just beneath the mantle layer. At any rate, the largest pieces of muscle seen in a mantle cell measured $10.5\ \mu$ (fig. 7, *m.f.'*), whereas a diameter of even $7\ \mu$ is exceptional. The average size is from 2.0 to $3.5\ \mu$.

Until the end of the eighth day, and in a few cases into the ninth day, this carriage continues. The dissolution and removal of the adductor are gradual. Portions remain unchanged until the very end of the transportive period, and, throughout the entire time, regressive and normal-appearing areas adjoin (figs. 6 and 7). Not until the eighth or ninth day do the first traces of the definitive adductor muscles appear; so it happens that the gradual, progressive removal of the larval adductor serves to provide adduction until the permanent muscles differentiate and replace it.

Attention may now be focused on the larval mantle and its content of muscle fragments. The cytoplasm, which loses its zymogen granules and early reduces the bitten gill to ordinary coarse granulations, again becomes very coarsely granular beginning with the third or fourth day (figs. 8 and 11, *m.f.'*). This is due to ingested muscle, the bits being of irregular size (2 to $3.5\ \mu$) and roughly resembling the zymogen globules now gone. However, they never stain so brilliantly; with eosin their color is a dull brownish-red and the tinctorial appearance of the mantle is flat. Incidentally, the coloration of these

[8] In a series of encysted *Lampsilis anodontoides*, which is parasitic on the gills of the gar-pike, and in some miscellaneous stages in the metamorphosis of Unio gibbosus, indications of the swelling and fragmentation of the larval adductor were likewise observed. Furthermore, in the Unio preparations there was not only evidence of muscle removal by amoeboid cells, but the reduced fragments were also demonstrable within the mantle cells. Thus, there is reason to believe that these processes are common for the entire hookless group.

particles and the degenerating adductor is identical and points further to their genetic relation.

By the fourth day the mantle cells are becoming tall and columnar, with rounded free ends and distinct cell boundaries (figs. 8 and 11, *i.m.*). Measurements show that this elongation is due to actual increase in bulk, and not to lateral crowding. At five days both the rounding of the free ends and the indenting at the cell junctions are pronounced; the tallest cells measure about 27 μ. Until the end of parasitism conditions remain much the same. The cells attain a maximum height of 30 μ and the nucleus lies below the middle level (compare figs. 8, 10, and 11). Toward the free surface the cytoplasm is densely packed and granular; it stains dully—a brownish-orange with eosin. The basal half of the cell is vacuolate, or clear, and is often crossed with reticulate strands (fig. 10). After the eighth day, pieces of muscle as distinct entities are usually no longer seen in the cytoplasm, although exceptions occur.

As metamorphosis proceeds, the larval mantle[9] is crowded toward the center of the valve by the developing permanent mantle which encroaches from below and on both sides (figs. 10 and 11, *d.m.*); it does not grow ventrad from the region of the gill buds, as in Anodonta. This reductional process is evident from the seventh day on. There is no mistaking the limits of the two mantles, for each is distinctive. The definitive one has shorter, columnar cells with the nuclei at the free ends, surrounded by dark basophilic cytoplasm; the basal two-thirds or more of the cell is clear and pale. In the early days of their appearance these cells are cuboidal, but the same characteristics dominate; throughout the parasitic period the cells are remarkably regular and block-like, and their boundaries are exceptionally distinct.

Toward the end of parasitism the larval mantle decreases in height and its cells then range from 9 to 18 μ. This diminution possibly may be due in part to pressure from the grow-

[9] This is homologous with the so-called mushroom body, found in the transforming hooked glochidium.

ing foot, but in addition there is doubtless some independent shortening. They also lose part of their distal granulation, and the cell walls again appear less distinct. Nevertheless, there is no general cell fusion before the metamorphosing mussel becomes free. Concerning the ultimate disapparance of the larval mantle I can make no statement except that it is retained for a period by the free-living juvenile.[10] This is an unexpected discovery, for in the hooked forms, to which attention has hitherto been confined, it is known to vanish toward the end of parasitism.

As the foot gains prominence, beginning with the seventh or eighth day, it comes in apposition with the larval mantle, and, with continued growth, presses against it. For the last third of the parasitic period the two are largely in contact. It might be suspected that the distally stored nutriment of the mantle is transferred in a soluble condition to the foot and thence becomes distributed to the growing organism. Although the foot is held to be absorptive in adults (Churchill, '16), this particular transfer in the larva cannot be detected microscopically by ordinary methods.

It is perhaps worth while to point out that the larval mantle, although ectodermal in origin, is involved in various nutritive functions which usually characterize the entodermal gut of animals. The somewhat similar adaptation of the ectodermal mammalian placenta is brought to mind.

Nutritive activities of the gut. The enteric canal becomes established as a tube at about the end of the first third of parasitism. What part, if any, does it take in the nutrition of the metamorphosing larva?

At the fifth day of my series the stomach contains material that stains precisely like the degenerating adductor muscle. It tends to be relatively bulky and to conform to the shape of the lumen, like a cast. In all the specimens examined this

[10] In Lampsilis anodontoides the mushroom-shaped mantle likewise is still prominent at the end of parasitism. In Proptera laevissima, which is exceptional in that it continues to grow on the gills for some time after metamorphosis, stages were observed which had increased forty-fold in volume yet retained a typical 'mushroom body.'

content had undergone partial digestive resolution, and accordingly had lost the distinctive character of muscle; this is also true of the disrupted adductor itself which is homogeneous in the same preparations. The presence of this eosinophilic mass antedates the formation of the coarse liver granules of similar stainability. It, therefore, is not a coagulum of liver secretion.[11] Rather than urge this interpretation it would be more logical to assume the reverse and make the stomach content the genetic antecedent of the liver granules, or at least of their eosinophilic component; yet I have no inclination to do so. From these findings, meager as they are, one can infer that a certain fraction of the provisional adductor is received into the developing alimentary tract and digested there.

Especially in the second half of the parasitic period, finely particulate material of uncertain origin is present in the enteric tube. It seems reasonable that tissue transudate from the host is taken up by the gut, but as to other specific elements there is no clue. The larval mantle, although atrophic, lasts beyond the period considered here. There is nothing in the stages examined to indicate its direct reception into the digestive canal.

Such facts seem to indicate that the gut is functional as a digestive organ during two-thirds of encystment. Its rôle may well be important, even though the visible evidence for this is slight and bespeaks rather a subordinate activity.

B. Hooked glochidia

If attention be now directed to the group of hooked glochidia, many comparable conditions are found. My observations are based chiefly upon a series of infections of Anodonta corpulenta, attached to the fins of the orange-spotted sunfish, Lepomis humilis, and upon other infections, both artificial and natural, of Hemilastena ambigua on the gills of its urodele host, Necturus maculatus. The Anodonta infection was carried out at a temperature of about 20°C.

[11] Toward the end of parasitism, however, large liver granules do find their way into the adjoining gut lumen.

The Hemilastena series were procured during colder weather, in the middle of October. These thermal differences must be borne in mind with respect to the time factor in order to reconcile the descriptions which follow with those of the preceding pages.

Fate of the host tissue. In Anodonta the bitten fin is locked firmly between the flexed valvular beaks and their stout spines (Arey, '24). This host material consists of epithelium several layers deep, and usually includes some of the firmer core of the fin, such as connective tissue, blood vessels, and fin rays. The amount is about half that taken by Lampsilis luteola. Similarly, Hemilastena bites on the stratified epithelium of the gill and, usually, on connective tissue and capillaries as well (fig. 2, *h.t.*).

In each, the mantle proper consists of large cuboidal cells, packed with acidophilic granules (figs. 2 and 3, *z.g.*); although coarse (1 μ), they are but one-third to one-half the diameter of the corresponding granules of Lampsilis luteola. At first the cell boundaries are indistinct, but sometimes they are clearly discernible; this masking is due to the zymogen granules. The nuclei are vesicular and contain a prominent nucleolus. The free surface, next the mantle cavity, bears a prominent cuticular membrane (figs. 3 and 4, *m.c.*); in Hemilastena it shows vertical markings, like canaliculi, which give the whole the appearance of a row of blocks. This membrane is much heavier than the delicate one, scarcely distinguishable, in Lampsilis luteola.

Shortly after encystment the zymogen granules begin to disappear from the mantle cells. In some specimens of Anodonta they had vanished completely by thirty-six hours; in others there was great depletion, but not total loss, at that time. Cell boundaries now show plainly; the cytoplasm is vacuolate and stains without brilliancy. In Hemilastena some mantle cells may lose all their granules while adjoining cells are intact (figs. 2 and 3); there is also a marked tendency for the distal granules to disappear before the basal ones do (fig. 2); the cytoplasm then appears slightly basophilic and the cell boundaries distinct.

While these changes have been taking place in the mantle, there is modification of the ingested mass as well. In Hemilastena the epithelium rounds up and separates from the connective tissue, still held by the hooks. The epithelial elements bitten by Anodonta begin to loosen and disintegrate soon after encystment. Apparently there is digestion in the mantle cavity, coincident with the loss of mantle-cell granulation; this is illustrated by the bare nucleus, *a*, in figure 2. At thirty-six hours, in the Anodonta series, all but the more resistant fibrous tissue or fin rays may be largely gone, and by the second day this dissolution of the softer elements is complete. The firm residue of tough tissue remains for the most part undigested and with only partial further reduction throughout the metamorphic period.

Besides the probable extracellular disintegration and digestion in the mantle cavity there is phagocytic ingestion by the mantle cells. Hemilastena demonstrates this clearly because of the huge size of the Necturus cells. In figure 2 a fortunate cut brings three stages of the process within the limits of a single section; at *a* is a nucleus, denuded of its cytoplasm, in contact with the mantle; at *b*, the cytoplasm of the mantle cell is protuberant and fused with that of a host cell. Stage *c* shows a cell completely ingested. As such cells are large they may equal or exceed the bulks of their captors; for example, cell *a*, figure 3, has a diameter of 13 μ. At *b* of the same figure is an ingested pigment cell. Similarly, in Anodonta, twenty-nine hours after encystment, the mantle contained much host material; these pieces measured to 9 μ in diameter.

It is important to inquire how ingestion occurs through such heavy cuticulae. Preparations were found in both Hemilastena and Anodonta which showed cells being taken in through breaks in the membrane (fig. 4, *a*). There was no appearance of artefact in these openings, and it must be the regular method of entrance, since the material obviously passes through in some manner. Just how the cuticula is opened would be highly instructive, but to this there is as yet no clue.

During the first two days of parasitism ingestion is steadily going on in Anodonta. The fragments and particles thus received are stored partly in vacuoles and partly in the compact cytoplasm. By thirty-six hours the vacuoles are abundant and may be huge, apparently by coalescence; they occupy much of the cell's base and contain one to several fragments. Smaller vacuoles are also present in the upper part of the cell. What is their significance? Are they like the food vacuoles in a protozoon, or are they caused by a liquefaction of the foreign tissue? In any event they are transitory—at least in their exaggerated expression; at seventy-two hours only small vacuoles persist, giving the cytoplasm an alveolar appearance.

During this period of ingestion the mantle cells increase in size and become taller as the result of food received and digested. The mantle cells of Anodonta put out elongate conical or finger-like processes which are often local and do not involve the whole surface of a cell. They differ from typical pseudopodia, inasmuch as a cuticula covers them. It does not seem probable that they result from crowding by the new mantle; their shape and distribution negative a mere mechanical distortion. These peculiar extensions are present while ingestion is going on.

The mushroom body. The incorporated host tissue is largely digested by the third day in Anodonta. By this time the so-called mushroom body has put in its appearance. It is nothing else than the larval mantle, forced toward the center of the valve both by the definitive mantle cells which creep centripetally from the periphery and by the gill buds and more of the mantle which likewise crowd ventrad from above. The larval mantle thus progressively becomes limited to the central regions of the valve and gradually assumes a pedunculated mushroom appearance. The component cells are tall ($15 \times 45\,\mu$ in vertical section), granular, vacuolate (especially at the base), and poorly acidophilic; their nuclei are vesicular, with a prominent nucleolus, and are located basally. At seventy-two hours the cells are still separate, though they

soon fuse. The free surface of the mushroom body extends in rounded cell tips, usually deeply notched at the cell junctions; this gives an irregular scalloped contour. Is the mushroom appearance caused by pressure from all sides? Probably in part—especially the constricted base—but the cells have also increased greatly in volume since encystment, as direct measurements show. The mushroom body is gradually reduced, and toward the finish of the parasitic stage it no longer is recognizable.

Involution of the larval adductor. The larval adductor is destroyed about the middle of parasitism. This is at the time when the mushroom formation is most pronounced. Although properly graded older stages were not available, indications in miscellaneous sections of other encysted hooked glochidia point to a later history similar to that already described for the hookless group. Furthermore, portions of the adductor are demonstrable within the mushroom body. It would thus seem that both mushroom body and adductor serve as food, and the former, although steadily regressing, is useful in the glochidial economy longer than has generally been supposed. During its decline it is still active in reducing the enclosed muscle fragments to more easily utilizable material.

While the involution of the mushroom body advances, the definitive mantle is growing correspondingly. On their first appearance its cells are short columnar to cuboidal in shape. The cytoplasm is basophilic and the nuclei stain so heavily as to mask the nucleoli.

SUMMARY AND CONCLUSIONS

The glochidia of fresh-water mussels undergo an internal metamorphic development while encysted upon appropriate aquatic vertebrate hosts. This growth and differentiation can proceed only when certain environmental requirements are fulfilled. Concerning some of these factors nothing is known, and of others there are mere intimations. Thus, it is sufficiently established that the parasitism is highly specific and demands definite hosts. Enough concerning the natural im-

munity of non-hosts and the acquired immunity of normal hosts has been discovered to show that delicate adjustments and relations exist (Arey, '23 b, '32 b); but as to the actual nature of the immunity, or even the factors involved, next to nothing is known. Again, it is apparent that a favorable proximity to the available oxygen supply is furnished by the superficial, ectoparasitic location of the larva. That the oxygen potential, even on gill filaments, is concerned with the inception or progress of metamorphosis seems reasonable, and even probable, yet such remains unproved.

On the other hand, a considerable amount of nutriment is surely necessary to condition the rather extensive development and differentiation that characterize metamorphosis. The sources of this raw nutritive material are several: The host tissue, bitten during attachment, supplies part; the larval adductor muscle is sacrificed as an additional supply, and the same is probably true of the atrophying mushroom body; direct transudation of the host's tissue juices doubtless accounts for the remainder.

The cellular host tissue enclosed between the valves of an encysted glochidium is removed in two ways. A portion is taken up by the cuticular-covered mantle cells and digested intracellularly. Further, there is sufficient reason to believe that the coarse granules that first pack the mantle cells are the precursors of a digestive secretion, which, at least in part, escapes into the mantle cavity; here it acts on much of the host tissue, causing its prompt dissolution and resolution into utilizable fluid nutriment.

Another source of nutriment is furnished by the provisional larval adductor muscle which undergoes degenerative changes in situ, then fragments, and finally is carried away bit by bit by amoeboid cells. These turn over their muscle content to the larval mantle, where the particles are further reduced beyond recognition.

The larval mantle increases greatly in thickness, but steadily diminishes in area, as the definitive mantle grows centripetally to replace it. In the hookless glochidia the larval

mantle, or 'mushroom body,' persists until after the end of parasitism; in the hooked forms it disappears toward the end of the parasitic period.

In general, the provisional mantle serves first as the bearer of sensory cells which are instrumental in securing attachment to a host (Arey, '21), and then for the dissolution and ingestion of the bitten host tissue. Next, it receives and reduces the degenerating adductor. Finally, the mantle remnant is sacrificed and doubtless serves as an additional source of nutriment.

Special vascularization of the host tissue to facilitate the passage of nutriment from host to parasite does not occur (Arey, '32 a), yet there is no reason to doubt that an appreciable part of the larval nutrition results from transuding tissue juices. This belief is strengthened by the apparent necessity for the removal of katabolic wastes along the same path but in the reverse direction. Since the internal transformation of a glochidium is relatively extensive, its metabolic slag surely does not remain at the site of origin.

The gut serves as an organ of nutrition throughout two-thirds of the parasitic period. It appears to admit and digest part of the adductor muscle and certain unidentified particulate matter. In addition, the gut, like the definitive mantle and other organs, doubtless absorbs tissue transudate from the host. What proportion of the total food is cared for by the gut cannot be determined at present.

These results supply information as to the food requirements of transforming mussels which should be considered in future attempts to rear commercial shells by culture methods in the absence of parasitism.[12]

[12] In the continuance of such experiments it may prove worth while to consider whether the epithelially embedded fin parasites, robust in structure and less intimately associated with the hosts' tissues, are not the most favorable type of experimental material.

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PLATE 1

EXPLANATION OF FIGURES

All the figures are photomicrographs of vertical sections cut dorsoventrally through both valves of the glochidia.

1 Lampsilis luteola completely encysted after an attachment of two and one-quarter hours on a gill filament of Micropterus salmoides. × 150.

2 Hemilastena ambigua encysted two and one-quarter days on the gill of Necturus maculatus. Three successive stages in the ingestion of red blood cells by the mantle are shown at *a*, *b*, and *c*. × 355.

3 Hemilastena encysted one day. The mantle has ingested a red blood cell (*a*) and pigment masses (*b*). × 710.

4 Hemilastena encysted one day. The mantle is receiving a blood cell of the host (*a*) through a gap in the prominent cuticula. × 710.

5 Lampsilis luteola encysted six days. The initial swelling of the larval adductor fibers is shown. × 355.

6 Lampsilis luteola encysted five days. Partial dissolution of the adductor and the arrival of transportive cells are illustrated. × 355.

7 Lampsilis luteola encysted four days. A nest of muscle fragments and transportive cells show. × 355.

8 Lampsilis luteola encysted four days. Transportive cells with muscle fragments are passing between the two mantle layers. × 700.

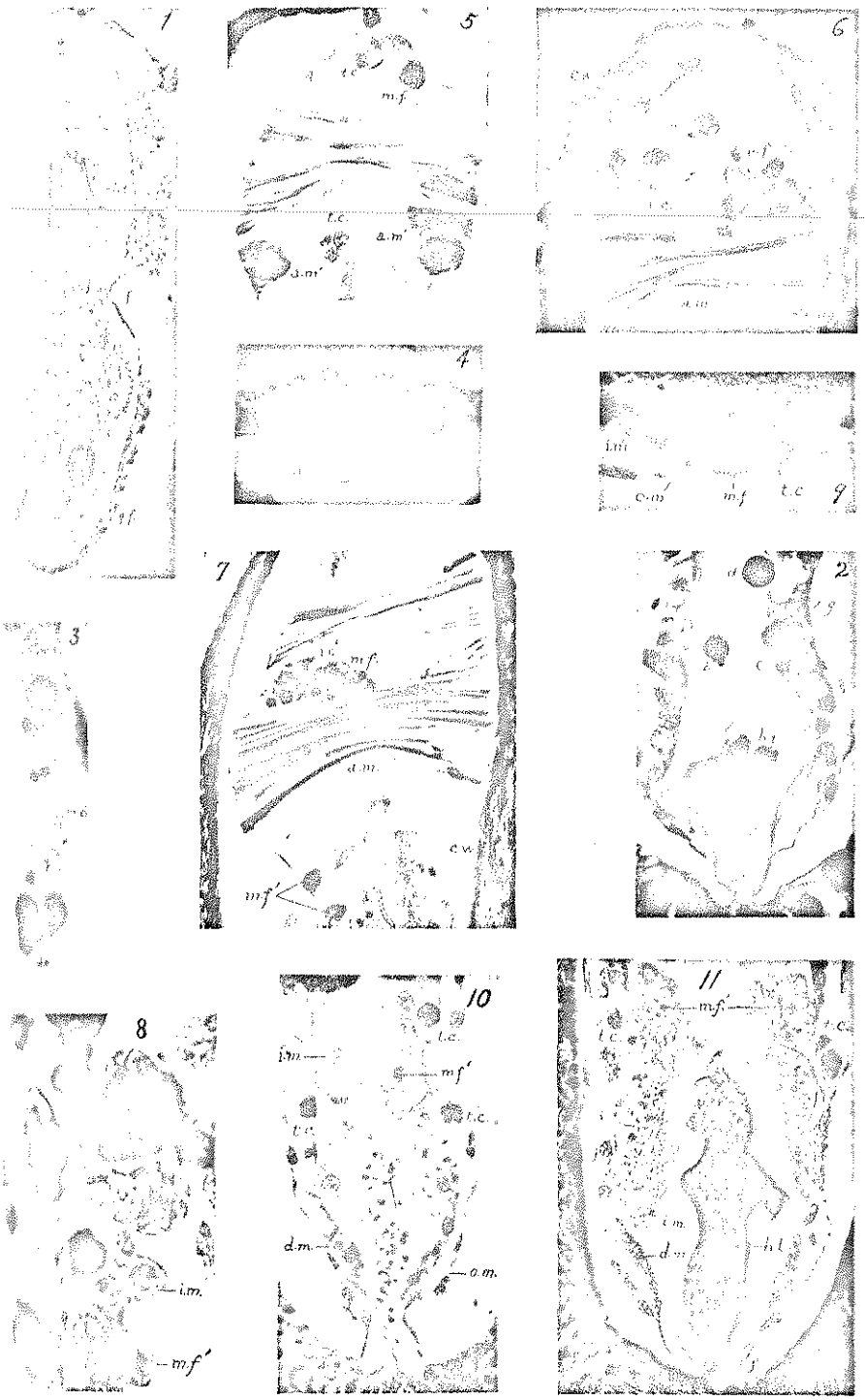
9 Lampsilis luteola encysted seven days. A transportive cell and its contained muscle fragment presses against the inner mantle layer. × 710.

10 Lampsilis luteola encysted seven days. The transportive cells and larval mantle show. × 355.

11 Lampsilis luteola encysted five days. The larval mantle contains many adductor fragments. × 355.

ABBREVIATIONS

a.m., adductor muscle
a.m.', swollen adductor fibers
c.w., cyst wall
d.m., definitive mantle
f. flange
h., hinge
g.f., gill filament
h.t., host tissue
i.m., inner mantle cells
m.c., mantle cuticula
m.f., muscle fragments
m.f.', muscle fragments in mantle
o.m., outer mantle cells
s., shell cuticula
t.c., transportive cells
z.g., zymogen granules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