

THE IMPLANTATION OF THE GLOCHIDIUM
ON THE FISH

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Three Plates

The present study, which has been made in connection with the investigations now being carried on at the University of Missouri for the U. S. Bureau of Fisheries on the artificial propagation of fresh-water mussels, was undertaken in order to determine the precise changes that take place in the tissues of the fish as a result of the parasitism of the glochidium.

The work has been done under the direction of Professors George Levevre and W. C. Curtis, and I wish to express my thanks for much help and advice that I have received from them.

THE PARASITISM OF THE GLOCHIDIUM

As has long been known, the larvæ of the *Unionidæ* do not complete their development unless they become parasitic on fishes. When they are discharged from the gills of the female mussel they fall to the bottom and remain there with the valves gaping widely open. Unless they succeed in reaching a host they soon die, but when once attachment to the fish has occurred, they quickly become enclosed in a cyst formed from the epidermis of the host, and there pass through the stages necessary for their transformation into young mussels. When the metamorphosis is completed, they free themselves from the cyst and after this time lead an independent existence.

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The investigators who first studied the glochidium during its parasitic life refer only incidentally to the relation of the larva to its host.

Leydig (11) in 1866 made the discovery that the glochidium lives for a time as a parasite on a fish.

Forel (6) in the following year succeeded in finding fishes with glochidia embedded on different parts of the body. He observed that the cyst in which the glochidium lives during the parasitic period is composed of epithelial cells and that after the larva attaches itself to the fish the cells of the epithelium around it begin to multiply and gradually enclose it. Forel estimated that the glochidium remains on the fish from three to four months.

Braun (1, 2) was the first to make an artificial infection by removing glochidia from the gills of the mussel and putting them with fishes in a vessel. He was able in this way to obtain material for studying the glochidium during the entire post-embryonic development. He found that the larval mantle cells grow out into the mantle cavity and form fungus-like bodies. Later investigators agree with him in believing that the function of these larval mantle cells is to take up, as food, the fish's tissues enclosed between the valves of the shell. In describing the formation of the cyst he says that by the irritation of a foreign body the cells of the fish's epidermis are caused to grow and rise up around the larva, completely surrounding it in three or four days. Braun also discovered that the temperature of the water affected the duration of the parasitic period.

Schierholz (14) made a number of infections in order to determine the length of time that the glochidium remains on the fish and found that it varied from fourteen days in July to five months during the winter. He states that the time required for the formation of the cyst varies with the size of the blood vessels which are ruptured by the larva when attachment occurs; the larger the vessels the more rapidly is the cyst completed. In some cases the glochidium is covered in two or three

hours, but usually about twenty-four hours are required and the cyst continues to grow thicker until the third or fourth day.

According to Schmidt (15) the epidermal cells of the fish begin to proliferate twenty-four hours after the infection and by the third day the complete cyst is formed.

Faussek (3, 4) devotes more attention than any previous observer to the changes brought about in the tissues of the fish as a result of the presence of the glochidium. He describes in detail the way in which the larval mantle cells act as an organ for taking up food. The formation of the cyst is regarded by him merely as a process of healing of the wound produced by the parasite. On studying the changes which take place in the cyst after its formation he observed the appearance of intercellular spaces arising in consequence of an accumulation of lymph.

Harms (7-10) more recently has made a detailed investigation of the post-embryonic development of three different species of mussels, and in connection with this work he records a number of observations on the general conditions under which parasitism occurs. Reference will be made further on to such of his results as bear upon my own observations.

MATERIALS AND METHODS

The glochidia of *Symphynota complanata*, *Lampsilis ligamentina*, *Lampsilis recta* and of *Unio complanatus* were used as material for the study of the relation of the parasite to the tissues of the host.

The first three belong to the group of mussels known as "winter-breeders" in which the glochidia are carried in the gills throughout the fall and winter and are normally discharged during the following spring and early summer. Glochidia are therefore easily obtained during the gravid period of the mussels, and if they are removed from the marsupium at any time during this period and fish infected with them, they will undergo the metamorphosis. This seems to indicate, as Harms (10) suggests, that the retention of the glochidia in the mar-

supium over winter is merely an adaptation for protection against cold.

Unio complanatus, on the other hand, is one of the so-called "summer-breeders," in which the embryonic development occurs in late spring and summer months and the glochidia are discharged as soon as they are fully formed.

There are two distinct types of glochidia, the hooked and the hookless. The glochidia of *Symphynota complanata* are of the former type, having at the apex of each valve a strong hook provided with numerous teeth on its outer surface. The glochidia of the other three forms studied, *Lampsilis ligamentina*, *L. recta*, and *Unio complanatus* are entirely hookless. As is usually the case with hooked glochidia, those of *Symphynota complanata* are much larger than the hookless ones and differ from them in shape. For example, the glochidia of the former measure 0.30 x 0.29 mm., while those of *Lampsilis ligamentina* and *L. recta* measure only 0.24 x 0.20 mm., and those of *Unio complanatus* 0.20 x 0.21 mm.

Hooked glochidia are best adapted for attachment to external parts of the fish, as the margin of the fins, fin-rays, operculum and mouth, but they are often found also on the gill-arches and rakers. Large numbers of glochidia of *Symphynota complanata* do quite often grasp the gills, but they are apt to tear entirely through the delicate filaments with their stout hooks, and even if they do succeed in becoming properly attached, their large size seems to make it somewhat difficult for them to become covered by the epithelium of the gill-filaments. If once completely embedded, they may remain in this condition until the end of the metamorphosis, but in many cases the wall of the cyst is apparently not strong enough to retain them on the filament, the cyst either rupturing and allowing the glochidium to escape or breaking off below the point of attachment of the glochidium which, in this event, falls to the bottom still surrounded by the epithelial cells. After such premature liberation from the fish, no further development occurs, and the glochidium soon dies.

Hookless glochidia are essentially gill-parasites. They cling to the tips and along the sides of the filaments in large numbers. They may attach to the fins, but their grasp in such situations is insecure, and they are usually brushed off before the completion of the metamorphosis.

The most successful infections were made on large-mouth black bass, *Micropterus salmoides*, on *Fundulus diaphanus*, and on the green and red-spotted sun-fishes, *Apomotis cyanellus* and *Lepomis humilis*, respectively. The crappie, *Pomoxis annularis*, was also used but it is difficult to keep these fishes alive in the laboratory until the completion of the parasitic period.

The ripe glochidia were carefully removed from the gills of the mussels and placed with the fishes to be infected in small aquaria or other vessels. A comparatively small number of glochidia were used in order to guard against the danger of over-infection which may readily occur if the glochidia present are too numerous, or the fish are exposed to the infection for too long a time. As Harms (9) says, the fish are seldom injured by infection of the fins, no matter how heavy it may be, but they are often killed by over-infection of the gills. However, if the glochidia are crowded together on the fins too closely, successful implantation is prevented and after a short time they drop off. An exposure of from five to thirty minutes was usually found necessary to insure an adequate infection. The glochidia were kept stirred up so as to distribute them as evenly as possible through the water. The gills of the fishes were examined with a hand-lens at frequent intervals during the exposure, and when the requisite number of glochidia had become attached, the fishes were quickly removed to a tank, into which running water was introduced.

According to Schierholz, the infection of the gills and fins takes place to about the same degree, but this is not true of my experience, since I have often observed that glochidia of both the hooked and hookless types will attach to the gills in large numbers, while at the same time the fins show only a very slight infection. If the glochidia develop normally, they remain

on gills and fins the same length of time, but, as before stated the hooked glochidia often fall off the gills before they complete the metamorphosis, while the hookless ones rarely ever remain on the fins more than a few days. For this reason, gill and fin infections must be studied separately.

Harms (9) found that large fishes became infected more heavily on the gills than on the fins, both by *Utaio* and *Anodonta* larvae, while small fishes were far more susceptible to fin-infection. This result is not in accordance with my own experiments, for I have found that infection of both gills and fins, by hooked and hookless glochidia, occurs more heavily in the case of large fishes than in that of smaller ones. Black bass become infected much more rapidly than the sun-fishes and crappie. In the case of the black bass, successful fin-infections have not been obtained with the glochidia of *Symphynota complanata*, although this fish is very susceptible to gill-infections. Occasionally, however, a few of these glochidia attach themselves to the fins, but they always drop off within a few hours. Both the bass and sun-fishes will bear a heavy gill-infection. Often more than one thousand glochidia become successfully implanted on the gills of a fish not more than four inches in length and complete their development there without injury to the host.

It has been generally held by previous observers that the duration of the parasitism varies inversely with the temperature of the water in which the development takes place. For example, Harms (8) found that the glochidia of *Anodonta* completed the metamorphosis in 80 days at a temperature of 8°-10° C, in 21 days at 16°-18° C, and in 12 days at 20° C, while in the case of *Utaio* the length of the parasitic period at a temperature of 16°-17° C was 26-28 days. He believes that the longer period required for the metamorphosis of *Utaio* is due to their less advanced stage of development at the time of the infection. Although my own experiments show a general correspondence with Harms' results for both the hooked and hookless types of glochidia, the relation between the duration

of parasitism and the temperature of the water has not been quite so definite. In *Symphynota complanata*, with a hooked glochidium, the time has varied from 9 to 15 days at temperatures varying from 16°-18° C, while in *Lampsilis ligamentina*, which has a hookless glochidium, the period has been somewhat longer at these temperatures, namely, 14 to 36 days. In the latter species, the rule that the parasitism is shorter, the warmer the water, is not uniformly applicable, for I have observed certain cases in which this relation did not hold. For example, I have recorded a period of 14-21 days at an average temperature of 16.4° C, while in other experiments at 19° or 20° C it has taken from 19 to 36 days for the development to be completed. In the latter case, although the temperature was three or four degrees higher, the period was considerably longer instead of being shorter as might have been expected.

In those infections which lasted from 9 to 36 days the young mussels were set free during a period of 4 or 5 days. This agrees very well with Harms' results, since he finds that the duration of the period of liberation is 5-6 days and that more mussels drop off during the middle days of this period than the first and last.

IMPLANTATION OF THE GLOCHIDIA

Sections of glochidia attached to the gills and fins, cut 5 μ thick, and whole mounts of glochidia on gill-filaments and on fins were studied. The living larvae, also, at various times after their attachment to the gills and fins were carefully examined.

At once upon attachment of the glochidium to the fish, the epithelium of the latter in the immediate neighborhood of the glochidium begins a rapid proliferation. Faussek (4) and Harms (8) believe that this proliferation of the epithelial cells is caused by the irritation produced by the wound, and that it is to be regarded merely as a healing process. At all events the glochidium exerts a stimulus upon the cells of the host which are thrown in consequence into active division. The cells divide

mitotically and in no case has any evidence of amitotic division been observed.

Cell division is most abundant near the level of the constriction caused by the glochidium, and the cells formed in this region of active proliferation push the ones above them up over the surface of the shell. The edges of this growing tissue ultimately fuse over the dorsal side of the glochidium, and the wall of the cyst is thus completed.

Implantation on Gills

Fig. 1 shows a cross-section through a glochidium which has been attached to a gill filament for fifteen minutes. The epithelial cells near the glochidium have already begun to proliferate. In this case, as in all the other infections I studied, the formation of the cyst began at once, and not, as Schmidt (15) observed, twenty-four hours after the infection. As stated before, the cells divide most actively just below the glochidium, and in this position a mitosis may be seen on each side in fig. 1. Dividing cells are rarely ever found in the upper part of the cyst during the early stages of its formation, but as the lower cells increase in number they push the ones above them up over the surface of the glochidium. Numerous red blood cells and leucocytes are often found around the glochidium before the cyst is fully formed (fig. 1). These come from vessels of the gill filament which have been torn by the glochidium in the process of attaching itself. Occasionally epithelial cells which have been sloughed off from the edge of the wound are found with these blood cells.

Fig. 2 is a cross-section through a glochidium thirty minutes after attachment. The numerous blood cells seen in the gill tissue just below the point where the glochidium is attached are due to an extensive hemorrhage caused by the tearing of vessels in this region.

The wall of the cyst continues to grow rapidly, and the progress made by the end of one hour may be seen in fig. 3. The glochidium usually bites entirely through the epithelium

with the result that the ventral border of the shell comes in contact with the underlying mesodermal tissue, which may, therefore, contribute to the formation of the lower part of the cyst and in which one often sees an indication of slight proliferation (fig. 3).

Fig. 4 represents a section through a glochidium which has been attached to the gill for three hours. The tissue growing up on either side is meeting above the glochidium.

After the complete implantation of the glochidium the cells composing the cyst continue to divide, and the region of proliferation is no longer limited to the lower part of the cyst, but mitoses may often be found higher up around the glochidium, as is shown in fig. 5, which is a section through a glochidium forty-four hours after attachment. The cyst has become thin and compact and the cells are closely pressed together, especially next to the glochidium where the nuclei of the cells flattened against the shell become elongated and oval in outline. Numerous mucus cells may be seen in the outer part of the cyst.

Fig. 6 is a section through a glochidium which has been attached to the gills of the fish for twelve weeks. The total period in this case was thirteen to sixteen weeks, so that the metamorphosis is at this time almost completed, and the tissue of the cyst has assumed the appearance which it retains until the liberation of the glochidium. The accumulation of lymph has brought about the formation of large intercellular spaces filled with this fluid. These spaces gradually increase in size and the epithelial cells become greatly elongated and connected by long protoplasmic processes. Many of the large spaces between the cells contain red blood cells and leucocytes. This vacuolated appearance is most marked just below the glochidium and in the outer part of the cyst, while the tissue next to the glochidium still remains compact and the cells lie closely pressed together. The gill-filament for some distance around the parasite is affected by this accumulation of lymph and presents much the same appearance. Even after the inter-

cellular spaces appear cell-division may take place and occasional mitoses are seen in the sections (fig. 6).

After this change takes place, the wall of the cyst is much weakened and pieces of the tissue composing it are often broken off, even before the glochidium has completed its development. Fig. 7 represents a section through a glochidium in which this has occurred, leaving the larva only partly enclosed. Sometimes the glochidium is in this way prematurely liberated. When the cyst wall is ruptured, the cells become swollen and the nuclei enlarged, while the intercellular spaces entirely disappear.

Fig. 8 is a section through a young mussel which is partly freed from the cyst. It is still attached to the tip of a gill-filament, but the cyst on one side has broken off and on the other the cells are degenerating.

The time required for the complete embedding of the parasite varies somewhat with the different types of glochidia. The smaller hookless ones usually become enclosed in two hours, but the tissue forming the cyst may grow more slowly and not form a complete covering for the glochidium until as much as six hours after attachment. In the case of the larger hooked glochidia of *Symphynota* the cyst on the fish is usually completed in from three to eight hours, but as has been mentioned before it seems to be somewhat difficult for these glochidia to become embedded on the slender gill-filaments and occasionally they may be found five days after attachment not more than half covered. When this occurs, the glochidium still clings to the gill and the tissue around it appears entirely normal.

A glochidium often attaches itself to two filaments lying close together and the epithelium of both filaments grows up around the parasite to form the cyst. This is especially apt to occur in the case of the large hooked glochidia of *Symphynota*.

The glochidium continues to grasp the gill tightly until it is completely embedded. During the first few days of the parasitism, after the cyst is fully formed, but while it is still loose,

the glochidium usually lies with the shell valves slightly gaping. During this time it often moves slightly by opening and closing the valves. It may even bite off the piece of gill-filament which it has seized and become completely turned around in the cyst. Even when this takes place the glochidia seem to develop normally, for they are often found in this position during the late stages of the parasitism.

Schierholz (14), Faussek (4), and Harms (7), all describe the cyst as being very thin at first, and gradually becoming thicker, but in the cases which I observed it reached its maximum thickness at once, and as the cells continued to divide became thinner and more dense during the first few days of the parasitism until it assumed the appearance shown in fig. 5.

The time at which the tissue of the cyst becomes vacuolated varies according to the length of the parasitic period. In those infections which last fourteen days the intercellular spaces begin to appear about the fourth day, while in those extending over a period of thirty days the tissue assumes this appearance on the eighth or ninth day. As stated before, after the formation of these numerous lymph-spaces the cyst is much looser and weaker, and many glochidia which have not yet completed the metamorphosis fall off during this time. Parts of the cyst may become broken off as shown in fig. 7, but more often the tissue breaks below the point at which the parasite is attached and the glochidium falls to the bottom still surrounded by the wall of the cyst. This happens most often in the case of the larger hooked glochidia of *Symphynota*, but the lighter glochidia of *Lampsetis* may become separated from the fish in the same way. Although the glochidia are usually alive at the time they leave the fish and may often be seen to move slightly, the development does not continue and they soon die. Occasionally a fully metamorphosed glochidium will fall off still covered by the tissue of the cyst. When this occurs, the foot may be seen to move around within the mantle cavity, but the glochidium seems never to be able to free itself from the surrounding tissue. The glochidia of *Lampsetis* are the most favorable for study at

this stage because they are much more transparent than those of *Symphrynota* and their structure can be observed more easily. Harms (9) describes a slight growth of the shell of *Unio* before the young mussel breaks through the cyst. I did not observe any external changes in any of the glochidia I studied.

Implantation on Fins

The proliferation of the epithelium of the fin around an attached glochidium is much less rapid than that of the gill. This is very evident from a comparison of figs. 1 and 9. Figs. 9 and 10 show cross-sections through glochidia which have been attached to a fin for one hour and the growth of the epithelial tissue is less advanced than that of the gill, shown in fig. 1, at the end of fifteen minutes. The blood-vessels of the gills are much larger than those of the fins and this probably accounts for the more rapid growth of the epithelium of the gill.

Sometimes the glochidium in attaching itself to the fin injures the cells around it by mechanical pressure, and they gradually become detached from the other cells and degenerate. The protoplasm appears hyaline and vacuolated and the nuclei contracted (fig. 10). Later, these cells are sloughed off. Immediately after attachment a few scattered blood cells which have escaped from torn vessels are usually found around the glochidium (fig. 9). In the intercellular spaces of the tissue forming the cyst and of the fin tissues lying within the mantle cavity are numerous leucocytes and red blood cells. They are easily distinguished from the nuclei of the epithelial cells because they are much smaller and darker.

As in the case of the gill tissue, when the proliferation of the cells to form the cyst first begins, mitoses are most often seen just below the glochidium (fig. 10).

The appearance of the tissue of the cyst at the end of three hours is shown in fig. 11. The shell hooks usually become turned in and pressed closely against the enclosed tissue of the fish, in this way making the grasp of the glochidium more

secure. Often, however, they become bent outward along the surface of the fin (fig. 12).

As Faussek (3) observed and described fully, the fish's tissue which is enclosed within the mantle cavity of the glochidium disintegrates and is taken up by the cells of the larval mantle and used as food during the early stages of the parasitic life. Fig. 12 shows a cross-section through a glochidium in which the mantle cells are sending out pseudopodial processes, and within these cells are numerous leucocytes and fragments of the fish's cells sometimes enclosed in large vacuoles. Harms (10), in describing the way in which these processes arise, states that as soon as the glochidium becomes attached to the fish all the larval tissues begin to grow actively and press on the mantle so that these cells are pushed out into the mantle cavity. Later, as the edges of the permanent mantle are formed along the borders of the shell, the large cells of the embryonic mantle become pressed together more closely and pushed further into the mantle cavity forming the fungus-like bodies first described by Braun (1).

The time required for the glochidium to become entirely covered on the fin varies from six to twenty-four hours. Figs. 12 and 13 both show cross-sections of glochidia which have been attached to the same fin for six hours. In fig. 12 the epithelium covers only about half of the glochidium, while in fig. 13 it has grown over the entire glochidium, completely embedding it.

The cysts formed on the fins and on the gill-arches are always much thicker than those on the gill-filaments. This is evident from a comparison of the drawings of cross-sections of glochidia which are entirely embedded on gills and fins, as figs. 5 and 14. The tissue of the cyst on the fin never assumes the compact dense appearance with flattened cells closely pressed against the shell, which is characteristic in cysts on the gills. This is evident from an examination of fig. 14, which is a cross-section of a glochidium which has been attached to the fin for forty-eight hours.

The changes which take place in the cyst after its complete formation are the same as in the case of the gills. The intercellular spaces appear at the same time, and when the metamorphosis is completed the glochidium ruptures the cyst and frees itself by movements of the foot and by slightly opening and closing the valves.

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EXPLANATION OF FIGURES

- All of the figures are from camera lucida drawings made with a 2 mm. oil-immersion objective.
- Figs. 1—8 are drawn from sections of glochidia of *Lampsilis* and *Unio* attached to gills of fishes.
- Figs. 9—14 are sections of glochidia of *Symphynota complanata* attached to fins of fishes.
1. Cross-section of glochidium of *Lampsilis ligamentina* 15 minutes after attachment, showing mitoses in epithelial tissue forming cyst.
 2. Cross-section of glochidium of *Lampsilis ligamentina* 30 minutes after attachment.
 3. Cross-section of glochidium of *Lampsilis ligamentina* 1 hour after attachment.
 4. Cross-section of glochidium of *Unio complanatus* 3 hours after attachment, showing the glochidium almost embedded.
 5. Cross-section of glochidium of *Unio complanatus* 44 hours after attachment, showing compact appearance of tissue of cyst at this stage.
 6. Cross-section of glochidium of *Lampsilis ligamentina* 84 days after attachment showing intercellular spaces in tissue of cyst.
 7. Cross-section of glochidium of *Unio complanatus* 11

days after attachment, showing premature liberation of glochidium.

8. Cross-section of glochidium of *Lampsilis recta* 114 days after attachment, showing the young mussel leaving the fish and the tissue of the cyst degenerating.

9. Cross-section of glochidium of *Symphynota complanata* 1 hour after attachment.

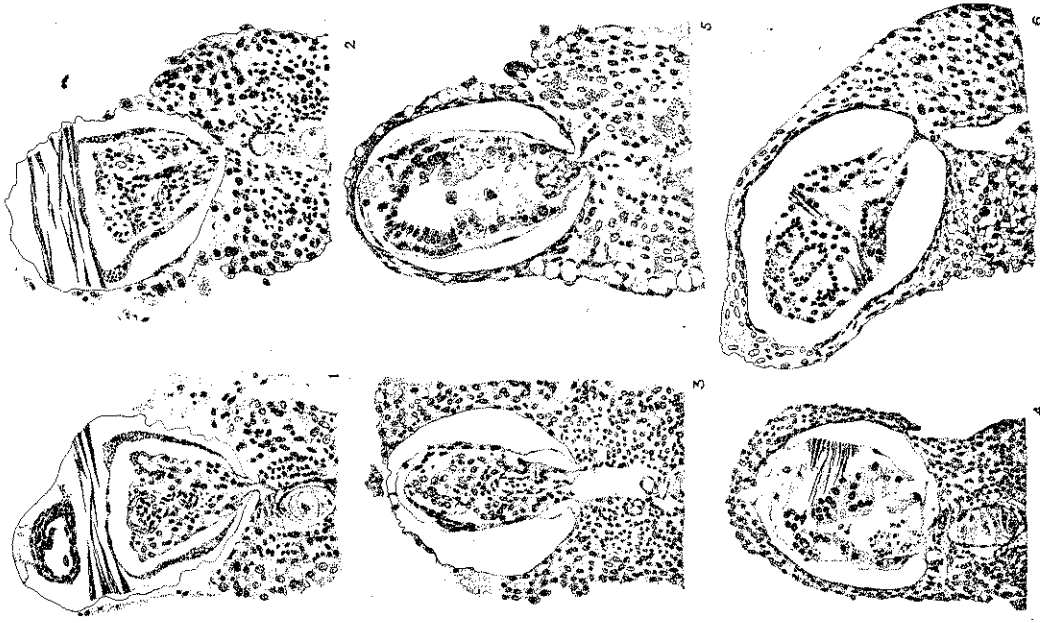
10. Cross-section of glochidium of *Symphynota complanata* 1 hour after attachment, showing mitoses and injured epithelial cells around glochidium.

11. Cross-section of glochidium of *Symphynota complanata* 3 hours after attachment.

12. Cross-section of glochidium of *Symphynota complanata* 6 hours after attachment, showing the cells of the larval mantle taking up the fish's cells enclosed between valves of glochidium.

13. Cross-section of glochidium of *Symphynota complanata* 6 hours after attachment, showing the glochidium completely embedded.

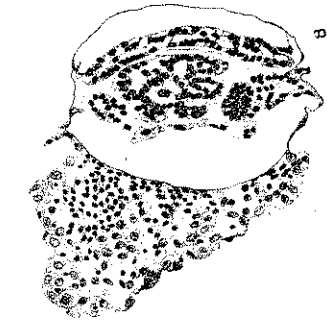
14. Cross-section of glochidium of *Symphynota complanata* 48 hours after attachment.



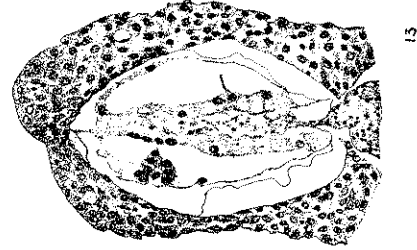
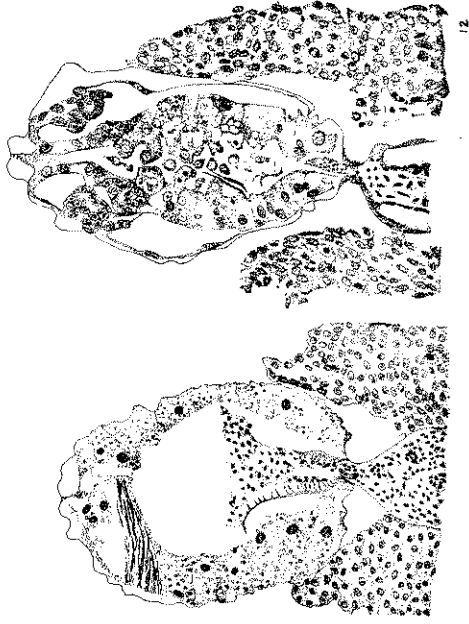
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PLATE 2

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