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Development and morphology of the glochidium larva of *Anodonta cygnea* (Mollusca: Bivalvia)

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(With 2 plates and 4 figures in the text)

The development of the parasitic glochidium larva of *Anodonta cygnea* L. is outlined, with reference to earlier studies on unionid larvae, and a comparison is made between the mature glochidia of the three genera, *Anodonta*, *Unio*, and *Margaritifera*.

The glochidium of *Anodonta cygnea* is anatomically and morphologically specialized in connection with its parasitic existence. The specialized structures are described, and their significance discussed.

Contents

Introduction

During a study on the physiology and behaviour of the mature glochidium larva of

Although the radiation of bivalves is extensive, only a few families have successfully invaded fresh water. During this process several changes have occurred in their life history. In most marine bivalves the eggs are fertilized externally, and the embryos develop as planktonic organisms. In contrast, almost all fresh-water bivalves incubate eggs and/or young. Among British species only *Dreissena polymorpha* Pall., which apparently colonized fresh-waters in the 19th century (Hass, 1929), has a free swimming veliger. The Sphaeridae retain the young in the inner demibranchs and release them as miniature adults; both trochophore and veliger stages being suppressed. The Unionidae also incubate their larvae in the demibranchs. These larvae, the glochidia, are, in most species, specialized for subsequent parasitic life.

Materials and methods

Glochidia were obtained as described elsewhere (Wood, 1974). Material for histological work was fixed in Bouin's alcoholic fluid and embedded in paraffin wax. Sections were cut at 5 to 7 μm and stained, usually with Heidenhain's haematoxylin and light green, or Mallory's triple stain (Humason, 1962).

The ^{14}C tracer experiment was carried out as follows. 0·25 ml of a 1 mc/ml aqueous solution of sodium bicarbonate was added to 100 ml of *Euglena* culture and left for 24 h at room temperature (20 C) to allow the algae to assimilate ^{14}C (as $^{14}CO_2$, presumably with the production of carbohydrates). A green sludge of *Euglena* was then obtained by centrifuging the culture. It was shaken in distilled water and re-centrifuged, then tested for its radioactivity by counting the number of disintegrations/second in a scintillator. 0·1 ml of sludge was introduced into each of 5 gravid adults (dry weight without shell approximately 4 g) which was equivalent to about 0·01 μc per animal. The sludge was introduced by "forcibly feeding" each mussel. The shell valves were forced slightly apart, a pipette containing sludge was introduced into the mouth of the animal and slowly emptied. Any algae which started to spill out of the mouth were moved in again on the ciliated tracts. Water was flushed through the mantle cavity for several minutes, to ensure that any algae which did not enter the mouth were washed away. The adults were then placed together in a tank through which fresh water constantly flowed. This prevented contamination of any part of the animals with faecal material. A specimen which had not been fed with radioactive algae was placed in the tank as a control.

Mussels were killed after 1, 2, 4, 8, and 11 days, and the control after 11 days, by removal from the shell and fixation in Bouin's alcoholic fluid. Each animal was later cut to a more manageable size to include digestive tissue, foot and demibranchs. These tissue slices were embedded in paraffin wax and sections of each were cut at 7 μm and transferred to clean glass slides prior to dipping in Ilford K5 Nuclear Research Emulsion. The slides were kept, after dipping, in dry light-proof containers for 7 days. The emulsion was then developed and fixed, and the slides thoroughly washed in distilled water. The sections were stained with nuclear fast red and light green, resulting in a contrasting background against which exposed silver bromide grains showed up clearly as black dots of metallic silver.

Fertilization

Histological examination of the foot of specimens collected in early summer showed most individuals to contain both eggs and sperm. The gametes were apparently mature and probably develop simultaneously in different regions of the same gonad.

In late summer eggs pass from the gonad into the interlamellar spaces. The sperm, however, pass from the gonad, through the exhalant siphon into the water, and are

subsequently drawn into the mantle cavity of nearby individuals. The percentage of eggs which are fertilized appears to be high. Soon after the adults had spawned, I examined the demibranchs of at least 80 gravid individuals, and rarely found non-developing eggs.

Development of the glochidium

Gravid specimens contained developing embryos during August and September, and apparently mature glochidia by the end of October at the latest. It is possible, however, that fertilization and glochidial maturation occur earlier or later in the year, according to

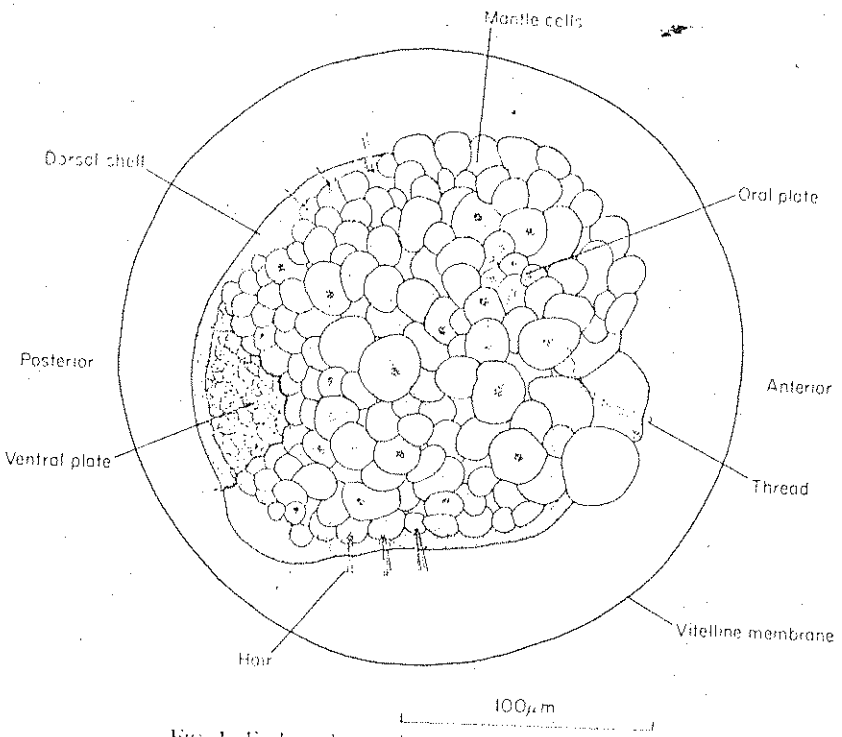

FIG. 1. Early embryo, viewed from the ventral side.

environmental conditions. The effect of temperature on development was shown by the following experiment. Twenty adults containing glochidia were collected in early October, and ten were transferred to tanks on the roof. The maximum temperature in these tanks was 19·0 C in October, 14·0 C in November, and 9·5 C in January. The other ten mussels were placed in tanks at 10 C. A few glochidia were removed from each adult weekly from October to January, and their developmental stage recorded. Glochidia on the roof developed normally, whereas those held at 10 C were apparently still in the same developmental stage in January as in early October.

In the following description of the embryo of *Anodonta cygnea* I use terminology taken from a paper by Lillie (1895) in which he described the early development in un-named *Anodonta* and *Unio* species.

The early embryo

Various regions can be identified in a September embryo (Fig. 1). Dorsally is an ectodermal region where large cells constituting the shell gland are secreting a cap-like shell. Ventrally, other ectodermal cells form the larval mantle, the oral plate (which will eventually give rise to the oesophagus), and the ciliated ventral plate (which will form the foot). The ventral plate is situated on the posterior lip of the blastopore and mantle cells are adjacent to the anterior lip. Ectodermal cells are also associated with and form the thread gland. There are six thread gland cells, situated just beneath the anterior end of the

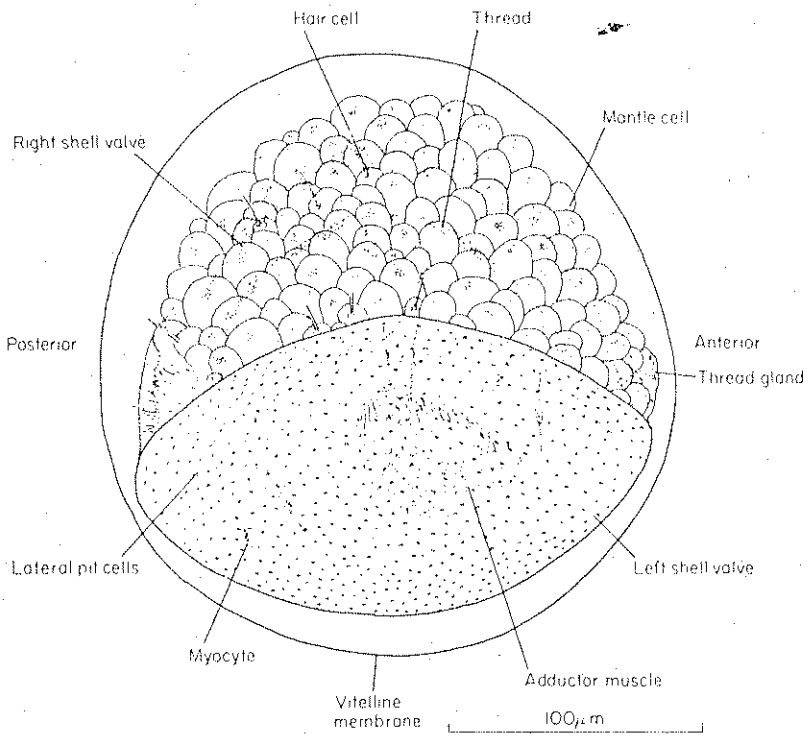

FIG. 2. Developing glochidium, viewed from the left side.

shell gland, and according to Lillie (1895) one gives rise to the thread itself and is surrounded by the remaining five. The hairs, which have developed from specialized ectodermal cells, can also be seen at this stage.

The larval adductor muscle, lying anteriorally and dorsal to the mantle cells, is formed together with the myocytes from mesoblast cells. There are several pairs of myocytes which, Schierholz (1888) suggests, play a mechanical part in development by contracting slowly and steadily to produce invaginations. Other mesoblast cells stretch from the outer walls of the embryo to the endodermal sac. These later form the pericardium, nephridia, and possibly other structures. They are not contractile.

The endodermal cells are situated posteriorally, dorsal to the ventral plate. Lillie observed that "the entoderm generally assumes the form of a sac, communicating by the blastopore with the exterior, but at other times no lumen is discernable".

Shell development and associated changes

A bivalve shell gradually develops from the original thin, cap-like shell and, as it does so, the mantle becomes bifid, and moves dorsally to line the inner surfaces of the valves. As the mantle cells move in they carry with them the ventral and oral plates which thus meet, unite, and come to occupy the whole of the posterior region on either side of the hinge (Fig. 2). The anterior part of the ventral plate is the basis for the formation of the

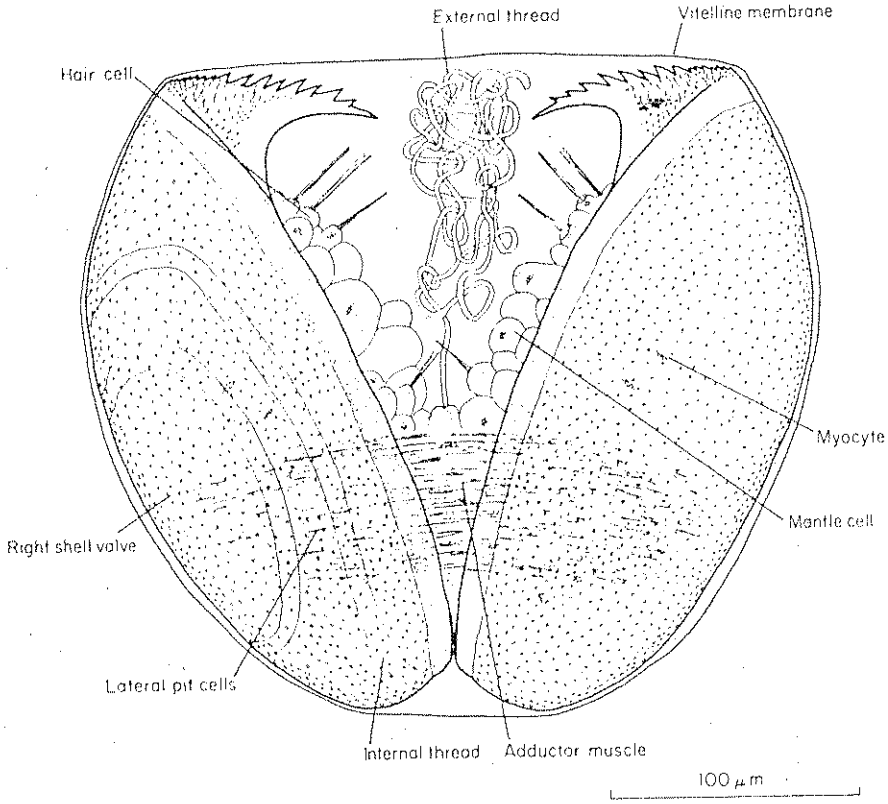

FIG. 3. Mature glochidium, within the vitelline membrane, viewed from the posterior end.

foot and pedal structures. Lateral parts of the ventral plate, known as the lateral pits, are reported by Schierholz (1888) to give rise to the gill filaments.

The mantle cells also carry the hair cells and thread gland to their definitive positions. The adductor muscle does not shift in position but after the shell becomes bivalve, it can, by contracting and relaxing, open and close the shell. The shell is pale and thin initially but when lined by mantle cells it becomes thicker and darker, the pores become more obvious and the spiny hooks grow (Figs 3 and 4). The developmental stage of glochidia is apparent without even opening the demibranchs, since the overall change in colour of the glochidial shell brings about a corresponding change in colour of the intact demibranch.

Growth of the thread

The thread gland appears to be formed from one central and five accessory cells, with the central cell primarily responsible for the production of the thread. During invagination of the mantle cells, the cells constituting the thread gland move posteriorly, so that they come to lie in a central position, below the shell hinge. A thread gland in the anterior position can be seen in Fig. 2, and in the definitive position in Fig. 4.

As the thread gland shifts in position, the central cell of the gland grows dorsally, into the mantle cavity. It moves backwards towards the hinge line to the posterior end of the

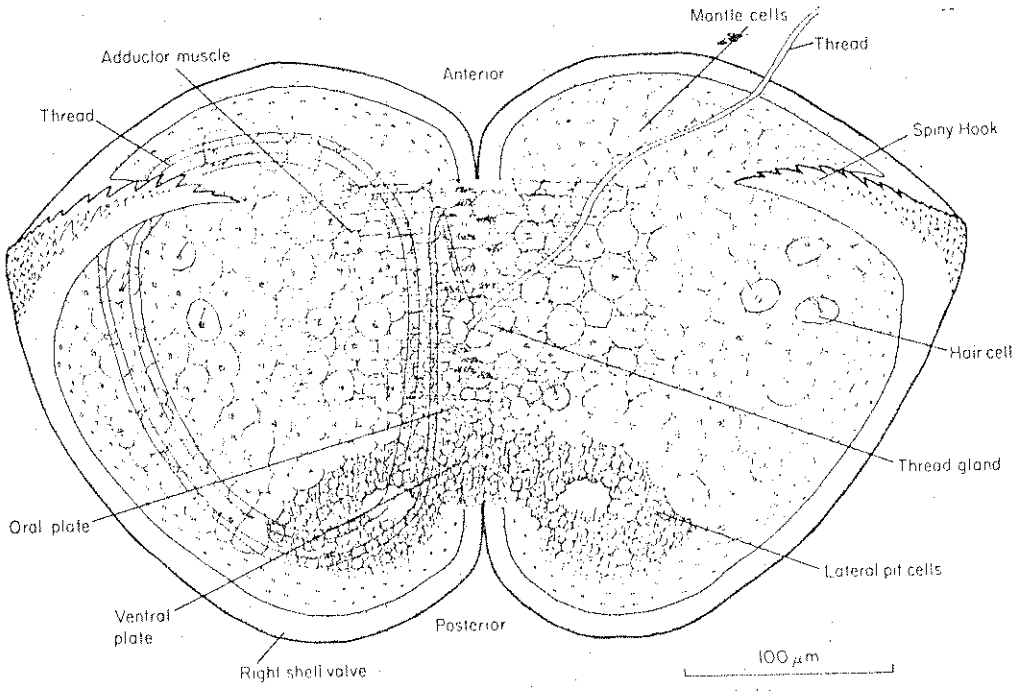

FIG. 4. Mature glochidium, viewed from the ventral side.

body, close to the endodermal sac, and then coils two or three times round the adductor muscle (Fig. 4). The way in which the central cell grows through the tissues is described by Lillie (1895) as follows. "The nucleus of the central cell migrates to its inner end, the greater part of the protoplasm following it; finally almost all of the cell lies within the primary body cavity, but is still connected with the spot it formerly occupied by a strand of vacuolated protoplasm. The vacuoles run together, and form a lumen running from near the exterior . . . the terminal nucleus persists for some time longer, but gradually dwindles and disappears. During its existence it no doubt controls the growth in length of the gland, but after its disappearance the further growth seems to be supported by the five large cells around its opening". He suggests that the thread, which lies in the lumen of the elongated central cell, is produced by "an actual metamorphosis of the substance of the cell", and that when this has begun, "numerous mesoderm cells apply themselves to the gland and completely encase it. The further growth and secretion seems to be supported by these cells".

After the thread has been formed within the glochidial tissues it grows out between the five accessory cells, to lie in the mantle cavity. Lillie states that "extrusion of the thread takes place", which implies that the thread which is coiled up inside the shell moves to the outside. This is not so, for a developed glochidium has both an external and an internal thread. Both take up the same histological stains (e.g. periodic acid Schiff) and have the same elastic properties (Wood, 1974). The external thread is retained initially inside the vitelline membrane (Fig. 3), but when this ruptures the thread is free, becomes tangled with other threads, and binds one glochidium to another.

Morphology of the mature glochidium

Tissue development

Examination of glochidia from December to March showed that, after the movements which accompany invagination had occurred, the position of cells and tissues did not alter. Neither was there any apparent change in structure and organization of individual cells. Lillie (1895) stated that "the cerebral ganglia have begun to form in some glochidia of *Anodonta* in February, just anterior to the stomodaeum". I have not, however, been able to identify nervous tissue with certainty.

It is possible that ultrastructural and physiological changes do occur during this period. The December developed glochidia are, however, able to infect fish, and behave in the same way as March glochidia towards prospective hosts and in various environmental conditions.

The shell

At maturity all the glochidial soft parts are enclosed within the shell, which is armed with a spiny hook on the apex of each valve (Figs 3 and 4). This consists of a solid ridge, and two delicate wings which stretch from the distal end of the hook to the rim of the shell (Plate I(a)). The hook is armed with numerous small spines at its base and a few, large, stout spines along the rest of its length (Plate I(b)). Lillie (1895) reported that "the hooks are joined to the valve proper by a hinge and are moved by special muscles (myocytes)". However, like Arey (1924), I found no evidence in support of this statement. Rather, it appears that movement of the hooks only occurs when one presses against the other, or against an object placed between the valves. This causes a flexure of each hook at its base so that it eventually lies at right-angles to its original position. The spines of one hook can then interlock with those of the other, and this may be an important factor in ensuring successful attachment, for, when they interlock, they also clasp host tissue (Plate I(c)).

The mantle cells

The mantle cells line the shell (Fig. 4), and Arey (1932) reports that during the early days of encystment they are active in digesting host tissue. It is also possible that they ingest food during the period when glochidia are living in the adult demibranchs. The endodermal cells, from which the gut will develop later, form only an indistinct sac at this stage, communicating with the exterior by means of a small opening, the blastopore.

No experimental evidence has been provided which shows conclusively that developing glochidia ingest food, and if they do, from where, and with which cells. In the hope of answering some of these questions, I fed adult gravid *A. cygnea* with ^{14}C labelled algae,

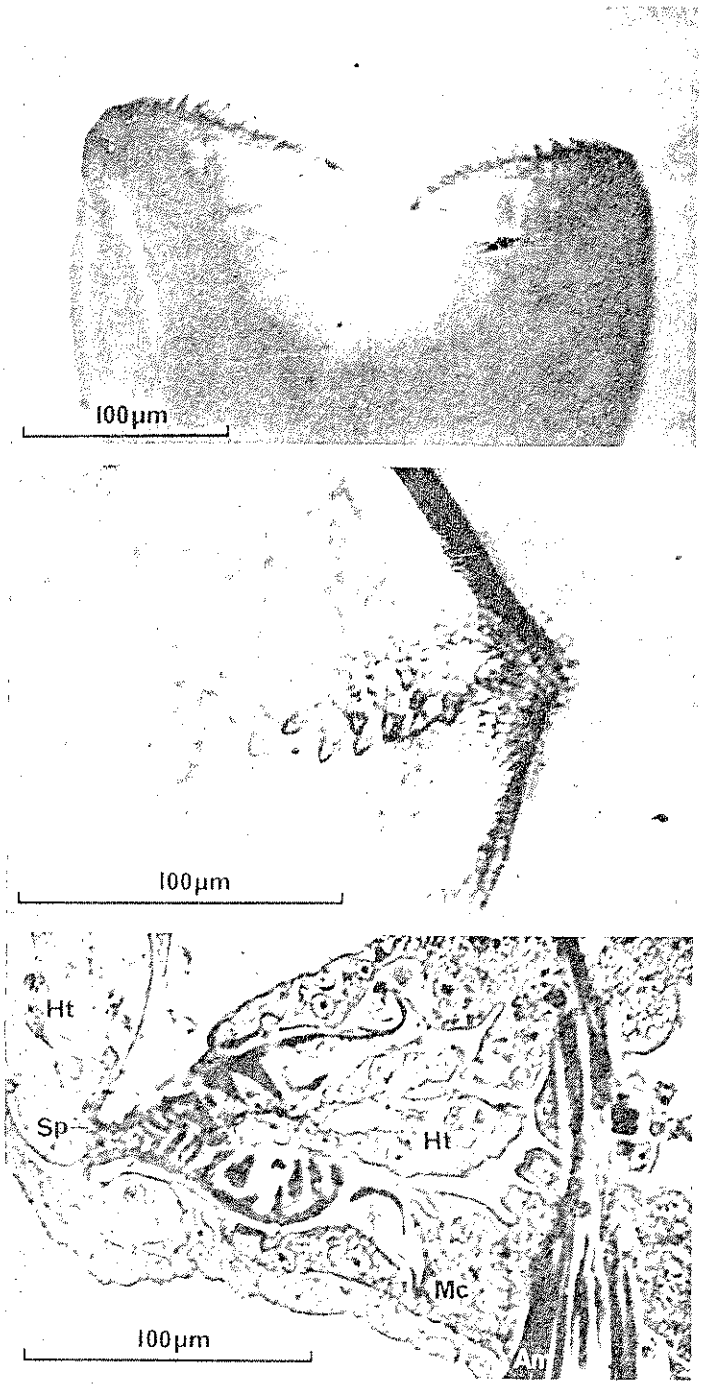

PLATE I. (a) The spiny hooks as seen from the anterior or posterior end. (b) Hook viewed from above. (c) Section through a glochidium attached to a host, to show the reflexed hooks and the interlocking spines. Am, Adductor muscle; Ht, host tissue; Mc, mantle cells; Sp, spiny hooks.

and prepared autoradiographs to find the spread of ^{14}C labelled substances throughout the tissues, and possibly the glochidia. The experiment was carried out in January when the glochidia appeared to be developed, but when they would still presumably require food for basic metabolic processes. The adults were fed with labelled algae (*Euglena* sp.), rather than injected with pure, labelled, organic compound, because algae are natural foodstuffs and are presumably readily assimilated. The assimilation products, some containing ^{14}C, would then be incorporated into many compounds, any of which might eventually reach the glochidia.

Mussels were killed 1, 2, 4, 8 and 11 days after they had been "forcibly fed", and autoradiographs prepared. In each animal ^{14}C was abundant in the gut and haemocoel, and also present in the interlamellar connections and glochidia. A quantitative estimation

TABLE I

The spread of ^{14}C in the adult demibranch, and in the glochidial cells of the mantle and lateral pit

Number of days between feeding and killing the adult	Number of exposed silver grains in 10 μm square grid (mean of three grids)			
	Background	Interlamellar connections	Lateral pit cells of glochidium	Mantle cells of glochidium
1	8	5	5	9
2	4	13	13	8
4	8	10	13	16
8	8	10	10	19
11	7	19	21	25
Control	7	8	7	8

of ^{14}C spread in the latter areas was made by counting the number of black dots (metallic silver) in a grid 10 μm square. A background count was made in the interlamellar spaces, since some silver grains develop even in an unexposed emulsion, and others may be developed by cosmic rays and other sources of ionizing radiation. When evaluating autoradiographs, these background values must be taken into considerqtion. Three counts were made in each area, and the mean value calculated (Table I).

The experiment, although with only a few adults, indicates that glochidia ingest food. This almost certainly comes from the adult, probably as mucus, secreted by cells in the interlamellar connections (Plate II).

Both mantle cells and lateral pit cells contained ^{14}C but, because assimilation products presumably spread to all the cells, it is not possible to say which are responsible for ingestion.

The hair cells

The highly specialized mantle cells bear what is possibly their effective cilia. They are chemosensory, and of extreme importance during the attachment and metamorphosis. Fine processes can sometimes be seen, stretching from the distal end of the hair cell towards the shell valve. The nature of these, and their function, are unknown. They could be myocytes

Section
Adductor

alternatively, some co-ordinating structure. The latter seems probable after consideration of the type of response elicited by stimulation of the hair cell (Wood, 1974).

The thread

The thread, the position and growth of which have already been described, possibly acts an attachment organ. Important properties are that it is long (up to 2 mm externally), sticky, and pliable, and will therefore become attached to any object with a slightly

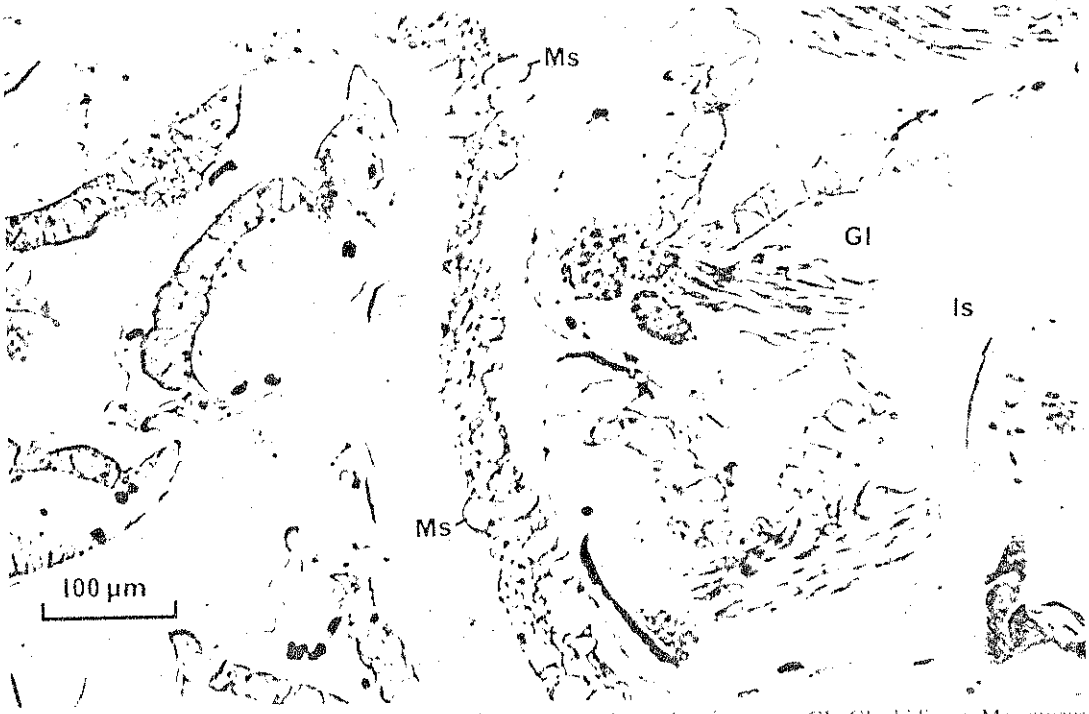

PLATE II. Section through part of a gravid demibranch of *Anodonta cygnea*. Gl, Glochidium; Ms, mucus secreting cell in interlamellar connection.

rough surface, such as the fin of a fish. I also found the thread to be extremely elastic and therefore, once attached, unlikely to break under the strain as a glochidium is towed through the water.

The adductor muscle

The single adductor muscle is situated towards the anterior end of the glochidium. Its apparently branched fibres stretch from one valve to the other, and contain a single centrally placed nucleus. When it contracts it jerks the shell valves together, and is capable of holding them in this position either momentarily or for several hours.

Other structures

The structures described above all serve a particular purpose in the parasitic phase of the glochidium. In contrast, the lateral pits, ventral and oral plates, and endodermal sac are rudimentary tissues and play no part in host attachment.

The glochidium of other unionids

The British Unionidae comprise three genera, *Anodonta* (3 spp.), *Unio* (2 spp.), and *Margaritifera* (2 spp.). All possess glochidia which, in structure and development, differ in the ways indicated in Table II.

There are many North American Unionidae. Most genera (e.g. *Lampsilis*, *Obliquaria*, *Unio*, *Quadrula*) possess glochidia with hookless shells, while some (e.g. *Anodonta*, *Strophitus*, *Symphynota*) possess glochidia with hooked shells. *Lampsilis alatus* does not fit either category because, although its glochidium possesses hooks, they are not, according to Lefevre & Curtis (1910), homologous with those of the *Anodonta* type. Furthermore, each shell is not triangular in side view, as it is in the typical hooked and hookless glochidia, but is the shape of an axehead.

TABLE II

Differences in glochidial development and morphology in the three genera, ***Anodonta, Unio,*** *and* ***Margaritifera***

	Genus		
	Anodonta	*Unio*	*Margaritifera*
Fertilization of eggs	August	March	First brood July; Second brood August
Release of glochidia	Following May	July	August; March
Glochidial size: length from hinge to ventral edge of one shell valve	360 μm	200 μm	50 μm
Hooks on shell	Present	Absent	Absent
Larval thread	Present	Present	Lost at a premature stage
Usual attachment area on host	Fins	Gill filaments	Gill filaments

Discussion

In Britain the unionids are the only bivalves with parasitic larvae. Representatives of this family are also found in North America and Australia. Direct development from glochidium to juvenile apparently occurs in two North American species, *Anodonta* (*Utterbackia*) *imbecillis* and *Strophitus edentulus* (Lefevre & Curtis, 1911). Glochidial development takes place "in a chord in the gill which forms a sort of placenta or nutritive body". *Strophitus edentulus* is also able to develop on a fish in the normal way. In Africa and South America parasitic larvae are produced by the freshwater family Mutelidae (Bonnetto, 1951; Fryer, 1961), but these differ in many ways to the glochidium of unionids.

The suppression of a planktonic stage in the development of freshwater bivalves may be advantageous in a river system where larvae are liable to be swept away to an unsuitable environment. The parasitic phase presumably affords a safer means of dispersal, and some protection from predators at an otherwise vulnerable time.

It is evident at an early stage in the development of *A. cygnea* that the embryo is

divergent from the trochophore type of larva, and that it is developing, not into a veliger, but into a highly specialized glochidium. I found that, with few exceptions, Lillie's descriptions of the embryology of unionid larvae were also applicable to *A. cygnea*. Ectodermal, mesodermal and endodermal cells can be identified in the embryo. The cells which secrete the shell are ectodermal, as are the hair cells, the mantle cells, the cells constituting the thread gland, and those of the oral and ventral plates. Mesodermal cells form the larval adductor muscle and the myocytes, and endodermal cells form the endodermal sac. It is possible to divide these structures into two categories; those directly involved in the parasitic phase, which later degenerate, and those which are rudimentary but develop later. The hair, mantle, and thread gland cells, myocytes and larval adductor fall into the former category, while the oral and ventral plates and endodermal sac fall into the latter. During metamorphosis all the former structures degenerate and the latter, former rudimentary structures develop. Two glochidial structures which degenerate, the mantle and the adductor muscle, are replaced by analogous adult structures (except that two adductor muscles are present in the adult). The rudimentary structures which develop are the ventral and oral plates and the endodermal sac. The ventral plate gives rise to the foot and gills, the oral plate to the oesophagus, and the endodermal sac to the gut.

Glochidia of *A. cygnea* are mature by October, but are not released until the following May. During this time they are sustained by the adult, probably by mucus secreted from the lining epithelium of the demibranchs. Once released they have quickly to become attached to a host, and the thread can act as an attachment organ. This thread is often referred to as a byssus (and the thread gland as a byssus gland), which suggests that it has affinities with the byssus of adult bivalves (e.g. *Mytilus*). It does not, however, appear to be homologous with this type of byssus which only develops in the adult, is situated on the foot, and has no internal structure comparable to that found in glochidia.

Summary

Anodonta cygnea, like other unionids, produces a parasitic glochidium larva. All unionid glochidia are essentially similar, but differ in certain aspects of development and structure.

Fertilization occurs in late summer and development takes place in the outer demibranchs of the adult mussel. The early embryo, enclosed within the egg membrane, consists of a rounded mass of cells, of which the mantle cells and the ciliated oral plate cells are particularly evident. A shell gradually develops and as it does so the mantle and associated cells shift in position until they come to line its inner surface. The shell becomes bivalve and is then opened and closed by the adductor muscle which stretches between the two valves.

The glochidium larva is mature by the end of October at the latest, but it remains in the demibranchs until the following May, when it is released to begin its parasitic phase. The thread, adductor muscle, sensory hair cells and hooked shell all play an important role in host attachment, but all except the shell degenerate during metamorphosis, when the juvenile tissues develop.

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