Dr. G. G. Kennedy with Mrs. P. Lyster Jameson's compts.

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ON THE ORIGIN OF PEARLS.

By H. LYSTER JAMESON, M.A., Ph.D., D. Sc.

[*From the* PROCEEDINGS OF THE ZOOLOGICAL SOCIETY OF LONDON,
March 4, 1902.]

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On the Origin of Pearls.

By H. LYSTER JAMESON, M.A., Ph.D.

(Plates XIV.–XVII.[1] and Text-figures 22–24.)

Most theories of Pearl-production have assumed that the "nucleus," whatever its origin may be, is the direct cause of the secretion of a true pearl, and that the latter arises as a result of the mollusc's endeavour to coat with carbonate of lime an irritating body.

I do not propose in this paper to give yet another complete historical survey of the various hypotheses, dating back to the time of Pliny, which have been propounded. These theories have been summarized over and over again by writers on pearl-formation. The more recent ones may conveniently be grouped under the following heads:—That pearls are (1) concretions of shell-forming fluid (Réaumur, 1717); (2) shell-substance deposited around bodies or concretions of internal origin (Küchenmeister, in part, 1856; von Hessling, 1858; Pagenstecher, 1858); (3) formed around an abortive or displaced ovum (Home, 1826; Kelaart, in part, 1857); (4) secreted to coat over a grain of sand; (5) the result of injury to or perforation of the shell; (6) caused by a parasite (Filippi and others); (7) formed in an ampulla in the tissues (Hessling, 1858; Diguet, 1899). Several writers have allowed the possibility of two or more of these causes.

The origin of the "grain-of-sand" theory is veiled in obscurity.

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[1] For explanation of the Plates, see p. 165.

It has had many supporters, and still maintains a prominent position in zoological text-books and popular compilations. It is doubtless largely due to a confusion of true pearls with "blisters" or pearly excrescences on the shell. There is no recorded instance of an undoubted sand-grain having been found in a pearl, although hundreds have been examined. All attempts to produce pearls by introducing such bodies into the tissues or between the shell and mantle have led, at best, only to the formation of "blisters." Such methods of obtaining the latter have long been known to the Chinese, and have repeatedly been applied in other countries. Chemnitz, Beckmann, and others (1791) regarded Linnæus's "secret process" as merely boring the shells. However, no subsequent boring experiments have yielded anything but blisters, and the popular notion of Linnæus's *modus operandi* is little more than a guess. A great step in the right direction was made when Filippi, in 1852, discovered the connection between pearls and the presence of *Distomum duplicatum* in *Anodonta*. Filippi regarded these Trematodes as encysted. In his later papers he allowed other forms such as *Atax ypsilophorus* to be occasional causes of pearl-formation. He recognized that the action of these parasites was specific, and compared it to the formation of plant-galls. Küchenmeister (1856) associated pearls in *Margaritana margaritifera* with the larvæ of *Atax ypsilophorus* van Beneden, which occur in the mantle, enclosed in cysts secreted by the mollusc. He held that other parasites, as well as bodies of internal origin, might also cause pearls.

Möbius (1857) found Trematode remains in pearls from the Pearl-Oyster of the West Coast of America (probably *Margaritifera margaritifera* L., var. *mazatlanica* Hanley[1]). Kelaart (1859) held that parasites played an important part in pearl-formation in *Margaritifera vulgaris* (Schumacher) in Ceylon, but did not associate any definite organism with it, although he found several species living in the Pearl-Oyster. Thurston (1894) confirmed the existence of platyhelminthan parasites in the same species, but did not assert that they had anything to do with pearl-production. Garner (1871) found that pearls in *Mytilus edulis* and *Margaritana margaritifera* were due to Distomids, against which the molluscs protected themselves by coating them with calcium-carbonate. Comba (1898), who claims to have discovered a method of producing free pearls by artificial means, says (p. 6) that the cause is "un parassito il quale viene dal mollusco avviluppato di strati di una bava che indurendosi forma la perla formando così una pustola ed una pallina che cresce in grossezza."

Dubois (1901) found in *Mytilus edulis* that the production of pearls was due to Distomid larvæ, to which (without description) he applied the name *Distomum margaritarum*. His account of the "*désagrégation*" of formed pearls, and the liberation, to repeat their life-cycle, of the parasites that form their nuclei, is quite at

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[1] For revised nomenclature of the Pearl-Oysters, see Jameson, 1901.

variance with my experience; and apparently presupposes that the Trematode can survive complete calcification, which would indeed be a very remarkable biological phenomenon. According to Dubois it is only certain pearls that, by the death of the *Distomum*, escape this annual disintegration and so reach greater dimensions.

Von Hessling (1858), it seems, was the first to ascertain that the pearl is formed inside an epithelial sac, and he emphasized the importance of this structure. He regarded the sac as being derived from the blood-cells.

This sac has been noted by Diguet (1899), who suggests that it may be due to the stimulation of a parasite. I can find no support for Diguet's view, that the formation of the pearl in this sac proceeds on different lines to those on which the substance of the shell is deposited.

The "vesicle or bag of the ovum" figured by Home (1826, pl. xiii.) may also be this sac.

Before entering upon an account of my own observations, I wish to express my thanks to Mr. H. H. Arnold Bemrose for kindly preparing the microphotographs which accompany this paper; to Baron Louis d'Hamonville for much valuable information concerning the pearl-bearing mussels of Billiers; to Mr. A. Scott, of the Lancashire Sea-Fish Hatcheries, for supplying me with abundant material from the Piel mussel-beds; and to Mr. W. Wells, Marine Superintendent at the Brighton Aquarium, for conducting experiments for me.

The distribution of pearl-producing individuals of *Margaritifera margaritifera* L., *M. maxima* Jameson, *Pinna nigrina* Lam., *Hippopus hippopus* L., and *Tridacna gigas* Lam., in New Guinea and Torres Straits, suggested to me that pearls were the result of a specific pathological condition, and that the circumstances necessary to ensure infection were present only in certain areas, often of small extent. I soon convinced myself, by a study of material that I brought home with me, that Trematodes formed the nuclei of some of the pearls in each of the above-named species, but that others contained nothing more than a few yellowish granules in the centre. The same results were obtained with specimens of *Mytilus edulis*, sent me from Lancashire by my friend Mr. James Johnstone. In all cases where the pearls had been preserved *in situ* in the tissues, they were found to be enclosed in a sac composed of an epithelium physiologically and histologically identical with the outer shell-secreting epidermis of the mantle. This observation at once accounted for the similarity in structure between the layers of the shell and those of which a pearl is composed. The obvious conclusion was that *this sac is the direct, and the Trematode the indirect, cause of pearl-production*, and that the key to the problem of the origin of pearls might be obtained by investigating the origin of the sac and its relations to the Trematode.

As it was not possible for me to return to the habitat of the true Pearl-Oysters, I selected the Common Mussel (*Mytilus edulis*) as a suitable species upon which to begin my observations. This mollusc produces pearls in many localities on the coasts of Europe, but it is only on certain beds that pearls are abundantly formed. The most favourable places seem to be estuaries or land-locked channels. In such situations pearls may be found in almost every example, except those which are attached to stakes or floating objects, and so raised off the bottom.

The pearls produced by the Common Mussel are, like the nacreous lining of the shell, lacking in lustre. They are generally white or silvery, but blue and brown examples are not uncommon. They have no value as gems, though, strange to say, a market seems to have existed for them in the first half of the last century ("D. C.," 1830).

They are mostly formed in the subcutaneous tissue of the dorsal body-wall or in the mantle-lobes. When they occupy a more deep-seated position they have probably been secondarily displaced.

In the little harbour of Billiers (Morbihan), situated on the estuary of the Villaine, there is a colony of pearl-bearing mussels, that has been described by d'Hamonville (1894). After reading d'Hamonville's account, I was struck with the idea that this colony should offer special facilities for investigating the causes of pearl-formation and the conditions for infection. D'Hamonville found that although the mussel is abundant all round the coast, pearls are only produced in the harbour itself, the beds being, at most, only a few acres in extent. Here almost every shell, if not too young, contains pearls.

I visited Billiers in August 1901 and again in December of the same year, and had no difficulty in finding the parasites and tracing the part played by them in pearl-formation. They were the larvæ of a Distomid belonging to the subgenus *Leucithodendrium* (Loos), and very closely resembling *L. somateriæ* (Levinsen), which in the mature condition inhabits the intestine of the Eider Duck. I found larvæ, very similar to these, in Sporocysts in *Tapes decussatus*, and subsequently proved the infection of *Mytilus* experimentally from these Sporocysts. In September of the same year I visited Piel, Lancashire, and found that there also pearls are caused by the same parasite, but that in this case *Cardium edule* acts as "first host" for the Sporocyst.

Finally, in December 1901, when I revisited Billiers, I examined five specimens of the Common Scoter or Black Duck, *Œdemia nigra* L., which is notorious in the Villaine for its depredations on the mussel-beds, and is locally called, on account of its habit of feeding on *Mytilus*, "Cane moulière." Every one of these specimens was teeming with *Distomum* (*Leucithodendrium*) *somateriæ*.

For histological work, pearls were decalcified *in situ* in the tissues and then sectioned. Others were decalcified, cleaned, and examined whole; while others again were ground down on a Water-of-Ayr hone, care being taken that the "Schliff" so made should pass as nearly as possible through the centre. The *Cercariæ* and Sporocysts were either examined entire, or sectioned *in situ*.

For decalcifying, it was found best to use pearls preserved in spirit, as those that had been kept in a dry state, although often giving good results when examined entire in oil of cloves, were unfit for cutting with the microtome, owing to the brittle and horny nature of the dry conchyolin. The most satisfactory effects were obtained by using very dilute (·5 to 1 per cent.) nitric acid in water. Stronger solutions often caused bubbles of carbon dioxide to be evolved in the residual conchyolin, but by using these weak solutions freely the gas was dissolved almost as soon as formed. The time required for this process varied from a few days to some weeks, according to the dimensions of the pearl and the proportion of conchyolin to salts.

Structure of the Mantle and Shell.

The tissues composing the mantle of *Mytilus edulis* are:—

(1) An external simple epithelium, which is a direct continuation of the dorsal body-wall;
(2) An internal ciliated epithelium, resembling the epidermis of the foot and gills; and
(3) A spongy connective tissue, in the meshes of which the blood circulates.

The external epithelium (Pl. XIV. figs. 1–4, Pl. XV. fig. 5, *ext.ep.*, and text-fig. 22) is composed of a single layer of flattened columnar cells with spherical or ovate nuclei. The outer surfaces of these cells are closely applied to the inner nacreous substance of the shell. The appearance of this epidermis differs considerably according to the degree of contraction and method of preservation. Its constituent cells are polygonal in surface view, and brick-shaped or columnar in sections at right angles to the surface.

Their protoplasm stains rather more strongly with hæmatoxylin than that of the connective tissue, and shows faint striæ perpendicular to the surface. Their bases are attached to the connective-tissue fibres. Scattered here and there throughout this epithelium are spherical cells which stain lightly. They may be the "Eirund körnige Zellen" that Tullberg describes (1882). They are never numerous, and I am inclined to attribute their presence largely to defects in preservation.

The epidermal lining of the mantle-cavity (Pl. XIV. fig. 2, Pl. XV. fig. 5, and text-fig. 22, *int.ep.*) is the typical glandular ciliated epithelium so common in the skin of invertebrates. It is composed of columnar ciliated cells and interstitial gland-cells, some of which project basally into the connective tissue. It is altogether a much more heterogeneous layer than that applied to the inner surface of the shell, and is usually rather thicker.

The connective tissue (Pl. XIV. figs. 1–4 ; Pl. XV. fig. 5, *c.t.*) is a meshwork of irregular or stellate cells with oval or spindle-shaped nuclei, and more delicate fibres the nuclei of which are relatively longer and narrower, and stain more deeply than those of the former. There is a perfect intergradation between the two kinds of cells, and their relative abundance varies in different individuals according to the condition of the gonads. The fibres are more numerous just under the epidermis than elsewhere. The blood circulates in the spaces between these cells and fibres, and in places these lacunæ are enlarged to form regular blood-sinuses. Numerous blood-corpuscles (*bl.*) can be seen in the meshes of the connective tissue, especially under the outer and inner epidermal layers.

Yellow refractive granular masses, showing traces of cellular structure, sometimes occur in the meshes of the mantle parenchyma, especially in old mussels. These may be the remains of the broken-down gonads of previous years, or groups of dead leucocytes.

In the connective tissue are embedded the nerves and muscle-fibres of the mantle, and the gonads when ripe extend into it.

The structure of the shell has been very thoroughly investigated by von Nathusius Königsborn (1877), Tullberg (1882), and Ehrenbaum (1885), to whose observations I can add nothing new.

The method in which the shell is laid down is of great interest on account of the identity in structure between the substance of pearls and that of the shell. Biedermann's recent paper (1901), which is full of new and significant facts and carefully summarizes previous observations, proves beyond all question that the organic basis of the shell (conchyolin), which is present also as the basis of pearls, is a true cuticular product, secreted or excreted by the underlying epidermis of the mantle. Biedermann shows that in both Lamellibranchs and Gastropods the calcareous substance of the shell *can only be deposited in such a cuticle.*

The cuticular conception of the conchyolin was, I believe, first propounded in this form by Huxley (1859). In sections of the decalcified shell and mantle, I find that the epithelium is generally applied closely to the conchyolin, and its cuticular outer surface is apparently directly continuous with the latter. If, during the process of fixing, the mantle has been separated from the shell, a certain amount of uncalcified conchyolin may be found attached to the epidermis. Moreover, if the mantle of a live mussel be carefully stripped from the inner surface of the shell, a delicate transparent membrane, like that which Huxley found in *Anodonta*, but less conspicuous, can be detected. This membrane tears away irregularly, some parts adhering to the mantle, others to the shell. This irregular tearing is a further evidence that the uncalcified membrane is in continuity with both shell and mantle. That the mantle can, however, detach itself from the inner surface of the shell is obvious in such forms as *Margaritifera*, *Pinna*, and *Tridacna*, where the mantle-margin is freely retractile.

The evidence adduced in support of the alternate theory of growth by intussusception, originated by Mery (1712), revived by von Nathusius (1877 & 1898), and supported by Felix Müller (1885), is not convincing. The facts supposed to lend weight to this hypothesis are quite explicable on the apposition theory.

Pearls, Blisters, and Concretions.

As some confusion exists as to the exact connotation of the word pearl, I propose to adopt in this paper the terms "pearl," "blister," and "free concretion" for three different kinds of structures that occur in molluscs.

Pearls.—A pearl consists of one or more layers of shell-substance (*i. e.*, conchyolin in which the crystals of inorganic matter are disposed in the same manner as in the shell), enclosing a central nucleus, and formed in a closed sac embedded in the tissues. This sac is composed of epithelial cells similar to those that form the outer mantle-epidermis. This sac is first formed around a parasite, which probably exercises a specific stimulation.

In *Mytilus edulis* and many other forms this parasite is a larval Trematode, but it is probable that certain other parasites can stimulate some molluscs to form such sacs. The parasite does not necessarily become the nucleus of the pearl, but may escape from the sac before calcification.

Any of the substances which form the different parts of the shell may be represented in a pearl. Thus we have nacreous pearls, prismatic pearls, the periostracum pearls of *Modiola modiolus* formed in the mantle-margin, pearls a part of which may be formed of the transparent striated substance which characterizes the attachment of the muscles to the shell, and pearls formed entirely or in part of the substance of the hinge-ligament. Large brown leathery hinge-pearls are occasionally found in Torres Straits in *Margaritifera maxima* Jameson.

A pearl may become secondarily fused to or embedded in the substance of the shell by the absorption of intervening tissues (text-fig. 22). These pearls are sometimes spoken of as attached pearls. Similarly two or more pearls may become fused together, forming double or compound pearls, of which a notable example is the celebrated "Southern Cross."

The various substances, when two or more are present, are not always arranged exactly in the reverse order of the layers of the shell, as sometimes stated. We may, indeed, have several alternations of nacre and prismatic substance, or of the latter and conchyolin. The kinds of shell-substance entering into the composition of a pearl are determined by the position of the latter. De Villepoix (1892) has shown that different parts of the mantle-epithelium are concerned severally in the formation of periostracum, prismatic and nacreous substance. Obviously the epithelium of the pearl-sac acquires the special characters of the adjacent part of the epidermis.

Blisters.—It is proposed to confine this term to internal excrescences of the shell, which are caused by the intrusion of foreign bodies between the mantle and the shell, or by the secretion of a nacreous cicatrix to close the perforations of boring molluscs, worms, or sponges. These are sometimes referred to as "attached pearls" or even as "pearls," but have a totally different mode of origin and should never be confused with the latter.

Concretions.—In many molluscs small free calcosphæritic bodies occur at times in the connective tissues, which, not being enclosed in epidermal sacs, cannot acquire the structure of the shell-substance. They are probably due to different causes in different molluscs. In *Tapes* they are frequent, and are due to the calcification of degenerated Sporocysts or of dead Cercariæ contained in the same. Similar concretions, which I found in *Pholas candida* at Billiers, were caused by dead Cercariæ of another species, contained in Sporocysts.

Old examples of *Mytilus edulis* L., *Modiola modiolus* L., *Hippopus hippopus* L., *Margaritifera vulgaris* (Schumacher), and *Anodonta* sometimes contain similar bodies, but their origin in these cases is uncertain.

In all instances that have come under my notice they are more or less spherical, and composed of needle-like prisms of carbonate of lime radiating from a centre.

Structure of Pearls.

A *Mytilus*-pearl examined entire often shows a darker spot in the centre, which corresponds to the "nucleus." The nucleus is always visible in a section ground from the pearl, though its size varies from ·1 mm. to ·7 mm.

It is often yellowish brown or black, the colour being imparted by the dead remains of the Trematode, or by the small amount of residual matter left, if the worm has escaped from the sac (Pl. XVII. figs. 12–16). The crystalline structure of the nucleus is quite different to that of the remainder of the pearl and to that of the shell. We find in the nucleus one (Pl. XVII. fig. 16) or more (Pl. XVI. fig. 8, Pl. XVII. fig. 14) centres of calcification, consisting of spherical masses of radially arranged crystals. Each centre of calcification, if more than one be present in the nucleus, shows a distinct black cross when viewed between crossed nicols. Sometimes the nucleus is irregularly or incompletely calcified (Pl. XVII. figs. 14–16). The resemblance which concretions and the nuclei of pearls bear to *Harting's bodies* (Harting, 1872) is interesting; for they are formed, so far as we can judge, in a similar manner, namely, by the slow precipitation of carbonate of lime in a viscous substance like albumen or decaying animal matter. On the other hand, the peripheral parts of the pearl are, like the shell, formed by the calcification of the cuticle of the living cells, and owe their structure to the special characters of that membrane or of the underlying epidermis.

A section of a decalcified pearl shows the nucleus, in which the cuticle and sometimes the suckers of the *Distomum* can be distinguished. Occasionally the outlines of the soft parts (*e. g.*, pharynx and digestive cæca) are still visible, as in Pl. XIV. fig. 1, *ph.* & *dig.* More generally, however, nothing can be seen but a mass of yellowish-brown granular substance surrounded by the cuticle (text-fig. 22).

There is often a certain amount of refractive granular matter associated with the remains of the worm, probably an excretion; and, if the parasite migrates out of the sac, this may form the inconspicuous nucleus of a pearl.

Just as the peripheral parts of a pearl present, when ground down to a thin section, a similar structure to that of the shell, so the conchyolin basis of a decalcified pearl shows the same characters. The outermost layer of the latter is uncalcified and continuous with the cuticle of the cells of the sac, just as the outer mantle-epidermis is attached to the inner surface of the shell (Pl. XIV. fig. 1, *con.'*).

There is no organic union between the conchyolin and the nucleus.

The sac containing the pearl is composed of a simple columnar epithelium (Pl. XIV. fig. 1 & text-fig. 22, *s.*), which in its histological structure, as well as in its power of secreting as a cuticle the conchyolin basis of the pearl, is indistinguishable from the outer epidermis of the mantle.

Blood-spaces, containing corpuscles, are well developed around the sac.

Such a pearl cannot then be compared—as some writers have suggested—with the concretions or calculi of cholesterin or other substances found in the vertebrate body, but rather with the structures sometimes found in epidermoid tumours and atheroma cysts.

Origin and Development of the Pearl.

The Trematode enters *Mytilus edulis* as a tailless Cercaria, and at first may often be found between the mantle and shell. It is probable that it reaches this position by boring through the mantle, but I have not yet been able to find one in the act of doing so. The larvæ creep about on the inner surface of the shell, and, after a while, again enter the connective tissue of the mantle, where they come to rest, assuming a spherical form. They seem to avoid the more muscular parts of the mantle—no doubt because the absence of a definite boring apparatus makes it difficult for them to pass through the latter. When embedded in the tissues they are visible to the naked eye as little yellowish spots, about $\frac{1}{2}$ mm. in diameter.

At first the worm only occupies a space lined by connective-tissue fibrils (Pl. XIV. fig. 2), but soon the tissues of the host give rise to an epithelial layer, which lines the space and ultimately becomes the pearl-sac (Pl. XV. fig. 5, *s.*).

This epithelium appears to arise quite independently of the outer epidermis, and is no doubt due to a specific stimulation on the part of the parasite, as other parasites, *e. g.* Sporocysts, Cestode larvæ, &c., are not surrounded by such a sac.

At first a few cells appear (Pl. XIV. figs. 2, 3, *pr.*), which proliferate and arrange themselves along the walls of the cavity. These cells are larger than the connective-tissue corpuscles, and more susceptible to stains. They are flattened and polygonal in surface view. Their nuclei (Pl. XIV. fig. 3, *n.*) are large and spherical, and show the conspicuous chromatin reticulum and distinct nucleolus that characterize the nuclei of embryonic or rapidly dividing tissues.

I have not been able to find the nuclei of these cells actually undergoing division. The proliferating sheet of cells ultimately surrounds the parasite and becomes the sac. From the first these cells are basally continuous with fibres of connective tissue (Pl. XIV. fig. 3, *c.t.*). Their transformation into the pearl-sac is a gradual one, and every step can be traced in sections of the parasites *in situ*.

If the Trematode larva completes its maximum possible term of life it dies, and the tissues of the body break down to form a structureless mass, which retains the form of the parasite owing to the rigid cuticle.

In this mass arise one or more centres of calcification (Pl. XVI. fig. 8), and the precipitation of carbonate of lime goes on until the whole larva is converted into a nodule which has the calcosphæritic structure already described for the nucleus. The granular matter surrounding the worm, if present, also undergoes calcification.

The epithelium of the sac then begins to shed a cuticle of conchyolin (Pl. XIV. fig. 1), and from this point the growth of the pearl probably takes place on the same lines and at the same rate as the thickening of the shell.

The sac sometimes begins to form pearly substance before the worm is completely calcified (Pl. XVII. fig. 16).

The Distomid larvæ sometimes leave the sac formed around them, and voluntarily migrate into other parts of the body before again settling down. Empty sacs may be found in the mantle, and old specimens of the larva (distinguishable from recently immigrated ones by their darker colour and laden excretory organs) sometimes occur free between the mantle and the shell.

The occurrence of pearls in which the nucleus is not a Trematode but merely a few refractive granules (Pl. XVII. fig. 13) can be accounted for in this manner.

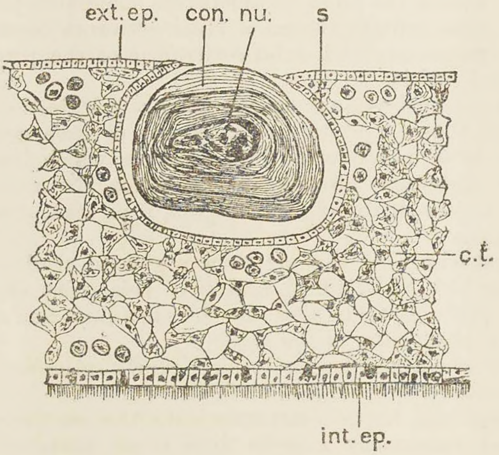
Some compound pearls are evidently formed by short migrations on the part of the Cercariæ, which leave a small amount of residual material in the sac, vacate it, and settle down in the immediate vicinity (Pl. XVII. fig. 15).

The residual matter in the first sac forms the nucleus of a pearl, and if the Trematode dies another is formed in the new one beside it. If these pearls grow and fuse a double pearl is formed, the nucleus of one half being obviously a Trematode, that of the other being merely granular matter.

I have traced three stages in the formation of such a pearl—the first, in which a Cercaria is found in a sac with a vacated sac close by; the second, in which a small pearl is close to the live Cercaria; and the third, in which two or more small pearls lie close together, only one having a Trematode for nucleus.

Dubois (1901) suggests that the death of the Distoma may sometimes be determined by Sporozoa, some members of which group are known to attack Trematodes. In one of the specimens that I sectioned there was a parasitic protozoon embedded in the tissues. If such parasites were to occur frequently they would of course facilitate and intensify the production of pearls. But they are not essential, any more than the presence of the dead Distoma in the sac is necessary for pearl-formation.

Text-fig. 22.



A Pearl about to become attached to the Shell.

nu., nucleus of pearl; *int.ep.*, internal ciliated epidermis of mantle; *ext.ep.*, external epidermis of mantle; *con.*, conchyolin basis of pearl; *s.*, epithelium of pearl-sac; *c.t.*, connective tissue. × 50.

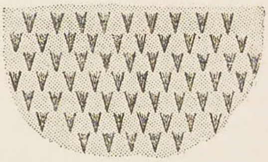
A pearl may increase in size until its diameter is considerably greater than the thickness of the mantle, so that it protrudes visibly. It may even break through the ciliated epidermis; for valuable pearls have been found in the branchial chamber and also outside the shells.

If it presses upon the tissues intervening between itself and the shell, these may become absorbed, in which case the epithelium of the pearl-sac becomes continuous with the shell-forming epidermis (text-fig. 22). The result is that the subsequently formed layers of the pearl are continuous with those of the shell, and an attached pearl is formed. The fusion of two or more pearls to form a compound pearl is effected in the same way.

Structure of the Trematode Larva.

With the exception of the female reproductive organs, which are as yet undeveloped, the larva presents all the characters of *Distomum* (*Brachycœlium* Dujardin, 1845, *Leucithodendrium* Loos, 1896) *somateriæ* (Levinsen, 1882), from the Eider Duck, Greenland. (For sub-classification of Dujardin's subgenus *Brachycœlium*, see Stossich, 1899.) The body (Pl. XV. fig. 6) is oval, blunter in front than behind, and tapering markedly in the last third. In the resting condition inside the sac it is nearly spherical. The average dimensions are ·55 mm. to ·7 mm. The extreme sizes seen were ·45 mm. and ·75 mm. The oral sucker is larger than the ventral one, the ratio of their diameters being usually about 4 : 3. But in this point there is a considerable amount of variation both in the larva and in the adult *L. somateriæ*. Except on the surfaces of the suckers, which are smooth, the cuticle is beset with small spines (text-fig. 23). These are arranged in transverse rows, the members of which also form diagonal rows, so that the cuticle in surface view appears to be divided up into little diamond-shaped fields. There are about two hundred transverse rows of spines on the dorsum. Immediately around the ventral sucker the spines occur in concentric circles. The connective tissue is the typical parenchyma of the Flatworms (Pl. XIV. fig. 2; Pl. XV. figs. 5, 7, *pa.*). It is richer in nuclei immediately under the skin than elsewhere, especially on the dorsal surface (Pl. XV. fig. 7, *pa.n.*). Some muscle-fibres are present in the connective tissue running from the body-wall to the suckers and pharynx (Pl. XV. fig. 7, *m.f.*). The musculature of the body-wall consists of an outer circular layer (Pl. XV. figs. 5, 7, *c.m.*) immediately under

Text-fig. 23.



Cuticle of the *Cercaria*, in surface view. × 700.

the cuticle, a deeper longitudinal layer (*l.m.*), and, on the ventral surface, a less defined tract of transverse fibres inside the longitudinal muscular coat.

The suckers are lodged in a slight involution of the cuticle (Pl. XV. figs. 5, 7). Their relative and absolute sizes in surface view are determined by the degree of contraction of their constituent fibres. The ventral sucker is about one-fourth or one-third of the total breadth of the body.

The mouth is situated in the middle of the anterior sucker, and generally appears triangular in sections (Pl. XV. figs. 6, 7, *m.*). The funnel-shaped buccal tube opens behind, by a narrow orifice, into the spherical muscular pharynx (*ph.*). This is followed by the short straight œsophagus (*œ.*), which, passing upwards and backwards, bifurcates to form the sac-like digestive cæca (Pl. XIV. fig. 2; Pl. XV. figs. 5–7, *dig.*), which are dorsal to the other organs. In the resting worm these cæca are greatly distended with yellowish granular material, doubtless derived from the tissues of *Mytilus*.

Even the œsophagus is often tightly crammed with food. The digestive system in this condition occupies the bulk of the body, anterior to the ventral sucker, but when empty is much smaller. The posterior end of the pharynx is provided with a group of salivary glands (Pl. XV. figs. 6, 7, *s.gl.*). The epithelium of the digestive system consists of very large flat polygonal cells with conspicuous nuclei (Pl. XV. fig. 7, *int.ep.*). The individual cells can sometimes be distinguished in pressure preparations. There is an ill-defined supra-pharyngeal nerve commissure (Pl. XV. fig. 7, *n.c.*) and a pair of lateral cords. The excretory system (Pl. XIV. fig. 2; Pl. XV. figs. 5–7, *ex.*) consists of two enormous tubular sacs, extending to the anterior end of the body and converging to form a pyriform median vesicle, which opens by a pore at the hinder end (Pl. XV. fig. 6, *ex.p.*). The excretory tubes are generally quite full of opaque spherical granules, presumably of excretory matter. When treated with hydrochloric acid they become transparent (Pl. XV. fig. 6).

In living specimens a few flame-cells can be seen in short, lateral, and apparently unbranched tubules given off by the excretory sacs. But the distended condition of the latter makes it difficult to ascertain their precise relations.

The female organs are not developed in the resting larva. The worm is protandrous, and the male genital organs reach a conspicuous size, even in the Sporocyst. The rudiments of the testes, vasa deferentia, and penis are very obvious in sections (Pl. XV. figs. 5, 7) and in stained preparations of the entire worm (Pl. XV. fig. 6). Being composed of young cells they stain deeply. In fresh specimens they are less obvious. The penis opens out at the genital pore (Pl. XV. fig. 7, *g.p.*), which is situated immediately in front of the anterior border of the ventral sucker. It is an elongated hollow pyriform body, lying in front of and dorsal to the sucker. The rudiment of the seminal vesicle receives the vasa deferentia (Pl. XV. fig. 6, *v.d.*), which can be traced back into the spherical testes (*te.*).

Biology.

When the larva first enters *Mytilus* it is somewhat smaller than the resting specimens and more transparent. The excretory organs, which are laden with granules while in the Sporocyst, are comparatively empty, and the gut is not yet distended with food. As it grows older both the excretory and digestive systems become more and more laden, so that they form the great mass of the body (Pl. XIV. fig. 2; Pl. XV. fig. 5). It is largely to the contents of the latter that the parasite owes its dark yellow colour, the cuticle being pale golden or straw-coloured.

The worm often excretes some granular substance, which may almost surround it in the sac. It is this stuff that serves as "nucleus" for a pearl, if the Trematode migrates to another part of its host.

It is interesting to note that at no period is this worm *encysted*, in the sense in which the Liver-fluke and so many other Cercariæ encyst. The dark colour of the epithelial sac, which can often be isolated with the worm, suggests, on casual observation, that the latter is encysted, but I have determined by sections that this is never the case.

In a certain sense it is a resting stage, but the distension of the alimentary system makes it obvious that it is also a highly assimilative phase in the life of the worm, which is storing up energy for the maturation of the gonads, on reaching the final host.

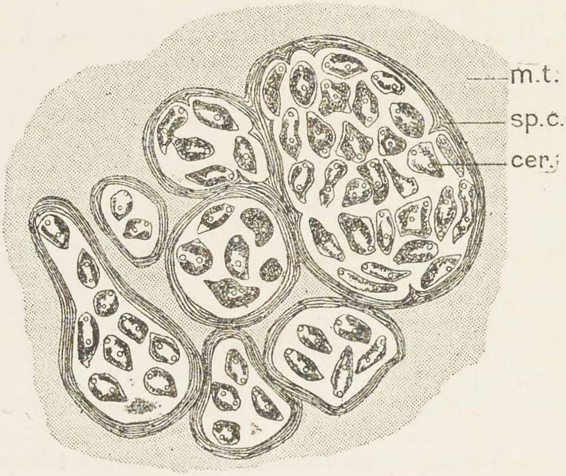
Life-history of the Parasite. The Sporocyst Stage.

After a laborious examination of most of the organisms which inhabit Billiers Harbour, I was rewarded by finding, in *Tapes decussatus* Gmel., Sporocysts containing tailless larvæ, almost identical with those which occur in *Mytilus* (Pl. XVI. figs. 9, 10, and text-fig. 24). They differed from the latter only in their smaller size, paler colour, more distended excretory organs and empty gut, and in the possession of special sense-organs and eyes. *Tapes decussatus* is extremely abundant in Billiers Harbour, living in burrows about six inches deep in the black gravelly clay that forms the bottom. The local name of this mollusc is *Palourde*, and it is extensively collected for food. I am informed that although *Tapes* occurs in small numbers all along the shores of the Villaine, it is nowhere found in sufficient quantities to be worth fishing, except in Billiers Harbour. Indeed, when I visited Billiers in December 1901, a lugger from the other side of the estuary came over specially to collect this shell and *Mya arenaria* for the markets, there being no supply on the south shore.

I found Sporocysts in every specimen of *Tapes* that I examined, numbering nearly two hundred. They occur in the muscular or connective tissue of the mantle-margin, where it is attached to the "pallial line," especially along the ventral border, and around the insertion of the siphonal musculature. The favourite place seems to be the dorsal side of the latter. The individual Sporocysts are embedded in and closely adherent to the bundles of muscle-fibres.

In young examples I found small, simple, spherical or oval Sporocysts, about ·5 mm. in diameter, and containing 6–10 Cercariæ, but in larger examples groups of Sporocysts occur (Pl. XVI. fig. 9, and text-fig. 24).

Text-fig. 24.



Tapes decussatus.

Group of Secondary Sporocysts, as seen in a pressure preparation.

m.t., muscular tissue of *Tapes*; *sp.c.*, Sporocyst; *cer.*, Cercaria contained in Sporocyst.

The individual Sporocysts in these cases are often larger than the simple ones. The groups are, from their position, their relations to one another, and their progressive increase in size and number of constituent cysts as *Tapes* grows older, evidently produced by budding or secondary division of the original simple ones. Their growth is very slow, and their duration of life must be practically co-extensive with that of their host. In large specimens of *Tapes* they appear to represent several successive infections; as we may have, in the same individual, large groups, probably several years old, smaller ones containing three or four cysts, and finally little single Sporocysts like those found in young individuals. On the other hand, in young *Tapes*, 10–20 mm. long, although the simple cysts and small groups occur, the large groups are not to be found.

Specimens of *Tapes* measuring 12 × 10 mm., with one marked *period* or interruption in the growth-rings on the shell, contained small simple Sporocysts, or, at most, groups of three or four secondary ones. Examples which averaged 17–21 mm. in length contained masses of seven to ten cysts, those measuring 27 mm. had still larger groups, while from that size upwards there was a steady progression in the dimensions and number of constituent units in the groups of Sporocysts. Some of those found in *Tapes* 40–50 mm. long measured 7 mm. in diameter, and contained as many as thirty secondary cysts, and a hundred Cercariæ or even more.

As the Sporocyst grows, it sometimes compresses the tissues that intervene between it and the shell, which apparently interferes with the secretion of fresh shell-layers. This leads to the development of white chalky spots on the inner surface of the valve. These patches, in old individuals, further prove that the large groups of Sporocysts are the descendants of the original small ones which are present when the molluscs are young. For we may see two or more white scars on the lining of the shell, marking the position of the cysts when the shell was younger. In fact, the Sporocysts may leave, imprinted on the shell, the history of their movements as the mantle-margin shifts outwards, just as the adductor muscles mark on the nacre the record of their migrations. The first or innermost of these scars is a small one, such as might result from a triple or quadruple cyst, the next is larger, while the group of Sporocysts in the mantle-margin is larger again. This plainly shows that as the mantle-margin followed the peripheral growth of the shell, the group of Sporocysts increased in size.

These compound Sporocysts are, of course, thicker than the normal thickness of the mantle, and stand out as opaque white granular eminences, obvious as soon as the shell is opened.

On the beds of Pearl-bearing Mussels in the Barrow Channel, opposite the Piel Fish-Hatchery, where every specimen of *Mytilus* is abundantly infected with the *Leucithodendrium*, and almost every specimen contains pearls, *Tapes* is not found. The Cockle, *Cardium edule* L., is common there, and acts as a host for the Sporocysts. Somewhat less than half the specimens of *Cardium* that I examined at Piel were infected. In *Cardium* the Sporocysts occurred in the mantle-margin, close to the anterior border of the anterior adductor muscle. Large groups, such as occur in *Tapes*, were not observed, but only single, triple, or quadruple cysts.

I have not yet been able to trace the infection of *Tapes* or *Cardium*. It is therefore impossible to say whether infection takes place by means of a free swimming *Miracidium* larva or not. The constant occurrence of the Sporocysts in exactly the same positions suggests that the eggs are carried into the digestive system of *Tapes* with the food-bearing current, hatch out in the alimentary canal, enter the circulatory system, and reach their destination *via* the posterior pallial artery, along the course of which they are distributed in *Tapes*. Moreover, the position in which I found the Sporocysts in *Cardium* at Piel corresponds to the end of the anterior pallial artery.

The Cercaria in the Sporocyst first appears as a little oval cellular ball, budded off from the wall, measuring about ·05 mm. During the early stages of its development it is transparent, and its structure can be made out without difficulty; but, as it grows, its excretory organs become gradually laden and distended with opaque granules, which conceal the other parts.

The fully formed Cercariæ in the Sporocysts measure ·15 mm. to ·3 mm. They are whiter than those found in *Mytilus*, but the arrangement of the spines on the cuticle is the same. They possess a pair of pyramidal or conical light brown eye-spots (Pl. XVI. fig. 10, *e.*), each provided with a lens. There are about six tactile papillæ (*t.p.*) at the anterior end of the body. These sense-organs are no doubt serviceable to the larva, during its free living stage, after leaving *Tapes*, and before entering *Mytilus*. The digestive system (Pl. XVI. figs. 9, 10, *dig.*) is empty, and occupies less space than in the *Mytilus* stage. The form and relations of the suckers and pharynx are the same. The penis (Pl. XVI. fig. 9, *pe.*) and testes are already developed, and have the same relations as in the *Mytilus* worm. In pressure preparations of the live worm they are not easy to discern.

The majority of Cercariæ in the Sporocysts are fully developed, young transparent ones being less common. They probably remain a considerable time before vacating it. A few on their way out may often be found free in the tissues of the mantle.

Search in the mud and with the tow-net at Billiers, failed to reveal the free living stage. I have, however, found examples in water in which *Tapes* had been kept for some days.

This Trematode is not provided with a cercarian tail at any stage of its existence, and it is only capable of creeping movements. The larva in the Sporocyst is rather more active than the later stage which occurs in *Mytilus*.

If a Cercaria dies while still in the Sporocyst, its remains become calcified; but, not being enclosed in an epidermal sac, secreted by the mollusc, it does not give rise to a pearl, but merely to a concretion. Again, an exhausted Sporocyst may undergo similar calcareous degeneration with the same result.

Artificial Infection of Mytilus.

On leaving Billiers in the beginning of September 1901, I brought with me about fifty infected examples of *Tapes*. I first placed these in a tank at the Piel Fish-Hatchery, which Professor Herdman and Mr. Scott kindly placed at my disposal. In order to test experimentally the infection of *Mytilus* from *Tapes*, I put in the same tank about seventy mussels, taken from the piles of the old pier at Piel. These mussels of which I examined a number, were practically without parasites. About one in every five of the largest examples contained a Cercaria, one had two

Cercariæ, and one contained a small pearl. It is apparently difficult for infection to take place, except on the bottom, owing to the absence of swimming-organs in the parasite. Hence the absence of Cercariæ in these examples.

Eleven days after they were placed in the tank I examined two of these *Mytili*, and found that the first contained one and the second two Cercariæ. These Cercariæ were recently immigrated examples, as they were small, rather transparent, and not yet surrounded by sacs.

I then transferred the experiment to Brighton, where Mr. W. Wells, the Marine Superintendent at the Aquarium, kindly kept the molluscs in a tank in his private office.

On the 18th of November, 1901, two months after the specimens were placed in this tank, I examined six of the mussels. Of these one contained six Cercariæ, another four, two had each three parasites, one contained two, and one was still uninfected.

When the experiment was transferred to Brighton I added about two dozen mussels that had been in the Brighton Aquarium for two years. I examined six such mussels before introducing the others, and found that none of them contained live Cercariæ, though four of them had one small pearl apiece.

On 5th April, 1902, I took up a sample of 10 mussels from this tank, comprising five of the specimens originally taken from the pier at Piel, and five of those that had been transferred from another tank at Brighton.

The following table suffices to show that in both cases infection had taken place:—

(*a*) Piel Pier mussels.
No. 1. Contained 7 Cercariæ.
No. 2. ,, 3 live and 2 dead Cercariæ, one of which was partly calcified.
No. 3. ,, 2 live and 2 dead Cercariæ.
No. 4. ,, 4 live Cercariæ.
No. 5. ,, 2 live Cercariæ.

(*b*) Specimens transferred from other tank at Brighton.
No. 6. Contained 19 Cercariæ.
No. 7. ,, 3 Cercariæ.
No. 8. ,, 2 Cercariæ.
No. 9. ,, 1 (dead) Cercaria.
No. 10. Still uninfected.

The Adult Leucithodendrium.

Although I have not had an opportunity of making a direct *feeding experiment* upon *Somateria* or *Œdemia*, there is hardly any doubt that the parasite that causes the formation of the pearl-sac, and consequently of the pearl, in *Mytilus edulis* is the larva of *Leucithodendrium somateriæ* (Levinsen), originally described from the Eider Duck (*Somateria mollissima* Linn.) in Greenland,

and rediscovered by me in the Scoter, *Œdemia nigra* L., from Bridlington Bay and the Villaine Estuary.

After finding the Sporocyst I made a careful examination of such fishes and gulls as I could secure at Billiers, but could find no parasite corresponding to the larva. However, on enquiring of the fishermen I was informed that the great enemy of the mussel in those waters is a diving duck, locally called "Cane moulière," which frequents the Villaine in winter. M. d'Hamonville, to whom I wrote on the matter, had no hesitation in saying that this bird was *Œdemia nigra*. On my visit to Billiers in December last I proved, by shooting an example and procuring four others that were taken in nets, that it was so. The name "Cane moulière" seems to be applied to another duck as well, probably the Scaup, *Fuligula marila* Linn. A few young Scoters remain on the Villaine during the summer months.

The Scoter is very common in winter at the mouth of the Barrow Channel, just opposite the pearl-bearing mussel-beds.

Before going to Billiers in December I had proved the occurrence of *L. somateriæ* (Levinsen), associated with *Levinsenia pygmæum* Lev., in a specimen of *Œdemia nigra* sent me from Bridlington by Mr. G. Williamson.

The Scoter received from Bridlington was in a rather advanced state, and I could only determine the presence of a few examples of *L. somateriæ* in the hinder part of the small intestine. But the five specimens procured on the Villaine were infested with *L. somateriæ* from the stomach to the anus and even in the cæca. I calculated that each specimen contained at least six thousand examples of the parasite. *Levinsenia pygmæum* Linn., which occurred abundantly in the Bridlington specimen, was not found at Billiers. The intestine of the Bridlington duck contained nothing but fragments of *Mytilus*-shells. The example which I shot at Billiers was feeding over mussel-beds, and the other four were caught in special nets which are placed on the beds of mussels at ebb tide, and in which the birds get entangled when they visit these beds with the flood, to feed.

These Billiers specimens had apparently been feeding exclusively on mussels, as no other food was found in them, except that one individual contained about half a dozen *Nucula* sp. and a broken *Littorina*-shell, in addition to *Mytilus*. In the crop and stomach some of the mussels were still entire, and specimens up to 40 millim. in length were found; in the stomach the shells are crushed, and pass through the intestine in small fragments at most a few millimetres in diameter.

The striking likeness, except for the matter of size, between the *Mytilus*-worm and *Leucithodendrium somateriæ*, and the occurrence of the latter in the two birds that are known to feed *par excellence* on mussels, is almost sufficient to prove their identity without the feeding experiment. I hope, however, to make this experiment if birds can be secured. So far I have been unable to purchase live examples, although I have made enquiries in all directions. My discovery in the small intestine of a Billiers Scoter, three inches behind Meckel's diverticulum, of a single immature example of the parasite, positively identical in size and all details with the Cercaria from *Mytilus*, practically proves the point.

The adults agree with Levinsen's description and figure, except that the genital pore (Pl. XVI. fig. 11, *g.p.*) is just in front, and not in the centre, of the ventral sucker.

Levinsen's observation on this point has been treated with scepticism by later writers, and, indeed, such a position for the opening of the penis and other genital tubes would not only be a novelty in Trematode anatomy, but would probably render the sucker useless as an adhesive organ.

The adult worm varies in size from ·2 mm. to ·55 mm. It is therefore only about half the size of the larva found in *Mytilus*. It is protandrous, and specimens in which the male organs are ripe are generally larger than egg-laden females.

The diminution in size accompanying sexual maturation is of great interest, and can be accounted for in this way, I think. The resting-stage in *Mytilus* is a highly assimilative phase in the worm's existence. The bulk of the body is greatly increased by the distended gut and excretory system. A reduction in bulk would be effected by the discharge of the contents of the latter, but still more by the absorption of reserve material required to mature the gonads. In fact, the reproductive organs seem to grow at the expense of the other tissues of the body. The gut, I may mention, is empty in the adult worm.

Specimens from *Œdemia* die very quickly as a result of the *post-mortem* cooling of the body of their host. The Scoter that I shot was still warm when I dissected it, but the parasites died in a few minutes when placed on a slide, and an hour after the bird had been opened every adult worm was dead. The immature specimen above mentioned survived the death of the host by twelve hours. The temperature of the room where I worked at Billiers was very low, and possibly in a well-heated laboratory death would not ensue so quickly.

Owing to the rapid death of the worms, the material that I preserved was not suited for detailed histological work, but the relations of the various organs to one another could be determined on sections, and by this means I have been able to check my observations on pressure preparations of fresh material at Billiers.

Plate XVI. fig. 11 shows the structure of an average individual in which the maximum number of eggs has not yet been reached, and the uterus is not too complicated to mask the other structures.

The arrangement of the cuticular spines is the same as that described for the larva. The suckers and the digestive and excretory systems are also the same. The genital pore (*g.p.*) is just in front of the ventral sucker. The penis (*pe.*) is pyriform. Its extremity seems to be beset with glands. Posteriorly it goes over into the large sac-like seminal vesicle (*s.v.*). This lies dorsal to and, as a rule, a little to the right of the sucker. At its hinder end it receives the vasa deferentia (*v.d.*). The testes (*te.*) are a little more comspicuous than in the larva. The uterus (*ut.*) opens out just in front of the penis. The arrangement of its convolutions depends upon the number of eggs contained (up to 100, or even more). It seems to begin on the left side, near the ovary (*ov.*), which is larger than the testes. It forms a series of convolutions on the left side, crosses in front of the genital opening to the right, where it forms further convolutions, passes forward as a loop to the anterior end of the body, and runs back to open out at the genital aperture. I am not convinced that this arrangement is always strictly adhered to.

The eggs in the uterus have the form depicted in my sketch and in Levinsen's figure. They measure from ·018 mm. to ·023 mm., the average dimensions being ·021 mm. × ·018 mm., but they differ considerably in different individuals. I can add nothing new to Levinsen's observations on the yolk-gland.

I sought in vain for this worm in *Bernicla brenta* Pall., *Tadorna cornuta* Gmel., *Querquedula crecca* Linn., *Colymbus arcticus* Linn., *Larus argentatus* Gmel., and *Rissa tridactyla* Linn. I have had no opportunity of examining other allied birds.

Being unable to secure live Eiders or Scoters, I tried feeding a male Pochard, *Fuligula ferina* Linn., with infected *Mytili*, but without results.

L. somateriæ will very probably be found in the other members of the genera *Somateria* and *Œdemia* when looked for.

The Conditions essential to Pearl-production.

The characters and life-history of the parasite suffice to account for the anomalous distribution of pearl-bearing mussels, and, by analogy, throw light on the cause of the differences in the number of pearls produced by the true Pearl-Oysters on various pearling and shelling grounds.

In order to be abundantly infected *Mytilus* must be on the bottom, for the tailless Cercaria or "*Cercariæum*" is dependent upon its limited creeping powers, and the chance of being transported by currents and deposited with silt, &c.

Hence mussels grown on stakes, like those on Piel pier, although right in the middle of the beds of pearl-bearing individuals, are practically uninfected.

Secondly, there must be an abundance of the first host (*Tapes* at Billiers, *Cardium* at Piel) in the immediate vicinity of the mussels, in order to ensure frequent re-infection. For I find on the coasts of the Villaine, where *Tapes* is scarce, the proportion of infected *Mytili* is small. Moreover, on the Roosebeck Scar, outside the Barrow Channel, where *Cardium* is not found, pearls do not occur frequently.

Thirdly, the beds must be near the feeding-grounds of the

Scoter (or another final host), and the set of the currents must favour the transportation of the larvæ or eggs (whichever it may be) to the beds where *Tapes* occurs.

Although it is only on certain beds that these conditions exist infection takes place to a small extent on very many beds. I have hardly ever examined a sample of mussels from any locality without finding here and there among them an infected individual.

Duration of Life of the Larva in Mytilus, *and Rate of Growth of Pearls.*

I am at present making experiments to test the longevity of the resting larva. I have, however, three facts to record that lead me to think it is less than two years.

While the mussels on the foreshore opposite Piel Fish-Hatchery are highly infected, those on the Roosebeck Scar, outside the Barrow Channel, are not so. When I was at Piel, Mr. Scott showed me a small patch of mussels on the pearl-bearing beds, and told me that these molluscs had been brought in from the Roosebeck by a fisherman about two years previously and thrown down there. I examined a number of these mussels, and each of them contained several small pearls. Some, indeed, had as many as ten, and all were infested with the Trematode. From the presence of pearls in these specimens, it is probable that the first Cercariæ to enter them had been dead some time. The dimensions of these pearls throw some light on the time required to produce pearls of a certain size. The five largest specimens weighed together 6·9 mg. (dried on filter-paper after being preserved in spirit), and measured respectively 1·3 × 1 mm., 1·5 × 1 mm., ·9 × ·85 mm., 1·2 × ·8 mm., and 2·1 × 1·15 mm. The last was obviously, from its form, a double pearl.

Again, as four out of the six specimens that I dissected after they had been about two years in the Brighton Aquarium contained each a small pearl, but no live Trematodes, it is probable that the latter were unable to survive two years in the Aquarium.

Moreover, at Piel and Billiers pearls are very seldom found in mussels less than 40 mm. long, which size is probably attained when the mussel is in its third year. I find Cercariæ, on the other hand, in specimens only 20 mm. in diameter.

The average size of the larger pearls found in old mussels at Piel is about 2 × 2 mm., but all sizes, from the dimensions of the parasite to 3·35 × 3·2 mm., were found. At Billiers they are usually smaller, as the mussels are regularly fished there and seldom reach a great age. The pearl-bearing beds at Piel are not fished, as the infected mussels are not marketable.

The sizes to which pearls grow in other molluscs differ very greatly for the several species and for the same species in different localities. Their growth is, in fact, regulated by the causes which control the thickening of the shell. Hence the white porcellaneous pearls of *Tridacna gigas* and *Hippopus*

hippopus, in which species the shell is thick and massive, are often as large as a cherry. Among the true Pearl-Oysters the large thick-shelled species, *Margaritifera maxima* Jameson and *M. margaritifera* Linn., produce the largest pearls, those yielded by the smaller *M. vulgaris* (Schumacher) on the Ceylon fisheries and elsewhere being usually small, and prized rather for their colour and lustre than for their dimensions.

And it is interesting to note that *M. vulgaris* in the Persian Gulf, where it attains larger dimensions and yields a more massive shell than in Ceylon, gives on an average larger pearls than the Gulf of Manaar pearl-oyster.

The general experience of everybody acquainted with pearl-fisheries is that the largest pearls are found in the oldest and thickest shells, which proves how intimately the growth of pearl and shell are associated. It is natural that such an association should exist, since, as is obvious from the results recorded in this paper, the mechanisms of both processes are the same.

Origin of Pearls in other Forms.

Two questions will naturally occur:—Are we warranted in assuming that the mechanism of pearl-formation is the same in other molluscs? and, Is it generally caused by Trematode larvæ?

In answer to the first question, I may say that in those cases where I have been able to examine pearls *in situ*, in *Margaritifera margaritifera* Linn., *M. vulgaris* (Schumacher), *M. maxima* Jameson, *Hippopus hippopus* L., and *Pinna nigrina* Lam., this sac is universally present. It has been noticed by von Hessling in *Margaritana margaritifera*, and by Diguet in *Margaritifera margaritifera* L., from California. But, apart from this evidence, it is safe to say that without such an epithelial sac to shed the cuticular conchyolin, the nacreous layers of the pearl could not be laid down at all.

To what extent other causes besides Trematode larvæ may be capable of inducing such sacs to develop, has yet to be ascertained. Trematodes have been unquestionably associated with pearl-formation in *Anodonta*, *Margaritana margaritifera*, *Mytilus edulis*, and *Margaritifera* (?) *mazatlanica* (see Introduction).

Besides these records, I have detected the remains of Trematodes in decalcified or sectioned pearls from the following species:—*Margaritifera margaritifera* Linn., *M. maxima* Jameson, *M. vulgaris* Schumacher, *Pinna nigrina* Lam., *P. euglypta* Hanley, *P. virgata* Menke, *Hippopus hippopus* Linn., *Tridacna gigas* Lam., and *Mytilus magellanicus*.

I examined pearls from several other molluscs, but had not sufficient material to ascertain satisfactorily. In *M. vulgaris* Schumacher, besides the Trematode, there seems to be a second organism, possibly a Gregarine, concerned in pearl-formation.

The periostracum pearls in the mantle-margin of *Modiola* are also associated with parasitic protozoa.

These data suffice to show that in many molluscs, including several of the species yielding the most valuable pearls, Trematodes are one cause, if not the exclusive cause, of pearl-formation. To what extent other parasites are capable of producing the same effects cannot be said at present. That the other causes to which pearls have from time to time been attributed play any part is a matter of the merest conjecture only, and has never, so far as I know, been demonstrated by experiment or investigation.

Possibilities of Economic Application.

The bearing of the facts recorded in this paper upon the problem of artificially producing pearls, and so meeting the difficulty presented by the increasing demand and exhausted fisheries, is obvious. It was indeed with the hope of throwing some light on this matter that I first took up the subject, about three years ago. The key to the realizing of this, so often regarded as an academic dream, lies obviously in the scientific study of the parasites which occur in the valuable forms. This was pointed out exactly fifty years ago by Filippi, but has been ignored by most subsequent writers.

The life-history of the Trematodes occurring in the genus *Margaritifera* probably agrees in the more essential points with those of other Digenea. Their adult stages may reasonably be expected to occur in the organisms that eat the pearl-oysters, notably such fishes as *Balistes*, while the first host will almost certainly be some mollusc occurring on the pearl-banks or shelling-grounds.

Having ascertained the first host, there is no reason why infection should not be performed by placing young pearl-oysters in company with it in more easily accessible waters. To attempt to establish the cultivation of pearl-oysters on new grounds without also cultivating and infecting the first host of the parasite would be futile. Needless to say, such methods of artificially promoting natural infection would be incomparably superior to any method of pearl-production by *operation* on the individual oyster, as millions of examples could be treated by the former method, while tens were being operated upon.

It is obvious from my Brighton experiment that infection can be induced in *Mytilus*, and I can see no reason to doubt that, in a couple of years, these *Mytili* will contain pearls, resulting from that artificially induced pathological condition.

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EXPLANATION OF THE PLATES.

PLATE XIV.

Mytilus edulis.

Fig. 1. *Mytilus edulis* L., Billiers. Section of a small Pearl, decalcified *in situ*, showing remains of Trematode as nucleus. *ext.ep.*, external epidermis of mantle; *c.t.*, connective tissue; *bl.*, blood-corpuscles; *s.*, epithelium of pearl-sac; *con.*, conchyolin basis of pearl; *con.'*, outermost uncalcified layer of same, attached to epithelium; *cu.*, cuticle of dead Trematode; *ph.*, pharynx, and *dig.*, digestive system of same. × 90.

Fig. 2. The Trematode larva in the connective tissue of the mantle, prior to formation of sac. *f.*, fibres of connective tissue; *pr.*, proliferating cells which ive rise to the epithelial sac; *cu.*, cuticle; *dig.*, digestive cæca; *ex.*, excretory organs, and *pa.*, parenchyma of the parasite. Other figures as above. × 130. The section passes between pharynx and ventral sucker. The ventral surface of the parasite is turned towards external epidermis.

Fig. 3. The cells of the proliferating epithelium which is destined to become the sac (*cf.* fig. 12). *nu.*, nuclei with chromatine reticulum; *c.t.*, connective tissue.

Fig. 4. Cells of the fully-formed sac which surrounds the Cercaria in fig. 2. *c.t.*, underlying connective-tissue cells and fibres.

Plate XV.

Fig. 5. The sac fully developed around the larva, which is cut through ventral sucker. *s.v.*, rudimentary seminal vesicle at base of penis; *c.m.*, circular musculature; *l.m.*, longitudinal musculature; *skr.*, ventral sucker. Other letters as in previous figures. × 130.

Fig. 6. The Cercaria as it occurs in *Mytilus*. From a specimen stained *in toto*. *cu.*, cuticle; *m.*, mouth; *a.s.*, anterior sucker; *p.s.*, posterior sucker; *ph.*, pharynx; *s.gl.*, salivary glands; *œ.*, œsophagus; *dig.*, digestive cæca; *ex.*, excretory system; *ex.p.*, pore of same; *pe.*, penis; *te.*, testes; *v.d.*, vasa deferentia. × 130.

Fig. 7. The same, in longitudinal section. *pa.*, parenchyma; *pa.n.*, nucleated subcutaneous layer of same; *int.ep.*, intestinal epithelium, in surface view. *g.p.*, genital pore; *c.m.*, circular musculature; *l.m.*, longitudinal ditto' *m.f.*, muscle-fibre in parenchyma; *n.c.*, supra-œsophageal nerve-commissure' Other letters as in fig. 6. × 130.

Plate XVI.

Fig. 8. Dead Cercaria in *Mytilus*, with three centres of calcification. × 130.

Fig. 9. Section of the muscular mantle-margin of *Tapes*, showing the Sporocysts with contained Cercariæ. *m.*, musculature of *Tapes*; *sp.*, sporocyst; *cer.*, Cercaria; *ex.*, excretory tubes; *dig.*, digestive cæca; *pe.*, penis *s.*, ventral sucker of same.

Fig. 10. Pressure preparation of the Cercaria from Sporocyst in *Tapes*. The specimen was examined alive, so sexual organs are not visible. *e.*, eyes; *t.p.*, tactile papillæ; *dig.*, digestive cæca; *ex.*, excretory system. × 700.

Fig. 11. The adult worm from *Œdemia nigra* L., River Villaine. *s.v.*, seminal vesicle; *ov.*, ovary; *ut.*, uterus; *g.p.*, genital pore. Other letters as in fig. 6. Stained *in toto*.

Plate XVII.

Fig. 12. *Mytilus edulis* L., Piel, Lancashire. Photo of a thin slice or "Schliff" through centre of pearl, showing calcified Trematode as nucleus. × 25.

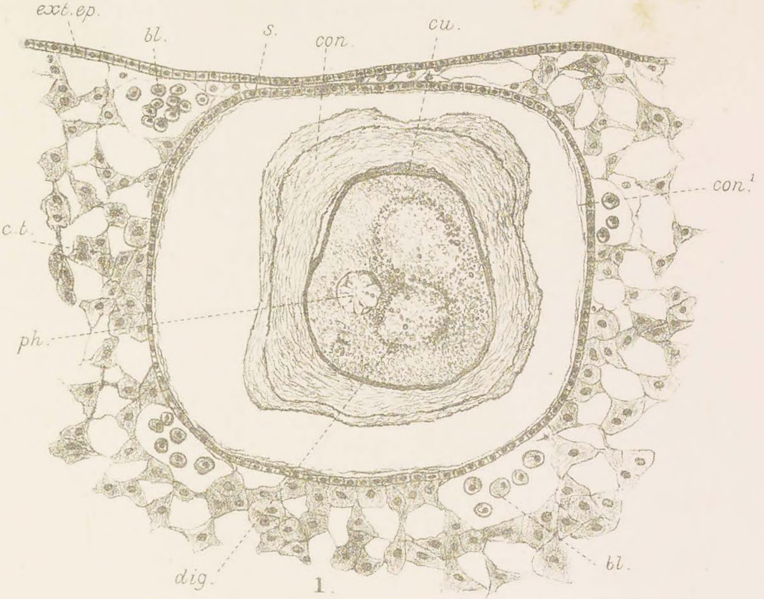
Fig. 13. Ditto. Pearl formed in sac vacated by Trematode. A few granules of residual matter have calcified to form nucleus. × 25.

Fig. 14. Ditto, showing several centres of calcification in the Trematode. × 25.

Fig. 15. Ditto, double pearl, one of constituents being formed around Trematode, the other as in fig. 13. × 25.

Fig. 16. Pearl from *Margaritifera margaritifera* Linn., New Guinea, showing imperfectly calcified Trematode, with radially arranged prisms. × 25.

P. Z. S. 1902, vol. I. Pl. XIV.



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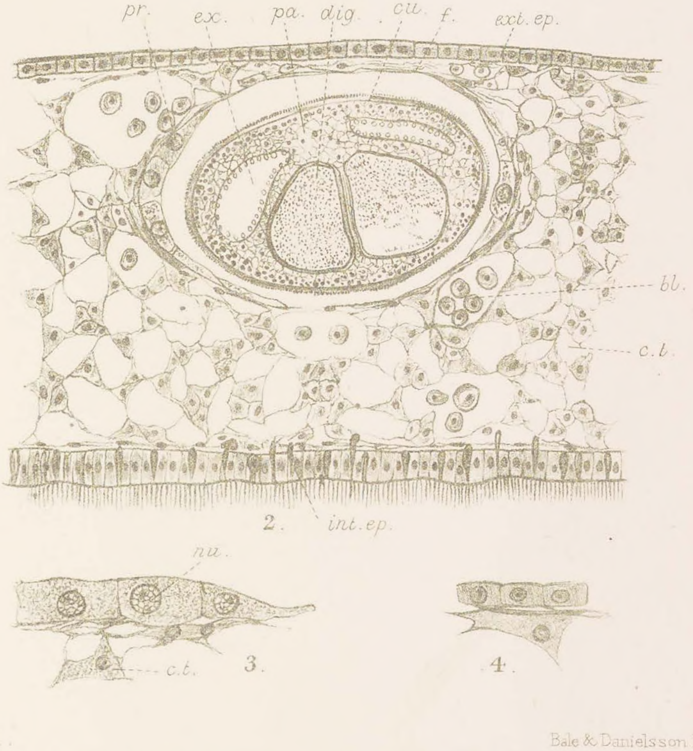

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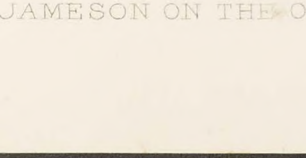

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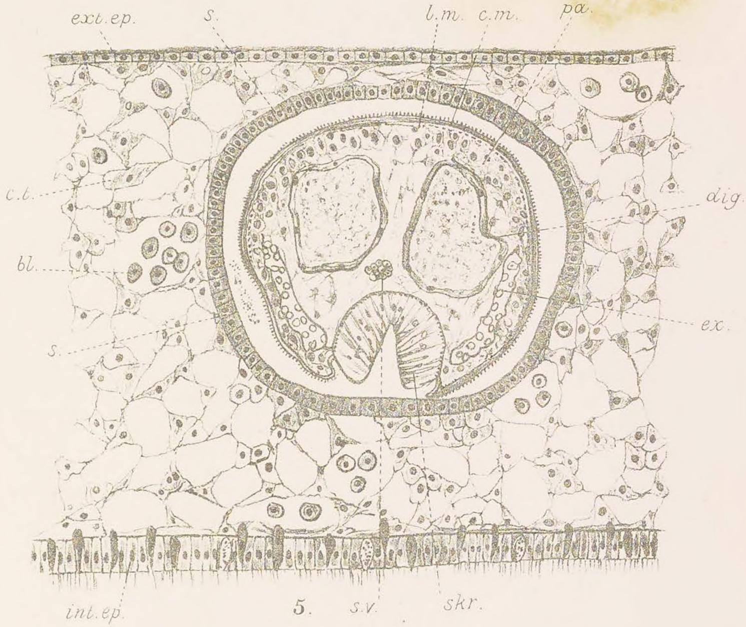

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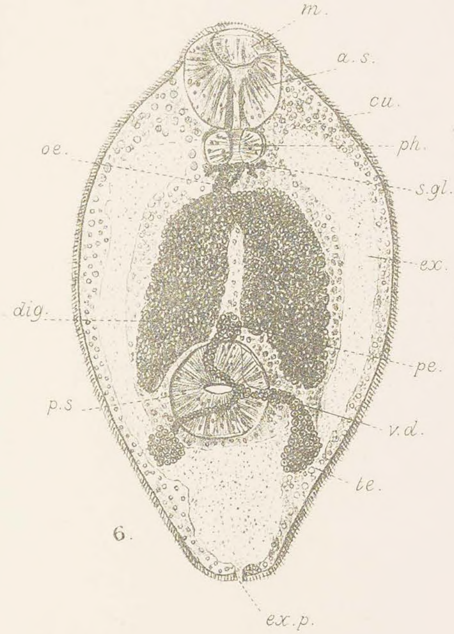

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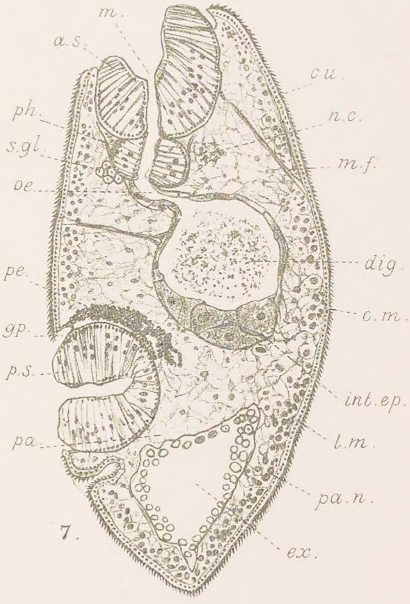

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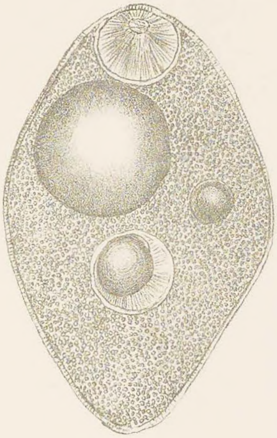

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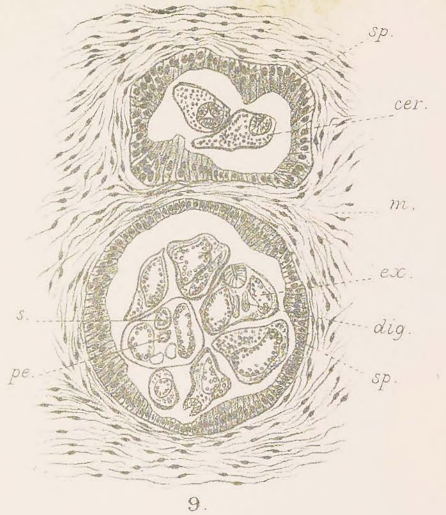

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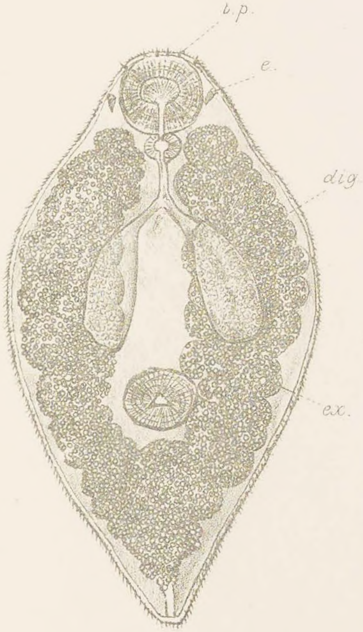

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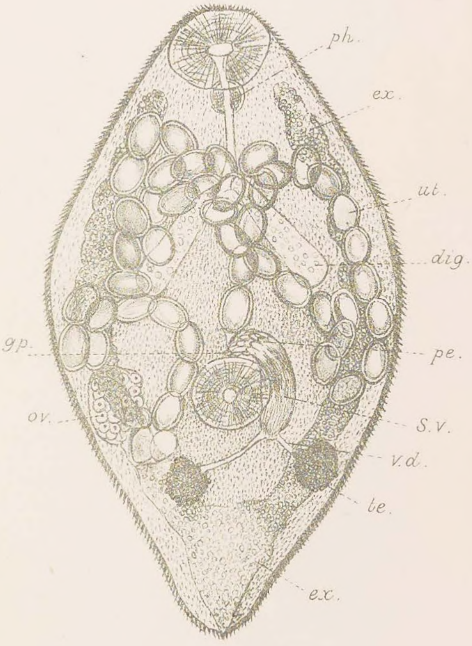

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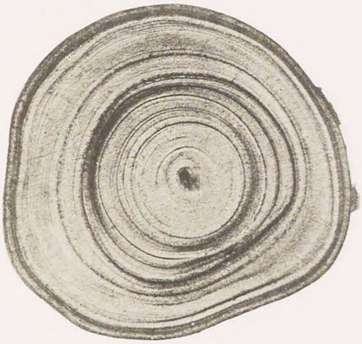

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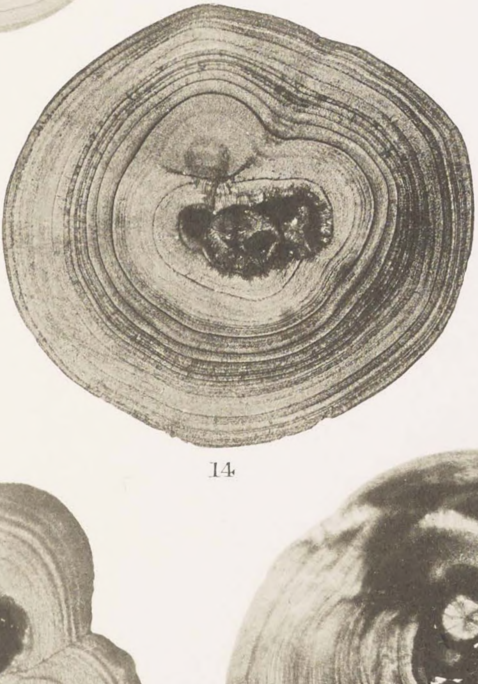

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