Studies on the Cultivation of Pieces of the Mantle of Modiolus modiolus.

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With Plates 29 and 30 and 5 Text-figures.

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I. INTRODUCTION AND PREVIOUS WORK.

Comparatively few papers have appeared on the cultivation of invertebrate tissue in artificial media, and of these the majority deal with the Mollusca.

Krontowsky and Rumianzew (1922) record the migration of lymphocytes from tissue of Anodonta cultivated in agar and tissue extract, and Zweibaum (1925 a) has cultivated the ciliated epithelium of the gills of Anodonta in modified Ringer. The explants of the gills were very active for some days, and those kept in diluted Ringer survived for 63 days; they tended to fragment into two or three smaller pieces, and to become spherical in 4–6 days. Pieces of gill, one to two millimetres in length, became more or less fixed in the medium, and after 24 hours an outgrowth of lymphocytes took place. In another paper Zweibaum (1925 b) states that the cut edges of the explant often come in contact and unite. The cells on the outside become regrouped, so that a spherical body is formed which is covered, wholly or in part, by cilia; the cells on the inside are not arranged in any order. There is a multiplication
of epithelial and lymphatic cells, in the interior of the spheres, which persists until the sixth day and occasionally until the tenth day. Migration of epithelial and connective tissue cells was not observed. Janda and Bohuslav (1934) attempted to cultivate pieces of the intestine of Anodonta; they found that the cells did not multiply, but maintained their normal size and number for a considerable period.

Cary (1931) states that active cultures were obtained from the gonads, liver, kidney, and gill of Ostrea argus, and from the cerebral and pleural ganglia, and the margin of the mantle of Strombus gigas. He does not give any details of the type of cells in the outgrowths. Federow (1933) obtained an outgrowth of nerve-fibres from fragments of the optic lobes of the Cephalopod, Rossia gulaeopios. Bohuslav (1933a), working on three species of Helix and on Arion empericornum, obtained cultures of the seminal vesicles which showed good growth. Preparations of the salivary glands underwent degenerative changes in a short time, although explants from the ducts gave an outgrowth of thick epithelial membranes. Explants from the wall of the stomach gave rise to metaplastic cysts of epithelial origin. Bohuslav concluded that, in general, the type of growth corresponded to that of vertebrate cultures, although typical fibroblast formation occurred only in preparations of the seminal vesicle, and that the greatest potential growth was in the epithelial elements. In a later paper Bohuslav (1933b) records the migration of amoebocytes and an outgrowth of connective tissue from pieces of the heart of Helix pomatia. Koníček (1933) also obtained a migration of amoebocytes from explants of the heart of Helix pomatia. Pieces of the mantle gave rise, at first, to an outgrowth of many kinds of cells which later degenerated leaving chiefly amoebocytes and mucoid cells; this was followed by a growth of connective tissue.

Gatenby (1932) describes the outgrowth of many types of cells from pieces of the roof of the mantle of Helix aspersa. There is, at first, a migration of amoebocytes, which is followed by sheets of epithelial cells and wandering cells other than amoebocytes. The epithelial cells are from the layer next to the shell, and continue to secrete crystals. Later, bladder-like out-
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growths, which contain several types of cells, occur around the explant; they become hollow with muscle-fibres on the inside. In about 20 per cent. of the preparations flat tail-like outgrowths, composed of connective tissue and epithelial cells, are present. This contribution was followed by a series of papers by Gatenby and his collaborators which contain further observations on the behaviour of the outgrowths and details of the structure of the cells.

So far as the author is aware no attempt has been made to cultivate the tissues of Lamellibranchs other than those of Anodonta and Ostrea. The behaviour of explants of Anodonta, as described by Zweibaum, do not appear to be in any way comparable to that of cultures of vertebrate tissues, while Janda and Bohuslav did not obtain cell multiplication in explants from the intestine. The writer concludes, therefore, that, except for Cary's work on Ostrea, and with the possible exception of Krontowsky and Rumianzew's preparations and of Zweibaum's cultures, in which a migration of lymphocytes took place, no true growth has been obtained from Lamellibranch tissue.

It occurred to the writer that the cultivation of the tissues of marine invertebrates, using sea-water as a medium, might not present so many difficulties as with terrestrial forms. In view of the success which has been obtained with certain Molluscs, it was decided to experiment with the mantle of Mytilus. Preliminary investigations were carried out with pieces of the mantle of Mytilus, and a migration of ameobocytes and, in some cases, an outgrowth of epithelial cells was obtained in sea-water, and in sea-water and tissue extract. The work on Mytilus, however, was abandoned as at the time of year at which the investigations were begun the mantle contained large quantities of reproductive elements, and these not only made observations difficult but hindered growth. Modiolus was finally chosen as the mantle was free of reproductive cells, and the animals could be collected in large quantities locally.

The use of sea-water as a culture medium has not been extensively investigated. Lewis (1916) working on the hermit crab obtained outgrowths using 90 per cent. sea-water with the
addition of 10 per cent. crab broth; an outwandering of cells also took place from pieces of Limulus tissue grown in a similar medium. Cary (1931) experimented with sea-water as a medium for the cultivation of the tissues of marine invertebrates, but states that growth appeared to be most active in a modification of Goldschmidt's solution. The addition of 5–10 per cent. Strombus broth increased the rate and extent of cell multiplication and migration.

The present paper deals with technique and with observation on the type of outgrowth obtained from explants of the mantle of Modiolus. The structure of the cells is described as seen in living preparations and by ordinary methods. It is hoped to record the findings of a cytological investigation of the cells of the outgrowths in a future paper.

II. MATERIAL AND METHODS.

The material for the present investigation was obtained from the mantle of Modiolus modiolus, collected at low tide on the Firth of Forth. Most of the tissue was obtained from individuals which had been recently collected, but, owing to unsuitability of the tides, it was desirable to keep a stock in the laboratory. Specimens were kept in a large tank to which fresh sea-water was added from time to time. The animals remained in a healthy condition for considerable periods, but only in a few cases were cultures made from individuals which had been in captivity for more than one month.

The preparations were made under strictly aseptic conditions. The glass ware and knives were sterilized in an air oven, and all other instruments in an instrument sterilizer. Cultures which had not been rendered sterile contained, by the second or third day, numerous bacteria. In these cultures the amoebocytes became rounded and the growth of the epithelial cells ceased. As washing in several changes of sterile sea-water is unreliable, it was decided to expose pieces of the mantle to the action of ultra-violet light. Murray (1931), working on planarians, obtained sterile tissue by exposing the animals for 4 minutes at a distance of 44 cm. from the burner of an ultra-violet lamp.
Hill (1934) obtained sterile cultures of the mantle of *Helix* by exposing pieces of tissue for 4 minutes at a distance of 40 cm. Pieces of the mantle of *Modiolus* were removed and placed in sterile sea-water in watch glasses contained in Petri dishes. The glasses were then placed 44 cm. from the burner of an ultraviolet lamp for 5 minutes, the pieces being turned after 2½ minutes. The tissue was transferred to several changes of sterile sea-water, and subsequently cut up into small pieces under a dissecting binocular to which a glass cover was fitted to prevent bacterial infection. Cultures were made by the hanging-drop method and the preparations sealed with paraffin wax. Only in a few cases were cultures prepared in this way non-sterile.

Some of the first preparations were set up in sea-water. Outwandering of amoebocytes took place, and in some cases an outgrowth of epithelial cells was obtained. It was found, however, that more extensive outgrowths of epithelial cells were obtained in a medium of sea-water with the addition of tissue extract. It was finally decided to use a modification of the medium described by Lewis (1916), substituting tissue extract for crab broth. The tissue extract was obtained from pieces of gonad and muscle of *Modiolus*, and was used in the proportion of ten parts to ninety parts of sterile sea-water. The sea-water was rendered sterile by autoclaving, and was subsequently stored in sealed glass vessels until required for use. The tissue from which the extract was made was exposed to the action of ultra-violet light, washed in several changes of sterile sea-water, ground up in sea-water, and finally centrifuged. The pH of the medium was adjusted to approximately 8·0, which is that of the water in which the animals live.

I spent the greater part of the summer vacation of 1935 at the Strangeways Laboratory, Cambridge, in order to learn the technique of tissue culture, and to carry out some preliminary work on the culture of invertebrate tissues. It is, therefore, a great pleasure to express my thanks to Dr. Honor B. Fell for granting me facilities to work and for her help during my stay at Cambridge. I also wish to thank Dr. Fell for reading the manuscript of this paper. My thanks are due to Mr. R. J. Fant for taking the microphotographs on Pl. 30. The work on
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III. Observations.

1. The Epithelial Cells.

A preliminary investigation of the first successful preparations showed that, in many cases, the explant, or part of the explant, was surrounded by an outgrowth of elongate epithelial-like cells many of which contained large globules similar to those present within the cells of the epithelium lining the shell. These cells grow out as a sheet which is continuous with the cells of the epithelium which secretes the shells (figs. 11-15, Pl. 30). As the result of the examination of both living and fixed preparations it was concluded that the cells are cells of the shell epithelium, which become elongate, grow out into the medium, and form a solid sheet of tissue. This type of growth was present in a certain number of preparations only. That the medium was not at fault was shown by the outwandering and behaviour of the amoeocytes in practically all the cultures irrespective of the outgrowth of the epithelial cells. Further investigations showed that an outgrowth of epithelial cells never took place from explants which were placed so that the layer of ciliated epithelium was uppermost, i.e. next to the cover-slip. It was subsequently found that if the shell epithelium was placed next to the cover-slip growth was obtained in practically all the preparations. Very often, however, the layer of ciliated cells becomes stretched and folded over the edge of part of the explant; growth never takes place in these regions. It is usual, therefore, to obtain cultures showing two or more regions of growth separated by areas in which growth has been prevented by the stretching and folding of the ciliated cells. It is evident that, in order to have successful outgrowths, a surface must be present such as that of the glass cover-slip to which the cells may readily adhere, and that the edges of the explant be free of ciliated cells.

The outgrowth is often present on the day after the preparations are made, i.e. in 24 hours or less, but frequently the only
change recognizable after a period of 24 hours is the elongation, towards the medium, of the epithelial cells at the edge of the explant. There is thus a variation in the actual time at which an outgrowth is visible.

An examination of a large number of cultures, at periods up to 96 hours after the preparations were set up, shows that growth takes place in the following manner. There is an elongation of the cells of the shell epithelium at the edge of the explant. The peripheral part of each cell is composed of hyaline cytoplasm, which may contain a few small globules or granules, but never large globules such as may be present in the middle and basal regions. The peripheral part stretches into the medium while the rest of the cell remains, for some time, in contact with the explant. This condition was frequently observed in 24-hour cultures. The periphery of the cell is drawn out into a number of fine hyaline processes which are often in contact with amoebo-cytes, and in the later stages, at any rate, appear to be firmly attached to the cover-slip. The region of growth in older cultures extends for some distance into the medium. The cells are closely packed together and form a fairly compact sheet (figs. 11-15, Pl. 30; Text-fig. 1). The cells are not arranged in definite layers, but, as a measure of the extent of the growth zone in young cultures, it was found that from its outer limit to the edge of the explant it was composed of two to four cells placed end to end. As in the earliest stages, the cells at the periphery of the zone of growth are provided with fine hyaline processes. In older cultures the growth region is often composed of about five to six cells placed end to end, but the extent of the outgrowth usually varies considerably in different regions of the same culture. The outgrowths also vary from preparation to preparation; thus in many cultures the extent of the zone of growth at about 48 hours was equal to that of 72 hours, or more, in other preparations (figs. 11-15, Pl. 30).

The most active growth takes place between about 24–72 hours; it may, however, continue and the cells, apparently, remain healthy for several days after this period, but for the purpose of the present paper cultures older than 96 hours are not described.
The foregoing description is based on the investigation of living cells; further details were obtained by the examination of fixed and stained preparations. There is considerable variation in shape and size amongst the cells present in a culture, and also in the proportion of cells of any one type in different preparations. In many cases the majority are columnar in shape, while others, especially those situated towards the periphery of the growth zone, possess expansions of clear cytoplasm at their distal ends. Often the cells situated in the immediate vicinity of the explant are less elongate. Large cells, which are irregular in shape and contain large globules, are situated in small groups amongst the elongate cells. In many cultures three types of epithelial cells, arranged in groups, may be recognized. In parts of the outgrowth the cells are elongate and closely packed together. Their nuclei contain one to two deeply stained nucleoli and granules of chromatin (fig. 6, Pl. 29). The cells towards the periphery of these regions are larger and have larger nuclei, while those situated at the edges of the growth zone have fine processes extending into the medium. In other regions many of the cells are less elongate and more irregular in shape, and possess oval or rounded nuclei (fig. 3, Pl. 29). Those situated at the periphery are provided with fine processes similar to those of the more elongate cells. Large irregularly shaped cells almost completely filled with globules occur in groups (fig. 7, Pl. 29).

The fine processes of the peripheral cells, as seen in fixed
material, are composed of hyaline cytoplasm. They originate from an expansion of clear cytoplasm which may contain a few small granules (figs. 3 and 7, Pl. 29).

Nuclei in undoubted stages of mitosis or of amitosis were not observed, but a few nuclei which appeared to be in the early prophase were present in some of the fixed preparations; they were very faintly stained and could not be interpreted with any certainty.

Another type of growth was frequently observed within the explant. Folds are often present on the surface of the explant; these result in the formation of hollows which are surrounded by shell epithelial cells on a different plane and in contact with the cover-slip. The epithelial cells become elongate and grow towards the hollows. On viewing, with the low power, an explant in which this type of growth is taking place, a number of centres of growth can be recognized, each of which is directed towards a hollow. These observations were carried out on living preparations only, but the changes in the form and structure of the cells appear to be similar to those taking place at the edge of the explant, except that cells with large globules are more numerous. It was possible to trace, with greater ease than in the outgrowths at the edge of the explant, the transition between the unaltered cells of the shell epithelium and those of the zone of growth.

2. The Amoebocytes.

A large number of amoebocytes are always present around the margin of the explant a few hours after the cultures are made (figs. 8, 9, and 10, Pl. 30). Amoebocytes make their appearance in the medium of some preparations in less than an hour, while in others they do not occur outside the explant for from 1 to 3 hours. Migration takes place in all cultures irrespective of whether the tissue is placed with the ciliated cells or the shell epithelium next to the cover-glass. If the explant is placed with the ciliated cells next to the glass, and the action of the cilia is strong enough to cause it to move, or to set up violent currents in the medium, then the amoebocytes do not adhere to the cover-slip, are rounded, and usually collect in large clumps. In cultures
in which strong currents are only present in certain regions, it is usual to find rounded cells, which are often clumped, in the vicinity of the currents, while in other parts of the preparation the amoebocytes are active and behave in the normal manner presently to be described. The majority of the preparations were not kept for longer than 5–6 days, a few, however, were left for from 10 to 12 days; after this period the amoebocytes were still active. In cultures which were undergoing degenerative changes, due to the action of bacteria or to other causes, the amoebocytes became rounded and were usually collected in clumps.

Observations on cultures, immediately after they are made, show that the outwandering of the amoebocytes takes place in the following manner. Amoebocytes within the explant can be seen undergoing changes of shape, and moving to the layer of tissue in contact with the cover-slip, and towards the edge of the explant. During these movements fine pseudopodim-like processes, composed of hyaline protoplasm, are formed and become attached to the cells of the mantle or to the cover-slip. Later, the cytoplasm moves forwards towards the pseudopodia and the cell takes up a new position. Movement does not always continue towards the edge of the explant, but ultimately most, if not all, of the amoebocytes leave the mantle and wander out into the medium. On reaching the medium the cells, by means of their pseudopodia, become attached to the cover-slip and move rapidly away from the explant; a few, however, may move back towards the mantle (figs. 8, 9, and 10, Pl. 30). The amoebocytes spread through the medium, but are most numerous within approximately 0.5 mm., or in some cases 1.0 mm. of the explant.

An examination of living preparations showed that the amoebocytes are composed of endoplasm, which may contain granules and vacuoles, surrounded by a thin layer of hyaline ectoplasm. The ectoplasm is capable of forming membrane-like expansions around the margin of the cell from which fine hyaline processes, or pseudopodia, originate (figs. 1 and 2, Pl. 29; Text-figs. 2–5). Occasionally a few small granules may be present in the expansions at the base of the pseudopodia.
The appearance of fine processes may sometimes be due to folds, or to optical sections of the membrane, but careful observations show that true processes are also present.

The amoebocytes are either rounded, or elongate and irregular in shape; the endoplasm of the irregularly shaped cells may be hyaline, but is usually finely or coarsely granular. The endoplasm of the coarsely granular cells contains clear vesicles and sometimes more solid bodies. Large vacuoles and bodies are absent from the finely granular amoebocytes, but cells with finely granular cytoplasm and containing a few small vacuoles or vesicles are present in some of the cultures. Finely granular and hyaline cells were kept under observation, and the changes in their contents noted. For instance, in one case, an irregularly shaped cell, when first observed, contained finely granular endoplasm and four small vesicles. After half an hour the vesicles were slightly larger, and the granules had increased in number. In about an hour the vesicles had increased greatly in size and in number, while granules and small vesicles were scattered through the endoplasm. Owing to the movement of the cells the vesicles may disappear from view and reappear.

**Text-Figs. 2-5.**

Living amoebocyte, drawn at intervals of approximately 15 minutes.

N., nucleus; V., vacuole.
again suddenly. Consequently it is difficult to determine the manner in which the vesicles arise. It is probable that material is taken up from the medium and, if in the form of solid particles, is at first visible as granules or larger bodies; later, these become dissolved and converted into vesicles. Further evidence in support of this view was forthcoming as the result of observations on fixed and stained cultures. In this material the granules are usually faintly stained, but the large bodies vary in intensity. The latter are sometimes deeply stained and homogeneous, but are often faintly stained and contain clear spaces and dark granules (fig. 4, Pl. 29). The bodies are frequently surrounded by a clear space, while some of the cells contain vesicles with deeply stained rims. It appears, therefore, that when the large bodies undergo changes prior to their disappearance small clear areas and, later, a clear central region is formed. The unstained central area spreads towards the periphery, producing a vesicle with a deeply stained rim; finally the rim disappears leaving a clear vesicle or vacuole. As the result of these observations it is concluded that the hyaline and finely granular amoebocytes are converted into coarsely granular cells by the accumulation of material engulfed from the medium.

The nucleus is sometimes visible in the living cell, but the presence of vesicles and other bodies in the endoplasm renders its detection difficult. In fixed cells the nucleus is usually rounded, but sometimes is elongate. The chromatin of the rounded nuclei appears to be in the form of granules or small masses, which are clumped in the central region, while granules are present on the inside of the nuclear membrane (figs. 4 and 5, Pl. 29). In the elongate nuclei the chromatin in the central region is not so closely clumped, but consists of granules arranged in rows, and sometimes of threads or rods. Granules are present on the inside of the nuclear membrane. The variation in the shape of the nucleus is probably due to pressure from the surrounding structures.

The endoplasm of the rounded cells is granular and contains vacuoles and large bodies; the latter stain in a similar manner to those of the elongate cells. The pseudopodia are usually short and blunt. The nucleus is rounded, and contains chromatin
clumped in the central region and granules on the inside of the nuclear membrane.

Several elongate cells, kept under observation for long periods, were seen to undergo rapid movements and changes of shape; they often became spherical or rounded, and later were elongate again (figs. 1 and 2, Pl. 29; Text-figs. 2-5). As these changes are constantly taking place it is evident that only one type of amoebocyte exists. However, when old cells become packed with vacuoles and other bodies they become rounded, and, although short pseudopodia are present, they do not appear to become elongate again. Such cells are often present amongst those situated farthest from the explant, but may also be present in other parts of the medium.

Movement takes place in the following manner. Pseudopodia are formed, usually at one side of the cell, and this is followed by a flow of cytoplasm towards the pseudopodia. The pseudopodia which were already present now become detached from the cover-slip, and the cell undergoes a movement, or contraction, towards the new pseudopodia.

As pseudopodia are constantly being formed, movement in any one direction does not continue for long. The end of a pseudopodium, in contact with the glass, often spreads out to form a broad terminal region, from which fine processes usually originate. The pseudopodia frequently become attached to adjacent cells, and may be very long and fine with one or two small thickened areas (fig. 5, Pl. 29). When the tension becomes too great they lose their connexion and rapidly contract towards the cell. A blunt projection usually remains for some time marking the place from which the pseudopodium originated. Several cells may come in contact, by means of their pseudopodia, and form a loose network (figs. 9 and 10, Pl. 30). Sometimes pseudopodia at opposite poles of a cell are firmly attached to the cover-slip, so that the cell becomes very elongate and consists of two terminal expansions and a narrow connecting region (fig. 4, Pl. 29).

Amoebocytes are often more numerous in certain cultures than in others made from the same animal. This is probably dependent on the number present in the explant at the time
when the preparations are made. When very numerous the pseudopodia of adjacent cells come in contact and form a loose network around the explant.

Several clumps of rounded cells are present in some of the cultures. Pseudopodia are absent from the cells in the middle of the clumps, but are present in those situated at the periphery. The pseudopodia of elongate cells may form a network around the clumps. These groups of rounded cells are probably formed by currents in the medium, due to the action of cilia, but in some cases may be due to a large number of cells forming a network and finally clumping.

Stages of mitosis or of amitosis were not observed, but a few cells with double nuclei were present in some of the fixed preparations.

IV. Discussion.

Findings on the method of cell division in invertebrate cultures are somewhat conflicting. Zweibaum (1925 b) states that amitosis takes place and that mitosis seldom occurs in preparations of the gills of Anodonta. Murray (1927), working on planarians, has observed both early and late stages of amitosis in her cultures, and believes that there is no evidence that mitosis takes place. Fischer-Piette (1931) records the occurrence of mitosis in cultures of the lymph gland of Homarus, while Bohuslav (1933 a) believes that both mitosis and amitosis occur in his cultures of molluscan tissue. Gatenby (1932), Gatenby and Hill (1934), and Gatenby, Hill, and Macdougald (1934) describe amitosis in their cultures of the mantle of Helix, and Gatenby and Duthie (1932) state that cell multiplication in cultures and in regeneration of the pulmonary cavity wall is by amitosis.

The writer has not observed definite stages of mitosis or of amitosis, but nuclei in what appeared to be early prophase stages were observed amongst the epithelial cells. It is of interest that Gatenby (1932) also records a few doubtful prophase stages.

The manner of growth of the cultures of the mantle of Modiolus is similar in many respects to that of vertebrate tissue. The outgrowth of an extensive compact sheet of cells, such as that obtained during the present investigations, appears
to be uncommon for the tissues of invertebrates. Bohuslav (1983b), describing an outgrowth of connective tissue from explants of the heart of Helix pomatia, claims that it is the first record of an extensive growth of solid connective tissue from explants of invertebrate tissue.

Zweibaum, as the result of observations on the spheres formed in the cultures of the gills of Anodonta (see p. 659), concludes that there is a well-marked biological difference between the tissues of vertebrates and those of invertebrates, and that cultures of invertebrate tissues constitute a form of vegetative life rather than a true culture.

The writer believes that the results of the work on the mantle of Modiolus show that, given a suitable medium, and freedom from violent currents or movements, it is possible to obtain outgrowths from the tissues of invertebrates which are comparable to those obtained in vertebrate cultures.

The failure of the shell epithelial cells to become attached to the cover-slip and grow out from explants placed with the ciliated cells uppermost, and from the regions where the layer of ciliated cells become stretched over the edge of the mantle tissue, is due in part to strong currents set up in the medium by the action of the cilia. It is evident that violent movements in the medium would prevent the cells of the shell epithelium from growing over or past the layer of ciliated cells and becoming attached to the cover-slip. The currents in the vicinity of the growth zone of preparations placed with the shell epithelium next to the cover-slip would not be so strong. It is probable that gentle currents in the neighbourhood of the outgrowth would be beneficial by continually changing the medium in contact with the cells.

The cells which grow out from the shell epithelium undergo changes of form, so that they become elongate, and the cytoplasm at their distal end becomes clearer and capable of forming fine hyaline processes. It is probable that the cells, as they migrate out from the explant, undergo a process of dedifferentiation, but as sufficient cytological observations have not been made it is not proposed to discuss the matter. It should be pointed out, however, that the presence of globules in some of
the larger cells indicates that the cells of the outgrowth do not lose their function.

The type of growth present at the folds within the explant is different from that which takes place at the periphery. The amount of growth is limited, for in no case was a hollow completely covered over. It is probable, therefore, that in these regions little or no cell division takes place, and that growth consists chiefly of the elongation and spreading out of the cells. It is a favourable region, however, in which to observe the changes in the shape of the cells, as all stages between the unaltered epithelial cells and the elongate cells with fine processes can be identified with ease. Although a solid surface to which the cells may adhere is necessary for the outgrowth at the edges of the explant, such a surface is not essential for the spreading of the cells over the hollows. As the hollows are usually shallow the cells may receive support from the underlying tissue.

Much work has been done on the amoebocytes of invertebrates, but as the present paper is only concerned with their structure and behaviour as seen in tissue cultures it is not proposed to review the literature. References to previous works on amoebocytes will be found in papers by Takatsuki (1934) and by Haughton (1934a).

The outwandering of amoeboid cells from explants of molluscan tissue have been recorded by Bohuslav (1933b), Konštek (1934), Haughton (1934b), Gatenby (1932), Gatenby and Hill (1934), and by Gatenby, Hill, and Macdougald (1934).

As the result of the present investigations the writer believes that the hyaline amoebocytes of *Modiolus* are readily transformed into the granular type, and that the amoebocytes can rapidly alter their shape. Only cells which have become clumped, or necrotic or are densely packed with vacuoles, granules, and larger deeply stained bodies retain their spherical form. That the amoebocytes remain in a healthy condition and retain their normal function in the medium is indicated by their movement and by the accumulation, as the result of their phagocytic activity, of granules and larger bodies within the cytoplasm.
Goodrich (1919) states that the figures usually given of the pseudopodia of invertebrates as fine tapering processes are in reality drawings of optical sections of membranous expansions of cytoplasm which thin out peripherally to a very fine film. He believes that in most invertebrates the membranous pseudopodia are normally expanded, and that fine pseudopodia are not present. The formation of the latter is abnormal, and is due to physico-chemical changes taking place in the fluid. Takatsuki (1934), working on Ostrea, agrees with these findings, and states that amoebocytes are often provided with fine filamentous pseudopodia; these, however, are probably formed as the result of abnormal conditions.

The present writer believes that in the amoebocytes of Modiolus membranous folds of the ectoplasm become spread out to form flat processes, and that fine pseudopodial-like structures may also be present. The latter are well seen in the long processes which sometimes stretch between two adjacent cells, or are attached to the cover-slip. A flow of cytoplasm was observed along these pseudopodia as the result of which the cells underwent rapid changes of shape and position. As the appearance of these formations was not followed by degenerative changes, and as they were absent from necrotic cells, it is concluded that their formation is not due to abnormal conditions.

Gatenby and Hill (1934) believe that the amoeboid cells which grow out and form a network in cultures of the mantle of Helix are identical with the connective elements of the tissue, and that the network seen in cultures is the normal arrangement of the cells in the tissue of the mantle wall. An examination of sections of the mantle of Modiolus showed that numerous cells similar in appearance to the amoeboid cells of the outgrowth were present. Many of these were provided with pseudopodia, while others were rounded. Connective-tissue elements and muscle-fibres were also present. It was concluded, therefore, that a large part of the connective tissue of the mantle of Modiolus is made up of amoebocytes.
V. Summary.

1. Pieces of the mantle of *Modiolus* were sterilized by means of ultra-violet light, and cultivated, by the hanging drop method, in sea-water, and in sea-water plus tissue extract.

2. An outwandering of amoebocytes takes place shortly after the preparations are made; this is followed by an outgrowth from the epithelium which secretes the shell. Three types of epithelial cells are present in the outgrowths.

3. Undoubted stages of mitosis or of amitosis were not observed amongst the epithelial cells.

4. The shell epithelium often becomes folded so that hollows are present on the surface of the explant. The cells at the margin of the folds become elongate and tend to grow over the hollows.

5. The amoebocytes, by means of membranous expansions of the ectoplasm and fine pseudopodial-like processes, undergo movements and change of shape. Clumped and necrotic cells are rounded.

6. Hyaline and finely granular amoebocytes, due to their phagocytic action, become filled with granules, vacuoles, and large deeply stained bodies.

7. The amoebocytes often form a loose network in the medium.

8. Stages of mitosis or of amitosis were not observed, but amoebocytes with double nuclei were present in some of the preparations.

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DESCRIPTION OF PLATES.

LETTERING.

E., explant; F., deeply stained material which has been engulfed from the medium; G., granules in epithelial cells; N., nucleus.

PLATE 29.

Fig. 1.—Living amoebocyte.
Fig. 2.—The same cell after an interval of approximately 10 minutes; it is now in contact with another amoeocyte and has become rounded.

Fig. 3.—Part of outgrowth to show epithelial cells. 96 hours. Bouin.

Fig. 4.—Amoeocyte. Bouin.

Fig. 5.—Amoeocytes in contact by means of pseudopodium. Bouin.

Fig. 6.—Part of outgrowth to show elongate epithelial cells. 48 hours. Flemming.

Fig. 7.—Part of culture to show large epithelial cells with granules. 96 hours. Bouin.

PLATE 30.

Microphotographs.

Figs. 8, 9, and 10.—To show living amoeocytes.

Fig. 11.—Part of outgrowth of epithelial cells. About 72 hours. Bouin.

Figs. 12-15.—Different parts of outgrowth in culture approximately 48 hours old. The cells at certain parts of the periphery of the zone of growth, and the processes extending into the medium, are but faintly stained, and consequently are not well shown. Bouin.