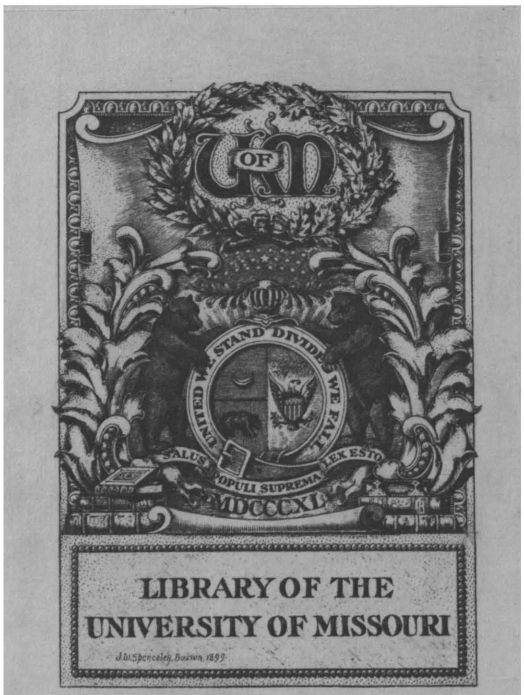

A HISTOLOGICAL STUDY OF THE MARSUPIUM OF THE UNIONIDAE.

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Thirty-seven figures

Four plates

CONTENTS

INTRODUCTION

In connection with the extensive investigation of the Unionidae which has been conducted by the Bureau of Fisheries for the past four years, with a view to determining the feasibility of artificial propagation of the mussel on a commercial basis, it was thought worth while by the writer to make a careful study of the marsupium of a large number of genera, including as many as possible of the distinctive types, as classified by Simpson ('00) in his "Synopsis of the Naiades".

The method of this investigation has been to study ma-

terial taken at various intervals, beginning with the marsupium in a pre-gravid condition, and progressing through the various stages of gravidity to the post-gravid condition. In this manner, it has been found possible to determine just what histological changes occur in the epithelial lining of the water tubes, which, as has long been well known, function as marsupial pouches for the embryos until the latter are discharged as glochidia.

The report as herewith presented is the result of investigations conducted for the Bureau of Fisheries during three summers at the Biological Station, Fairport, Iowa and also at the Marine Biological Laboratory, Woods Hole, Mass.

In presenting this report, it gives me pleasure to acknowledge my indebtedness to Prof. Geo. Lefevre and to Prof. W. C. Curtis of the University of Missouri, under whose direction the work was commenced, when the writer was a student at the above institution. I desire also to acknowledge indebtedness to Dr. R. E. Coker, Director of the Fairport Biological Station, whose unflagging interest in the work has made it possible to carry on the investigation of this subject.

STRUCTURE AND FUNCTION OF THE MARSUPIUM.

The morphological structure and function of the marsupium in the important genera of the Unionidae has been fully stated by Ortmann: "Monograph of the Najades of Penn. 1911", and in this paper I will merely refer briefly to the general nature of the marsupial gill. As was well known before Ortmann's paper, the gill of the mussel is composed of an inner and

outer lamella, connected by interlamellar junctions which divide the interior space into water tubes, which communicate with the water outside by means of small ostia perforating the lamellar surface. The surface of the interlamellar junctions is covered with a layer of glandular epithelium. Into the water tubes of the marsupial gill the eggs are passed, and here develop into the young larval shell, known as the glochiduim.

While Ortmann has touched briefly upon the gross change in the marsupium, he has not worked this subject out in any detail, and his photographs give a very incomplete idea of the conditions existing. Moreover he has not touched upon the histological changes which it is the primary purpose of this paper to set forth.

In the following pages, we shall take up in order each of the type forms studied, discussing in detail, with accompanying plates, both the changes in general structure and the histological changes, emphasizing especially the latter, which have not been heretofore described.

1. Type Homogenae, Form--Unio Complanatus.

The material, from which the study of the marsupium of Unio complanatus was made, was obtained in the summer of 1909, from a fresh water stream near Onset Junction, Mass. Beginning the latter part of June and extending thru August, at which time the females were discharging glochidia, sections from the outer gill, which functions as the marsupium in this genus, were made at right angles to the

long axis of the gill. These were killed in Gilson's Fixing Fluid, and later, sections were made in the medium portion at right angles to the water tubes. These sections were stained with Delafield's Haematoxylin and counterstained with Erythrosin. Examination with both the low power of the microscope and the oil immersion lens reveal certain facts from which the following observations, as to general structure and the histology of the epithelial lining of the water tubes, are based. Careful drawings have been made from camera lucida projections; the low power drawings, showing the general structure of the water tubes, with a magnification of 87 diameters, the high power drawings, showing the histology of the epithelium of the water tubes under the oil immersion lens, magnification of 475 diameters.

General Structure.

I shall describe first, the changes which take place in the general structure of the marsupium beginning with the pre-gravid condition, thru the various stages of gravidity to the post-gravid condition. While no startling changes have been observed, certain interesting modifications take place which I shall now indicate below.

Fig. 1 in Plate I shows a section in the pre-gravid gill, killed in late June, before any eggs had been passed into water tubes. The water tubes are small and show the epithelial lining thrown into deep convolutions. This latter fact is of significance in relation to the changes which later take place. The interlamellar partitions, separat-

ing the water tubes, are extremely thick, being nearly twice the width of the water tube itself. Numerous blood vessels are shown in intimate contact with the lamellar folds.

Fig. 3 shows a section made from a gill, into which the eggs have recently been passed, the embryos showing an early cleavage stage. The most superficial study will show that considerable change has taken place in the general structure of the water tubes , in a very short space of time, since the eggs have been in the gill only a few days. We can understand now, the significance of the deep folds into which the epithelial lining of the water tubes is thrown in the pre-gravid gill. As seen in Fig. 3, the water tubes are greatly enlarged in size as we should expect to find them as they are now gorged with embryos. While in Fig 1 the water tubes show a width of 2 c.m. and a breadth of ½ c.m., in Fig. 3, the width is 7 c.m. while the breadth is 2. Further, as a natural consequence of this enlargement in size of the water tubes, the interlamellar junctions, which were so thick in Fig. 1, have become much drawn out, are thinner, and the folds of the epithelial lining are tending to disappear. In addition, even under the low power magnification, we are able to find traces of the appearance of differentiated cells in the epithelium. Fig. 3 also shows clearly the presence of ostia connecting the water tubes with the outer lamella.

Our next section, as seen in Fig. 5, is made in a later

stage of the development of the embryo, which is now in the early gastrula. Considerable changes have taken place in the appearance of the water tubes, the most striking of which, as we should expect, being in the interlamellar junctions. These are now extremely thin and all convolutions of the epithelial lining have practically disappeared, although the actual size of the tube itself shows no great change over the preceding section. Another striking change is the presence, in greater number and increasing size, of the differentiated cells, which we began to observe in the preceding section, in the epithelial lining of the water tubes. These are the most striking changes in the general structure which this section shows.

The next section studied, as seen in Fig. 7, shows the water tubes at a much later stage in the development of the embryo, which are now in the glochidial stage. Although the drawing shows only a few of the glochidia, the section shows the water tube completely filled with them, together with a few embryos which are not so fully developed. We will observe that the stretching of the interlamellar junctions shows no appreciable advance over the preceding stage although the water tubes are slightly larger than when the marsupium was filled with embryos in early gastrula. Our attention, however, is immediately attracted to the large vacuoles in the glandular epithelium which, even under the low magnification, present all the appearance of mucus vacuoles. These are now very large and very numerous, being the most conspicuous feature of the interlamellar junction.

Numerous sections, made from a large amount of material, taken at about this stage, all present practically the same appearance as that of the water tubes, shown in Fig.7.

All during the latter part of the month of August, the gravid females are found discharging their glochidia from their water tubes, thru the supra-branchial cavities, and to the outside, thru the excurrent siphon.

As a result of this discharge of their contents, the tension on the walls of the water tubes is removed and we should naturally expect a diminution in size. Fig. 9 shows a section thru such a gill, from which the glochidia have just recently been discharged. Our attention is at once called to the striking similarity existing between the condition of the water tubes in the post-gravid and pre-gravid gill. As in the pre-gravid gill, we find small water tubes, with thick interlamellar junctions, and the epithelial lining of the water tubes thrown into deep folds, these being even more conspicuous than in the pre-gravid gill. All of the above changes may be easily explained on a purely mechanical basis, since we should expect an expanded tissue to relax when cause of that expansion is removed. Without doubt the most significant change that has taken place is the complete disappearance of the large vacuoles in the layer of glandular epithelium, lining the water tubes. As seen under low power of magnification, absolutely no trace of such vacuoles are to be found. We must conclude, therefore, that the contents of these differentiated cells are discharged, previous to the post-gravid condition, in all probability, at the time when the embryos are expelled

from the water tubes.

Histology.

Having discussed the changes in general structure which occur in the marsupial water tubes during the gravid period, we are now in a position to study, in detail, those histological changes which our previous study has clearly shown us must take place. The structure of the epithelial lining of the water tubes is well known and presents no unique features. In their article in the "Journal of Experimental Zoology", Vol. IX,No.1, entitled "Reproduction and Parasitism of the Unionidae", Lefevre and Curtis have discussed, in detail, the histology of the marsupium. No work, however, has been done upon those evident changes which take place in the epithelium, producing the development of the large mucus cells, and I shall now attempt to describe, in as much detail as is possible, these histological changes. All of this material was studied under the oil immersion lens with a magnification of about 475 diameters.

As stated by Lefevre and Curtis, the glandular epithelium is found chiefly on the surface of the interlamellar junctions, resting upon a base of connective tissue and smooth muscle fibers. Fig. 2 shows a small portion of this glandular epithelium, thrown into conspicuous ridges. Two of the cells show the presence of vacuoles, filled with a clear mucus-like, colorless fluid. The nuclei are large and conspicuous and show no trace of karyokinetic changes. The cells of the epithelium are practically uniform in size.

Our next drawing, Fig. 4, shows a corresponding portion

of the epithelium of the water tube, when the embryos are in the early cleavage. Marked changes have taken place in the epithelial cells, which show differentiation. While some of the cells show no increase in size, the majority are considerably larger, some being almost completely occupied by the vacuoles containing the mucus-like fluid. Within the connective tissue, can be seen a smaller and darker nucleus, which is probably the nucleus of a leucocyte. My observations on this point verify the conclusions of Lefevre and Curtis, who say "There can be little doubt that the epithelium becomes infiltrated with wandering blood cells, from the underlying sinuses in the interlamellar junctions. Many indications are present that seem to show that these cells actually wander thru the epithelium into the cavities of the water tubes, but what their ultimate fate is, if this be the case, we are as yet unable to say. Possibly they may be ingested by the glochidia and used as food". We find numerous instances which would verify the above. Although we have not actually found specimens which show the presence of the leucocytes penetrating the epithelium into the cavities of water tubes, my material does show what appears to be ingested leucocytes in the sections of the glochidia in the early stage. However, I have not yet absolutely demonstrated the fate of the leucocytes.

The next drawing, Fig.6, shows the condition of the epithelium in the early gastrula stage of the embryo, Fig. 5. The only distinct change from the preceding stage consists

in the lowering of the epithelial ridges, the cells now resting flat upon the base of muscle fibers and connective tissue.

A careful study of material shows no appreciable histological change, until we come to the glochidial stage. Reference to the low power drawing, Fig. 7, will show, even under the magnification of 52 diameters, the very conspicuous mucus cells of the epithelium. When magnified 475 diameters, as shown by Fig. 8, very interesting changes are seen to have occurred. The cells are enormously enlarged and almost completely filled with the mucus vacuoles. A single cell is now as large as one of the complete ridges shown in Fig. 2. Further, the nuclei show a considerable increase in size and we find the nuclei of several leucocytes, both within the connective tissue and in the mucus cell itself. The above appear to be all of the changes which take place in the glandular epithelium. There has been no proliferation of the epithelium at all, so far as we have been able to observe. In Fig. 10, we have a return to practically the same condition of the epithelium as seen in Fig. 2, in the pre-gravid gill. This drawing shows a portion of the epithelium in the post-gravid gill, when all of the glochidia have been discharged. A very careful study of a large amount of material of this stage, shows no trace whatever of the large mucus vacuoles which were so conspicuous in the preceding stage. The cells show a remarkable decrease in size and as in the pre-gravid stage are thrown into deep folds and ridges. The nuclei have also decreased in size.

Although I cannot state the exact amount of time which has elapsed since the glochidia were discharged from this gill, I am quite certain that it cannot have been very long in the post-gravid condition.

Summary.

The foregoing is the only detailed study which has been made of the development of the large mucus cells in the glandular epithelium of the marsupial water tubes of the Unionidae, the presence of which had been known for a long time. I have shown that beginning with small undifferentiated cells in the pre-gravid condition of the marsupium, thrown into deep folds and ridges, a two-fold change takes place. First, as a result of mechanical process of stretching, due to the action of the introduction of the embryo into the water tube, folds and ridges disappear and the epithelium lies flat upon its base of muscle fibers and connective tissue. Secondly, I have shown the differentiation which takes place, resulting in the production of the enormous mucus cells in the glochidial stage, which disappear absolutely at the time when the glochidia are discharged from the water tubes. Although I have no material which actually shows the discharge of the mucus cells, I have material which suggests it, and I am reasonably safe in concluding that the contents of the mucus vacuoles are discharged with the outgoing glochidia, forming the mucilaginous matrix which surrounds them. We can see no other interpretation of their formation and disappearance. Since the glochidia remain in the water tubes such a short time

after their development, there can hardly be any relation between the presence of these mucus cells and the nutrition of the embryo, although this theory was suggested previous to the time of this investigation. As will be seen, our study of the histology of the marsupium during the various stages of the gravid period has disclosed no startling changes in structure or function. We have been able to demonstrate, however, all the stages in the formation of the large mucus cells and their ultimate fate in disappearing at the time of the discharge of the glochidia. We also believe that we are correct in our interpretation of their function as just discussed above.

2. Type Tetragenae, Form--Quadrula metanevra

The genus Quadrula, of which metanevra is a good example, is, like Unio, a summer incubator. The eggs are passed into the water tubes during May and early June. As early as June 10th, we have found embryos in the gastrula stage; as in Unio, the glochidia discharge from the gill during the latter part of August and early in September. The period of incubation seems to be somewhat longer in Quadrula than in Unio.

Although both summer incubators, Quadrula differs from Unio in the nature of its marsupial modifications, since in this type, all four gills are functional marsupia. In studying this form, sections were made of the water tubes from material taken from both gills at corresponding levels. A careful study of this material has shown that both gills present the same features in the structural and histological changes

which take place during the gravid period, so that the following description will apply to either gill.

When brought into the tanks in the laboratory, Quadrula almost invariably aborts its embryos and in the early stages, as described by Lefevre and Curtis, they are found to be in peculiar flattened masses known as conglutinates, the embryos in this state being firmly bound together by a mucilaginous secretion from the epithelium of the water tubes.

The material, from which the study of the following forms is made, was obtained from the Mississippi river, near the Fairport station during the summer of 1910. The same methods of fixing material, cutting and staining sections, was employed as described for Unio. Sections were made from the gills of the following species--metanevra, plicata, ebena, trigona. Metanevra may be taken as a typical form of this genus, as far as regards the general structure and histology of the marsupium.

General Structure.

Following the plan used in Unio, we shall now describe those changes which occur in the general structure of the water tubes during the gravid period. Although we should naturally expect changes, similar to those which take place in Unio, the two forms being so much alike in the marsupia, we find very interesting differences.

Fig. 11 shows a section taken thru the outer gill of a pregravid female, killed about June 25th. As in Unio, we find the thick interlamellar partitions, the epithelial lining of which is thrown into conspicuous folds. The water tubes here

are relatively larger than in Unio, a given water tube being about the same width as that of the interlamellar partition. A very conspicuous feature of this section is the large blood vessels in the interlamellar junctions. So far, we have noticed no particular differences from the structure of the Unio gill, but there is one very striking difference which exists. This consists of the presence of very obvious mucus cells, in the glandular epithelium, which are as numerous and as large as in the glochidial stage of Unio. Also they seem to be confined to a more restricted area, toward the center of the interlamellar partition.

Fig. 15, shows a section taken at a corresponding level of the water tube, in a gill which contains embryos in early cleavage stage. We do not find here just what we might have expected, if the structural changes are purely mechanical. Although the water tube is now somewhat larger than in the pregravid gill, no such increase in size has taken place, as in the corresponding stages in Unio. The interlamellar partition is somewhat thinner than in the pre-gravid gill, being now about one half the width of the water tube. Further, the epithelial folds are not so pronounced: the large mucus cells are no more numerous than in the preceding stage although they show a small increase in size, not particularly noticeable.

A careful study of material, taken at various intervals in the further development of the embryo, shows no appreciable change until we come to the water tube, containing fully developed glochidia. Reference to Fig. 7, will show that in this stage in Unio, the interlamellar junctions were extreme-

ly thin, almost the entire epithelium consisting of the large mucus cells. A comparison of Fig. 17, with Fig. 7, will show a striking difference. In the first place, the interlamellar junctions in the glochidial stage of Quadrula are practically as thick as they were in the pre-gravid stage, being about six or eight times as thick as the Unio partition. Secondly, the mucus vacuoles are considerably larger than in the Unio section and occupy a specialized area, the epithelium being thrown into very sharp ridges.

Our next figure shows a section thru the post-gravid gill, after the glochidia have been discharged from the water tubes. The condition, as seen here, is similar to that in Unio, though not so pronounced. The water tubes show a decrease in size, with a corresponding thickening of the interlamellar partitions: the epithelial lining of the water tubes is again thrown into deep folds.

Histology.

Our low power study, of the epithelial lining of the water tube in the pre-gravid gill, has shown the presence of numerous mucus vacuoles. When we come to the detailed study of this epithelium, under the oil immersion lens, as seen in Fig. 13, we find that the general arrangement of the cells is quite similar to that of the pre-gravid stage in Unio. The epithelium is thrown into folds, resting upon a base of connective tissue and smooth muscle fibers. The cells here, are about the same size as they are in the corresponding stage of the Unio gill, the principal difference being the presence of numerous and well developed mucus vacuoles. We are not sur-

prised to find this, since we know that, at the time the egg is introduced into the water tube of the gill, a gelatinous secretion of the epithelial cells furnishes the matrix for the conglutinates, as stated by Lefevre and Curtis. This secretion must take place shortly after the eggs are introduced into the water tubes, since the conglutinates shows the eggs in early cleavage stages.

Fig. 14 shows a corresponding section of the epithelium taken from the water tube of Fig. 15, when the embryos are in the early cleavage stage. Not much change has taken place in the arrangement or appearance of the cells--the epithelium is still thrown into folds, the cells are about the same size and the mucus vacuoles appear to be just about as abundant and scarcely any larger. Many of the small leucocyte nuclei can be seen here both in the connective tissue and out in the epithelial cells.

In our study of the histology of the Unio gill, we found an enormous increase in size of the epithelial cells in the marsupium containing glochidia. In Quadrula also, we find this same enlargement. Fig. 18 shows this condition. The cells are now resting almost flat upon their muscular base and are almost completely occupied by the large vacuoles. In all this, we find practically the same condition as in our Unio gill: the histology represents practically no new feature. As we should also expect from our previous study, the post-gravid stage shows a return to conditions practically identical with that of Unio, the epithelium again being thrown into deep folds, the cells small, with no ap-

parent indication of mucus vacuoles.

Summary.

Our study of Quadrula, as given in the preceding pages, shows that in the nature of the general structural modifications of the marsupium, and in the histology of the epithelium, this type of marsupium is quite different from that of the Unio. Assuming that Unio represents the simplest and most primitive form of marsupium, we may conclude that Quadrula represents a modification of this primitive type. We have found two striking differences to exist. In the first place, as regards general structure of the water tube, the Quadrula gill does not show the thinning of the lamellar junctions which was so conspicuous a feature in the latter stages of the Unio gill, such as in the glochidial stage, Fig. 8. Secondly, as regards the histology, the epithelial lining of the water tubes, in the Quadrula gill, has well differentiated mucus vacuoles in the pre-gravid stage, whereas none were observed in the corresponding stage of Unio. These differences may be explained as due to difference in function. The presence of the well defined mucus cells in the pre-gravid gill of the Quadrula, is clearly related to the discharge of the glutinous secretion which forms the matrix of the conglutinates. Since it is quite evident that this discharge of mucus occurs at the time when the eggs first pass into the water tubes, we should naturally expect to find the mucus cells well developed in the pre-gravid stage.

We have shown in our above study, that no particular dif-

ferentation further takes place in the epithelium until we come to the glochidial stage. Here, just as in Unio, the epithelial cells are enormously enlarged, being almost completely filled with the large mucus vacuoles. Since these disappear in the post-gravid stage, we believe that we are justified in concluding that their contents are discharged at the time when the glochidia leave the water tubes. We can see no other explanation of their disappearance, since they are not found in the post-gravid stage.

3. Type Heterogenae, Form--Lampsilis ventricosa.

In studying Lampsilis ventricosa, we have a form of the Unionidae which differs both from the Unio and Quadrula. As regards structure, only the posterior end of the outer gill is differentiated as marsupium and further, in respect to length of gravid period, Lampsilis belongs to the class of winter incubators. The eggs are passed into the water tubes of the gill, early in September--glochidia are formed in midwinter and remain in the gill until early summer, when they are discharged. We have found gravid females with the embryos of the previous year as late as July 1st.

An examination of the low power drawings will show the changes in the general structure of the water tubes. Lampsilis shows no striking difference from what we have already found to be true in Unio: the structural changes more closely resemble the Unio than they do Quadrula. As we should naturally expect from an examination of the greatly swollen Lampsilis gill, our sections show the water tubes to be enor-

mously enlarged. In the pre-gravid gill as seen in Fig. 28, the interlamellar junctions are extremely thick, the epithelium not being thrown into conspicuous folds as in the corresponding stage in the Unio gill. As in the pre-gravid stage of the Quadrula, the mucus vacuoles are large and rather conspicuous. In the succeeding stages, we find practically a repetition of what we found in Unio; the water tubes have become greatly enlarged with a corresponding thinning of the interlamellar junctions. These changes are shown in Figs. 23 and 25. In these two stages the mucus cells are not seen and the epithelial cells rest flat upon their base of muscle fibers. In the glochidial stage, the epithelium presents practically the same appearance that it did in Unio although the mucus vacuoles are not nearly so large nor so conspicuous. What attracts our attention here, is the entire absence of the filaments of the outer lamellae; no trace remaining except a layer of flat epithelial cells, and there is no indication of the chitinous rods, which form so conspicuous a feature of the gill of the Unionidae. None of the other genera which I have studied shows any indication of such modification. However, I have examined several different species of the Lampsilis, including L. rectus, ligamentinus, anadontoides, and they all show this peculiar modification of the lamellae. Examination of the two preceding drawings, Figs. 23 and 25, clearly shows an indication of the beginning of this change in the lamellae. Just what the significance is of this peculiar modification, I am as yet unable to state. There can be no doubt, however, that it is a characteristic of the Lampsilis marsupia.

The post-gravid gill shows a return to the condition of the pre-gravid with a decrease in the size of the water tubes and a thickening of the lamellar junctions. There is no trace here of the presence of any mucus cells.

Histology.

The histology of the marsupium of Lampsilis during the gravid period is similar, in some respects, to that of Unio and Quadrula yet there are conspicuous differences. In both Unio and Quadrula the epithelial cells show their most striking differentiation in the water tube of the glochidial stage, at which time we find the largest cells and most conspicuous vacuoles. Our study of the Lampsilis gill, however, shows a striking difference in this respect, for the largest cells are found in the pre-gravid gill, shown in Fig. 29. A comparison of Fig. 29 with other high power drawings of this series, Figs. 20, 21, 24 and 27, will show this quite clearly. This condition, we believe, may be explained as due to the fact, already noted in Quadrula, that the eggs, as soon as passed into water tubes, become surrounded with a gelatinous matrix forming the conglutinates. Since the amount of the matrix in these conglutinates is considerable, we should quite naturally expect to find large mucus cells in the epithelium which furnish the secretion.

During the early stages in the development of the embryo, the epithelium shows no differentiation and there is an entire absence of mucus vacuoles. It is not until we come to the glochidial stage that we find any trace of the mucus cells and even in this stage, the cells are only slightly larger

than in the early cleavage stages, with very few mucus vacuoles. The few mucus vacuoles which do appear, as seen in Fig. 27, are small, conspicuously different in appearance from those enormous vacuoles as seen in the Unio gill in this stage of development. Fig. 27 represents a section taken from a gill killed about June 25th, just before the time for the discharge from the water tubes. The study of material taken at frequent intervals during the preceding winter months, beginning at about November shows that this is a typical section, representing the condition of the epithelium during this entire period.

The condition of the post-gravid gill is shown by Fig. 20 and is quite similar to that in the other form we studied. Here the undifferentiated epithelial cells are thrown into a series of ridges and show no trace of any mucus vacuoles.

Summary.

Our study of Lampsilis, which may be taken as representative of that group of the Unionidae known as the Heterogenae, has shown us, both in the changes in general structure and the histology of the water tubes, that we have here a type of marsupium which differs both from Unio and Quadrula. As regards changes in general structure, Lampsilis is more like Unio in the enlargement of water tubes which takes place, with a corresponding decrease in the thickness of the interlamellar partitions. It is unlike both Unio and Quadrula in the disappearance of all trace of both outer lamellae and chitinous rods, which Lampsilis shows in the glochidial stage.

The histology here also presents unique features. Whereas

both Unio and Quadrula show the development of the epithelium of the water tube into enormous cells, most of which are filled with mucus vacuoles, Lampsilis shows no indication whatever of such a differentiation. The epithelium shows the largest cells to be present in the pre-gravid stage. We have explained this condition as due to the secretion at the time that the eggs are passed into the water tubes, of the substance which forms the matrix for the conglutinates. During the glochidial stage, in which condition the embryos are kept in the water tubes, this period lasting during the winter months, the epithelium presents practically the appearance of the early gravid stages: the cells are very little larger and the mucus vacuoles, while present, are not conspicuously developed.

4. Type Mesogenae, Form--Obliquaria reflexa.

The fourth form studied, Obliquaria reflexa, is a summer breeder, although Ortmann classifies it as a winter breeder. There can be no doubt of this, since in late June or early July, we have found, in abundance, all stages of the embryos, from early cleavage to early glochidia.

As stated by Lefevre and Curtis ("Reproduction in the Unionidae", 1910; page 85), the marsupium consists of a differentiated portion in the middle region of the outer gill, a variable number of the water tubes being specialized for this purpose. The portion of the gill, both in front and behind the marsupium, retains its respiratory nature. When gravid, the water tubes of the marsupium are greatly swollen, being conspicuously distended by the solid cord of conglu-

tinates.

Although a summer incubator, both the changes in general structure of the marsupial water tubes and the histology of the epithelium are so conspicuously similar to what we have already found in Lampsilis, that we shall discuss this form very briefly. Reference to Plate IV will show this similarity quite clearly. Practically all that we have said of Lampsilis, will apply equally well to Obliquaria. As seen in Fig. 31, we find small water tubes and thick interlamellar partitions. As soon as eggs have been passed into water tubes and conglutinates formed, the water tubes become distended, this being most strikingly shown in glochidial stage as seen by Fig. 36. As regards the histology, the facts concerning this are clearly shown in Figs. 32, 33, 35 and 37. Since the changes here shown are identical with those in the marsupium of Lampsilis, we shall not further discuss them.

CONCLUSION.

Our study of the histology of the marsupium of the Unionidae, in the four types as discussed above, has disclosed certain interesting facts in regard to the general structural changes which take place in the water tubes, as well as histological changes in the epithelial lining.

As regards the general structural changes in the water tubes, during the gravid period, our investigation seems to indicate that those changes which take place in Unio may be regarded as typical for most forms. These changes consist briefly in an enlargement of the water tubes during the time

when they are filled with embryo, with an accompanying decrease in the thickness of the interlamellar partitions. During this period also the epithelial lining of the water tubes which, in the pre and post-gravid condition is thrown into a series of deep folds, is found to rest flat upon its base of muscle fibers and connective tissue. The only exception which we have found, to this general rule, is Quadrula. In this form we fail to find the thinning of the interlamellar partition in the latter stages of gravidity.

As regards the histological changes which take place in the marsupium, Unio seems to present distinctive features. Lampsilis and Obliquaria are practically alike, while Quadrula is not wholly like either of the above, exhibiting some of the characteristics of Unio and some of Lampsilis. If we take Unio as the most primitive form of marsupium, all the other forms seem to show modifications of this typical form. In Unio, as we have seen, we find in the pre-gravid and post-gravid gill, an undifferentiated epithelium lining the water tubes. During the development of the embryo, thru the glochidial stage, changes take place which result in the production of large mucus cells occupying almost the whole of the epithelium. These mucus cells show their first appearance during the early stages of the development of the embryo and become gradually larger thru succeeding stages, culminating in an enormous development; in the marsupium containing glochidia. Since the post-gravid gill contains no traces of these mucus cells, we conclude that their contents are discharged either at the time of the discharge of the glochidia,

or some time just preceding this. No other explanation of their disappearance can be given. As to the function of this secretion, it seems reasonable to infer that it may furnish a gelatinous covering for the embryos as they discharge into the water.

While in Quadrula we find the same enlargement of the epithelial cells, with their mucus vacuoles in their glochidial stage, we also find a condition which differs from Unio in the pre-gravid gill. At this stage in Quadrula, well differentiated mucus cells are found in the epithelium of the water tubes. Their presence at this stage, we should naturally expect, since at the time the eggs are passed into the water tubes, a secretion occurs which forms the matrix of the conglutinates. Necessarily we should expect at this early stage to find the cells which had this function.

In both Lampsilis and Obliquaria, as is well known, the conglutinates are very characteristic and conspicuous and we have shown that the secretion, which constitutes the matrix, is discharged at the time that the eggs first enter the water tubes. This fact being already known, it was naturally to be expected that our investigation would disclose the presence of well developed mucus cells in the marsupium, which is being prepared for the developed embryos. Both Lampsilis and Obliquaria show the largest mucus cells in the pre-gravid gill, and in no case does the epithelium show any indications of the development of such enormous mucus cells as we find in Unio. If the function of the mucus cells were to furnish nourishment for the developing embryos, we should have expedted

to find a form like Lampsilis, with its long period of incubation, to show the most conspicuous development of its mucus cells in the glochidial period, in which condition they remain in the marsupium for several months in the winter. Yet a very careful study of material taken at frequent intervals during the winter months, shows the epithelium to exhibit very little differentiation, the cells being very slightly larger and in the post-gravid gill, showing hardly any mucus vacuoles. We therefore feel justified in concluding, finally, that as far as our present knowledge extends, there is no relation between the presence of the mucus vacuoles, in the epithelial lining of the water tubes of the marsupium, and the nutrition of the embryos. The main function of the mucus cells appears to be the secretion of the gelatinous substance which forms the matrix of the conglutinates.