1959S—PUCKETT—CRAYFISH OVIDUCT

HISTOLOGICAL OBSERVATIONS ON THE
OVIDUCT OF CAMBARUS LONGULUS LONGULUS
GIRARD

By

Dillon Hugh Puckett, B. S.

Approved by: Horton H. Hobbs, Jr.
Major Professor

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B. E. Frye
R. D. McKinsey

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INTRODUCTION

No detailed study of the histology of the oviduct has been made for any crayfish. Herrick (1909) noted that cyclic changes connected with ovulation occur in the oviduct of the lobster, but no work had been done to determine if similar cyclic changes associated with ovulation occur in the crayfish.

A study of the histology of the vas deferens of Cambarus l. longulus (Girard 1852), was made by Johnson (1958) in which striking changes associated with the reproductive cycle were noted. The present study was undertaken to complement Johnson's work by disclosing the histological nature of the oviduct of this species and to determine, also, to what extent, if any, cyclic changes are associated with reproduction in the female crayfish.

Review of the Literature

Herrick (1909) in his study of the natural history of the American lobster reported and described observations of cyclic changes, associated with ovulation, occuring in the oviduct of the lobster. He reported that at the time the eggs are ready to be laid the oviducal epithelium is glandular in type, that its cells become greatly elongated and distended, that when treated with common fixing and staining reagents the cytoplasm and the nuclei are clear, that the nuclei lie close to the basement membrane and are elongated in the direction of the long axes of the cell. He observed that after ovulation the cytoplasm of the cells is "more vesiculated", the nuclei are more granular and are further removed from the basement membrane, and that the nuclei are more deeply stained and oval in

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A search of the literature failed to reveal reports of any other work that had been done on the histology of the crayfish oviduct.

Materials and Methods

The crayfish were collected from several localities in Virginia. Some were taken from Moorman's River near Charlottesville, some from Racoon Creek near Palmyra, and others were taken from Catawba Creek at Catawba, and from the Roanoke River north of Blacksburg. All the streams from which collections were made, with the exception of those from the Roanoke River, form a part of the James River drainage system.

Collections were made during the following months: February, March, April, May, July and August of 1958 and in June and July of 1959. These collections over a period of seven months provided specimens from at least two months before and after the period of ovoposition (see Smart, 1957). During the present study the period of ovoposition was apparently terminated before April 26.

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Preparation of Collected Specimens for Microscopic study of the Tissues of the Oviduct

Most crayfish were dissected as soon as they were brought into the laboratory, and no animals were kept for more than nine days before dissection. A few entire animals, punctured to permit penetration of the fixative, were preserved in Bouin's fixative at the time of collection and were kept for dissection and sectioning at a later date.

The length of the carapace and the length of the areola of all the crayfish dissected were measured with the Vernier caliper and these measurement were recorded. The length of the ovary was recorded for all the animals from which the ovary was removed. The condition of the ovary at the time of dissection was also recorded. Those ovaries which were removed were preserved in 70% alcohol. These data were helpful in the determination of the approximate length of time before or after ovoposition.

Most of the dissections consisted of removing the coxae of the third periopods together with the attached oviducts. In a few animals, however, the ovary, and coxae were removed intact.

After dissection, those tissues which were to be prepared for histological study were placed in Bouin's fixative for two hours. In order to soften the exoskeleton of the coxae they were transferred to a 2% nitric acid solution in 70% alcohol for a period of seven days during which time the solution was changed each twenty-four hour period. When the treatment with the acid alcohol was completed the

tissues were passed through the alcohol series, cleared in xylene, and embedded in paraffin.

Sections, cut at ten microns, were stained in Heidenhain's Iron Haematoxylin with eosin as a counter stain, Mallory's Triple connective tissue stain, or the Periodic Acid Schiff reagent, some of the latter of which were counterstained with haematoxylin and Orange G. Other stains utilized were Alcian Blue, Toluidin Blue, Weigart's Resorcin Fuchsin, and Sudan Black.

Approximately fifty-five specimens were examined and serial sections of the oviducts of thirty-one crayfish were studied for the preparation of this manuscript. The serial sections were treated for study according to the staining techniques mentioned above. The remainder of the specimens were used in the determination of the gross anatomy of the oviduct and for study of the oviduct in various whole-mount preparations, as, for example, in determining the presence of smooth muscle fibers by birefringence using polarized light.

THE GROSS ANATOMY OF THE FEMALE SEX APPARATUS

The gross structure of the sex apparatus of the female Cambarus l. longulus was determined by dissection and the use of sections through the thoracic region.

The ovary is located in the thorax and is clearly visible, when the carapace of the animal has been removed, as a white to brownish organ lying along the sagittal plane of the body immediately ventral to the heart and dorsal to the intestine and the posterior lobes of the liver. The ovary is trilobed, consisting of two anterior lobes, each of which comprise about one-third of its length, and a single, longer, posterior lobe, which, except for a short period following ovoposition, extends posteriorly above the intestine into the anterior part of the abdomen.

The oviducts arise laterally at the junction of the anterior and posterior lobes of the ovary, are transparent and barely visible in an animal which has recently laid but are seen as moderately heavy white tubes in one which is approaching the egg-laying season. They pass laterally over the posterior lobes of the liver where they turn ventrally along the lateral surfaces of the liver lobes toward the coxae of the third walking legs. As they approach the coxae they pass between the fifth and sixth sternal apodemes and the IX and X pleural apodemes (Snodgrass; 1952). As the oviduct passes into the coxa it follows a course lateral to the flexor muscles of the abdomen and the Musculi ventrales superficiales thoracis (Schmidt; 1915) attached to the fifth and sixth sternal apodemes.

As the oviduct enters the coxa it passes between the broader head of Musculus levator basipoditis medialis (Puckett, et al.) and the posterior head of Musculus promotor (Schmidt, 1915). It passes venteromesially along the anteromesial edge of the former and thence along the anteromesial edge of the broadest head of Musculus depressor basipoditis posterior (Puckett, et al.) between it and the ribbon like head of Musculus levator basipoditis medialis (Puckett, et al.) and Musculus depressor basipoditis anterior (Puckett, et al.), lying just posterior to the two latter muscles. The distal end then passes immediately anterior to the origin of the mesial head of Musculus depressor basipoditis posterior, (Puckett, et al.), where the oviduct is attached, on one side, to the movable vestibular membrane covering the external orifice and elsewhere along a heavy chitinous ridge which bounds the oviducal orifice.

The external orifice of the female genital tract is usually obscured by an oval, cuticular membrane on the ventral posteromesial surface of the coxa of the third pereiopod. The orifice is an arc-like slit lying along the anteromesial margin of the membrane and its size is controlled by the position of the membrane; i. e., if the membrane is pulled up into the coxa by the muscle which controlls it the opening is enlarged and if the muscle controlling the membrane is relaxed the membrane resumes its position, closing the external orifice. Because the orifice is an arc-like slit the opening is never circular but assumes a spindle-like shape when the membrane is pulled into the coxa (Figs. 1, 2, 5, and 6).

The muscle, <u>Musculus vestibularis</u> (new name), which controlls the membranous covering of the oviducal orifice consists of a thin band of fibers which originate on the posteromesial wall of the coxa and extend anteriorly to be inserted on the inturned margin of the vestibular membrane. There is evidence that at the time of ovoposition this muscle draws the free border of the vestibular membrane proximally into the vestibule allowing the passage of eggs through the oviducal orifice.

The oviduct and the muscle, <u>Musculus vestibularis,</u> are attached to the inturned margins of the cuticle forming the oviducal orifice. The inturned edge of the vestibular membrane bears a heavy cuticular phragma-like projection to which the muscle is attached by tonofibrils. The cuticle on the opposing wall is delicate and not thickened.

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HISTOLOGICAL OBSERVATIONS ON THE OVIDUCT AFTER OVOPOSITION

General Description. -- The oviduct is a tubular organ the contour of the lumen of which is made irregular by longitudinal folds. The height of these folds reflects the diameter of the lumen; i. e., when the walls are distended the folds are scarcely discernible but when they are in a relaxed state the folds may be as much as 310 micra in height. In the vicinity of the ovary there is an irregular arrangement of the folds (Fig. 7). The walls of the oviduct consist essentially of two layers, an epithelial lining resting on a basement membrane surrounded by a highly vascularized fibrous connective tissue coat in which are embedded longitudinally or obliquely arranged smooth muscle fibers.

The Proximal End (Figs. 7 and 8). -- At its proximal end the oviduct is firmly attached to the ovary, and an ostium, in the usual sense, is lacking. The connective tissue coat of the ovary is continuous with that of the oviduct and the follicular epithelium of the former is separated from the lining epithelium of the latter only by a very thin fibrous connective tissue between the basement membranes of the two layers. Thus the proximal end of the lumen of the oviduct appears to end blindly.

In this area the epithelial lining is simple columnar, varying in height from 18.7 micra to 30.6 micra. The cytoplasm contains acidophilic, P.A.S.-positive granules and many of the cells are highly vacuolated. Some of the granular material in the cytoplasm of the cells gives a positive test for glycogen (c. f. Figs. 9 and 10).

Tests with toluidin blue confirm the polysaccharide nature of some of the granules. This epithelium rests upon a basement membrane and is surrounded by a connective tissue layer of varying thickness. The single, oval nucleus of each cell ranges in size from 5.1 micra by 6.8 micra to 6.8 micra by 8.5 micra and contains three to five prominent chromatic granules irregularly scattered among the smaller ones (Fig. 4). These cells are secretory in function and are responsible for the large amount of P.A.S.-positive secretion within the lumen of the oviduct. The fact that the secretion within the oviduct is both P.A.S.-positive and acidophilic suggests that mucin is at least one of its components. Alcian Blue preparations indicate the presence of mucin within the cells and in the lumen of the duct. Staining of the tissues with Sudan Black indicated that no tightly bound lipids remained in the tissues which could account for the staining results observed with the other techniques used. No Sudan Black preparations of fresh tissue were made although these are contemplated in future work in order to help determine the presence of lipids in the tissues or secretions of the oviduct.

The connective tissue layers of both the oviduct and ovary have the same histological characteristics and, as mentioned in the general description, form a continuous covering over both organs. This is a loose, acidophilic, highly vascularized, fibrous tissue that fails to stain with Weigart's Resorcin Fuchsin. Within it are oblique to longitudinally arranged smooth muscle fibers, and many ovoid to spindle-shaped nuclei. There are two types of nuclei in the muscular connective tissue layer. Those of the connective tissue

Tests with toluidin blue confirm the polysaccharide nature of some of the granules. This epithelium rests upon a basement membrane and is surrounded by a connective tissue layer of varying thickness. The single, oval nucleus of each cell ranges in size from 5.2 micra by 6.2 micra to 6.6 micra by 8.5 micra and contains three to five prominent chromatic granules irregularly scattered among the smaller ones (Fig. 4). These cells are secretory in function and are responsible for the large amount of P.A.S.-positive secretion within the lumen of the oviduct. The fact that the secretion within the oviduct is both P.A.S.-positive and acidophilic suggests that mucin is at least one of its components. Alcian Blue preparations indicate the presence of mucin within the cells and in the lumen of the duct. Staining of the tissues with Sudan Black indicated that no tightly bound lipids remained in the tissues which could account for the staining results observed with the other techniques used. No Sudan Black preparations of fresh tissue were made although these are contemplated in future work in order to help determine the presence of lipids in the tissues or secretions of the oviduct.

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The Distal End (Figs. 5 and 6). -- A general description of the external orifice of the oviduct with its covering is given in the section on, "The Gross Anatomy of the Sex Apparatus". The epithelium of the oviduct with its muscular-connective tissue coat is attached to the infolded margins of that portion of the exoskeleton which forms the lining of the oviducal vestibule, immediately proximal to the external orifice. Here the epithelium of the oviduct appears to abut that underlying the cuticle. The oviduct in this region of the coxa is surrounded by a loose fibrous vascularized tissue which contains in some areas rather large blood sinuses. At the junction of the oviduct and the cuticular lining of the oviducal vestibule the connective tissue coat is affixed to the cuticle by strands of fibers that converge to form tonofibrillar-like attachments. Additional connective tissue fibers, immediately distal to the oviducal attachment, radiate from the walls of the vestibule to the marginal wall of the coxa (Fig. 5).

The limiting membranes of the epithelial cells of the oviduct, in its distal portion, are completely broken down and the lumen of the oviduct is filled with a secretion not unlike that in the more proximal regions. No cell membranes are discernible, and the nuclei, variable in outline, are closely appressed to the basement membrane. This condition range in size from 4.25 micra by 5.1 micra to 5.1 micra by 8.5 micra and those of the muscle fibers from 3.4 micra by 8.5 micra to 4.25 micra by 11 micra. Strands of the connective tissue layer extend into each of the oviducal folds and at the base of each fold there is a prominent blood sinus frequently containing blood cells and coagulated plasma. (Figs. 3, 4, and 11).

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The Oviduct Near Midlength. — Although there is a rather marked difference in the areas at the extremities of the oviduct, as is seen from the preceeding discussion, there is a gradual transition along its length from the condition observed at the proximal end to that seen in the distal portion of the duct. The prominent difference at the ends of the oviduct, seen in an animal that has laid, is the breaking down of the cell membranes near the distal region. The cells of the proximal region, although having intact cell membranes, after ovoposition are highly vaccuolate and have relatively few granular elements.

The Muscle of the Vestibular Membrane. — The fibers of Musculus vestibularis are striated and exhibit alternating Q and I bands with distinct H and Z lines. Connective tissue associated with these fibers bind the extremities of the muscle to the cuticle by well-defined tonofibrils.

HISTOLOGICAL OBSERVATIONS ON THE OVIDUCT BEFORE OVOPOSITION

The preceeding discussion describes the histological nature of the oviduct in an animal which had, as judged from the condition of the ovary at the time of dissection, recently passed through the egg laying season although there were no eggs attached to the abdomen. The histology of the oviduct in an animal just prior to egg laying is somewhat different as might be expected. Most of the epithelial cells along the entire length of the oviduct in an animal just before egg laying are distinctly columnar and are from one-half to two-thirds longer than those in an animal which has laid. Both before and after ovoposition the epithelial cells near the ovarian end are more distinctly columnar than those at the distal end. The epithelial cells of the longitudinal folds, however, after egg laying, maintain their columnar form (c.f. Figs 3 and 4) but have a distinctly smaller volume.

The cytoplasm of the epithelial cells before egg laying is extremely granular (Fig. 10). No prominent vacuoles are present in the cytoplasm as they are after ovoposition. That the granular elements contain a polysaccharide was indicated by the fact that they were both P.A.S.-positive and also stained with Alcian Blue and Toluidin Blue. Sudan Black preparations were made to ascertain whether or not the apparent polysaccharides were tightly bound lipids which had not been removed by the lipid solvents used. That many of the granular elements were glycogen was clearly demonstrated (c.f. Figs. 9 and 10). In view of the fact that some of the granules are P.A.S.-positive and also stain with Alcian Blue and Toluidin Blue there is strong indication that mucin is present.

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The lumen of the oviduct before egg laying is almost completely filled with the secretion mentioned earlier. Little if any of this secretion is present in animals which have recently laid, however, it begins to reappear prior to the first molt after egg laying. The large amount of secretion produced by the epithelial cells before egg laying probably accounts for their vacuolar nature after egg laying and, also, for the fact that the cells are much less granular.

There are no discernible differences in the connective tissue and muscular coat before and after ovulation.

In the elongate cells of the oviduct of an animal which has not ovulated the nuclei are located in the cell at a point from one-half to three-fourths the distance of the long axis from the basement membrane, whereas, in the oviduct of an animal which has laid they lie closely appressed to the latter membrane (Figs. 3 and 4)

DISCUSSION

In Bronns', "Klassen und Ordnungen des Tierreichs" (1944) it is reported that, Mayer (1877), in work on the crab Eupagurus, observed a chitinous continuation of the outer integument which acts as a valve over the oviducal orifice to close the orifice. Mayer was unable to demonstrate a musculature which controlled the action of this valve-like continuation of the outer integument and it was postulated that the eggs opened it by dilation of the oviduct. A covering, similar to the one reported by Mayer, was observed in the crayfish in the present study and is referred to as the vestibular membrane (Figs. 1,2,5, and 6). A musculature controlling the movement of this membrane or "valve" was observed in the crayfish and is referred to as the vestibular muscle (Figs. 2, 5 and 6). It is believed that this muscle, by contracting, draws the flap of the vestibular membrane up into the coxa and thereby opens the oviduct for passage of the eggs to the outside.

Herrick (1909) reports that in the lobster the cells of the oviduct become greatly elongated and distended and that after egg laying they are shrunken to less than one-fourth their former size. This is in agreement with the present work on the crayfish oviduct. He further reports that before egg laying the cytoplasm of the oviducal epithelium is clear, its nuclei are also clear, and that the nuclei lie well down toward the basement membrane; and that after ovulation the cytoplasm is vesiculated, the nuclei are more granular and are farther removed from the basement membrane.

In this study of the crayfish oviduct it was found that the

cells of the oviducal epithelium before egg laying are highly granular rather than clear and that the nuclei of this epithelium are also quite granular in appearance. It was, also, observed that the nuclei do not lie close to the basement membrane until after ovoposition. The cytoplasm, after ovoposition has occurred, appears less granular than before and is vacuolate (c.f. Figs. 3 and 4).

It is assumed that the abundant secretion, found in the oviduct from several months before ovulation up to the time of ovulation, plays some important role in the formation of a covering about the eggs at the time they are laid. The foregoing assumption is based upon the fact that this secretion is most abundant at the time of ovulation and is almost lacking just after ovulation. This secretion is produced in such abundance just prior to ovoposition that in those areas where it has retracted from the wall of the oviduct the outer margins of the epithelial cells appear ciliated because of strands of the secretion pouring from them. That the production of the secretion is important in so far as the process of ovoposition is concerned cannot be doubted. The discovery of the part it plays in ovoposition will, however, require further work.

The smooth muscle cells within the connective tissue coat were definitely identified with the aid of a phase microscope which was equipped with a light polarizing unit. Stains employed were not sufficiently selective to enable one to determine the limiting membranes of these fibers. The nuclei of these fibers are readily distinguished from those of the connective tissue by their more distinctly spindle-shape despite

their otherwise close resemblance.

It is apparent that cyclic changes associated with reproduction do occur in the oviduct of the crayfish. Basically these changes involve a building up of the epithelial cells of the oviduct, a period of very active secretion with a concomitant loss in their volume and extreme degradation in the distalmost portion, followed by a regenerative process. Immediately after ovoposition the epithelium of the oviduct appears depleted in that the cells are vacuolar, contain very few granular elements, and in the distal end the limiting membranes of the cells are destroyed. The lumen of the oviduct immediately after ovoposition contains very little secreted material. Gradually the limiting membranes of the lining epithelium of the distal portion of the oviduct are reconstituted, and like the more proximal epithelium becomes granular in nature. Repair of the cell membranes at the distal end is accomplished within about two months after ovoposition. Although, at this time, the cells are still partly vacuolar, especially near the proximal end, their apparent complete restoration is accomplished by two months prior to the next period of ovoposition.

In Cambarus l. longulus, the period of ovoposition occurs in the spring when the epithelium is depleted of its secretory granules, becomes vacuolate, reduced in volume, and partially destroyed. During the summer and winter the regenerative process is completed.

SUMMARY

The oviduct of the crayfish is a tubular duct the walls of which consist of an inner folded columnar epithelium resting on a basement membrane that is surrounded by a fibrous connective tissue layer in which are embedded longitudinally directed smooth muscle fibers.

Prior to ovoposition the cells of the oviducal epithelium are elongated, contain many granular elements, and throughout the length of the oviduct the epithelial cells have complete limiting membranes. The nuclei of the cells at this time lie about one-half to two-thirds of the distance of the longitudinal axis of the cell from the basement membrane. In the lumen of the oviduct there is, just prior to ovoposition, an accumulation of secreted material sufficient to distend the walls of the oviduct.

The process of ovoposition leaves the epithelial cells of the oviduct with a depleted appearance. Most of the cells of the epithelium are not so elongated as they are prior to ovoposition, are only slightly granular, are highly vacuolate, and in the distal area of the duct the limiting membranes of the cells are broken down. The nuclei, after ovoposition, though not considerably reduced in size, are closely appressed to the basement membrane upon which the lining epithelium rests.

A period of regeneration in the epithelial cells of the lining of the oviduct follows ovoposition during which the cells are gradually restored to the condition described just prior to the process of ovoposition.

The blind proximal end of the oviduct abuts the connective tissue surrounding the ovarian follicles, and the distal end is guarded by a cuticular membrane that is controlled by a small muscle consisting of

striated fibers. This muscle is opposed by the elasticity of the cuticular membrane.

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FIGURE 1. Ventral view of the coxa of the third pereiopod with the external orifice of the oviduct visible at the lower left. (X 29)

FIGURE 2. Interior of the coxa from the proximal end. (X 29)

am - articular membrane
bp - basipodite
cba - coxobasipodal aperture
cx - coxa
vm - vestibular membrane
vmms - Musculus vestibularis

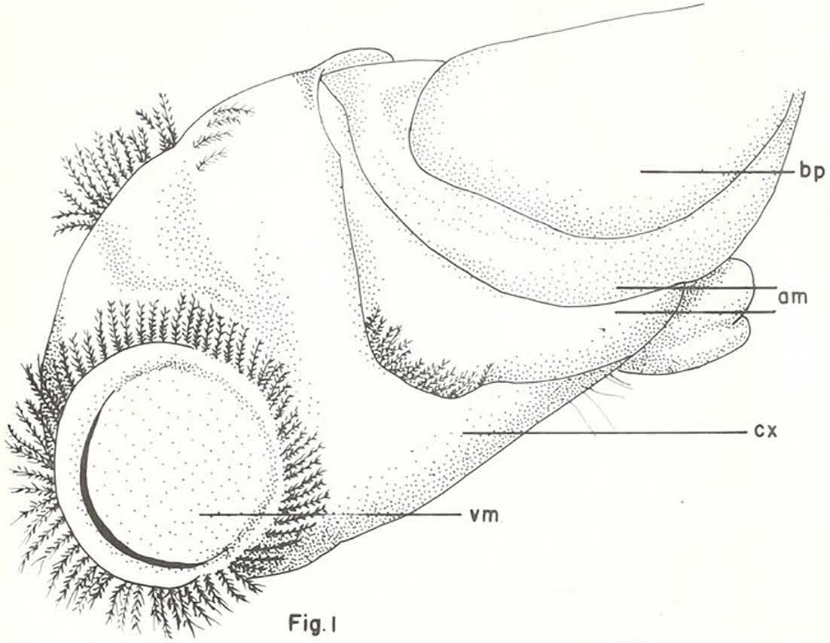

Fig. 1

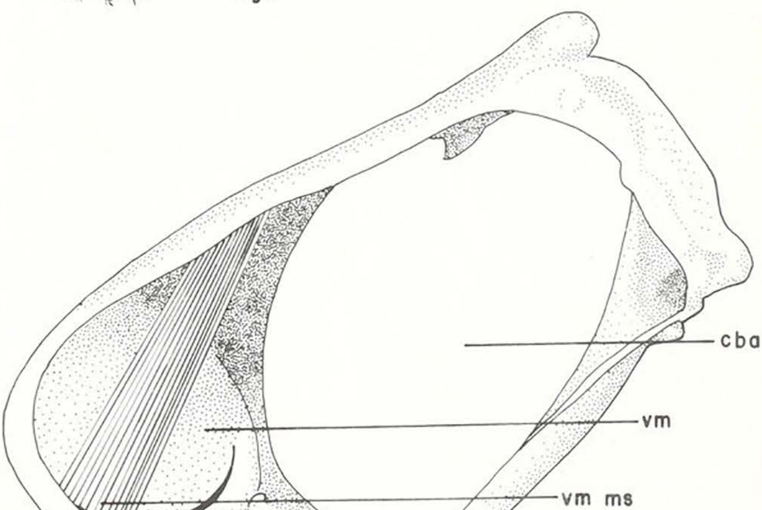

Fig. 2

FIGURE 3. Camera lucida drawing of a section of the oviduct of a crayfish before egg laying (X 554).

FIGURE 4. Camera lucida drawing of a section of the oviduct of a crayfish after egg laying (X 543)

FIGURE 5. Diagram of the distal portion of the oviduct with the oviducal orifice, the vestibular membrane, and *Musculus vestibularis*

Explanation of abbreviations

bc - blood cell
bm - basement membrane
bs - blood sinus
ct - connective tissue
cu - cuticle
ep - epithelial cell
epdn - epidermal nucleus
lo - lumen of the oviduct
ov - oviducal vestibule
smn - smooth muscle nucleus
vm - vestibular membrane
vmms - *Musculus vestibularis*

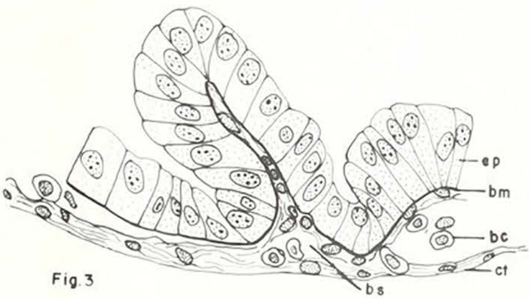

Fig.3

Fig 4

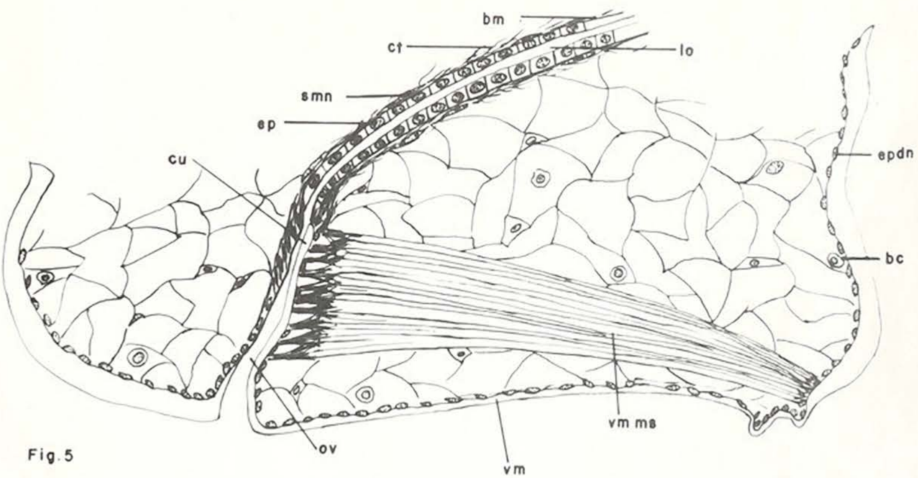

Fig.5

FIGURE 6. A section through the coxa of the third pereiopod with the oviducal vestibule visible to the left (X 180). The attachment of the oviduct may be seen near the middle of the section. The vestibular membrane and its controlling muscle, _Musculus vestibularis_, are visible in the lower portion of the section.

FIGURE 7. A section through the oviduct and a small portion of the ovary, visible at the upper left of the figure (X 100). The irregular nature of the folds in this area may be seen in the oviduct.

FIGURE 6

FIGURE 7

FIGURE 8. A section through the proximal portion of the oviduct with a part of the ovary visible at the right. Two ovarian follicles may be seen in the ovary and the lumen of the duct is filled with secreted materials. (X 180)

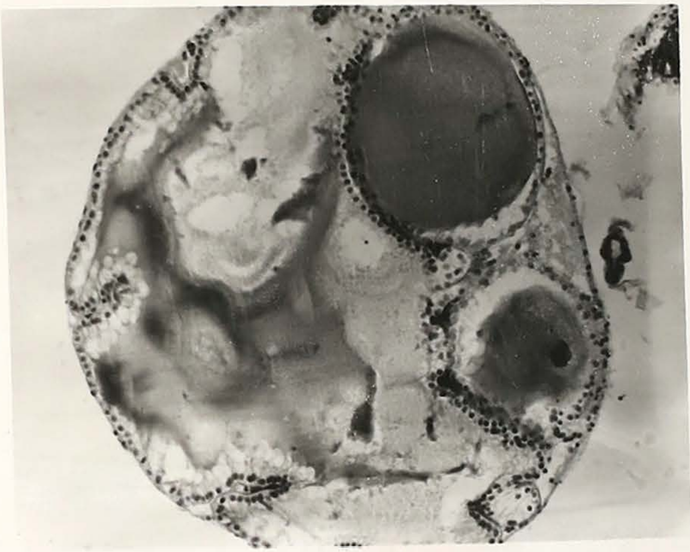

FIGURE 8

FIGURES 9-10. Sections which were stained with Periodic Acid Schiff reagent. Figure 9 was treated with saliva and used as a control testing for the presence of glycogen. Figure 10 was stained simultaneously and in the same manner but was not subjected to saliva. (X 617)

FIGURE 9

FIGURE 10

FIGURE 11. A cross section of the oviduct with the connective tissue layer visible in the folds and the blood sinuses present at the base of the folds. The connective tissue layer appears thickest where the folds are deepest and thinnest where the wall is distended. (X 301)

FIGURE 11