ANATOMY OF THE MALE REPRODUCTIVE SYSTEM OF THE BLUE CRAB, *CALLINECTES SAPIDUS* RATHBUN

By

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INTRODUCTION

Basic information on the normal anatomy of the male reproductive system of the commercially important blue crab has not previously been developed. An understanding of the activities and limitations of the male in continuation of the species is important for planning of the most intelligent conservation of this animal. A series of problems, including the post-embryonic development and differentiation of the parts of the system, spermatogenesis, physiology of copulation, total reproductive capacity, effect of winter hibernation on the reproductive system, and the time of onset and nature of senility has been planned at the Chesapeake Biological Laboratory. The basis for these studies lies in a thorough understanding of the adult anatomy.

The gross morphology of the male reproductive system of the blue crab has been briefly described by a number of authors, beginning with the original treatment of the structure of this animal by Brooks (1882), and followed by Churchill (1919), Beaven (1932), Truitt (1939), and Newcombe (1945). Each of these authors has given a brief description of the system accompanied by a drawing, and the general anatomy is well enough known to be available on a commercial chart (Turtox Class-Room Chart C R 11.731) and in amateur teachers' guides. Snodgrass (1936) and Cochran (1935) have described the gross anatomy of the external genitalia quite thoroughly. The rest of the descriptions of this species are superficial and in several cases inaccurate, and none offers details of structure. Therefore, the form and distribution of all of the parts of the adult male reproductive system is offered.

Many writers have considered some phase of the male reproductive system. Detailed accounts of all of the organs of a species are rare,

however, and comparisons must be based in most cases upon fragments gleaned from broad descriptions or from intensive comparative consideration of single organs. This paper has been planned to present the gross and histological anatomy of the entire system of one species. No previous similar treatment has been made.

The gross morphology of the male decapod reproductive system has been extensively investigated by Faxton who describes Cancer productus, C. magister, Hyas lyratus, Oregonia gracilis, Hemigrapsus nudus, Telmessus cheiragonus, Epialtus productus, Pugettia gracilis (1915) and Cancer gracilis, C. oregonensis, Scyra acutifrons, Lophopanopeus bellus, Lopholithodes mandtii, Hapalogaster mertensii, Rhinolithodes wosnessenskii, Petrolisthes eriomerus, Pagurus setosus, Astacus leniusculus, Homarus americanus, and Pamalirus interruptus (1917a). He also presents the histological anatomy of the vas deferens of representative Anomura, Brachyura, and Macrura from among the species listed (1917a). Henri-Heldt (1932) describes the reproductive system of the penaeids Aristeomorpha, Aristeus, Penaeus, Parapenaeus, and Sicyonia. Binford (1913) briefly discusses the organs of Hemippe mercenaria. The monographic treatments on Cancer pagurus by Pearson (1908), Eupagurus bernhardus by Jackson (1913) and Homarus americanus by Herrick (1909) each describe the reproductive system. The treatise of Calman on the class Crustacea (1909) is useful as a source of generalizations.

The decapod testis has been studied by a number of authors, including Grobben (1878), on several species; Sabatier (1885) on Astacus, Carcinus, Crangon, Pagurus, and Scyllarus; Gilson (1885, 1886) on a great many Arthropods including fourteen decapods; Carnoy (1885) on Squilla, Crangon, Portunus, and Pagurus; Hermann (1890) on Astacus fluviatilis, Maia,

Stenorhynchus, Homarus vulgarus and others; Miyama (1926) on Panulirus japonicus and other writers. There is a wealth of literature on spermatogenesis in Decapoda, but the present problem has not been concerned with it directly. Many of these papers may include some description of testis anatomy.

The vas deferens has been described by a number of the writers listed above, and especially by Faxton (1917a). Additional sources include Mouchet on Diogenes pugilator and other paguridæ (1930) and Spalding on Carcinus maenas (1942).

The penis has been frequently mentioned in previous papers, but rarely described or discussed. Duvernoy (1850) provided an early description and made useful contributions concerning its modifications and function. Andrews (1910) gives an account of the gross and histological structure as found in Cambarus.

The male pleopods of Decapoda are described for many species in taxonomic treatments such as these of Ordway (1863) and Rathbun (1930), and in monographs on individual species. Bell (1905) has made certain comparative generalizations on their structure.

MATERIALS AND METHODS

Adult male blue crabs, Callinectes sapidus Rathbun, from the Chesapeake Bay were used in this study. No criterion of maturity had previously been established although Truitt (1939) and other writers had judged from field observations on mating behavior that male crabs are sexually mature during the last three growth stages. This would mean that in the Chesapeake Bay area most crabs above 2½ inches in width would be mature. Commercial soft crab dealers who retain large numbers of crabs on floats have noticed that males as small as 3-3½ inches in width will frequently exhibit preliminary courtship activities and even present the appearance of normal copulation. However, observations on the condition of the gonads with respect to body length, width, depth, and other characteristics strongly suggested that only males greater than 5½ inches in width are likely to be mature. Specimen study and histological tissue selection were, therefore, based only upon large males. Among these, only those which were fully hard and which were "fat" but not old were used. Most of the histological tissues were collected in mid-summer in 1943 and 1944.

For purposes of study, the reproductive system (figs. 3 and 4) was divided into (1) mid-lateral testis, (2) testis at junction with the anterior vas deferens, (3) coiled anterior vas, (4) massive median vas, (5) slender posterior vas just behind the median vas, (6) posterior vas at bar anterior-dorsal to thoracic segment VIII, (7) posterior vas between this point and the exoskeleton, and (8) the external penis. Portions of each of these regions were fixed in the several fixatives employed and prepared for histological examination. Most of these parts were also secured from males in mid-winter and from immature males for comparison. The first pleopods of two large adult soft males were fixed in Bouin's.

Preliminary examination of tissues fixed in Bouin's demonstrated that the contents of the vasa deferentia are most difficult to section. Accordingly, tissues from a series of individuals were fixed in Bouin's, Carnoy-Lebrun, Worcester, Gilson, and General (alcohol 3 parts, formalin 2 parts, and glycerine 1 part). The results, on the basis of success in sectioning and acceptable staining reaction, are summarized below.

Table 1. Relative quality of fixation

Tissue	Bouin's	Gilson	Carnoy-Lebrun	General	Worcester
Testis	+	0	0	+ 1.	+
Anterior vas	0	0 2.	0	0	+
Median vas	-	-	-	-	-
Posterior vas	-	0	0	0	0
Posterior vas near penis		+	+		0
Penis	0	+		0	+
Pleoped 1	0				

+ = good; 0 = fair; - = poor

1. Fixation in General results in strongly chromophilic nuclei and nearly achromatic cytoplasm.

2. Spermatophores are best preserved in Gilson (see fig. 22) but may be somewhat shrunken.

The most effective of these fixatives was Worcester's, which yields excellent preparations from several parts and usable sections from all except the median vas.

All tissues were cleared in 1,4-dioxane and embedded in tissuemat (54-56° C.). Most of them were sectioned at 10 mu, with a few testis preparations cut at 6 and 4 mu. Alternate serial slides were stained in Heidenhain's Iron Haematoxylin and Azan.

Figures 3, 4, 5, and 6 are based on dissections of fresh and preserved animals. Figure 7 was prepared from a series of camera lucida sketches at table level employing a 40 mm. objective and 10x ocular. Photomicrographs were prepared by the author at the magnifications indicated.

OBSERVATIONS

1. Gross Anatomy

External Characters. The adult male blue crab (figs. 1 and 2) is easily distinguished from the female. The abdomen or "apron" of the male contains but four movable segments since the second abdominal piece is formed by the fusion of segments XIV - XVI, and is narrow and "T" shaped. The abdomen of the immature female is triangular and that of the mature female is composed of the full six segments and is broad and semicircular. The male abdomen bears only two sets of pleopods, modified to form an intromittent complex. Females, both immature and mature, carry four pairs of pleopods which function for egg attachment in the adult. In addition, the male has characteristic heavy chelae, shorter lateral spines, more blue color on the claws, and a general trimness in contour in contrast to the more bulky female. No biometric comparisons have been made because of the previous lack of a criterion of either male maturity or instar number, but the sexes are sufficiently dimorphic to be easily recognized.

Reproductive system. The reproductive system of the adult male consists of the paired testes, vasa efferentia, vasa deferentia (with distinguishable anterior, median, and posterior portions), the external penes, and the highly modified first and second abdominal appendages or pleopods. All of the internal organs, except portions of the testis and posterior vas lie in the central body region between the large muscle masses at the base of the five pereiopods. The internal organs are rather markedly variable in size, perhaps in relation to effective functioning. No information is available as yet regarding the cause of these variations, so this description will be sufficiently general to include virtually all mature males not in copula.

Testis. The testis has the appearance of a slender white convoluted cord (figs. 3 and 4, T). It lies beneath the hypodermis of the carapace and on the anterio-dorsal surface of the hepatopancreas. The distal end is near or slightly posterior to the base of the lateral spine. The surface is irregular and the organ is opaque. The spermatic artery is visible along most of the length of the testis. Near its median end, the testis lies on top of the median vas deferens and passes medially around the dorsal end of the mandibular leader and posterio-dorsal to the stomach. A short cross-bar joins the testes near their proximal end. It is five to ten centimeters long and is identical in appearance with other testis tissue.

Vas efferens. The vas efferens is very small and is embedded in the mass of the anterior vas deferens. It is not macroscopically distinguishable.

Vas deferens. The vasa defferentia may occupy a large part of the central body cavity (figs. 3 and 4, A.V.D., M.V.D., and P.V.D.). Like the ovary of the female they vary in distribution in relation to other visceral organs. Each vas is divisible by both anatomy and physiology into three distinct portions, and these will be described separately.

Anterior vas deferens (A.V.D.). The anterior vasa are tight white coils on either side of the median line posterior to the dorsal part of the stomach and anterior to the pericardium. Each rests on the respective large median vas.

The most anterior coils are slender, translucent, and delicate. They are bound together by a thin but strong membrane and cannot be separated or straightened by dissection. Rupture of these coils releases small white rod-shaped masses which slide easily from the tubes. These

are probably sperm masses in process of subdivision into discrete spermatophores.

The coils increase in size posterio-ventrally. Here they are thicker, white, and quite opaque. Rupture releases a large number of spermatophores and the viscous white fluid in which they are distributed. Spermatophores are white, opaque, and usually proportioned quite like a bird egg. A fine membrane limits the sperm, which appears as a fine closely packed mass. Spermatophores vary from 500 x 300mm to 200 x 150mm in size and average about 300 x 225mm. Little is known of the physical or chemical nature of the surrounding medium other than its staining reactions in Azan and HIH. Spermatophores retain their shape and appearance after 20 hours in 2% NaCl, but swell markedly in 1.5% NaCl. Rough determinations place the chlorinity of the blood of this species at about 13-15 o/oo, corresponding to a salt concentration of 2.5 - 3.0%.

The largest and most posterior coils of the anterior vas deferens contain spermatophores in numerous lobule-like subdivisions of the tube and in the same kind of white thick matrix. This is the most important storage region for completed spermatophores in the entire system. These coils increase in size and lead into the median vas.

Median vas deferens (M.V.D.). The vas turns anteriorly and laterally to become the large pink median vas. It is the most massive and obvious part of the system. It lies along the side of the stomach and has the position and appearance shown in figures 3 and 4. It is bright pink in the adult male and the surface is very irregular and pebbled. The largest portion, near the anterior vas, is followed by an abrupt posterior turn, and the vas becomes smaller and paler in color toward its posterior end.

Rupture of the median vas opens a number of pockets containing a white cohesive cotton-like fluid. Spermatophores are sometimes present in a few of these pockets or lobes near the anterior vas, but they are not characteristic of the median region. The thick substance is present to the point where the median vas is much reduced in diameter and merges into the posterior vas.

Posterior vas deferens (P.V.D.). The posterior vas is a long convoluted tube extending from the median vas to the base of the penis. Its disposition may differ between individuals, perhaps in relation to the course of the even more variable hepatopancreas, but the arrangement shown in figure 4 is typical. It forms a large mass ventral and slightly anterior to the heart and pericardium, passes the sternal artery and has several coils in the region of the hind-gut caecum between the heavy muscle masses at the base of the swimming legs. Thence it turns abruptly ventrad and enters the large foramen medio-dorsal to the eighth thoracic segment. It passes through the muscle mass of the fifth pereiopod to the base of the exopodite as the ductus ejaculatoris.

The posterior vas varies in length and in diameter. It may be 175mm. long and 3-5mm. thick in a large male, but most frequently it is of smaller dimensions. The first inch is white and opaque, and the rest of the vas within the central body region is pale green and translucent. The surface is rough and presents the appearance of a mass of thin-walled blisters on a larger central tube. These projections are the lateral pouches described in the histological portion. Rupture releases a thin translucent liquid from the center of the vas which is heterogeneous in appearance under low magnification. No spermatophores have been seen in the posterior vas.

Lateral to the bar from the median plate to the internal posterior edge of segment VII, the posterior vas or ductus ejaculatoris is slender, smooth, translucent, and relatively straight.

Penis. The penis is a slender, weak tube in Callinectes (figs. 3 and 5, PEN.). It arises from a slight lipped mound at the mesal point on the ventral surface of the coxopodite of the last pereipod. It is 1-2mm. in diameter and 10-12mm. long. Fine hairs surround the basal portion. The gonoduct can be seen as a white opaque tube at the center. The distal tip is closed by a turned-in lip.

The penes lie in the cavity between the ventral surface of the thorax and the under-turned apron. Each passes into the anterior proximal foramen of the first pleopod.

Pleopod 1. The highly specialized first pleopod of the male is the functional intromittent organ. Its external anatomy is illustrated in figure 6, and its relation to the other parts of the "copulatory complex" is indicated in figure 5. It receives spermatophores and semen from the small penis and acts as a tube of transport in copulation, carrying these materials into the paired oviducts and seminal receptacle of the adult female. Snodgrass (1937[6]) has described the external genitalia, and the following description employs his terminology.

The first pleopods are normally concealed beneath the flexed abdomen in the median ventral groove. Each consists of a broad flat coxopodite attached to the sternum of the first abdominal segment and a long tapering telopodite or "flagellum." These cross each other once proximally and twice distally in the normal position. They extend to the end of the terminal segment of the abdomen - a specific character within this genus. The coxopodite is partially membranous and bears many fine feathery hairs. The telopodite is folded to form a tube-like passage for the sperm and

has a large central cavity near its base. This cavity is entered anteriorly by a large proximal foramen, which receives the end of the penis (fig. 5). Posteriorly, a membranous flap covers a second foramen into the central chamber, which receives the distal portion of the second pleopod. The posterior foramen is continuous with a groove which passes along the length of the telopodite as the external indication of an internal duct. The groove opens mesally near the slender transparent tip of the flagellum. The distal two-thirds of the length of the telopodite is slightly pitted, and the distal one-third bears spines which point away from the tip and probably serve to hold the organ in position during insemination. Cochran (1935) reports the following internal musculature: promotor coxopoditis, abductor basipoditis (telopoditis of Snodgrass), adductor basipoditis, and abductor flagelli. The method of insertion has not been described.

Pleopod 2. The second pleopod is much smaller than the first and is specialized to function as a pumping organ which forces semen and spermatophores from the bulbular base of the first pleopod (fig. 6). It is composed of a large flat coxopodite, a small middle basipodite, and a short curved flagellum. The coxopodite is partially membranous and bears a number of fine hairs. About three-fourths of the flagellum lies within the distal posterior foramen of the first gonopod. The flagellum terminates in a tiny slipper-shaped transparent tip which bears spines. This pleopod contains the same muscles as the first, except that it lacks the abductor basipoditis.

OBSERVATIONS

2. Histological Anatomy

Testis. The testis, from the mid-lateral portion to the lateral tip, has the histological structure shown in figure 8. It is incompletely divided into lobes, each of which consists of a group of seminiferous tubules limited by an investment and perforated by seminiferous ducts.

The wall of the testis has two layers - a delicate surface membrane and a well developed basement of fibrous connective tissue. The peritoneal epithelium varies greatly in thickness, since it is quite thin on the external surface of the lobule but crosses the peripheral spaces between lobules with greater depth. This tissue stains orange in Azan, and scattered red ovoid nuclei of approximately 4-8mu diameter can be seen. No intercellular membrane is visible. The epithelium is not clearly delineated where it is thick, and is sometimes obscured by the adherence of body fluids. It is, however, found consistently and is considered a normal constituent.

A cremated layer of fibrillar connective tissue is present in the peripheral wall of the testis and in the interlobular walls. It shows characteristic affinity for blue in Azan and stains from brown to black in HIE. The layer varies from 3 to 10mu in thickness at the periphery, from 2 to 6mu between lobules, and may be more diffuse in the larger multilobular junctions. In all regions it bears nuclei which vary from spheres to rods in shape. These are from 1 x 4.5mu to 3 x 10mu in size, and are oriented with the long axis parallel to the wall bearing them. The average nucleus is ovoid, about 1.5 x 6mu, stains red in Azan and black in HIE, and contains scattered chromatic granules. No cell membranes are visible.

Occasional large storage cells are present in the walls of the lobules (fig. 10). They are somewhat ovoid in shape and average 6.5 x 9mu. Each contains a nucleus of 4.5 x 3mm surrounded by deeply chromophilic particles which are red and black in the stains employed. An individual cell may contain few or many inclusions but it is not clear whether these represent stages of development or of release. Storage cells usually appear singly, in any part of the connective tissue, although more may be present at the junction of several lobules.

The wall around and between lobules bears the minor blood vessels which supply the testis tissues.

Complete seminiferous lobules are shown in figure 8. Each has a wall of connective tissue around most of its periphery, but always shows confluence of its contents with that of other lobules or of ducts. Thus, the spermatogenetic cells of all lobules have access to the seminiferous duct either directly or by passage through neighboring lobules. Lobules vary greatly in distribution and in size. The maximum dimensions are about .45-.50mm. x .20-.30mm. in cross section and .45-.50mm. in length along the course of the testis. Direct measurement is made difficult by the frequent confluence of lobules. One lobule may be open at four or five points to converge with contiguous lobules or with the duct.

Groups of large cells are frequently observed along the walls of lobules or at the tips of the interlobular wall (fig. 9). These cells may be spermatogonia, but their identity is not yet certain. They are easily identified by their large size and distinct extra-nuclear cytoplasm. The spherical nucleus has a diameter of 4-8mm and the complete cell measures about 5-10mm. Mitotic figures are present in some of these cells, but the nature of their activity in the adult has not been determined.

Two cell types are present within the distal seminiferous lobules - developing spermatocytes and accessory nuclei (fig. 10). All spermatocytes within a specific lobule are usually in the same stage of development although some exceptions occur. They fill the entire lumen of the lobule and do not usually show any zonation or increased differentiation from the wall to the center. The stages are generally uniform in all the lobules in the distal part of the testis, and more varied in those near the vas efferens. They vary from 6-8mu in the early stages to 1.5-2mu in the completed sperm. They will not be described in detail in this paper.

The accessory nuclei are generally peripheral in distribution. They are not always attached to the wall, but are usually near it. Most are ovoid and about 4.5 x 7.5mu, but many are quite irregular in contour and as large as 5 x 10mu. Each contains one or two chromophilic granules and other fibrillar and granular particles which, together with their larger size, distinguishes them from the nuclei of the wall. No cell membranes are visible.

The seminiferous ducts of the testis are highly variable (figs. 7 and 8). One to fifteen portions may appear in a section, but it is probable that all of them form a single system, as indicated in the reconstructions in figure 7.

The duct is essentially a tube. It opens to receive sperm from adjacent testis lobules, but is otherwise lined with a distinct epithelium (figs. 9 and 10). This epithelium is tall and marked at some part of the periphery, but it rarely completely encloses the lumen. Where it is neither collecting from the testis nor lined with columnar or cuboidal cells, the duct is closed by a thin squamous epithelium.

Ducts vary in width from 40 to 300mu and are usually quite irregular in shape. The columnar portion of the epithelium rests upon connective tissue and is backed by strands of longitudinal striated muscle. The cells are 8-10mu high, with basal ovoid, spherical, or rod-shaped nuclei which average 3 x 6mu. There is no evidence of ciliation.

The functional structure of the apparently complex testis can be stated quite simply. A seminiferous duct and its branches collect sperm from a large number of seminiferous tubules. The tracing of serial sections proves that every lobule of the testis has access to the duct, either directly or indirectly. The duct carries sperm over a devious route but basically toward the proximal vas efferens and vas deferens.

The physiology of the testis lobule is not so clear. Each lobule usually contains a mass of spermatocytes in the same stage of differentiation, as has been pointed out. However, completed sperm are to be found in the center of lobules in which the predominant type is any of several stages in spermatogenesis. These sperm are usually in process of passing into the duct, but some have no connection with the duct. These are apparently held in the midst of the surrounding spermatocytes for a period of time.

Thus far, description has applied chiefly to the average structure in the mid-lateral testis. The complete organ is composed of a series of lobes which succeed each other along the course of the testis. Each lobe contains a group of lobules, but has continuity with other portions through some convergence of contents, through the ramblings of the seminiferous duct, and by investment. Characteristically, all of the lobules within a specific lobe contain spermatocytes or spermatids in about the same stage of differentiation. Since these stages differ markedly in

density and in staining reaction, the lobes are often easily distinguishable. The spermatocytes in the distal lobes of the testis, however, are usually in the same stage of development, and this region has a uniform appearance (fig. 8).

A striking modification occurs in certain of the seminiferous lobules. As the spermatids undergo differentiation, the wall of the lobule is modified to form a cuboidal or low columnar epithelium (figs. 11, 12, 13 and 14). This change is most frequently observed near the proximal end of the testis, but it may also occur in more distal parts. There, epitheliated lobes may be succeeded proximally by lobes containing earlier sperm stages and showing typical lobule structure.

This change does not occur in isolated lobes. A lobe in which the lobules are all columnar is adjacent to several in which the modification is in process. These, in turn, are usually next to regions showing progressively less change until this specialization is no longer apparent.

The following steps occur in the development of a lobule:

1. The accessory nuclei migrate peripherally until they are all at the wall of the lobule (fig. 11). They do not show any orientation in relation to the wall nor is any cell membrane visible. Many show no connection with the wall. The spermatids are still relatively large (4-5mm), and in early stages of differentiation.

2. The accessory nuclei form into a low group, 6-10mm high, and cell membranes appear around them (figs. 11 and 12). There is as yet no free surface. This tissue may overlay the so-called spermatogonia along the lobule wall. The spermatids are closely adjacent to the differentiating cells and have

undergone some cytological change, best indicated by their increased blueness after Azan staining. The stage achieved by the spermatids is not closely correlated with the degree of development of the epithelium. Usually, the spermatids show an increased clumping and are more compact at this stage.

3. The epithelium is completely differentiated and may show a free unciliated border (fig. 14). The average height is 20mu, and cells may be 12-15mu wide. Nuclei are most frequently median in position and generally spherical, about 7mu in diameter, although they show considerable variation. The extra-nuclear cytoplasm contains many deeply staining granules and perhaps vacuoles. Spermatids are almost completely differentiated and appear as a compact mass at the center of the lobule. No muscle is present in or around the wall. All lobules still empty their contents into the seminiferous duct.

One remarkable and puzzling observation was made. The seminiferous duct collects sperm from lobules which contain any one of a number of spermatogenic stages (figs. 9, 10, 11, 12, 13 and 14). Sperm are received from the distal testis or from lobules in any of the stages described above, although all sperm in the duct are uniform and apparently completely differentiated. No intermediate stages are seen even in lobules which contain only differentiated sperm and early spermatocytes.

Vas efferens. A short muscular vas efferens transports all of the mature sperm from each testis (figs. 15 and 16). It receives sperm from a single point along the course of the seminiferous duct and passes it into the anterior vas deferens.

The entire vas efferens of an adult male of 61 x 135mm. was 2.63mm. in length, and follows an exaggerated S convolution. Five points on the vas of this specimen were selected for histological study: (1) at the junction of the vas efferens and the seminiferous duct, and (2) 650mu, (3) 1200mu, (4) 1900mu, and (5) 2300mu from that junction. The following description is based on those points and on the sections of incomplete vas obtained from other animals.

The vas passes from the center of a testis lobe, out of the lobe, thence through connective tissue and into the mass of the fine coiled anterior vas deferens (fig. 15). The tissue surrounding the vas after it leaves the testis is composed chiefly of blood sinuses and connective tissue strands. It contains large strongly chromophilic bodies of 30-40mu which may be storage or waste reservoirs (fig. 15).

The vas shows a general increase in diameter from distal to proximal portions. A slight lip can be seen at the point of juncture with the seminiferous duct. Near this point the vas of the crab under observation has a diameter of about 100mu, which increases as follows: at point (2) 123 x 143mu, (3) 100 x 165mu, (4) 215 x 255mu, and (5) 200 x 270mu.

The outer wall of the vas proper consists of rather dense connective fibers and associated nuclei (fig. 16). A variable ring of striated muscle lies within this. Most of the fibers are circular, but occasional bundles of longitudinal fibers occur, usually inside of the circular set. The connective and muscular investments increase in quantity along the course of the vas. Both are light and incomplete near the testis and heavy and entire near the proximal end.

The vas efferens is characterized by the presence of several typhlosole-like projections of the wall into the lumen (figs. 15 and 16).

These appear about 150mm from the distal end of the vas and are present in all subsequent parts. Near point (2), 650mm from the anterior end, the largest of these contains a large blood sinus, but none other shows any cavity. Two to four large projections are present most of the length of the vas. These may be 70mm high and are often bi- or tri-furcate. Two or three lower portions of tall epithelium are usually found between the taller protrusions.

Each large projection has a core of connective tissue and muscle fibers which is continuous with the wall of the vas (fig. 16). Frequently, a few strands of muscle and connective tissue extend nearly to the free tip.

The internal epithelium is highly variable. It may be quite low, 4-6mm, between the projections, and it may be 50mm high on the projections. Near the testis, very tall cells with slender nuclei cover the projections, but in more proximal portions the epithelium is less variable and generally lower. Nuclei are located near the free end of the cells, and vary from spheres of 4-6mm in diameter to rods of 1.5 x 8mm. No cilia have been observed.

All vasa efferentia seem appear to be inactive. The typhlosolic projections occupied a major portion of the lumen, and very few sperm cells were present. Sperm were, however, quite abundant in the seminiferous duct and in the anterior vas deferens, and there is continuity of their lumens with that of the vas efferens. Each end of the vas is marked by a slight lip, and these may have been relaxed in the process of fixation.

Vas deferens. Anterior. The coiled anterior vas deferens differs markedly in histological structure from the vas efferens. Most of it

bears a tall epithelium, and its modifications for secretion, spermatophore elaboration, and spermatophore storage as well as for transport make it quite different from the simpler transporting vas efferens.

All of the anterior vas deferens has the same basic investment. A thin superficial membrane covers the organ, and all parts of the greatly convoluted coils of the vas are within it. It is quite like the peritoneum of the testis, but is based on a heavier layer of connective tissue. The testis, vas efferens, and vas deferens have a continuous covering, and the coils of the vas are not separable because of it.

The interlobular spaces are filled with large blood sinuses, smaller blood vessels, connective fibers and nuclei, and large chromophilic bodies like those described near the vas efferens. All parts of the anterior vas are supplied with circular striated muscle, but the layer is not so heavy here as around the vas efferens. A thin stratum of connective tissue surrounds each of the coils.

There are no points of sharp differentiation between subsequent portions of the anterior vas, but certain changes occur gradually in progression from the most anterior part toward the median vas. The tube increases greatly in size, as indicated by figures 3, 4, 17, 18, 19, 20 and 21; the epithelium varies in size and in nature of secretion, and the contents of the lumen are modified. These variations are great enough and of sufficient importance to merit detailed attention.

The most anterior coils are slightly larger than the vas efferens, with a diameter of 200-300mm, and have the structure shown in figure 17. They have a very tall feathery epithelium, of 40-60mm, which is secretory. The cells of this epithelium stain less densely than those of subsequent

coils, and show a finely granular cytoplasm with indistinct indications of vacuolization. Nuclei are median or slightly distal, and variable. Most are ovoid and about 3 x 6mu in diameter. The cell walls are not distinct, but nuclei in the same plane are at intervals of about 15mu, suggesting that as the cell width.

The sperm within these anterior coils are rather densely packed and highly chromophilic, staining red in Azan and black in HIH. A brilliant-staining secretion is liberated from the epithelium of this region, even in its most anterior part (fig. 17). This material is scarlet red after Azan and jet black after HIH. For convenience and because it shows a stain reaction similar to the secretion described by Spalding (1932), this material will be designated Substance A. It is abundant around the homogeneous mass of sperm and at the surface of the epithelium.

The tube presents the appearance illustrated in figure 18 when it reaches a diameter of about 400-500mu. The epithelium is higher, 70-80mu, and denser. Its nuclei are larger, averaging 12-15mu, median or slightly distal in position in the first coils and basal in later portions. The cells are vacuolated and 14-24mu wide. Substance A is present and more abundant, and is partially mixed with a second product. This Substance B (Spalding 1932) is less homogeneous and stains bright blue in Azan and gray to brown in HIH. Slender lines of Substance B can be seen extending across the lumen through the sperm mass (figs. 18 and 19), and these are apparently the first stage in the separation and elaboration of the spermatophores. The two secretions of this region cannot be associated with any distinct cell types, since all of the epithelial cells appear to be uniform. Both products occur along the free surface of the epithelium and in varying abundance in deposits around the periphery of the lumen.

Subsequently, the epithelium becomes much lower, averaging 15-25mu in height (fig. 20). It is very actively secretory, and apparently produces Substance B, since only that is present at the epithelial surface. Nuclei are reduced in size, 3 x 4.5mu, and consistently basal. All the nuclei of the vas deferens contain deeply-staining inclusions and these are the most marked. Nuclei are frequently full of bodies of various sizes. Sperm masses are rather clearly delineated, and some appear to have a thin investing membrane. Very little of Substance A is visible.

The last coils considered to be part of the anterior vas are quite large and contain completed spermatophores (figs. 21 and 22). Cross-sections show a coil diameter of 1-3mm., and may contain fragments of scores of spermatophores. The epithelium is low, 20-30mu, and shows little or no evidence of secretory activity.

Spermatophores are ovoid (fig. 21), and the largest seen measured 340 x 250mu. In each, sperm are tightly packed within a thin envelope. This membrane stains red in Azan and black in HIH. The sperm mass is often shrunken away from the envelope in stained material, and may reveal a uniform light blue substance between the sperm and the membrane (fig. 22). The spermatophores lie free in a quantity of very heterogeneous material. The base of this matter appears as fine grains or droplets, blue or grey-brown in these stains. It has a great many inclusions (fig. 22). These may be spherical, ovoid, rod-shaped, or irregular in outline. They reach a maximum of 50mu in greatest dimension, and most of the larger are in the form of rods. These particles stain variably. Most are blue after Azan, but occasionally all are red and various intermediate conditions were seen. This stain is somewhat difficult to apply with perfect consistency, and it is not known whether the variations seen resulted from

alterations in technique or from a near-neutrality of the substance, which might result in visually striking changes in stain susceptibility in almost consistent treatment.

This portion of the anterior vas and all of the median vas are extremely hard after fixation, making satisfactory sectioning impossible. The spermatophores and the surrounding mass shatter badly and make complete analysis difficult. Descriptions of these parts are based on the fragments obtained and on observations of the faces of sectioned blocks by vertical illumination as employed in the Ultropak microscope.

Median. The median vas deferens is the most massive portion of the system (fig. 4). It is composed of several very large coils of irregular shape enclosed as a single mass. A thin superficial membrane surrounds the coils, and blood vessels, blood sinuses, and connective tissue occupy the spaces between coils. It was not possible to ascertain the complete morphology of the organ, and it is not determined whether the coil is single or multiple at this point. The wall of each coil is, as in more anterior portions, based upon a stratum of connective tissue and has a light muscularis.

The epithelium is relatively low - about 15mu, with nuclei of 6 x 10mu. These cells show a rather dense cytoplasm after the techniques employed, and analysis for secretory activity is difficult. Some signs of vacuolization were present, however, and the simplest form of the lumenary content occurs at the surface of the epithelium. It seems probable that the cells secrete actively.

Each coil has a large lumen which may reach several millimeters in diameter. Most of these are filled completely with a mass of particles which have the appearance of very fine fluid droplets. They vary in size

up to about 7mu and stain homogeneously blue in Azan and brown in HIH. The same material is present at the surface of the epithelium, in the form of tiny particles, suggesting these cells as the source.

Some coils contain spermatophores. These are apparently nearest the anterior vas and contain some of the heterogeneous matter found in that region. Only occasional spermatophores occur in other coils of the median vas.

Posterior. The posterior vas deferens is a complex tube (figs. 23, 24 and 25). It is characterized, in the intercopulatory condition, by the presence of one or two major cavities and a great number of smaller lateral pockets. The lateral pockets fuse with each other or with the central portion, so that the lumens of all parts are in confluence. There is considerable variation in the wall, the epithelium, and the contents of the lumen, but the occurrence of peripheral pouches is consistent.

The anterior end of the posterior vas is much like a reduced portion of the median vas. A number of large parts of the duct appear in any one section, with smaller branches around and among them. The epithelium is typically low and cuboidal, of 20-25mm, with large median to basal nuclei of about 10-15mm. It does not appear to be secretory. Each branch is lightly muscled and the group of tubules is embedded in connective tissue and bound by a thin peritoneum.

Homogeneous and fine material fills all of the pockets. It is like that found in the median vas, and shows the same staining reactions typically blue after Azan but occasionally orange or red.

The mid-region of the posterior vas shows some modification. The large central cavity is more consistently single and the lateral pockets are smaller and more uniform. The epithelium is taller, about 15-30mm,

denser, and appears to be actively secretive (fig. 23). The muscularis is heavier, and scattered longitudinal bundles are present, especially around the large central portion of the duct. The central cavity and the larger peripheral cavities contain the uniform material described above, although some heterogeneous particulate chunks may be present. Fine droplets or vacuoles are visible near the surface of the epithelium.

At the bar anterio-dorsal to segment XII, the posterior vas has the structure shown in figures 24 and 25. The entire organ is 2-3mm. wide at this point. It has the fine peritoneal lining described for other regions, and connective tissue and blood vessels around the duct. The largest tubule may appear as two or three separate portions or may be fused to form a central vessel. All tubules have a muscularis of circular fibers, and longitudinal bundles are increasingly abundant near the central tubule.

The epithelium is variable (fig. 25). It is typically tall (30-60mu) with a crenated surface in the central tubules and lower (15-30mm) and more even in the peripheral pockets, but the distinction is never sharp. Nuclei are uniformly ovoid and about 3 x 6mm. All of the epithelium shows a "brush" border which is probably ciliated. The abundance of very fine droplets and granules at the surface indicates secretion by this epithelium. The contents of the lumen are variable. Most of the material is fine and uniform, especially near the wall, but the same heterogeneous mixture encountered in more anterior sections may be present in the central and larger lateral tubules. Large cavities sometimes appear within the fluid in the lumen, and these contain very fine highly chromophilic particles.

The last inch and a half of the posterior vas, the ductus ejaculatoris, is simpler and more muscular (fig. 26). Near the bar, the vas has a diameter of 1-3mm., which is reduced to about .5-1.0mm. where the vas

passes through the shell. The vas is embedded in connective tissue and is often attached to muscular, vascular, or other tissues of this region, or it may be free. The muscularis is increasingly well developed, is accompanied by a great deal of fibrous tissue, and is well supplied with blood vessels. The lumen is simplified in this portion and is single and nearly round near the shell. The epithelium is 15-20mu high and has a distinct brush border. Nuclei are median, ovoid, and average 3 x 6mu.

The contents of the lumen are rather uniform. Fine vacuoles or droplets are present at the surface of the epithelium, which appears to be finely ciliated. Again, most of the central material is uniform, but a mixture may be present.

A ductless accessory gland accompanies the posterior vas deferens from near the bar almost to the outside shell (fig. 27). It may be a single mass or divided into several major portions, and reaches a maximum size of 1.00 x .3mm. The cells are arranged in intertwining cords of 6-15mu width. Cords are usually composed of a single linear group of cells. Connective tissue separates and marks off the individual cords. Cells vary in size, with the maximum at about 9 x 15mu. Nuclei vary accordingly, but are most frequently spherical and 5-6mu in diameter. Each has one or two large chromophilic inclusions, as well as other granulations. The gland is richly supplied with blood vessels, which may branch within the gland. Many of the same vessels also reach the epithelium and muscularis of the vas. No function of the gland is determinable from direct observation.

Penis. The penis is a slender external tube which passes spermatophores from the vas deferens into the base of the first pleopod or copulatory cirrus. The proximal two-thirds of it has the histological

structure shown in figure 28. It is .5 to 1.5mm. in width. Expressed most simply, it is a very muscular epitheliated duct lying in parenchymatous tissue and protected by an external cuticle. The organ is complex, and merits further histological description of the average structure. Modifications along its course will be considered.

The internal epithelium and adjacent tissues are shown in figure 29. The cells are of variable height, and average 20-40mu. Nuclei are ovoid, vary from 4 x 5mu to 3 x 8mu, and are median in position. Granules can occasionally be seen at the distal end of the cells, and the border is markedly ciliated (figs. 29 and 30). There is no indication of secretion, and the lumen contains only a small amount of achromatic material.

The epithelium is closely applied to a very dense stratum of circular connective tissue fibers. The layer is about 5-8mu thick, and is bright blue or brown after the stains applied.

The muscularis is heavier here than in any other part of the system. It may be present as a mass 90-100mu deep around the epithelium. Most of the muscle fibers are circular, in bundles 3-15mu wide. Longitudinal bundles are interspersed among the circular fibers, and are often grouped to form a large strand at one point near the sub-epithelial connective tissue stratum. No cytological description will be given here, but this muscular tissue, as well as that from other regions in this species, stains quite brilliantly and offers excellent material for cytological study.

Parenchyma occupies most of the penis between the muscularis and the cuticle. It is loose and spongy, consisting basically of open fibrous connective tissue. Blood sinuses and blood vessels are plentiful. Certain interesting cells occur which apparently represent stages

in the development of large storage depots. The largest of these are 25-30mu in diameter, are "signet" shaped with a peripheral nucleus and cytoplasm, and contain a large volume of stored material in the center. This substance may be granular or quite uniform. Smaller and probably younger cells of this series are scattered through the parenchyma.

Hypodermis underlies the cuticle. The layer is 10-20mu thick, and is rarely more than two cells deep. Cells are quite uniform, about 5-10mu, with nuclei of 4-6mu. The hypodermis is quite closely associated with the inner margin of the cuticle. Its cells show increased density near the cuticle, and may appear vacuolated at their more proximal border.

The cuticle averages 18-20mu in thickness. Fine striae parallel the surface and the cuticle increases in density from the hypodermis to the surface. It stains increasingly deep shades of blue from the hypodermis peripherally, and has a bright red outer margin after Azan.

Distally, the internal tissues of the penis change considerably. The muscularis gradually is reduced in size and almost disappears. The lining epithelium is abruptly replaced by invaginated cuticle with a modified hypodermis. This terminal lining is shown in figure 31. The internal cuticle stains uniformly, and is 7-15mu thick. The underlying hypodermis is formed of very slender cells, with maximum depth of 120mu and average width of 10mu. These cells have elongate nuclei placed medially and averaging 4 x 8mu. The distal cytoplasm of these cells is finely granular.

The cuticle is continuous with that of the external surface. The hypodermis of the internal lining is in continuity with that of the periphery and apparently is modified from it. The parenchyma, external hypodermis, and external cuticle are essentially as described above.

but the organ reduces in diameter toward the tip. The invagination penetrates the distal end for a distance of approximately .75mm.

Pleopod 1. The general internal anatomy of the first abdominal pleopod is illustrated in figure 32, a cross section near the basal cavity of the telopodite. The internal tube is seen to be quite well closed by the extensive overlapping of the edges and lines with a cuticulum continuous with that of the external surface. It should be noted that this section is from a soft crab in which the exoskeleton is incompletely hardened and that the cuticulum is therefore not typical of the adult hard crab.

Hypodermis, one to three cells in depth, lines the surfaces. It is quite active in this stage.

Loose fibrous connective tissue, blood vessels and blood sinuses occupy much of the internal region of the pleopod. Muscles are present in more basal sections but are absent at this level.

Many rosette glands are present around the cavity. Small stages appear as masses of nuclei grouped in a compact bundle 15-20mu in diameter (fig. 33). Large well developed glands are 60-70mu in diameter. Nuclei are larger, 4 x 6mu and peripheral in wedge-shaped cells. These cells stain pale blue in Asam. A very small duct, 2-4mu wide, is present at the center of some glands. No connection with the surface is visible. These glands are located in only the basal one-third of the pleopod.

Figure 34 shows a section near the distal tip of the pleopod. The cuticle, hypodermis, connective reticulum, blood vessels, and blood sinuses are retained, but the rosette glands of the basal region are absent. The internal cavity is still well closed.

DISCUSSION

1. Gross Anatomy

Internal reproductive organs. The internal reproductive organs of Callinectes sapidus follows the pattern found, with some modification, in all Decapoda. The testes are paired and connect at their median end, and the glandular vasa deferentia transport sperm to the exterior, as is usual in members of this order (Snodgrass 1936, Calman 1909). The arrangement of these organs differs, however, from that of the Anomura, where they are usually confined to the abdomen and often unsymmetrical (Calman 1909, Faxton 1917a, Jackson 1913), and from the Astacura and Panilura, where the testes are not distributed anterio-dorsally (Herrick 1909, Snodgrass 1936). The organs of all Brachyura previously described, however, resemble closely those of C. sapidus. The descriptions and illustrations of Faxton on twelve Brachyuran crabs of the West Coast (1915, 1917a) of Binford on Menippe mercenaria (1913), of Pearson on Cancer pagurus (1908), and of Spalding on Carcinus maenas (1942) all conform with this species in structure and distribution.

There are, however, several distinctive features present. The testis is more slender in proportion to body size than that of previously described forms, and the modifications of the vas deferens are more marked. Several writers have mentioned some difference between the anterior and posterior ends of the vas, but none are as striking as the specialization seen in the coiled anterior vas and massive median vas of this species.

Spermatophores are stored in quantity only in the anterior vas deferens of C. sapidus. This fact will be of value in further studies of maturity in this species. As pointed out by Calman and Spalding, the

Anomura usually employ small pedunculate spermatophores and the Astacura large non-pedunculate spermatophores. The first two generally store the completed bundles over the length of the vas. Some Brachyura, Cancer (Pearson, Faxten 1917a and Kelliker 1841) and Carcinus (Spalding, Busseta 1926) retain spermatophores in the entire length of the tube, whereas C. sapidus stores spermatophores in a specialized anterior region.

Penis. The short external penis of some Decapoda is an interesting modification among those which have occured to permit accurate sperm deposition or insemination in the absence of a true intromittent penis. Duvernoy (1850) divides the Decapoda into two sub-orders on the basis of the position of the penes or "verges" - "Brachygastres" possessing an external penis and "Macrogastres" having an internal coiled penis. He was apparently the first to understand and write upon the functioning of the entire intromittent complex of these Crustacea. Penes are present in all Brachyura, certain Paguridae (Calman), and some Astacura. Spalding describes it on Carcinus as the "genital papilla" which is quite like that of Callinectes. Andrews (1911) figures a short conical papilla of Cambarus affinis which had been misunderstood by previous investigators. It is highly probable that the penes of all Decapoda which possess the intromittent complex are permanently in position in the first pleopod and not inserted with copulation, as had been suggested.

Pleopod 1. The modifications present in the first and second pleopods in male Decapoda are often so great as to be of taxonomic character. Duvernoy described certain of these and explained their purpose. Brachyuran crabs differ from most other Decapoda in the presence of paired internal seminal receptacles in the female, and the "intromittent complex" of the male is specialized to effect insemination. In this

group the first pleopod is a tube with foramens admitting the penis and pumping second pleopod. C. sapidus has unusually long and completely tubular first abdominal appendages, but otherwise its "copulatory cirri" are much like those of Cancer (Pearson, Bell 1905), Carcinus (Spalding), and other Brachyura. Ordway (1863) first used the relative length of this appendage as a specific character within this genus, and it is included in the present authoritative taxonomic organization of Rathbun (1930). The pleopod of sapidus is longer than that of any other Callinectes.

Pleopod 2. The second abdominal appendage of this species has a characteristic shape, but differs in no important feature from other Brachyura. Its function is probably entirely that of a plunger forcing semen and spermatophores from the base of the first pleopod.

2. Histological Anatomy

Testis. The testes of Decapoda fall into two types, depending upon the nature of spermatogenic activity. Many species show the presence of a primitive germinal cell type at the periphery of the testicular follicle with progressive spermatocytic differentiation toward the open center of the lumen. Astacus fluviatilis, the European crayfish, has been most thoroughly examined by Sabatier (1855), Gilson (1886), Grobben (1878), and Hermann (1890). A brief summary of the observations of these workers will serve to illustrate the first type.

The testis is composed of a large number of small pockets, variously termed "cul-de-sac testiculaire", "acinus", "follicle of the testis", "spermatogonie" and, in the present paper, seminiferous lobule. A plasmodium with coarse granular nuclei lines the lobule and envelopes large less granular cells. The nuclei of the plasmodium increases in size and segregate as cells termed "cellules-mère", "métrocytes", "Kreatzkeime", "protospermatoblasts", or "germes de remplacement" and corresponding to spermatogonia of more modern nomenclature. These apparently differentiate directly to become sperm cells free in the open center of the lobule and pass back to the vas deferens.

This general method of development occurs in Decapoda of several different groups. Minippe mercenaria, the stone crab of southeastern United States, is described by Hinford as producing sperm by differentiation in successive waves from one wall of the "testicular tubule" toward a simple duct along the opposite wall. In Paralithodes camschatica, the edible Japanese crab, ". . . .the spermatogonial cells are found lining the inner walls of the cyst while the deeper portions are occupied by the maturing cells in different grades of development

. . . ." (Miiyama 1926, p.22). Eupagurus bernhardus, the hermit crab, buds off "gonadial elements" into the lumen of the tubular testis. These proliferate and differentiate as they pass down the long testis to the vas deferens (Jackson 1913). According to Komai (1920) the stomatopod Squilla oratoria produces sperm by a similar method.

Several decapods follow the same type of testis physiology evident in C. sapidus, where the mass of spermatocytes within a specific lobule shows a uniform stage of maturation. Panulirus japonicus, the Japanese spiny lobster (Miiyama), Homarus vulgaris, the European lobster (Hermann), and the brachyurans Carcinus maenas (Spalding), Maia squinado, and Stenorhynchus (Hermann) all have a single spermatogenic stage present within a lobule at one time. There is apparently no evolutionary significance in this particular physiological feature, since several different sections within the same Sub-order are represented under each type.

The accessory cells present in the tubules of C. sapidus are difficult to homologize with testicular components found in other species. Two cell types are present within the seminiferous lobules of all species where histological description is adequate. Binford figures a scattered cell of nucleus in the wall of the follicle of Menippe and between succeeding groups of spermatocytes, but does not discuss it. Hermann describes large agranular cells which have no function in sperm production in Astacus. Gilson, basing his discussion on Grobben, mentions a cell type inactive in spermatogenic function which secretes the "plasmas spermatiques." Hermann figures and describes several lobules from Homarus vulgaris which bear several striking resemblances to sections from C. sapidus. One of these is the presence of small nuclei among spermatocytes and near the periphery. Paaten mentions "nutritive cells"

in the lobules of the testis of Cancer magister (1918) and Loxhoconcha bHelling (1926). Both arise from the primary spermatogonia. These are reminiscent of the "indifferent cells" of the developing ovary of C. sapidus, which arise from primitive oogonia but never function as oocytes (Cronin 1962). The origin of these nuclei and of the large cells presumed to be spermatogonia in the male C. sapidus can only be determined by studies of early development of the system.

A single brief record in the literature has bearing on the final disposition of these accessory nuclei in a distinct epithelium around the periphery of the lobule. Minford says: "The outer wall and sometimes these inner partitions which border the regions containing mature spermatozoa, become thick and columnar in structure." (p.149), but his figures of this region show no similarity to the stages of testis epithelialion illustrated in figures 11-14 of this paper. No conclusion is warranted concerning the purpose of this epithelial development. It may mark the termination of function of the specific lobule involved.

Very few decapod testes have, apparently, collecting tubes comparable with the seminiferous ducts of C. sapidus. Minford implies that spermatozoa are "pressed" out of the testis by the developing spermatocytes and by the production of fluid. He figures an open cavity at one side of the follicle lined with a plasmodium-like group of nuclei except in one of four illustrations, where the cavity has a distinct low columnar epithelium. No muscle or other means of locomotion is described. Hermann, in the figure of several lobules of Homarus vulgaris referred to above, has drawn two "canalicules excréteurs" which closely resemble the seminiferous ducts of C. sapidus. He does not discuss nor describe them further. Corresponding ducts should be observed in any species when they are

present, and it is not easy to accept the conclusion that a feature of this sort is absent in most of the decapods having an otherwise similar system.

Vas efferens. Calman names a ". . . .narrow efferent duct leading from the testis. . . ." (p.289) as a typical division of the decapod of vas deferens. No other mention was found in the literature reviewed concerning any structure comparable to the vas efferens of the blue crab. Other descriptions of the male reproductive system are, however, rather general and most frequently incidental to a discussion of spermatogenesis, comparative anatomy among groups, or monographic presentation of a broad field of facts concerning a species. If the size of the organ in this species is at all typical, the vas efferens may easily have been entirely overlooked. On the other hand, few decapods have a highly differentiated vas deferens, and the vas efferens of C. sapidus may also be a relatively rare incident of specialization.

The vas has one interesting feature in its modification for expansion, as indicated by its typhlosole-like projections of the epithelium in the apparent absence of either secretory of absorptive function. The extension of the muscularis and tunica fibrosa of the vas into the projections further suggests their use in expansion. Perhaps sperm is released from the testis in considerable quantity at intervals and passed rapidly through the vas efferens with the contraction of its muscularis.

Vas deferens. Calman makes the following statement concerning the vasa deferentia of Decapoda:

> The vas deferens presents typically three divisions. . . . (1) a narrow efferent duct leading from the testis; (2) a glandular part, with wider lumen, often convoluted; (3) a terminal ductus ejaculatoris with muscular walls. In Brachyura (except Dromiidae) the distal portion of the second or glandular division is provided with caecal diverticula which in some cases are very numerous, forming a large glandular mass. (p.289)

Survey of the rather brief literature confirms this generalization but calls attention to the wide variation in structural and functional detail existing among decapods. A brief summary of several groups will serve to illustrate the relationship between C. sapidus and other Crustacea.

The penaid shrimp described by Henri-Heldt (1932) have three divisions within the vas deferens, modified for (1) reception of sperm, (2) secretion, and (3) molding and storage of spermatophores. In most of this, the secretion is not released around the sperm mass but in a parallel and sometimes contiguous chamber along the vas, which fuses with the region containing sperm near the posterior end of the duct.

The vasa deferentia of Anomura are modified for the elaboration of pedunculate spermatophores attached to a short strip of membrane. Histological studies are very brief, but several reports include some description. Heuchet (1930) lists for Diogenes pugilator (1) a secretory "hélice," (2) a coil with a large lumen where spermatophores are segregated, (3) a region where the spermatophores are attached by a pedicle to the lining membrane, and (4) a portion where segments of this membrane break off and are stored in a mucous secretion. In Eupagurus bernhardus Jackson describe an anterior region with tall epithelium followed by a straight portion where segmentation occurs and by a long storage duct. A muscular ductus ejaculatoris is present. Gilson figures the spiral part of the "conduit déférent" of Pagurus callidus as being quite like the most anterior coils of the vas of C. sapidus, with tall epithelial cells having a median nucleus. He also presents a more posterior section containing spermatophores and "plasma coagulé" reminiscent of more posterior portions of the anterior vas of C. sapidus.

Pasten (1917) has given the only comprehensive histological description of the vasa deferentia of the major groups of Decapoda. He describes the vasa of the anomurans Pagurus gibbus, and Lopholithodes mandtii and lists serosa, fibrous subserosa, circular and longitudinal muscularl, and a cuboidal secretory epithelium as the components of the wall. The epithelium of Lopholithodes is thin and generally uniform, but that of Pagurus becomes concentrated, tall columnar, and strongly ciliated at one point.

The same author has presented the histological anatomy of the vasa in three Astacura, Astacus leniusculus, Homarus americanus, and Panulirus interruptus. These have (1) a fibrous connective tissue coat, (2) circular layer of smooth muscle and (3) an inner columnar epithelium which is ciliated and usually secretory. The vas of Homarus is differentiated longitudinally, having columnar epithelium in the proximal slender end and a highly convoluted internal epithelium, although less so than C. sapidus, in the distal end where it bears fine cilia on tall cells. The vas of the spiny lobster, Panulirus, contains a large glandular typhlosole-like structure which secretes the viscid fluid used in fixation of spermatophores on the ventral surface of the female. The vasa of Astacus are nearly uniform over their entire length.

Brachyuran crabs have the same basic layers in the vas: (1) outer thin fibrous tissue, (2) thin circular smooth muscularis, and (3) prominent epithelium. Pasten has described Cancer magister, C. oregonensis, and Lophopanopeus bellus (1917a). The proximal end in both species of Cancer contains a tall vacuolated and convoluted epithelium which is secretory. The distal vas is larger, filled with spermatophores, and has a low epithelium. The vas of Lophopanopeus is uniform and like the posterior

portion of Cancer. This "black-clawed crab" produces a large single spermatophore after the manner of the Astacura.

Binford, writing on Mithrax, says: "The deferent duct is lined with a layer of columnar epithelium which secretes the substance that forms the walls of the spermatophores." (p.149).

The only previous detailed description of the elaboration of the spermatophore has been that of Spalding on Carcinus maenas, the green crab (1942). The process in this species is much like that of C. sapidus, but differs in several details. Spalding describes four regions of the vas deferens:

(1) Near the testis, the wall is low columnar, non-secretory, and thinly muscled. Only free sperm are present.

(2) In a region producing substance "A", erythrophilic in Mallory's, the epithelium is cuboidal. The secretion penetrates the sperm mass and spermatophores are moulded by "churning movements" of the vas.

(3) Where substance "B", blue after Mallory's, is produced, the epithelium is tall and uneven, being composed of large bulbous cells which are very actively secretory.

(4) Most of the length of the vas stores spermatophores. A low cubical epithelium with little differentiation is present.

The spermatophores of C. sapidus appear, on the contrary, to be isolated by the blue secretion and then enclosed in a capsule which stains red. No evidence was obtained on the chemical nature of these fluids.

The degree of specialization exhibited by the vas deferens of C. sapidus is high among Decapoda. The anterior vas is a well developed

structure for the transportation of sperm and for the elaboration and storage of spermatophores. The function of the massive median vas has not been determined. The seminal receptacle of a recently impregnated adult female contains a quantity of unidentified material superficially of the same appearance as that in the median vas. The long retention of viable sperm in this receptacle is interesting and is not understood and the possibility of the production of sustaining material by the median vas should be investigated. The posterior v_{as}, including the ductus ejaculatoris, is secretory but otherwise apparently functions only at the time of copulation.

The presence of cilia in the posterior vas and penis is of interest since, as Pasten pointed out, many general texts list the total of usual absence of cilia as a general attribute of all Arthropoda (1917a, 1917b). Cilia line part of the gonoduct of Astacus, Pagurus, Homarus, Panulirus, and Callinectes, and ". . . .further investigation in the Arthropoda will in all probability show that cilia are more generally present among the members of this phylum than has hitherto been assumed." (Pasten 1917a, p.294).

Penis. The only previous histological description of the penis encountered is that of Andrews. He describes and illustrates sections of the penis, called simply papilla, of Cambarus affinis. The basal portion is much like figure 31 of this paper, with an internal epithelium, a large region of connective tissue and blood vessels, and an enclosing light cuticle. He does not describe the epithelium in any detail, but mentions it as a "secreting lining." The distal portion is also much like that of Callinectes (figure 31). The lumen is closed by a "great longitudinal ridge" surfaced by inflected cuticle. Few muscle strands

are present. He ascribes to this ridge the function of closing the tip of the gonoduct, and suggests that it would be forced open only by the passage of semen.

He shows a number of small tubular glands in the wall of this distal portion which open into the central duct. They are not present in C. sapidus.

The penis of Callinectes sapidus appears to be employed only for transport since secretion does not occur. The heavy muscularis and the cilia both probably assist in passage of semen and spermatophores into the intromittent pleopod.

Pleopod 1. Spalding (1942) presents the only available histological description of the first copulatory pleopod of Decapoda. Spalding describes and illustrates that appendage in Carcinus maenas. He demonstrates the nature of the cavity of the pleopod, which is less completely closed than in C. sapidus, and describes the region of rosette glands around the basal cavity. According to his description, the ducts of these glands open into the central cavity of the pleopod. These ducts were seen only within the glands in C. sapidus. Yonge (1938) showed that glands in Homarus comparable to these produce the body cuticle and secrete the cement which binds the eggs to the pleopods of the female. Their function has not been established in Callinectes.

SUMMARY AND CONCLUSIONS

1. The gross and histological anatomy of the parts of the adult male reproductive system of Callinectes sapidus Rathbun is described.
2. The internal organs consist of paired anterior testes, fused at the median end, and paired central vasa deferentia. The vasa are differentiated into coiled anterior, massive median, and slender posterior regions.
3. The external organs consist of short penes situated on the basal coxopodite of the last pair of legs and the modified first and second abdominal appendages. The first pleopod is a tube which receives semen and spermatophores from the penis and carries them to the seminal receptacle of the female. The second pleopod is a pumping organ inserted into the base of the first.
4. The testis is subdivided into a series of lobes. A lobe contains a number of seminiferous lobules. Each of these is filled with a mass of developing spermatocytes which are all usually in the same stage. Accessory nuclei and cells regarded as spermatogonia are present in all lobules.
5. Seminiferous ducts collect spermatozoa from the lobules and transport them to the vas efferens. These ducts are epitheliated and lightly muscled with striated fibers.
6. At the termination of spermatogenic activity within a lobule, the accessory nuclei gather at the wall and form a cuboidal to columnar epithelium.
7. A short muscular vas efferens, with typhlosole-like projections which provide for expansion, carries sperm to the vas deferens.
8. The coiled anterior vas of this species has a generally tall epithelium and is lightly muscled. It secretes two fluids which appear to

delineate masses of sperm and enter into the formation of the capsule of the ovoid spermatophore.

9. Most of the completed spermatophores are stored in the larger coils of the anterior vas.

10. The massive median vas is essentially a storage region for a heterogeneous substance of unknown function. This material is probably carried into the seminal receptacle as part of the semen.

11. The long posterior vas deferens is a complex tube with a number of lateral pouches. It is ciliated, probably secretory, and has a muscular wall.

12. A muscular ductus ejaculatoris passes sperm to the penis.

13. The penes are weak chitinized tubes containing the gonoduct embedded in parenchymatous tissue. The gonoducts are heavily muscled and provided with a well ciliated epithelium. The external cuticle is inflected at the tip on a large ridge which closes the end of the penis between periods of copulation.

14. The first pleopod possesses a closed groove for transport of the genital products. Rosette glands may add to the semen.

15. All of the muscular tissue of the male reproductive system is striated.

16. The male reproductive system of Callinectes sapidus is comparable to that of the Decapoda and particularly to that of other Brachyura. However, it shows a relatively high degree of differentiation in the presence of the seminiferous ducts, the vas efferens and the specialized portions of the vas deferens.

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FIGURES

Figure 1. Dorsal view of mature male. Note heavy claws. X5

Figure 2. Ventral view of mature male. Note structure of abdomen. X5

Figure 1.

Figure 2.

Figure 3. Dorsal dissection of male reproductive system.

T - Testis

A.V.D. - Anterior vas deferens

M.V.D. - Median vas deferens

P.V.D. - Posterior vas deferens

PEN. - Penis

Figure 3.

Figure 4. Medio-dorsal view of male reproductive system after median sagittal dissection and removal of the carapace. Positions of the digestive system, heart and sternal artery are indicated by dotted line.

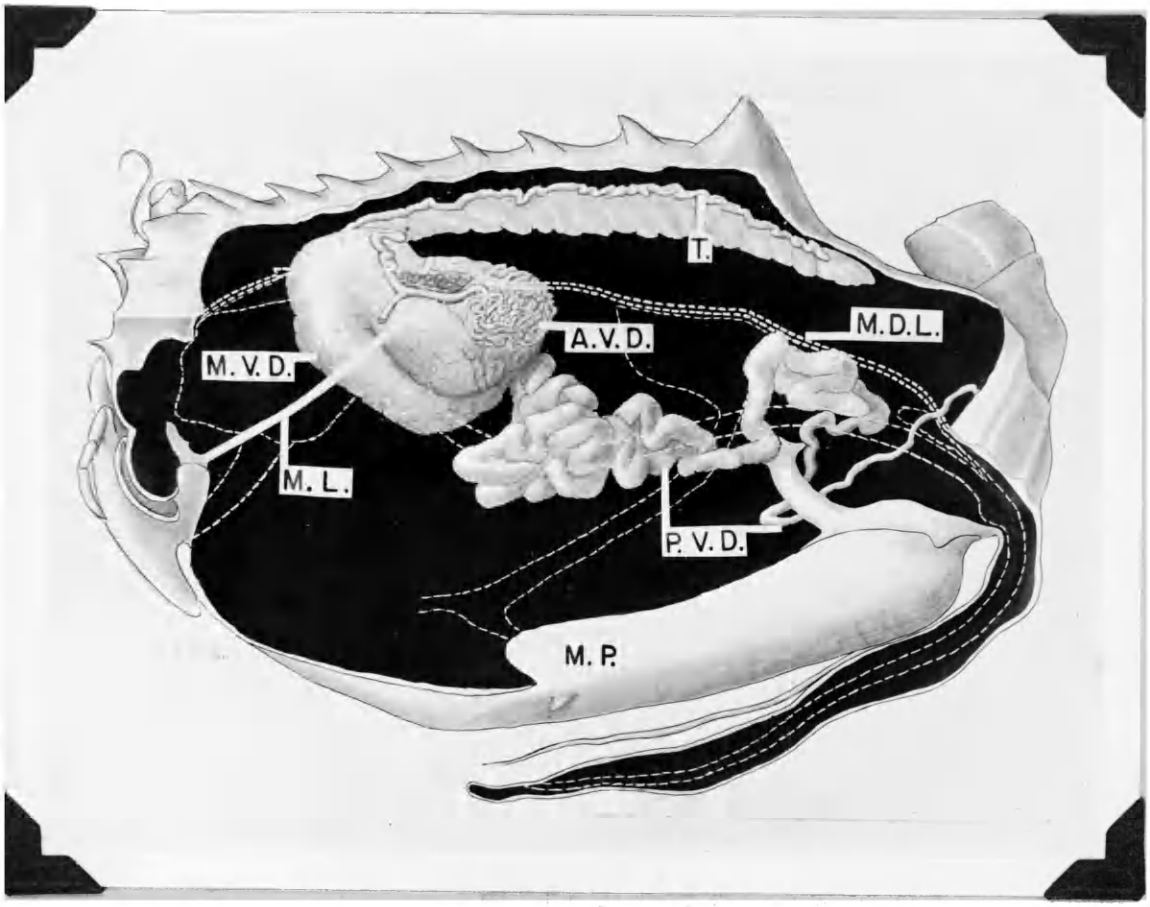
M. L. - Mandibular leader

M. D. L. - Median dorsal line

M. P. - Median plate

Other labels as in Figure 3. (Drawing by Hazie M. Smith)

Figure 5. Posterio-ventral view of external genitalia after partial extension of the abdomen to show the insertion of the penis (PEN.) and second pleopod (PL.2.) into the base of the first pleopod (PL.1.). COX.XII is the coxopodite of the last pereopod. (Drawing by Hazie M. Smith)



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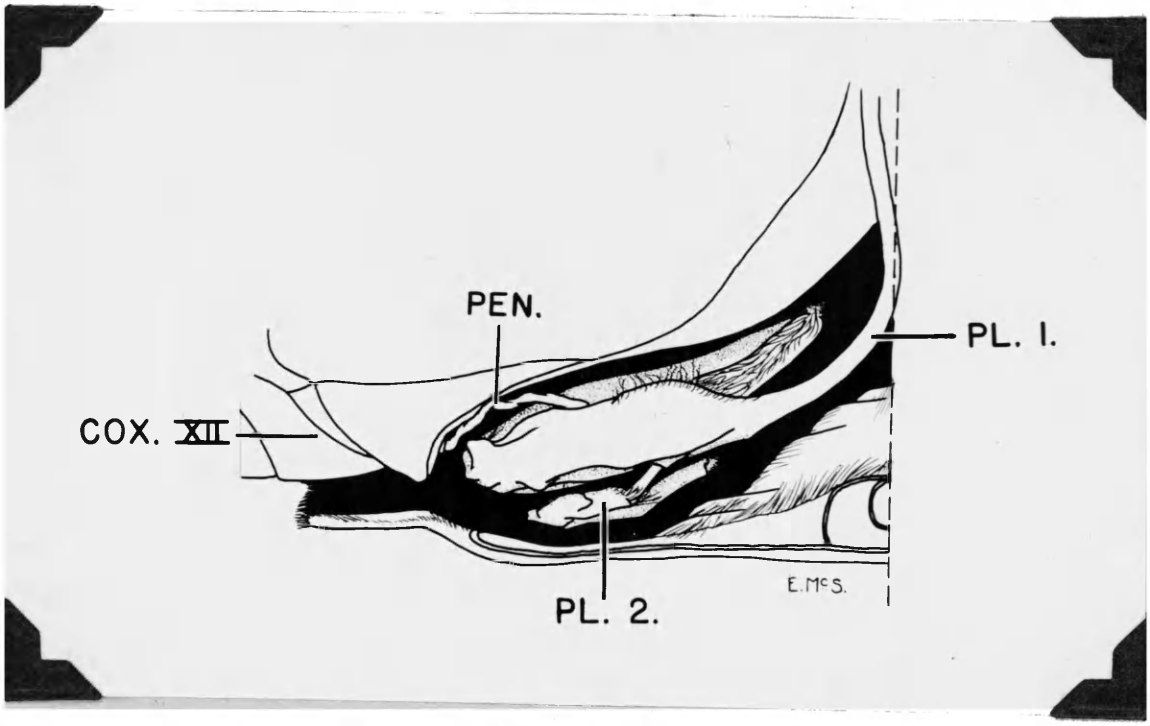


Figure 5.

Figure 6. Anatomy of the first and second abdominal appendages.

S - Spine of telopodite

GR. - Groove in telopodite

P.F. - Posterior distal foramen

A.F. - Anterior proximal foramen

COX.XIII - Coxopodite of first pleopod

COX.XIV - Coxopodite of second pleopod

BAS. - Basipodite

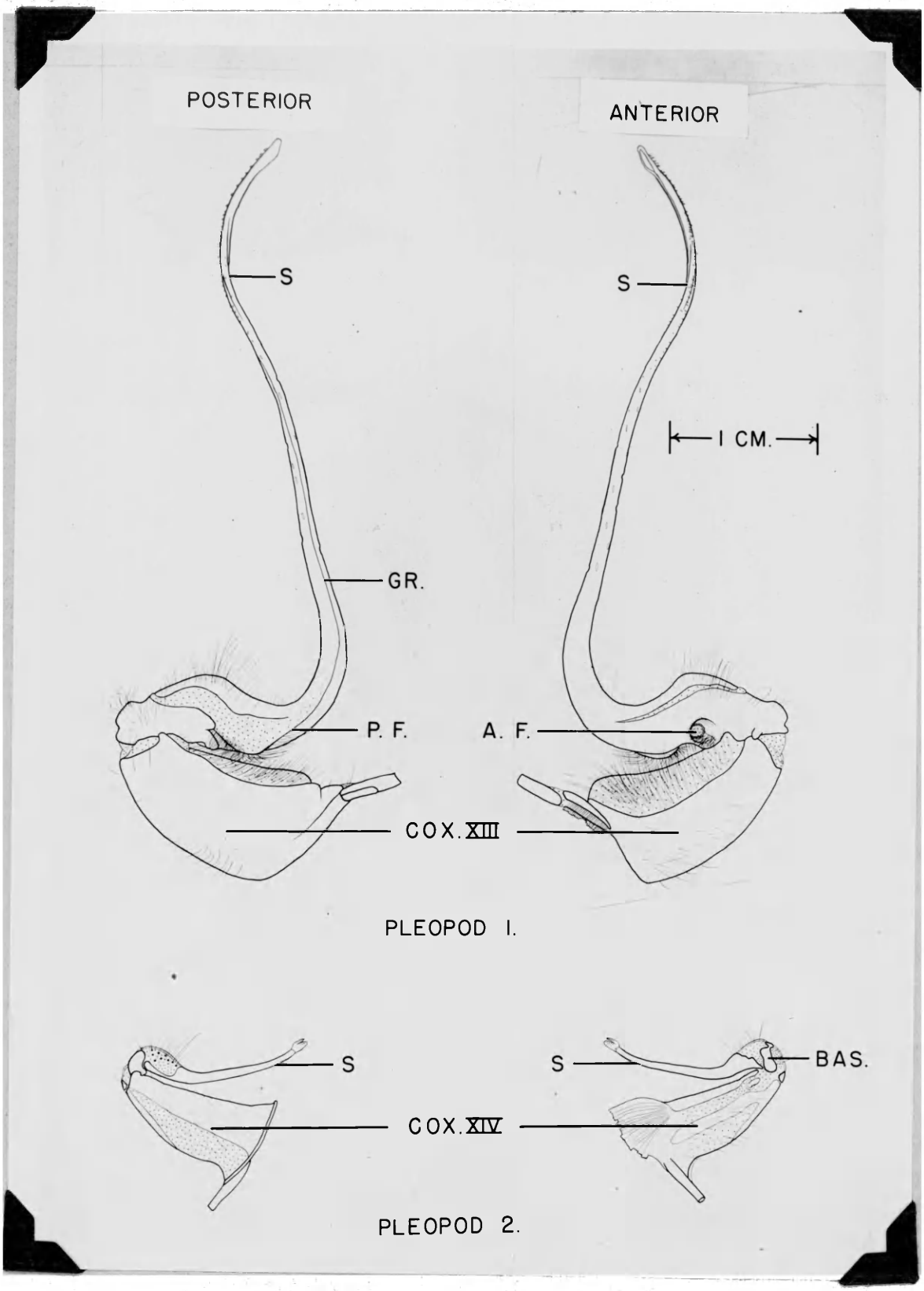

POSTERIOR
ANTERIOR
S
S
1 CM.
GR.
P. F.
A. F.
COX. XIII
PLEOPOD 1.
S
S
BAS.
COX. XIV
PLEOPOD 2.

Figure 6.

Figure 7. Reconstruction drawings of seminiferous duct in three lobes of the testis. Note marked irregularity of course and numerous small projections which give appearance of numerous ducts in figure 8.

Figure 8. Cross section of mid-lateral testis. Note large lobes subdivided into lobules and perforated by seminiferous ducts containing dark sperm masses. Worcester's fixative, Azan stain, B filter. X32.8

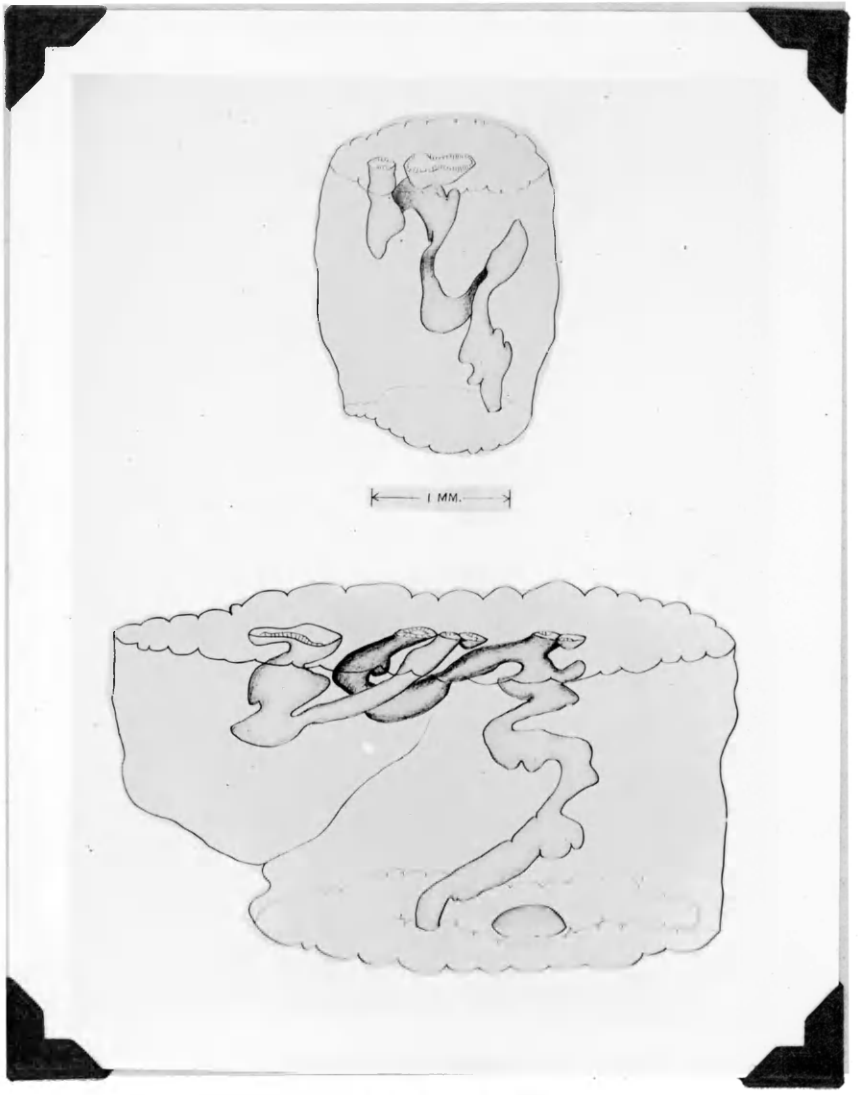


Figure 7.

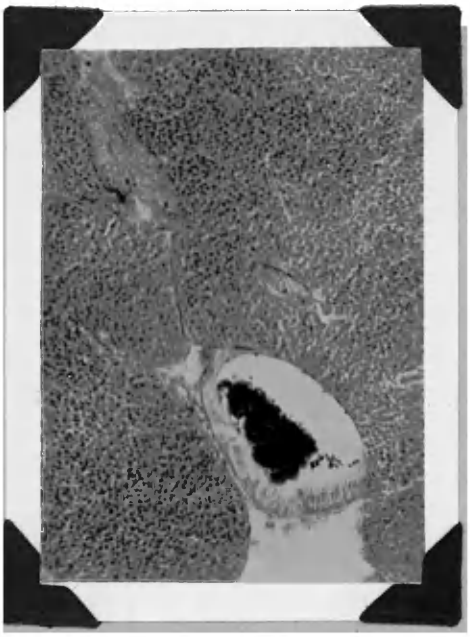
Figure 8.

Figure 9. Cross section of mid-lateral testis. Note light group of spermatogonia in upper left corner and seminiferous duct in lower right corner. Worcester's fixative, Azan stain, B filter. X188

Figure 10. Cross-section of mid-lateral testis. Note scattered darker accessory nuclei in upper portion, dark storage cell in interlobular wall and epithelium of the seminiferous duct. Bouin's fixative, Azan stain, B and E filters. X400

Figure 11. Portion of testis showing early stage of epitheliation. Compare with 12, 13 and 14. Azan stain, B filter. X400

Figure 12. Accessory nuclei gathered at lobular wall. Carnoy-Lebrun fixative, Azan stain, B filter. X400



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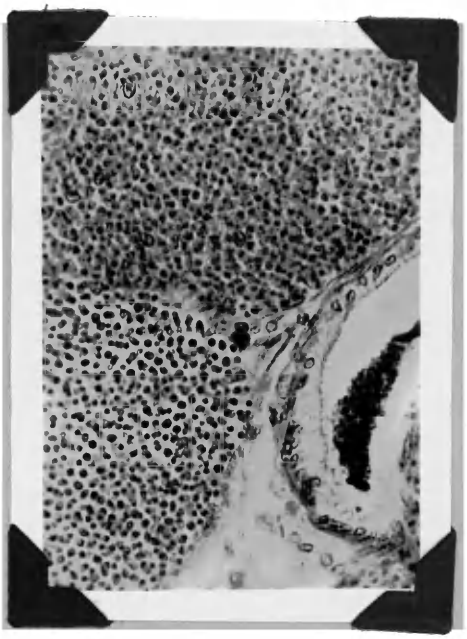


Figure 10.

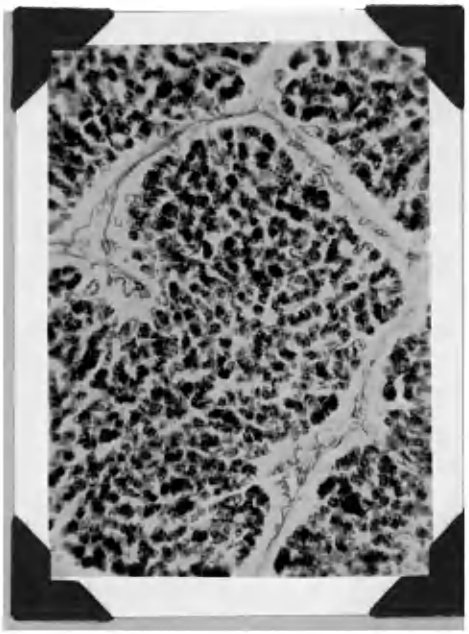


Figure 11.

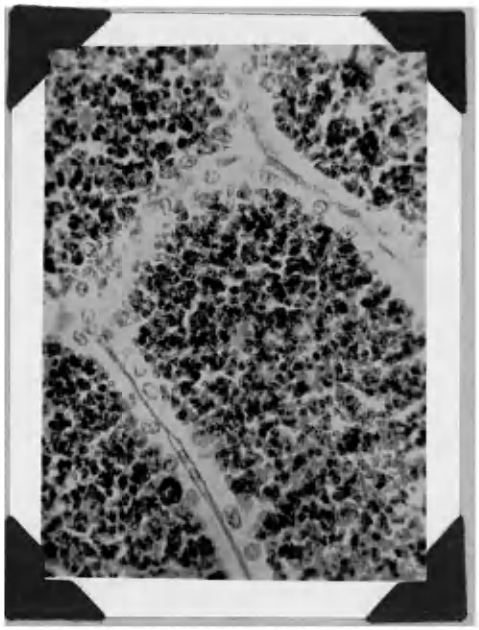


Figure 12.

Figure 13. Advanced stage of epithelium development. Carnoy-Lebrun fixative, Azan stain, B filter. X400

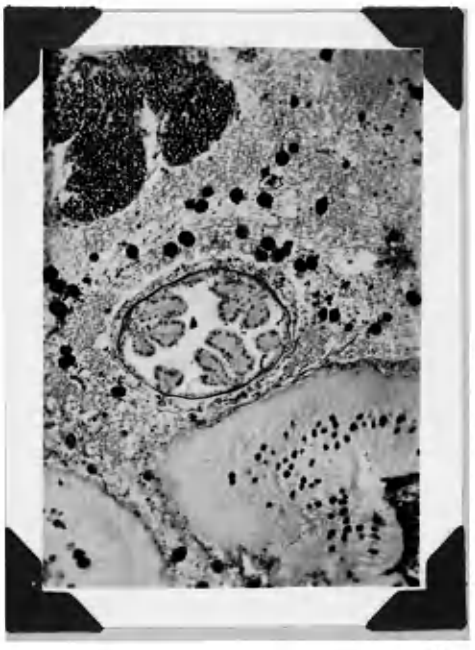
Figure 14. Final form of epithelium in testis lobule. Carnoy-Lebrun fixative, HIH stain, B filter. X400

Figure 15. Vas efferens in cross section three-fourths of its length from the testis (upper left) to the anterior vas deferens (lower right). Note large dark storage deposits. Worcester's fixative, Azan stain, B filter. X90

Figure 16. Cross-section of vas efferens. Note muscularis and typhlosole-like projections. Worcester's fixative, Azan stain, B filter. X400

Figure 13.

Figure 14.



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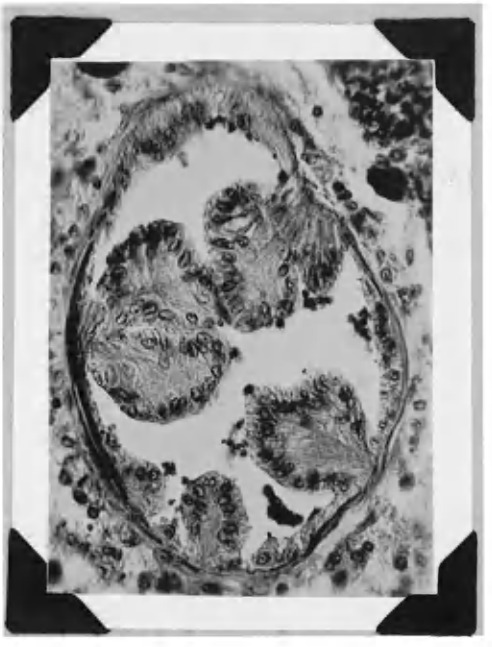

Figure 16.

Figure 17. First portion of anterior vas deferens. Note median nuclei of epithelium and deeply chromophilic secretion with sperm in lumen. Worcester's fixative, Azan stain, B filter. X188

Figure 18. Coil posterior to that shown in figure 17. Note distal nuclei, light and dark secretions and line of substance "B" marking out sperm masses. Worcester's fixative, Azan stain, B filter. X188

Figure 19. Region slightly posterior to figure 18. Nuclei are now nearly basal. Worcester's fixative, Azan stain, B filter. X188

Figure 20. Final stage in small coils of anterior vas. Note basal nuclei and the secretion products. Worcester's fixative, Azan stain, B filter. X188

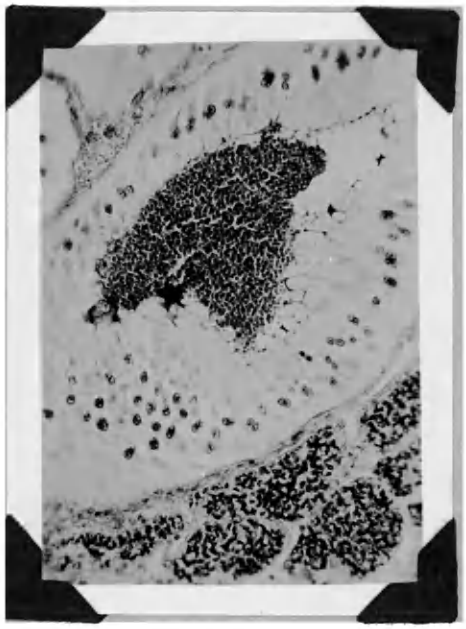

Figure 17.

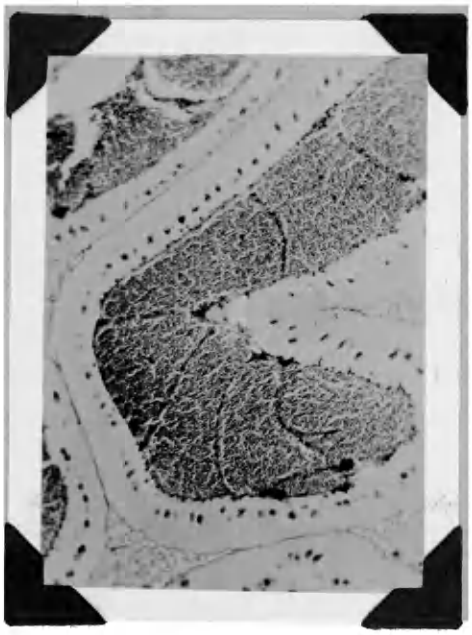

Figure 18.

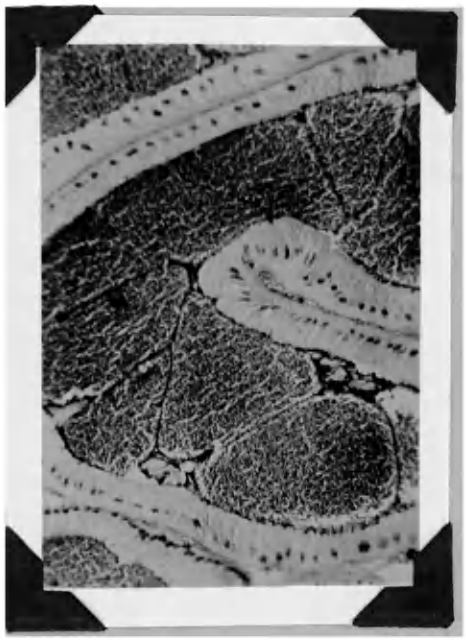

Figure 19.

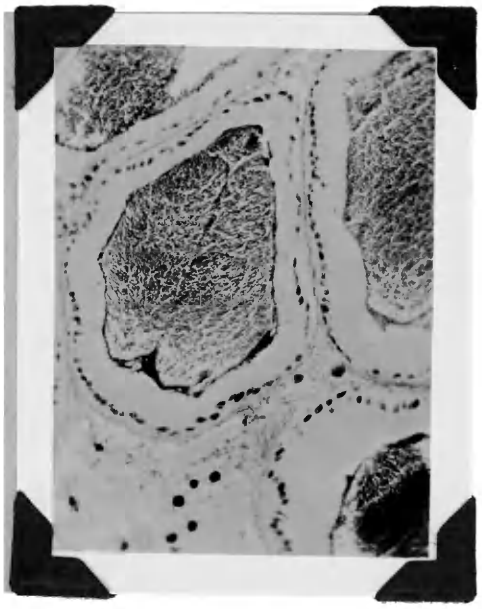

Figure 20.

Figure 21. Large coils of anterior vas deferens, showing group of formed spermatophores, surrounding matrix and epithelium. Carnoy-Lebrun fixative, Azan stain, G and E filters. X90

Figure 22. Portion of spermatophore and matrix. Note fine spermatozoa, spermatophore envelope and very heterogeneous matrix. Gilson's fixative, HIH stain, B filter. X198

Figure 23. Cross-section at the middle of the posterior vas deferens. Note lateral pouches and contents of central tube. Gilson's fixative, HIH stain, G filter. X90

Figure 24. Cross-section of posterior vas deferens near ductus ejaculatoris. Note complexity of the tube and of lateral pouches. General fixative, HIH stain, no filter. X32.8

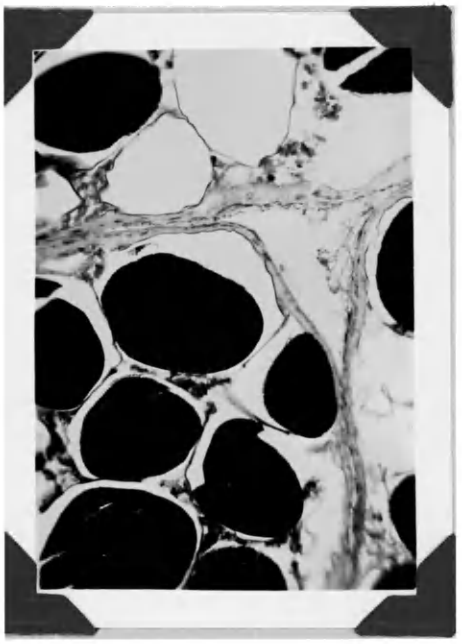

Figure 21.

Figure 22.

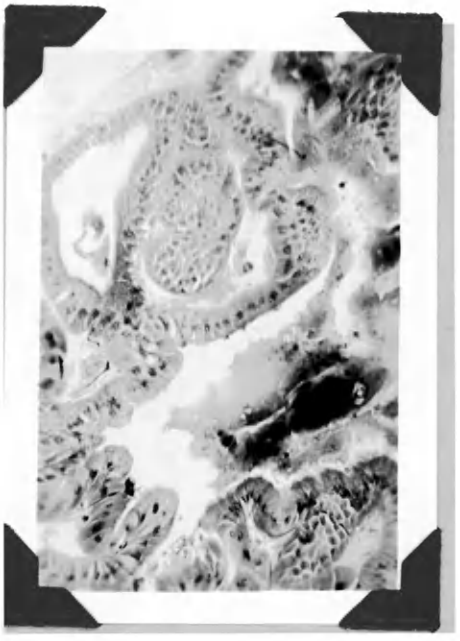

Figure 23.

Figure 24.

Figure 25. Epithelium of posterior vas deferens near ductus ejaculatoris. Taller epithelium is on central tube, lower on lateral pouch. Note muscle fibers, appearance of ciliation, and contents. Worcester's fixative, MIH stain, B filter. X198

Figure 26. Cross section of ductus ejaculatoris. Note heavy connective tissue, muscularis and empty lumen. Gilson's fixative, Azan stain, B and V filters. X90

Figure 27. Glandular tissue occurring along ductus. Note cords of cells. Bouin's fixative, Azan stain, B and E filters. X400

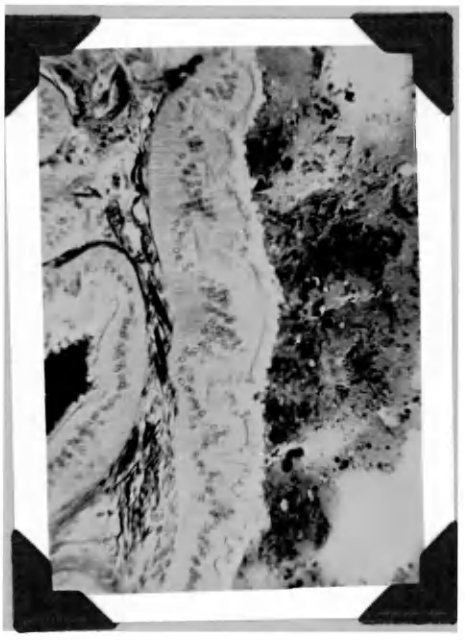

Figure 25.

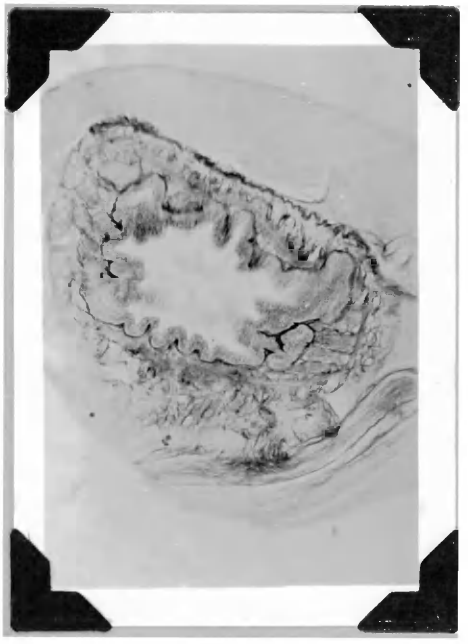

Figure 26.

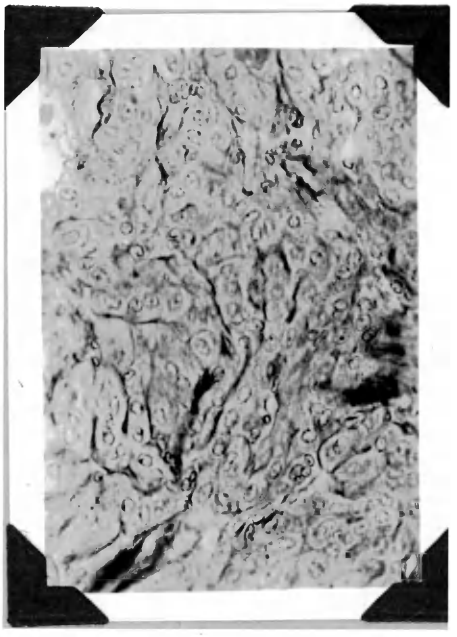

Figure 27.

Figure 28. Cross-section of penis near the proximal end. Epithelium, muscularis, and parenchyma containing storaged epositæ are present. Worcester's fixative, HIH stain, C5 and H filters. X90

Figure 29. Epithelium and heavy muscularis in basal part of the penis. Bouin's fixative, HIH stain, C5 and H filters. X400

Figure 30. Portion of Figure 29 under oil immersion. Note cilia. Data as above. X900

Figure 31. Cross-section of the distal end of the penis, illustrating inflected cuticle on typhlosole-like ridge bearing modified epidermis. Worcester's fixative, Azan stain, H filter. X90

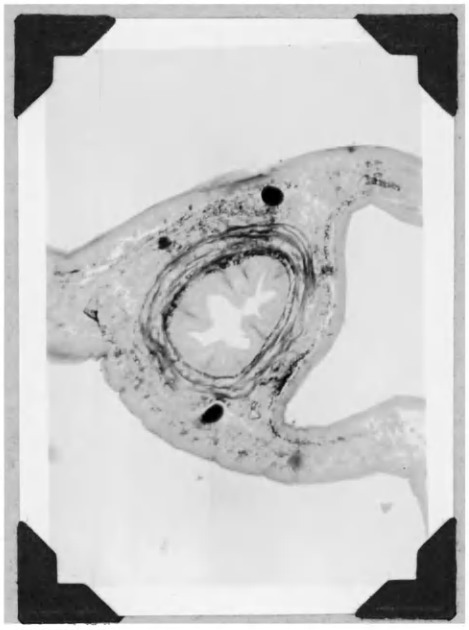

Figure 28.

Figure 29.

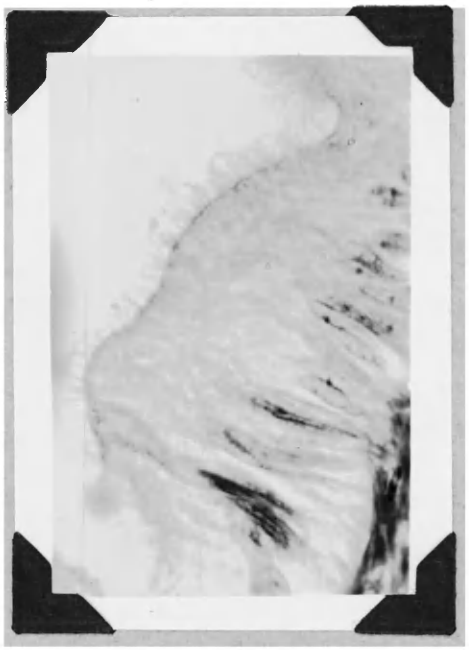

Figure 30.

Figure 31.

Figure 32. Cross-section of pleopod 1 near proximal end. Note closure of groove and varied rosette glands. Bouin's fixative, Azan stain, B and G filters. X32.8

Figure 33. Rosette glands in basal portion of pleopod 1, illustrating diffuse and compact stages of gland. Bouin's fixative, Azan stain, B and G filters. ~~X32.8~~ X400

Figure 34. Cross-section of distal part of pleopod 1. Note groove and absence of rosette glands. Bouin's fixative, HIH stain, no filter. X90

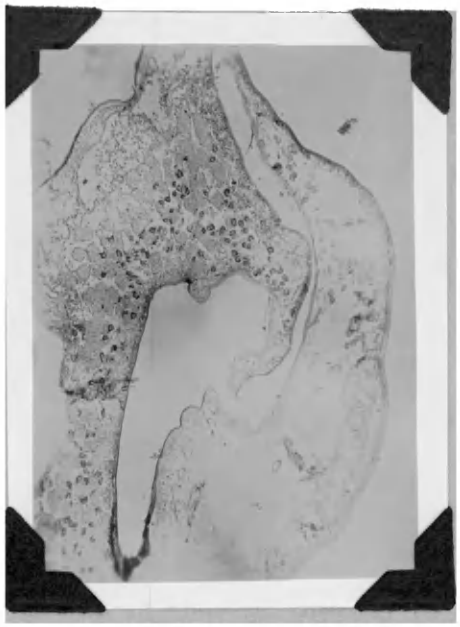

Figure 32.

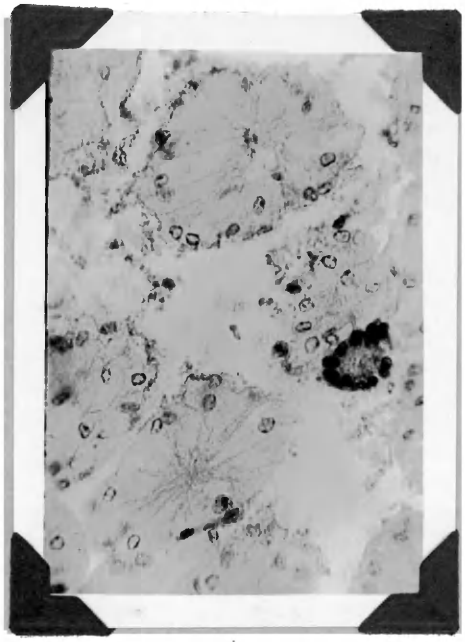

Figure 33.

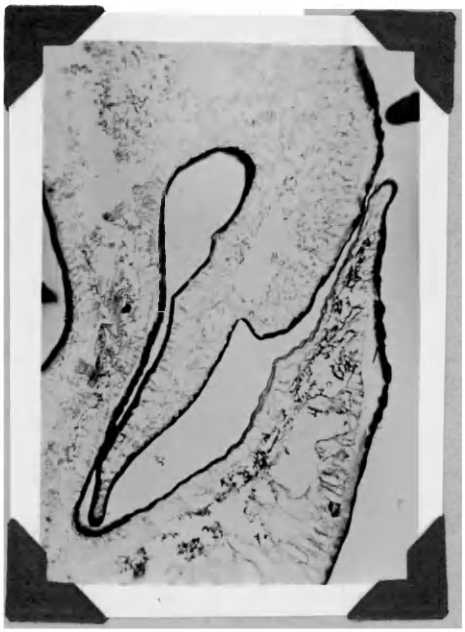

Figure 34.