STUDIES ON THE GENITAL SYSTEMS AND REPRODUCTION IN THE CHIMAEROID FISH *HYDROLAGUS COLLIEI* (LAY AND BENNETT)

by

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INTRODUCTION

Hydrolagus colliei is placed in the Family Chimaeridae, Order Chimaeriformes and Class Chondrichthyes (1). The term Holocephali may be used as a subclass designation. Although the members of this group are similar to the Elasmobranchii in having a cartilaginous skeleton, the magnitude of difference in many other anatomical features is sufficient to place them in a separate subclass.

Investigations on the urogenital systems in chimaeroid fishes have been mostly on a gross anatomical level and relatively incomplete, due partly to the difficulty of obtaining specimens. Franz Leydig, in 1851 (19), gave an account of the gross anatomy of one male and two female specimens of *Chimaera monstrosa*. In this study, he distinguished between the anterior and posterior portions of the adult male kidney. He designated the anterior parts as "accessory sex glands", later called "Leydig's glands" by other investigators. In 1854, Joseph Hyrtl (13), extended Leydig's work on *Chimaera* by giving some finer details. He stated that the epididymis ("Nebenhoden") extended the full length of the abdominal cavity. Hyrtl could not find connections between testis and epididymis. He stated that no urinary sinus is found in the male, but described the urogenital sinus, which apparently he did not consider to be homologous with the urinary sinus of the female.

Semper gave only a few additional details on C. monstrosa (32). He incorrectly described a Leydig's gland in the female. He stated that there is, in the female as in the male, a sharp distinction between Leydig's gland and the more posterior urinary kidney. Semper thought that the accessory genital gland (which lies between the posterior ends of the female reproductive tracts) was homologous to the rectal gland of elasmobranchs, but Howes (12) considered this homology unlikely since he thought that the accessory genital gland was absent in male chimaeroids. Mazza and Perugia (23), and more recently Clothier (7), have shown that the homologues of the rectal gland of Selachii are found as several separate glands embedded in the wall of the hind gut in C. monstrosa and H. colliei. The accessory genital gland is apparently a separate entity.

Mazza (22) indicated a seasonal cycle of growth and development in the testes of C. monstrosa and provided a histological analysis of the testicular ampullae. He denied the existence of direct connections between testis and deferent duct.

Histological details on the ovarian follicle and auxocyte in C. monstrosa were given by Giacomini (10). He described the cell types of the follicle and the increase in thickness of the follicle followed by a later decrease in its height.

Redeke (31) divided the kidney of Chimaera and Callorhynchus into three zones. A cranial zone, forming an epididymis, is present in the male only. A middle zone forms more epididymis in the male and additionally secretes urine. In the female, the middle

zone is urine producing, while in both sexes, the caudal kidney zone functions only as a urine producer. Redeke found no "vasa efferentia" and no sexual kidney (Geschlechtsniere) in chimaeroids. He distinguished both a urinary bladder and a digitiform gland (accessory genital gland) in the female alone.

The first detailed cytological work on C. monstrosa was that of Stephan (33) in which he concentrated on the disposition of the centrosome during spermiogenesis.

Wallace (35) gave further details on both the ovarian ova and the follicles of C. monstrosa. In this paper, he proposed that the larger cells of the auxocyte follicle are nutritive in function and that they evolve from the smaller follicle cells. He noticed processes extending from the nutritive cells through the vitelline membrane and into the auxocyte cytoplasm.

In a large monograph on chimaeroid fishes, Bashford Dean (8) included an exhaustive bibliography and discussed evolutionary relationships. Dean described the early embryology of H. colliei along with the external features of older embryos, and provided figures of the female reproductive system. In his Figure 2, Plate 1, he incorrectly labels the opening of the accessory genital gland as the urinary opening; the latter opening is not shown. An excellent account, along with beautiful illustrations, is provided on the formation of the egg capsule. He also included some histological data on the ovarian membranes.

Parker and Burlend (28) described efferent ductules and spermatophore formation in C. monstrosa. They believed that no

portion of the adult kidney functions as an outlet for spermatozoa, but that the sex cells pass from the efferent ductules directly into the "spermiduct" (ductus deferens).

The following year, Burlend (5) presented anatomical observations on both adults and late immature stages of Chimaera, but had little to add to the work of previous investigators.

In 1920, Morgera (25), made some observations on the efferent ductules and Leydig glands. These details differ greatly from those described by others for the same species. Many homologies with the efferent testis systems of amniotes are claimed, but without supporting evidence.

Kolmer and Scheminzky (15) and recently Marshall and Lofts (20) have reported interstitial cells of Leydig to be present in the testis of Chimaera.

Parker and Haswell (27) reported the presence of spermatophores (apparently in Callorhynchus).

A detailed study of the renal glomerulus of C. monstrosa and of other fishes was made by Bargmann (3), and these were found to be comparable to the glomeruli of higher vertebrates.

In a textbook review, van den Broek (34) includes some personal observations on C. monstrosa and concludes that the chimaeroids resemble Selachii in their efferent testis channels, but he gives neither details nor illustrations.

De Lacy and Chapman (9) described the egg cases of Hydrolagus and made exact measurements of them.

Legendre (16) gave a little information on the reproductive organs and reproductive cycle of C. monstrosa, but most of his details are a repetition of the observations of previous investigators.

A recent study by Prasad (29) on the shell glands of various fishes includes a brief and general description of these organs in H. colliei. He concludes that these glands differ in no essential features from those of elasmobranchs.

Hisaw and Hisaw (11) have very recently studied corpus luteum formation (both ovulatory and atretic) in H. colliei and also in several species of elasmobranchs. These investigators suggest that the corpus luteum may have originally been a device functional in ingesting both yolk and cell debris from ovulated and atretic follicles; only later in evolutionary history did it acquire an endocrine function.

Because of the rather comprehensive studies by Leigh-Sharpe (17, 18) and others, a consideration of the secondary sex characters is omitted in this paper.

It may be said in summary, that most of our knowledge of the urogenital systems of chimaeroid fishes depends upon the fragmentary and rather divergent studies on C. monstrosa. Limited studies on H. colliei and other species have added only a few details. A plentiful supply of animals of various ages provided the present author an opportunity to study in some detail the urogenital systems in another member of the Subclass Holocephali.

MATERIALS AND METHODS

Between June 23, 1959 and February 8, 1961, about 3,000 specimens of H. colliei of sizes ranging from the hatching stage to maturity were collected. Of this number, about 200 selected specimens were preserved for anatomical studies.

Most specimens were collected by means of an otter trawl operated from the 55 foot motor vessel Hydah owned by Captain Cleave Vandersluys of Friday Harbor, Washington. A few fish were collected at Friday Harbor by means of a setline extending into the bay from a point near the University of Washington Marine Laboratories. The collection site most often used was an area of 27 fathoms in depth in Lopez Sound on the eastern side of Lopez Island, San Juan Archipelago, Washington. A five or ten minute drag along the bottom of this depression usually yielded several hundred specimens of Hydrolagus along with a few dogfish (Squalus suckleyi), skates (Raja), and various teleosts. The numbers of H. colliei taken in six major collections are summarized in Table 1 (p. 11). Other sites of major collections included a 40-fathom hole immediately north of Upright Head, Lopez Island, and a 53 - 57 fathom site about 4 nautical miles due south of Kings Point, Lopez Island.

Since the attenuated tip of the tail of Hydrolagus is often missing, a measurement from the tip of the snout to the anterior rim of the anus (snout-vent length, hereafter referred to as S-V length) was used as a more reliable scale upon which to base

the relative maturity of the animals. In selecting animals for relative development of the reproductive system, the S-V length was found to be more reliable than the total length. The tails of H. colliei vary considerably in length in animals of equivalent maturity.

Specimens for anatomical and histological studies were fixed in 10% formalin, Bouin's fluid, Zenker's-acetic fixative, Zenker-Formol (Maximow's) fluid, and 70% ethyl alcohol. After dehydration in dioxane, clearing in benzene and embedding in paraffin, sections cut at 5 - 10μ were stained in Masson's trichrome stain, Weigert's iron hematoxylin, and MacCallum's modification of Verhoeff's elastic fiber stain. Some formalin-fixed specimens were macerated in 10% KOH to facilitate microdissection (alcohol-fixed specimens would disintegrate in KOH).

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OBSERVATIONS AND DISCUSSION

A. The reproductive cycle

Six large collections of animals were made between August 1959 and February 1961. Data on the reproductive cycle, obtained from the specimens gathered, are summarized in Table 1.

A total of 2,659 fish were collected on the first five occasions. The sex ratio in this population sample was about three males to two females. The largest male collected was 62 cm long and its S-V length was 24.5 cm, while the largest female measured 67 and 31.5 cm respectively. These specimens are presumed to approximate the maximum sizes for this species. Only five females (0.47%) had egg cases in utero, although from 12 to 35% of the adult females had large ovarian follicles, indicating that they were reproductively active. The failure to find a larger number of gravid females, when sampling the deeper areas where high concentrations of this fish are found, might be explained on the supposition that females ready to spawn migrate to other areas. Observations by others (6, p.72; 14) indicate that both eggs, and mature females about to lay eggs, have been seen in inter-tidal or subtidal areas. In this study a high percentage of the females caught on the Friday Harbor setline were gravid. Thus the fact that a somewhat lower percentage of females possessed large ovarian follicles on August 15, 1960 (Table 1), may reflect a migration of such females to shallower waters. A peak of reproductive

activity may actually occur in the summer months, as Dean indicated (8, p. 23), instead of in the winter season as the data in column five, if taken alone, might imply. On the basis of these facts, it would be fair to say that reproductive activity continues throughout the year in both sexes, although only a third or less of the adult female population is active at any one time.

Figure 1 presents weights of ovaries plotted against S-V length. Curves through the outer points on the graph are drawn to indicate the wide range of weights which mature ovaries may exhibit in various states of activity. This figure indicates that the female ratfish becomes sexually mature at an S-V length of 24.0 - 25.0 cm. The ovaries show a great increase in weight due to the growth of a few follicles to mature size. After spawning occurs, the ovary returns to a lower weight of 2.0 - 7.0 g. Twenty-two ovaries with large follicles averaged 16.5 g in weight and ranged from 7.8 - 30.3 g.

All information on the male indicates that when adulthood is attained active sperm production continues throughout the year. This circumstance would assure the insemination of a high percentage of ovulating females. Figure 2 demonstrates that most males reach sexual maturity at an S-V length between 18.5 and 20.0 cm. Duplicate points on this graph were placed as close to the actual point as possible. Figure 3 presents a curve constructed by averaging the testis length (from Fig. 2) on lines representing each 0.5 cm of S-V length; points between these lines were averaged with those of the nearest 0.5 cm S-V lines. When

the testis has reached a length of 3 - 3.5 cm, both the epididymis and ductus deferens contain spermatozoa, and the mid-section of the ampulla ductus deferens nearly always contains the blue-green secretion characteristic of the adult. Rarely, an adult male was found with a narrowed ampulla ductus deferens containing little or no blue-green secretion. These animals are thought to have copulated very recently. Figure 4 indicates a progressive and steady increase in the weight of the testis after an S-V length of 18 - 19 cm is attained.

In column seven of Table 1, the average weight of gonadal material per individual male (testes 1 cm in length and longer), indicates a fairly constant weight of about 16 g (8 g/testis) the year around.

Table 1

Date	No. of males collected	No. of females collected	No. of females with egg cases in utero	% of adult females with large follicles	Average wt. of total ovarian tissue per female in grams	Average wt. of total testicular tissue per male in grams
8/7/59	180	152	-	34.8		
8/13/59	367	224	-	35.8		
12/29/59	452	185	1	29.8		
3/16/60	314	129	-	21.9	14.23	16.39
8/15/60	286	370	4	12.1	9.8	16.06
2/7/61			-		16.68	15.78
Totals	1599	1060	5			

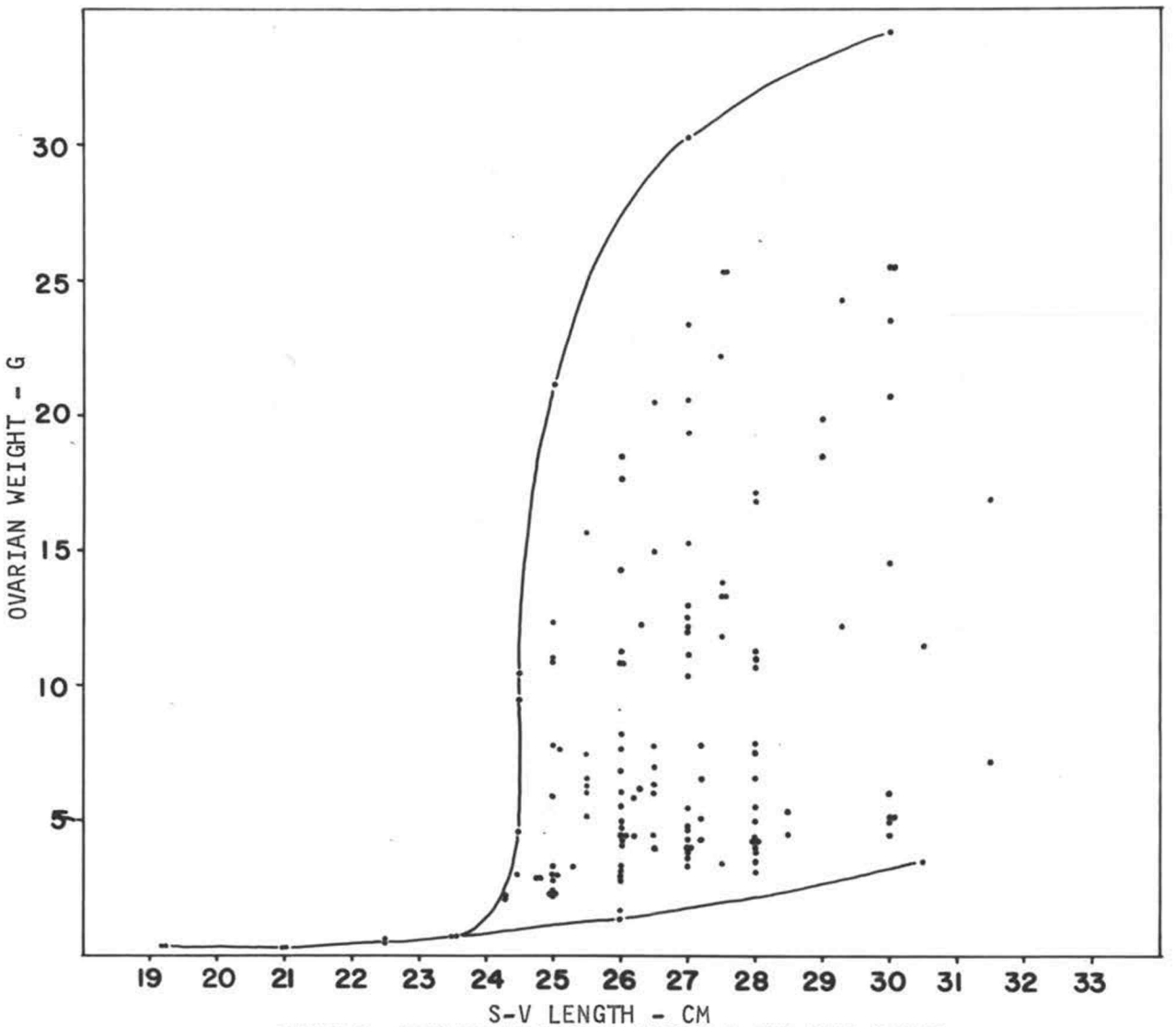

FIGURE 1. OVARIAN WEIGHT PLOTTED AGAINST SNOUT-VENT LENGTH.

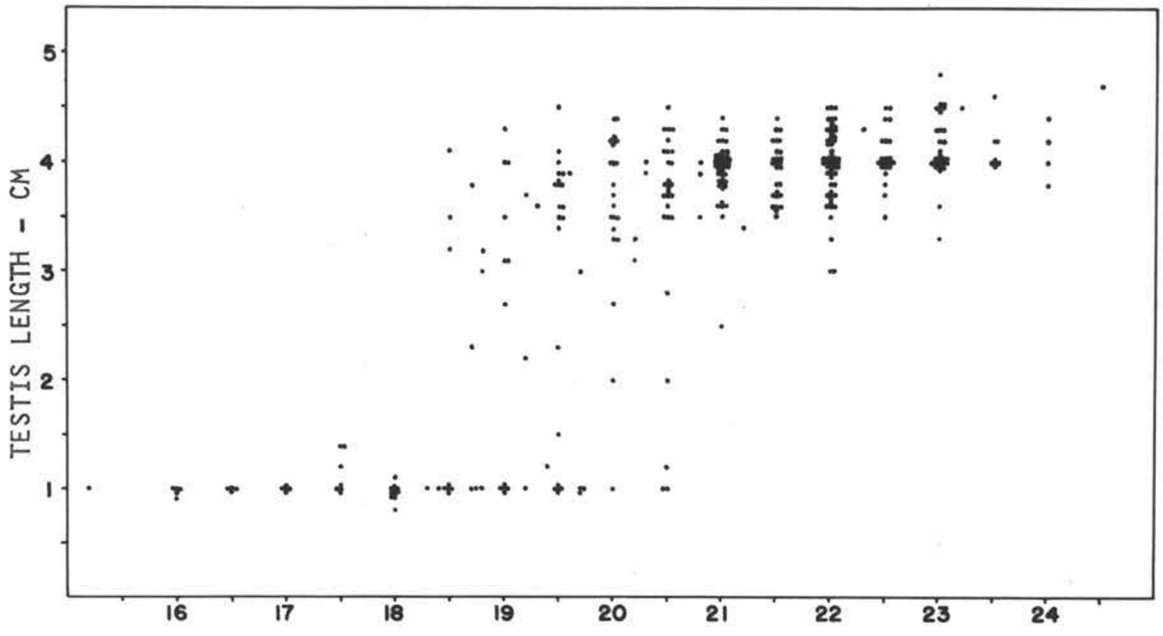

FIGURE 2. TESTIS LENGTH PLOTTED AGAINST SNOUT-VENT LENGTH.

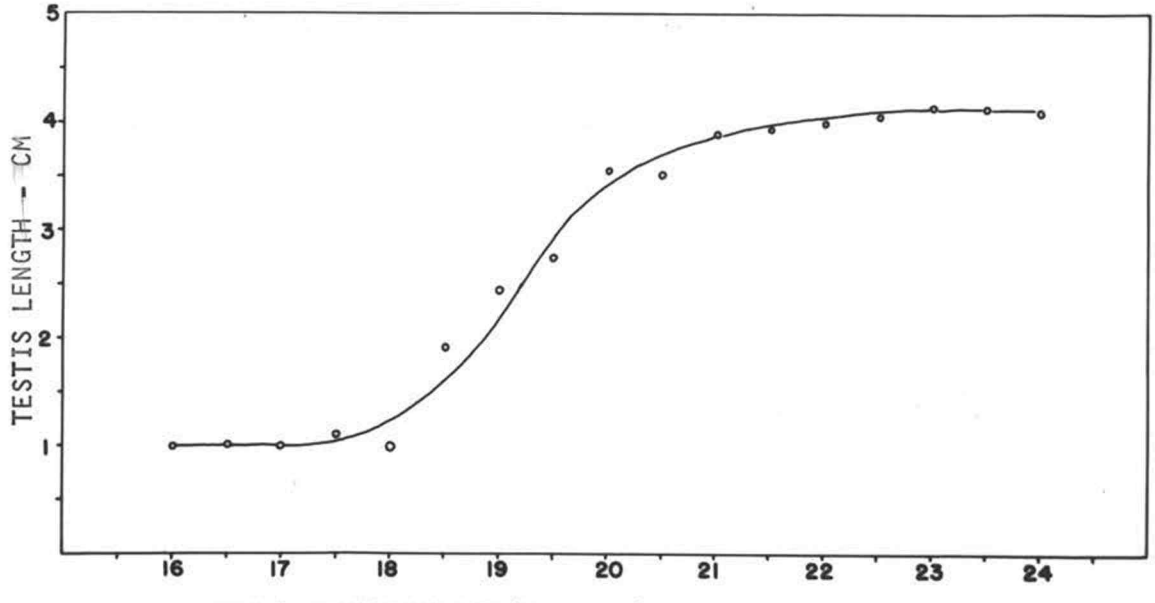

FIGURE 3. AVERAGE TESTIS LENGTH (FROM FIGURE 2) PLOTTED AGAINST SNOUT-VENT LENGTH.

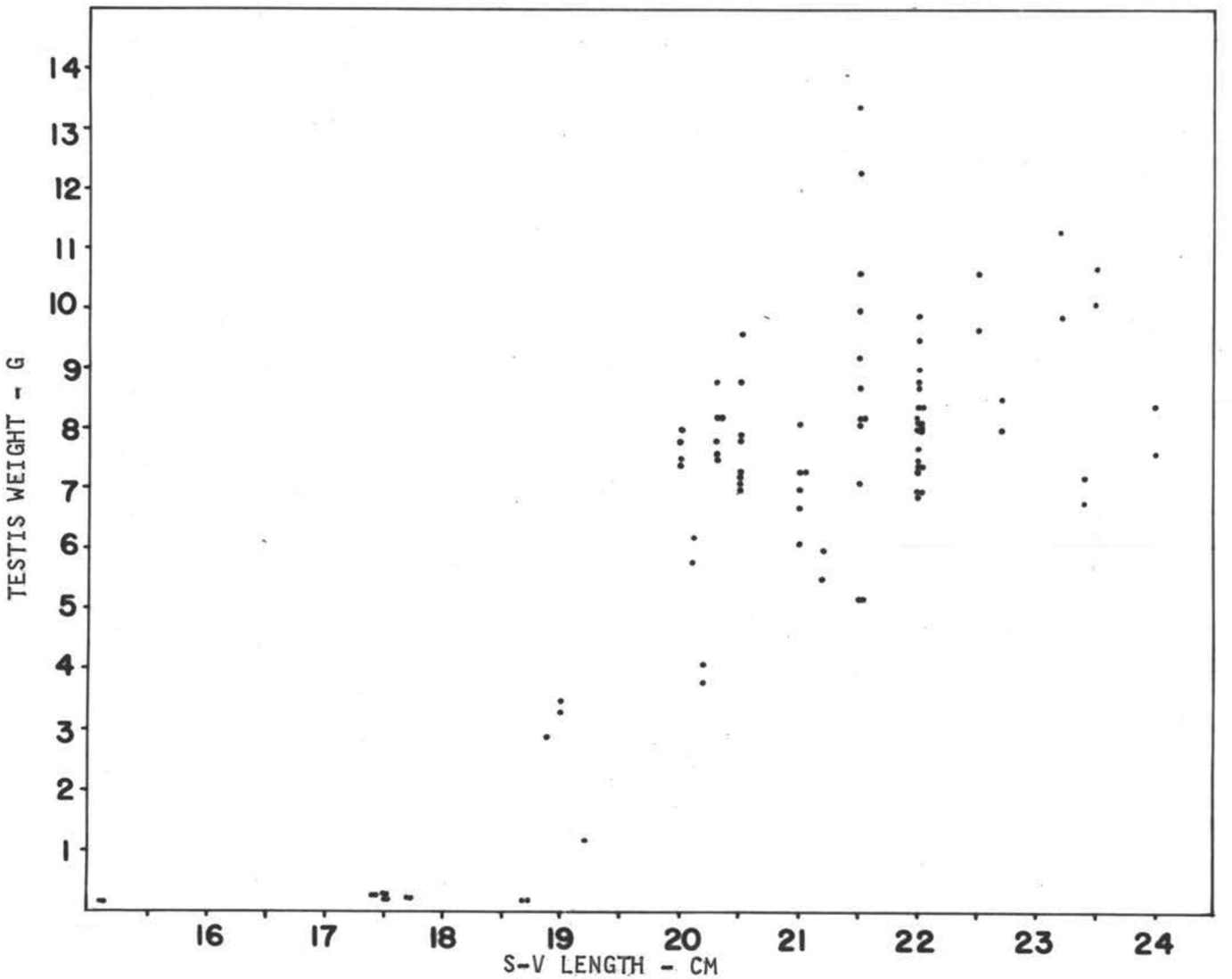

FIGURE 4. TESTIS WEIGHT PLOTTED AGAINST SNOUT-VENT LENGTH.

B. The structure and differentiation of the male urogenital system

1. Testis

In the newly hatched male (S-V 41mm) the testis is a small, flat disc of germinal tissue attached by its medial edge to the dorsal body wall. The lateral edge of the testis is produced into a prominent, caudally bent projection (Plate1, fig. 5) which retains its identity throughout life and continues to be the source of new testis tissue. Long ligaments extend both cephalad and caudad from the testis and are also attached to the body medially but remain free laterally. The germinal tissue at this time occupies a roughly circular area of about 1 mm in diameter.

The testis is covered dorsally and ventrally by a thin, simple squamous epithelium continuous with the peritoneal lining. The parenchyma contains a network of cords of smaller dark-staining cells among which are dispersed the larger (14-17μ) primordial germ cells (spermatogonia). This network lies in the central region of the testis and is embedded in a matrix of diffuse mesenchymatous cells, while the germinal projection consists of closely packed cells with few fibers. The cord network is in communication with the outer epithelium only at the caudal, basal region of the germinal projection. Dorsally at this site, lie a few larger cells similar to the germ cells. In parts of the ventral epithelium, large germ cells are also occasionally seen. The larger blood vessels lie between the germinal cords and the ventral

epithelium of the testis (Plate 2, fig. 9).

Those germinal cords near the medial edge of the testis acquire a lumen and the proximal portions of five or six of these pass transversely a short distance before opening into the single longitudinal testis canal (central canal of testis) which varies in diameter from 17-25μ. This latter channel extends along the medial border of the testis. The longitudinal canal consists of a simple cuboidal to columnar epithelium 4-7μ high, each cell with a few long cilia.

In an older animal (S-V length 82 mm), the testis, except for the germinal projection, has thickened somewhat, especially medially where the testis cords are acquiring lumina and where some germ cells and smaller cord cells are organizing into ampullae. The process of ampulla development appears to involve a general cavitation of the cord, followed by multiple evaginations along the length of the cord (Plate 8, fig. 34). These diverticula become vesicular and their lumina subsequently become discontinuous with that of the cord. In these blind vesicles the germ cells may be seen dividing. Those gonial cells in the medial section of the testis are up to 28μ in diameter and have a large nucleus (21μ); those in the lateral portions are smaller (14-15μ). Even these smaller germ cells are many times larger than the cord cells (6-7μ in diameter) and are therefore easily distinguishable from the latter.

At a later stage (S-V length 88 mm) the testis is about 1 mm

thick due to its expansion ventrally, This leaves the lateral germinal process more toward the dorsal body wall (Plate 2, fig. 10). This projection alone contains the germ cells within primitive testis cords, and at the base of this process, new ampullae are forming. The more advanced ampullae of a graded series are seen toward the medial side of the testis. When the ampullae are fairly well formed, the germ cells are visible exclusively within them and not in the adjoining duct. The terminal branches of the duct system consist of slender solid cords of small cells. These thin cords extend from the ampullae to portions of the duct system which have a lumen and thin walls of low cuboidal epithelium. These latter chambers, scattered throughout the testis substance, are connected to other similar chambers by slender ductules and finally, are connected to the longitudinal testis canal by ductules possessing very narrow lumina. Cilia are absent from the duct system except in the longitudinal testis canal and the ductules immediately adjoining it. The largest ampullae (medial) at this stage are from 35-53μ in diameter and are composed, as before, of two types of cells, the larger germ cells (around the periphery) with contracted nuclear substance and the smaller Sertoli elements arranged around the central lumen. These designations agree with the literature on Selachii, which bear close resemblance in both spermatogenesis and ampulla development to Hydrolagus. At the extreme ventro-medial portion of the testis lies a zone of degenerating ampullae and matrix tissue. This zone

marks the beginning of a change in symmetry anticipating the adult condition. In the adult this zone of degeneration is the site of discharge of spermatozoa from the mature ampullae into the collecting duct system. The remnants of the ampullae regress and are resorbed.

The testis at this stage is bean-shaped and about 3.5 mm long, 2 mm wide (exclusive of the germinal projection) and 1 mm thick. It has a lateral recess from which the germinal process projects. The dorsal surface of the testis is flattened against the dorsal body wall, while the ventral surface is extremely convex. Due to the expansive growth of the testis ventrad, the anterior and posterior ligaments are left attached to the testis very near the dorsal aspect.

A specimen of 103 mm S-V length shows little change except for the absence of degenerate tissue medially. Whether this is a common feature of testes of this size has not been determined. The germ cells at the tip of the germinal projection are smaller than those at its base. Medially the germ cells are again smaller due to division after being incorporated into ampullae. Many division figures, especially affecting the germ cells, are seen in the ampullae. The epithelium on the dorsal aspect of the testis is low cuboidal, that on the ventral surface is squamous.

A still older specimen (S-V length 135 mm) has a testis with much degenerating tissue in its medial portion. Ampullae develop to a stage in which all Sertoli cells are found lining the ampulla

lumen with the larger gonial cells peripheral to the Sertoli elements. These ampullae have moved to the zone of degeneration.

In a specimen of S-V length 178 mm, the testis is about 9 mm long, 5 mm wide, exclusive of the germinal projection, and still about 1 mm thick. The medial region of the testis consists of degenerating tissue. The largest ampullae border the degeneration zone and are 50-60μ in diameter. They have developed to the stage in which Sertoli cells either line the lumen or are migrating peripherad among the gonial cells.

The new ampullae which are forming at the base of the germinal projection now form as solid balls of intermingled germ and Sertoli cells. The ampullae are attached to solid slender cords and secondarily attain lumina. It is presumed that the lumen of an ampulla arises through a rearrangement of the composing cells, although some central cellular debris was noted in a few cases. The germinal projection is now definitely attached to the testis more dorsally than laterally (Plate 2, fig. 11). Successively older ampullae radiate out from the base of this projection toward the zone of degeneration.

In specimens measuring from 185 mm S-V length upward, the testes show much growth in size and are becoming mature. Two specimens (S-V length 187 and 188 mm) presented a much thinner zone of degeneration and ampullae in which mature-appearing spermatozoa occur in bundles, both of which are adult features. These testes, 12 and 16 mm wide respectively, have both a more

rounded dorsal side which bears the germinal projection (now relatively minute) and a more flattened ventro-medial surface, both of which conditions are seen in the adult.

A mature animal has testes which measure up to 4.5 cm in length and weigh as much as 13.4 g. The ventro-medial aspect of the testis is nearly flat, while the dorso-lateral surface (carrying in its center the germinal process) is rounded (Plate 2, fig. 12). The germinal process has remained very small and projects above the testis ligaments now represented as small ridges on the dorsal side of the testis (Plate 1, fig. 7). The testis has grown in length especially in the cephalic direction. This shifts the anterior testis ligament from the original anterior margin of the testis to a position dorsal to the testis and somewhat caudal to its anterior pole. All primitive germinal tissue is restricted to the germinal projection, especially near its caudal concave portion; new ampullae form at its base. As the ampullae grow in size, they spread out cephalad, caudad and laterad, and at the same time move toward the flat ventro-medial zone of degeneration. By the time the ampullae have reached the ventral side, their contents have developed into bundles of mature spermatozoa associated with large Sertoli cells. These ampullae then empty their content of sperm bundles into the slender exit ductules to which they are attached and which have newly acquired lumina. The spermatozoa proceed to the dorso-medial edge of the testis and thence into the longitudinal testis canal to be carried to the epididymis. The partially collapsed

ampullae with the retained Sertoli cells then degenerate, and a new series of ampullae mature behind them.

It is interesting to note that none of the developing ampullae degenerate until they arrive, in the matured condition, on the ventral side of the testis. This contrasts with the testis of Squalus suckleyi in which some early ampullae are noted to degenerate, especially within a band slightly removed from the germinal line (Plate 2, fig. 13). This difference may be due to the fact that the germinal tissue in Hydrolagus is concentrated into one pointed projection so that growing ampullae can spread out anteriorly, posteriorly, and laterally. In Squalus, new ampullae are produced from a germinal line (not projecting above the testis surface) extending along nearly the whole length of the testis. Therefore, the growing ampullae in Squalus can fan out only in a lateral plane; crowding evidently forces some ampullae to degenerate. It is also interesting to compare the testis of the skate Raja rhina in which the germinal tissue is located neither in a single projection, as in Hydrolagus, nor in a line as in Squalus, but rather in many sites over both the dorsal and latero-ventral surfaces (Plate 2, fig. 14). From each of these pits (indented instead of projecting), the growing ampullae can expand outward in nearly all directions, and no degeneration is seen except in the outer, mature, emptied ampullae.

Matthews (21, p. 274) states that in Cetorhinus maximus, the testis is constructed of lobes with the germinal tissue in the

center of each lobe; all ampullae gradually grow peripherally and empty their sperm into ducts surrounding the lobes. The entire testis of either Hydrolagus or of Squalus appears to correspond to a single wedge-shaped lobule of the testis of Cetorhinus.

The testis epithelium in Hydrolagus is simple squamous except in the germinal projection area where it is cuboidal to columnar and with large spaces between the cells. Only a very thin tunica of collagenous tissue underlies most of the epithelium, but this tunica is thickened at the base of the germinal projection.

Interstitial cells of Leydig are stated by Marshall and Lofts (20, p. 704) and by Kolmer and Scheminzky (15) as quoted from Rauther (30, p. 189) to occur in Chimaera. The author of the present paper has demonstrated interstitial cells distinct from connective tissue fibroblasts in Hydrolagus.

The general features of spermatogenesis in Hydrolagus appear to be similar to those described by Matthews in Cetorhinus. Several germ cells in Hydrolagus accompanied by small Sertoli elements become incorporated into an ampulla. The cells increase in number and become arranged so that a single layer of Sertoli cells occupies the area next to the lumen, and another single layer of larger gonial cells occupies the periphery. At this stage there appear to be about equal numbers of Sertoli and gonial cells. The gonial cells then continue to divide until this layer is three to four cells thick and the ampulla lumen is almost obliterated. The Sertoli elements begin a migration peripherally through the gonial layers and take up their final position in the

outer wall of the ampulla. This ampulla is surrounded by a thin layer of collagenous tissue. Gonia then undergo meiotic divisions and produce spermatids. These divisions are synchronous within small groups but not quite synchronous throughout the ampulla. As spermiogenesis begins, the spermatids become arranged into cup-shaped groups, the open end communicating with the central lumen. Each cup-group appears to be intimately associated with one, now much enlarged, Sertoli cell. As the spermatids take on the long, slender form of the mature spermatozoa, they are gathered into bundles in which the spiral sperm heads lie parallel and very close together. These sperm bundles in the mature ampulla are arranged radially around the periphery, and each bundle is apparently embedded in a Sertoli cell. The heads are all directed peripherad with the tails extending into the lumen.

Of great interest is the fact that each bundle nearly always contains exactly 64 spermatozoa. In 23 double-checked counts of carefully selected cross-sections through the sperm heads of the bundles, 22 counts gave a total of 64 spermatozoa, while only one gave a count of 63. This great constancy of number leads one to suspect that the Sertoli cells always become associated with a constant number of gonia. It is perhaps easiest to account for the constancy of final number of spermatozoa if one assumes that one Sertoli cell becomes associated very early with one spermatogonium. Indeed, it has already been mentioned that at an early stage the Sertoli cells are arranged contiguously with an apparently equal number of gonial cells. If the association occurs at this

time, thereafter the gonial cell would undergo four mitotic divisions to produce sixteen spermatogonia, which transform into sixteen primary spermatocytes. Then, two meiotic divisions would produce 64 spermatids which transform into mature spermatozoa.

The above explanation would seem to be the simplest for both the constancy of number and the fact that the number is 64. Electron microscopy of early ampullae might provide further clarification. Another possibility is that the Sertoli cell becomes associated with a larger number of gonia as it makes its way from the lumen to the periphery through the layer of spermatogonia, at that time about four cells thick. This however, implies that the Sertoli cell has either an ability to associate itself with a selected and constant number of gonia or, that in associating with a variable number, it has the ability to regulate divisions in such a manner that the final number of spermatozoa is always 64. The one Sertoli-one spermatogonium hypothesis is a simpler explanation.

The Sertoli cells in mature ampullae are 10-15μ in diameter and have a number of large nucleoli or similar bodies. One of these is very large (3μ); one or two smaller bodies (1.5 - 1.6μ) and several additional much smaller bodies are seen. With the technique used, these bodies usually stain somewhat differentially; the largest one is basophilic and some of the smaller ones are somewhat acidophilic.

At maturity the ampullae reach a diameter of about 0.23 mm, with the sperm bundles spaced about 0.018 mm apart, center to

center. Using the formula $4\pi r^2$ the surface area is calculated to be 0.16 mm^2. This would correspond to a square 0.4 mm on a side. Since the sperm bundles are about 0.018 mm apart, there would be 22 bundles along a side or 484 bundles over the area. Multiplying by 64 gives an estimate of 30,976 spermatozoa in each mature ampulla, all stemming from the 6 - 12 germ cells incorporated into the early ampulla.

A few similar counts of sperm bundles in mature ampullae of Squalus suckleyi and Raja rhina also gave a number of 64. This number is possibly quite common to Chondrichthyes in general.

It is of interest to note also that nowhere in the testis of either young or adult Hydrolagus does there appear any obvious hemopoietic tissue (epigonal organ) as is found in many elasmobranchs. The mature testis of Squalus suckleyi has an extensive zone of hemopoietic tissue along the entire medial (mesorchial) side. Raja rhina has a flat zone of this tissue over most of its ventro-medial and posterior surfaces (Plate 2, figs. 13, 14).

In a sample of 33 males, the total combined weight of the right testes was 255.2 g, while the weight of the left testes totalled 255.8 g. There is therefore no significant asymmetry between the testes in Hydrolagus as in many species of Selachii. The greatest difference between the weights of the paired organs was found in an individual whose testes had a weight ratio of 0.86 to 1.

2. Efferent ducts and epididymis

Several incomplete investigations have been made on the efferent duct system of the testis which have resulted in various interpretations. It is apparent that none of the authors have fully clarified the problem. Working with a limited number of specimens of Chimaera monstrosa, Leydig (19, p. 265) described several "vasa efferentia" extending from the testis toward the kidney segments but found no connections. Hyrtl (13, p. 1079) found no efferent ducts but assumed that there were such ducts as occur in the Selachii. Mazza (22, p. 311) denied the existence of efferent ducts and thought that spermatozoa (and even whole ampullae) leave the testis through pores to enter a pocket formed by the peritoneal folds. From here he thought the sex products passed into the coiled ductus deferens but could find no openings. Redeke (31, p. 12), finding no vasa efferentia, thought that there was nothing in the Holocephali homologous to the sexual kidney (Geschlechtsniere) of Selachii, although he designated a large part of the anterior portion of the male opisthonephros as an epididymis. Among other comments, Dean (8, p. 132) includes his belief in the "absence of the Geschlechtsniere in Chimaeroids", but gives no details. Parker and Burlend (28, p. 331-336) described five or six efferent ducts joining the testis to a longitudinal canal running forward and leading directly into the coiled spermiduct (ductus deferens). They state that no connections exist between the ductuli efferentes and the mesonephric tubules.

Burlend in 1910 (5, p. 533) stated additionally that the sexual kidney is represented in Chimaera by a combination of the rete testis, efferent ducts, and the longitudinal collecting duct of the testis. It was his view that an epididymis "as usually defined", is absent in these forms. One may assume that he meant by the phrase "as usually defined", the inclusion of opistho-nephric tubules as sperm channels. In 1920 Morgera, (25, p. 52), apparently unaware of the works of Parker and Burlend, described only a single duct from the testis directly across to Leydig's gland where it branched into three before entering the ducts of Leydig's gland. He stated further that the ducts which Leydig saw were only blood vessels. Morgera declared that Leydig's gland corresponds, in its greater part, to the true epididymis of other vertebrates especially that of the amniotes. Morgera's paper is quite divergent in many details from previous works on Chimaera as well as with the situation that obtains in H. colliei.

Van den Broek (34, p. 53) described in C. monstrosa five ductuli efferentes joining a Nierenrandkanal. The sperm then pass by a large number of ductuli epididymidis to the ductus epididymidis (ductus deferens). He stated that the pathways of sperm discharge from the testis in Holocephali agrees with that in the Selachii and that Holocephali do not occupy a unique position in this respect. He gives, however, only general statements with no detailed description or illustrations of the relationships of this duct system.

It is not difficult to understand why previous authors have had difficulty in delineating the entire efferent duct system of chimaeroids. Not only are these ducts very small, but they also form a complex network which is intermingled with both blood vessels and the exit ducts of Leydig's glands. This study enjoyed the advantage of a large supply of specimens of all sizes. By removing the ventral half of the testis (after formalin preservation), scooping out most of the parenchyma, and clearing for about two days in 10% KOH, the duct system could be made out very clearly. Even so, to delineate the system in full, very patient teasing with needles and fine forceps was necessary. In addition, serial sections of this region in young specimens complemented the investigation.

In H. colliei the collecting tubules of the mature ampullae form an extensive network over the flat ventro-medial face of the testis. Near the mesorchium the tubules are gathered into a variable and smaller number of ducts (usually 4 to 7) which in turn enter a more or less distinct longitudinal testis canal, (called by van den Broek the Mierenrandkanal, 34, p. 53). The canal lies in the mesorchium at the base of the testis and extends from a point in the epigonal ligament (postgonal in position) somewhat posterior to the testis parenchyma, forward to the anterior kidney level. This longitudinal testis canal described by Parker and Burlend (28, p. 331) is of variable diameter and splits for short distances to form small loops. From the dorsal side of the longitudinal testis canal, smaller ducts (45 - 65μ in

diameter) cross from the base of the testis to the kidney substance and sometimes form another, less well defined longitudinal duct buried between the kidney segments and the highly coiled ductus deferens. This arrangement is highly variable, sometimes forming only loops connected serially with the longitudinal testis canal. Without embryological evidence it may be tentatively assumed that this second longitudinal canal is the kidney-edge canal (Nierenrandkanal) described for the Selachii (2, p. 284). Van den Broek (34, p. 53) spoke of only a single canal (which he called Nierenrandkanal) in C. monstrosa, but there appear to be two fairly distinct longitudinal canals in Hydrolagus, a longitudinal testis canal and a kidney-edge canal. At three or four places along the latter canal (or loops) small, short ducts course to the lateral halves of the anterior three or four kidney segments and open into a Bowman's capsule in each segment (one tubule per segment). The fact that the Nierenrandkanal and these capsular ducts (ductuli epididymidis) were completely missed by previous investigators, with the possible exception of van den Broek, gave rise to the belief that chimaeroids were essentially different from the Selachii in the disposition of their efferent duct system, and that no sexual kidney exists in this group. The present study shows that Hydrolagus at least, conforms well to the elasmobranch pattern, and that the anterior few kidney segments do form (anatomically) a sexual kidney or "epididymis" as in Selachii. It appears however, that spermatozoa coming from

the testis do not flow into any kidney duct except that of the first kidney segment.

The kidney segments (metameres) consist anteriorly of a medial and lateral part, each of which is a fine and highly coiled tubule (Plate 3, fig. 15). Each tubule (medial and lateral) joins with its partner at the distal end of the segmental tubule. The more posterior segments have several coiled tubules joining a single segmental tubule. All segmental tubules join the segmental duct which extends caudad to the cloaca. Near the first kidney metamere, the longitudinal testis canal and the kidney-edge canal join to form one duct and this continues directly into the single duct of the lateral first kidney segment (Plate 3, fig. 15). After a much coiled course, this canal is joined by a single one from the medial part of the first kidney segment, and as one duct (first Leydig duct), continues directly into the large ductus deferens proper. As the animal approaches sexual maturity, both the canal of the lateral portion of the first kidney segment and the first Leydig duct (segmental tubule) enlarge greatly and become folded postero-ventrally over the medial portion of the first kidney segment and over both parts of the second kidney segment (Plate 3, fig. 16). The medial part of the first kidney segment remains small and becomes a portion of Leydig's gland. Its duct flows into the now enlarged first Leydig duct. The lateral part of the first kidney segment along with the first Leydig duct, if not also the anterior highly coiled portion of the ductus deferens and parts of the efferent ductule complex as

well, should together be termed epididymis, since they appear homologous with the epididymis of amniotes. The ductus deferens receives, as it passes posteriorly, a duct from each Leydig gland segment; the anterior ducts run from the Leydig gland segments laterally through the loops formed by the ductuli efferentes (Plate 3, fig. 17).

Thus it has been determined that spermatozoa flow in succession from the testis into the longitudinal testis canal, forward into the lateral half of the first kidney segment, through the first Leydig duct and into the ductus deferens. The lateral part of the first kidney segment as well as the first Leydig duct enlarge and become secretory storage vessels as does the ductus deferens. No spermatozoa flow into either the kidney-edge canal or into the ductuli epididymidis of the next posterior kidney segments as is the case in some Selachii, but both of these structural elements often remain patent, and thus Leydig gland secretions apparently could flow in the opposite direction to join the sperm-filled longitudinal testis canal.

In the adult the longitudinal testis canal is 160 - 200μ in diameter and has a simple columnar ciliated epithelium 7 - 10μ high. The canal is filled with spermatozoa, mostly in small bundles, with cellular debris and perhaps with some secretion from Leydig's glands. The epithelial tube of the canal is closely surrounded by a thin layer of heavy collagenous fibers. The epithelium here does not appear to be secretory, and is therefore mainly conducting in function.

As the longitudinal testis canal passes into the lateral tubule of the first kidney segment, it enlarges to a diameter of 0.45 - 0.75 mm, equal to that of the ductus deferens proper, and its epithelium transforms into the ductus deferens type. In this region the epithelium is about 32μ in height and is composed of two cell types, nonciliated secretory cells with basal nuclei, and ciliated cells with slender nuclei closer to the lumen. Here the lumen is filled with spermatozoa both singly and in packets, with cell debris and with secretion droplets as in the longitudinal duct.

As the kidney segment region passes into the first Leydig duct and ductus deferens (0.45 - 0.6 mm in diameter) the epithelium becomes lower (14 - 21μ) and the spermatozoa appear clumped into larger bundles, the heads lying closely parallel with the spirals in phase. Parker and Burlend (28, p. 334) speak of these bundles as spermatophores, and Parker and Haswell (27, p. 188) state that "the sperms become aggregated into spermatophores in the form of small ovoidal capsules surrounded by a resistant membrane and full of a gelatinous substance in which bundles of sperms are imbedded". No such membrane or gelatinous contents have been distinguished in Hydrolagus. The epithelium of the ductus deferens is highly secretory, often showing large masses of secretion in the cytoplasm. The lumen of this duct receives also the secretion of the metamerically arranged Leydig's glands. All ducts have a thin coating of collagenous tissue externally against the epithelium and a much thinner tissue to bind the coils together. No elastic fibers are seen except along blood vessels and immediately beneath the peritoneum.

The ductuli epididymidis in the newly hatched male are well developed and their lumina communicate with the lumen of a Bowman's capsule in the lateral portion of the kidney segment (Plate 8, fig. 36). Capsules occur also in the medial portions of the kidney segments but ductuli epididymidis do not communicate with them. The lateral capsules have also another duct, the true opisthonephric duct, whose coils make up the lateral kidney segment. In a few cases ducts connecting with either the kidney-edge canal or with the longitudinal testis canal end blindly before reaching the kidney. These ducts occur especially in a position posterior to the ductuli epididymidis and may represent abortive ducts of the latter type.

As growth continues, both the ductuli epididymidis and glomeruli of Bowman's capsules tend to degenerate and at maturity neither Bowman's capsules nor glomeruli are present except in the posterior 6 or 7 segments of the male kidney. The lumina of the ductuli epididymidis may become discontinuous in places. At all events, their functional significance appears to be minor even when they do communicate via their very small lumina with the ducts of Leydig's glands.

Already at the hatching stage (S-V 41 mm) the anterior one or two kidney segments show degenerating lateral-most renal corpuscles. The glomeruli are small, and apparently as they contract and degenerate, the outer wall of Bowman's capsule forms into a short segment connecting the ductus epididymidis with the

opisthonephric (Leydig's) tubule. Farther posteriorly the renal corpuscles possess glomeruli which nearly fill the capsule and do not as yet show degeneration.

3. Leydig's glands

By the time of sexual maturity, the anterior portion of the opisthonephros (about 23 segments) has lost all evidence of renal corpuscles and renal tubule differentiation. The tubules are now 100 - 115μ in diameter anteriorly, and a little larger posteriorly (135 - 150μ), and possess a simple columnar epithelium 35 - 50μ in height. This epithelium consists of two cell types, non-ciliated secretory cells with round nuclei close to the base, and a few ciliated cells with elongate nuclei closer to the tubule lumen. Some dark-staining secretory material is present in the lumina and cytoplasm of the secretory epithelium.

Leydig gland secretions are conducted from the secretory tubules into a single duct for each segment, and thence into either the coiled part of the ductus deferens or more caudad into the ampulla ductus deferentis. These collecting ducts are from 70 to 105μ in diameter, and consist of simple cuboidal epithelium 10 to 17μ high, all cells of which possess long cilia. There is no evident secretory activity in these ducts. As with the epididymal coils, there is a thin sheath of heavier collagenous tissue around the Leydig gland epithelial tubes, and a thinner collagenous tissue binding the coils together. Leydig and others have referred to

the secretion in these glands as being of a fatty nature. Evidence for this has not been found in Hydrolagus; on the contrary, the secretion appears to be a basophilically staining non-fat fluid.

4. Ampulla ductus deferentis

While the anterior half of the segmental duct shows some coiling even in a newly hatched male, and becomes increasingly more coiled as growth proceeds, the posterior portion remains straight and acquires a thick sheath of fibroblast cells interlaced with thin collagenous fibers. When the animal has an S-V length of between 165 and 180 mm, this posterior portion begins to show differentiation into an ampulla ductus deferentis. It is first near the anterior end of the straight ductus deferens that the wall of the epithelial tube takes on a wavy configuration, the waves being directed in a lateral plane (Plate 4, fig. 19). The entire tube is about 1 mm in diameter at this stage, while the inner epithelial tube is 150 - 200μ in diameter. The connective tissue sheath becomes thickened dorsally, and Leydig ducts, arising from Leydig gland segments at a more anterior level, pierce the dorsal wall of the ampulla to open into its lumen.

There is next an outpouching of the epithelium in a dorso-lateral direction to form a longitudinal series of small chambers separated by vertical transverse walls. Their chambers communicate broadly with the ventral through-channel. The Leydig ducts are

not pushed outward, but remain deep in the lumen, while the pouches grow dorso-laterally around them. This process continues to form a fan-like array of three or four separate pouches on each side at any one level; thus longitudinal radiating walls form between pouches in addition to the transverse walls. The longitudinal walls however, do not occur in the anterior section. As development proceeds and the chambers become larger, the intervening walls become thinner and the epithelium lining the chambers lowers from a height of 40 - 50μ in the earlier stage to 10 - 20μ at maturity.

During growth the ductus deferens serves solely to conduct urine posteriorly to the vent region, since nearly the whole opisthonephric region from the testis back is a urine producing organ, and since the ductus deferens receives the exit ducts from the opisthonephros. However, as the anterior opisthonephric section loses its urinary function and transforms into an accessory genital gland of Leydig, a new secretion is poured into the ductus deferens to be added to the secretion produced by the ductus deferens epithelium itself. The ductus deferens then becomes exclusively a carrier of germ cells and accessory fluids.

The ampulla ductus deferentis increases uniformly in size until a diameter of about 3 mm is attained. At this stage one section ceases to increase in diameter while the rest of the duct continues to grow. This results in a constriction between the anterior two-thirds and the posterior one-third portion of the ampulla.

In the mature male the portion anterior to the constriction is about 35 mm long and 5 to 9 mm in diameter. Following a short constriction (3 mm diameter) the posterior section attains a length of 20 mm and a maximum diameter of 10 - 15 mm. Posterior to this the duct narrows again to 2 - 3 mm and opens separately from its mate into the antero-lateral aspect of the urogenital sinus (Plate 4, fig. 18). This sinus appears to be formed by a fusion of the posterior enlarged ends of the deferent ducts.

The first 14 mm of the length of the anterior ampulla appears an opaque white in the mature fresh specimen; the color is due to the content of bundles of spermatozoa. The next 17 mm section of the anterior ampulla is a translucent blue-green occasioned by its content of a blue-green secretion, firm and gelatinous in its consistency. The terminal 4 mm of length of the anterior ampulla is a translucent white and bears a clear fluid. The posterior ampulla ductus deferentis is light grey and translucent enough to reveal the interior chambers which contain a clear fluid.

Ducts from Leydig gland segments enter the dorsal aspect of the ampulla ductus deferentis, course within a wall between chambers, and open directly into the through-channel on its dorsal side. The number (commonly twelve) and exact distribution of these ducts varies somewhat in different specimens. Nine ducts generally enter the anterior ampulla ductus deferentis, one enters into the constriction, and two or three enter into the posterior ampulla. The urinary exit ducts of the functional adult

kidney all enter directly into the urogenital sinus, occasionally one entering the ductus deferens just before it in turn enters the sinus.

The anterior ampulla ductus deferentis possesses a very distinct median vertical partition running longitudinally over all but its anterior and posterior ends. This partition is incomplete to provide a through-channel which lies dorsally at the anterior end (Plate 5, fig. 20) but is located dradually more ventrally toward the posterior end of the ampulla. Burlend (5, p. 517) described the through-channel in Chimaera as being located in the dorsal midline for the full length of the ampulla. The anterior-most sperm-filled section is divided by vertical cross-partitions extending from the outer wall obliquely caudad to fuse with the median vertical partition. The slight notch in the medial edge of the cross-partition forms the through-channel (Plate 5, fig. 21) and communication of the latter with the side chambers. No secondary longitudinal partitions are developed in this section. The coiled ductus deferens (of small calibre) expands to form the anterior pointed end of the ampulla ductus deferentis (Plate 4, fig. 18). The through-channel begins with a relatively large lumen but becomes relatively narrow posteriorly. The narrowness of this channel coupled with the fact that the next posterior sections are filled with a rather solid secretion is probably responsible for the sperm being driven into the side chambers. No spermatozoa were seen in the through-channel farther back, although a few may be seen in the blue-green secretion masses in

the middle section. From this, one may assume that spermatozoa are both stored and matured in this anterior section as well as in the epididymis.

Histologically, the chambers of the sperm storage section exhibit a simple columnar epithelium uneven in height from 8 to 23μ. Two cell types are developed, the most numerous being unciliated secretory cells having dome-like luminal ends. With the technique employed, the cytoplasm has many clear vacuoles. Scattered among the secretory cells are less numerous non-secretory cells with long cilia.

The outer wall of this anterior ampulla section has a thin layer of collagenous tissue immediately surrounding the epithelial tube. This tissue along with a few muscle fibers forms the central parts of all partitions. External to this layer is a very thick smooth muscle tunic 450μ thick dorsally which gradually thins ventrally to 150μ. Nearly all muscle fibers course circularly in this section. Contraction of this heavy muscularis presumably ejects the sperm and seminal fluids at the time of copulation. Next in order is another layer of collagenous tissue grading off to the loose connective tissue located between the ductus deferens and either the kidney or the body wall.

In the portion of the ampulla containing the blue-green secretion, the internal structure is slightly more complex due to the radially arranged longitudinal partitions which here are in addition to the transversely arranged cross-partitions. Thus the

interior is divided into many chambers radiating from the central through-passage (Plate 5, figs. 22, 23, 24). The median ventral partition is split to form a trough-like through-passage into which all chambers open dorsally. This trough also develops small and incomplete cross-partitions. Posteriorly in this portion the trough-like through-channel lies progressively more ventral and with a correspondingly broader dorsal median partition above it. This means that at the posterior end of this portion all the side chambers radiate from the ventral median line. Here the chamber passages turn caudally and run parallel to the through-channel a short distance before joining it on its dorsal surface (Plate 4, fig. 18). The dorsal muscle layer of the outer wall becomes progressively thinner cephalo-caudally until it nearly matches in thickness that of the ventral wall.

In stained sections, the blue-green semi-solid eosinophilic secretion proves to be produced by the walls of the ampulla, although a number of ducts from Leydig's gland segments penetrate this section and empty into the through-passage. The simple columnar epithelium is non-ciliated but has a distinct brush border at the lumen. It is uneven in height (15 - 20μ), and the tallest cells are filled with an eosinophilic secretion similar to that in the lumen. Most cells have a light-staining cytoplasm, while the cells containing secretions are scattered and of lesser number. The outer circular muscularis layer is as much as 500μ thick in the antero-dorsal region and only 75μ ventrally. The collagenous fiber capsule is correspondingly thicker ventrally

(150 μ) and thinner dorsally (60 μ). Most of these collagenous fibers are very heavy and longitudinally arranged.

A short rear portion of the anterior ampulla contains no blue-green secretion. The epithelium becomes gradually lower (to 14 μ) and even in height, but with little evidence of secretory activity. There is a graded transition from the anterior secretory to the posterior non-secretory region (partly ciliated.) In this region some abnormal spermatozoa occur; these have swollen heads and some cellular debris is seen.

At both the posterior end of the anterior ampulla ductus deferentis, and in the posterior ampulla, there is no continuous median vertical partition; all chambers radiate from the median ventral through-passage. Toward the posterior end the two deferent ducts bend toward the midline and twist slightly so that the through-channel lies ventro-medially (Plate 5, fig. 25). This circumstance results from a narrowing of the body posteriorly so that the ampulla lies below the kidney instead of lateral to it. From the posterior end of the anterior ampulla ductus deferentis and caudad, all exit channels from the chambers course posteriorly along the dorsal side of the through-passage before joining with it.

The epithelium of the posterior ampulla ductus deferentis is 25 - 35 μ high and has a pseudo-stratified columnar construction. Most cells appear both ciliated and to contain secretion vacuoles. The secretion is only moderately eosinophilic.

A circularly arranged smooth muscle layer 225 μ thick makes up the outer wall of the posterior ampulla and is bounded by a thinner collagenous layer 75 μ thick. Ventrally around the through-channel, smooth muscle fibers are mixed with very heavy collagenous fibers, the latter making the tube quite rigid. Collagenous fibers and scattered smooth muscle cells make up the partitions between sheets of epithelium.

In C. monstrosa, Parker and Burlend (28, p. 334) noted mainly free spermatozoa in the ductus deferens ("epididymis"), but "spermatophores" in the anterior portion of the ampulla ductus deferentis. They thought that the green gelatinous secretion may aid in dissolving spermatophores. Burlend (5, p. 517) declared that free spermatozoa together with Leydig gland secretions were found in the posterior ampulla ductus deferentis. The specimens of Hydrolagus currently investigated present no spermatozoa behind the anterior sperm storage area except for a few abnormal ones in the last few chambers of anterior ampulla ductus deferentis.

The ampulla development in Hydrolagus is more elaborate than that in Squalus suckleyi. The ampulla ductus deferentis of the latter species compares well with an immature specimen of Hydrolagus (s-v 187 mm).

5. Urinary kidney and urogenital sinus

The entire kidney extends from a level corresponding to the anterior pole of the gonad to a point somewhat caudal to the anus.

The kidney segments (metameric) usually number 29 (based on the number of segmental tubules) but occasional counts of 28 and 30 were made in both males and females. The adult urinary kidney segments of the male are much larger than those of the Leydig gland but comprise only about six segments. These are pressed together so that segmentation is difficult to discern, but usually six ureters leave the dorsal aspect of the kidney, course ventrally along the lateral surface between kidney substance and body wall musculature, and in immature males all ureters fuse into two or even a single duct before emptying into the lower neck region of the urogenital sinus. In the adult male the sinus has expanded so that most of the ureters open separately into it at its antero-dorsal aspect close to the openings of the deferent ducts on each side (Plate 4, fig. 18).

The urinary kidney is rich in renal corpuscles and the tubules are differentiated into a variety of cell types and tubule diameters similar to those found in elasmobranch fishes. In the midline between the kidneys there is a strip of interrenal tissue, usually in the form of one long rod (10 - 20 mm long) along with smaller isolated masses both in front and behind it (Plate 4, fig. 18).

The ureters have a simple columnar unciliated epithelium 17 to 35μ in height. The oval nuclei occupy the basal half of the cells. A dense collagenous envelope about 45μ thick surrounds the epithelial tube along with a few smooth muscle fibers.

In immature animals, the urinary sinus as already mentioned appears to be formed by the expansion and fusion of the posterior ends of the two deferent ducts. It forms a single median chamber situated dorsal to the intestine and just ventral to the urinary kidney. Its lower end is a narrowed urogenital passage opening to the exterior on a small prominence posterior to the anal opening (Plate 7, fig. 28). This sinus is lined in the young animal by a stratified cuboidal epithelium the surface cells of which are large and resemble somewhat the transitional bladder epithelium of mammals. At maturity however, the epithelium has thinned to the simple or pseudostratified columnar condition over much of the sinus, but there is an abrupt change at the neck to a stratified cuboidal epithelium. The epithelium is enveloped by a tunica propria of loose collagenous connective tissue. The next layer in order is composed of a smooth muscle layer 300μ thick; some of its component cells are obliquely and others longitudinally oriented.

From the neck of the urogenital sinus, a short passage (retaining the compound cuboidal epithelium of the skin) leads to the exterior. On its anterior surface, this passage possesses a small epithelial out-growth which is homologous with the accessory genital gland of the female. The posterior ends of the vestigial paramesonephric ducts end blindly and separately just dorsal to this outgrowth.

Leydig (19, p. 264) ascribed to the genus Uricolaria the ciliated protozoa which he noted in the urinary ducts and uterus

of C. monstrosa. But the urogenital protozoa noted in H. colliei have been provisionally placed in the genus Trichodina by Dr. Ivan Pratt, professor of parasitology at Oregon State University. Members of this genus are usually external parasites of fresh water fishes.

The absence of hemopoietic tissue from all parts of the urogenital system of Hydrolagus contrasts with the condition in Selachii; this consideration emphasizes a marked divergence between Holocephali and Selachii in this respect.

6. Cloaca

There is present in the newly hatched male a very shallow cloaca. It consists of a slight depression bounded by a low ridge of tissue which includes within its borders the anal and urogenital apertures (Plate 7, fig. 28). With further growth, the cloacal ridge flattens out, and the anus and urogenital sinus open at the external body surface.

7. Paramesonephric ducts and accessory genital gland

Paramesonephric ducts persist in their entire length as very thin and apparently functionless structures throughout the life of the male. Anteriorly they fade to very tiny ducts which open separately to the coelom on either side. The epithelium remains simple cuboidal to columnar, unciliated, and 8 to 18μ in height. The paramesonephric ducts in males never open posteriorly into the

urogenital passage. In the young specimen, these ducts terminate in a small bulb which is separated from the lumen of the urogenital passage by two thin sheets of epithelium (its own epithelium and that of the urogenital passage). In the adult, the ducts are somewhat swollen near the posterior portion but end blindly and with a small diameter at the posterior end.

8. Accessory genital gland

A small glandular organ, present in both sexes, is located between and ventral to the posterior ends of the paramesonephric ducts (Plate 7, figs. 28, 29). In young animals the gland opens into the terminal portion of the urogenital sinus. This organ has been variously called "receptaculum seminis," "digitiform gland," and accessory genital gland. The accessory genital gland rudiment in males grows up to 6 mm in length, acquires a small lumen, but appears never to be functional. The existence of the male accessory genital gland rudiment was missed by most previous investigators and its existence was specifically denied by others. The rudiment cannot be easily seen except in sections of young animals, but in the adult it does appear as a small protuberance between the posterior extremities of the uteri and the hind gut. It can be readily seen at the posterior end of the abdominal coelom.

Along the middle portions of the paramesonephric ducts

slender tubular outpocketings appear, some of which are not connected with the ducts (Plate 4, fig. 19).

C. The structure and differentiation of the female urogenital system

1. Ovary

The ovary in a young female at hatching stage (38 - 40 mm S-V) is a flat plate of germ tissue (like the testis) midway along an extensive genital fold. The ovary does not exhibit one large projection as does the testis, but rather several small projecting masses of epithelial cells. These give the ovary a rough lateral outline (Plate 1, fig. 6).

The primordial germ cells (about 17μ in diameter) are nearly all located on the dorsal side where the epithelium comprises one-fourth to one-third of the total thickness of the ovary. The ventral epithelium is a thin single layer of squamous cells. Between these two epithelial layers, the ovarian medulla is composed of loose mesenchyme permeated with blood vessels. A few isolated segments of tubules are found medially in the mesovarium, and these apparently represent vestiges of either the efferent ductules or the longitudinal duct system or both. There is a very distinct collagenous basement membrane underlying the germinal epithelium, and the medullary tissue is of progressively looser construction toward the ventral side.

Germ cells are found in individual clusters of about a dozen cells each; these nests are separated from others by small epithelial cells. Some of these epithelial cells are elongate and

extend dorso-ventrally between nests and span the entire width of the epithelial layer. In the central regions, the germ cells appear necrotic; their cytoplasm appears clear and the nuclear material is contracted into a tight knot (Plate 8, fig. 38). Peripherally, normal appearing germ cells are seen.

With further growth (S-V 46 mm), the nest arrangement disappears and only single germ cells are seen. Some have grown to about 28μ in diameter, and each cell has acquired a single layer of small, flattened epithelial cells. The medullary margin of the dorsal epithelium acquires an irregular outline due to the growth in size of the germ cells and pushing of medullary cells in the reverse direction against the epithelium.

At a length of S-V 75 mm, the largest germ cells are 32 - 42μ in diameter each with a well developed simple follicle of squamous to cuboidal cells 5 - 7μ thick. The larger follicles extend into the medullary region but are still connected to the epithelium by a broad stalk of epithelial cells. Also scattered about the epithelium, especially on its outer surface, are smaller germ cells, some without a distinct follicle layer. The general dorsal epithelium is 30μ or less in thickness between the larger follicles. In the larger follicles, some of the component cells are greatly enlarged. As the larger follicles push ventrally into the medulla, they carry with them the collagenous basement membrane which then surround the layer of follicle cells. As yet there is no sign of a membrane between the follicle cells and the contained auxocyte.

In ovaries from a specimen of S-V 88 mm length, the largest follicles were up to 140μ in diameter. The follicle layer was 7 - 14μ thick with a few larger cells among the more numerous smaller cells. Medullary tissue forms a layer of flattened cells about 14μ thick external to the basement membrane. The largest follicles reside in the center of the ovary with smaller ones to the periphery.

The ovary at S-V length 110 mm is about 0.55 mm thick and contains follicles whose auxocytes measure up to 150μ in diameter and whose germinal vesicles (60μ diameter) are placed near the ventral side. The cellular follicle attains a maximum thickness of 60μ ventrally, but is somewhat thinner dorsally where it is still attached to the dorsal epithelium by the stalk of epithelial cells. Where large follicles are attached, the surface of the ovary may be somewhat indented. The dorsal epithelium is now only 17 - 21μ thick; this implies that the medullary tissue is growing into the epithelium at the same time that the follicles from the epithelium are pushing down into the medulla. There is an indistinct collagenous acellular vitelline membrane around the largest auxocytes. A zona radiata inside the vitelline membrane is becoming distinct. The cellular follicle has larger cells (up to 28μ in diameter) mixed with the more numerous small follicle cells which comprise several layers. The largest follicle cells make up a single layer. External to the cellular follicle is a layer of fibrous collagenous tissue. Up to this stage, the ovary

has had a rather solid construction, but now distinct lymph spaces appear around the larger follicles. These spaces are the first signs of a general cavitation of the ovary.

When the female has attained an S-V length of 125 mm, the dorsal germinal epithelium has thinned to a simple cuboidal to columnar layer of 7 - 10μ thick. Blood vessels are enlarged and more numerous, coursing in great numbers among the larger follicles; in this area the lymph spaces continue to enlarge.

In a somewhat older animal (S-V 172 mm), the lymph spaces have expanded so that they occupy most of the ovarian substance, leaving only thin walls of medullary tissue between the spaces (Plate 8, fig. 39). Each lymph chamber surrounds one or more growing follicles which are covered by a thin layer of collagenous connective tissue. The largest follicle measures nearly 1.1 mm in diameter.

In a follicle whose auxocyte measures 197μ the surrounding collagenous membrane stains densely, is about 1.5μ in thickness, and cuts across the stalk attachment. That portion of the basement membrane under the dorsal germinal epithelium has become indistinct. The vitelline membrane surrounding the auxocyte stains lighter and appears stratified. At about this time, what are presumed to be the yolk nuclei, appear in the egg cytoplasm. These dark-staining bodies are mostly on a level even with that of the germinal vesicle. When the follicle is about 1 mm in diameter the vitelline membrane is thicker (1.5 - 3μ) and more distinct than that

surrounding the cellular follicle. A distinct zona radiata 6μ thick lies immediately within the vitelline membrane. Many faintly distinguishable processes, apparently from the follicle cells, penetrate 12 to 15μ into the auxocyte. The cellular follicle is now thickest near the dorsal side of the ovary and thinnest in the region next to the germinal vesicle.

In a female of S-V length 235 mm (nearing sexual maturity), the largest oöcyte is 2 mm in diameter. It is contained in a much thickened follicle 60 to 75μ thick, which is however, only about 7μ in thickness immediately adjacent to the germinal vesicle. The cellular follicle is made up of larger cells (21 - 25μ) closest to the oöcyte and many smaller cells peripherally. The vitelline membrane is about 4μ thick but the zona radiata within it is reduced to 1.5μ and is less distinct than it was earlier. Yolk nuclei are seen near the periphery of the oöcyte cytoplasm.

In this ovary the first atretic follicle was noticed. It was about 0.75 mm across, its smaller follicle cells lying innermost and with some apparently invading and breaking down the peripheral oöcyte cytoplasm.

The mature ovary (in relatively inactive state) measures about 38 mm long X 30 mm wide X 10 mm thick, and contains follicles up to 6 mm in diameter. The average weight of 44 such ovaries was 4.6 g with a range of 2.3 to 7.8 g. This represents the basal size and weight of the inactive mature ovary, although during sexual activity the presence of large follicles may increase the

weight to at least 34 g (Fig. 1). Inactive ovaries of larger animals are a little heavier, probably due to the accumulation of atretic follicles and corpora lutea during the period of the reproductive life, to continued growth of the organ itself, or to both.

The right ovaries of 59 females totaled 533.3 g, while the combined weights of the left ovaries equalled 506.7 g. This variation (larger than in males) may be expected in the light of the existence of a wide range of ovarian weights which correlates with the individual reproductive cycle. The two ovaries often differ widely in weight in adult females. The largest difference in this sample was found in an animal in which the lighter ovary had a weight only 24.6% of the weight of the larger ovary. Of the 59 fish in this sample, the left ovary was heavier in 29 cases and the right ovary heavier in 30 cases. Although an individual female may show asymmetry in the ovarian weights, the above data indicate no tendency toward gonadal asymmetry in females as a whole.

In a follicle of 3.5 mm diameter, very tiny droplets are being laid down in the peripheral cytoplasm. The droplets are smallest immediately beneath the zona radiata and are progressively larger centrally. Droplets are not yet seen more than 50μ from the surface. The larger follicle cells are clustered while some areas of the follicle exhibit only small cells.

A 4-mm follicle presents its cells more evenly distributed,

with the larger ones closer to the vitelline membrane and with most small cells peripheral. The smaller follicle cells are dark-staining while the largest cells are lighter and have a very light-staining nucleus nearly filling the cell.

In a 7.5-mm follicle there is a marked thinning of the cellular follicle layer to about 28μ. The follicle now consists of several different parts. External to the thick vitelline membrane lies the cellular follicle layer whose cell boundaries are indistinct. Dean (8, p. 43) assumed that the follicle layer becomes syncytial. Peripheral to the cellular follicle layer lies a thin collagenous membrane, then a layer of flattened cells, and next another collagenous membrane almost as heavy as the vitelline membrane. Outside of the latter is a single layer of regular cuboidal cells followed finally by another layer of flattened collagenous tissue bordering the lymph cavity.

A fully grown follicle 18 to 20 mm in diameter, is completely filled with large yolk granules, except at the area opposite its surface attachment to the dorsal ovarian epithelium. Here the germinal vesicle lies in a small disc of protoplasm. The germinal vesicle reaches a maximum size of about 400 by 547μ, the longer dimension being in the plane of the oöcyte surface. The cellular follicle layer generally ranges from 10 to 25μ in thickness and consists of a simple layer of cuboidal to columnar cells whose nuclei lie close to the outer margin. Cell boundaries are fairly distinct, and the cell layer is thinnest next to the germinal

vesicle. All follicle cells are now similar in size and staining reaction. The tallest cells are at the cicatrix region and may be as much as 45μ in height. The vitelline membrane is much thinner than before and shows no distinct zona radiata within it. A very thin collagenous membrane surrounds the follicle layer followed by a layer of collagenous connective tissue bearing numerous capillaries. Between this connective tissue and the simple cuboidal layer is a thick collagenous membrane. This latter membrane is the thickest one in the follicle but is absent or indistinct in the cicatrix region where the simple cuboidal outer cellular layer is discontinuous. The cicatrix is a circular area about 4 mm across in the mature follicle. The cicatrix consists of only a thin collagenous connective tissue layer and a simple squamous germinal epithelium outside of the inner cellular follicle layer. In the cicatrix, only capillaries are seen and they are few in number. The numerous blood vessels over the rest of the follicle anatomose to form a vessel which encircles the periphery of the cicatrix. This red ring-vessel contrasts with the conspicuous clear yellow of the cicatrix area in fresh specimens. It is here that the oöcyte breaks through the ovarian wall at ovulation.

A follicle from which the oöcyte has recently been ovulated, collapses into a small, highly folded bag open to the abdominal cavity. The lining is of follicle cells five to ten cells thick. The outermost collagenous membrane which lies within the simple cuboidal cell layer is now as much as 7μ in thickness. Some cells

and debris are found in the follicle cavity. Hisaw and Hisaw (11) give further details on atretic follicles and on corpus luteum development.

It has been mentioned that the largest normal follicles (and ovulated ova) are about 20 mm in diameter. These have each a volume of about 4.7 ml. Occasionally, large atretic follicles are found which measure 30 mm in diameter, 10 ml in volume, and are of a much more fluid consistency than normal follicles. This doubling of the volume is apparently due to an increase in fluid content.

To summarize the general events of follicle development, the oöcyte grows from a primordial germ cell or oögonium about 17μ in diameter to a maximum of 20 mm. A simple layer of thin cells arrange themselves around the auxocyte. These thin cells multiply until there is a compound layer up to 75μ thick composed of cells of various sizes and staining reactions. Thereafter this follicle layer becomes steadily thinner until at full growth it is simple cuboidal and from 7 to 25μ in thickness. The vitelline membrane and zona radiata develop into thick, distinct membranes, but in later development become thinner and less distinct. In later stages of growth, a thin layer of collagenous connective tissue and a simple cuboidal outer cell layer become differentiated. A collagenous membrane between them develops into the heaviest of such membranes present in the mature follicle.

2. General discussion of the paramesonephric ducts

The paramesonephric ducts are simple tubes up to about 300μ in diameter at the hatching stage. The posterior regions are both flatter and a little larger in diameter than the anterior. Other than this there is no sign of the later regional differentiation. The ducts consist of a simple columnar epithelium surrounded by a thick layer of dense fibroblasts interlaced by a few thin collagenous fibers.

At maturity, the paramesonephric ducts have developed several distinct regions (Plate 6, fig. 26). An anterior slender oviduct extends from the common oviducal opening on the forward abdominal wall to the enlargement of the shell or nidamental gland. The gland is about 40 mm long, has a maximum diameter of 30 mm, and represents the widest part of the female reproductive tract. Posterior to this gland the uterus (narrower than the gland) extends about 60 mm and ends in a hard, narrow sphincter region followed by an enlarged final segment 40 mm long. One is inclined to label this latter segment as the "vagina" in keeping with the designation made in the elasmobranchs (34, p. 7). In the adult, the vaginae open independently to the exterior immediately behind the anus.

3. Oviduct

In a female just prior to hatching, the oviducts are slender

oval tubes 50 to 90μ in diameter and possess a simple columnar unfolded epithelium surrounded by a dense collagenous sheath 14μ thick. In an older animal (S-V 75 mm) the epithelium is cast into several low, longitudinal folds. With further growth, these folds become progressively more complex, and in a mature female they are very extensive, reaching nearly to the center of the lumen. At maturity, the oviducts are 3 to 5 mm in diameter and about 60 mm long. The simple columnar epithelium has increased in height to about 25μ and has developed cilia which always appear relatively late in development (near sexual maturity). A collagenous tunica propria surrounds the epithelium and provides a connective tissue core with many blood vessels between the epithelial folds. Surrounding the tunica, the heavy muscularis consists of inner circular and oblique smooth muscle fibers and outer longitudinal fibers.

On the posterior face of the septum transversum, the oviducts bend toward the midline, broaden and open by a single opening which is directed ventrad. The histology of this region is similar to that of the oviducts except for the fact that most muscle fibers run longitudinally (transversely with respect to the long body axis). Very coarse collagenous fibers of the septum transversum invade the muscle layer. In this region of the oviduct also, cilia are late in appearance, being first noticed in the nearly mature animal.

4. Shell gland

The shell gland region in young animals is distinguished from the oviduct region by its broader and flatter outline in section. At an S-V length of 170 mm, the epithelium is tall, simple to pseudostratified columnar 31 - 35μ in height, and has a series of transverse low folds on its dorsal and ventral sides. A thick tunica propria of collagenous tissue surrounds the epithelial tube; the portion next to the epithelium is very dense, becoming markedly looser in construction peripherally. A few longitudinal smooth muscle fibers lie outside the tunica, followed by a loose adventitia of collagenous connective tissue.

In an older animal (S-V 192 mm) the cross-folds (lamellae in adult) of the epithelium have deepened somewhat so that the epithelial waves are about 75μ in height. The alternating ridges and grooves are best developed at the anterior end of the shell gland and gradually diminish in height posteriorly to the smooth luminal epithelium of the uterus. Cells and nuclei on the ridges are very narrow and elongate, exhibiting a pinched appearance, while the cells of the grooves are oval with nuclei crowded toward the periphery (away from the lumen).

In a still older animal (S-V 225 mm) the epithelial lamellae are about 150μ in height. Short simple tubular outgrowths of the epithelium are seen at the oviduct-shell gland junction and also at the lower end of the shell gland. As yet, no outgrowths are produced in the region of the cross-lamellae. A dense layer of

collagenous tissue immediately adjoins the epithelium, enclosed in turn by a looser layer of collagenous tissue whose fibers course in all directions. An outer layer of collagenous tissue possesses fibers which are all longitudinally arranged.

In a nearly mature female (S-V 235 mm), the oviduct extends into the expanded shell-gland region and opens at the tip of a conical posteriorly-directed projection. Just anterior to its opening the oviducal lumen is expanded by a number of circular grooves into the bases of which open small tubular glands. Short simple and branched glands also open into the lumen both above and below this point.

Narrower gland tubes (35 - 50μ in diameter) open into the lumen of the shell gland proper between low folds at the base of the oviducal cone. From this point posteriorly, the transverse lamellae are seen. These lamellae are low anteriorly and gradually increase in height to about 0.5 mm in the central regions. In the posterior region the grooves between adjacent lamellae become gradually shallower, though the general shell gland lumen remains the same in diameter. In the lamellar region, glands up to 1.0 mm long and 0.08 - 0.85 mm in diameter open into the deepest parts of the grooves at the tips of short projections. This general structure of the shell gland of H. colliei agrees with that of Scyliorhinus canicula (24, p. 232-234) and of Chiloscyllium griseum (26). Although the glands of the above two species are described as simple tubular, those of H. colliei are branched

tubular. The development and branched nature of these glands in H. colliei agree closely with other elasmobranchs as described by Borcea (4, p. 432).

Posteriorly, where the lamellae become shorter and finally disappear, there follows a region in which most tubules are simple and of two distinct parts. The deeper portions of the gland tubes are 35 - 40μ in diameter and possess an epithelium 14μ in height, while the luminal halves are 25 - 28μ in diameter and have an epithelium only 7μ in height. This differentiation anticipates the contrasting histology and nature of the secretion of the two parts in the adult. The tubules of this posterior section open onto the flat luminal surface. The glands end abruptly at the site of transition from shell gland to uterus.

Some gland tubules have ciliated luminal surfaces, especially in the lamellar region, but the posterior tubules are mostly non-ciliated. Many division figures are seen, most numerous at the distal ends of the tubules. In this region the tubules show one or two generations of branches but these are short in comparison with the single neck portion of the glands.

At sexual maturity the glandular tubules have differentiated into several different secretory types similar to those found in elasmobranchs as noted by Prasad (29, p. 54). He describes an anterior albumen and mucous-secreting region, a broad middle shell-secreting region marked internally by the lamellae, and a posterior zone of shell and mucous secretion. In the latter zone the distal ends of the gland tubules secrete shell material, while the luminal

halves secrete mucous. There is an abrupt line of demarcation near the mid-section of these tubules, between shell-secreting and mucous-secreting cells. This is a striking example of a single gland tubule differentiated into two very distinct and separate zones. At maturity these posterior tubules are also branched.

The diameter of the shell glands in mature fish varies in relation to the reproductive state of the ovary, and not to the length of the animal. In a mature female which is reproductively inactive (small follicles), the shell gland measures about 13 by 17 mm in transverse section, and the longest tubules are about 10 mm in length. When the ovary contains large follicles, the shell gland has increased to 20 by 25 mm in section, while a shell gland in active secretion is up to 30 mm in diameter. In the latter case, the tubules are only slightly longer (probably due mostly to an increase in cell size) but the central shell gland lumen is wider. In the fully developed female, the shell gland is 35 to 40 mm in length. The shell material appears in the gland cells and lumina as droplets which soon coalesce to form into sheets which are laid down in strata as also described and figured by Borcea (4, p. 422) for elasmobranchs.

All shell gland tubules are circular in cross section. Most nuclei are round or oval and located at the base of the cells nearest the periphery. Other cells, fewer in number, have oblong nuclei closer to the gland lumen. Nalini (26, p. 207) stated that in the young stages of Chiloscyllium griseum all cells are ciliated, but that in adults, only the cells with nuclei closer to the gland

lumen retain cilia; the others become non-ciliated secretory cells. In Hydrolagus, even in secreting adults, all cells of the gland tubules appear to be ciliated.

5. Uterus

In young animals the uterus remains rather undeveloped. It is still only 2 by 6 mm in section in a fish of S-V 235 mm (nearing mature size). The uterus of the nearly mature animal is a flattened tube with simple columnar non-ciliated epithelium 17 to 28μ in height. Dorsally and ventrally a very heavy collagenous membrane 3 to 5μ thick lies against the epithelium. This membrane is absent at the lateral edges. Many capillaries lie between the epithelium and the collagenous membrane. The collagenous capsule is of loose construction at middle levels and denser peripherally. Poorly developed muscle cells, few in number, are found at the outer levels intermingled with small elastic fibers.

In a mature female whose ovaries have only small follicles, the uterus measures 4 by 10 mm in section. The dorsal wall is markedly thicker (1.5 mm) than the ventral (0.3 - 0.45 mm), and possesses a distinct medial longitudinal furrow. Other shallower longitudinal grooves mark the dorsal and ventral walls. The epithelium is simple columnar (35μ in height) and consists of two cell types. Light staining non-ciliated mucous cells with basal nuclei alternate, in about equal numbers, with darker staining ciliated cells whose nuclei are closer to the lumen. The luminal surface appears to be almost solidly ciliated due to the expansion of the luminal

surface of these ciliated cells at the expense of the mucous cells at this border.

Many capillaries lie closely applied to the epithelium and some have even penetrated between the basal ends of epithelial cells. Dense collagenous connective tissue surrounds the epithelium immediately adjacent to the capillary level. External to this is a tunica of loose collagenous tissue becoming denser toward the periphery where the muscularis is interlaced with very heavy collagenous fibers. Smooth muscle fibers form an inner circular and an outer longitudinal layer. At the lateral edges of the uterine tubes less collagenous tissue is found, and the wall is much thinner, being composed mainly of epithelium and muscularis.

The uterus of a gravid animal with partially completed egg cases in the shell glands, is much expanded, measuring 10 by 20 mm in cross-section. The dorsal wall is thickened to 5 or 6 mm, and the ventral wall is increased to 1.5 mm in thickness. This thickening appears to be due mainly to fluid imbibition in the tunica propria which now is an extremely loose collagenous tissue. All epithelial grooves are smoothed out except for the deep mid-dorsal one, which according to Dean (8, p. 176), molds the mid-dorsal keel of the egg case. Mucous-secreting goblet cells are more numerous than in the uterine epithelium of the non-gravid animal, and they are especially numerous in the longitudinal recesses of the lateral walls.

Blood vessels are especially plentiful; between the muscularis and peritoneum they form extensive sinuses bathing the uterine

surface. In the young animal the uterus is entirely retroperitoneal in position, but in the mature animal it is suspended along its dorso-medial border by a double-walled mesotubarium. Elastic fibers are mostly concentrated beneath the peritoneum and in the muscularis, but a few thin fibers are found in the tunica propria as well.

Near their posterior ends the paramesonephric ducts of the mature female show a markedly different structure from the more anterior regions of the ducts. About 45 mm from the external openings, the uterus becomes both narrower and nearly circular in outline. The collagenous layers gradually decrease while the muscularis increases in thickness (Plate 6, fig. 26D). The epithelium becomes tall, pseudostratified and ciliated with a decreasing number of goblet cells. The muscularis forms a powerful sphincter of inner longitudinal, middle circular and outer longitudinal layers, all of which are interlaced with very heavy collagenous fibers. Many well-developed elastic fibers are found in the muscle layers. The simple uterine epithelium changes abruptly to a compound cuboidal type characteristic of the vagina. Posterior to the utero-vaginal sphincter the reproductive tracts expand into chambers about 40 mm long. Some investigators have termed similar chambers as vaginae in elasmobranch fishes. The epithelium is compound cuboidal to squamous, 2 mm thick (75 - 100 cells) and thrown into a few large longitudinal folds (Plate 6, fig. 26E; Plate 8, fig. 40). External to this is a dense layer of collagenous tissue 0.45 mm thick followed by a muscle layer composed

of inner circular and outer longitudinal muscle fibers; this layer is also 0.45 mm in thickness. No cilia are found in the vaginal section. The epithelial cells are mostly spindle-shaped. The luminal cells have their long axes in the plane of the surface, while deeper cells are radially oriented. Heavy walls 3μ thick separate the cells. Oval nuclei are present in a light-staining cytoplasm. In the walls of these vaginal chambers where they open externally, are found heavy elastic fibers.

Plate 7 illustrates the development of the posterior region of the reproductive tract. In a very young animal (S-V 46 mm), the paramesonephric ducts end blindly and separately at the anterior wall of the urogenital sinus immediately dorsal to the accessory genital anlage. Following a constriction of their enlarged posterior ends, the paramesonephric ducts form a small bulb which is pressed against the forward wall of the urogenital sinus. The epithelium of the bulb is distinct from that of the urogenital sinus, and there may be connective tissue cells between the epithelia of the ducts and urogenital sinus. In a specimen nearing the hatching stage (38 mm S-V) the posterior tip of the bulb has cells of a glandular appearance (Plate 8, fig. 35), but in an animal of S-V length 46 mm, the terminal epithelium of the bulb is squamous. As growth continues (Plate 7, figs. 29-33), the portion of the urogenital sinus wall immediately around the paramesonephric bulb becomes extended into a duct. This extension becomes the vaginal portion of the adult reproductive tract. The bulb region remains closed during its shift relatively more

anteriorly; the plate between the uterus and vagina does not break through until the animal is approaching maturity. Thus it becomes apparent, that the lower or vaginal portion of the female reproductive tract is not differentiated from the paramesonephric ducts, but is rather an extension of the forward wall of the urogenital sinus. This circumstance also accounts for the compound nature of the vaginal epithelium as contrasted with the simple epithelium of the paramesonephric duct derivatives.

6. Accessory genital gland

The accessory genital gland anlage in newly hatched females, is a solid epithelial outgrowth from the forward wall of the urogenital sinus (Plate 7, fig. 29) just as in newly hatched males (Plate 7, fig. 28). With further growth in size, it gradually acquires a lumen, and at maturity it is a digitiform, heavy-walled structure 8 to 10 mm in diameter and 37 mm in length (Plate 7, fig. 33; Plate 6, fig. 26). The lumen has a narrow portion about 10 mm long, which leads into the urogenital sinus. The inner lining of the gland is a compound squamous epithelium 8 to 10 cells in thickness which in turn is surrounded by a thick layer of dense collagenous connective tissue 0.38 to 0.45 mm thick. External to this lies a muscularis as much as 0.6 mm thick, which is composed of inner circular and oblique muscle cells intermingled. Outside of this is a longitudinal smooth muscle layer. A thin collagenous adventitia and the peritoneal epithelium complete the wall in the anterior two-thirds where the gland projects into the coelomic

cavity. Posteriorly, the gland is surrounded by collagenous tissue and body wall musculature.

The gland in nearly all mature females possesses a semi-solid secretion body which fills the lumen of the gland. This body is milky white, of gelatinous consistency, and contains desquamated epithelial cells in its peripheral areas. Desquamation appears to be responsible for the production of this body.

Leydig (19, p. 269) surmised that the gland was accessory to the female genital system, while Hyrtl (13, p. 1086) thought that it was a seminal receptacle and contained spermatozoa. The gland was thought by Semper (32, p. 287) to be homologous with the rectal gland of Selachii but Mazza and Perugia (23) and more recently Clothier (7, p. 21-22) demonstrated the untenable nature of this concept. The latter three authors have found the rectal gland homologues embedded in the wall of the hind gut. The presence of spermatozoa in the accessory genital gland has been disproven. The present author found that the secretion had no apparent attraction for males under laboratory conditions. Furthermore, females possess glands full of secretion even when the ovaries are in the inactive state. Until further evidence is forthcoming, this structure may be provisionally considered an accessory genital gland, though its function remains obscure.

7. Urinary kidney and sinus

The kidney in the young female consists of 29 metameres as

in the male. As growth continues the anterior few segments degenerate to variable degrees. At maturity, about twenty-six segments can be counted, but the anterior nine or ten are extremely thin and probably nearly functionless. Progressively toward the posterior end of the body, the kidney segments are larger, but only the last nine or ten are well developed enough to be effective urine producers. The last one or two kidney segments are somewhat smaller than those immediately preceding them.

All segmental tubules from the kidney segments enter the longitudinal segmental ducts except for the last four or five, which fuse into a single duct on either side and these join the segmental ducts posteriorly. The latter ducts open into the neck of the urinary sinus antero-laterally (Plate 7, fig. 33). Previous authors reported that a urinary sinus is absent in the male, or implied that the urogenital sinus of the male was a different organ from the urinary sinus of the female. While the sinus of the female is a little longer than that of the male, the position, histology and connections of these two organs argues in favor of their homology. The essential difference appears to be only functional, in that the segmental duct of the adult male carries semen instead of urine as is the case in the female. The adult male segmental ducts (ductus deferens) enter the antero-lateral aspect of the urogenital sinus on either side, while in the female, the segmental ducts pass to the neck of the urinary sinus where they open after having received all of the segmental tubules (ureters) from the kidney metameres. The structure in

the immature female homologous with the entire urogenital sinus of the male is both anatomically and functionally distinguishable in an antero-dorsal urinary sinus and a postero-ventral urogenital sinus. In the adult female, however, this postero-ventral portion is flared and becomes incorporated as a part of the exterior which surrounds the apertures of the accessory genital glands, the vaginae, and the urinary sinus (Plate 7, figs. 29-33).

The histology of the urinary kidney and ureters in the female agrees closely with that of comparable male structures. The urinary sinus epithelium remains in the earlier compound cuboidal state, with many of the luminal cells transformed into mucous-secreting goblet cells. Eosinophils in large numbers invade the epithelium from below. A large distinctive cell type in much smaller numbers is also found (Plate 1, fig. 8; Plate 8, fig. 37). These cells usually occur at the base of a small epithelial pit and extend from the base of the epithelium to the lumen. They bear a large oval nucleus and a dark-staining cytoplasm. At the luminal surface, these cells have a tuft of fine processes which possibly are cilia. Their appearance suggests that they may be neuro-epithelial in nature. The presence of these cells in the urogenital sinus of males as well as in the urinary sinus of females, both young and adult, is strong additional evidence of the homology of these two sinuses.

A fairly heavy tunica propria followed by a muscularis of obliquely and longitudinally disposed smooth muscle fibers comprise the outer layers of the urinary sinus.

8. Cloaca

In the newly hatched female, the cloacal relationships are the same as those found in newly hatched males. A very low ridge of tissue demarcates the shallow cloaca, and also includes within its boundaries both the larger anal opening anteriorly, and the smaller urogenital opening posteriorly. During the course of further growth, the cloaca disappears as such, and the lower urogenital sinus gradually widens to allow all of the urogenital structures to open separately to the exterior. In the adult female, the anal opening is followed in the mid-line by the opening of the accessory genital gland. Behind this are the paired vaginal openings, and the opening of the urinary sinus is located last in order. There is thus a disappearance of the shallow cloaca in both male and female. Additionally in the female, there is a flaring of the urogenital sinus (comparable to the lower portion only of the male urogenital sinus) which does not occur in the male.

SUMMARY AND CONCLUSIONS

1. A study of the anatomy and histology of the urogenital organs of Hydrolagus colliei from hatching stage to maturity is presented. The urogenital systems of this species correspond well with those of elasmobranch fishes.

2. Data are given which indicate that this species spawns at all seasons of the year. From 12 to 35% of adult females are reproductively active at any given time, while adult males are continuously active.

3. The largest females reach a total length of about 67 cm, while the largest males are only about 62 cm in length.

4. There is a high degree of symmetry in the weight of the gonads in either sex, although the two ovaries of an individual female may differ widely in weight.

5. New germ cells are incorporated into ampullae at the base of a single, small germinal projection on the dorso-lateral aspect of the testis. Maturing ampullae migrate progressively across the testis and discharge their matured products into the efferent ductules at the flat medio-ventral face of the testis.

6. An hypothesis is advanced to the effect that there is a very early association of one Sertoli cell with one spermatogonium, and that a constant number of mitotic and meiotic divisions produces the uniform number of sixty-four spermatozoa found in each bundle.

7. The efferent duct system of the testis of H. colliei corresponds with that in the elasmobranch fishes. A longitudinal testis canal and a kidney-edge-canal (Nierenrandkanal) are differentiated to varying degrees. Connections exist between the latter canal and the anterior few segments of the opisthonephros. Only a single one of these connections (most anterior) in Hydrolagus is transformed into a part of the epididymis, the sexual portion of the kidney (Geshlechtsniere).

8. The chambered ampulla ductus deferentis is very complex and its lining epithelium is differentiated into several different secretory portions. The ampulla develops a heavy, circularly arranged muscularis, presumably functioning in the ejaculation of spermatozoa and seminal fluids at the time of copulation.

9. The opisthonephros in the young male contains renal corpusles which apparently excrete urine. During the growth of the animal, the anterior portion of this kidney loses the capsules and glomeruli; at maturity, this region forms an accessory genital gland (Leydig's gland).

10. A distinct single median organ develops as a urogenital sinus in males and as a urinary sinus in females; these are apparently homologous. Distinctive cells, possibly of a neuroepithelial nature, are included within the epithelial lining of these two organs.

11. In the young of both males and females there is an extremely shallow but distinct cloaca, which is no longer distinguishable in

the mature animal. In the mature female, the urogenital sinus also flares out so that the accessory genital gland, the paired reproductive tracts, and also the aperture of the urinary sinus open independently to the exterior.

12. There is a nearly complete representation of both the male and female reproductive tracts which persists in the adults of both sexes.

13. The paramesonephric ducts of the female differentiate into four regions, that of the ostium, the oviduct, the shell gland, and the uterus.

14. Each uterus is connected to the exterior by a vaginal segment of urogenital sinus origin.

15. The differentiated regions of the paramesonephric ducts in the adult female retain the simple lining epithelium, while the vaginal segments exhibit the compound epithelium of the urogenital sinus.

16. The shell gland develops first as a series of transverse folds on the dorsal and ventral walls of the paramesonephric ducts. Later, outgrowths arise from the grooves between the folds and develop into the branched glandular tubules; these differentiate into albumen, mucous, and shell-secreting types. Some tubules have two distinct regions, each producing a characteristic secretion.

17. A gland accessory to the genital system (ventral to the vaginae) and functional only in the adult female, is present also in the male. It is developed from the forward wall of the

urogenital sinus and it secretes a white gelatinous body molded to the shape of the gland lumen.

18. The cellular layer of the follicle of young auxocytes is at first simple, later becoming multilayered and of different cell types. As the follicle nears maturity, this layer becomes simple again and of a single cell type.

19. Yolk deposition begins when the auxocyte is 3 to 3.5 mm in diameter, and continues until the oöcyte is about 20 mm in diameter (at maturity).

20. No hemopoietic tissue could be distinguished in either the gonads or in any other portion of the urogenital systems of either sex.

21. Ciliate protozoan inhabitants of the urinary sinuses and ducts in Hydrolagus are provisionally assigned to the genus Trichodina.

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LIST OF ABBREVIATIONS

aa, anterior ampulla ductus deferentis

ag, accessory genital gland

an, anus

ao, opening of accessory genital gland

bv, blood vessel

bw, body wall

c, coelom

ca, constriction of ampulla ductus deferentis

cd, coiled ductus deferens (Leydig's duct)

cr, cloacal ridge

ct, connective tissue

de, ductulus efferens

dg, dorsal median groove of the uterus

dz, zone of degeneration of testicular ampullae

ed, system of efferent ductules from testis

eo, eosinophil

ep, epididymis

gc, genital cords

gf, genital fold

gl, glomerulus

gp, germinal projection

he, hemopoietic tissue

ir, interrenal tissue

k, kidney

ke, kidney-edge canal (Nierenrandkanal)

la, lamellae

lc, longitudinal testis canal

lg, Leydig gland

lp, lateral portion of kidney segment

lt, Leydig tubule (s)

mp, medial portion of kidney segment

mu, mucous cells

ne, neuroepithelial-like cell

o, ovary

oa, ostium tubae abdominale

oc, oviducal cone

ov, oviduct

ot, opisthonephric tubule

pa, posterior ampulla ductus deferentis

pb, paramesonephric duct bulb

pd, paramesonephric duct

pv, postcardinal vein

rw, radial wall of ampulla ductus deferentis

sd, segmental duct

sg, shell gland

sp, utero-vaginal sphincter

st, segmental tubule

sz, zone of maturing spermatozoa

t, testis

ta, testicular ampulla

tc, through-channel of ampulla ductus deferentis

ti, zone of spermatids

u, uterus

ug, urogenital sinus

uo, urinary opening

ur, ureters

us, urinary sinus

v, vagina

vo, vaginal opening

vw, vertical wall of ampulla ductus deferentis

EXPLANATION OF PLATES

Plate 1

Figure 5. Ventral view of the anterior portion of the left side of the urogenital system in a newly-hatched male. X40.

Figure 6. Ventral view of the anterior portion of the left side of the urogenital system in a newly-hatched female. X40.

Figure 7. Dorso-lateral view of mature testis. X1.

Figure 8. Section through the epithelial lining of the urinary sinus in an adult female showing a large neuroepithelial-like cell. X1500.

Plate 2

Figure 9. Transverse section through the testis region of a newly-hatched *Hydrolagus* (S-V 41 mm). X50.

Figure 10. Transverse section through the testis region of *H. colliei* (S-V 88 mm). X40.

Figure 11. Transverse section through the testis region of *H. colliei* (S-V 175 mm). X6.

Figure 12. Transverse section through the adult testis region of *H. colliei*. X2.

Figure 13. Transverse section through the adult testis region of *Squalus suckleyi*. X1.5.

Figure 14. Transverse section through the adult testis of *Raja rhina*. X1.5.

Plate 3

Figure 15. Diagram of the organization of the epididymal region in a newly-hatched male H. colliei. Those ductules shown in broken lines are more highly coiled than here represented, and take up the entire space within the solid line enclosing each part. Ductuli efferentis (DE) connect the longitudinal testis canal (LC) with the kidney-edge canal (KE). Each ductulus epididymidis connects the kidney-edge canal with a Bowman capsule in the lateral portions of the anterior opisthonephric segments. A glomerulus in the first opisthonephric segment has not been identified with certainty.

Figure 16. A diagram of the organization of the epididymal region in an adult male H. colliei. The glomeruli have degenerated. The tubule of the lateral portion of the first kidney segment (LP) together with the first segmental tubule (ST) have enlarged and folded back to lie ventral to the small medial part of the first kidney segment (MP) to form part of the epididymis.

Figure 17. A drawing to scale showing the actual configuration of the epididymal region on the left side of a nearly mature male. Ducts labelled LP, ST, and SD are shown less coiled than in life. Their coils are closely packed and comprise the entire space within the solid lines. The medial part of the first kidney segment and all the more posterior kidney segments shown are transformed into Leydig gland segments. X3.

Plate 4

Figure 18. Diagram drawn to scale and representing a longitudinal section through the ampulla ductus deferentis in an adult male. Numbers at broken lines indicate positions of transverse sections shown in Figures 20 - 25 on Plate 5. X3.

Figure 19. Ventral view of the middle urogenital region (on the right side) in a male of S-V length 180 mm and showing the early wavy configuration of the anterior ampulla ductus deferentis. The paramesonephric duct has numerous small processes of unknown significance. X7.

Plate 5

Figures 20 - 25. Transverse sections through the ampulla ductus deferentis corresponding to the positions marked by broken lines in Figure 18, Plate 4. X3.

Plate 6

Figure 26. Scale drawing of the right side of an adult female reproductive tract (ventral view). Position of ovary is shown in broken lines. A, B, C, and D are views which correspond to sections made at representative positions along the length of the genital tract as indicated by the corresponding letters. X1.

Figure 27. Scale drawing of the reproductive tract of the female (left side exclusive of oviduct) split along its side and laid open with the dorsal side of the tract to the right. About X1.

Plate 7

Figure 28. Sagittal section (to scale) through the vent region in a newly-hatched male (S-V 43 mm). X15.

Figure 29. Sagittal section (to scale) through the vent region in a newly-hatched female (S-V 46 mm). X15.

Figure 30. Sagittal section (to scale) through the vent region in a female of S-V length 75 mm. X5.

Figure 31. Sagittal section (to scale) through the vent region in a female of S-V length 88 mm. Note the vaginal outgrowths of the forward urogenital wall. X5.

Figure 32. Sagittal section (to scale) through the vent region in a female of S-V length 125 mm. X4.

Figure 33. Sagittal section (to scale) through the vent region in an adult female of S-V 250 mm. The lower urogenital sinus has flared to bring the openings of all ducts to the exterior. About X1.

Plate 8

Figure 34. Section of testis in a young male showing the formation of a new testis ampulla as an evagination from a testis duct. X600.

Figure 35. Transverse section of the posterior ends of the para-mesonephric ducts in a hatching-stage female. X600.

Figure 36. Section through renal corpuscle in an anterior kidney segment of the hatching-stage male. The kidney-edge canal is connected to the Bowman capsule in the next section posterior

(connection shown by broken lines). The opisthonephric tubule is shown connected with the Bowman capsule. X600.

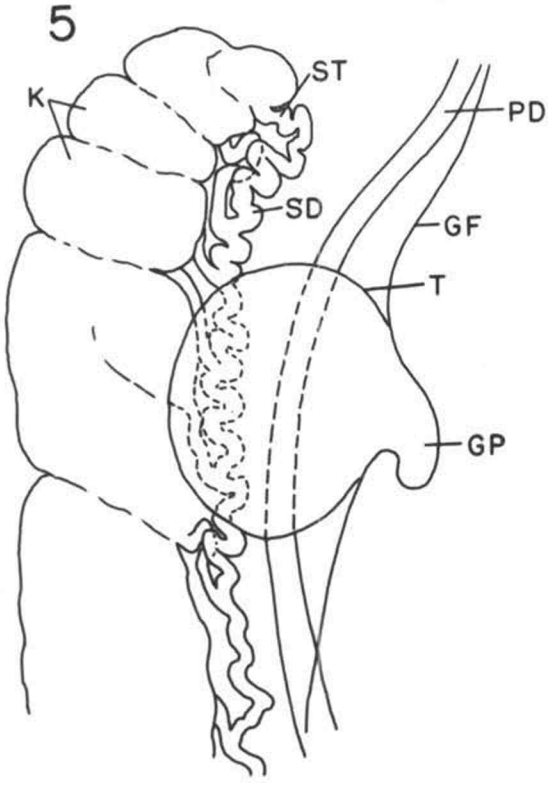
Figure 37. A neuroepithelial-like cell at the bottom of a pit in the urinary sinus epithelium of a mature female. X980.

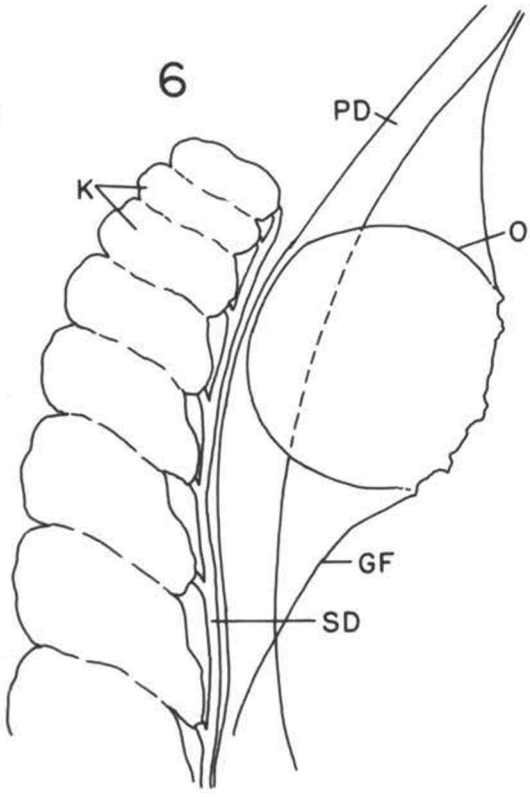
Figure 38. Section through the germinal epithelium at the dorsal side of the hatching-stage ovary showing nests of germ cells with contracted nuclei. X400.

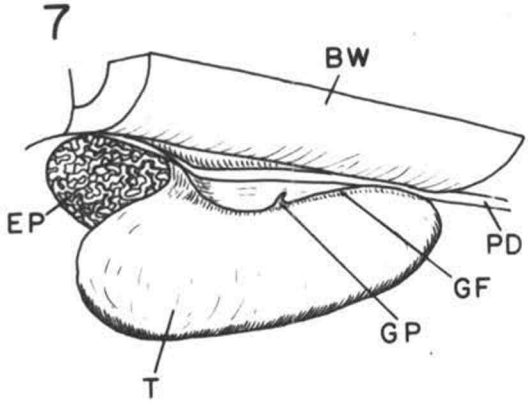
Figure 39. Section through the dorsal side of the ovary in a female of S-V 172 mm, showing two small follicles and the extensive lymph (?) chambers surrounding them. X100.

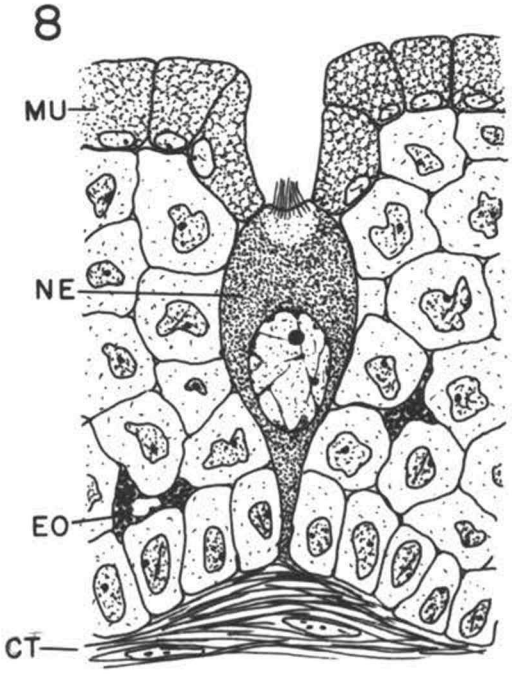
Figure 40. Section through the epithelium of the vagina. X400.

PLATE I









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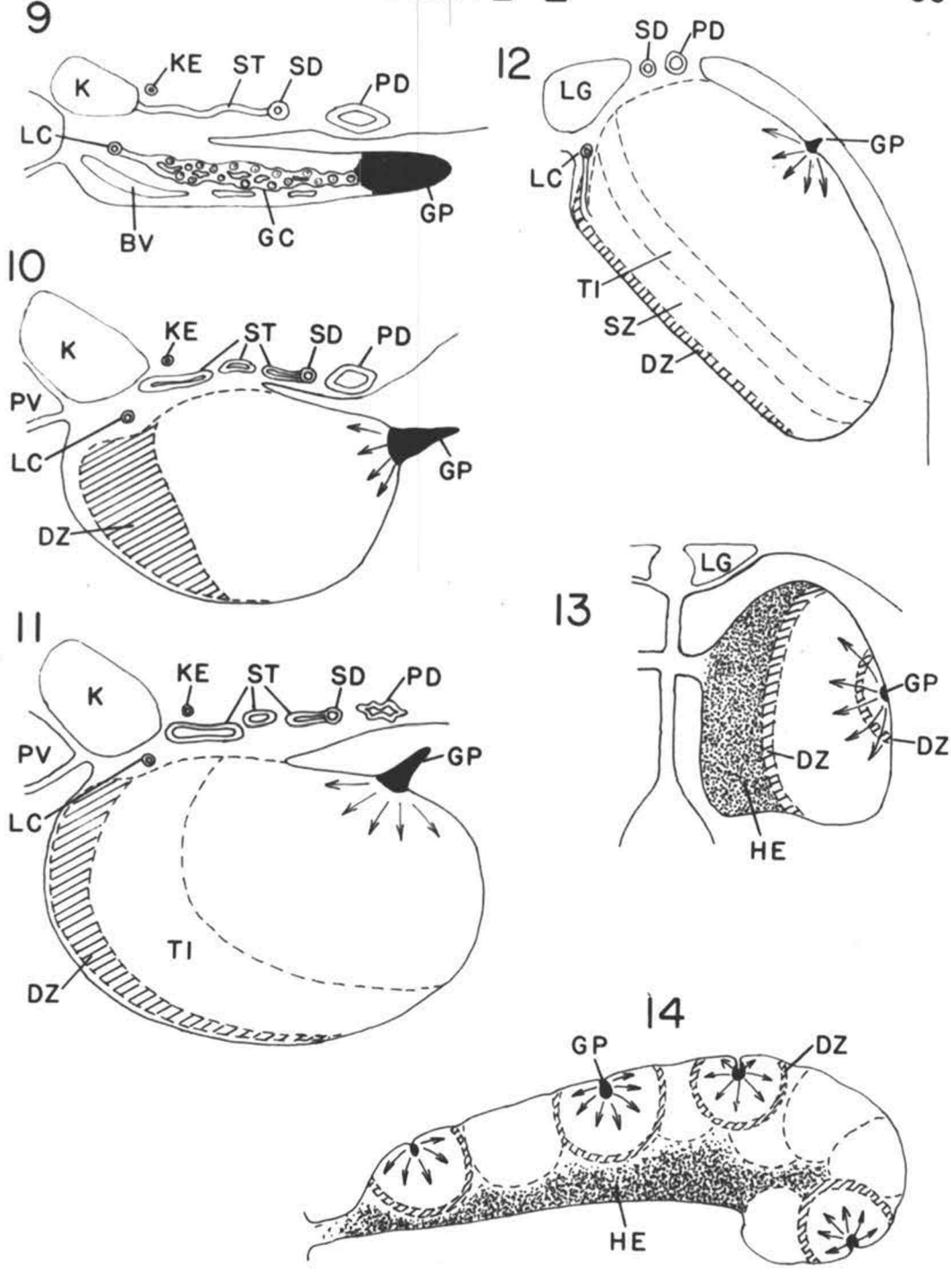
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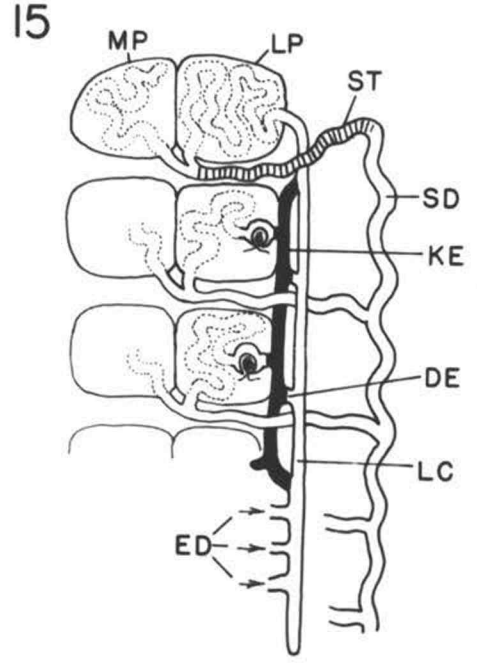
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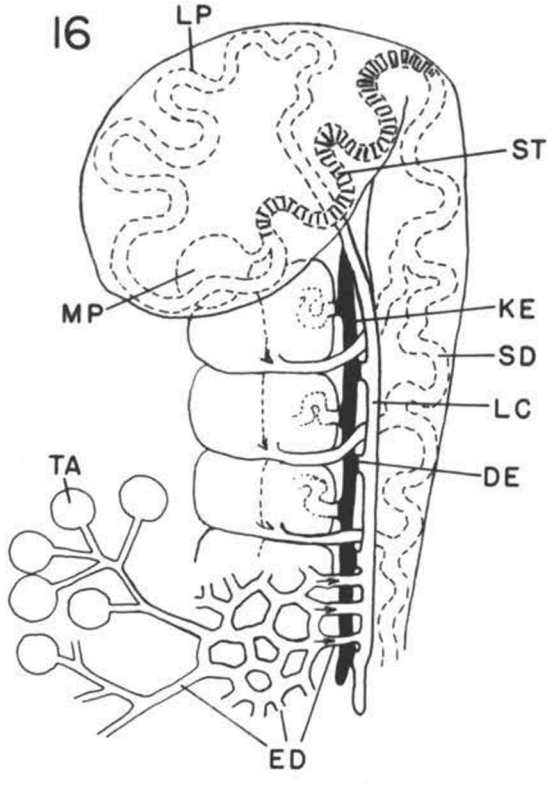
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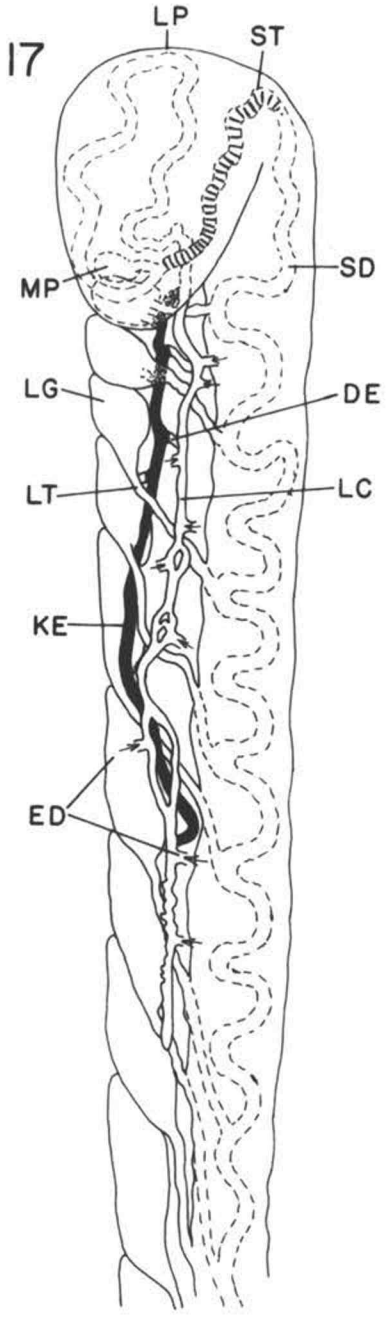
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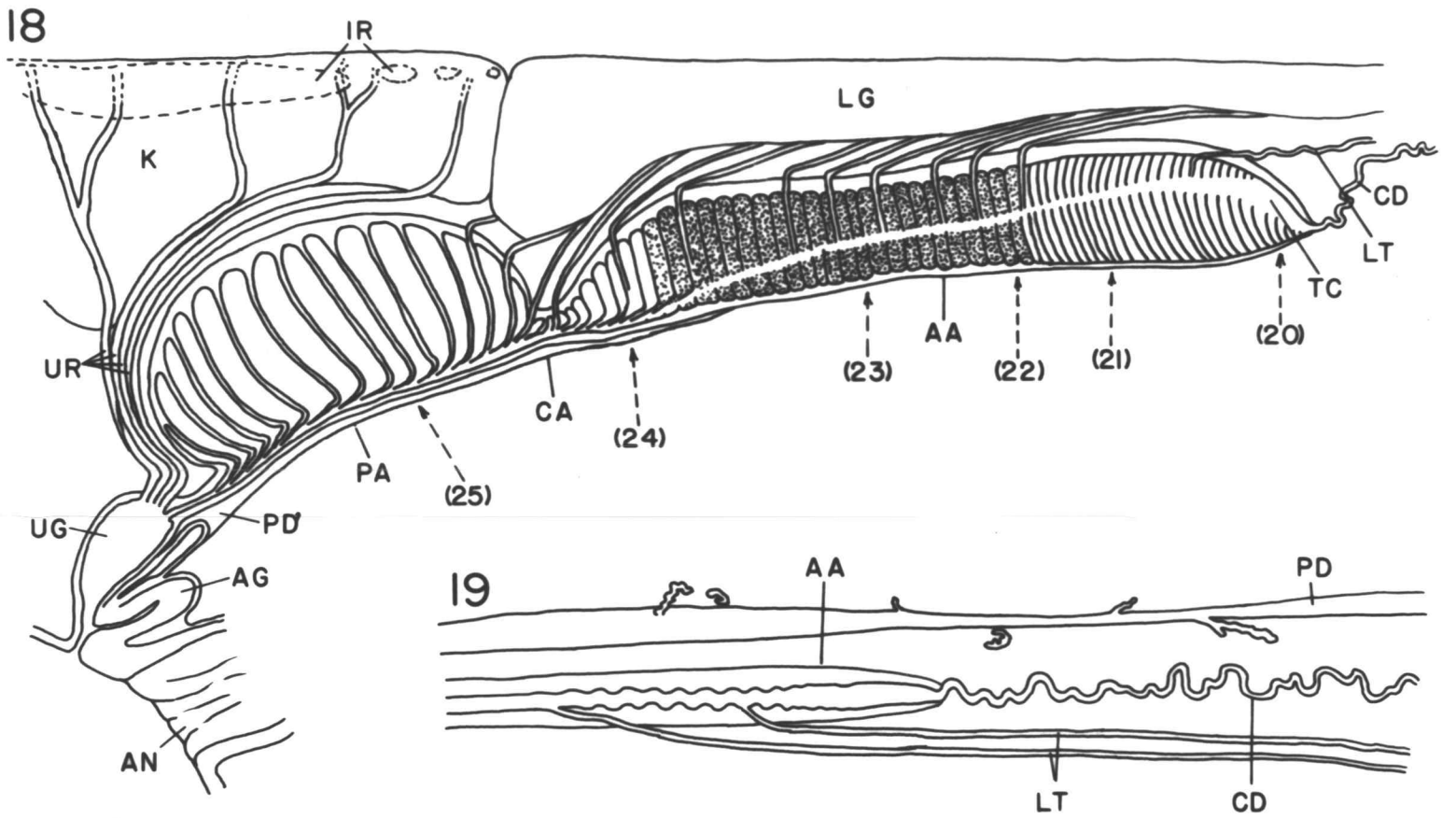
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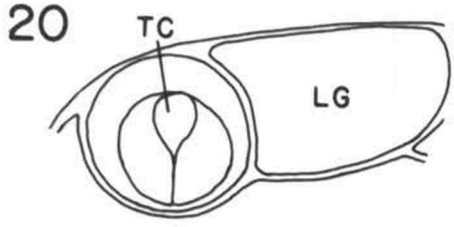
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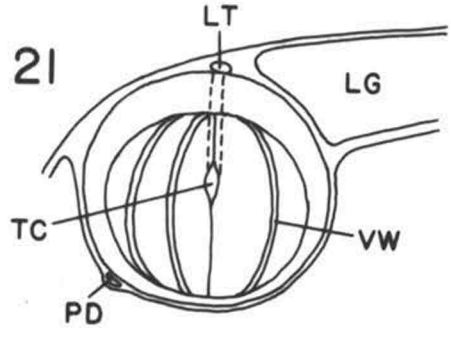
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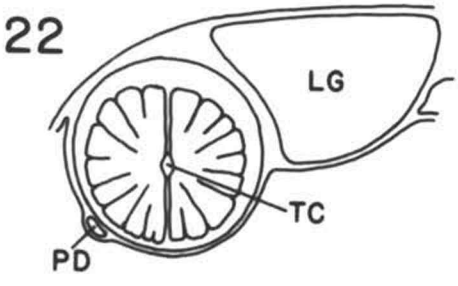
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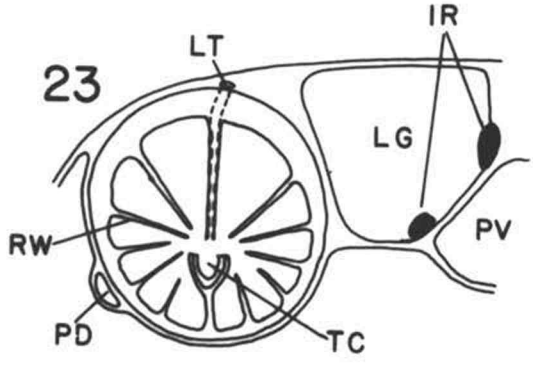
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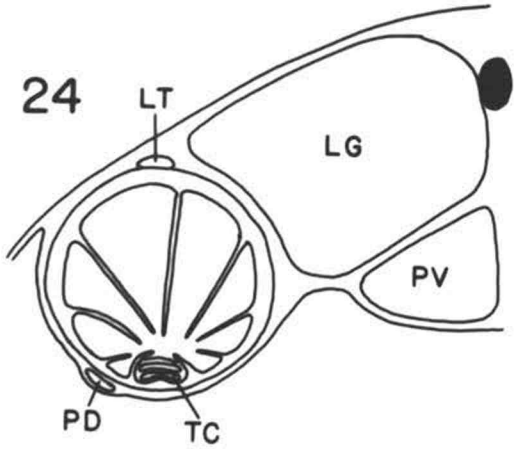
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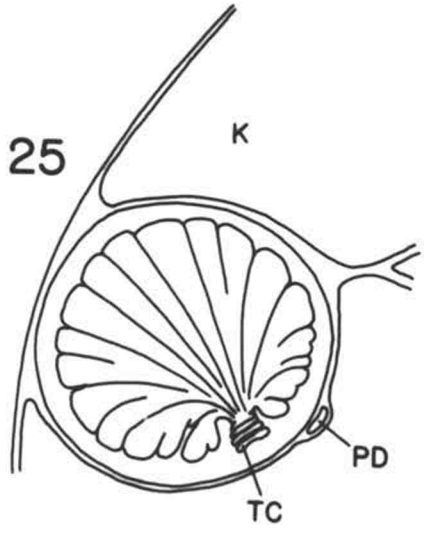
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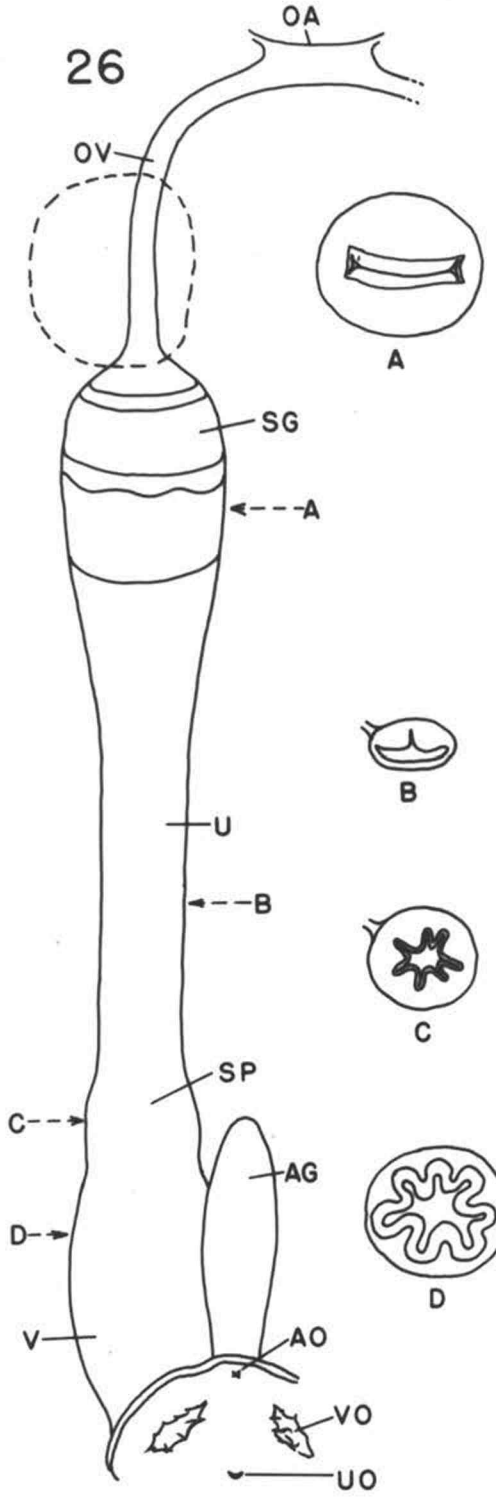

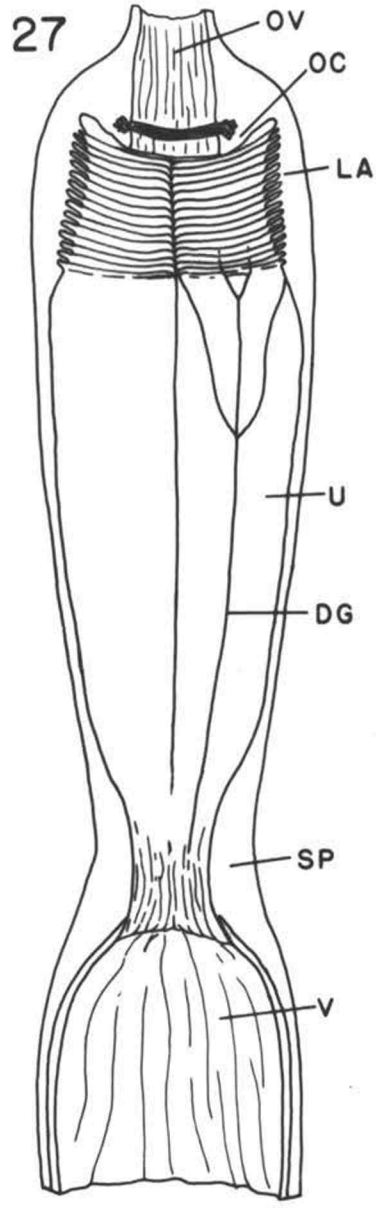

PLATE 7

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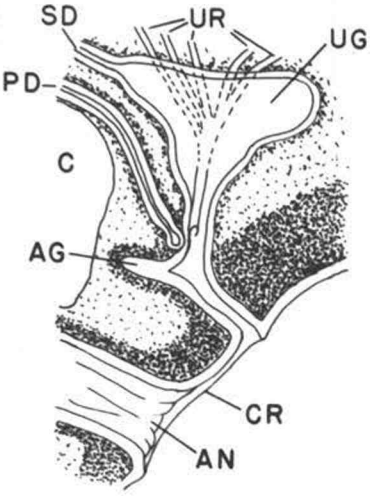

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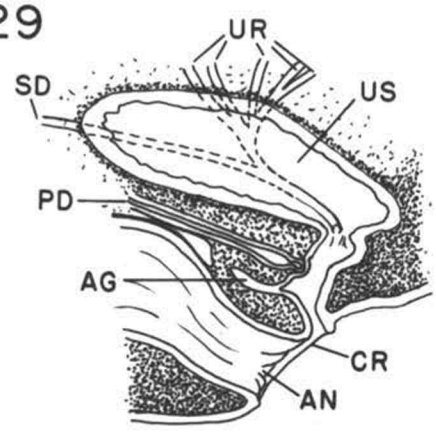

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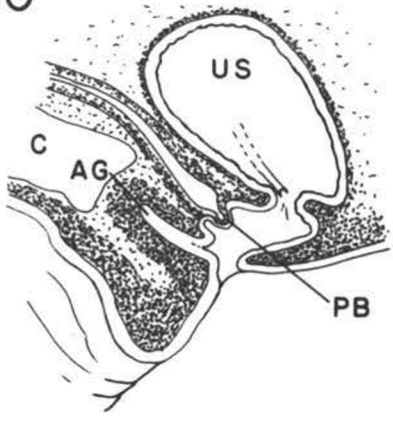

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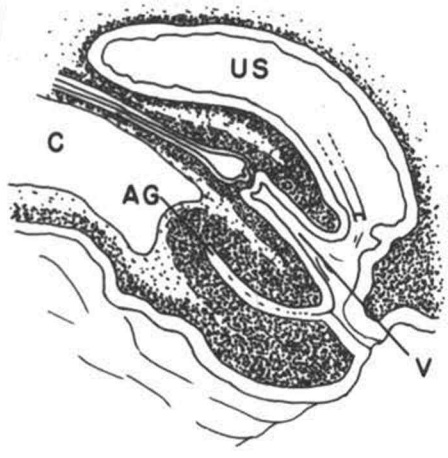

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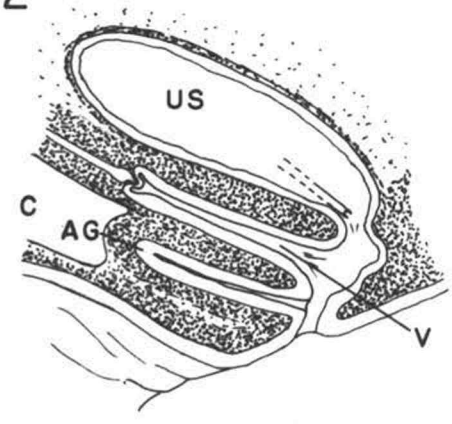

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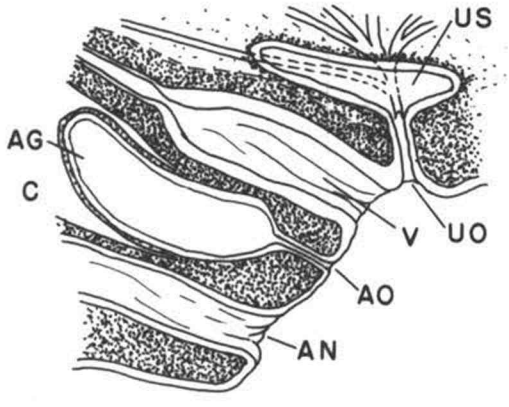

PLATE 8