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The thirty-fifth summer session was a continuation of a program offering research facilities to competent investigators for varying periods from a few days to three months. Subsidies covering living expenses are available to persons of competence, ability and experience.

Dr. Robert J. Schoffman of Griffin High School, Springfield, Illinois, spent his twenty-eighth consecutive season studying the growth rate of various fishes by scale analyses.

Dr. John S. Mackiewicz, Associate Professor of Biology, State University, Albany, New York, investigated the systematics of the Caryophyllidea, i. e. the unsegmented cestodes of fishes.

Eugene Cypert, Mississippi Valley Biologist with the U. S. Fish and Wildlife Service, Samburg, Tennessee,

was a frequent visitor and made an intensive survey of the possible effects of the increase of the curly-leaved pondweed in Reelfoot Lake on the lake and fishing.

The usual groups of summer students and teachers representing summer institutes and research programs visited the Station for brief periods.

The Station continues to be available to investigators during June, July and August although our facilities are limited to living quarters for six people, two boats, collecting equipment, chemicals, glassware and transportation around the lake. Isolated from the usual crowds of picnickers, campers, fishermen and hunters, the general atmosphere is conducive to concentrated research and reflection.

THE UROGENITAL SYSTEM OF THE MALE *NECTURUS MACULOSUS*

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INTRODUCTION

Necturus maculosus maculosus (Rafinesque), commonly called mud puppy or water dog, is the vertebrate used to illustrate the transition from water to land (Adams 1926, Hyman 1942, Eddy, *et al.* 1947). If any salamander may be said to be represented in college comparative anatomy texts as the "typical" urodele, it is *Necturus*. Yet such diversity exists in the six families of urodeles that none can be said to be typical (Baker and Taylor 1964). *Necturus* has probably become accepted as such because of its wide usage in the comparative anatomy laboratory. That usage, rather than implying "typicalness," is the result of an interplay of factors: wide distribution, size, simple organization, and "a curious mixture of specialization and primitiveness with the latter predominant" (Adams 1926). The general purpose of a course in comparative vertebrate anatomy is to learn the general anatomy of

several vertebrates and to understand the relations of the homologous structures in the illustrative forms (Eddy, *et al.* 1947). This study is made with that purpose in mind. It is the purpose of this paper to reiterate and clarify the finer morphological details of the urogenital system of the male *Necturus* with emphasis on the homology and evolution of the kidney, testes, and their associated ducts.

It is necessary to exercise caution in assuming phylogenetic trends from the ontogeny of existing animals (Fox 1963). The task of reconstructing origins and relationships becomes especially difficult in the order Urodela as the investigator must at once contend with the several instances of neotonous individuals. To further complicate phylogeny the perennibranchiate salamanders are incompletely metamorphosed as illustrated in retention of incompletely metamorphosed aortic arches (Baker 1949), gills, tail fin and other larval traits throughout their lives. Thus *Necturus*, *Amphiuma*, *Cryptobranchus* and *Siren* are designated as paedogenetic rather than neotonous; paedogenesis being a

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genetically fixed condition in which tissues fail to respond to the secretions of the thyroid gland that brings about metamorphosis (Goin and Goin 1962). Therefore, if one is to envision definite evolutionary trends among the Amphibia, one must study those systems most likely having the ability to attain maturity in the outwardly immature animal. Obviously phylogenetic relationships in the urodeles cannot be firmly established on those grounds commonly and judiciously used for other vertebrate orders, namely external characteristics, methods of fertilization and miscellaneous details in the degree of development of the nervous and skeletal systems. The urogenital system is in this respect a qualified basis for phylogeny. Several authors (Smith 1959, Smith 1960, Torrey 1962, Romer 1956) affirm this conclusion but fail to give reason for doing so.

Smith (1959) and Romer (1956) have held that vertebrates first evolved as adaptations to fresh water and that evolution is a reflection of changes of the kidney adapting by various means to balance internal with external environs (Smith 1959). Baker and Taylor (1964), observing *Necturus* as semi-larval, and *Amphiuma*, *Siren* and *Cryptobranchus* as incompletely metamorphosed, state that retention of embryonic or larval features by the urogenital system should be expected. However, since the kidney of the urodeles is generally agreed to be an opisthonephros which is an adult structure (Hyman 1942, Moog 1949, Weichert 1956, Smith 1959, Jollie 1962, Romer 1956), this is doubted in the fully developed individual. In addition the genital system is fully matured at sexual maturity (Romer 1956). Thus these systems, excretory and genital, interwoven as a result of embryonic propinquity and subsequent utilization of excretory tubules for genital purposes, may be seen as worthy bases of chordate evolution.

Rarely is it profitable or possible to study the urinary system in development and evolution with disregard to the genital system and vice-versa. Thus the terms urinary (excretory) system and genital (reproductive) system have been united into the urogenital system in the light of their intimate anatomical association in the adult and the marked interrelationships and dependencies during development (Nelsen 1953). Though their functions seem radically different, nature has integrated the two in ontogeny and evolution. The embryonic tissues contributing to the urogenital system lie close to one another in the walls of the dorsal portion of the trunk of the coelomic cavity and are: the nephrostomic plate, coelomic tissue underlying the nephrostomic plate during its development, endodermal lining and surrounding mesoderm at the caudal portion of the digestive tube, the ectoderm of the integument where the urogenital opening occurs, and the primordial germ cells (Huettner 1949, Nelsen 1953, Patten 1964, Balinsky 1960). In the course of development the testis comes to utilize that part of the urinary system embryologically designated as the mesonephros for transport of its products to the exterior, often by means of the mesonephric duct. The abduction of that duct and its accompanying "uriniferous" tubules has caused marked modifications of the urinary system (Romer 1956).

Though the urogenital system is basically similar in the modern Amphibia, these overlappings emphasize the differences in the degree of compactness or elongation of the structures and in the extent to which a segmented arrangement persists (Parsons and Williams 1963). In detail, the more significant morphological aspects of the urogenital system of *Necturus* are, as stated by Baker and Taylor, (1964): "The relation of the kidney to the opisthonephros and metanephric homologues, the origin of the longitudinal canal (Bidder's canal) and its relation to the epididymis, the rudimentary Mullerian duct and the decrease in number of urinary tubules and vasa efferentia with development."

There is a tendency for a confusing overlap of terminology in reference to the kidney. Oftentimes the embryological terms, pronephros, mesonephros, and metanephros, are confusing to the student when discussed in relation to the adult structure. In a short description of the adult animal, one must view the embryology of the urogenital system in retrospect. Along the nephrotomic plate there may be imagined three general areas, in order, anterior to posterior. (1) the pronephros or head kidney, earliest to develop embryologically; (2) the mesonephros or middle kidney, next to develop in the middle portion of the plate; and (3) the metanephros or "true" kidney, occupying the latter one-third of the nephrotomal plate. The pronephros is the kidney of embryo and probably adult of only the myxines (Fig. 1, B). Reptiles, birds and mammals, whose embryos utilize a mesonephros, have a metanephros in the adult state (Fig. 1, D). In these groups, the pronephros is vestigial and nonfunctional in early embryos (Nelsen 1953). Thus the terms pronephros and metanephros are used in reference to both embryo and adult. Mesonephros, on the other hand, is a purely embryological term and should not be confused with the remaining two adult forms, holonephros (term coined by Price (1904), in his discussion of *Bdellostoma*) and opisthonephros (introduced by Kerr 1919).

The holonephric kidney is unknown in any existing vertebrate. It would functionally encompass the full length of the nephrotomic plate (Fig. 1, A). The pronephros and metanephros were probably evolved independently of the opisthonephros from the holonephric basic form. The opisthonephros is the adult kidney of all adult anamniotes except myxinoids, a few teleosts, and, presumably, the ostracoderms. This type is derived directly from the holonephros and may be considered to utilize all those parts of the nephrotomic plate except the pronephric portion (Fig. 1, C). Embryos of animals possessing the opisthonephric kidney have a functional pronephros which is nonfunctional in the adult (Smith 1961).

This study presents a description of the morphology of the urogenital system of the male *Necturus* and attempts to utilize that description to illumine the phylogenetic relation of *Necturus* in reference to other members of the order Urodela.

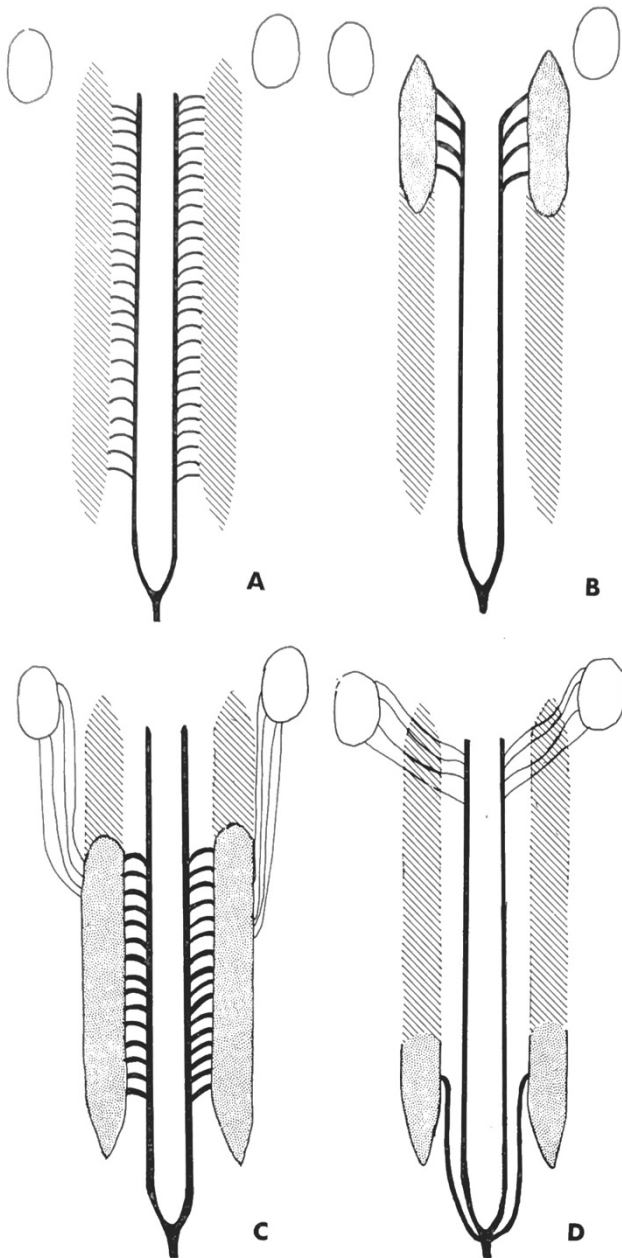


Fig. 1. The various types of kidneys of Vertebrates. (Key: diagonally cross-hatched: non-functional in adults. Stippled: functional in adult.)

A. Archinephros (holonephros). The primitive kidney found in embryo hag-fishes and supposed to represent the ancestral prototype of vertebrate kidney; from different areas of this primitive structure three kidneys, B, C, and D have successfully developed in the evolution of the vertebrates.

B. Pronephros. The functional kidney in the adult hag-fish, embryonic cyclostomes, fishes and amphibians; non-functional and transitional in reptiles, birds and mammals.

C. Opisthonephros. The functional kidney in most fishes, and amphibians; transient functional organ in reptiles, birds and mammals.

D. Adult kidney of reptiles, birds and mammals.

MATERIAL AND METHODS

Necturus maculosus maculosus is a relatively large caudate amphibian, reaching a maximum length of 17 inches (432 mm.). It is rust-brown in color, marked with round spots of blue-black. The male may be distinguished from the female by an external examination

of the vent, enlarged in the male during the breeding season. This male vent is a longitudinal slit, wrinkled at the margins, crossed posteriorly by a crescentric groove and is provided with two nipple-like papillae directed backward. The female vent is a simple slit (Bishop 1943).

Necturus is common in the central and eastern United States from the Gulf Coast to the southern regions of Canada. Its natural habitat is clear water and streams but it may be found in muddy and weed-choked bays, coves, in canals and drainage ditches. Although it may bite on a hook and is frequently caught, our specimens refused food. *Necturus* normally feeds on small aquatic animals and plants. It is easily recognizable because of its three pairs of bushy, blood-red external gills and two pairs of gill slits opening into the pharynx (Eddy, *et al.* 1947).

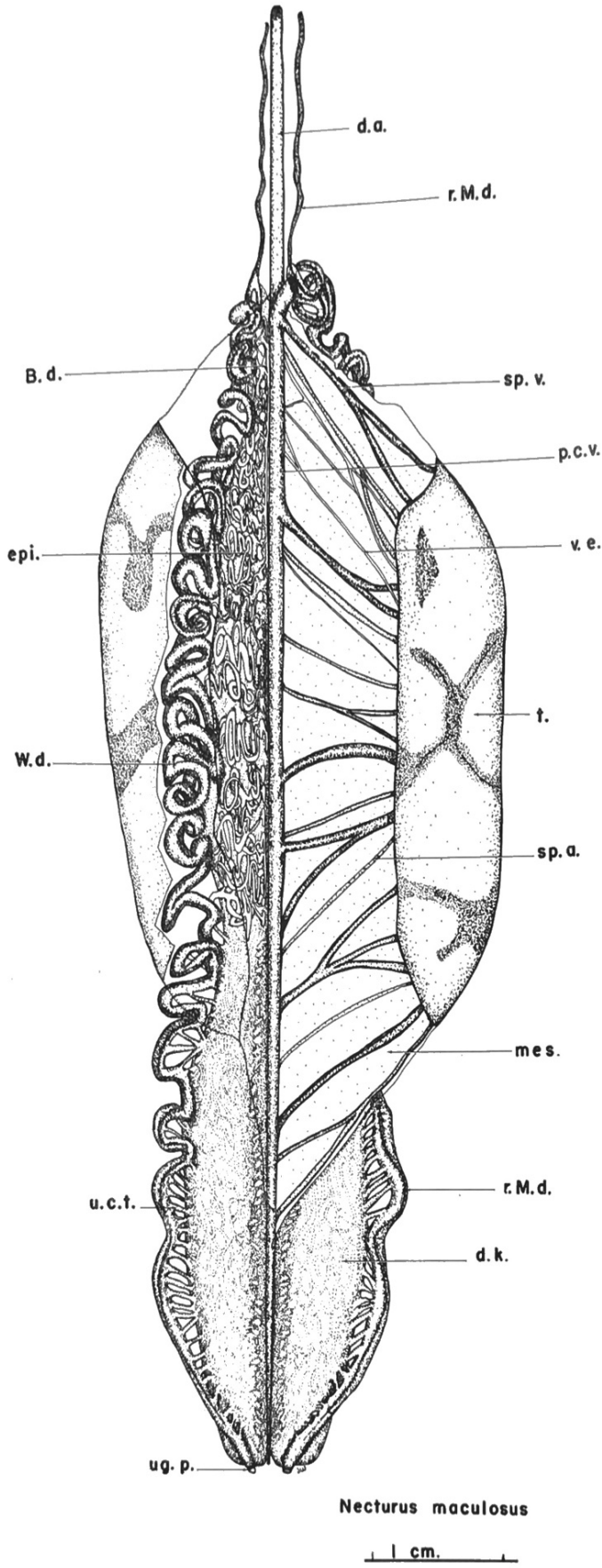
Thirteen live male *Necturus* were obtained from Wisconsin and sacrificed over a period extending from September to mid-February. A total of twenty-nine male urogenital systems were available for study, providing a wide range of observation. These systems were removed *in toto* and fixed in Bouin's solution. Fourteen systems were preserved in 85% ethanol; six, two of which had been injected with red and blue latex, in 6% formalin. Three systems were cleared and preserved in methyl salicylate. Six systems were serially sectioned and stained with standard hematoxylin and erythrosin. The sections were cut 25 micra thick. Studies of these cross sections and reconstructions based on sections were correlated with whole preserved systems. These various preparations complemented one another and greatly assisted determination of the interrelationship of the kidney and testis.

OBSERVATIONS

In general, the testes (Fig. 2) of *Necturus* are ovoid, elongate organs, supported by dorsal mesenteries, the mesorchia (mes.), which are continuous anteriorly with the mesentery that supports the lungs. The testes normally lie slightly ventro-lateral to the opisthonephroi, which are lengthy, occupying approximately 65% of the snout-vent length. The male opisthonephroi are bounded laterally by the convolutions of the Wolffian duct (W. d.) and mesially by the posterior vena cava (p.v.c.) and the dorsal aorta (d.a.). Each opisthonephros is divided into two portions, morphologically and physiologically, the anterior being the epididymis (epi.) the posterior, the definitive kidney (d. k.). Both portions are suspended from the dorsal body wall as they are enveloped by a fold of the visceral peritoneum. This same membrane supports the Wolffian duct (Chase 1923).

The paired, non-lobed testes are asymmetrically situated in the abdominal cavity, ventro-lateral to the opisthonephros. Each is supported from the ventro-medial portion of the opisthonephros by the mesorchium. Since the mesorchium is continuous with the supporting mesentery of the lung, its anterior limit is considered to be the most anterior spermatic vein

d. a.	dorsal aorta
d. c.	distal convolution
d. k.	definitive kidney
ef. epi. d.	efferent epididymal duct
epi.	epididymis
glom.	glomerulus
mes.	mesorchium
p. c.	proximal convolution
p. c. v.	post-caval vein
r. M. d.	rudimentary Mullerian duct
sp. a.	spermatic artery
sp. v.	spermatic vein
t.	testis
u. c. t.	urinary collecting tubule
v. e.	vas efferens
W. d.	Wolffian duct



Necturus maculosus

Fig. 2. The urogenital system of the male *Necturus maculosus maculosus*. The right testis is partially excluded in order that the underlying epididymis and Wolffian duct can be seen.

ABBREVIATIONS USED IN FIGURES 2, 3, and 4

af. epi. d.	afferent epididymal duct
B. d.	Bidder's duct
c. t. c.	central testicular canal

(Sp. v.). The posterior limit is well marked by another spermatic vessel inserting at a point approximately bisecting the definitive kidney. The testes are usually well pigmented, but there is no consistency of testicular markings. They vary from 21 to 54 mm. in length, the average being 38 mm. The width varies from 3 to 14 mm., the average being 8 mm. The size varies with seasonal reproductive activity, the larger size predominating during the winter months.

In the mesorchium may be seen 9 to 11 spermatic arteries (sp. a.) arising from the dorsal aorta and entering the testes. These arteries are about one-half the diameter of the spermatic veins. There are usually five large spermatic veins and 11 to 15 smaller ones emptying into the posterior vena cava. The lateral ducts of the testicular net (rete system), the vasa efferentia (v. e.) are four in number and are also in the mesorchium. The first two vasa efferentia leave the longitudinal collecting canal of the testes (Fig. 3, D c. t. c.) and anastomose before entering the longitudinal collecting duct, Bidder's duct (B. d.). Each is approximately .3 mm. in diameter. The posterior two vasa efferentia are approximately one-half this size. Since these vasa efferentia are so small they are rarely seen in whole mounts. All four vasa efferentia originate from the anterior one-third of the testis, the first being continuous with the longitudinal collecting canal of the testis. This longitudinal canal is continuous throughout the length of the testis. Many smaller tubules radiate into the testis (Fig. 3, c.t.c.). They serve to transport sperm from the ampullae where they mature. Development within an individual ampulla is synchronous. These ampullae are expendable in that as sperm are discharged into the epididymis at maturity, the series of ampullae concerned are resorbed to be replaced by others which have developed meanwhile (Romer 1956). The path of the sperm to the epididymis is thus: from ampulla to central testicular canal to vas efferens, to Bidder's duct, thence to an epididymal nephron.

The opisthonephros of *Necturus* is long and narrow. The length varies from 69 to 95 mm., averaging 81 mm. The width varies from 315 to 7 mm., the average being 4.5 mm. It narrows at the junction of epididymis and definitive kidney. This constriction almost bisects the opisthonephros, the epididymal or genital kidney being slightly longer than the definitive or excretory kidney. The average length of the epididymis is 42 mm. while that of the definitive kidney is 39 mm.

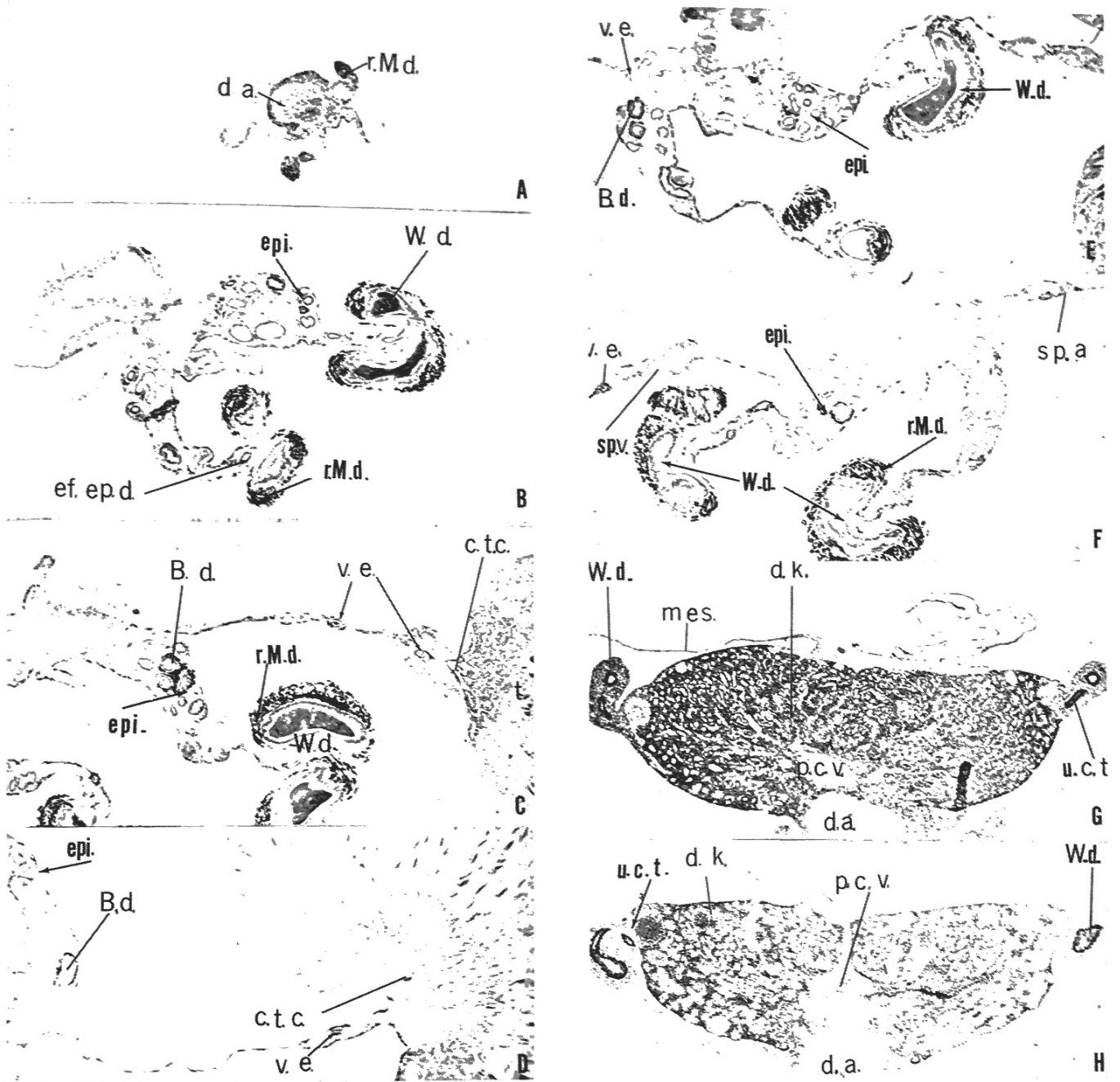


Fig. 3. Typical sections through the urogenital system of the male *Necturus*.

A. A section from the region anterior to the epididymides showing the rudimentary Mullerian ducts with open lumen lateral to the dorsal aorta.

B. A typical section through the anterior portion of the epididymides. Note the Wolffian duct packed with sperm and the efferent epididymal duct which connects the epididymis to the Wolffian duct.

C. A section through the anterior portion of the testis and epididymis. Note the intratesticular network and the two vasa efferentia in the mesorchium. These two vasa efferentia are the most anterior. Contrast their size with the third vasa efferens shown in D.

There are approximately 26 nephrons in each epididymis, each provided with an efferent epididymal duct (ef. epi. d., Figs. 3, B and 4). No nephrostomes were noted in the epididymis. Each nephric unit possesses a glomerulus and there is no anastomosing evi-

D. A typical section through the anterior one-third of the testis. Note the central testicular canal which allows sperm to pass into the vasa efferentia and thus to Bidder's duct in the epididymis.

E. Note the tiny vas efferens in the epididymal mass, just anterior to Bidder's duct. Bidder's duct is discontinuous in this region and the vas efferens opens directly into a glomerulus.

F. A section through the posterior end of the epididymis. Contrast the size of the vas efferens and the spermatic vein.

G. A section through the posterior portion of the definitive kidney of an immature specimen with the opisthonephroi fused.

H. A section through the posterior definitive kidney of a mature specimen. Contrast this with Figure G.

dent but overlapping of units is characteristic. The length of the units is not constant but is approximately 3 mm. Each consists of a glomerulus (glom.) into which an afferent epididymal duct (af. epi. d.) may open in the anterior units, ciliated collecting tubule,

proximal and distal convolutions (p. c. and d. c.) and efferent epididymal duct. The lumen of the tubule in the proximal convolution is much larger than that of the distal end.

Sperm have been observed in the anterior epididymal nephric units. They reach this unit by way of Bidder's duct, the longitudinal collecting canal of the kidney. This duct does not extend the entire length of the epididymis and its extent varies with the amount of degeneration it has undergone (Chase 1923). In its continuous anterior portion, Bidder's duct is demonstrable in whole mounts cleared with methyl salicylate. Ideally, four vasa efferentia would transport sperm into

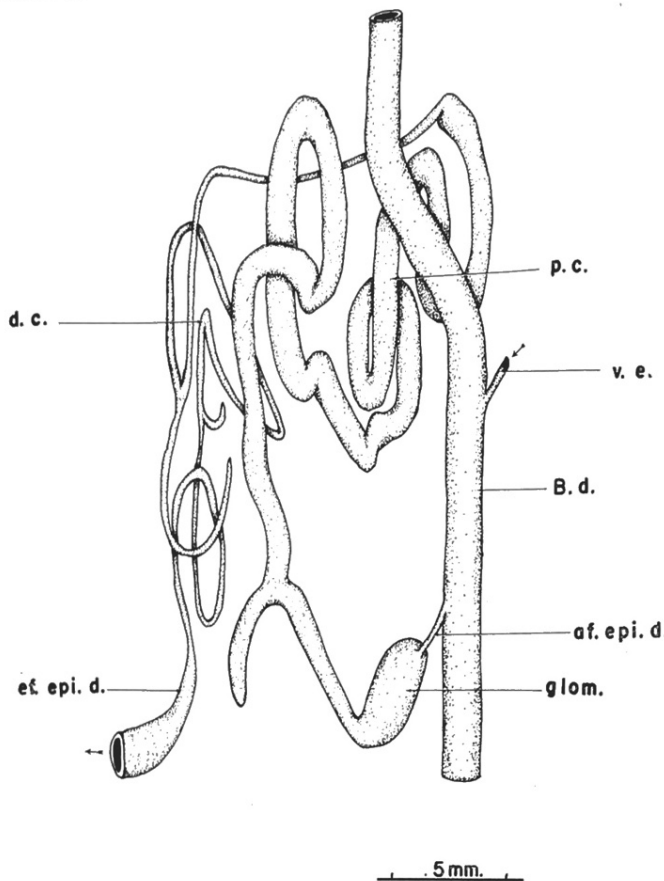


Fig. 4. A reconstruction of an epididymal nephron. Each of these units is about 3 mm. in length. Note the proximity of Bidder's duct to the glomerulus.

this duct and twenty-six would connect with the epididymis. This is, however, not the case. Usually only the first two vasa efferentia have sperm and join the longitudinal duct (Bidder's) at a level somewhat anterior to the level of the anterior tip of the testis (Fig. 2). This duct is usually lacking or non-functional posterior to the entrance of the third vas efferens. Oftentimes there is no afferent epididymal duct to the posterior nephrons and no longitudinal duct posteriorly. Evidently the posterior units do not function in sperm transport.

Clearly, then, to reach the Wolffian duct, the sperm must pass from the longitudinal testicular collecting canal through the vasa efferentia to the longitudinal (Bidder's) duct, through an efferent epididymal duct

to an anterior epididymal unit and finally out of the epididymis to the Wolffian duct via the efferent epididymal duct.

The Wolffian duct of *Necturus* is highly convoluted in the epididymal region, much less so in the anterior portion of the definitive kidney, and is almost straight prior to entrance into the cloaca. Along its lateral edge is a dark stripe of pigment, identifiable as the rudimentary Mullerian duct (Fig. 3 B, C) which continues anteriorly where it may possess a slight lumen (Fig. 3A). There are approximately 70 to 80 tubules opening into the Wolffian duct, about 26 from the epididymis and 40 to 50 from the definitive kidney.

The transition from genital to definitive kidney is gradual and the exact boundary is difficult to ascertain. Since both primary and secondary tubules were observed in the epididymal region of an immature specimen, it is possible that that portion of the adult is formed of only primary tubules, the secondary ones degenerating. Also, as both genital and anterior definitive kidney probably developed from the same embryonic mesonephros, complete and clear division of the two is not expected.

The definitive kidney is much more complex than the epididymis, the majority of the opisthonephric units being concentrated in this locus. On its ventral median surface a longitudinal row of glomeruli may be seen beneath the kidney's peritoneal covering which are not nephrostomes. The nephrostomes are funnel shaped openings into the body cavity of approximately .3 mm in diameter. These feed into the ventral nephric units in close proximity to their glomeruli. Then, contents of the nephrons of the definitive kidney flow into the collecting tubules which in turn empty into the Wolffian duct.

In the adult there is no fusion of the caudal portion of the definitive kidney as noted in *Sirenia* (Willett 1965) but such a fusion is observed in immature specimens.

Omitted from this discussion and the accompanying figures are such accessory structures as fat bodies, adrenal glands, cloacal glands and the urinary bladder.

DISCUSSION

The purpose of this report is to compare and contrast the urogenital system of the proteid salamander, *Necturus*, with that of other urodele families and establish the phylogenetic position of *Necturus* among the urodeles.

Among the six families of Urodela (seven if *Siren* is so considered), there is a great variation in the opisthonephric schema. As a result of this diversity, none should be called typical of the entire order (Baker and Taylor 1964). There is, however, a pattern of diversity which provides a basis for phylogeny of the families.

The Wolffian duct of *Necturus* is truly urogenital combining the morphological and physiological properties of both vas deferens and ureter. Such combination is probably a primitive feature (Willett 1965). In

contrast to *Necturus*, Baker (1965) reports that the Wolffian duct is totally emancipated from urinary function in the salamandrid, *Desmictylus*. Adams (1940) had reported this. The plethodontid, *Gyrinophilus* (Strickland 1966), and the salamandrids, *Taricha* and *Cynops* (Baker 1965) have 4 to 20 primary tubules which converge to form a short ureter which opens with the Wolffian duct into the urogenital sinus. The primary tubules vary from 12 to 34 in the Ambystomidae depending upon the species, arising on the dorso-lateral surface of the definitive kidney and converging into a single short ureter which drains into the cloaca in close proximity to the Wolffian duct (Baker and Taylor 1964). Francis (1934) illustrates a similar condition in *Salvadora salmoustra*. *Cryptobranchus* has 9 to 11 ureters, each of which opens independently into the cloaca (Ratcliff 1965). On the other hand, the Wolffian duct of *Siren* and *Pseudobranchius*, primitive trachystomes, is dual in function like that of *Necturus*. The Wolffian duct of *Necturus* is not enclosed in the epididymal mass as is that of *Siren*, but lies laterally with respect to the opisthonephros.

Along the surface of the Wolffian duct of *Necturus* is the pigmented rudiment of the Mullerian duct. This is a solid cord of cells (von Wittich 1853) which extends to the Wolffian duct and at that level may possess a visible lumen (Chase 1923). This is comparable to the *Plethodontidae* (Strickland 1966) but dissimilar to the *Ambystomidae* (Baker and Taylor 1964) and *Salvanderidae* (Francis 1934, Baker 1965). In the latter two families, the rudimentary Mullerian duct becomes confluent with the Wolffian duct and is embedded in its walls, not visible externally. No such duct is described for *Siren* (Willett 1965) while that of *Cryptobranchius* (Ratcliff 1965) is similar to that of *Ambystoma*. The presence of this duct is not unusual as it would be a remnant of the indifferent stage of development of the embryo (Witschi 1956, Romer 1956). Its origin in embryogeny is, however, a moot point. Most comparative anatomists and embryologists describe it as formed by a longitudinal split of the pronephric duct (Hall 1904, Eaton 1951, Goodrich 1930, Weichert 1959, Romer 1956, Harrison 1964). The pronephric duct is formed in a longitudinal thickening and folding off of the mesoderm of the nephrotomal plate region, growing out freely at its extreme posterior end, joining the cloaca to serve the pronephros and later, as the Wolffian duct, joining the mesonephros (Mollier 1890, Field 1894). Hoffman (from Strickland 1966) contends that in the male only the more anterior portion of the Mullerian duct arises from the pronephric duct. According to Smith (1960) present evidence strongly indicates that the sperm duct and oviduct are of independent origin, arising *de novo* from the dorso-lateral coelomic wall. In any case, the oviduct grows to a functional condition only under the influence of the ovary (Christensen 1929, Adams 1930, Noble 1931). Moog (1944) suggests that the rudimentary Mullerian duct is present in all amphibians but degenerates completely in some adult males.

Bidder (1846) first described the longitudinal collecting duct of the kidney; hence, its name, Bidder's

duct. It is believed to be formed by the longitudinal unstriated of the rete testis (Goodrich 1930) and to function in distributing sperm throughout the epididymis. In earlier description, Baker and Taylor (1964) termed the ducts leading to the epididymis from Bidder's duct the afferent epididymal ducts, those leaving the epididymis to join the Wolffian duct, the efferent epididymal ducts. The afferent epididymal ducts are those termed "transverse trunks" by Hilton and Parker (1932). If there is no degeneracy or fusion of epididymal units, the number of afferent ducts should equal that of the efferent ducts and this is the case in immature *Necturus*, but posterior-anterior degeneracy is characteristic (von Wittich 1853). Baker and Taylor (1964) describe an afferent and efferent duct for each nephric unit of *Ambystoma*, the number of units varying from 8 to 30 according to species. The *Salvanderidae* and *Plethodontidae* show various states of inequality of ducts. *Salvadora* (Francis 1934) has a ratio of 8 afferent epididymal ducts to 18 efferent; *Desmictylus* 4 to 6 afferent to 7 to 11 efferent; *Taricha* 5 to 9 afferent to 14 to 19 efferent; *Cynops* 5 to 6 afferent to 16 efferent (Baker 1965). In the latter three there are an equal number of efferent epididymal ducts and epididymal nephrons. In the plethodontid *Gyrinophilus* (Strickland 1966) no longitudinal duct is observed. This corroborates Spengel's (1876) statement that in *Spelerpes*, *Batrachoseps*, and *Heltholten*, the vasa efferentia pass directly to the renal corpuscles without intercalation of the longitudinal duct. As in *Necturus* the longitudinal duct of *Ambystoma* (Baker and Taylor 1964) and of *Cryptobranchius* (Ratcliff 1965) is in a ventro-medial position in the intertubular epididymal tissue. The longitudinal duct of the *Salvanderidae* is in the mesorchium (Spengel 1876, McCurdy 1931, Gray 1932, Francis 1934, Kindahl 1938, Adams 1940, Baker 1965), while in *Siren* it is absent (Willett 1965).

The division between epididymis and definitive kidney is not well delimited in *Necturus*, *Ambystoma* or *Cryptobranchius*, but the anterior limit of the caudal opisthonephros is distinct in the *Salvanderidae*, *Gyrinophilus* and *Siren*. Such a distinct separation coupled with the development of a short ureter certainly is similar to and foreshadows the amniote condition (Witschi 1956).

Further investigation of these structures in the *Plethodontidae* and *Amphiumidae* is encouraged to complete a comparative study of the entire order and clarify the evolutionary relationships.

PHYLOGENY

Though it is dangerous to attempt to assume phylogenetic relationships from the ontogeny of existing species (Fox 1963), it has been suggested that the *Proteidae*, of which *Necturus* is a genus, and *Sirenidae* are somewhat aberrant groups, the adults of which have retained a number of distinctive characteristics not shared by the other salamanders (Bishop 1943). The modern model is possibly derived from the ancient *Lepospondyli* which appeared in the late Paleozoic Era during the Carboniferous and early Permian Periods,

nearly 400 million years ago (Romer 1956). Goin and Goin (1962) have postulated that there were two main stocks which gave rise to the modern Urodela, the aristopod-neotridian and the microsaurian. They concluded that the Sirenidae are of the former type and should be in a separate order, the Trachystomata. It is probable that *Necturus* is of microsaurian origin, but as the most primitive urodele derived from that group, it has many characteristics in common with *Siren*.

The progressional development of the urogenital systems of the opisthonephros type is expressed in figure 1. The various stages of the evolution of the two functionally distinct ducts, the ureter serving the kidney, and the vas deferens, the testis, have been outlined by Willett (1965). As the animal develops on the evolutionary scale, there is emancipation of the Wolffian duct through a decrease in the number and a degeneracy of connections with the caudal opisthonephros, foreshadowing the amniote condition of a vas deferens whose sole purpose is sperm transport (Smith 1960, Romer 1956). Also in relation to the Wolffian duct, the upward trend is towards fewer afferent epididymal ducts as a result of postero-anterior degeneracy and fusion of the epididymal units. Complementing posterior epididymal degeneration, the number of functional vasa efferentia is reduced in the same manner until finally, only the most anterior portion of the epididymis and rete testis serve to carry sperm to the Wolffian duct (Maschkowzeff 1935).

Of unknown phylogenetic significance in the urodeles is the longitudinal duct which serves as the sperm duct in Teleostei (Kingsley 1917, Goodrich 1930), and as a ureter in the frog *Alytes* (Jollie 1962) and in *Discoglossus* (Noble 1931). Logically, the longitudinal duct would decrease in length as the epididymal units undergo postero-anterior degeneration. There is, however, no evidence of such in *Necturus*. *Siren*, which has no longitudinal duct, is considered more primitive than true urodeles (Willett 1965), but Spengel (1876) and Strickland (1966) note none in the Plethodontidae, the most advanced of all urodeles. The position of the longitudinal duct also varies as previously indicated. Further research is encouraged to reveal the evolutionary significance of this structure.

SUMMARY

The Wolffian duct of *Necturus* is truly urogenital as it serves both urinary and reproductive functions.

All urinary tubules from the definitive kidney open into the Wolffian duct.

Necturus has at most four vasa efferentia which are not readily visible in whole mounts except when filled with sperm.

There are approximately 26 epididymal units in each genital kidney, only the anterior two of which appear functional in sperm transport.

A prominent rudimentary Mullerian duct extends anteriorly from the proximal arch of the Wolffian duct.

On the basis of the male urogenital system, *Necturus* is more primitive than other urodeles and more advanced than the Trachystomata.

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