

THE MALE UROGENITAL SYSTEM IN CRYPTOBRANCHUS*

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The urogenital system of male urodeles has been discussed largely with reference to a "typical" system fashioned after an illustration by Spengel (1876). Though generally the urogenital systems in the six families of urodeles are similar (Parsons and Williams, 1963), detailed observations show that a great diversity exists among specific structures, and it is this diversity which produces new insights into the phylogenetic relationships (Baker and Taylor, 1964).

The holonephric kidney of cyclostomes gave rise to the opisthonephric kidney which develops from the lower two-thirds of the nephrotomal plate, thus differing from the mesonephros which develops from the middle third of the nephrotomal plate (Smith, 1960). The term "opisthonephros" was first used by Kerr (1919). A double functioning opisthonephric kidney (i.e. urinary and reproductive) first appeared in the actinopteran fishes (Romer, 1956; Smith, 1960), and though these same two functions are found also in the opisthonephric kidney of urodeles, differences in structure do occur (Nelsen, 1953; Romer, 1956). The posterior urinary portion of this opisthonephric kidney is termed the "caudal opisthonephros" and precedes the metanephric type of kidney found in reptiles, birds, and mammals (Romer, 1956; Smith, 1960).

The anterior sexual portion has been mentioned without reference to the term "epididymis" (Romer, 1956; Weichert, 1958), while Nelsen (1953) described the epididymis as the body composed of the tortuous epididymal duct and efferent ducts of the testis. Evidently he presumed that the epididymis was nothing more than the highly coiled anterior end of the sperm duct. A detailed study of the epididymis has been made for only three of the urodelan families: the Proteidae, from which Chase (1923) made his well known study on *Necturus maculosus*; the Salamandridae, from which Gray (1932) chose to work out the system of *Triton vulgaris*; and the Ambystomidae, from which Rodgers and Risley (1958) chose *Ambystoma tigrinum* and on which Baker and Taylor (1964) added details for three species.

The function of the Wolffian duct has been assumed to be dual by a number of anatomists (Francis, 1934; Weichert, 1958; Smith, 1960; Goin, 1962; Jollie, 1962); however, Baker and Taylor (1964) showed that the Wolffian duct of ambystomids has only reproductive function and thus the generalizations incorporated in many textbooks are wrong, often misleading and confusing to students.

It is therefore the purpose of this paper to describe

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in detail the urogenital system of the male *Cryptobranchus alleganiensis bishopi* (Grobman) and to further the understanding of the individual differences in the structure of this system among the families of the urodeles. With these differences clearly established, students will be better informed and phylogenetic relationships more precisely developed. The system in *Cryptobranchus* has been described briefly by both Nelsen (1953) and Jollie (1962), but no detailed information is available. Van den Broek (1938) described *Megalobatrachus* which is closely related to *Cryptobranchus*, and by comparison, *Cryptobranchus* and *Megalobatrachus* have been found to be quite similar in structure, though *Megalobatrachus* is considerably larger than *Cryptobranchus*.

MATERIALS AND METHODS

Cryptobranchus alleganiensis bishopi, a large salamander commonly called a Hellbender, is limited in distribution to the cold water rivers and streams of northern Arkansas and southern Missouri. It is a partially metamorphosed amphibian (Baker, 1949; Goin, 1962) which spends its entire life in an aquatic environment.

The animal in its own habitat is well camouflaged, usually brown to slate with reddish spots on its dorsal surface. After being held in captivity, the original vividness fades and the red spots become dark blotches. The specimens for this study were obtained from Spring River, near Mammoth Springs, Arkansas, just below a hydroelectric dam. Each animal was sacrificed as needed. The urogenital system was studied and sketched intact, after which it was removed, fixed in Bouin's solution and sectioned at 25 micra. Five complete sets of serial cross-sections of urogenital systems, varying in size, were studied. One set, cut at 20 micra, was prepared from tissue near the cloaca in order to show urinary tubule entrances. The sections were stained with standard alum-hematoxylin, counterstained with fast green or erythrosin, and mounted permanently with Kleermount. From these, reconstructions were made to verify the evidence obtained from visual studies of the whole system. One set of serial sagittal sections was prepared in the same manner as the cross-sections to determine how the Wolffian duct and urinary tubules opened into the cloaca.

OBSERVATIONS

The testes of *Cryptobranchus* are paired with a medial fold at the point of attachment of the vasa efferentia (Fig. 1,3). The size of the testes varies considerably with the season of the year and the size of

the animal. During the spawning season, which occurs about October, the testes enlarge in all planes while retaining their original proportions, although they do take on a "puffed" appearance. The sperm, developing in the seminiferous tubules, mature as they move centrally toward the lumen where they are transported through the intratesticular network to the vasa efferentia. The entire testes is active in sperm production.

The anterior portion of the opisthonephros, associated with reproductive functions, is known as the epididymis (epi). It is a highly coiled elongated mass of nephric tubules covered by a mesentery and having several ducts on each side. There are no definite nephric units. At some places along the epididymis, however, the number of tubules does decrease to as few as 7 or 8 in a cross-section. At other places, the variation increases to as many as 28 to 30 tubules. Because these variations are not regular, no system of separate nephric units can be postulated. The size of the individual epididymal tubules varies also with no appreciable regularity, though the larger tubes tend to remain toward the side of the epididymis where the Wolffian duct is located (Fig. 2,A). There are smaller tubes intermingled among the larger ones in many places along the length of the epididymis, but in some sections may be found only larger or smaller tubules exclusively. Recognizable nephric units with glomeruli are confined mainly to the definitive kidney, but are found to a lesser extent in the epididymis. Approximately 20 glomeruli were seen in a small specimen while only 6 were found in a larger and more adult specimen.

The lower portion of the opisthonephric kidney, the "caudal opisthonephros", is the definitive kidney and is approximately twice as wide as the epididymis and slightly shorter. The tubules of the definitive kidney are considerably coiled about one another, being for the most part smaller than the smallest epididymal tubules (Fig. 2,C,D). There were no observable differences in the lower and upper portions of the definitive kidney and so no distinction could be made between the possible meso- and metanephric tissues. The definitive kidney terminates posterior to the cloaca and contains functional nephric units for its entire length. There is

ABBREVIATIONS USED IN FIGURES

adrenal gland	ad. gl.
afferent epididymal duct	af. ep. d.
cloaca	cl.
definitive kidney	d. ki.
dorsal aorta	d. a.
efferent epididymal duct	ef. ep. d.
epididymis (genital kidney)	epi.
longitudinal canal	lo. c.
glomerulus	gl.
nephric compile	ne. c.
nephrostome	neph.
postcaval vein	pc. v.
rudimentary Mullerian duct	r. M. d.
testis	tes.
testicular fold	tes. f.
urinary tubules	ur. tu.
urogenital papilla	pt.
vas efferens	va. eff.
Wolffian duct	W. d.

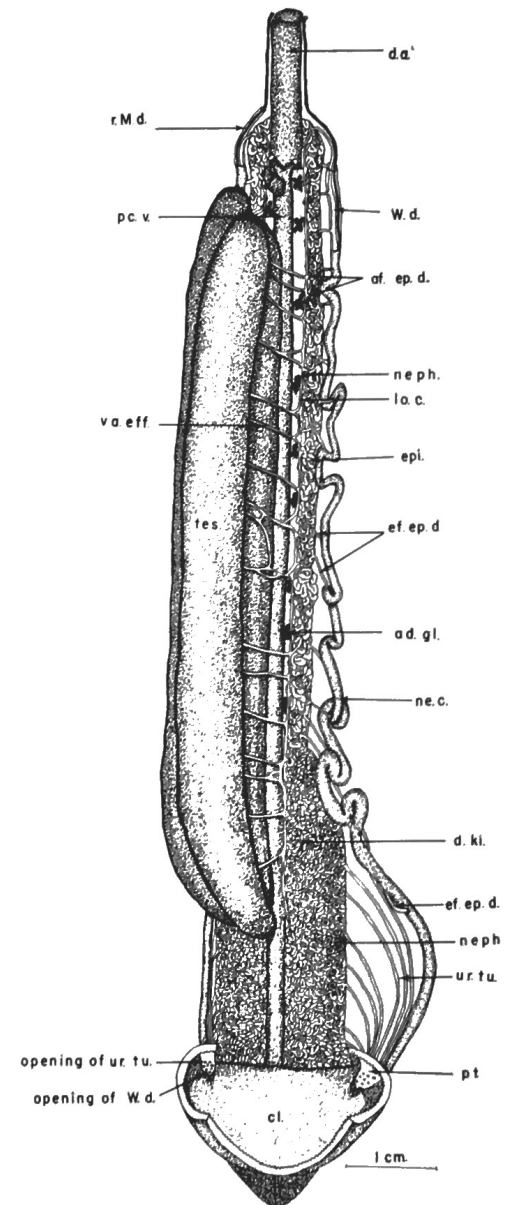


Fig. 1. Urogenital system of the male *Cryptobranchus alleganiensis bishopi*. The left testis is deflected to the right to show the underlying structures.

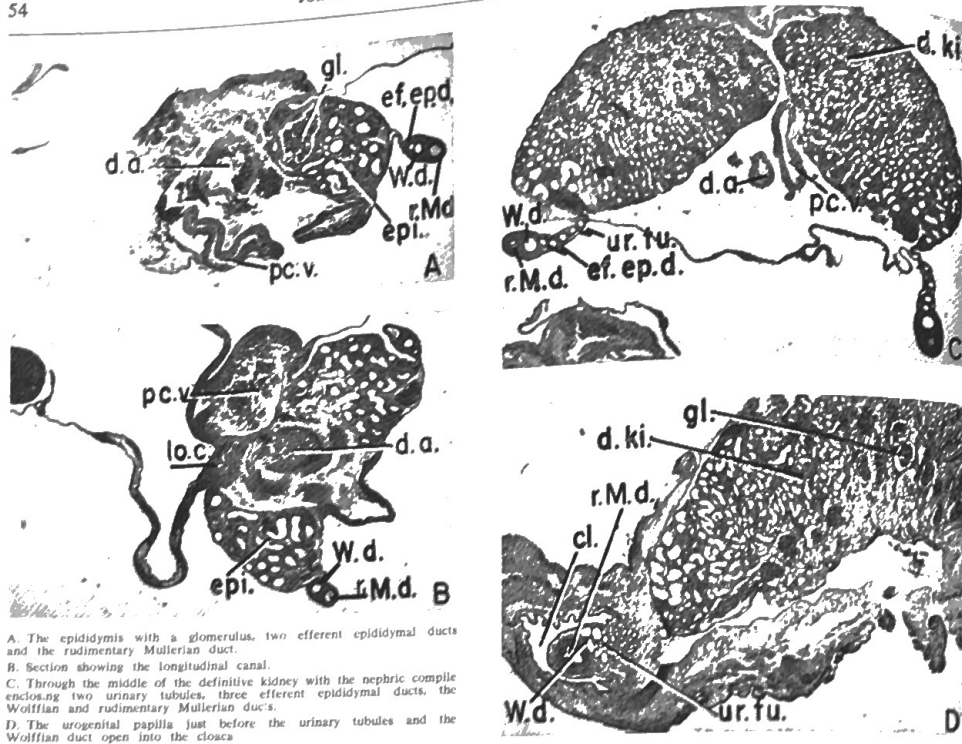


Fig. 2. Representative cross sections of the urogenital system.

also a relatively large number of nephrostomes found in the tissue of the definitive kidney as observed in both whole mounts and cross-sections.

The intratesticular network terminates in the longitudinal duct, a canal running longitudinally along the line of the testicular fold (not to be confused with the longitudinal canal). The sperm leave the testes and enter the vasa efferentia which, along with the longitudinal canal (lo. c.) and afferent epididymal ducts (af. ep.d.), comprise the rete system (testicular network). These tubules (the vasa efferentia) carry the sperm to the longitudinal canal (marginal canal) which lies in close association with the epididymis. The number of vasa efferentia varies from 11 to 17, this variation being found in seven specimens with the average number of tubules being 13. In every case there is a vas efferens at both the extreme anterior and posterior portions of the testis, with the remaining tubes distributed relatively evenly throughout its length. Paralleling each vas efferens from the testicular collecting duct (longitudinal duct) to the longitudinal canal is a spermatic blood vessel. Connecting with the longitudinal canal are the afferent epididymal ducts which traverse the short distance between the longitudinal canal and the epididymis proper. They range in num-

ber from 18 in a small animal to only 10 or 12 in the larger ones. There are no afferent epididymal ducts found connected to the definitive kidney. The efferent epididymal ducts (ef. ep.d.) transport the sperm, which have passed through the maze of epididymal tubes, to the Wolffian duct which then carries them to the cloaca. They differ in number from 11 in an older animal to 16 in a younger animal.

Both the Wolffian duct and the rudimentary Mullerian duct are bound in a common connective tissue so as to make them indistinguishable except in cross-sections. It is for this reason that what appears externally as a duct disconnected from the epididymal mass is often misnamed the Wolffian duct. It is actually this "nephric compile" (ne. c.) containing both the Wolffian duct and the rudimentary Mullerian duct (Fig. 2,C) that receives the efferent epididymal ducts. These ducts enter the Wolffian, contained in the nephric compile, alongside the definitive kidney giving the impression that they are urinary tubules and are therefore often misnamed such. There are no tubes either entering or leaving the rudimentary Mullerian duct.

The urinary tubules (ur. tu.), arising from the tissue of the definitive kidney, enter the cloaca separately from the Wolffian duct, leaving sperm transport as the

sole function of the Wolffian duct. The urinary tubules range in number from 20 in younger specimens to an average of 7 in older ones. Both the urinary tubules

and the Wolffian duct enter the cloaca from a papilla, a finger-like projection extending into the cloaca (Fig. 1). The urinary tubules enter a common connective tissue mass with the Wolffian duct just before its entrance into the papilla (Fig. 2,D), but at no time is there an anastomosis of the tubules. The rudimentary Mullerian duct ends blindly after entering the papilla.

DISCUSSION

With phylogeny as an ultimate goal, morphological studies must include interrelationships of all structures as well as relationships with structures of other animals and probable embryonic origins.

In a discussion of the testes, it will be recalled that the vasa efferentia are distributed evenly along the testicular fold, this condition being very different from that found in the elasmobranchs. The sharks and skates for instance have a rete system restricted entirely to the anterior portion of the testes (Goodrich, 1930). The condition displayed by the Cryptobranchidae has been called primitive (Goodrich, 1930). The vasa efferentia lead to the longitudinal canal whose origin is largely speculative. The vasa efferentia may join near the epididymis to form the canal (Goodrich, 1930), or it may be formed by solid outgrowths of the capsules of the malphigian bodies.

The opisthonephric kidney of urodeles is a fully formed adult kidney rather than merely a developmental stage and it incorporates both mesonephric and metanephric tissue (Moog, 1949; Romer, 1956; Jollie, 1962). The division among the separate portions of the opisthonephros becomes more marked as the animal develops. In younger animals the epididymis and kidney tubules are not found to be so clearly differentiated as in older ones. In a young *Cryptobranchus* two of the efferent epididymal ducts did not enter the Wolffian duct, but rather emptied directly into the cloaca. This evidences the incomplete differentiation of younger animals as opposed to the older Cryptobranchidae, where there were no cases of this type of structural arrangement. In larval stages there is a functional pronephros (Quiring, 1950) but this soon degenerates leaving only remnants in the opisthonephric kidney (Smith, 1960). The Wolffian duct, moreover, is a functional remnant of this early pronephric tissue (Smith, 1960) and remains as the vas deferens. In the line of urodelean development, the Wolffian duct retains only the function of sperm transport and loses all relationship with the urinary processes. This is the line of evolution followed by the amniotes (Goodrich, 1930) as opposed to the higher fishes. In the Chondrostei and Holosteii, the Wolffian duct retains only urinary functions and the longitudinal canal develops as a sperm duct (Goodrich, 1930). In another more isolated case, the nephric duct of the frog, *Alytes*, becomes the vas deferens, and the longitudinal canal loses its connections with the testis and functions as a ureter (Jollie, 1962).

In the urodele, *Necturus*, Chase (1923) described the urinary tubules as opening directly to the Wolffian

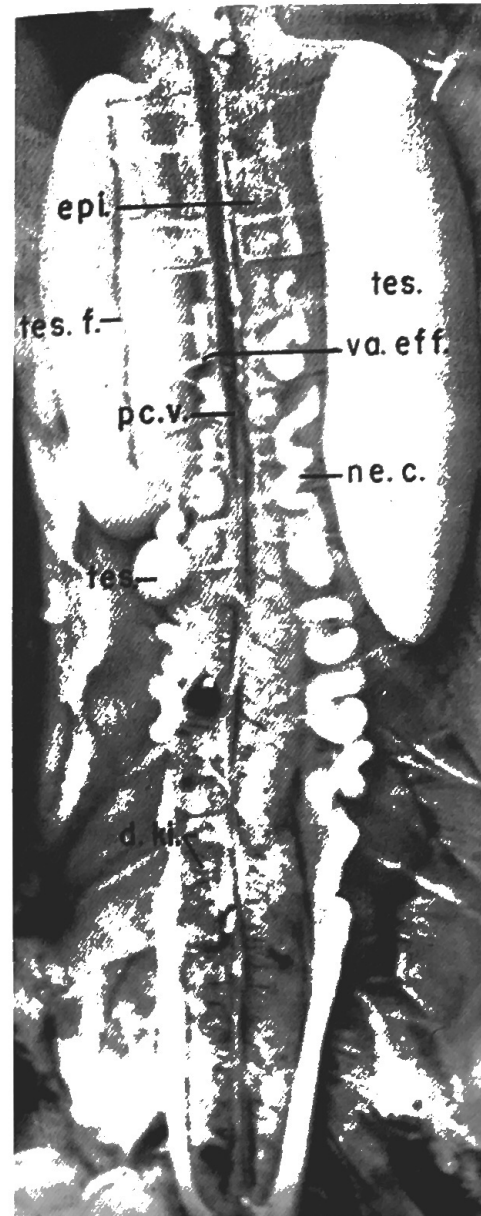


Fig. 3. Photograph of the urogenital system of a typical *Cryptobranchus*.

duct. Furthermore, he found between 70 and 80 tubules in each animal. With this in mind, and with the structural differences between younger and older cryptobranchids clearly established it can be seen that *Cryptobranchus* probably follows a developmental pattern resembling, at some stage, the adult *Necturus*. After differentiation of the opisthonephros, the urinary tubules may connect directly to the Wolffian duct, as in the case of *Necturus*. Upon further development, however, the urinary tubules move posteriorly toward the cloaca and become detached from their connection with the Wolffian duct. This detachment, along with a reduction in number, is found in the adult *Cryptobranchus*. In young *Ambystoma tigrinum* the urinary tubules drain directly into the Wolffian duct. At approximately 8 months, they shift posteriorly and lose all connection with the Wolffian duct. This feature was described as the "caudal migration of attachments" by Rogers and Risley (1938). McCurdy (1931) observed that in *Taricha torosus* only the first 3 or 4 tubules from the definitive kidney empty into the Wolffian duct. The last 18 or 20, he reports, pass to the cloaca where they unite in a short ureter which joins the Wolffian duct as it enters the urogenital papilla. In these two examples we can see possible intermediate stages in the emancipation of the Wolffian duct.

The relative size of the Mullerian duct seems significant as a basis for phylogeny. In *Cryptobranchus* the Mullerian duct of the younger animal is as large or larger than the Wolffian duct (Fig. 2.B). Only in the larger, older animals does the Mullerian duct degenerate to any great extent. In animals prepared in October, the Wolffian duct was enlarged considerably with a corresponding decrease in the size of the Mullerian duct. This is due to the fact that sperm are released at this time for mating and thus stimulate the expansion of the Wolffian duct. In the plethodon, *Gyrinophilus danielsi dunnii*, the Mullerian duct is only a pigmented cord paralleling the Wolffian duct and at no point is it open (Strickland, 1963). This feature is a striking contrast for two animals whose urogenital systems have been represented by one "typical" diagram in many texts (Kent, 1954; Romer, 1956; Weichert, 1958; Goin, 1962), and one which establishes further the degree of differentiation between the members of the order Urodela.

The vasa efferentia are the only ducts which are not reduced in number during the developmental period of *Cryptobranchus*. Though there is a variation from animal to animal, the age does not seem to be the controlling factor as animals of various ages often contain an equal number of vasa efferentia.

Jollie (1962) has reported that in *Cryptobranchus* a few urinary tubules drain directly into the cloaca, while others pass into the Wolffian duct indicating an incomplete condition. In each specimen studied it was found that one or two of the efferent ducts paralleled the nephric compile for a great distance and entered it at a point midway or more along the length of the definitive kidney (Fig. 1). For this reason, Jollie may

have concluded that these efferent epididymal ducts were actually urinary tubules entering the Wolffian duct.

The phylogeny of the urodeles is extremely difficult to determine because of the fact that several of the urodelan families do not reach an adult stage (Baker and Taylor, 1946). This is shown by *Necturus* which remains in a larval form, and by *Amphiuma*, *Cryptobranchus*, and *Siren* which only partially metamorphose (Baker, 1949). The order Urodela, however, is known to be more closely related to the Dipnoi than to the Anura (Kindahl, 1938) on the basis of pronephric tubule number and position, but beyond this relationship, the relations among the members of the urodelan order is uncertain.

Baker (1949) described the development of the aortic arches and the degree of metamorphosis among urodeles and found the order of increased development to be *Siren*, *Cryptobranchus*, *Necturus*, *Amphiuma*. Boyden and Noble had used these same four animals for serological tests (Dodson, 1952). The blood from *Necturus* was used as an antigen and injected into a guinea pig. The antibody produced was then mixed with a small measured amount of blood from each of the four animals and the amounts of precipitate compared. From this test they concluded that *Necturus*, *Siren*, and *Amphiuma* were more closely related to each other than to *Cryptobranchus*. Goin (1962), however, on the basis of the tail vertebral structure, lack of teeth, and the lack of a pelvic girdle has put *Siren* in an order altogether removed from that of the urodeles. Work being done currently on the morphology of the urogenital system of the Sirenidae substantiates this idea, the evidence being the absence of any form of Mullerian duct or longitudinal canal, as well as the kidney and epididymal structures. With these facts it can be seen that much contradiction exists as to the degree of relationship of these four genera. *Siren*, *Amphiuma*, and *Necturus* are shown to be closely related by one source, while another removes *Siren* entirely from its connection with the urodeles. If morphology were to be used as the principal source for explaining phylogenetic relations, one problem is whether *Necturus*, which does not metamorphose, should be considered in its existing adult stage or whether speculation should be made as to what degree of development it would attain should metamorphosis occur. However, even though metamorphosis does not occur in *Necturus*, the urogenital system must be completely developed as it serves a completely adult function, that of reproduction. The problem of the non-metamorphosed animal is thus removed when studying the morphology of the urogenital system. The urogenital system of *Cryptobranchus* is surely more advanced than that of the adult *Necturus* on the basis of the emancipation of the Wolffian duct. If the serological tests of Boyden and Noble can be considered as conclusive, *Cryptobranchus*, whose blood differs greatly from that of the other three animals, must also be more advanced than *Siren* or *Amphiuma*.

Studies of the *Siren* urogenital system complement this theory, but nothing conclusive can be stated until *Amphiuma* has been studied and described. There is still some question as to the validity of this conclusion, however, for two reasons. *Necturus* has cloacal glands and spermatophores which imply internal fertilization, a practice not carried on by *Cryptobranchus* (Bishop, 1943). Also, the relatively large number of nephrostomes found in the *Cryptobranchus* kidney points to a more primitive condition.

SUMMARY

Both the Wolffian duct and the rudimentary Mullerian duct are contained in a common nephric compile which parallels the opisthonephros. The Wolffian duct is associated exclusively with sperm transport and is therefore a true vas deferens. Nitrogenous waste is removed by way of several urinary tubules which enter independently into the cloaca through a urinary papilla. The number and size of these tubules is determined by the degree of development of the animal.

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LITERATURE CITED

- Baker, C. L. 1949. The comparative anatomy of the aortic arches of the urodeles and their relation to respiration and degree of metamorphosis. *Rep. of Reelfoot Lake Biol. Stat.*, 13: 12-40. (Reprinted in *Jour. Tenn. Acad. Sci.*, 24:12-40.)
- Baker, C. L. and W. W. Taylor, Jr. The urogenital system of the male *Ambystoma*. *Rep. of Reelfoot Lake Biol. Stat.*, 28: 1-9. (Reprinted in *Jour. Tenn. Acad. Sci.*, 39:1-9.)
- Bishop, S. C. 1943. *Handbook of salamanders*. Comstock Pub. Co. Inc., Ithaca, N. Y. 555 pp.
- Chase, S. W. 1923. The mesonephros and urogenital ducts of *Necturus maculosus*, Rafinesque. *Jour. of Morph.*, 37:457-532.

NEWS OF TENNESSEE SCIENCE

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Dr. Stelmon E. Bennett, associate professor of entomology, has been appointed head of the newly created Department of Agricultural Biology in the University of Tennessee College of Agriculture. In announcing the appointment, Dean of Agriculture Webster Pendergrass said that the new department combines the activities of the former departments of entomology and plant pathology in the Agricultural Experiment Station. Responsibilities of the new department will include, along with a limited amount of teaching, research dealing with various insects and plant diseases that affect the economic picture of the state's agriculture.

- Dodson, Edward O. 1952. *A textbook of evolution*. W. B. Saunders Company, Phila. 419 pp.
- Francis, E. T. B. 1934. *The anatomy of the salamander*. Oxford Univ. Press, London. 381 pp.
- Goin, C. J. and O. B. Goin. 1962. *Introduction to herpetology*. W. H. Freeman & Co., San Francisco. 341 pp.
- Goodrich, E. S. 1930. *Studies on the structure and development of vertebrates. Vol. II. The MacMillan Co.*, N.Y.C. 351 pp.
- Gray, Peter. 1932. The development of the amphibian kidney. Part II. The development of the kidney of *Triton vulgaris* and a comparison of this form with *Rana temporaria*. *Quart. Jour. Micr. Sci.*, 75 N.S. 425-465.
- Jollie, Malcolm. 1962. *Chordate morphology*. Reinhold Publ. Corp., N.Y.C. 478 pp.
- Kent, G. C. 1954. *Comparative anatomy of the vertebrates*. Blakiston Co., N.Y.C. 530 pp.
- Kerr, J. G. 1919. *Textbook of embryology. Vol. 2 Vertebrata*. MacMillan Co., London.
- Kindahl, M. 1938. Zur entwicklung der exkretionsorgane von Dipnoern und Amphibien mit anmerkungen bezuglich Ganoiden und Teleostier. *Acta Zool. (Stockholm)*, 19:1-190.
- Moog, Florence. 1949. *Structure and Development of the vertebrates*. Prentice-Hall, Inc., N.Y.C. 170 pp.
- McCurdy, H. M. 1931. Development of the sex organs in *Triturus torosus*. *Amer. J. Anat.*, 47:367-403.
- Nelson, O. E. 1953. *Comparative embryology of the vertebrates*. The Blakiston Co., N.Y.C. 982 pp.
- Parsons, T. S. and E. E. Williams, 1963. The relationships of the modern Amphibia. A re-examination. *Quart. Rev. of Biol.*, 38:26-53.
- Quiring, D. P. 1950. *Functional anatomy of the vertebrates*. McGraw-Hill Book Co., N.Y.C. 624 pp.
- Rodgers, L. T. and P. L. Risley. 1938. Sexual differentiation of urogenital ducts of *Ambystoma tigrinum*. *Jour. of Morph.* 63:119-141.
- Romer, A. S. 1956. *The vertebrate body*. W. B. Saunders Co., Phila. 486 pp.
- Smith, H. M. 1960. *Evolution of chordate structure*. Holt, Rinehart and Winston, Inc., N.Y.C. 529 pp.
- Spengel, J. W. 1876. *Urogenitalsystem der Amphibien*. *Arb. d. Zool. Zoot. Inst. Wurzburg* 3:1-114.
- Strickland, Priscilla L. 1963. The urogenital system of the urodele; *Gyrinophilus danielsi dunnii*. (Honors thesis unpublished) Library, Southwestern at Memphis.
- Van den Broek (A. J. P.) 1933. Gonaden und Ausfuhrungsgaenge in: *Handbuch der vergleichenden Anatomie der Wirbeltiere*. de Bolck, Goppert, Kallius et Lubosch. T.6. Urban u. Schwartzberg, Berlin.
- Weichert, C. K. 1958. *Anatomy of the vertebrates* (2nd ed.). McGraw-Hill Book Co., Inc. N.Y.C. 899 pp.

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