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THE ANATOMY AND EMBRYOLOGY OF THE HEMIPENIS OF *LAMPROPELTIS, DIADOPHIS* AND *THAMNOPHIS* AND THEIR VALUE AS CRITERIA OF RELATIONSHIP IN THE FAMILY COLUBRIDAE*

HUGH CLARK

INTRODUCTION

Purpose of the Investigation

Evidence for a natural relationship among species, genera and higher groups of snakes has come principally from studies in comparative anatomy and geographical distribution. Fossil remains have yielded very little toward the solution of problems of interest to the taxonomic herpetologist, and genetic work with snakes has only recently been undertaken.

The hemipenis has been used extensively in the classification of snakes. The present work was undertaken with the hope that possibly a comparative study of the developmental features of this organ might throw some light on the relationship among certain sub-families of the Colubridae which could not be ascertained by a study of its superficial characters alone. Even though this hope might not be realized it was felt that a comparative study of the morphology and development of the hemipenis in itself was highly desirable since no such work of a comprehensive nature had previously been undertaken.

GENERAL DESCRIPTION OF THE HEMIPENIS

The anatomical relationships of the hemipenis are shown in Plate I, both in the everted and resting positions. It is a paired organ, each member of which lies at the base of the tail on the ventral side of the body. It is held in place by two longitudinal muscles, the large retractor and the small retractor; the latter lies dorsomesial to the former in the anterior end of the tail. The hemipenis, the retractor muscles and the scent glands, which lie dorsally and slightly mesially to the small retractors, are surrounded by a circumferential layer of muscles, the propulsors. These muscles, originating on the vertebrae and intervertebral tissue and inserting on the midventral connective tissue, form a circular band from the anus to a point just caudal to the origin of the large retractors. They seem to be used in squeezing blood out of the tail into the hemipenis and also in forcing the secretion from the scent glands. The entire complex just outlined is covered by a layer of parietal muscles divided longitudinally into three groups. Since these do not form an integral part of the hemipenial apparatus a discussion of them is not included.

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Each hemipenis is supplied with blood by a pudendic artery arising from the dorsal aorta and by an incurrent system of veins originating in the tail. It is drained by an excurrent penial vein which anastomoses with its fellow from the other side, forming a common sinus which empties into the renal portal veins either by a single or a paired vessel. The organ is innervated by a large pudendic nerve composed of both sympathetic and purely spinal elements.

The external form of the hemipenis varies in different species; it may be in the form of a simple cylinder, or it may be bifurcate, or even quadrifurcate as in the Cuban boid, *Tropidophis.* Those of the species used in this investigation are shown in Plate II, Fig. 2. Along its ventral and lateral surface a groove, the sulcus spermaticus, extends from the cloaca to its tip. This groove may be forked or single; if forked, it may vary in its degree of bifurcation, often independently of the division of the hemipenis as a whole.

Besides the sulcus, the surface of the protruded hemipenis possesses a number of other features. A large part of the organ may, in some forms, be covered by transverse flounces, as in the Boidae. These flounces are, in most of the higher families, replaced by spines, which are usually largest near the base of the organ and decrease in size toward the apex. The apex may be naked or covered with minute spines or with small superficial pockets, the so-called calyces. Some of the calyces may be fringed, and the fringes may be spinose, or the lips of the calyces may be fused to form a free ring around the tip of the organ, as in the capitate type. Occasionally the calyces may be modified to form a collar-like structure, the hemipenial disc, which is present in certain xenodontine snakes. Between the proximal row of spines and the cloaca there is a naked connecting area. For purposes of description this region will be called the base, the region bearing the larger spines the middle, and the most distal part, which is variously ornamented, the apex or tip.

THE FAMILY COLUBRIDAE

The classification of the subfamilies of the Colubridae by Cope (1895) was based primarily on hemipenial structures, although his conclusions were corroborated by a study of other characters, such as the vertebrae and dentition. Eight subfamilies were described by Cope. Dunn (1928) felt that the character of grooved teeth was given too much weight and therefore proposed a simplification of Cope's system, in which he recognized four sub-families of the Colubridae, in the Western Hemisphere. These are Xenodontinae ,the Colubrinae, the Natricinae and the Sibynophiinae. The last named, represented by a single genus, having species in Central America, Southern Asia and Madagascar, is omitted from this study.

The xenodontine hemipenis (Plate II, Fig. 2) is characterized by a bifurcate sulcus and calyculate tip, the natricine type by a single sulcus and spiny tip, and the colubrine type by a single sulcus and calyculate tip. The Xenodontinae constitute the predominant group of the Colubridae in South and Central America. A few relict xenodontine genera exist in North America, but the Colubrinae and

Natricinae comprise the most abundant groups of colubrid snakes in this continent. Colubrine genera of South America are mainly of the racer group. Natricines have invaded Central America only slightly and are not found in South America at all.

The relegation of the split-sulcused forms to the southern continent in the New World is paralleled in the Old World, where the forms with a double (bifurcate) sulcus, constituting the "lycodontine" type, are most abundant in Africa and Southern Asia. (Charles M. Bogert (1940) of the American Museum of Natural History has made a study of the colubrid snakes of Africa and concludes that no true subfamily grouping can be recognized. The term *lycodontine* will, however, be used to refer to the split-sulcused type of hemipenis with apical spines. The subfamily name "Lycodontinae" is used in the discussion for convenience in comparing groups and refers to those snakes having this type of hemipenis.) However, in contrast to the calyculate or disced tip of the xenodontine hemipenis, the lycodontine is spinous. Continuing the parallel, the colubrines and natricines are most prevalent in Eurasia, just as they are in North America. The split-sulcused forms ar therefore tropical, an dthe single-sulcused forms holarctic. Superimposed upon the bifurcate character are the two alternative characters, calyculate and spiny tip, which also have significant taxonomic value. These problems will be given further consideration later. The relationships are shown in the following table:

Table I

Distribution of Hemipenial Types

Hemipenial Characters		Type	Area of Dominance			
			Eurasia	N. America	S. America	Africa
Sulcus Single	Tip calyculate	Colubrine	x	x		
	Tip spinous	Natricine	x	x		
Sulcus Split	Tip calyculate	Xenodontine			x	
	Tip spinous	Lycodontine				x

NATURAL RELATIONSHIPS AMONG SNAKES

In seeking phyletic relationship among groups of animals through a comparison of their morphological characters, nonadaptive structures are the most significant because others may have resulted from evolutionary convergence. Adaptive modifications may be useful, particularly in a group whose range is sharply delineated, but similar conditions in widely separated geographical regions might enable comparable or even identical changes in a character to appear and persist, thus giving a false indication of kinship.

Comparative anatomy and geographical distribution have been the principal sources of evidence for natural relationships among species, genera and higher groups of snakes. Fossil remains have yielded very little toward the solution of problems of interest to the taxonomic herpetologist, and genetic work with snakes has only recently been undertaken.

The skull might be expected to offer some traits of taxonomic value, but a detailed analysis of cranial morphology of more than 70 colubrid genera (unpublished data of Mr. T. P. Haines) reveals a great amount of adaptive modification and many cases of apparently convergent evolution. For this reason it would be unwise to assign any considerable phylogenetic significance to variation in skull pattern in small groups, such as the subfamilies of the Colubridae. The most valuable osteological character, although it also shows variations even within a single species (Bogert), is the posterior abdominal hypapophyses, characteristic of the Natricinae.

Accessory sex organs have been used frequently in classification as an indication both of taxonomic differences and phylogenetic kinship. The value of hemipenial characters in snakes has been recognized for some time, though Bogert challenges the propriety of classification on this basis alone. Although some intermediate types are available the hemipenes of the American subfamilies of the Colubridae are so different that there is seemingly little possibility of determining the true relationships in the family by examination of its superficial adult morphology alone.

Since the hemipenis has been used so extensively in snake classification it was felt that an analysis of its structure and development, both superficial and deep, might add some facts pertinent to ophidian taxonomy. It was understood from the beginning that the embryonic history is in no complete sense a repetition of the phylogenetic but it was hoped that some leads might be discovered which would bring one closer to a classification in accordance with a natural relationship among these groups of snakes.

Conceivably the single sulcus type (Table I) may have been the original one which became bifurcate during the process of evolution; if this were true the Xenodontinae might, if judged by this character alone, have been derived from the Colubrinae. On the other hand, if the sulcus arose in bifurcate form and secondarily became single, then an origin of the Colubrinae from the Xenodontinae would be suggested. Again, if calyces arose by a fusion of small spines phylogenetically then both of the calyculate types might have been derived from a previously spiny-tipped ancestor; but if the spiny-tipped hemipenis arose in calyculate form, then the natricines might be supposed to have been derived from the colubrines. It is recognized, of course, that any of the changes, here suggested as phylogenetic, may have taken place independently in different groups. In addition to these superficial characters, the anatomy and development of the vascular supply, innervation and musculature of the hemipenis were also studied in each of the subfamilies.

REVIEW OF LITERATURE

Previous comparative studies have been made in an attempt to homologize the hemipenis of the plagiotreme reptiles with the penis of cyclotremes, birds and mammals. A comprehensive review of this subject was published by Gerhardt (1909). Johannes Muller (1836) recognized the hemipenis as a sex organ and described it as an eversible sac with retractor muscles inserted at its tip. Leydig (1855) described the hemipenial spines underneath the epidermis as of a bony nature.

The embryonic hemipenis of *Natrix natrix* L. was described and figured by Rathke (1839), but his study was confined merely to the external form. The internal structure was studied by Unterhössel (1902) in his search for additional data on the comparative anatomy of the genitalia. He discovered a second retractor muscle and observed the formation of the sulcus and spines but a study of the vascular supply was omitted because of paucity of material. His main contribution was the observation that each member of the hemipenial pair arose as a solid, conical outgrowth in the region of the anus, with which it later retained a functional connection even when it was in a resting position in the tail of the adult.

After a lapse of about thirty years there appeared a comprehensive study by Wöpke (1931) on the genital organs of the lizard, *Lacerta agilis* L. Beuchelt (1936) published a lengthy treatise on the structure, function and development of the hemipenis in the European *Vipera berus* L. and *Natrix natrix* L. Comparison of the present findings with those of Beuchelt will be made in appropriate places.

MATERIALS AND METHODS

Methods of Obtaining Series

The xenodontine and colubrine representatives in this study are both ovoviviparous forms in which the embyro has reached only an early stage of development when the eggs are laid. Embryonic series were easily obtained from laboratory snakes by transferring the eggs, immediately after they were laid, to damp rotted wood in covered containers.

Successive stages in the series were obtained by removal of embryos at arbitrary intervals. The males are easily identified, since the hemipenis is in an everted position from its origin until the time of hatching. All the eggs of a clutch were kept together at the same temperature to insure a uniform rate of development and therefore, predictably different growth stages.

With regard to the garter snakes, which give birth to living young, it appeared at first necessary to kill an animal each time an embryonic stage was desired. In order to obviate this waste of material, embryos were removed from the mother by a series of Caesarean operations. The technique of these operations has been described elsewhere (Clark, 1937).

Histological Technique

All the embryos were fixed in Bouin's fluid, transferred to 70% alcohol for a brief period and stored in 82% alcohol. The garter snake embryos were embedded in celloidin and paraffin, but this method was found to have no advantage over the regular paraffin method, which was therefore used in all other series. Sections were cut uniformly at 10 micra.

Pasini's stain was used for the older Thamnophis embryos. It consists of a hematoxylin nuclear stain and an orcein-wasserblau cytoplasmic stain. It was used to differentiate the advance of keratinization and as a test for the horny nature of the spines. All of the xenodontine and colubrine material, as well as the younger specimens of the garter snake series, were stained in Heidenhain's iron hematoxylin with very satisfactory results.

Species Used

The Xenodontinae are represented by the ringneck snake, *Diadophis punctatus edwardsii* (Merrem) collected at Hatt Island in the Straits of Mackinac; the Colubrinae by the milk snake, *Lampropeltis triangulum triangulum* (Lac.) from Ann Arbor, Michigan; and the Natricinae by the garter snake, *Thamnophis sirtalis sirtalis* (L.) from Ann Arbor, Michigan and Pawling, N. Y. Although most of the work was confined to these species supplementary data were obtained in each of the subfamilies from a study of the spreading adder, *Heterodon contortrix* (L.) from South Kent, Connecticut and Carbondale, Illinois for the Xenodontinae; the blue racer, *Coluber constrictor flaviventris* (Say), for the Colubrinae from Ann Arbor, Michigan; and the water snake, *Natrix sipedon sipedon* (L.) from Cheboygan, Michigan for the Natricinae.

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ANATOMY AND DEVELOPMENT

SUPERFICIAL CHARACTERS

In the adult the hemipenis in all the species considered is covered near the base by large spines, which diminish in size toward the apex. The tip of the organ in the garter snake is naked, that of *Diadophis* is calyculate, whereas the colubrine hemipenis is calyculate over its distal third. The sulcus spermaticus arises in the cloaca and runs along the mesial side of each hemipenis to the distal end where it turns to the dorsal side. It is a single channel in *Lampropeltis* and *Thamnophis*, but in *Diadophis* the groove bifurcates and sends a ramus to each side of the apex (Plate II, fig. 2).

The hemipenis makes its appearance in the embryo, approximately three weeks after fertilization, as a conical protuberance on the ventrolateral surface of the body (Plate III). Its base covers roughly the area to be occupied by the future cloaca. Its surface is ectodermal and it is filled with a rather loose mesenchyme (Plate IV, figs. 6, 7).

The blunt conical primordium of the hemipenis grows much more rapidly in length than in diameter, so that approximately two weeks after its first appearance it has assumed the shape of a cylinder. Accompanying this change in shape there is a shift from the ventrolateral to the ventral side of the body, which position it occupies in the adult. During the fourth week after the appearance of the hemipenis a subterminal increase in its diameter occurs, making the organ club-shaped. Besides this modification in *Diadophis* there is an apical bifurcation. Subsequent changes in its form in any of the types under consideration consists merely of an increase in size and ornamentation.

Spines. Accompanying the foregoing modifications of form and position minute excrescenses appear on the surface in approximately transverse rows (Plate IV, fig. 8). These are the precursors of the spines, which have a similar origin in all the forms studied. Internally, the peripheral mesenchyme becomes oriented so that the aggregate nuclei of the cells destined to form spines appear like small elongated ovals perpendicular to the surface (Plate IV, figs. 9, 13). Such modifications continue until fundaments of spines are distributed throughout the entire subepidermal tissue of the middle region of the organ. Two or three of those near the base are larger than the rest and are destined to give rise to the characteristic, large basal spines of the adult. In general appearance the structure of the adult spines resembles that of compact bone, but, since there was no difficulty in sectioning even adult hemipenes, the matrix probably is not osseous. The epithelial covering of the spines is retained even in the adult, although it becomes flattened with age to a squamous type (figs. 10 and 11).

Calyces. The transverse ridges, which in the middle of the hemipenis bear spines, continue to the tip of the hemipenis, but here the spines are lost. Some of the spineless, apical ridges cross each other and merge so as to form large irregular pockets which appear in sections as peripheral indentations.

These pockets become subdivided as the embryo grows older. Their walls are reenforced so that stout epidermal cups, or calyces, are formed. In *Diadophis* these are confined to the most distal portion, but in *Lampropeltis* and *Coluber* they cover about the distal third of the hemipenis. Furthermore, in *Diadophis* the edges of the calyces receive additional support by the formation of spines in the fringes.

Sulcus. Concurrently with the development of spines the sulcus spermaticus appears. It begins as a proliferation of epidermal cells on the median surface near the base (Plate IV, fig. 12). From here it extends proximally toward the cloaca and distally toward the tip of the hemipenis. The walls of the sulcus extend dorsad along the cloaca and actually seem to continue onto the anal plate. On the dorsolateral side of the cloaca, there is an expansion of the sulcus, which has been called by Beuchelt the dorsal cloacal gland, the function of which has not been established.

In *Diadophis*, the sulcus bifurcates shortly after the two tips of the apical region of the hemipenis have become distinct. In figure 13 (Plate IV) a complete bifurcation of the retractor muscle is shown, but there is only a suggestion of a bifurcation of the sulcus. This undoubtedly signifies that the splitting of the sulcus is secondary to, and possibly contingent upon, the forking of the large retractor muscle and the other tissues of the apex. There is no indication of a forking in either of the forms which have a single sulcus, even though the hemipenis itself is obviously forked in *Natrix*.

Though the walls of the sulcus are appressed in the early embryo they begin to expand at the base in later stages, forming a T-shaped invagination as seen in cross-section (Plate IV, fig. 10). Shortly before birth a groove appears in this invaginated mass. By virtue of a juxtaposition of the lips of this groove during copulation a tube is supposedly formed for carrying sperm from the male to the female cloaca. This is indeed quite possible, for the connective tissue under the sulcus is more dense than in other parts of the hemipenis and might very well furnish the support necessary for such a procedure.

THE HEMIPENIAL MUSCLES

Adult

In the adult there are two sets of hemipenial muscles, the propulsors and the retractors (Plate I, fig. 1). The propulsors consist of arciform fibers originating on the dorsomedian skeletogenous septum, which pass lateral to the scent glands and hemipenes and insert in the median skeletogenous septum on the ventral side as far forward as the sixth caudal segment. Anterior to this point they insert at the lateral edges of a triangular muscle mass just caudal to the anus, called tentatively the *retractor cloacae*. The retractors consist of two pairs of long muscles, the *retractor penis magnus* and the *retractor penis parvus*. The former originates on the caudal vertebrae 26-28 in the species studied and inserts in the tip of the hemipenis; the latter originates on the first vertebra caudal to the scent glands, runs abruptly ventrad and continues parallel and dorsolateral to the large retractor into the hemipenis, and inserts on the subepidermal connective tissue of its base. A third retractor, the *retractor penis basalis*, is described by Beuchelt (1936) for the European species as running from the base of the hemipenis lateral to the anus on each side and meeting in the midventral line anterior to the anus. This muscle has not been found in either sectioned embryonic material or by dissection in adults of the American species used in the present investigation.

Development

The Retractor Muscles. Two weeks after the conical anlage of the hemipenis has made its appearance there is an elongation of the mesenchymal cells in the center of the hemipenis forming the promuscle mass or forerunner of the large retractor muscle. No sign of the small retractor is visible at this time.

The primary muscle cells of the large retractor make a right angle turn from the hemipenis and continue caudad into the tail. Part of this muscle therefore appears to be derived from hemipenial mesenchyme and part from the mesenchyme of the tail. The development of the muscle is accompanied by a surrounding epimysial sheath. About a week before hatching the muscle becomes subdivided into bundles by the invasion of connective tissue from this sheath, and this condition is retained in the adult.

No sooner has the large retractor grown into the tail than small blood vessels appear among its cells. Vascularization increases with the caudal extension of the muscle, and in an older embryo these vessels are greatly branched and occupy fully a third of the cross-sectional area of the muscle. Obviously, by comparison with other striate muscle such a large vascular supply is not necessary under ordinary physiological conditions, and it seems certain that its presence is associated with the eversion of the hemipenis. The venous connections of these vessels will be described later. Segmental branches of the caudal artery also supply the muscle.

Shortly after the connective tissue sheath has formed around the large retractor, and after the muscle has begun its growth into the tail, there appears dorsal to it a second and smaller condensation of mesenchyme. This is the forerunner of the small retractor. It is a separate entity and does not form by delamination from the large retractor, as might be inferred from its adult position. Developmentally it is more closely associated with the propulsors than with the large retractor, although its fibers are perpendicular to those of the propulsors.

There is more connective tissue between the fibers of the small retractor, but it does not divide it into fascicles as in the large retractor; nor is it as highly vascularized. It inserts at the base of the hemipenis lateral and slightly ventral to the large retractor. It is noteworthy that the small retractor in *Lampropeltis* and *Coluber* does not reach the base of the hemipenis before birth, as it does in *Thamnophis* and *Diadophis*, and consequently cannot aid in the prenatal withdrawal of the hemipenis. Its embryonic origin and position, however, is the same in all the forms studied, and it is well developed in all adults seen. The significance of the lag in development in the Colubrinae is not clear.

In all of the subfamilies the small retractor inserts on the base of the hemipenis. In the Natricinae the muscle originates on several vertebrae, thus forming a broad fan-like sheet between the vertebral column and hemipenis, reminiscent of the pattern in the Boidae.

The Propulsor Muscles. In *Diadophis* the anlage of the propulsors is first indicated by a definite aggregation of nuclei dorsal to the anal gland, approximately four weeks after the initial appearance of the hemipenis. In sections of embryos five days older the muscle has assumed its typical arciform character. The muscle fibers are at this stage short, apparently mononucleate, and without any suggestion of cross-striation. The change from a loosely arranged condition to a definitive one requires about a month. Shortly before hatching the muscle consists of a thick band of fibers extending laterad from the midline dorsal to the scent glands. These fibers interdigitate with fibers of a thinner lateral mass of propulsor muscles which insert on the midventral line. The line of insertion of these muscles is a heavy longitudinal bar of connective tissue, triangular in cross-section, which is located between the large retractors. The apex of the triangle extends upward and is drawn out to form a thin sheet, separating the retractor muscle and anal gland of one side from those of the other side. It expands again dorsally and forms a dorsal anchorage between the vertebrae for the propulsor muscles. Immediately posterior to the anus, in the region occupied by the retractor cloacae muscle, the propulsors insert at the lateral edge of this triangular mass, rather than in the midline. After birth the muscle fibers elongate so that in cross-sections of the tail single fibers extending from the middorsal to the midventral line are not uncommon. At this stage they are plainly striate and multinucleate, with the nuclei located peripherally.

The development of this band of muscles closely parallels that of the anal glands. These arise on each side as invaginations of the body ectoderm just anterior to the base of the hemipenis and grow dorsocaudad from that point. They make their appearance approximately six weeks after the estimated fertilization date. As soon as they begin to extend into the tail the surrounding unorganized mesenchyme becomes modified into muscle cells. Development continues until the anal glands have attained their full length, at which time the propulsor muscles also have reached their maximum prenatal development.

The development of the propulsors in *Lampropeltis* and *Thamnophis* does not deviate appreciably from the pattern outlined for *Diadophis*.

HEMIPENIAL BLOOD VESSELS IN THE ADULT

Since there are differences in the vascular structures of the genera studied, each will be discussed separately.

Diadophis (Plate V). The arterial supply in *Diadophis* consists of two pudendic arteries, one to each hemipenis, arising by a common trunk from the dorsal aorta at about the level of the urogenital papilla. The right branch proceeds almost directly to the hemipenis, but gives off a parietal branch to the lateral body wall as it turns abruptly ventrad above the cloaca. The left one, close to the point of separation from its fellow, gives off a median branch, the cloacal artery, to the dorsal side of the cloaca, after which it continues ventrad and supplies the lateral body wall with a branch symetrical to the one on the other side. The main artery then continues into the base of the hemipenis along the connective tissue at the bottom of the sulcus. It follows the path of the sulcus toward the tip of the organ, but about midway to the tip it enters the connective tissue of the large retractor muscle and extends to the apical limit of the muscle. During its course through the hemipenis it gives off several small branches to the connective tissue and to the erectile tissue. When the muscle bifurcates at the tip of the hemipenis the artery does likewise, but in doing so becomes greatly reduced.

At the base of the hemipenis the pudendic artery gives off a branch which extends ventral to the scent glands and supplies them with blood. This vessel, which was not mentioned by Beuchelt, will be referred to as the anal gland artery.

The incurrent veins of the hemipenis are the retractor muscle vein, located in the large retractor muscle, and the tumescent vein, which runs parallel to the large retractor. The latter arises in a system of venous sinuses back of the scent glands. Posteriorly it is a relatively small vein, but by virtue of tributaries along its course it enlarges as it approaches the hemipenis. It empties into the large basal erectile sinus which is confluent with the entire sinusoidal system of the hemipenial erectile tissue. The basal sinus is drained by a central penial vein, which has two main tributaries from the hemipenis proper. Upon leaving the hemipenis the penial vein parallels the pudendic artery and runs anterodorsad to join its fellow from the other side in the common penial sinus. This is drained by the common penial viens, which extend toward the renal portals. Each one, as it approaches the renal portal of its side, bends at an angle of 90° and parallels it for a considerable distance, during which its lumen is gradually reduced so that the opening into the portal vein is quite small (fig. 3A). This funnel-like entry serves to reduce the flow into the portal circulation.

Thamnophis. (Plate V). The pudendic arteries, like those of *Diadophis*, arise by a common trunk and run a short distance caudad before branching. There is no median branch to the cloaca, however. Each artery gives rise to a parietal branch which runs dorsad under each renal portal vein, thence above the dorsal musculature of the body to the lateral side. The pudendic artery itself continues ventrad along each side of the cloaca and enters the hemipenis beside the penial vein, without sending a branch to the anal glands. This latter finding agrees with that of Beuchelt for the common European grass snake. The hemipenial distribution is like that of *Diadophis*, with the exception that there is no apical bifurcation.

The retractor muscle veins of *Thamnophis* are much larger than those of either *Diadophis* or *Lampropeltis*, and the tumescent vein is correspondingly reduced. The reduction in size of the tumescent vein results from the absence of a system of sinuses posterior to the scent glands. Otherwise the course and relationships of this vein are similar to those of *Diadophis*. Hemipenial drainage also follows the same pattern with one notable exception. The common penial sinus is drained by only one common penial vein, namely, the left. The right one is absent even in the youngest embryonic stages. Although there is an obvious reduction in size of the left common penial vein as it approaches the renal portal there is no valve present. Beuchelt describes a funnel-shaped valve at the junction of the penial vein with the common penial sinus in the Old World natricine, *Natrix natrix* L., but this too is absent in the American garter snake.

Lampropeltis. (Plate VI). The arterial supply is essentially the same as that for *Diadophis* except that the anal gland artery is larger and extends farther caudad.

The tumescent vein follows the pattern previously outlined, but its tributary sinuses posterior to the scent glands are more abundant. No well defined vascular branches to the penial vein could be identified, and it seems to drain the large basal sinus only. The common penial sinus is bicornuate as in *Diadophis*. The right and left common penial veins do not show any appreciable diminution in size as they join the renal portals, but instead, a conical valve is formed in each at the junction.

In each genus studied there has been found a mechanism for reducing the rate of flow of blood into the portal circulation. This is advantageous in maintaining turgidity of the hemipenis during copulation and thus of insuring its retention in the female cloaca. These devices are shown diagrammatically in Plate II.

THE CAUDAL VEINS

In all three genera the renal portal veins anastomose in the region of the anus. The left caudal vein, emerging caudally from the anastomosis, is well developed in all the species studied, but, in *Thamnophis*, the right caudal vein is much reduced. In *Diadophis* it is abortive, extending for only a short distance along the vertebral column, and in *Lampropeltis* it is lost entirely.

THE ERECTILE TISSUE

The hemipenial erectile tissue consists of a meshwork of confluent sinuses between the epimysial sheath of the retractor muscle and the subepidermal connective tissue. The sinuses are supported everywhere by travecular extensions from the two layers of connective tissue. Many of the trabeculae originating on the epidermal side of the sinuses are associated with spines and might serve to erect the spines when the sinuses become turgid. Those which are attached between the spines would not perform such a function.

Blood reaches the sinuses through the retractor muscle veins, the pudendic artery and, to some extent, through the tumescent vein. It leaves the sinuses through the bulbus penis, which is sparingly supplied with trabeculae and consequently swells during copulation to form an enlargement which has been referred to as the base of the hemipenis.

The only discernible difference between the erectile tissues of the three groups studied is that the trabeculae are finer and more numerous in *Lampropeltis* and *Coluber* and more massive and less numerous in Diadophis and Thamnophis respectively.

NOMENCLATURE

The relationships of the vessels as described for the American species is such that a different terminology must be employed from that used by Beuchelt in his descriptions of the European species. The tumescent vein originates in the tail and empties directly into the bulbus penis, and not by a *Vena implens* as described by Beuchelt. The sinus dorsal to the anus, formed by the right and left penial veins is called the common penial sinus to replace the term *common tumescent sinus* used by Beuchelt, since the tumescent veins do not continue past the hemipenis, but empty directly into it. The excurrent *Vena penis* of Beuchelt, when it is present (absent in *Lampropeltis*), has been referred to as a tributary to the true penial vein which drains the entire hemipenis. The right and left penial veins join to form a sinus, which has been called the common penial sinus, and the vessels draining it have been called the right (when present) and left common penial veins rather than *right and left common tumescent veins.*

Because the major artery leaving the dorsal aorta in this region goes directly to the hemipenis this artery is called the pudendic artery rather than *common cloacal artery.* The *internal cloacal artery,* described by Beuchelt, becomes the cloacal artery since there is no external branch to the cloaca; and his *external cloacal branch,* which actually supplies the body wall, becomes the parietal artery. The right and left pudendic arteries arise from the aorta by a common trunk, and this is called the common pudendic artery. This condition apparently does not exist in either *Vipera berus* L. or *Natrix natrix* L., described by Beuchelt.

DEVELOPMENT OF THE HEMIPENIAL BLOOD VESSELS

The plan of vascular development for the three genera studied in detail is essentially the same. Reference to *Thamnophis* is intended throughout, unless otherwise indicated.

At an early age, before the mesenchyme has differentiated into the large retractor muscle, small spaces appear between the cells. Within the hemipenis these represent the precursors of the erectile sinuses and have a peripheral distribution. Outside the hemipenis they are oriented in a dorsoventral line toward the renal portal veins, indicating the path of the future penial veins (Plate VI). At this stage they contain no blood since there are yet no arterial connections. Soon, however, branches extend from the dorsal aorta toward the hemipenis. These are the paired primordia of the pudendic arteries which arise from the ventrolateral sides of the aorta (Plate VI).

After two weeks of development the pudendic arteries have migrated to the ventral side of the aorta and extend to the tissue of the hemipenis. Consequently some of the erectile sinuses contain blood. Other sinuses have developed in the hemipenial tissue also, pointing to the fact that the erectile system is embryologically independent of the vascular system and should not be regarded as a modification of either the arterial or venous vessels.

From the bulbus penis a vessel can be traced at this time which runs caudad, dorsal to the large retractor muscle. This is the tumescent vein. The penial veins likewise have appeared, extending anterodorsally to form a small sinus, the common penial sinus, which is already connected with the renal portal of the left side by the common penial vein. Thus, two weeks after the hemipenis has made its appearance as a ventrolateral conical projection, the circulation is established in its definitive form except for the vascularization of the large retractor muscle. This takes place during the following week.

Up to the stage just described the right and left caudal veins are about equally developed in *Thamnophis*, but the right one undergoes retrogression up to the time of birth. In *Lampropeltis*, the right caudal vein is absent from the beginning, and in *Diadophis* is never more completely developed than it was in the description of the adult condition.

INNERVATION

Adult

Thamnophis (Plate VII). Three segments contribute to the innervation of the hemipenis, numbered I, II and III from the anus forward. The most anterior of the nerves is a purely spinal one, having no sympathetic component. After giving off dorsal and ventral rami to the body wall it continues ventrad through the dorsal musculature, and, at the point of origin of the pudendic artery, it gives off a lateral, parietal branch. The main trunk continues caudad, parallel to the renal portal vein, supplying the body wall with a second small branch a short distance posterior to the parietal branch mentioned above. In the region of the common penial sinus, the trunk bifurcates, and both divisions run parallel to the penial vein and pudendic artery. One division goes directly to the hemipenis, but the fibers of the other intermingle with the nerve from segments I and II.

The nerve from segment II is likewise purely spinal. It courses ventrad through the dorsal musculature and then turns sharply caudad. In its ventral course it supplies the lateral body wall with a small branch. Just anterior to the anus it gives off a small median branch to the cloaca, and at the same point a large lateral branch joins the nerve from segment III described above. The main trunk joins the nerve from the next posterior segment (segment I) at the anterior end of the scent glands. The combined nerves of segments I and II separate for a short distance but later continue as a joint nerve in a ventrocaudal direction, giving off a small branch which runs parallel to the long axis of the body and innervates the propulsor muscles. A little farther caudad the two components again separate, and one part joins the nerve from segments II and III and runs into the hemipenis along the base of the sulcus together with the pudendic artery and penial vein; the other part continues caudad into the tail and innervates the large retractor.

The nerve from segment I passes ventrad through the muscles of the body wall and acquires a sympathetic component. The mixed spinosympathetic nerve turns sharply caudad above the cloaca in the region of the anus. At the anterior end of the scent glands it joins the main trunk from segment II. The course of the combined nerve from the two segments has been described.

Diadophis (Plate VII). In this form the nerve of segment I is again a mixed one. Before acquiring its sympathetic component the nerve gives off two parietal branches, one dorsal to the epigastric vein and one ventral to it. The ventral branch runs to the muscles anterior and lateral to the scent glands. The principal nerve continues ventrad to a position above the cloaca, turns sharply caudad and joins the nerve from segment II. The combined nerve runs parallel to the pudendic artery and penial vein into the hemipenis. The nerve of segment III supplies only the parietal and dorsal cloacal muscles.

Lampropeltis (Plate VII.) In *Lampropeltis* the nerve of segment I gives off its dorsal and ventral rami and then acquires a sympathetic component. This mixed nerve passes through the dorsal musculature and turns in a ventrocaudal direction, at the same time joining the nerve from segment II. The combined trunk runs lateral to the glandular horns dorsal to the cloaca and then ventrally into the hemipenis together with the pudendic artery and penial vein. The nerve from segment I gives off only a small lateral branch. The one from segment II gives off a large branch to the dorsal and lateral muscles of the body wall anterior to the anus as well as numerous branches to the cloaca. As in *Diadophis* segment III does not contribute to the hemipenial innervation, but simply supplies the parietal tissues and cloaca of its region.

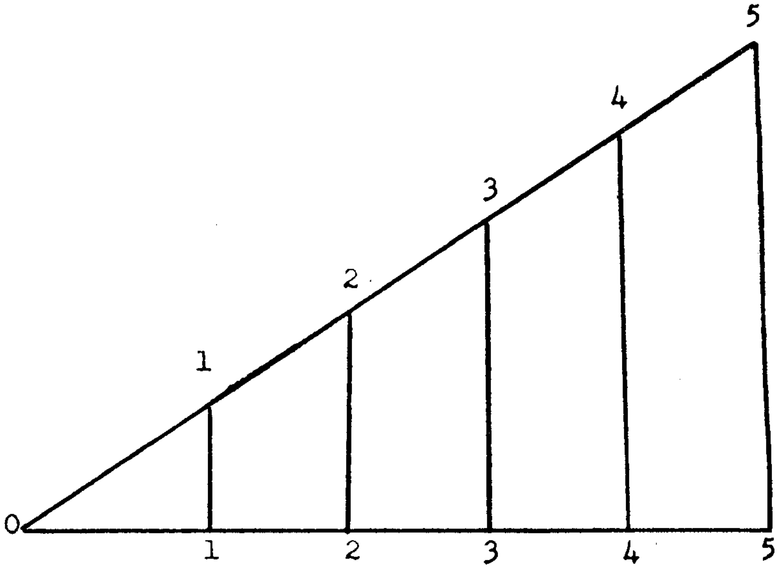
Development

Nerves are not discernible until about the third week of hemipenial development. At this time the nerve from segment I is present although it is not possible to distinguish its sympathetic component. The nerve from segment II is also developed, but it has not yet joined that from segment I, and the two nerves enter the hemipenis separately. Details of the distribution of the nerves could not be followed until the last prenatal stage, from which the foregoing descriptions were made.

DISCUSSION

PHYLOGENETIC ORIGIN OF THE HEMIPENIS

The absence of positive indication of genetic kinship in the groups studied from an embryological point of view suggests two possible conclusions. Either the hemipenial types have had an independent origin, or if they have had a common one, all recapitulatory steps in their embryonic history have been lost. The absence of such recapitulatory steps might be due to the great phylogenetic age of the hemipenis which has resulted in a compression of developmental stages so that intermediate structures are no longer recognizable. But the extensive variation in the xenodontine hemipenis alone shows that the organ is far from being evolutionarily static. The alternative to such a concept is the possibility that the mutation or mutations which brought about divergent types of hemipenes have had their effects in the early embryo. The meaning of this will be apparent from the accompanying diagram (text fig. 1), in which the triangle represents the complete development of the hemipenis. The adult organ is represented by the line 5——5, and successively earlier stages by 4——4, 3——3, etc. If, in a species "A" a mutation occurred which had its effect at 5, the new species, "B", would then show the same embryonic development from 0 to 5 as "A"; if the mutation became apparent at 4, then development from 4 to 5 would be different in the two species, and development from 0 to 4 would be similar or identical. Now, if the mutation is effective at stage 1 the development for the two species will be different from 1 to 5, and there will be no recognizable repetition of stages in the two species. Such possibilities have been suggested by DeBeer (1930) as being applicable in general where recapitulation does not occur. Admitting this possibility, it would not be logical to expect transitional stages of certain characters in the embryo, as for example, between spines and calyces or between single and bifurcate sulci, since each appears in its final form at its inception.



The question remains as to the possibility of a multiple origin of the hemipenial types. Camp (1923) came to the conclusion that the most likely origin of snakes was from anguimorphine lizards of the platynotid stock, but offered little evidence for a polyphyletic origin. There is still the possibility that the hemipenis underwent radical modification in the early phylogenetic history of snakes. Because of the rarity of ophidian fossil material of any sort and the absence of hemipenial casts all conclusions pertaining to hemipenial types must be drawn from extant material.

THE BASIC HEMIPENIAL TYPE

The hemipenis of lizards is flounced, often calyculate and, rarely, (Diploglossa) spinous. That of the Varanidae, the lacertilian group which is most closely related to snakes (Camp, 1923) is flounced and has a split sulcus. Those families of snakes usually considered closest to the basal stock, including the Boidae, Aniliidae, Uropeltidae and Xenopeltidae, have hemipenes with flounces and forked sulci. This sort of evidence therefore indicates that flounces and split sulci are primitive characters, and since there appears to be no reason to this is most likely the common type from which the others have been believe that the several types of hemipenis originated independently, derived.

ORIGIN OF THE SUPERFICIAL CHARACTERS

Calyces

Calyces are very similar histological structure to flounces in that both are ectodermal folds with a supporting loose connective tissue. Flounces are circumferential, overlapping folds (Plate VIII) whereas calyces are pockets surrounded by marginal folds and present a reticulate appearance when seen as a group. The proximal calyces often bear spines at their borders. Since calyces are found in association with flounces in supposedly primitive types of hemipenis they cannot be regarded as resulting from crowding of the flounces at the apex. It is more likely that they have been derived from flounces by the formation of strengthening bars or synapticulae between them. Such a process would permit the development of calyces well toward the middle of the hemipenis in such forms as *Coluber* and *Lampropeltis* and would provide a reasonable explanation of the phylogenetic origin of the individual calyx.

Spines

The synapticulae between the flounces might have given rise to spines also, by a process of ossification. Such a process would not imply that spines developed because of a need for strengthening the flounces at certain points, but merely envisions a fortuitous genetic mutation which manifested itself early in the embryo. It is further assumed that the mutation for local ossification in the flounces was modified in some way for a decreased spinosity from base to apex. This conception is suggested by the appearance of spines in circumferential bands in the embryo in which the larger spine primordia are located near the base and the progressively smaller ones toward the apex, the most apical folds being simply ectodermal ridges. It allows us to regard ornamentation as equally valuable (or valueless) to all species and hence persistent modification of it being fortuitous and genetically constant for the species. It will account for:

1. The large size of the basal spines, decrease in size of the spines toward the apex, and their complete absence in some forms (*Anoplophallus, Calamaria, Boidae* and members of the *Prosymna-Psammophis* group). The reason for the absence may differ with the different groups, however. In the Boidae, having a flounced hemipenis, presumably the flounces have not yet been replaced by spines, whereas the absence of spines in the other genera mentioned is almost certainly a secondary loss, the spines having been superseded genetically by other hemipenial modifications, as for example, the telescoping in the Prosymna hemipenis. The absence of the large basal spines in *Coluber constrictor constrictor*, which are prominent in other subspecies of this species, is likewise regarded as a secondary loss. Similarly the postnatal development of spines in *Clelia clelia* and *Alsophis* sp. must be a new condition which has been superimposed on the ordinary pattern of spine development as seen in most species of snakes. The fact that larger spines are present in the middle of the hemipenis of *Rhadinea steinbocki* is plainly a modification which has been made possible by the relatively enormous length of the hemipenis, and it alters in no way the conception of phylogenetic spine formation which was outlined above;

2. The occurrence of spines in the fringes of the proximal calyces of some calyculate species, as Diadophis; and

3. The appearance of spines at the apex of the penis in certain species of the Xenodontinae (*Rhadinea steinbocki* and *Xenodon* spp., specimens of which were seen through the kindness of Dr. J. R. Bailey), whose apical ornamentation otherwise consists of calyces or the apical disc. If this conception is valid, it implies that spines at the present time are invading the calyculate area of the hemipenis, and it necessitates the corollary that the calyces are receding in orthogenetic fashion; conversely, completely spinous forms, such as the Natricinae, would never give rise to a calyculate type. Consequently neither the Colubrinae nor the Xenodontinae could have been derived from a spiny-tipped ancestor without a reversal of the evolutionary processes.

Sulcus

Whereas calyces and spines are morphologically discrete, the split and single sulci are but geometrical variants of the same fundamental structure. It has been shown how the spines and calyces might have been derived from a common ancestral structure. The ancestral type must also have had potentialities for both split and single sulcus. In *Diadophis* the bifurcate sulcus appears only after the point of insertion of the large retractor muscle has divided. Although by no means conclusive proof, this is an indication that the change from the bifurcate sulcus to a single one accompanied the loss of the major internal bifurcation phylogenetically.

POSSIBLE PATHS OF EVOLUTION OF THE HEMIPENIS

It is possible that the flounced hemipenis with split sulcus, considered primitive on the basis of evidence reviewed above, gave rise to the single-sulcused, flounced type. Then, in view of the extant types, spinose and calyculate modifications must have proceeded in each of the two flounced basal stocks. If this were so, there should be, among the primitive groups of snakes, some having flounced and single-sulcused hemipenes, but actually there are none. Furthermore, if the flounced and single-sulcused organ were the primitive type, it would create the possibility that the colubrines are ancestral to the Xenodontinae, and the natricines to the "Lycodontinae". However, the holarctic distribution of the natricines and colubrines would suggest that they are the new forms rather than the ancestral ones; conversely, the xenodontines and "lycodontines" are primarily tropical.

An alternative evolutionary pattern is presented schematically in Plate VIII, which outlines a more probable course of evolution of the four present-day colubrid types from the primitive, ancestral type. Hemipenes resembling each of the hypothetical stages can be found among living species. Calyces, such as are found in lizards and boids in company with flounces, are believed to have arisen at the apex. Following this modification of the simple flounced hemipenis, the process of spine formation at the base of the organ was initiated in at least one group, resulting in the replacement of flounces by (or their combination with) spines, and thus establishing a basal stock of xenodontoid snakes. A derivative of the xenodontoid stock was modified by an invasion of the apical calyces by spines, or the flounces were replaced by spines directly. Either change would result in the "lycodontine" type, the original persisting as the xenodontine. Each of these groups was then modified in the same way, namely, by a fusion of parts.

The fundamental change in the series necessary for the transformation of split to single sulcus must have occurred in the muscle; contingent upon this were changes in blood vessels, nerves, connective tissue and superficial characters. Not only are the types, here outlined, found among living species, but also transitional stages are abundant. It is noteworthy that the hemipenial type which has been considered primitive is found in snakes which are supposedly primitive on the basis of other morphological and zoogeographical grounds. The fact that exceptions (e.g., spiny-tipped xenodontines) and anomalous forms are found is quite within the realm of expectation, since the hemipenis has been subject to so much phylogenetic change.

ORIGIN AND RELATIONSHIPS OF THE GROUPS STUDIED

Evidence From the Hemipenis

Although little evidence was obtained for relationships between the several groups from a strictly embryological point of view, analysis of some of the anatomical findings is more suggestive. A similarity is expressed between the xenodontine and colubrine hemipenial innervation in that two segments contribute to the innervation of the hemipenis of *Diadophis* and *Lampropeltis*, whereas that of Thamno*phis* is supplied by three segments.

Similarity in the form of the retractor cloacae in the colubrine and xenodontine species could hardly be a reflection of similar habits in the genera *Lampropeltis* and *Diadophis*. Again, the extensive retractor cloacae of these genera is markedly different from the short triangular mass, characteristic of this muscle in *Natrix* and *Thamnophis*.

The bulk of the anatomical evidence is derived from the blood vascular system of the hemipenis and related parts of the body. In *Thamnophis* there are two caudal veins, and in *Lampropeltis* and *Diadophis* there is but one unless the anal gland vein of *Diadophis* be construed as a degenerate caudal vein. It will be recalled that the paired caudal vein of the natricines was paired from the beginning, and the single caudal vein of the xenodontine and colubrine genera was never double at any time in its embryonic history. *Diadophis* and *Lampropeltis* possess an anal gland artery, which is absent from *Thamnophis*; likewise the cloacal branch of the pudendic artery, present in the two former genera, is absent from *Thamnophis*.

The hemipenial venous system apparently is not of great value in determining likenesses, but it may be useful in expressing differences. The method of draining the common penial sinus ,Plate II) differs in all the forms studied, and moreover, each of the devices previously outlined differs from that reported by Beuchelt for *Natrix natrix*. In view of these facts, the venous pattern may be a new and important source of recognizing specific differences.

It is also interesting to speculate on the significance of the hemipenial types in elucidating some of the phylogenetic history of the groups concerned, even though conclusions from this point of view may be at variance with those based on other criteria. Both of the Colubrid subfamilies with the more primitive type of hemipenis, Xenodontinae and "Lycodontinae", are predominantly tropical. Since a monophyletic origin of snakes seems to be the most probable they must have had a common place of origin. The "lycodontine" hemipenis has progressed farther along the path of evolution, insofar as it no longer retains calyces. But the xenodontines also show a tendency in that direction for in many species the proximal calyces have spinose fringes. It is necessary to postulate that the original xenodontoid stock of the Old World has been exterminated, since xenodontine hemipenial types are now virtually confined to the Western Hemisphere. Exceptions are found in Asia (*Pseudoxenodon*, *Heli-*

cops, Trirhinopolis, Dinodon), Africa (*Boaedon, Pseudoboodon*), and Madagascar— "*Sibynophis*". The progeny of the stock has persisted principally in the Old World as the "lycodontine" type and in the New World as the xenodontine type.

It is possible that the more dynamic colubrine derivatives of the original Old World xenodontine stock and the more active natricine derivatives of the "lycodontine" stock have been factors in the extermination of the xenodontines in the Old World as well as in the northern continent of the New World. Whether or not this be true, it is obvious that the natricines and colubrines have been more successful in the palearctic and nearctic regions, although the latter have begun to assume a prominent place in the herpetofauna of the Neotropical region. Of the two single-sulcused types the colubrines should be regarded as the older group on the basis of hemipenial evolution, for the natricines have superimposed upon the single sulcus complete spinosity, replacing the calyces to the apex. Therefore, on the basis of hemipenial evidence alone, it must be concluded that the Xenodontinae represent the oldest group, that they probably have given rise to the Colubrinae (unless the colubrine hemipenis were independently derived) and that the natricines are not properly placed in this evolutionary line. They would be related to the Colubrinae (or the Xenodontinae) only through a common ancestor.

Evidence From Other Sources

Bogert (1940), however, has made it amply clear that phylogenetic conclusions based exclusively on the hemipenis, not only are not valid but may even be misleading when applied as subfamily criteria. Therefore, it is necessary to draw upon data from other sources in order to get a true picture of relationships of the groups in question.

Of the American subfamilies under discussion the Xenodontinae are apparently oldest. Not only the hemipenial structure, but also the wide morphological variations and the variety of ecological habitats occupied by them connote great age for the group. They are predominantly neotropical. A few genera are endemic in North America (*Rhadinea, Farancia, Abastor, Carphophis, Diadophis and Heterodon*), and some which are apparently of this group are found in Asia and Africa.

The neotropical xenodontines either must have originated in the western hemisphere, or must have migrated hence from the Old World. Such a migration must have been either by way of a northern route (Behring Land Bridge) or by a southern route involving some geologically remote Afro-American bridge, evidence for which does not exist.

Assuming for the moment that the group had its center of origin in the neotropical region, it is difficult to understand how the secretive and ecologically specific snakes of the genera *Heterodon* and *Diadophis* have attained such a wide distribution, for *Heterodon* is found in most of the states east of the Rocky Mountains, and *Diadophis* ranges from coast to coast and from Canada to Mexico. *Carphophis* has a slightly more restricted distribution than *Heterodon*; *Farancia* and *Abastor* are confined to the southeastern portion of the United States. *Rhadinea*, existing only as far north as Mexico, may be a reentrant in the northern continent, but the other North American genera of the Xendodontinae are plainly relict genera of a once widespread group rather active and progressive representatives which have recently migrated from a center of dispersal.

Moreover, the postulation of a South American origin of this group of Colubridae necessitates a polyphyletic origin of snakes, or at least of the family Colubridae. The families Amblycephalidae, Aniliidae, Boidae and Uropeltidae, which are least specialized with respect to genital as well as other somatic characters, are predominantly Old World snakes, and it is most feasible that these groups have had their origin in the Eastern Hemisphere. It is, indeed, quite possible that the ancestors of the Colubridae migrated to the New World and subsequently gave rise to this family there, presumably in the Nearctic region. If this be the case, we are still confronted with the problem of the apparently relict xenodontine genera of North America. It will be recalled that Camp (1923) decided in favor of a monophyletic origin of snakes, regarding some anguimorphine lizard of the platynotid stock as ancestral to the Serpentes. *Varanus* is therefore the genus of extant lizards most closely related to snakes, and it is an Old World genus. However, the oldest known varanid is *Saniwa* from the Eocene of North America. The fact that this fossil of the varanid group happens to have been found in the Western Hemisphere in no way belies the eastern origin of the Varanidae, but rather indicates that it was a widespread group, capable of modification along the line of serpentine evolution.

In light of the foregoing statements it would be patently erroneous to designate South America as the place of origin of the Xenodontinae, particularly after consideration of the occurrence of African and Asiatic species. They must therefore, have arisen in the Old World and must have come to America by either a Northern or Southern route. If the migration had taken place by a southern route it would be virtually imperative that closely allied genera be found in Africa and South America. *Boaedon and Pseudoboodon erlangeri* and *Lamprophis* are African snakes having the xenodontine type of hemipenis, and *Helicops* is an American genus having the "lycodontine" type. Such a paucity of African "xenadontines" and American "lycodontines" does not indicate a great amount of migration. Africa was presumably not connected to the Eurasian mainland until after the xenodontines had disappeared; the absence of lycodontines from South America might be explained by the contention of Schuchert (1935) that no Afro-American land bridge has existed since the paleozoic era.

It is also possible that evolution in the xenodontine group has proceeded at such a rate in the Old World that the original stock is no longer recognizable. It would therefore be necessary to postulate that all of the speciation in the New World has been in a different direction from that which eliminated the original stock in the Old World. The chance that this has taken place is extremely remote, but even granting it were so, it means merely that South America is to be regarded as the western center of dispersal, rather than the site of origin, of the Colubridae, and the problems of distribution which were previously mentioned are still to be explained.

There is apparently no alternative to the conclusion that the xenodontines originated in the Old World, reached the New World by way of the Behring Land Bridge, occupied a wide area in the Nearctic region and left the six relict genera in the North American continent. These genera have presumably maintained their North American existence because of close ecological adaptation which relieved them of the biotic and climatic pressures which exterminated the other genera which occupied the Nearctic region. The members of the group which has extended their range into South America, being more active and progressive and free from the disastrous competition in the north, became modified and adapted in the numerous ways that are now apparent in the group as a whole.

Morphological and zoogeographical facts suggest that the colubrines are a rather new group, for they are undergoing rapid evolution, show a narrower range of hemipenial variation, are successful over a wide area, are extending their range into South America and already outnumber xenodontines in Central America. These facts seem to imply that the Colubrinae are older in this hemisphere than the Natricinae. The latter have not yet reached South America and are far outnumbered in Central America by the Colubrinae.

Both Natricinae and Colubrinae are represented in large numbers in the palearctic region. Here they are apparently not undergoing evolutionary change so rapidly as in the New World and are more generalized than the American species. For this reason, as well as those outlined in discussing the origin of the xenodontines, natricines almost certainly originated in the Old World.

SUMMARY

1. The hemipenes arise in the embryo as paired, solid protuberances on the ventrolateral surface of the body just anterior to the anus.

2. The muscles of the hemipenis develop in the following order: large retractor, small retractor and propulsors. The *retractor penis basalis* described by Beuchelt was not found in either adults or embryos.

3. Arterial blood is supplied by a pair of pudendic arteries, arising from the aorta separately in the embryo but by a common root in the adult.

4. The erectile sinuses arise independently of the arteries and veins. By confluence at the base of the hemipenis they form the bulbus penis.

5. Incurrent veins are the retractor muscle veins and the tumescent vein; excurrent veins are the right and left penial veins, which join above the cloaca to form the common penial sinus.

6. A device exists in each of the genera studied for reducing the rate of flow from the common penial sinus. In *Lampropeltis*, valves exist at the junction with the renal portal; in *Diadophis* the valves are replaced by funnel-like strictures; in *Thamnophis*, the right common penial vein is lost.

7. Hemipenial innervation in *Lampropeltis* and *Diadophis* consists of a mixed spino-sympathetic nerve from the first segment anterior to the anus and a pure spinal nerve from the next segment forward. In *Thamnophis*, three preanal segments contribute nerves but only the first segment anterior to the anus includes a sympathetic component.

8. Spines arise by mesenchymal reorganization to form blunt cylinders, appearing superficially in transverse rows on the hemipenis. Calyces appear later in development as irregular depressions in the epidermis. The sulcus spermaticus is an ectodermal invagination along the ventral side of the hemipenis, sometimes single and sometimes forked, presumably acting as a conduit for sperm during copulation.

9. Evidence is presented which indicates that the primitive hemipenis was split-sulcused and flounced. Formation of apical calyces in this group gave origin to the xenodontine ancestral type; formation of apical spines gave rise to the "lycodontine" ancestral type.

10. By modification toward singleness, the xenodontine type formed the colubrine and the "lycodontine" type formed the natricine.

11. Xenodontinae are considered the oldest of the three subfamilies studied. They are replaced to a great extent in North America by the colubrines, which also encroach upon their range in Central and South America. The Natricinae are probably the most recently evolved of the three subfamilies.

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PLATE I

Fig 1.. Dissection of tail of *Thamnophis*, showing the left hemipenis everted and the right one in its caudal, resting position. Hp., hemipenis; Lg. Ret., large retractor muscle (displaced to show small retractor muscle); Par., parietal muscles; Prop., propulsor muscles; Ret. Clo., retractor cloacae; Sc. Gl., anal scent glands; Sm. Ret., small retractor muscles, displaced to the left to show scent gland. x 1.5.

PLATE II

Fig. 2. Hemipenes of Colubrid types. A, *Thamnophis;* B, *Diadophis;* C, *Lampropeltis.* From Cope.

Fig. 3. Diagrams of the junction of the common penial veins with the renal portal veins. A, *Diadophis;* B, *Thamnophis;* C, *Lampropeltis.*

PLATE III

Fig. 4. Stage I, *Natrix*, superficial view from right side. Aud. Ves., auditory vesicle; Br. Cl., branchial cleft; Hp., hemipenis; Mes., mesonephros. x 13.

Fig. 5. Stage I, *Natrix*, superficial view from left side. Li., liver; Sept. Tr., septum transversum. Other abbreviations as above. x 13.

PLATE IV

Fig. 6. Photomicrograph of cross-section of *Diadophis* embryo at level of hemipenes, Stage I. Clo., cloaca; Dor. Ao., dorsal aorta; Epig., epigastric vein; Hp., hemipenis; Neur., neural tube; Noto., notochord. x 40.

Fig. 7. Hemipenis of *Diadophis* between stages I and II, to show solid mesenchymal structure of primordium, and, in center, beginning of organization of large retractor muscle. x 400.

Fig. 8. Hemipenis of *Diadophis*, following stage II. Note the large basal spine, the spines of the middle in transverse rows and ectodermal transverse ridges of apex. x 20.

Fig. 9. Photomicrograph through outside of *Diadophis* hemipenis, Stage II. Two spines are shown with the cells arranged in circular fashion; peripheral to this, at the upper side of the figure, note stratified epithelium. Erectile sinuses with red blood corpuscles appear at the lower left in the photograph. x 400.

Fig. 10. Cross-section of inverted hemipenis of adult *Thamnophis*. Note lumen in center, surrounded by dark spines. At right, below center, is the anchor-shaped sulcus spermaticus. x 40.

Fig. 11. Large spine bordering lumen in upper portion of fig. 10. The cellular structure and extent of matrix are clearly shown, as well as the squamous epithelium bordering the lumen. x 300.

Fig. 12. Sulcus spermaticus. x 300.

Fig. 13. Developing *Diadophis* hemipenis, showing the sulcus at the top of the figure. Note that the bifurcation of the sulcus is barely indicated, while the retractor muscles in the center of the photograph are completely divided. x 105.

PLATE V

Fig. 14. Diagram of hemipenial vascular supply in *Diadophis*. A. Gl. Art., anal gland artery; Caud. V., caudal vein; Clo. Art., cloacal artery; Com. Pen. Sin., common penial sinus; Com. Pu. A., common pudendic artery; Dor. Ao., dorsal aorta; Erect. Sin., erectile sinus; L. Com. Pen. V., left common penial vein; L. Pen. V., left penial vein; Par. Art., parietal artery; Pud. Art., pudendic artery; Retrac. V., retractor muscle vein; Seg. Art., segmental artery; Trab., trabecula; Tum. V., tumescent vein.

Fig. 15. Diagram of hemipenial vascular supply in *Thamnophis*. Caud. V., caudal vein; Com. Pen. Sin., common penial sinus; Com. Pu. A., common pudendic artery; Dor. Ao., dorsal aorta; Erect. Sin., erectile sinus; L. Com. Pen. V., left common penial vein; L. Pen. V., left penial vein; Pud. Art., pudendic artery; Retrac. V., retractor muscle vein; Seg. Art., segmental artery; Trab., trabecula; Tum. V., tumescent vein.

Fig. 16. Diagram of hemipenial vascular supply in *Lampropeltis*. Abbreviations as for figure 14.

PLATE VI

Fig. 17. Stereogram showing extent of vascularization and innervation at Stage I. Dor. Aa., dorsal aorta; Erect. Sin., erectile sinus; L. Epig. V., left epigastric vein; L. Ren. Por., left renal portal vein; Rec., rectum; Ur., ureter; V. def., vas deferens; Seg. I, segment I (first segment anterior to anus). x 40.

Fig. 18. Stereogram showing extent of vascularization and innervation at Stage II. C. Pen. Sin., common penial sinus; L. Epig. V., left epigastric vein; L. Pen. V., left penial vein; L. Ren. Por., left renal portal vein; Rt. Pud. Art., right pudendic artery; Seg. II, segment II (second segment anterior to anus, the first being unlabeled). x 40.

PLATE VII

Fig. 19. Diagram of innervation of hemipenis in *Thamnophis*. Lft. Caud. Vein, left caudal vein; Rt. Epig. Vein, right epigastric vein; Rt. Caud. Vein, right caudal vein; Rt. Ren. Por., right renal portal vein; I, II, III, first, second and third segments anterior to the anus.

Fig. 20. Diagram of innervation of hemipenis in *Lampropeltis*. Abbreviations as for figure 19.

Fig. 21. Diagram of innervation of hemipenis in *Diadophis*. Abbreviations as for figure 19.

PLATE VIII

Fig. 22. Schematic representation of interrelationships of hemipenial types discussed in this paper.

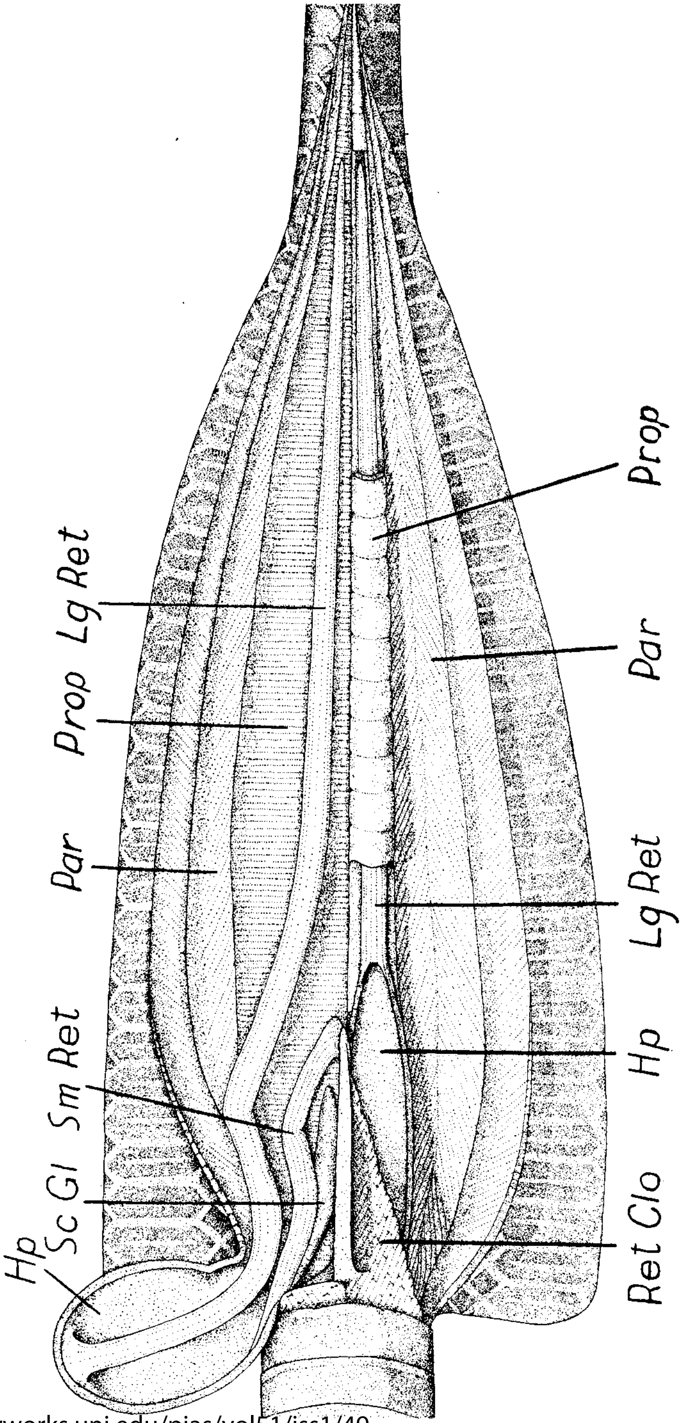


Plate I

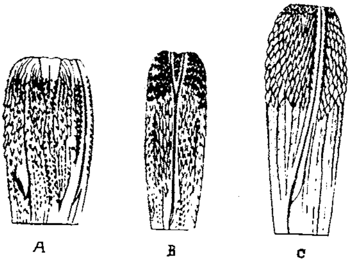


Figure 2.

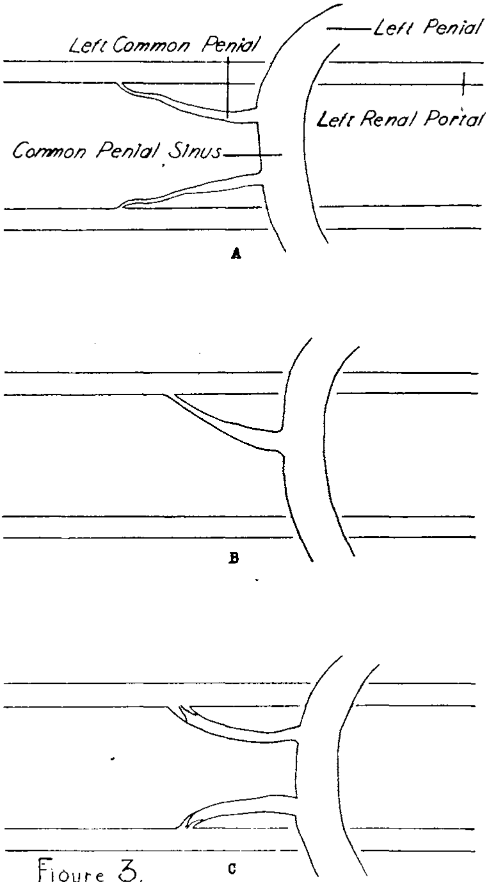


Figure 3.

Plate II

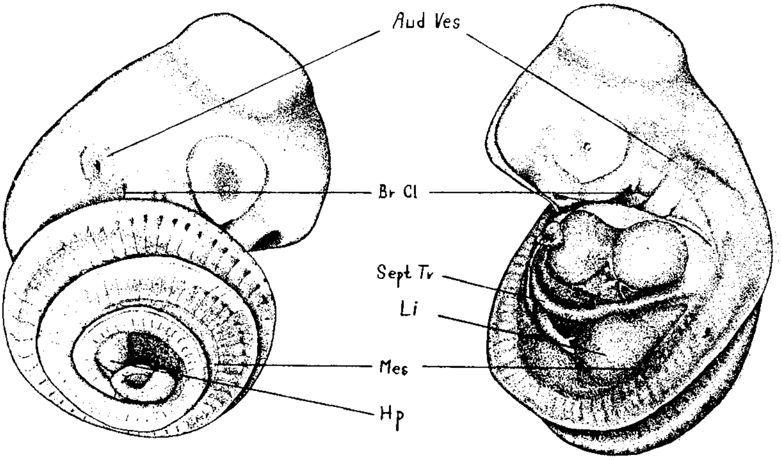


Fig. 4

Fig. 5.

Plate III

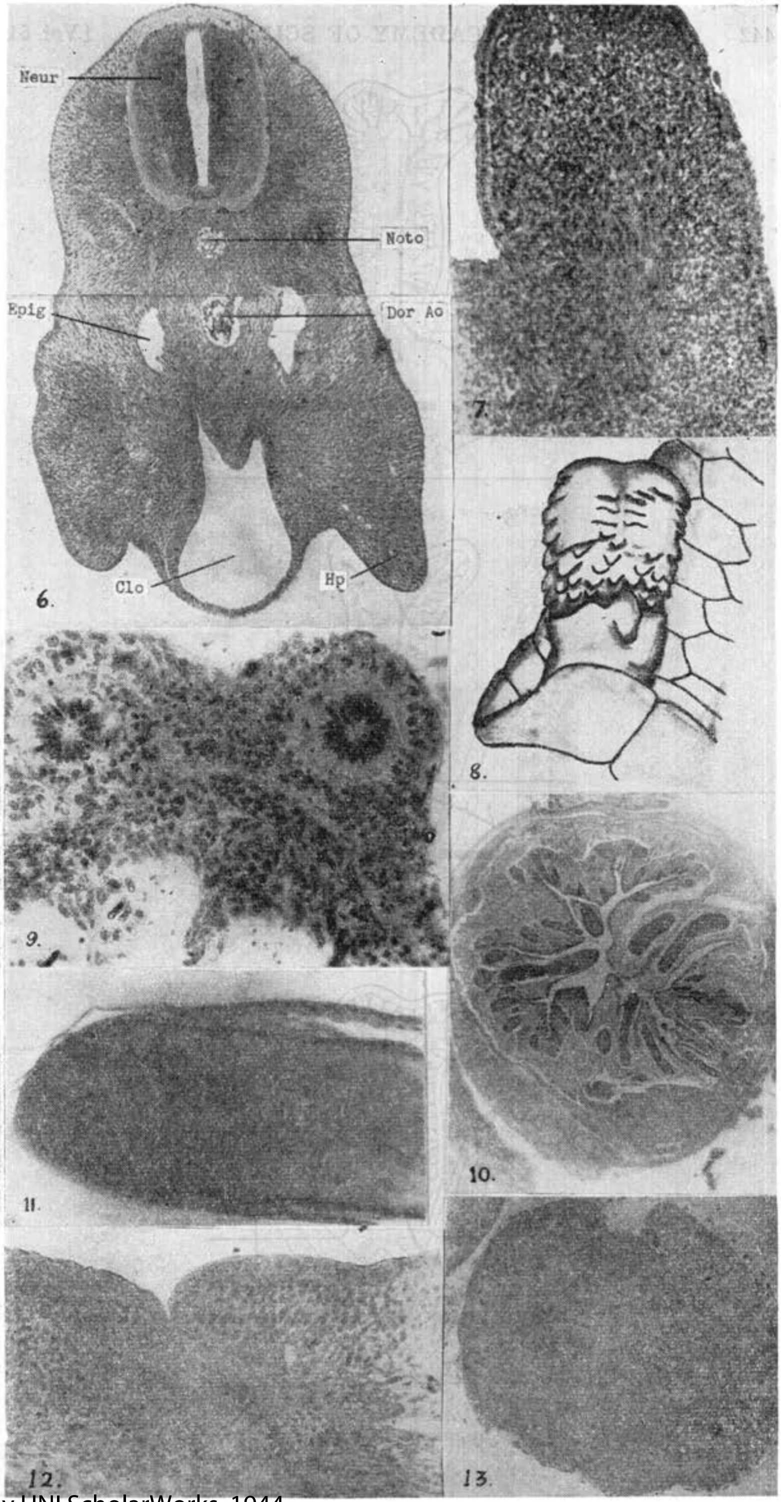


Plate IV

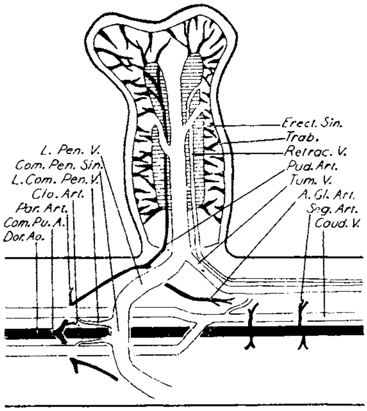


Figure 14 *DIADOPHIS*

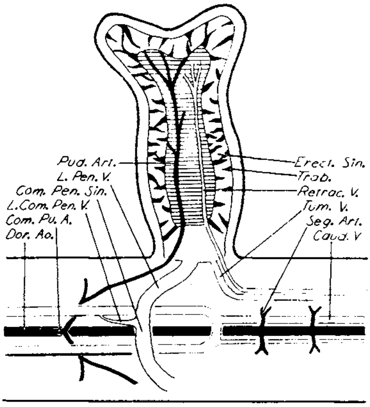


Figure 15 *THAMNOPHIS*

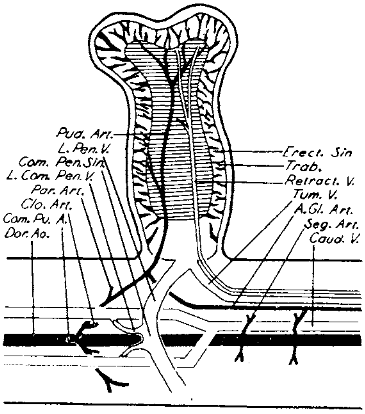


Figure 16 *LAMPROPELTIS*

Plate V

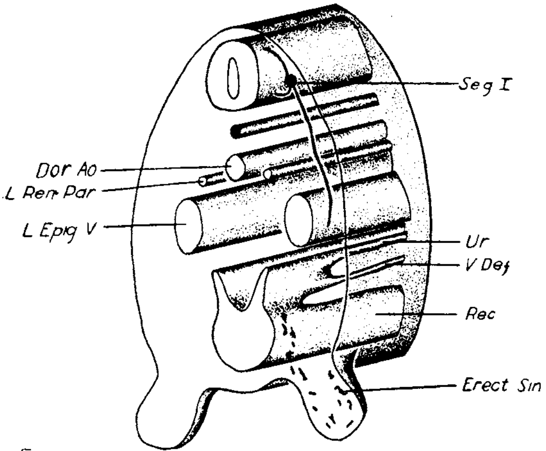


Figure 17.

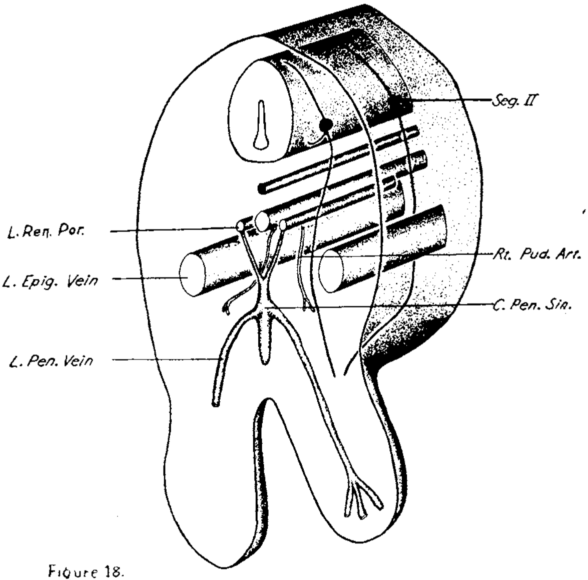


Figure 18.

Plate VI

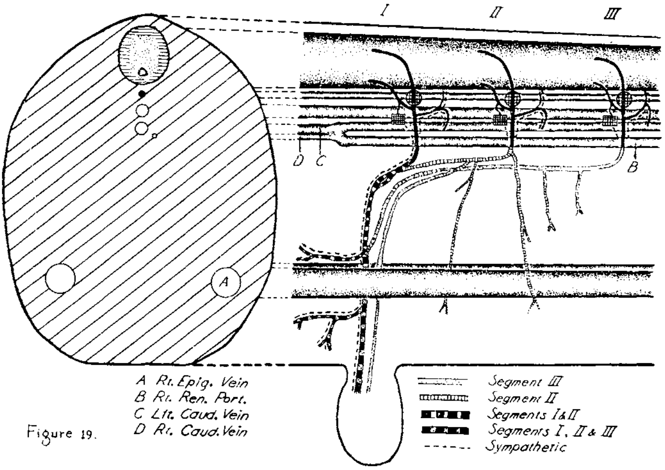
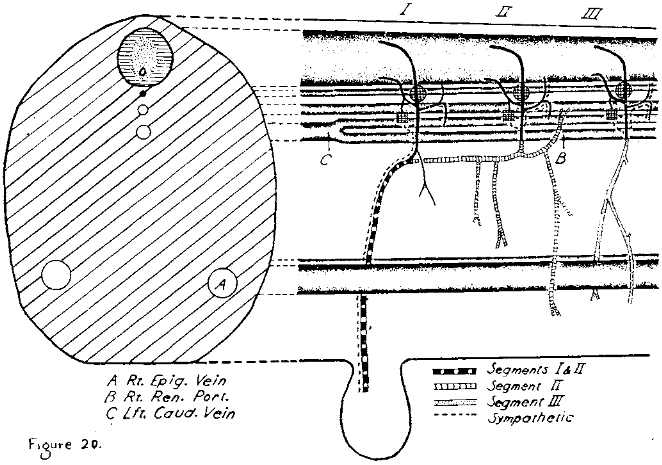


Figure 19.



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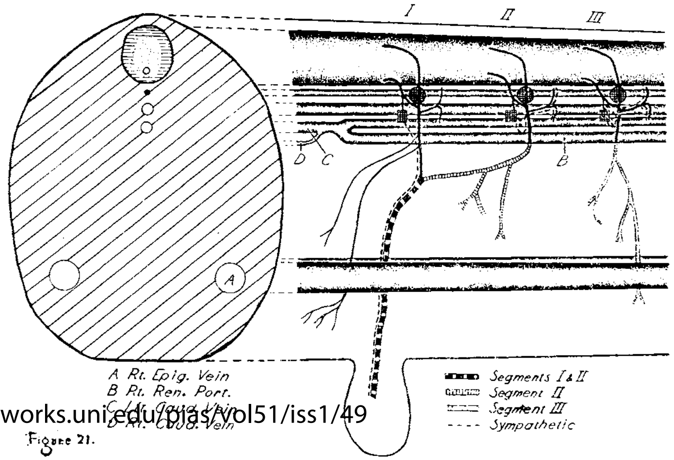

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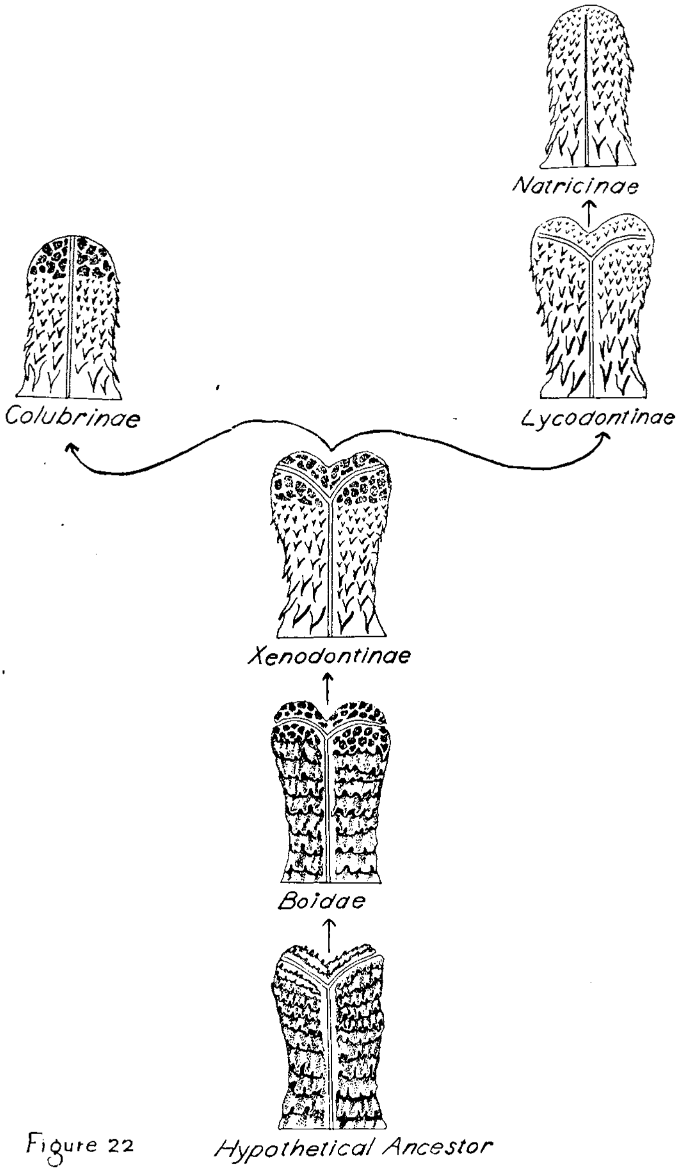

Figure 22

Plate VIII