A STUDY OF THE PHARYNGEAL AND ASSOCIATED ARTERIES
OF SIX FAMILIES OF URODELA WITH A DISCUSSION OF
PHYLOGENETIC IMPLICATIONS

A THESIS

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Rezneat Milton Darnell Jr., B.S.

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INTRODUCTION

In the evolution of the vertebrates no chapter is more distinct than that which deals with the abandonment of their aquatic habitat by certain fishes and the morphological changes which this group was forced to undergo in its attempt to cope with the vicissitudes imposed by the terrestrial environment.

Many of the forms, unable to meet the demands of land life have long since become extinct. Others remained for a while on land and then reverted to their ancestral home, having to readapt themselves to an aquatic habitat. Others adapted themselves more completely to terrestrial life and have managed to persist in this environment, undergoing remarkable adaptive changes.

Consequently in the urodeles, the lowest of the living vertebrates above the fishes, various degrees of adaptation to terrestrial life and readaptation to aquatic life have given rise to a perplexing array of characters. Although certain of the urodeles comprise remarkably well-defined groups, the relationships of these groups to each other is, in most cases,
obscure. Improper interpretation of the existing information on the subject has, in the past, led to a great deal of confusion among taxonomists and anatomists.

The circulatory system is, perhaps, more subject to change than is any other major system of the vertebrate body. With every loss or shift of a muscular, skeletal, or glandular element, a corresponding change in the blood supply must occur. Consequently in the circulatory systems of the urodeles great modifications have taken place which reflect the evolutionary history of the group to a remarkable degree.

Because of inadequate methods and procedures, the value of the circulatory system in tracing phylogenetic development has generally been overlooked in the past. The procedure has generally been to deduce from previously established phylogenetic relationships what changes have occurred in the vascular system of vertebrates.

Employing modern methods and materials a study of the arterial circulation of the pharyngeal region has been undertaken on six of the eight recognized families
of urodeles, and the results of this study are reported herein.

The present work has as its purposes the following: (1) to establish the merits and to point out the disadvantages of the new methods employed in the study of the circulatory system, (2) to give accurate descriptions with original drawings of the pharyngeal arteries of the urodeles considered, (3) to establish the value of the circulatory system in ascertaining evolutionary relationships, and (4) to discuss the phylogenetic implications of the data secured in the present study.

A comprehensive review of the literature dealing with the circulatory systems of the urodeles is not included herein since an excellent bibliography dealing with the Amphibian circulatory system has already been compiled by Francis (1934).

In the present discussion new terminology is kept at a minimum in considering the different arteries, and an attempt is made to standardize the existing nomenclature by adopting the terminology used by Francis (1934) in his comprehensive treatise on Salamandra. The arteries herein described are named according to their homology with those vessels described by Francis.
MATERIALS AND METHODS

The types used in this study to represent each of the six families of Urodela are indicated in the classification at the end of this section. The source of each species was as follows: large *Amphiuma tridactylum* and *Siren lacertina* were obtained from Dr. C. L. Baker, Department of Biology, Southwestern College, Memphis, Tennessee; *Necturus maculosus* and *Triturus viridelescens* were obtained from General Biological Supply House, Chicago, Illinois; *Plethodon glutinosus* were collected in Austin, Texas; and *Ambystoma texanum* was secured on collecting trips in and around Houston, Texas. Additional specimens of *Triturus viridelescens* were donated by Richard Ethridge.

Injections of the circulatory systems were made with polymerized vinyl acetate dissolved in acetone. Several portals of entry for the injection fluid were tested with varying results. Best results were achieved in large specimens (10-40 inches long) by injecting directly into the dorsal aorta, sending the fluid against the normal direction of blood flow. If the animal was particularly large (over 25 inches in
length), it was found desirable to enter the dorsal aorta only an inch or two posterior to the apex of the ventricle. If the dorsal aorta is entered further posteriorly in large specimens, it is difficult to obtain complete injections of the cranial region because the elasticity of the aorta greatly reduces the pressure on the fluid inside the vessels. On smaller specimens it was found that injections into the ventricle yielded the best results if sufficient pressure was applied.

Inasmuch as the plastic begins hardening immediately when it is injected, it was found desirable to apply as much pressure as possible without breaking the walls of the vessels. A single injection was sufficient in most types to leave all the desired vessels injected, the one exception being *Necturus*. In this form double injection was necessary, injections being made first into the dorsal aorta as described above and then into the ventricle.

The calibre of the needle used was determined by the size of the specimen, and in this study # 18, # 20, # 26, and # 27, guage needles were used.

Using the methods described above, good injections
were secured on salamanders whose length varied from 1.9 inches in the smallest *Triturus* to 37 inches in the largest *Amphiuma*.

After the injection was completed it was allowed to 'set' for several minutes, and then, after the posterior portion was removed, the injected anterior end was placed in a concentrated aqueous solution of potassium hydroxide. In from three to seven days all but the largest specimens were completely dissolved away from the plastic replica of the circulatory systems.

The specimens were observed periodically as they were being macerated and in some cases dissections were carried out on injected specimens. But the latter procedure was not followed in all cases since this study was not intended as a description of the various peripheral ramifications of the arteries discussed.

During the course of the study it was found desirable to cause *Siren* to lose its gills. For this purpose 'Proloid', an extract of the thyroid gland, was employed. Success was achieved by four subcutaneous injections of 100 mg. of the extract in
3 cc. of water at intervals of 7-8 days. Two weeks after the last injection when the gills were completely non-functional and almost completely absorbed, the amphibian was anesthetized and the circulatory system was injected with plastic.

All injections were thoroughly studied, using a binocular microscope for examination of small specimens and for details of the larger ones. Special attention was paid to the point of origin and method of origin of the various arteries. Original composite drawings were made from the injections. The more usual conditions were reproduced in the drawings, and all observed major or frequent variations are recorded at appropriate places in the text.

The first advantage of the plastic injection method employed in this work is that it allows the examination of far greater numbers of specimens than has been possible by any previously known method. This ease of injection and examination removes a great part of the labor involved in the study of the circulatory system and permits a fair sampling of the population. Because of this, anomalies may readily be recognized as such and, particularly if they are frequent, may even provide valuable information concerning changes
that are currently taking place in the population or which have occurred in the past history of the race.

The second advantage of the present method is that the size of specimens which can be examined depends only on what sizes can be injected. In the present study the smallest specimen secured (1.9 inches from tip of nose to tip of tail) was successfully examined after injection with a #27 hypodermic needle, even though the diameter of the ventricle was only slightly greater than that of the needle. With a smaller needle, even smaller specimens could be injected and studied. This makes available for study larval and embryonic forms which were previously subject to study only by serial sections.

The third advantage of the method is that it permits the examiner to view the system as a unit from the outset, leaving until later the study of the component parts. In the past the reverse has been true and has often led to distortion of the picture (and illustrative figures).

The fourth and, perhaps, the greatest advantage
of this method is that it presents a permanent, detailed, three-dimensional replica of the human of the circulatory system. This replica shows all internal structures such as septa and valves and in good injections all branches, regardless of size, are apparent immediately upon inspection. Moreover, the exact method of origin of branches may be ascertained, i.e., from which side of a given vessel the branch arises, its immediate direction (which may be diametrically opposite from its ultimate direction), and the presence or absence of a partial partition or constriction at the origin of the branch. It also reveals the partial separation of fused vessels which have arisen separately in the individual's ontogeny. Examination of such fused vessels (as when the 'third' arch of Triturus becomes confluent with the systemic) reveals that the vessels are in reality connected only by a long slit, and that in cross-section the fused vessels would appear as a figure '8'. 
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DESCRIPTION OF THE PHARYNGEAL ARTERIES
OF URODELA

AMBLYSCIDAE
(Plate 2)

The following description has been prepared from examination of injections of five medium-sized Ambystoma texanum.

In Ambystoma four arches are present, the carotid or 3rd aortic arch (A.III), the systemic or 4th aortic arch (A.IV), the "third arch" or 5th aortic arch (A.V), and the pulmonary or 6th aortic arch (A.VI).

The carotid, the most anterior of the aortic arteries, passes laterally from the truncus arteriosus, gives off the external carotid, and passes dorsally, then anteriorly to the cranium as the internal carotid. The external carotid supplies the middle of the lower jaw and arises as a dorso-mesial branch of the carotid bulb (or carotid gland) which is a perforated enlargement of the carotid arch and which will be discussed in detail later.

The systemic passes laterally to the level of the carotid bulb somewhat paralleling the carotid, dips dorsally, and then loops mesially to fuse with its fellow from the opposite side. The fused systemics pass
toward the tail in the mid-dorsal line as the dorsal aorta. Three major arteries are given off by the systemics before they fuse. The ascending pharyngeal arises from the dorso-lateral aspect of the systemic loop and passes anteriorly and, after a way, somewhat ventrally to supply the periphery of the lower jaw. Shortly before the systemic fuses with its mate to form the dorsal aorta, it gives off a branch, the palatino-nasal, which passes to the roof of the pharynx and mouth. The vertebral artery arises from the systemic at the very base of the palatino-nasal and proceeds posteriorly for a short distance before dipping into the vertebral column.

The third arch parallels the systemic, receives the ductus Botalli, bends dorsally, and immediately joins the systemic just before the latter gives off the ascending pharyngeal.

The pulmonary leaves the truncus arteriosus a short distance posterior to the anterior arches and does not quite parallel them, passing in a more posteriorly direction. At its lateral extremity the pulmonary gives off the ductus Botalli and then proceeds caudally and somewhat mesially to the lungs. At about the level of the forelimb the pulmonary artery gives rise to a large branch, the esophageal artery, which passes
anteriorly to supply the esophagus.

Major variations from the above were (1) in one individual the third arch was partially fused with the systemic on both sides, and (2) in one individual the ascending pharyngeal anastomosed with the internal carotid, the two arteries separating further on.

No previous description of the branchial and pharyngeal arteries of Ambystoma has been found in the literature.
Fourteen injections of large and medium *Plethodon glutinosus* were examined in preparation for this study.

The pharyngeal arteries are remarkably similar to those of *Ambystoma*, and only the differences and certain other major points will be stated here.

Normally four aortic arches are present, although occasionally a specimen will have the 'third' arch fused with the systemic.

The carotid arch gives off the internal and external carotids as in *Ambystoma*, although no vestige of a carotid bulb has been observed in any specimen examined. At its origin the external carotid directs itself mesially, then makes a posterior loop before continuing anteriorly to the lower jaw. The distribution of the external and internal carotids are as in *Ambystoma*.

The systemic arch passes laterally, dorsally, and then mesially as in *Ambystoma* and turns posteriorly before joining its mate to form the dorsal aorta. The ascending pharyngeal in this form arises from the systemic somewhat closer to the mid-line than in *Ambystoma* and seems to originate in a posterior direction before
turning anteriorly. In well injected specimens a branch is seen to arise from this artery a little behind the angle of the jaw which immediately divides into an anterior and posterior ramus which parallel the ascending pharyngeal superficially for a short distance. The palatino-nasal and vertebral arise as before except that the former now arises from the anterior rather than the dorsal surface of the systemic like in Ambystoma.

The 'third' arch parallels the systemic, receives the ductus Botalli, and joins the systemic sooner than in Ambystoma.

Just after leaving the truncus arteriosus, the pulmonary arch in Plethodon gives off two branches to the esophagus. It gives off the ductus Botalli sooner and directs itself more definitely posteriorly than in Ambystoma. After giving off the ductus Botalli, the pulmonary in a wide curve comes to lie parallel with the dorsal aorta and somewhat ventral to it. In this lungless form the pulmonary supplies the esophagus and intestine. No homologue of the esophageal of Ambystoma has been observed in Plethodon.
Although no description has been found in the literature of the circulatory system of Plethodon, of interest in this connection is the work of Seelye (1906) on Desmognathus fusca (also of the family Plethodontidae). In her drawings Seelye figures the ascending pharyngeal (which she calls the maxillary artery) as receiving a branch from the external carotid before passing to the angle of the jaw. The artery which she labels the ascending pharyngeal stems from the pulmonary arch and is homologous with the esophageal artery of Plethodon. The ascending pharyngeal, in reality, stems from the systemic arch.
The following description has been made from examination of six injections of *Triturus viridescens*.

The pharyngeal arteries of *Triturus* are remarkably similar to those of *Ambystoma* and *Plethodon*, and only the differences and certain other major points will be mentioned here.

In the present study no specimen of *Triturus* has been observed to have more than three branchial arteries, the 'third' having fused with the systemic.

The carotid bulb is present, and the external and internal carotids arise as in *Ambystoma*.

In *Triturus* the systemics proceed posteriorly a good distance behind the level of the pulmonary before fusing to form the dorsal aorta.

The ascending pharyngeal arises more mesially even than in *Plethodon*. The vertebral arises on the cranial side of the systemic in conjunction with the palatino-nasal, and it may be confluent with it for a short distance anteriorly before turning posteriorly and dipping into the vertebral column.

In one specimen the ascending pharyngeal was ob-
served to have anastomosed with the internal carotid.

The pulmonary gives rise to the short ductus Botalli which joins the systemic much proximal to the junction in either Ambystoma or Plethodon. After bending posteriorly the pulmonary gives off the esophageal artery slightly anterior to the origin of this same vessel in Ambystoma.

Francis' (1934) description of the pharyngeal arteries of Salamandra (also of the family Salamandridae) is almost identical with that given above for Triturus. However, Francis finds one or two small cutaneous branches as well as the lateral pharyngeal artery arising from the ductus Botalli; these branches have not been observed in the present study.
The following study was made from examination of injections of three large *Amphiuma tridactylum*.

The pharyngeal arteries of *Amphiuma* are somewhat different from those of the three previously described urodeles. Three aortic arches persist, and no remnant of the 'third' arch has been found.

The carotid arch is directed antero-laterally at about a 45° angle. A well developed carotid bulb is present, and the external carotid takes origin from the dorso-mesial edge of the latter structure. The internal carotid makes its loop dorsally and then proceeds toward the head region. The internal carotid receives a large branch from the systemic which parallels the internal carotid a short distance before anastomosing with it, somewhat posterior to the angle of the jaw.

The large systemic arch parallels the carotid as it leaves the truncus arteriosus. This arch makes its dorsal loop and then directs itself posteriorly to a position slightly anterior to the fore limbs where it fuses with its mate from the opposite side to form the dorsal aorta. No ascending pharyngeal is present as
such. However, the large aforementioned branch of the systemic which fuses with the internal carotid takes origin from the same locus as the ascending pharyngeal and has a large side branch supplying the region normally supplied by the side branch of ascending pharyngeal in Plethodon. The vertebral in Amphiuma arises as a posterior branch of the palatino-nasal after the latter has progressed in an antero-mesial direction for quite some distance from its origin on the systemic.

The pulmonary arises from the truncus arteriosus at the level of the fore-limb and immediately loops posteriorly. No ductus Botalli is present.

In his description of the circulatory system of Amphiuma, Baker (1945) correctly shows the three aortic arches, the carotids, vertebral, and complete absence of a ductus Botalli. However, his drawings do not clearly show the connection between the systemic and internal carotid which has been found in the present study.
The following description of the pharyngeal arteries of Siren lacertina was made from study of injections of six large specimens.

The vascular systems of Siren and of Necturus are of particular interest because of the information which they yield regarding the ontogeny and phylogeny of these amphibians and which will be discussed in detail later.

In Siren three aortic arches are present, and no trace of a 'third' arch has been found in any specimen examined. External gills are normally present in adult Siren, and in the following discussion the carotid, the systemic, and the pulmonary arches will be referred to as afferent and efferent branchials I, II, and III.

The first afferent branchial artery arises from the truncus arteriosus as in Amphiuma and passes antero-laterally to the first gill where it gives off generally nine paired lateral branches which in turn give off lateral capillaries within the filaments of the gill. Before reaching the gill the first afferent gives off from seven to nine small connections to the ventro-mesial continuation of the first efferent.
Proximal to the gill by-passes the first efferent turns anteriorly as the external carotid and gives off a mesial branch which parallels the first afferent to the mid-ventral line where it anastomoses with its homologue from the opposite side and continues posteriorly supplying the walls of the truncus and the conus arteriosus with blood. The efferent rami in the first gill unite into one vessel which joins the ventral loop of the first efferent distal to the gill by-passes. After receiving the efferent branch from the first gill, the first efferent branchial makes its dorsal loop, heads mesially and somewhat posteriorly and about halfway to the mid-line it suddenly turns anteriorly as the internal carotid. At the angle of origin of the internal carotid a branch is received which takes origin from the fused second and third efferents.

The second afferent branchial parallels the first and passes to the second gill, giving off paired side branches as in the case of the first gill. The second efferent arises from small efferent rami in the gill and after receiving the third efferent, progresses mesially and then posteriorly to where it fuses with
its mate from the opposite side to form the dorsal aorta. Just after leaving the gill the second efferent receives blood by means of a large loop which arises by from seven to nine small connections with the second afferent immediately proximal to the gill. Just after the second efferent receives the third efferent it gives off the large aforementioned branch to the internal carotid which is probably homologous with the ascending pharyngeal. (See Plate 1.A.)

As in Amphiuma the vertebral arises as a posterior branch of the palatino-nasal after the latter has progressed anteriorly for some distance.

The third afferent arches anteriorly and then posteriorly into the third gill which has the same sort of structure as the first two gills. The third efferent originates as ram in the gill and after leaving the gill in a mesial direction, turns abruptly forward to where it fuses with the second efferent. The pulmonary artery arises as a mesial continuation of the third efferent just after the latter emerges from the gill. It presently turns posteriorly to supply the lung. Normally four rather large connections
proximal to the gill unite the *third afferent* with the *third afferent* and *pulmonary artery*.

It should be noted that for every gill, shunts or *gill by-passes* are present which permit the blood from the heart to reach the general circulation without having to enter the gills.

Cope (1889; Plate XXI) shows the three aortic arches in *Siren*. Although his drawing is accurate in what is included, Cope omits the *vertebral* and *external* and *internal carotids* in his drawing, which is greatly out of proportion.
This description has been made from examination of eight injections of medium-sized specimens of *Necturus maculosus*.

The *first afferent* passes laterally and then dips posteriorly into the first gill where it gives off 10 to 15 unpaired branches from its ventral aspect. Each of these branches breaks up into a considerable number of bush-like capillaries in the gill filaments. The structure of the *efferent* in the gill closely resembles that of the *afferent*. After passing from the gill the *efferent* continues on the ventral side in an antero-mesial direction as the *external carotid* to supply the middle portion of the lower jaw. A short distance proximal to the gill the *external carotid* receives a small connection from the *first afferent*, which may be multiple (see below). This, obviously, is the persisting *gill by-pass* which in *Siren* is always multiple. About midway between the gill and the *gill by-pass* the *external carotid* gives rise to a small, but often conspicuous vessel which
passes directly anteriorly supplying the lateral muscles of the pharyngeal region. Midway between this latter vessel and the first gill the first efferent turns dorsally, arches posteriorly receives a branch from the fused second and third efferents and continues anteriorly as the internal carotid.

The second and third afferents are fused at their origin and after proceeding antero-laterally for a short distance, arch somewhat posteriorly, divide into two branches and proceed to the second and third gills respectively. The second and third gills resemble the first in the structure of the afferent and efferent rami. After leaving the gills the second and third efferents unite to form the dorsal systemic arch. This arch proceeds anteriorly for a short way, and then posteriorly to the level of the fore-arm to fuse with its mate from the opposite side forming the dorsal aorta. Just before joining the third efferent, the second efferent receives a branch from the second afferent, a gill by-pass. Immediately after the junction of the second and third efferents, a posterior branch is given off, the pulmonary artery which supplies the lung. This vessel sends numerous small side-branches
to the esophagus. Shortly before the dorsal systemic arch swings posteriorly, it gives rise to a rather large vessel which passes antero-laterally to fuse with the internal carotid. This vessel and a similar one in Siren are evidently homologous with the ascending pharyngeal of the metamorphosed urodèles. About half way between this vessel and the mid-ventral line the dorsal systemic arch gives rise to the palatino-nasal. This artery, after proceeding anteriorly for some distance, gives off a large posterior branch, the vertebral.

Miller (1905) correctly figured and described the pharyngeal circulation of Necturus, but his main drawing is somewhat confusing in that it shows the external carotid arising from the first afferent rather than the first efferent branchial arch on the left side. His drawings also show multiple gill bypasses; in all specimens examined in the present study they were either single or absent.

Noble (1931) reproduced the drawing of Miller, but he labelled the external carotid only on the left side, which shows it stemming from the afferent, and in the context he described the vessel as arising from the
afferent. Subsequent authors (Hyman, 1947), (Eddy, Oliver, and Turner, 1947), (Turtox quiz sheet), etc. incorrectly show the external carotid stemming from the afferent rather than from the efferent.

In the present study four specimens of Hecturus were injected by way of the ventricle alone. In these injections none of the efferent arteries or their branches (including the external carotid) were found to be injected, even though the afferents were completely injected. Injections by way of the dorsal aorta alone left only the efferents and their branches injected. In these injections the external carotid was completely injected. Double injection with contrasting colors showed definitely that the external carotid stems from the efferent, although it may closely parallel the afferent dorsally for a good distance before continuing to the lower jaw.
COMPARISON OF THE PHARYNGEAL AND BRANCHIAL ARTERIES
OF URODELA

In the present section each of the pharyngeal arteries will be discussed separately, and comparisons will be made of the modifications which have occurred in the six families herein considered. Special attention will be directed to those features which appear to be of phylogenetic significance.

Ontologically the urodeles constitute an extremely heterogeneous group. (See Table I). On the basis of structure and physiology Noble (1931) considers Siren a form which has "ceased to differentiate beyond a very early stage in larval life;" Necturus, as having "reached a later stage of urodele ontogeny;" Amphiuma, a form which has "nearly completed" metamorphosis, and Triturus, Ambystoma, and Plethodon, forms which have reached and remained in the adult state. It is highly desirable in the present comparison to consider the urodeles in the above-mentioned order. For when considered in this order, few postulations are necessary to complete the picture of the ontogenesis (and, perhaps, the phylogensis) of the aortic arches and their derivatives in the urodeles.
It should be noted, however, that even though *Siren*, *Necturus*, and *Amphiuma* are sexually mature forms which in most other respects have not completed their metamorphosis, they are all retrogressive forms whose ancestors were fully metamorphosed and terrestrial. This phenomenon of 'larvation' is not at all uncommon among the urodeles, but has occurred independently to greater or less degree in some forms of every existing family.

Nor is the degree of larvation the same in all neotenic forms, or even, necessarily, in all portions of the same individual. Sexually mature *Siren*, for instance, exhibits the long, keeled tail, absence of hind limbs, and pelvic girdle, the hypobranchial apparatus, and certain cranial ossifications characteristic of a very early larval form, while possessing fully metamorphosed skin. In addition, its gills are not those of a larva (which *Siren* develops and loses before it is five inches long), but are redeveloped gills, (Cope, 1885) different in structure and texture from those of any other urodele larva.

It is often difficult to distinguish between those characteristics which are larval, primitive, degenerate, specialized or advanced. Particularly is this so in
the urodeles in which the ontological stages are arrayed so haphazardly and since they have not given rise to any so called 'higher' group of organisms. Before proceeding, therefore, it is well to define these terms. **Primitive** refers to a condition present in the adult of an ancestral form. **Larval** refers to the condition of a structure in what is normally considered an immature form. A larval structure may be practically identical with its homologue in the adult of an ancestral form. More often, however, it resembles the condition present in the larva of an ancestral form. For this reason it is far more profitable to compare individuals in the same ontogenetic state of development than it is to compare the larva of one with the adult of another. **Degenerate** is used to refer to regressive characteristics, loss or atrophy of certain elements normally present and functional in the ancestral form. **Advanced** will be used in this discussion to refer to characters which are present in adult, terrestrial forms of *Ambystoma*, *Plethodon*, and *Triturus*, since they are farthest removed from the ancestral condition. **Specialized** refers to a characteristic which is adaptive in nature and in particular cases may be synonomous with any of the
other four terms.

Primitively the urodeles possessed four aortic arches, the 3rd, 4th, 5th, and 6th, although the number has been reduced to three independently in several different groups. This reduction may generally be regarded as a specialization. Among the forms considered Siren, Necturus, Amphiuma, and Triturus have all independently lost the 'third' arch, presumably in adapting themselves to their long life on land.

If attention is directed to the external carotid, it will be seen to arise in Siren and Necturus as the ventral continuation of the efferent portion of the 3rd aortic (carotid) arch. Proximal to the gills it receives connections from the afferent. These connections are always multiple in Siren, and at least sometimes multiple in Necturus. After the urodeles has lost its gills, these gill by-passes may enlarge somewhat and become twisted around each other forming a labyrinth-like structure which serves to reduce the pressure of the blood going to the head. This structure is here called the 'carotid bulb' in preference to the term in common use, the 'carotid gland', because the
latter term implies that the structure is glandular in nature, which it surely is not. When the gills are lost at metamorphosis, the afferent joins the efferent directly through the carotid bulb, and the external carotid of the metamorphosed form stems from the mesial aspect of the bulb. (See Plate I.B,C,D,E.)

Passing to the systemic, it may be seen that in Siren and Necturus, gill by-passes are also present here to take over the function of connecting the afferent with the efferent when the gills are lost. This connection is normally multiple in Siren, but single in Necturus. In Amphiuma, Ambystoma, Triturus, and Plethodon, the afferent is continuous with the efferent, and the connection is single as in Necturus, not multiple as in Siren which has recently lost its gills.

The ascending pharyngeal artery is not present as such in Siren, Necturus, and Amphiuma. Instead, in these forms, a vessel, presumably homologous with the ascending pharyngeal, takes origin from the systemic and, after a distance, joins the internal carotid. This vessel is probably a persistence of the dorso-mesial continuation of the primitive 3rd aortic arch, which has remained to connect the carotid with the systemic after the mesial portion of the arch has
become fused with the **systemic**. In *Siren* the length of this connection is only slightly greater than its diameter, but in *Necturus*, it is somewhat longer. In both of these forms, it proceeds laterally from the **systemic** as would be expected if the vessel were a persistence of the primitive 3rd **aortic arch**. In *Amphiuma* this connection is much longer than in either of the two previous forms, and it closely parallels the **internal carotid** for a distance before anastomosing with it shortly behind the angle of the jaw. This vessel sends off a side branch in *Amphiuma* which in turn divides into an anterior and a posterior branch which parallel the **internal carotid** peripherally. In *Plethodon* a similar branch has been observed to arise from the **ascending pharyngeal artery**. In *Siren*, *Necturus*, and *Amphiuma* the **internal carotid** supplies not only the cranium, but also the periphery of the lower jaw. In *Ambystoma*, *Plethodon*, and *Triturus* the aortic ramus has become completely separated from the **internal carotid**, and this vessel, now the **ascending pharyngeal**, relieves the **internal carotid** of its duty of supplying the periphery of the lower jaw. The factors responsible for the ultimate separation
On the basis of these considerations, the conclusion has been reached that the differences which exist between the groups are constant differences and that the alterations which have been noted are the result of slow, long-range transformations which may be correlated with major changes in the structure and in the physiological make-up of the organisms involved.

There has been demonstrated in the present study a fairly continuous series which includes the more primitive state of the pharyngeal arteries as exhibited by *Siren* and *Necturus*, the intermediate condition as found in *Amphiuma*, and the more advanced condition as found in *Ambystoma*, *Triturus*, and *Plethodon*. And even among the last three, small but constant differences have been found to occur.

While the series may be regarded as purely ontogenetic, this interpretation presents the following difficulties. (1) Sexually mature *Siren*, *Necturus*, and *Amphiuma* presumably represent different stages of ontogenesis, yet they exhibit marked similarity in the condition of the vertebral and palatino-nasal arteries. In all these forms the vertebral passes anteriorly for a good distance before looping posteriorly to dip
of this vessel from the carotid are not immediately apparent. However, it should be pointed out that the internal carotid of Siren, Necturus, and Amphiuma receives blood from two sources, from its own ventral connection with the carotid bulb, and from the systemic by way of the connecting ramus previously referred to.

In Siren, Necturus, and Amphiuma the vertebral artery passes forward for a good distance before turning posteriorly and dipping into the vertebral column. An anterior branch of this vessel supplies the roof of the mouth. In Plethodon and Ambystoma this vessel has migrated posteriorly to assume the status of a separate artery, the palatino-nasal, which now arises from the systemic independently of the vertebral. The vertebral in these two forms passes directly posteriorly from its origin on the systemic. Triturus exhibits a condition which is intermediate between that in Amphiuma, Siren, and Necturus, on the one hand, and in Ambystoma and Plethodon, on the other. Its condition, however, is much closer to that of the latter two.

The 5th aortic or 'third' arch is variable, and its persistence in Ambystoma and Plethodon may be regarded as vestigial.
The 6th aortic or pulmonary arch is present in all forms studied, although in Necturus the proximal portion has been lost in correlation with the failure of the last branchial cartilage to develop. (Noble, 1931). The primitive condition is well exemplified in Siren which possesses external gills, gill by-passes, a ductus Botalli, (which is the efferent portion of the pulmonary arch), and pulmonary artery which is a posterior continuation of the efferent portion of the pulmonary arch. Necturus is similar to Siren except that no gill by-passes have been noted, and the pulmonary artery stems from the fused efferents of the 'third' arch and pulmonary arch. Amphiuma, Ambystoma, Triturus, and Plethodon have all lost the gills, hence the efferent joins directly with the afferent and continues posteriorly as the pulmonary artery. The ductus Botalli is present in all these forms except Amphiuma.
The vessels under consideration in this study represent the main trunks of the arterial system. These trunks lie deep within the body and, for the most part, are not associated with supplying blood to any specific muscle, bone, or gland, but to general regions of the body. Because of their function and position, these vessels are far more stable and less subject to changes brought about by slight modifications in the other body components than are the peripheral vessels.

In this study remarkably few variations have been encountered, even though they have diligently been sought, and most of those which have been found are of minor import, such as slight shifts in point of origin or junction of certain of the vessels. Since in most cases significant numbers of specimens were used and relative constancy was found among practically all specimens, it is felt that error due to anomalies is reduced to a minimum.
into the vertebral column. At its anterior extremity the vertebral gives rise to the palatino-nasal.

(2) Sexually mature Triturus, Ambystoma, and Plethodon have all reached the adult state of ontogenesis, yet they differ among themselves in the degree of confluence of the vertebral and palatino-nasal and in the method of origin of the vertebral. In Triturus the two vessels are confluent for a short distance forward before separating. In Ambystoma the vessels arise together from the dorsal aspect of the systemic (rather than the anterior aspect as in the four proceeding forms), and separate immediately after leaving the systemic. In Plethodon they arise separately, the vertebral stemming from the posterior and the palatino-nasal from the anterior aspect of the systemic.

(3) Necturus and Siren, although representing different ontogenetic stages, exhibit almost the same degree of separation of the ascending pharyngeal and internal carotid. In both of these forms the ascending pharyngeal is fairly short, and it progresses in an antero-lateral direction, that is, it is 'aimed' in the direction of the external carotid. In all the other forms it is either a separate artery, or it
parallels the internal carotid before the junction.

(4) Preliminary investigation of the axolotl, or neotenic Ambystoma, indicates the adult condition of the vertebral, palatino-nasal, and possibly of the ascending pharyngeal.

Although thorough examination of the immature forms of all the types used will be necessary before a final judgement can be made, preliminary evidence indicates that the characteristics observed are not entirely determined by the ontogenetic state of the individuals, but that the phylogenetic state is, at least in part, indicated.

On the basis, primarily, of the status of the ascending pharyngeal, the vertebral, and the palatino-nasal, the following relationships are suggested.

All the urodeles are descended from a single terrestrial ancestral line. The earliest departures from this line were Siren and Necturus. Siren was, perhaps, the first to stem away from the main urodele line, but Necturus seems to have followed shortly, and both have undergone considerable change since their divergence began. Amphiuma was the next to leave the main line. In becoming adapted to its unique habitat it has also undergone considerable specialization.
Ambystoma, Triturus, and Plethodon form a fairly homogeneous group, but even among these forms some differences exist. Triturus and Ambystoma seem to be at approximately the same advanced phylogenetic stage, while Plethodon seems to be even further removed from the condition of Siren, Necturus, and Amphiuma. This similarity between Triturus and Ambystoma argues for a fairly recent common ancestry of the two groups.

The views herein presented find support from the work of other investigators on the subject, although different views have also been presented. Reed (1920) on the basis solely of the morphology of the sound-transmitting apparatus put forth the suggestion that the urodeles comprise two 'legions', the first embracing the Ambystomidae, Sirenidae, and Salamandridae, the second including the Necturidae (=Proteidae), Amphiumidae, and Plethodontidae. Dunn (1922), however, reviewed the work of Reed and pointed out that Reed's observations are subject to a different interpretation. On the basis of further work he reached the conclusion outlined below which, he says, "seems to agree quite as well with the otic apparatus and far better with other anatomical features."

"The Sirenidae are the most isolated group. Scarcely
a character can be found to ally them with any one or another of the main stocks.

"The Proteidae are only slightly less isolated.

"It is quite possible that this genus (*Amphiuma*) is descended from primitive salamandrids.

"Within the superfamily *Salamandroidea* the *Ambystomidae* and the *Salamandridae* are about parallel.

"It is probable that some primitive Salamandrid gave rise to the much degenerate *Plethodontidae*.

Noble (1931) in his textbook on the *Amphibia* prefers to include the *Amphiumidae* with the *Salamandridae* and *Plethodontidae* within the suborder *Salamandroidea* and to assign each of the other families, *Ambystomidae*, *Proteidae*, and *Sirenidae* to separate suborders. Although Noble's book has received wide publicity, it was admittedly, "written primarily to introduce the student to the biology of both frogs and salamanders," and his conclusions are certainly not considered inviolable.

Inasmuch as the present work upholds and adds to the conclusions drawn by Dunn, his classification, slightly modified, is reproduced here as the best existing interpretation of the evidence concerning the relationships of the urodeles. (See Plate 1.F.)
Suborder I..................Meantes
  Family 1..................Sirenidae
Suborder II...............Proteida
  Family 2..................Proteidae
Suborder III.............Amphiumoidea
  Family 3..................Amphiumidae
Suborder IV..............Salamandroidea
  Family 4..................Ambystomidae
  Family 5..................Salamandridae
  Family 6..................Plethodontidae
SUMMARY

By means of a new and improved injection technique a study has been made of the pharyngeal and branchial arteries of representatives of six families of the order URODELA.

Descriptions are given of the branchial and pharyngeal arteries of each of the six families, and a comparison is made of the condition of the arteries in the different families.

Phylogenetic evidence is pointed out which supports the view that the Sirenidae and the Proteidae were the first of the existing forms to stem from the main urodele line, that the Amphiumidae came off somewhat later, and that the Ambystomatidae, Salamandridae, and Plethodontidae comprise a rather homogeneous group of recent divergence.
BIBLIOGRAPHY


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A. The Afferent Branchial Arteries of *Siren*  
(Note the short, thick connection between the internal carotid and the systemic.)

B. The First Aortic Arch of *Siren* which has lost its gills  
(Note the reduction in number, the thickening, and the twisting of the gill by-passes.)

C. The Second Aortic Arch of *Siren* which has lost its gills

D. The Third Aortic Arch of *Siren* which has lost its gills

E. The First Aortic Arch of *Amphiuma*  
(Compare with Fig. B.)

F. Phylogenetic Tree of Urodela
The Pharyngeal Arteries of *Ambystoma*  
(Note the presence of the ascending pharyngeal; palatino-nasal and vertebral arise separately.)
PLATE 1

SALAMANDRIDAE

PLETHODONTIDAE

AMBYSOMIDAE

AMPHIUMIDAE

SIRENIDAE

PROTEIDAE

B. E.

C.

D.
PLATE 2.
The Pharyngeal Arteries of *Plethodon*
(Note the presence of the ascending pharyngeal; palatino-nasal and vertebral arise separately; esophageal and pulmonary arteries supply esophagus in this lungless salamander.)
The Pharyngeal Arteries of *Triturus*  
(Note the presence of the ascending pharyngeal; palatino-nasal and vertebral arise together.)
PLATE 4.
The Pharyngeal Arteries of Amphiuma
(Note the absence of the ascending pharyngeal, but the presence of the long connection between the internal carotid and the systemic; vertebral arises as a posterior branch of the palatine-nasal.)
PLATE 5.
The Pharyngeal and Branchial Arteries of Siren
(Compare with PLATE 1.)
(Note the absence of the ascending pharyngeal, but the presence of the short, thick connection between the internal carotid and the systemic; the vertebral arises as a posterior branch of the palatino-nasal.)
The Pharyngeal and Branchial Arteries of *Necturus*
(Note the absence of the *ascending pharyngeal*, but
the presence of the short, thick connection between
the *internal carotid* and the *systemic*; the *vertebral*
arises as a posterior branch of the *palatino-nasal.*)