THE NEURO-HUMORAL CONTROL OF CIRCULATION

or

THE MORPHOLOGY AND PHYSIOLOGY OF THE VASCULAR CHANGES INVOLVED IN GILL REDUCTION IN SALAMANDERS.

By

Frank Henry John Figge

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ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

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INTRODUCTION

The most fundamental fact concerning the physiology of the vascular system is that the blood travels in a circle or circulates. This discovery was made by William Harvey (1628). It is the more remarkable, since it is evident that Harvey demonstrated the functional existence of capillaries long before they could be shown to exist anatomically (Malphighi, 1661). The problem that presented itself as a result of Harvey's discovery, was to determine the mechanisms by which the circulation of blood is controlled. Considerable advance has been made in the solution of the problem, but it is far from solved and each new discovery seems to complicate, rather than simplify, our knowledge of the mechanisms that control circulation. While we cannot aspire to definitely clear up this fundamental problem; it is hoped that the following work will contribute a few facts that will share in its final elucidation.

The circulatory system of a vertebrate may be divided, according to our present day conceptions, into a number of subordinate systems or organs. This division is based on anatomical and physiological considerations. There is in every case a propulsive organ, the heart; a distributive organ, the system of arteries; an organ for interchange of substances, the capillaries and lymphatics; and an organ for collecting

blood and returning it to the heart, the venous system, (Krogh, 1929). Much work had been done to determine the rôle played by each of these individual parts in controlling the circulation of blood. The factors by which these subordinate parts of the system are controlled, are being extensively investigated at the present time. Most of this work concerns itself with the mechanisms responsible for variations of short duration in the relative size or diameter of small and large vessels, in the rate of flow of blood, and of the pressure in the various parts of the system. Comparatively little physiological work has been done to show what factors operated to bring about some of the drastic morphological and physiological changes in the vascular system that occurred during phylogeny.

One of the most interesting episodes in the phylogeny of vertebrates deals with the change from water to land forms. This entailed a profound modification of the vascular system. It meant the development of a new respiratory organ and the loss of the old one. Fortunately, there exist to-day a number of forms of Amphibia that reproduce for us this chapter in our phylogenetic history. The period in the ontogeny of an amphibian that depicts this chapter in phylogeny is known as the metamorphic period. Most of the changes that take place during this period are all well known and will not be discussed here. Corresponding to this change in Amphibia, there occurs during the development of nearly every pulmonate vertebrate, a shifting of the respiratory function to the lungs.

Many of the vessels which carry blood low in oxygen content before this shifting, afterwards carry oxygenated blood and vice versa. The resulting vascular changes are usually explained by the assumption that they are due directly to the alteration in blood pressure or other mechanical processes such as negative pressure in the thorax.

While the shifting of the site of oxygenation of blood occurs during the ontogeny of all vertebrates above fishes, it is in the Amphibia that we find the most complete picture of this chapter of our development. When an amphibian changes from an environment of water to one of air, it is necessary to shift the respiratory surfaces from the gills to other organs. This involves the most radical modification of the vascular system. An attempt will be made in this work to determine some of the factors or mechanisms that bring about these ontogenetic morphological changes in the vascular system.

Before venturing into the physiology of these changes, it is necessary, first, to have a clear picture of the most minute details of the morphology of the vascular and respiratory systems in the larval amphibian; and second, to know precisely what morphological changes occur. The first part of the paper will, therefore, be a detailed description of the vascular and respiratory systems of larval and adult forms. The morphological relations of the nervous system to the vascular system, in so far as it has been demonstrated, will also be given in this

part of the paper. The second part of the paper will describe the physiology of the branchial circulation: The first section will deal with the nervous control of branchial circulation, the second section with the humoral control of circulation.

It is hoped that a study of the various parts of the vascular system involved in the changes, and their reactions to nervous and humoral stimuli will lead to certain facts that might enable us to discover the causes for these changes. Specifically, then, these problems have been formulated:

1. What are the factors that cause the gill vessels to be obliterated and other vessels to take their place as respiratory vessels; and

2. What relationship is there between the morphogenetic process of gill reduction and the vascular reactions?

MORPHOLOGICAL REVIEW

I. THE MORPHOLOGY OF THE RESPIRATORY PORTION OF THE BLOOD VASCULAR SYSTEM IN AMBLYSTOMA TIGRINUM LARVAE.

The physiological section of this paper would be quite unintelligible without a knowledge of the morphology of the animal used in these experiments. For this reason, a few of the essential morphological facts will be reviewed. While the entire description is based upon the investigations and dissections of the author, many of the facts to be presented here have been described previously. An attempt will be made to give credit where credit is due. The few facts which have not been described elsewhere, to the author's knowledge, will be summarizd at the end of the morphological section.

A. The Heart.

General

The heart of a fish is usually described as a two-chambered heart. It receives only reduced (venous) blood and has the task of pumping this in one continuous circuit through the gills and systemic vessels. The Dipnoi are exceptions, in that the atrium and bulbus cordis are partially divided in correlation with the development of lungs. The division of the atrium and bulbus cordis is carried even farther in Amphibia. In the Reptiles, the heart is almost completely divided into the four-chambered heart, such as found in Birds and Mammals. It is clear that the phylogenetic development of septa in the cardiac tube was an attempt

to separate pulmonary from systemic blood. This was carried to completion only in Birds and Mammals. Benninghoff (1933) summarizes this as follows:

1. Branchial heart, (Fishes).
2. Branchial-lung heart, (Dipnoi).
3. Branchial-lung-skin respiration heart, (Amphibia).
4. Lung-heart with limited respiratory capacity of lungs, (Reptiles).
5. Lung-heart with full respiratory capacity of lungs, (Birds and Mammals).

Specific.

The morphology of the heart and aortic arches in A. tigrinum have been briefly described in previous papers. (Boas, 1882 and Figge, 1930). These will be reviewed and certain details added that are necessary to appreciate the physiology of these structures.

A. tigrinum, there is a septum that divides the atrium into two unequal parts. The systemic and cutaneous veins empty by way of the sinus venosus into the larger more cephalad portion (right atrium), while the pulmonary veins open into the smaller portion. There is no septum in the ventricle, but the trabeculae carnae form a reticulum in the cavity of the ventricle which tends to minimuze the mingling of the two types of blood. (Züllich, 1930; Kingsley, 1917). The bulbus cordis is also separated into a pulmonary and systemic portion by a spiral fold. This causes the blood from the right atrium to be sent to the lungs, while most of the blood coming from the lungs is sent to the carotid arch, (Brücke, 1852; Sabatier, 1873). Quite recently, Züllich has described the heart of A.

maculatum. It is a much more detailed description than the above, but coincides at all points. On the basis of observations made on the living heart, he also concludes that the spiral fold tends to keep the two kinds of blood (pulmonary and systemic) separated. It was to emphasize this point that a description of the heart was included in this paper, because many of the conclusions and hypotheses are based on this fact.

B. The Aortic Arches in the Larva.

In the larval form, four aortic arches are present on each side. They correspond to arches three, four, five, and six of the vertebrate scheme. Arches three, four, and five carry blood to a gill, while the sixth arch forms a direct communication between the heart and the lung. All of the aortic arches are interrupted by an anastomosis. The size and arrangement of the vessels forming this anastomosis vary, but are characteristic for each arch. They will be described later. The aortic arch or primary circuit consists of a large ventral vessel taking origin from the bulbus arteriosus, and a large dorsal vessel that joins one of the radix aortae, and an intermediate communicating anastomosis. The afferent and efferent branchial vessels are connected to the arch near the ventral and dorsal connections of the anastomosis respectively, (See Figs. 3, 5, 18) The afferent branchial or distributing vessel of each gill sends an arteriole into each gill filament. This arteriole extends as a continuous vessel around the

margin of the filament. A large number of capillaries connect the afferent and efferent limbs of this arteriole, forming a capillary network between the two limbs of the arteriole loop. (See Figs. 10, 11, and 18). The efferent arterioles unite to form the efferent branchial vessel which conveys the blood to the dorsal portion of the aortic arch. This break-up and re-collection in the external gill is known as the secondary or branchial circuit.

The individual aortic arches and gills will be described in detail. It has already been mentioned that the aortic arch or primary circuit consists of large ventral and dorsal portions connected in the larvae, by an intermediate anastomosis that varies in form and size in different arches of the same animal, (See Figs. 4, 5, 6 and 7). The anastomoses on corresponding arches of different animals, however, show considerable uniformity.

The Third Arch Anastomosis.

The anastomosis on the third aortic arch is better developed and more extensive than any of the others. This is the carotid arch and is associated with the first gill. The anastomosis consists of a number of small arterioles that take origin from the large ventral portion of the arch. Two or three of these may unite to form a larger trunk; or a single arteriole may extend from the large ventral vessel to a large vessel that lies just dorsal to it. This large vessel must also be considered to be a portion of the intermediate anastomosis. Its dorsal end communicates with the efferent branchial vessel which,

at the point of junction, becomes the dorsal portion of the carotid arch. The ventral end of this large anastomotic vessel communicates with the ventral or external carotid. (See Fig. 4)

The Fourth Arch Anastomosis.

The intermèdiate anastomosis of the fourth aortic arch, or the second gill arch, is also well developed. (This anastomosis later forms a part of the large systemic arch). The anastomosis consists of a number of small arterioles that take origin from the ventral portion of the arch. These unite to form a vessel of considerable size that passes dorsally to communicate with the junction of the efferent branchial artery of the second gill and the dorsal portion of the fourth aortic arch. (See Fig. 5).

The Fifth Arch Anastomosis.

The fifth arch anastomosis is not well developed, and this arch is eliminated at the time of metamorphosis. It consists of only a few small arterioles, sometimes only two or three take origin from the ventral portion of the arch. These may unite to form a vessel that communicates with the junction of the efferent branchial and the dorsal portion of the aortic arch; or it may unite with one of the collecting branches of the efferent branchial vessels. (See Fig. 6)

The Sixth Arch Anastomosis.

The anastomosis on the sixth or pulmonary arch consists, in the author's opinion, of two portions: a bi-polar anastomosis that corresponds to a branchial circuit, and a uni-polar anastomosis that corresponds to the true intermediate anastomosis of the sixth aortic arch. The ventral portion of the pulmonary arch divides, as soon as it comes in contact with the visceral arch, into a large vessel that ramifies in the gill blade, and one or two small arterioles that follow the visceral arch. The large vessel that ramifies in the gill blade is considered to be homologous to the afferent branchial vessel. The numerous branches of this vessel reunite in the dorsal portion of the gill blade to form another large vessel which is homologous to an efferent branchial vessel. This joins the dorsal portion of the aortic arch. The ventral and dorsal portions of the arch are also connected by the small vessels that accompany the visceral arch. These are smaller and appear to be constricted where they communicate with the ventral portion of the arch. The pulmonary artery takes origin from the dorsal portion of the aortic arch. The extreme dorsal portion is called the ductus arteriosus. (See Fig. 7).

The following facts are significant and will be referred to later. In the larval state, the anastomosis on the carotid or first gill arch is best developed and has the greatest capacity for shunting blood directly from the ventral to the dorsal portion of the arch. The fourth or systemic arch is

next in this respect, while the anastomosis on the fifth arch is able to transmit only a relatively small quantity of blood.

C. Gills.

The only work that deals specifically with the gills of A. tigrinum is one published in 1895 by Clemens. He made an historical survey of the work on gills in general. From this and his own work, he compared the different forms of external gills in both fishes and amphibians; and he contrasted them to the internal gills in these forms. In spite of the generalnature of the work, the description of the gills in A. tigrinum was given in considerable detail, and agrees at all points with the following description which was written independently.

The gills consist of two main parts: the gill stem and the gill filaments. The gill stem is a lateral projection from any gill arch. (See Fig. 23). It is made up of skin and loose hydrated connective tissue, in which the afferent and efferent branchial vessels are found. (See Fig. 8) Branches of the vagus and glossopharyngeal nerves pass out into the stem of the gill. These nerves have been traced to the skin of the stem, to the large branchial vessels, and even into the filaments. Large nerve branches also supply the levator and depressor arcuum muscles. The depressor arcuum muscle extends almost to the tip of the gill stem. (See Fig. 8) It is attached to the skin and vessels

along the course of the afferent branchial artery and its branches. The function of these nerves and muscles will be considered later.

The anatomy of the gill filament has been studied in great detail. The filaments are attached to the stem of the gill in two main rows. They are widest at the point of attachment which will be called the base. They are long and flat and taper toward the free end which will be referred to as the tip of the filament. The arteriole which takes its origin from the afferent branchial vessel and passes around the margin of the filament will be called the arteriole loop of the filament. The limb which carries blood to the filament will be called the afferent limb of the arteriole loop. The other limb will be called the efferent limb of the arteriole loop. (See Figs. 10 and 18).

Each filament is composed of this marginal arteriole loop with a network of communicating capillaries. (See Figs. 10 and 18.) These vessels, along with a mesh or network of connective tissue cells, form the core or framework of the filament. The melanophores are located in the central part of this; they are most numerous near the efferent limb of the arteriole loop at the margin of the filament. This core of vessels is covered by a continuation of the epidermis in the form of a simple ciliated epithelium. (See Fig. 9)

In making direct observations on the blood vessels of the filaments, it was noted that small particles that

floated in the water would, when they floated near a filament, suddenly dart away from it. More careful observations on the particles in the water showed that definite currents of water bathed the gill filaments. The physiological significance of these currents is easily seen; but what morphological structures were responsible for the currents? It was the search for the cause of the currents that revealed the cilia on the epithelium of the gill filament. Later it was found that Clemens (1895) mentioned that the epithelial cells covering the filaments of external gills are ciliated. He also discussed the physiology of these; he stated that the current of water created by the action of the cilia would enhance the respiratory exchange.

After many unsuccessful attempts, nerve fibers were demonstrated in the gill filaments, by means of a methylene blue technique. One of the factors that makes it difficult to demonstrate these nerves is that they are, in many cases, found only in a sheath formed by melanophores. This difficulty was partially overcome by injecting adrenalin to contract the melanophores, before attempting the nerve stain. The nerve fibers demonstrated in the filament appear to be unmyelinated or Remak's fibers. (See Fig. 12) No nerve endings were found, either because the nerve fibers observed end in the melanophores, or because the continuity of the fiber is not evident in the region where a melanophore is

located. More work is necessary before any conclusions can be drawn with regard to these nerves.

II. THE RELATION OF THE VAGUS AND GLOSSOPHARYNGEAL NERVES TO THE BRANCHIAL VESSELS IN A. TIGRINUM LARVAE.

The vagus and glassopharyngeal nerves have been described in detail in Amblystoma by Herrick (1894) and Coghill (1902). Herrick used A. punctatum; Coghill used A. tigrinum. The cranial nerves of other Amphibia have been described by Strong (Anura), (1895) and Kingsbury (Necturus), (1895). The terminology used by these investigators will be adhered to in the following description:

The vagus and glossopharyngeal take origin from the medulla oblongata, very near each other. As a matter of fact, it is impossible to distinquish between these nerves at the origin. Just caudal to the origin of the acoustic nerve is a large nerve root. This is, from Coghill's description, taken to be the lateralis or first root of the vagus. Just caudal to this, there are several (four) routlets which come out of the medulla at right angles, and one rootlet, which originates from the medulla, caudal to these at an acute angle. This receives very fine nerve filaments from the medulla. According to Coghill, the root just caudal to the lateralis root of the vagus is the glossopharyngeal. The four remaining roots are all vagus roots. All of these nerve roots apparently unite, or at least, pass through the chondrocranium along with the jugular vein. (See Fig. 13)

Immediately after leaving the chondrocranium, there is found a large ganglion. At this point, the nerves are so closely bound together that they are separated with difficulty. A few small nerve twigs take origin from this ganglion and pass dorsally to the skin in that region. Distal to the ganglion we find four main nerve trunks: One which is called the truncus glossopharyngeus; the two trunks just caudal to this are called the first and second branchial vagus trunks; the most caudal and largest branch contains the abdominal and cardiac portions of the vagus. The first and second branchial vagus trunks may arise from the ganglion as a common trunk, in which case there are only three main nerve trunks taking origin from the ganglion. Figure 13 is a diagram of the vagus and glossopharyngeal nerves in which the ganglion gives rise to four trunks. These four trunks will be described separately.

The Truncus Glossopharyngeus.

The glossopharyngeal divides near the otic capsule into two main branches. One of these passes cephalad as the Ramus communicans IX + X to VII. The other large branch follows roughly the internal carotid artery or dorsal portion of the third aortic arch. It gives off some small branches to this vessel, and then passes to the dorsal end of the third visceral arch. Here it gives off a branch to the first gill. It then continues along the ventral portion of the third aortic and visceral arch as the Ramus post-trematicus IX. Its ventral distribution will not be discussed. The branch

to the first gill sends branches to the levator and depressor arcuum muscles of the first gill and communicates with the branch of the first branchial vagus trunk to this gill. It sends numerous twigs to the afferent and efferent branchial vessels. Vagus fibers have been traced into the filaments of the first gill.

The First Truncus Branchialis Vagi.

The more cephalad or first branchial trunk of the vagus, after giving rise to the few fine nerve fibers to the dorsal portion of the fourth aortic arch, a motor branch to the second levator arcuum muscle, and a pharyngeal branch, passes dorsal to the dorsal end of the fourth visceral arch. At this point, it gives off a branch to the first gill and another to the second gill. The branch to the first gill communicates with the corresponding branch of the glossopharyngeal. The combined nerve enters the first gill, and supplies the levator and depressor branchii muscles. It follows the main blood vessels in the gill and sends many small nerve twigs to these vessels. Some fine branches of this nerve in the first gill have been traced into the gill filaments, and to the skin of the gill stem. The branch to the second gill communicates with a similar branch from the second branchial vagus trunk and will be described later. The first branchial vagus trunk, after crossing the dorsal end of the fourth visceral arch and giving off the two gill branches, follows the visceral arch cephalad and ventrally as the first Ramus post-trematicus of the vagus.

The Second Truncus Branchialis Vagi.

This is distributed in the same manner as the first truncus branchialis vagi. It sends one large branch into the second gill which communicates with the branch of the first Ramus branchialis vagi to the second gill. It then becomes the third post-trematic and follows the fifth visceral arch. The two branches of the first and second branchial vagus trunks to the second gill supply the levator and depressor branchii muscles and, as in the first gill, contribute numerous fine twigs to the blood vessels and filaments. The second truncus branchialis vagi also sends a large branch to the third gill. This communicates, as in the other gills, with another branch to this gill. This other branch, which supplies the third gill, is derived from the nerve to the sixth aortic and visceral arch, which will be described in connection with the truncus visceralis vagi.

The Truncus Visceralis Vagi.

The fourth large branch which passes caudally from the ganglion soon gives off a number of small branches. Two of these are larger than the others, one is the nerve that passes laterally and follows the sixth visceral and aortic arch. This gives off a nerve of considerable size which follows the pulmonary artery. Another branch of this supplies the ductus arteriosus. After giving rise to the branch to the third gill, it then continues ventrally to the sixth visceral arch. The second larger branch passes caudally and medially to become the lateral line branch of the vagus.

The main trunk or intestinal ramus of the vagus continues caudally, following the jugular vein. This gives rise to, in addition to the branches mentioned above, the cardiac branches and a large muscular branch which takes origin caudal to the sixth arch. This is the recurrent branch and supplies the constrictor arcuum and laryngeal muscles.

III. THE CHANGES THAT TAKE PLACE DURING METAMORPHOSIS.

This description was necessarily confined to a study of the gross changes in the parts of the vascular system that are concerned in respiration. In addition, an histological study of gill reduction was made.

The study of the gross changes in the heart and aortic arches was started several years ago. Four hundred animals in larval, metamorphic, and adult stages were killed and dissected. A summary of the findings was published in 1929 (Gilmore and Figge). This description is based largely on this work and the examination of approximately one hundred additional animals. Many of the latter group were dissected while anesthetized. The findings were essentially the same by this method, but many details and observations of a physiological nature were possible. Some of the details observed earlier have become more significant in the light of experimental observations.

The gross changes in aortic arches that occur during matamorphosis are shown in Figure 3. On the right side are aortic arches of a larva, on the left is a diagram of the aortic arches of a recently metamorphosed adult. For the sake of simplicity, the visceral arches were omitted. It is not shown in the diagram, but it should be mentioned that the aortic arches in the larva are associated with their respective visceral arches and are, therefore, separated from each other by a gill slit. In the adult, the gill slits are

fused and some of the visceral arches are reduced. The aortic arches lose their connections to visceral arches and migrate caudally.

Perhaps the most conspicuous change in the aortic arches takes place in the intermediate anastomosis which unites the ventral and dorsal portions of an arch. During metamorphosis, the relatively small arterioles composing this, increase in diameter and form a large arterial trunk. In the adult it would be impossible to differentiate between this vessel, which is formed from the anastomosis, and any other portion of the aortic arch. The remnants of the afferent and efferent branchial vessels, however, give an indication of its extent. The significance of the substitution of a large arterial trunk in the place of the anastomosis will become evident later. It is easily seen that this alone would stop the flow of blood thru the gill. (See Fig. 18)

The carotid, systemic, and pulmonary arches increase in diameter during metamorphosis. The fifth arch decreases in size and is eventually almost obliterated. (See Figs. 1, 2 and 3.) The reason for this selectivity is not known. Evidence will be presented later to show that this is related to the morphology of the anastomosis in this arch. It will be remembered that the anastomosis on the carotid and systemic arches are much better developed and have a much greater capacity than that on the fifth arch.

During the larval stages, most of the blood going to the lungs is derived from the ductus arteriosus, this has passed

through the gills, and is, therefore, relatively high in oxygen content. When the anastomosis on the sixth aortic arch is replaced by a large arterial trunk, most of the blood flowing thru the pulmonary artery comes directly from the heart and is, therefore, relatively low in oxygen content. In the adult, little blood flows thru the ductus arteriosus and this vessel decreases in size. (See Figs. 1 and 2)

The vascular changes in the skin and pharynx are also significant, but have not been studied in great detail. There is not so much change in the cutaneous vessels themselves at this time, but these acquire a more superficial position due to the shedding of skin. Skin shedding thus increases the efficiency of oxygen intake thru the skin. Blood seems to circulate more freely in the cutaneous network of the adult than in the larva. This may only be apparent since it is more difficult to see blood circulate in the cutaneous network of the larva because of the relatively thick covering of epidermis. That the cutaneous vascular network is well developed in the larva, is evident from the photograph of the skin of the operculum. These vessels were injected with Berlin Blue gelatin mass. (See Fig. 14)

No detailed study was made of the pharyngeal vessels. It is significant that one of the largest arteries supplying the pharyngeal epithelium is derived from the sixth aortic or pulmonary arch. This vessel reaches its highest development in the lungless salamanders where the pharynx is one of the chief respiratory organs. As early as 1898, F. Maurer noticed

that in adult amphibians, the pharyngeal epithelium is invaded by capillaries that pass between the basal cells of this and form a network of anastomosing capillaries just deep to the superficial layer of ciliated epthelial cells.

S. Gage and S. Phelps Gage (1890) had previously studied the development of ciliated epithelial cells in the alimentary tract of amphibians. They found that the ciliated epithelial cells in the pharynx were developed some time during metamorphosis, because they were present in adults, but absent in larval and perennibranchiate forms. They, therefore, assumed that the pharyngeal cilia were related in some way to air-breathing.

The vessels in the lungs change little, if any, during metamorphosis. In watching the circulation in the living larval lung and comparing it with the circulation in the adult, one usually finds differences. The larval lung is frequently collapsed and the circulation is poor. The adult lung is usually distended with gas and blood circulates rapidly. During the experiments, it was found that if the collapsed larval lung is cannulated and distended with a gas, circulation through the lung is greatly enhanced. The larval lung is occasionally found distended naturally, but even so, the circulation is not as good as in the adult lung. One reason for this is that the lung vessels of the larva are separated from the heart, either by the gill vessels, or the anastomosis on the sixth arch. The resistance to flow through these relatively small vessels greatly diminishes the

pressure peripherally. In the adult, a large arterial trunk is substituted for the anastomosis on the sixth aortic arch. The variations in pressure produced by the action of the heart are thus transmitted directly to the pulmonary artery. This makes possible a higher pressure in the pulmonary artery and accounts for the better cerculation.

The Study of a Normal Gill Reduction.

If the gills of an animal that is just beginning to show metamorphic symptoms are examined, it is frequently seen that the filaments appear quite large and distended. For this reason, the gill is very large before gill reduction starts. The first evidence that gill reduction has started is seen in the development of spiral-shaped filaments. This gives the gill a fluffy, crinkled appearance. The spiral-shape of the filament at this stage is, probably, due to constriction of the gill capillaries and the arteriole at the tip of the filament. This would result in a much higher pressure in the afferent limb of the arteriole loop than in the efferent limb. The high pressure and continuous pumping on the afferent limb of the filament would cause this side to grow longer. The other side would remain the same or even grow shorter. As the lengths of the two sides of the filament become unequal, it begins to assume a spiral-shape.

At the same time or shortly after this, the arteriole at the tip of the filament begins to become convoluted. Eventually, these convoluted arterioles form little knobs of convoluted

vessels at the tip of each filament.

Shortly after these knobs are formed, the epithelium on the gill filaments takes on a peculiar grayish appearance. The adjacent layers of epithelium on neighboring filaments then begin to fuse. This process usually begins at the base of the filaments and at the base of the gill. Soon the filaments have not only shortened, but have fused together into a solid mass. One cannot help but wonder, at what stage of this fusion the cilia ceased to function, and whether this is a factor in the tendency of filaments to stick together.

Observations made on living animals during metamorphosis show that blood flows thru the gill vessels during any stage of gill reduction. I have even observed blood flowing through the gill nodules that are completely covered by skin. Blood cells are also found in the vessesl in sections of the black pigment mass which is the remains of a gill in a metamorphosed animal. But the branchial vessels in which blood is seen to flow during metamorphosis are the remains of the arteriole loops of the filaments. These are, in many cases, extremely dilated and, under normal conditions, blood flows very slowly through these, especially near the completion of metamorphosis. It was noted very recently that any anesthetic which stops the respiratory movements, and, thus, interferes with oxygenation of the blood in the lungs and pharynx, will cause an accelerated flow of blood through the metamorphosing gill.

The capillaries in the gill filament are closed relatively early. Shortly after the filaments fuse, the

capillaries are oblitereated, probably, by phagocytosis. If the latter is true, then the capillary walls and the surrounding connective tissue cells are the only parts of a gill that are removed by phagocytosis.

The histological study of gill reduction shows, contrary to prevalent assumptions, that the process of gill reduction is not so much a process of histolysis and phagocytosis, as it is a process of the migration of cells to new positions. Photographs of representative cross-sections, taken through gills in different stages of reduction, will serve to substantiate this statement and facilitate the description of the process.

A cross-section through a larval gill shows the continuity of the skin and the epithelium covering the filaments. It also shows the centralposition of the melanophores. The melanophores appear in such a cross-section in rows corresponding to the section of a filament. They are, however,widely scattered. (See Fig. 8)

The next two photographs are of sections through gill stubs after the filaments have fused together. (See Figs. 15 and 16) The arrangement of melanophores indicate the positions of the fused filaments. In many cases one can clearly see two fused layers of epithelial cells, separating two rows of melanpphores. It will also be observed that this fused mass of filaments is surrounded by a capsule that grows thicker in the later stages. This capsule is formed by the migration of the epithelial cells that originally covered

the filaments and which, at this stage, are located between the rows of melanophores. The evidence for this is as follows:

1. No mitotic figures are seen during the formation of the capsule which eventually surrounds only a black mass of pigment.

2. The layers of cells which form this capsule are continuous with the layers of cells between the rows of melanophores.

3. The cells forming the capsule are identical with the cells between rows of pigment cells.

There is no evidence that any of the epithelial cells disintegrate or are destroyed by phagocytosis. They all apparently migrate to take part in the formation of the capsule. Only the pigment cells remain. These clump together in an almost solid mass, as shown by a photograph of a gill nodule. (See Fig. 17) These black masses of pigment cells persist for some time. They are connected to the aortic arches by remnants of the afferent and efferent branchial vessels. In these black pigment masses there are found the remains of the branchial nerves and some of the arterioles connecting the stubs of the afferent and efferent branchial vessels.

Morphological Speculation.

From the study of the distribution of the vagus nerve in the larval and adult urodeles and in mammals, it is interesting to speculate as to which components of the

vagus nerve in mammals are homologous to the branchial components in urodeles. The attempt to homologize nerves is complicated because nerves are usually used as criteria for the homology of other organs. This method should also work in the reverse direction; but the homologies between the branchial elements in the lower forms and their vestiges in the higher forms (if they exist) are not definitely known. For example, there is no known homologue of a gill, as such, in mammals. Consequently, we cannot say definitely which nerves in a mammal correspond to those branches going to gills in urodales.

It appeared probable that the carotid body in man might be a vestige of the first gill in urodeles. The evidence for such a hypothesis is that this organ is not found in the branchiate vertebrates and does not appear in an amphibian until metamorphosis. Moreover, the carotid body and the first gill have the same nerve supply. Both of these receive one branch from the glossopharyngeal and one from the vagus. The carotid body's position and blood supply is also in agreement with the possibility that this structure is the vestige of the first gill in urodeles.

The following table gives the list of nerves in mammals that are thought to be homologous to the branchial elements of the vagus and glossopharyngeal nerves in urodeles:

Table of Homologies

Urodele Larvae		Mammals.
Branch of glossopharyngeal to 1st gill	- -	Branch of glossopharyngeal to carotid body and sinus.
" " 1st truncus branchialis vagi- to first gill	-	Branch of superior laryngeal to carotid body and sinus.
" " 1st or 2nd truncus branchialis- vagi to 2nd gill arch.		Branch of vagus to aortic arch or the aortic nerve.
Vagal branches to 3rd gill arch	- -	? ? ?

These homologies are based solely on morphology. The physiology of these nerves are well known in mammals as aortic and carotid nerves, which reflexly control blood pressure, respiration, and heart rate. There is some evidence that the nerves to the gills function in the same manner in urodeles and fishes. It was shown by Lutz and Wyman (1932) that cardiac inhibition follows sudden increase of pressure within the gill blood vessels of Squalus acanthus and Necturus. More physiological, as well as morphological, evidence is necessary before it may be concluded that the branchial nerves are homologous to the aortic and carotid nerves in mammals.

MORPHOLOGICAL SUMMARY

1. The anatomy of the anastomoses at the base of the gills and on the sixth aortic arch were photographed and described.

2. It was suggested during this work that the small number of minute arterioles making up the anastomosis at the base of the third gill, might be related to the fact that the fifth aortic arch is obliterated during metamorphosis.

3. Remak fibers were demonstrated in the gill filaments.

4. An histological study of gill reduction was made. This appears to be a process involving, for the most part, the migration and transformation of cells. Histolytic processes are undoubtedly of minor importance in gill reduction.

(Note:- This summary includes only contributions of the author)

PHYSIOLOGY

I. THE NERVOUS CONTROL OF CIRCULATION IN THE GILLS OF AMBLYSTOMA TIGRINUM LARVAE.

Two methods were used in an attempt to gain some insight into the physiology of the branchial elements of the vagus and glossopharyngeal nerves. One was the method of cutting or stripping these nerves out, in the larval stages, to note whether or not this would have any immediate effect on the circulation in the gill, or whether it would later have any influence on metamorphosis or gill reduction. The second method tried was that of stimulating these nerves and noting the changes, if any, in the gill vessels, heart rate, and respiratory movements.

A. The Effect of Branchio - and Total Vagotomy.

1. On Normal Metamorphosis of A.tigrinum larvae.

Theoretical Discussion.

The sensory elements in the vagus and glossopharyngeal that are concerned in the reflex control of blood pressure, cardiac rhythm, and pulmonary ventilation have been investigated by numerous physiologists. Two branches of the vago-glossopharyngeal nerves have been shown to have similar functions: One is the nerve to the carotid sinus, Hering, 1924; Heymann, 1928) the other is the so-called depressor nerve, (Cyon and Ludwig, 1866).

As has been mentioned in the morphological discussion, it is interesting that both of these nerves have their sensory endings on or near the remnants of the aortic arches. The endings of the nerve to the carotid sinus are in the vicinity of the carotid sinus and carotid body or third aortic arch anastomosis in Amphibia; and the endings of the mammalian depressor nerve are on the arch of the aorta which is the remnant of the fourth aortic arch in lower forms. The third and fourth aortic arches in Amphibia are associated with the gills. The fifth or remaining gill arch of Amphibia is not represented in adult Birds and Mammals. Morphologically, the depressor nerves must be homologous to some component of the branchial branches of the vagus and glossopharyngeal nerves, because it is these branches that supply the aortic and visceral arches in the lower forms.

Little is known regarding the function of the vagus branches to the gill arches except the contribution of Lutz and Wyman (1932). They found that increased pressure in the gills causes cardiac inhibition. Little more is known regarding the fate of the nerves that supply the gills when the gills are reduced, as is shown by a glance at text-books of comparative anatomy. "In the transition to terrestrial life the vagus group suffers naturally the loss of the branchial elements"; (Wilder, 1923). "With the absence of gills in the amniotes and their loss in the higher Amphibia, the lateralis elements disappear and

the branchial nerves are reduced, though parts are present as pharyngeal nerves." (Kingsley, 1917). This vague way of referring to the homologies of these elements is typical. A study of the physiology of the branchial branches of the vagus was, therefore, made, in the hope that this would help to homologize the branchial nerves and the so-called carotid and aortic nerves. In addition, it was thought that if the branchial nerves were involved in the control of blood pressure, heart rate, and respiration, that these nerves might be related, in some way, to gill reduction or the metamorphic process in general, It was not found possible to demonstrate, with the methods employed, that these nerves have any influence either on gill reduction or on metamorphosis.

Methods and Materials.

The animals used in this experiment were A. tigrinum larvae collected in Colorado in August, 1932. Animals of all sizes were selected, ranging from 38 mm. to 86 mm. in body length.

The operation was performed under chlorotone anesthesia. The animal was pinned down in a wax dish. A small slit was cut through the skin at the angle formed by the third gill and the body. From this, an incision was carried cephalad along the base of the gills. Another incision was made in a cephalad and medial direction, outlining a V-shaped flap. When this flap was raised, the branchial branches of the vagus were easily seen. In

order to fully expose the vagus and glossopharyngeal where they leave the chondrocranium, as was done in some cases, it was necessary to also sever the levator arcuum muscles. With this exposure, it was a simple matter to cut or pull out centrally and peripherally any one of the branches to the gills. Various nerves and combinations of nerves were cut in sixty animals. Twenty-six animals of corresponding sizes were used for controls.

Results.

The animals will be listed in groups according to the operation. Observations made on the gill vessels before and immediately after cutting the vagus branches showed no appreciable changes in the calibre of these vessels. The nerves were not simply cut, but as much of the nerve as possible was removed by pulling it out centrally and peripherally. The nerves were recut when some were found to have regenerated. Twelve different types of operations were performed. It was hoped that at least one or more of these operations might show an inhibition of metamorphosis, but this was not the case.

It is evident, by merely glancing at the list of metamorphosed animals, (See Tables I and II) that animals in all groups metamorphosed. It is true that four of the animals are still living and larval; but so are two control animals. In general, the operated animals metamorphosed at the same rate as the controls. The death of larval animals (6 controls; 16 operated) was due in most cases to infection

TABLE I,

Control A. tigrinum in Branchio-

and Total Vagotomy Experiment.

Nov. 1, 1932. Animal Number	Metamorphosed in	Died Larval in
Ca 2		303 days
Ca 3	221 days	
Ca 4	223 days	
Ca 5	205 days	
Ca 6		281 days (transferred & sacrificed)
Ca 7	215 days	
Ca 8	221 days	
Ca 9		86 days (escaped)
Ca 10		(Still Alive - 495 days)
Ca 11	221 days	and Larval
Ca 12	230 days	
Ca 13		73 days
Ca 14		62 days
Ca 15	150 days	
Ca 16	215 days	
Ca 17	230 days (infection)	
Ca 18		334 days (disappeared)
Ca 19	223 days	
Ca 20		(Still Alive and Larval - 495 days)
A 1	181 days	
A 2	150 days	
A 3	150 days	
A 4	181 days	
A 5	150 days	
A 6	181 days	
Mean -	197 days	

TABLE II.

The Effect of Branchio- and Total Vagotomy.

Animal Number	Date Operated 1932.	Recut in	Metamorphosed in	Died Larval in
Removal, bilaterally, of the 3 large vagus and glossopharyngeal trunks and the 6th visceral arch nerve.				
B 2	Oct.26			212 days
B 3	Oct.26	248 days		269 days
B 22	Nov.17	199 days	201 days	
B 23	Nov.17		190 days	
B 50	Nov.21		193 days	
B 51	Nov.21	197 days	210 days	
Three large vagus and glossopharyngeal trunks to all gills.				
B 3x	Oct.25	220 days		383 days (disappeared)
B 4x	Oct.25	220 days	237 days	
B 5x	Oct.29		10 days	
B 6x	Oct.29		216 days	
B 7x	Oct.29	218 days		252 days
B 1	Oct.26			34 days
B 4	Oct.26	221 days		267 days
B 5	Oct.26			50 days
B 6	Oct.26	221 days		475 days
B 7	Oct.26	221 days		269 days
B 26	Nov.17 (left)	221 days		(Alive and Larval-477 days)
B 27	Nov.17(right)	221 days	214 days	
B 17	Nov.2 (left)		179 days	
B 18	Nov.2		228 days	
B 38	Nov.18			18 days
B 39	Nov.18		196 days	
Removal of glossopharyngeal trunk.				
B 31	Nov.18		196 days	
B 43	Nov.21		161 days	
Removal of first branchial vagus trunk.				
B 32	Nov.18		164 days	
B 44	Nov.21	197 days		(Alive and Larval-473 days)
B 2x	Oct.25	220 days		311 days
Removal of second branchial vagus trunk.				
B 10	Oct.26	219 days		477 days
B 33	Nov.17		188 days	
B 45	Nov.21		131 days	
Removal of first and second branchial vagus trunks.				
B 8	Oct.26			20 days
B 9	Oct.26	221 days	227 days	
B 11	Oct.26			20 days
B 21	Nov.17	196 days	198 days	

TABLE II.
(continued)

Animal Number	Date Operated 1932	Recut in	Metamorphosed in	Died Larval in
Removal of vagus branch to 6th arch.				
B 12	Nov.1	215 days	221 days	
B 13	Nov.1	215 days	298 days	
B 34	Nov.18	200 days		(Alive and Larval
B 46	Nov.21		130 days	in 476 days)
Bilateral 1st gill denervation.				
B 1x	Oct.25	220 days		315 days
B 35	Nov.18		165 days	
B 47	Nov.21		186 days	
Bilateral 2nd gill denervation.				
B 36	Nov.18		29 days	
B 36s	Dec.23		178 days	
B 48	Nov.21		51 days	
B 48s	Dec.23		46 days	
Bilateral 3rd gill denervation.				
B 37	Nov.18	201 days	205 days	
B 49	Nov.21		160 days	
Removal of intestinal ramus, including lateral line and 6th visceral arch branches.				
B 15	Nov.2	213 days		215 days
B 16	Nov.2 (left)		211 days	
B 14	Nov.2	213 days	- not regenerated	(Alive and Larval
B 19	Nov.11		124 days	in 494 days)
B 20	Nov.11			180 days
Complete vagotomy, (oncluding glossopharyngeal, lateral line, branchial, and intestinal branches of vagus.				
B 24	Nov.17		197 days - nerves	had regenerated
B 25	Nov.17	201 days		227 days
B 30	Nov.18		190 days	
B 40	Nov.21	196 days	201 days	
B 41	Nov.21	196 days		259 days
B 42	Nov.21			died first day from bleeding.

or high temperatures.

Denervation of the second gill seemed to stimulate metamorphosis. Animals B 36 and B 48 metamorphosed in twenty-nine and fifty-one days respectively. These animals were replaced by B 36s and B 48S. One of these again promptly metamorphosed in forty-six days, but the other required 178 days. The average number of days required for controls to metamorphose was 197. Since the same nerves were cut in several other groups and no acceleration of metamorphosis was observed in these groups, the evidence is not sufficient to draw the conclusion that cutting the nerves to the second gill accelerates metamorphosis.

Summary.

The removal of the branchial portions of the glossopharyngeal and vagus nerves has no effect on the metamorphosis of A. tigrinum larvae. Animals in which the glossopharyngeal and all parts of the vagus (branchial, intestinal, cardiac, and lateral line) were removed, metamorphosed at the same rate as controls.

Conclusion.

The evidence presented here indicates that the morphogenetic processes involved in the metamorphosis of A. tigrinum larvae are not dependent upon the vagal reflexes.

A. Effect of Branchio - and Total Vagotomy.

2. On Metamorphosis Produced by Thyro-activator (A. L. Hormone).

It was found in the previous experiment that the vagus

branches will regenerate after a certain period. Even though these were removed at intervals, it was thought that the regenerated nerves might have been responsible for the negative results.

To eliminate this possibility, a number of animals were branchio-vagotomized, others completely vagotomized. To make certain that the nerves would not regenerate, only a short period for operative recovery intervened between the operation and the injection of anterior lobe. The operated animals, along with a number of controls, were injected in the usual manner with anterior lobe extract prepared and extracted according to the Uhlenhuth method. (Uhlenhuth, and Schwartzbach, 1928.) The method of operation was the same as described in the previous experiment. Six types of operations were performed on twelve animals. The operations were all made on the same day, January 2, 1933.

Fourteen days later, the injections of anterior lobe extract were started. The animals were injected on alternate days for twelve days, a total of six injections were given. Each injection contained the extract of 100 mg. of dried anterior lobe powder in 0.5 cc of acid ringer, (neutralized). Five normal animals were used as controls. Three of these were injected with the operated animals. In addition, a hypophysectomized animal was injected as a control.

Results.

The size of the animals, the type of operation, and the results are shown in table III. The three normal control

TABLE III.

The Effect of Branchio- and Total Vagotomy on the Metamorphosis Produced by Anterior Lobe Injections.

Animal Number	Body Length (mm)	Operation	Results	No. of Days From 1st Injection.
A 1	73	Control	Metamorphosed	24
A 2	75	"	"	26
A 3	72	"	"	24
A Hyp	79	" (hypophysectomized)	partial meta.	30
A 4	69	" (not injected)	no change	35
A 5	70	" " "	" "	35
B 1	71	2nd gill denervation	metamorphosed	19
B 2	72	" " "	"	20
B 3	70	6th aortic and visceral	"	20
B 4	70	arch.	"	26
B 5	66	Complete branchio-vagotomy	"	33
B 6	73	" " "	"	25
B 7	71	Glossopharyngeal and 2 large	"	22
B 8	70	branchial vagus trunks	"	19
B 9	69	complete unilateral vagotomy	left partial	33
B 10	75	" " "	right meta.	33
B 11	71	" bilateral	" metamorphosed	33
B 12	69	" "	" larval	33

animals were metamorphosed with six injections of anterior lobe extract in twenty-five days, and the hypophysectomized animal had partially metamorphosed in the same length of time. The vagotomized animals again metamorphosed at about this same rate, with one exception. A completely vagotomized animal, B 12, did not metamorphose. However, another animal operated in exactly the same manner metamorphosed.

Conclusion.

1. Neither branchio-vagotomy nor complete vagotomy inhibits the metamorphosis produced by thyreo-activator in A. tigrinum larvae.
2. This is presented as evidence that the vagal and glossopharyngeal reflexes are not necessary for the morphogenetic process of gill reduction.

B. The Effect of the Removal of the Cervical Sympathetic Trunk and Ganglia on the Metamorphosis of A. tigrinum Larvae.

When a complete vagotomy, involving also the glossopharyngeal, is performed, many of the sympathetic fibers to the gills are also destroyed. There is, however, a possibility of sympathetic fibers passing from the cervical sympathetic along the radices aortae, or the dorsal portions of the aortic arches, and thus to the gill vessels. In order to determine whether these hypothetical branchial sympathetic fibers had any influence on gill reduction, the cervical sympathetic trunk and ganglia were removed in two animals.

In one of these, E 3, the cervical sympathetic was removed from both sides; in the other, E 2, the operation was performed only on the right side. Both of these animals were injected at the same time and with the same dosage as the animals in the preceding experiment.

Results.

Both animals metamorphosed as rapidly as the controls, in twenty-two days. Gill reduction was normal in both animals and no difference could be noted between the two sides of the animal having the cervical sympathetic intact on one side.

Conclusion.

This is tentative evidence that the cervical sympathetic trunk and ganglia are not necessary for the metamorphic process which is induced by anterior lobe injection in A. tigrinum larvae.

C. The Effect of Stimulation.

1. Of the Vagus Branches to the Gills.

A number of attempts were made to gain some information through stimulation of the branchial nerves. If a vagus branch to the gills is cut and stimulated peripherally, the gills which it supplies are drawn down in the shape of a crescent. During this time blood stops flowing through the gill. The fact that blood stops flowing through the gills was established both by direct observation of the

flow of blood, and by the perfusion of the isolated gill. Stimulation of the isolated gill nerve preparation caused a cessation of the flow of perfusion fluid through the gill. This demonstrated, either that the branchial trunks of the vagus contains constrictor fibers to the gill vessels, or that the action of the depressor branchii muscle is capable of completely stopping the flow of blood through a gill. The latter appears to be the most probable. Since the vagus nerve of amphibians contains sympathetic fibers, it might be supposed that stimulation of these was responsible for the constriction of the branchial vessels. It will be shown in another part of this work that adrenalin dilates the branchial vessels. In view of this, it does not appear probable that it is a matter of stimulating sympathetic fibers that accompany the vagus branches to the gills. The attempts to paralyze the depressor branchii muscle with curare were unsuccessful. Animals, that were deeply paralyzed otherwise, would still wave their gills, and stimulation of the vagal branches to the gills gave the same reaction as without curare. Stimulation of the central end of a branchial vagus trunk causes movements of the gills on the opposite side and cardiac inhibition. This latter observation conforms to the findings of Lutz and Wyman (1932) on Elasmobranchs and Necturus.

2. Of the Cervical Sympathetic.

It was impossible to isolate and stimulate the cervical

sympathetic. Stimulation of the nerve and adjoining muscles, brought about movements of the animal that made observations impossible.

General Conclusions.

1. The physiological evidence in favor of the homology of the branchial elements of the vagus in urodeles and the aortic and carotid nerves in mammals, is that the stimulation of the central ends of the branchial trunks inhibits the heart and induces respiratory movements.

2. No evidence for the nervous control of branchial circulation was obtained. This does not mean that the nerves do not regulate the gill vessels, but only that it has been impossible (with the methods employed) to demonstrate that they do.

II. THE HUMORAL CONTROL OF CIRCULATION IN THE BRANCHIAL VESSELS OF A. TIGRINUM LARVAE.

In order to investigate the humoral mechanisms that might have an influence on the branchial circulation or on gill reduction, three methods were tried. These will be described and their limitations pointed out.

The first method to be tried was that of measuring the blood pressure in the arterial system. This method has great limitations and is exceedingly difficult. It was, therefore, abandoned very early. The second method to be tried was the perfusion of gill vessels. This also has great limitations and is difficult technically; but, nevertheless, yielded some very interesting results that were substantiated by the third method. The third method is much simpler and consists of making direct observations on the reactions of the vessels in question. The reactions to humoral stimuli applied externally to certain parts of the gill vessels could be studied and compared with similar non-treated vessels which served as controls. Moreover, the same substances, used to stimulate gill vessels by diffusion through the branchial epithelium, could be introduced into the blood stream. The gill vessels proved to be ideal for making the observation, perhaps even better than the classical web or tongue of the frog or the ear of the rabbit.

Because there are a considerable number of circulatory hormones and substances, it was necessary to decide whether to attempt to investigate all of the substances superficially, or only a few of these in a relatively thorough manner. The latter course was chosen in spite of a strong desire to include in this paper the effect of all known circulatory hormones and substances.

A. Blood Pressure Experiments in the Larval Salamander.

An attempt was made to determine the reactions of the branchial vessels to various substances and stimuli by taking the blood pressure ventral to the gills. To the author's knowledge, no one has ever tried to measure and record on a kymograph the aortic blood pressure of the larval salamander. This proved quite difficult. In the first place, the entire animal has only three or four cubic centimeters of blood. This blood has a very great tendency to clot. The cannula used must be quite small; yet large enough to allow sufficient blood to flow in and out to record diastolic and systolic pressures; but even with small cannulae, the process of cannulation is not simple. The manometer must be extremely sensitive and yet require only a minute amount of blood to charge its level. All of these difficulties were finally overcome only to find that if the blood pressure is taken at only one point, it means very little, if anything.

Method and Materials

The apparatus which finally gave results and enabled the recording of diastolic and systolic pressures on a kymograph, consisted of a mercury manometer of small bore, (2 mm. inside diameter.) This was connected by glass and rubber tubing to a cannula inserted in the fourth aortic arch, ventral to the gills. The smallest diameter of the cannula was large enough to permit blood to flow in and out of the cannula rapidly enough to record diastolic and systolic pressures. The changes in the level of the mercury were so slight that it was necessary to magnify these by means of a balanced lever attached to the balance wheel of a clock.

The animals were first anesthetized with cholotone, then ether was used during the experiment. Heparin was injected to prevent the coagulation of blood. This was also added to the saline solution in the cannula.

Results.

Blood pressures were recorded in only five animals. The pressure ranged from 4 to 10 mm. of mercury. Stimulation of the gills or central ends of nerves to the gills caused no change in blood pressure. Occasionally these stimuli caused a momentary cardiac inhibition and movements of the gills. The striking result was the fact that the injection of a very large dose of adrenalin had no effect on the blood pressure ventral to the gills. The reason for this, which became evident later, is that adrenalin, while it constricts the systemic vessels, dilates the gills vessels. If the pressure

is taken between the heart and the gills, as it was in these cases, the constriction of systemic vessels is offset by the dilatation of the branchial vessels.

Conclusion.

From what has been said, it will be evident that one can conclude very little from the blood pressure reactions of the whole animal, especially if this is recorded at only one point. Because of this and the complexity of the method, this method was abandoned.

B. Perfusion of the Branchial Vessels.

1. The Effect of Adrenalin.

Since it was impossible to determine the reactions of the branchial vessels by recording the blood pressure, the perfusion method was tried. This method was used quite successfully by Keys and Bateman in determining the effect of adrenalin on the branchial vessels in eels (1932) and even previous to this in fishes by Krawkow (1913). Both of these investigators perfused all the gills of one animal at the same time. The in-flow cannula was placed in the ventral aorta. The outflow from a cannula in the dorsal aorta was measured (Keys and Bateman) or the drops that fell from the dorsal ends of the branchial arches were counted (Krawkow). Both investigators reported that adrenalin increased the rate of flow through the gill. This same method of perfusion was tried on the branchial vessels of A. tigrinum, but was soon abandoned in favor of a method which permitted the perfusion of individual gills and parts of gills.

Methods and Materials

In making up the perfusion fluid, that was used in the following experiments, several conditions had to be satisfied. It was necessary:

1. That it be equi-osmotic with the blood of a larval salamander.
2. That it be balanced ionically to conform to Ringer's solution.
3. That it contain enough sodium bicarbonate as a buffer and maintain a pH near that of blood.

4. That it contain a simple carbohydrate such as dextrose for nourishing the vascular tissues.

Jona (1912) had determined the freezing point depression of frog blood and adult salamander blood. For Rana esculanta he found it to be 0.465° C and for Salamander maculata to be 0.479° C. Both of these being adult forms, this gave no exact information as to the freezing point depression of larval salamander blood. It was necessary, therefore, to determine the osmotic pressure of larval salamander blood by the cryoscopic method.

Three larval salamanders were anesthetized in chloretone. A small quantity of heparin was injected into one of the aortic arches of each animal. The heparin was dissolved in a solution having a freezing point depression 0.450° C, as near as possible to that estimated for larval blood. Each animal received 0.5 cc of this heparin solution. After a short time, blood was taken with a syringe from one of the aortic arches. A total of 9 cc. was obtained from the three animals.

The freezing point was determined with the Beckmann apparatus. Five determinations were made. The results of each determination and the calculations are given below:

Freezing point of larval salamander blood. Beckmann thermometer.

1st determination	4.065° C
2nd " "	4.065° C
3rd " "	4.065° C
4th " "	4.062° C
5th " "	4.063° C
Average	4.064° C
Correction for super-cooling +	.007
	4.071° C

Freezing point of distilled water. Beckmann thermometer.

1st determination	4.539° C
2nd " "	4.538° C
3rd " "	4.539° C
4th " "	4.539° C
Average	4.539° C
Taking the difference	4.071° C
We obtain	.468° C

as the freezing point depression of larval salamander blood.

The next problem was to make up a perfusion fluid that would have the same freezing point depression as the larval salamander blood, and still satisfy the other requirements mentioned above. To do this, stock solutions of sodium chloride, potassium chloride, and calcium chloride were prepared. The amount of any one of these substances necessary to lower the freezing point of a liter of water 0.410° C was calculated and carefully weighed. Since all the stock solutions of these salts had the same freezing point depression, they could be mixed in any desired proportion and the resulting solution would still have the desired osmotic pressure. The perfusion fluid was prepared by mixing the stock solutions immediately before use; 950 cc. of the sodium chloride solution was mixed with 30 cc. of potassium chloride and 20 cc. of calcium chloride. Sodium bicarbonate, dextrose, and urea were added in the following amounts:

0.4 gm. --- sodium bicarbonate per liter
0.9 gm. --- dextrose per liter
0.5 gm. --- urea per liter

The last three substances were found to lower the freezing point about .056° C.

Theoretically, the final perfusion fluid should have

lowered the freezing point 0.466° C, but seven determinations with the Beckmann thermometer, showed a freezing point depression of 0.468° C which is exactly what was found for larval salamander blood. The following table summarizes the method and the concentrations of the various salts:

Salt	concentration per liter	molality of sol.	Δ f.p.	Am't used per liter
NaCl	6.9263 gm.	.1185	0.410° C	950 cc.
KCl	8.8341 gm.	.1185	0.410° C	30 cc.
$CaCl_2.2H_2O$	13.2723 gm.	.0903	0.410° C	20 cc.

The perfusion fluid as used, contained the following substances stated in grams per liter:

NaCl	6.5800 gm.	per	1000	cc.	water.
KCl	.2650 gm.	"	"	"	"
$CaCl_2$	.2004 gm.	"	"	"	"
$NaHCO_3$	.4 gm.	"	"	"	"
Dextrose	.9 gm.	"	"	"	"
Urea	.5 gm.	"	"	"	"

This is essentially the same as a mammalian Ringer-Locke solution except that the salts are only about two-thirds as concentrated, and in addition, it contains a small amount of urea. The perfusion fluid was made up fresh each day. Adrenalin solutions as well as other test solutions were made up just before use.

The cannulae that were used for perfusion of parts of individual gills were quite small. These were bevelled and annealed at the tip with a shoulder near the tip. The cannula was usually greater in diameter than the vessel to be perfused. The vessel was split for a short distance and by grasping each half with a small mouse-toothed forceps, could

be stretched over the expanded end of the cannula, Ca. (See Figs. 20,21, 22). It was then tied in place. The capacity of the cannula at the pressure used was measured in each case to make certain that this was much greater than the capacity of the gill that was being perfused.

The perfusion was carried out at constant pressure of 250 mm. of water. This is slightly higher than the normal blood pressure. To determine the rate of flow, the inflow was measured. The preparation was tested for leaks at the end of an experiment by placing a dye in the solution. By observing the outflow, the slightest leak could be detected. A leak was found in only a few cases and these results were discarded. In order to maintain a constant pressure and determine the rate of inflow, the following method was devised: (See Fig. 19) The cannula (Ca) was connected, by means of rubber tubing (RT) and a T-tube for a bubble-catcher, (B.C.) to the bottom of a test tube, (TT). The upper end of the test tube was stoppered with a one-hole stopper, into which a six-inch length of 8 mm. glass tubing (GT) was placed. This entire system was filled with perfusion fluid up to a mark near the middle of the glass tube. A burette (B) was arranged so that the tip drained into the upper end of the glass tube (GT). The burette was constantly adjusted so that as fast as the perfusion fluid left the system, it was replaced by fluid from the burette. The burette readings were taken at short intervals, and the amount of fluid necessary to maintain a constant level in the tube was calculated from these. The results were then

plotted.

To perfuse an isolated gill (without the anastomosis), the external gill was cut off just distal to the anastomosis at point F, in figure 18. The afferent branchial artery was then split for a distance of about 2 mm. to facilitate cannulation. As soon as this vessel was cannulated, it was tied in place by a double ligature. The perfusion fluid was allowed to slowly escape from the cannula during the cannulation. The whole process of cannulation was carried out in a wax-bottomed dish filled with perfusion fluid. As soon as the ligatures were tied, the full perfusion pressure was turned on. The perfusion fluid escaped through the cut end of the efferent branchial artery. In order to reach the outflow vessel the perfusion fluid had to pass through the gill filaments. (See Fig. 21) Two isolated gills were perfused simultaneously, one with perfusion fluid alone, the other with perfusion fluid plus adrenalin. After a certain time, the two tubes were changed from one cannula to the other so that the one that had been perfused with perfusion fluid alone was, after the exchange of tubes, perfused with perfusion fluid plus adrenalin and vice versa.

The technique used in perfusing an isolated anastomosis was as follows: In an anesthetized animal, the aortic arch was ligated ventrally as close to the bulbus arteriosus as possible, dorsally it was ligated close to the radix aortae. The whole gill arch was then cut out. The gill was severed from the visceral arch just distal to the anastomosis, leaving only a few filaments on the visceral arch portion. The ventral

end of the aortic arch was then cannulated and any blood contained in the vessels was forced out before the afferent branchial artery was ligated just distal to the anastomotic vessels. All the filaments on the preparation were included in the ligature. The only outflow vessel left open was the dorsal end of the anastomotic vessel. (See Fig. 22)

The 1:1000 solution of adrenalin chloride used in the perfusion experiments was obtained from Parke, Davis, and Company.

Results.

a. Perfusion of All Gill Arches Simultaneously.

In a preliminary experiment, the method employed by Keys and Bateman, and Krawkow of perfusing all the gills at once was tried. It was found in these experiments that the rate of perfusion was not dependent entirely on the size of the vessels perfused, but was influenced by the heart rate or, if the cannula was placed in the truncus arteriosus, by the rhythmic contractions of the bulbus arteriosus. Moreover, the rate of perfusion did not change appreciably on addition of adrenalin. These negative results suggested that one set of vessels in the branchial circulation reacted by dilatation with adrenalin while another constricted. Because of this, the perfusion of a single gill arch was attempted.

b. Perfusion of a Single Gill Arch.

In another preliminary experiment, a whole gill arch (external gill plus the anastomosis at the base), was perfused. (See Figs. 20, 24) In this experiment it was

found that changing the perfusion pressure from 300 mm. to 200 mm. of water caused the perfusion rate to fall from 45 cc. to 28 cc. per hour. During the following 30 minutes, the rate gradually decreased to 23 cc. per hour. At this time, the anastomosis at the base of the gill was ligated. The perfusion rate dropped from 23 to 8.5 cc. per hour. The ligature was then released and the perfusion rate increased to 20 cc. per hour. When the ligature was re-tightened and tied securely, the perfusion rate fell to 3 or 4 cc. per hour. After the anastomosis was ligated, only the external gill was perfused. Adrenalin was added to the perfusion fluid and the rate of perfusion promptly increased to 15 cc. per hour. This rate was maintained for about an hour, then the gill was perfused with perfusion fluid alone. The rate of perfusion gradually fell to 6 cc. The ligature was then removed from the anastomotic vessel and the entire branchial arch was now perfused. The rate of perfusion increased to 30 cc. per hour. Adrenalin was then added to the perfusion fluid and this had little, if any, effect. From this preliminary experiment, it was evident that in order to test the effect of any substance on the gill vessels, it would be necessary to perfuse the gill and the anastomosis separately.

c. Perfusion of the Isolated External Gill.
(Without the Anastomosis)

Adrenalin was used in concentrations ranging from 1:50,000 to 1:5,000,000. It was tried seventy-two times on

TABLE IV.

Effect of Adrenalin on Rate of Perfusion

in an Isolated Gill.

Exp. No.	Gill Number 1st	2nd	3rd	Concentrations	Effect on Perfusion Rate	No. of Times Repeated
20 b		x		1:50,000	increased	3
21 b			x	" "	"	2
22 b	x			" "	"	2
23 b	x			" "	"	3
24 b		x		" "	"	3
25 b		x		" "	"	2
1 b		x		1:100,000	"	1
2 b		x		" "	"	2
3 b		x		" "	"	2
4 b			x	" "	"	2
5 b			x	" "	"	2
34 b			x	" "	no action - old perfusion fluid	
35 b		x		" "	increased	2
6 b			x	1:500,000	"	2
7 b		x		" "	"	2
8 b			x	" "	"	4
9 b			x	" "	"	4
14 b		x		" "	"	1
15 b		x		" "	"	1
16 b		x		" "	"	2
17 b		x		" "	"	2
18 b	x			" "	"	2
19 b	x			" "	"	2
10 b			x	1:1,000,000	"	3
11 b		x		" "	"	2
12 b			x	" "	"	2
13 b		x		" "	"	2
30 b	x			" "	"	2
31 b	x			" "	"	1
32 b			x	" "	"	2
33 b		x		" "	"	3
26 b		x		1:5,000,000	"	2
27 b			x	" "	"	2
28 b		x		" "	"	2
29 b			x	" "	"	1
					Total -	72

thirty-five individual gills. The gills were cannulated distal to the anastomotic vessels as shown in figure 21. The results are summarized in Table IV. In every case, except one, the addition of adrenalin to the perfusion fluid increased the rate of perfusion. In this case, perfusion fluid a week old had been used.

The effect of the addition of adrenalin to the perfusion fluid may be described more quantitatively by the use of graphs. The results obtained from two gills that were perfused at the same time are plotted on the same paper. It will be seen that when a gill, that had been perfused with perfusion fluid alone, was perfused with perfusion fluid plus adrenalin (1:1,000,000), there was a distinct increase in the rate of flow amounting sometimes to a two hundred percent increase. This gill was again perfused with perfusion fluid alone, and the rate of perfusion dropped to the original level. (See Figs. 25, 26, Gills 10b, 11b, 12b, 13b.)

All concentrations (1:50,000 to 1:5,000,000.) used were found effective and had the same action. It was noted that there was a tendency for the rate of perfusion to gradually decrease during an experiment lasting three to six hours. This gradual decrease in the rate of perfusion was more evident during perfusion with perfusion fluid alone, but was evident even during perfusion with perfusion fluid plus adrenalin. In view of this, it was desirable to know what would happen if we perfused with perfusion fluid alone

over a long period of time.

The results obtained from perfusing gills with perfusion fluid alone for a period of five hours may be seen in figure 27, (Gill 14b). During the first hour the rate of flow decreased rapidly from 36 cc. per hour to 10 cc. per hour. After this, the decrease was more gradual. At the end of five hours, the rate of flow was only 1.2 cc per hour. At this time, when adrenalin was added to the perfusion fluid, the rate of flow increased to 29 cc. per hour and then gradually decreased to 20 cc. per hour.

The results obtained from perfusion of a gill with perfusion fluid plus adrenalin over a period of four hours may be seen in figure 28 (Gill 16b). The perfusion was started at 11.00 a.m. At first there was a marked increase in the rate of flow, as is always the case with perfusion with adrenalin solutions. For twenty minutes, the rate of perfusion was 99 to 100 cc. per hour. From that time on, the rate of perfusion varied some, but gradually fell to 42 cc. per hour. At 3.06 p.m., about four hours after the beginning of the experiment, the gill was perfused with perfusion fluid alone. The rate of perfusion dropped from 42 cc. to 18 cc. per hour. At 4.34 p.m., perfusion with perfusion fluid plus adrenalin (1:500,000) was again started. The rate of perfusion increased from 18 cc to 40 cc. per hour.

Discussion.

These results prove beyond a question of a doubt that adrenalin dilates some of the gill vessels. It was impossible

to determine by this method just which vessels are affected. It might be either the capillaries or the arteriole at the margin of the filament. It is difficult to determine the diameter of the vessels unless they are filled with blood or some other easily visible fluid. Adding washed red blood cells to the perfusion fluid necessitates an oscilating perfusion pressure or a pump-like perfusion. This method has not been tried, as yet, because of the complicated technique and apparatus required. Adding India ink or dyes, such as methylene blue, to the perfusion fluid had such a pronounced effect on the rate of perfusion that this method of making the vessels visible was out of the question.

No reason can be given as to the cause of the gradual decrease in the rate of flow during a perfusion experiment. It was thought that this might be due to the fact that the perfusion was carried out at constant pressure. A pumping action during the perfusion inhibited this gradual decrease in the rate of flow but did not eliminate it. It was concluded, therefore, that the gradual decrease in rate of perfusion was not due entirely to the fact that the method of perfusion did not simulate the pulsatile nature of the blood stream.

A study of the records of Keys and Bateman on perfusion of branchial vessels in eels, gives additional support to this conclusion. While Keys and Bateman do not mention it in their paper, their graphs showing perfusion rates indicate that they also obtained a gradual decrease in the rate of

perfusion during a two or three hour perfusion experiment. Since they used a pump which simulated the heart action, we must look elsewhere for an explanation for the gradual constriction of the branchial vessels.

It was possible that this reaction might be due to the fact that all the nerves to the gill had been cut. A gill was, therefore, perfused with the nerves intact, but the rate of perfusion gradually decreased just as in an isolated gill.

When a gill was perfused for an hour or more with perfusion fluid alone, the rate of perfusion fell almost to zero. In such a preparation it should be possible to localize the point of constriction by gradually paring off the vessels from the efferent side toward the afferent. This was tried. The efferent limbs of all the marginal arterioles were cut near the bases of the filaments (at point K in figure 18.) No increase in the flow resulted. The arterioles at the margin of the filaments were then cut successively at points J and I, but the perfusion rate was not affected. The filaments were then split lengthwise with the same negative results. Even cutting the arteriole at point H and complete removal of the filaments did not increase the rate of flow through the preparation. The explanation for these negative results will be found in the part of this paper which deals with the effect of mechanical stimulation of these vessels. It was discovered later that such intense mechanical stimulation, as that produced by cutting the

vessels, causes a complete constriction of the vessels stimulated.

Later work on the effect of oxygen and carbon dioxide on the branchial vessels of the intact animal suggest that one of the reasons for the gradual constriction of the branchial vessels during perfusion may be that the oxygen and carbon dioxide tension of the perfusion fluid was not properly regulated. This hypothesis will be tested at a later date. These results obtained by perfusion of urodele gills coincide with those of Krawkow, and Keys and Bateman in fish gills.

Conclusion.

The branchial vessels dilate when perfused with adrenalin solutions, as evidenced by the increased rate of perfusion. Just which branchial vessels dilate could not be ascertained by this method.

d. Perfusion of the Isolated Anastomosis

Results

When the anastomosis at the base of a gill was isolated and perfused by the technique described, the detached gill was placed on another cannula and perfused at the same time. (See Fig. 21 and 22) In order to compare the reactions, the rates of perfusion were plotted in the same graph (See Fig. 29). It was observed that the reaction of the anastomotic vessels to adrenalin is just opposite to that observed in the gill. When adrenalin was added to the perfusion fluid the arterioles making up the anastomosis constricted and caused a decrease in the rate of perfusion. The effect was not as marked as the dilatation was in the case with the isolated gill, but

was, nevertheless, quite distinct. During the perfusion of anastomoses, it was noted that the rate of perfusion through the anastomosis of the first gill arch was very high. The rate of perfusion was almost as high in the second gill arch anastomosis. In the third gill arch anastomosis, it was lowest. In the first two gill arches, the capacity of the anastomosis was always greater than that of the gills on those arches. The capacity of the third gill arch anastomosis was always less than the gill on that arch.

The results of perfusion of a third gill and corresponding anastomosis may be seen quantitatively in figure 29. (10a and 23b). The curve 23b respresents the rate of perfusion in the third gill. As stated previously for the isolated gill, perfusion with perfusion fluid plus adrenalin (1:50,000) caused an increase in the rate of perfusion, while perfusion with perfusion fluid alone, caused a marked decrease in the rate of perfusion. The rate with adrenalin was 50 to 60 cc. per hour. When perfused with perfusion fluid alone the rate dropped to 34 cc. per hour. Adrenalin solution was again perfused and the rate went up to 50 cc. per hour. A change to perfusion fluid alone caused a decrease in the rate of flow to 18 cc. per hour.

During the time that the gill was being perfused with adrenalin solution, the anastomosis was being perfused with perfusion fluid alone. When the tubes were exchanged on the cannulae, the gill was then perfused with perfusion fluid

TABLE V.

Effect of Adrenalin on Rate of Perfusion in an Isolated Gill Arch Anastomosis.

Exp. No.		Anastomosis on Aortic Arch No. 3	4	5	6	Concentration	Effect on Perfusion Rate
5	a			x		1:10,000	decreases
6	a		x			" "	"
7	a			x		1:50,000	"
8	a			x		" "	"
9	a	x				" "	"
10	a	x				" "	"
11	a		x			" "	"
12	a		x			" "	no change
3	a			x		1:100,000	" " - leak
4	a		x			" "	" " - "
21	a			x		" "	" " --old perfusion
22	a		x			" "	decreases fluid
1	a			x		1:500,000	"
2	a			x		" "	no change - leak
16	a	x				1:1,000,000	decreases
17	a	x				" "	"
18	a				x	" "	"
19	a			x		" "	"
20	a		x			" "	"
13	a		x			1:5,000,000	"
14	a		x			" "	"
15	a			x		" "	slight decrease

alone and the anastomosis with adremalin solution. The reaction of the anastomotic vessels to adrenalin was just the opposite to that of the gill. The gill vessels constricted when they were perfused with perfusion fluid alone, while the anastomotic vessels constricted when they were perfused with adrenalin solution. The rate of perfusion in the anastomosis during a fifty minute period averaged about 20 cc. per hour. When perfused with adrenalin solution, the rate dropped to 7.5 cc. per hour. A change to perfusion fluid alone increased the rate to 15 cc. per hour. The rate of perfusion then gradually decreased to 12 cc. per hour. When adrenalin was again perfused, it fell to 7.5 cc. per hour. Perfusion without adrenalin solution then increased the rate to over 20 cc. per hour.

The experiment was repeated forty times on twenty-two gill anastomoses. Table V shows which arches were perfused, the concentration of adrenalin used, and the results in each case. Only five anastomoses did not respond to adrenalin by constriction. Three of these were found to have a leak in the system. One was perfused with perfusion fluid a week old. Why there was no response in the one anastomosis (12 a) is not known.

Discussion.

The decrease in the rate of flow through the anastomosis when perfused with adrenalin is not nearly as great as the increase in perfusion rate which adrenalin produces in the gill. It is not the magnitude of the reaction that is remarkable, but the fact that in the same gill arch, we find

some vessels which constrict, while others apparently dilate when perfused with adrenalin. If we should perfuse both sets of vessels at the same time, the reaction of one set would tend to negate the reaction of the other set. This explains why it was found impractical to perfuse all gills and gill arches at the same time. In the latter part of this paper, which deals with the effect of oxygen, this differential reaction of the vessels of a branchial arch will become even more significant.

Conclusion

The arterioles in the anastomosis at the base of the gill constrict when perfused with solutions containing adrenalin of 1:10,000 to 1:5,000,000.

2. The Reactions of Branchial Vessels to Pitressin.

a. On the Isolated Anastomosis and Isolated Gill.

The effect of pitressin on branchial vessels of the eel was investigated by Keys and Bateman in 1932. They concluded from four experiments that this hormone in the concentrations they used (25-50 units per liter) had little effect on the mean calibre of the branchial vessels. It will be shown here that pitressin (100 units per liter of perfusion fluid) constricted the anastomotic vessels as well as the branchial vessels.

Method and Materials.

The method of perfusion was the same as that described for adrenalin except that pitressin was added to the perfusion fluid. The pitressin was obtained from Parke, Davis, and Company, in 1 cc. ampoules containing twenty pressor units per cc. One cc. of this plus 199 cc. of perfusion fluid gave a solution containing 100 pressor units per liter. A whole branchial arch was removed from the animal. This was divided into its component parts by cutting off the gill proper just distal to the anastomosis. Each part was then cannulated and perfused.

Results

The effect of pitressin on the gill vessels was difficult to determine by this method because the perfusion fluid alone gradually constricted the vessel. When pitressin was added to this, it hastened the constriction and perfusion fluid soon ceased to flow through the gills. Only two of the gills were perfused for any length of time; and it was necessary to produce a pump-like action on the perfusion pressure in order to do this.

The anastomotic vessels invariably reacted to pitressin by constricting. This reaction was obtained eight times on four gill arches. These reactions are shown quantitatively in the accompanying graph. (See Fig. 30)

Discussion.

It must be admitted that the eight experiments reported here, four on gills and four on anastomoses, are not

sufficient to be conclusive. The conclusions are, therefore, only tentative. It is interesting, however, that while adrenalin constricts one set of vessels and dilates the other, pitressin constricts both sets. It is also interesting that adrenalin constricts the melanophores on the gill filaments while pitressin expands these.

Conclusion.

Pitressin constricts both the anastomotic arterioles and the gill capillaries.

C. Direct Observations on the Reaction of Branchial Vessels to Substances or Stimuli Applied Externally to Gill Filaments.

It was definitely established by the perfusion method that adrenalin in all concentrations dilated the gill vessels; but it could not be determined whether the increased rate of flow with adrenalin was related to the dilatation of the arterioles or the capillaries. The method of making direct observations on the vessels of the gill of the intact animal solved this problem as well as many others.

Methods and Materials

The animals used were, unless otherwise stated, normal A. tigrinum larvae, 1 to 3 years of age, (Colorado race).

The anesthesia used in most cases was "Nembutal", the dose depending on the size of the animal. This was injected subcutaneously in the midline just caudal to the gills, or in other words, into the most cephalad part of the dorsal body fin. In some cases, curare was used, in others, the animals were not anesthetized. In the latter case, the animal was placed in a condom. This was then pinned down at various places in a wax-bottomed dissecting dish. The animal was almost completely immobilized in this way and struggled very little after the first few minutes. A slit was made in the condom near the base of the animal's gills and these were pulled out through the slit. By placing a

gill, or an individual filament between a white piece of paper and the objective of a binocular dissecting microscope, it was possible to determine the diameter of any vessel in the gill filament, even without the aid of artificial light. Many observations were made without anesthesia and without artificial light, but in most cases an arc light was used to facilitate the observations. The light from this was cooled and filtered by passing it through thick glass and a light blue solution of copper sulphate. This light had little, if any, effect on the vessels.

The animal was kept in shallow water and a continuous stream of water was passed through the dish. The temperature was kept at approximately 20° - 22° C. This was found to be an important factor since the temperature, more than anything else, regulates the heart rate. The heart rate was checked before and after many experiments to make certain that this factor was not responsible for the changes observed in the gill vessels.

The substances to be tested were applied in various concentrations to individual filaments or groups of filaments or to one gill at a time. The other filaments and gills thus served as controls. Several methods of application were employed. In some cases tiny crystals of the substances in question were applied for a short time to a definite group of vessels in an individual filament. This is one of the methods devised by Krogh. In other cases the substances were dissolved in the modified Locke-Ringer solution and applied in various concentrations to

individual filaments or gills.

Theoretical Considerations.

Many of the reactions of the gill vessels were puzzling until the hydrodynamic principles involved in the gill circulation became more clear. These principles are intimately related to the relative sizes and arrangement of the vessels. A diagram of an aortic arch with the branchial vessels; and a discussion of the possible hydrodynamics will greatly facilitate the presentation of the results. (See Fig. 18)

From the diagram, it is evident that there are three ways for blood to pass from the heart to the dorsal aorta:

1. It may pass through the ventral portion of the aortic arch, A, thence through the intermediate anastomosis, B, C, D, to the dorsal portion of the aortic arch, E.
2. It may pass from A to the afferent branchial artery, F, and thence through the arteriole, G, H, I, J, K to the efferent branchial vessel, L, which communicates with E, the dorsal portion of the aortic arch.
3. Or it may pass through F, to the afferent limb of arteriole, G, H, and then through any capillary in the capillary bed, M, N, O, to the efferent limb of arteriole, J, K, and thence to L and E.

Under normal conditions in the larval animal some blood travels from the heart to the dorsal aorta by all three of these routes. In order for any blood to flow through the capillary bed, M, N, O, of the filament, the pressure must be greater in the afferent limb of the arteriole, G and H,

than it is in the efferent limb J, K.

The morphological conditions that make possible the differences in pressure in the two limbs of the arteriole are as follows: Both the afferent and efferent limbs of the arteriole loop of the filament taper toward the tip, where they communicate directly with each other; so that at point I, the diameter of the arteriole is much less than at either G or K. If the arteriole loop could be straightened out, it would be evident that if a liquid was forced through this tube from G, to K, the pressure in the tube would be greater at G and H than at J and K (Bernoulli's theorem). Then in addition to the tapering of the arteriole, there is the viscosity of the blood and the fact that the arteriole is bent upon itself, that tend to make the pressure lower in the efferent limb, J, K, than in the afferent limb, G, H.

By far the most important mechanism which controls the difference in pressure in the two limbs of the arteriole loop, is the anastomosis at the base of the gill. As long as the vessels making up the anastomosis have a small diameter, the pressure in the dorsal pprtion of the aortic arch, E, and the efferent branchial artery, L, may be much lower than the pressure in the ventral portion of the aortic arch and the afferent branchial vessel. If the vessels making up the anastomosis at B dilate, there is a tendency to increase the pressure in L, E, and K, and at the same time, to decrease at at A, F, and G. Dilatation of the anastomotic vessels B, has a pronounced tendency to equalize the pressures in both limbs of the arteriole loop

of the filament; and thus to stop the flow of blood through the filaments.

There are, then, three factors that regulate the flow of blood through the capillaries in the gill filament. One is the tone or diameter of the capillaries themselves (M, N, O); another is the tone or diameter of the arteriole at the tip of the filament I; and last, but not least, there is the tone or diameter of the vessels in the anastomosis at the base of the gill. It must be remembered that this concept of the gill circulation is based on the anatomical considerations, and it remains to be seen whether or not this rather speculative, but logical, concept will be supported by the physiological results or observations.

Results.

Through a rather fortunate accident it was found possible to test some of the postulated hydrodynamic principles. In manipulating a gill filament with two dissecting needles, one of the limbs of the arteriole lpop was accidently stimulated by the point of one of the needles. The arteriole was seen to gradually constrict, until it had completely stopped the flow of blood through that part of the vessel. After a few seconds the constricted portion gradually dilated.

Shortly after recording these observations, it was realized that this reaction might be useful in studying the effect of a change in the diameter of any part of the arteriole loop. The following is a description of

the effects produced by causing a constriction at various points in the filament.

1. Effect of Mechanical Stimulation on Branchial Vessels

An arteriole was stimulated at point K, or the efferent limb of the arteriole near the base of the filament. The resulting constriction eventually stopped the flow of blood at that point. This stopped the flow of blood through the whole filament. Blood was seen to move forward and backwards with each heart beat in the arteriole as well as in the capillaries. This fluctuating motion of the blood was greatest in the afferent limb of the arteriole G, H, and gradually diminished toward the constriction at K, but was seen even in the capillaries.

Stimulation of the midpoint J, of the efferent limb of the arteriole also caused a cessation of the flow of blood at that point. Near the constriction, on the side toward point G, or toward the base of the gill, the blood fluctuated back and forth with each heart beat. In the capillaries, at the base of the gill, M, the blood flowed rapidly. Contrary to expectations, blood also flowed through the capillaries in the tip of the filament O, but more slowly than at the base. The flow of blood through the capillaries at the tip of the filament was made possible by a reversal of the direction of the flow of blood in the capillaries near the constricted arteriole. That is to say, blood passed through the capillaries in

the tip of the filament O, entered the efferent limb of the arteriole and passed toward the constricted portion of this. The flow through the constricted arteriole being impossible, blood passed back into the capillaries, then flowed toward the base of the filament through the capillaries. After circumventing the constricted portion of the arteriole, it again returned to the efferent limb of the arteriole and resumed its usual course.

The arteriole was next stimulated mechanically at the tip of the filament. This constricted, and the constricted area traveled for a short distance in the opposite direction of the flow of blood, or along the afferent limb of the arteriole. As would be expected, the blood did not stop flowing through the capillaries, except those near the tip of the filament when the constriction had migrated toward the base of the filament. The blood fluctuated back and forth in the efferent limb near the constricted arteriole at the tip of the filament. Some of the blood cells could be seen to pass back and forth from the efferent arteriole near the tip, into the capillaries, and then back into the efferent arteriole. The circulation through the capillaries may have increased, due to the constriction of the arteriole at the tip, but this could not be definitely ascertained.

Mechanical stimulation of the afferent arteriole near its middle or at point H, caused the usual constriction at that point. The capillaries proximal to this were open. Distal to the constriction, blood did not flow through the capillaries, but fluctuated between the efferent arteriole

and the capillary bed with each heart beat. This fluctuation was also evident in the arteriole at the tip of the filament, and even as far proximal in the afferent limb as the constriction. All of the above observations were made a number of times on six different animals.

To determine whether or not the capillaries, as well as the arterioles, were endowed with the ability to contract independently, the same method of mechanical stimulation was tried on the capillary bed of a filament. A filament was supported by a glass slide and both were but slightly submerged in water. A slight pressure was exerted on the capillary bed of the filament with a pin point. The capillaries in the region stimulated, closed down almost immediately. This reaction soon spread in all directions. Eventually a relatively large circle in the capillary bed was seen where no blood circulated; while blood in the capillary bed of the same filament near this area, circulated normally. The extent to which the reaction spread depends on the sensitivity of the animal and the strength of the stimulus.

To produce a strong mechanical stimulus, a capillary bed of a filament was pierced by the needle. The reaction to this stimulus was quite uniform. If the needle pierced the filament near the middle or near points H and J, of the arteriole, spreading of the constriction of the vessels involved, not only capillaries, but also the arterioles on both sides of the filament. If both of the arterioles closed completely, as happened occasionally, no blood

circulated in the distal portion or tip of the filament. The filament became narrow or wasp-waisted in the area where the vessels had constricted. At the height of one of these reactions, the width of a filament was measured by means of an occular micrometer:

Just proximal to the area constricted, it measured	0.76 mm.
At the area constricted, it measured	0.40 mm.
Just distal to the area constricted, it measured	0.50 mm.

When the capillary bed of the filament was pierced by a needle, two or more of the capillaries at the point stimulated were usually torn open. Only a few blood corpuscles escaped from these openings, because of the rapid constriction of the surrounding vessels. This is, undoubtedly, a protective mechanism to prevent bleeding, which is extremely important in such a delicate and easily injured structure as a filament on an external gill. When the vessels surrounding the point of injury again dilated, no bleeding occurred. This explains why it was impossible to localize the part of the filament that closed up during perfusion of the gill. It will be remembered, that in an attempt to solve this problem, the arterioles and capillaries were cut with scissors in various places. This intense mechanical stimulation caused these small vessels to constrict to such a degree that it was impossible to force the perfusion fluid out of the cut ends of these with the pressures employed.

Discussion.

From all the work presented here, on the mechanical stimulation and injury of blood vessels in various parts

of the filament, what should be emphasized above all else is, first that contractile elements exist in a gill filament that are capable of altering the diameter or tone of capillaries or arterioles. Second, that these elements may regulate the diameter or tone of individual capillaries or a small part of the arteriole in any filament. Third, that if the capillaries and arterioles in a certain part of the filament constrict to the point of stopping the flow of blood, the filament becomes narrow or constricted in that region. In general, it may be said that when the vessels in a gill filament are dilated, the filament is much longer and wider than when they are constricted.

The search for contractile elements in the gill filament, that was prompted by these observations has been unsuccessful so far. The only cells that could be responsible appear to be connective tissue cells, and it is doubtful that these have the ability to contract.

2. The Effect of Adrenalin on Branchial Vessels as Determined by Direct Observation.

The method of making direct observations on the branchial vessels was used to confirm the results obtained by the perfusion experiments. It was possible, by this method, to definitely establish the fact that, when a gill was treated with adrenalin solutions, the capillaries dilated tremendously.

Methods and Materials

The anesthesia and technique of arranging the animal was the same as described previously. It was found possible to place one of the animal's gills in a special observation dish or tray. It could thus be immersed in solutions containing any test substances in any desired concentration. The corresponding gill on the other side was used as a control gill, Eventually a technique was devised which permitted the application of a test substance to a single gill filament. By this method, control filaments could be observed simultaneously.

Results.

The effect of adrenalin was tested thirty times on twelve different animals. The concentrations ranged from 1:2,000 to 1:1,000,000. The effect observed was always an unmistakable dilatation of the capillaries of the gill filaments. In addition to the observations that the capillaries increase in diameter, there is other evidence in favor of a capillary dilatation.

While observing the capillaries in the tip of a gill filament 0.2 cc of a 1:3000 solution of adrenalin was injected into one of the aortic arches. Strangely enough the blood stopped flowing through the vessels at the tips of the filaments. That this was not due to constriction of the vessels was evident. Moreover, the vessels at the tip were filled with blood that seemed to fluctuate back and forth with each heart beat. Why the blood should stop

flowing through the tip of the filament was indeed perplexing until it was observed that the capillaries at the base of the filament had dilated to such a degree that it was possible for all the blood to pass through a few capillaries located near the base of the filament. (See M Fig.18) Here it flowed quite rapidly, following the easiest and shortest route through the filament.

Conclusion.

By the method of direct observation, it was possible to show conclusively that adrenalin, in all concentrations tested, produces an extreme dilatation of the gill capillaries. It was not possible to observe any change in the diameter of the arterioles.

Other Substances.

A number of other substances were tried in the same manner as described for adrenalin. These results will be described briefly:

3. The Effect of Pitressin on Branchial Vessels.

Pitressin (1:10 in salamander Locke-Ringer) constricted the capillaries and arterioles. .2 cc. full strength pitressin plus .2 cc of the above mixture, constricted the capillaries and arterioles. Pitressin (1:15) constricted both the capillaries and arterioles. These results were repeated six times on different filaments and invariably the capillaries and arterioles constricted. Compared with the action of adrenalin, the effect of pitressin is very

transitory. .1 cc pitressin, injected intravenously, constricted both the capillaries and arterioles.

4. The Effect of Acetic Acid on Branchial Vessels.

When acetic acid was applied in concentrations of 1:200 and 1:1000, it produced a dilatation of the branchial capillaries and constriction of the arterioles. It has much the same action as adrenalin. This reaction was obtained in three experiments.

5. The Effect of Lactic Acid on Branchial Vessels.

Lactic acid was employed in the same concentrations as acetic acid and the same number of times. This had exactly the same action as acetic acid.

6. The Effect of Acetylcholine on Branchial Vessels.

Acetylcholine, 1:1000, when applied externally to a filament, dilated the arterioles and probably the capillaries. The dilatation of the arteriole at the tip of the filament was sometimes so great that all the blood flowed through the arteriole at the margin of the filament, none of it went through the capillary bed. It was certain that the capillaries were not constricted, but under the circumstance, it was difficult to determine that they dilated actively. This reaction was obtained ten times in ten experiments. The injection of .0003 grams of acetylcholine killed the animal almost instantly. All vessels were dilated. The injection of .000125 grams caused all vessels to dilate: branchial, systemic, arterioles, and capillaries.

7. The Effect of Histamine on Branchial Vessels.

Histamine, 1:1000, dilated the branchial capillaries. Only three experiments were made with histamine.

8. The Effect of Nicotine on Branchial Vessels.

Nicotine was tested sixteen times. Nicotine, 1:1000, constricted the arterioles. When applied to a single filament, it produced a twitching of the filament. It caused the afferent limb of the arteriole loop of the filament to shorten. Because of this, the filament assumed a crescent shape. After the initial stimulus had worn off (in a few seconds), the filament sometimes became straight again. This was frequently accompanied by a bursting of the arteriole at the tip of the filament. Blood spurted from the tip for some time and then it suddenly stopped.

9. The Effect of Urethane on Branchial Vessels.

Very fine crystals of urethane were applied directly to the capillary bed of a filament for a short time. (This method was devised by Krogh for use on the frog's tongue.) Urethane caused great dilatation of capillaries and arterioles; and eventually produced stasis of long duration. Urethane was tested five times.

10. The Effect of Butyn on Branchial Vessels.

Butyn (0.5%) dilated the branchial capillaries in three experiments.

11. The Effect of Cocaine on Branchial Vessels.

Cocaine (0.5%) had a tendency to constrict the gill capillaries in five tests.

12. The Effect of Caffein on Branchial Vessels.

Caffein was tried several times, but even when pure crystals were applied, little, if any, effect was noted.

13. The Effect of Methylene Blue on Branchial Vessels.

Methylene blue, 1:2000 to 1:100,000, produced a definite constriction of the gill capillaries. This was demonstrated in twenty perfusion experiments and four times by direct application to the gill filament.

14. The Effect of Tissue Extract on Branchial Vessels.

Tissue extract (vagotonine or kalikrein) was tried only twice, but it seemed to have little, if any, effect on the branchial vessels.

15. The Effect of Crystalline Thyroxine on Branchial Vessels.

Crystalline thyroxine was also tested, both by application of pure crystals and solutions, but it had no observable effect on the branchial vessels. The observations were, however, of short duration.

16. The Effect of Changes in Oxygen and Carbon Dioxide Tension of the Blood on Branchial Vessels.

In an earlier paper on the effect of ligating the pulmonary arch, (Figge, 1930) it was predicted that a change in the oxygen tension of the blood supplying the gills was at least partly responsible for gill reduction. The following quotations are taken from the discussion of the paper: "It is, therefore, thought that the reduction of gills in the controls is due in part at least to this change from a low to a relatively high oxygen content of the blood supplying them" - - - "This recalls the attempts of von Chauvin and of Boulenger to enforce metamorphosis by enforced air breathing, or by purely external non-glandular factors. Many of these attempts were doubtless successful, but later investigators have obtained varying and conflicting results. One is given the impression, however, that there is something in the external environment (oxygen perhaps) that plays some rôle in the production of metamorphic changes. The possible relationship that may exist between these external factors and the internal secretions is not fully understood at the present time". - - - - "It is perhaps needless to point out that it may be of extreme importance to the developing amphibian to have an arrangement whereby the gills are not reduced until oxygen is available from another source". For the entire chain of reasoning that led to this hypo-

thesis, it will be necessary to refer to the earlier paper. It may be summarized briefly as follows:

1. The gills are not reduced in any amphibian until there is developed an adequate oxygen intake at points other than the gills.

2. When blood is oxygenated at these other points, lungs, skin, or pharynx, it returns to the heart and is sent to the gills.

3. The hypothesis is that when the gills are thus supplied by oxygenated blood, they will be reduced.

The method devised to test this hypothesis is not only practical, but simple and the results are conclusive.

Methods and Materials.

The animals used were normal A. tigrinum larvae, one to three years old. These varied in size, (body length 70 - 140 mm.)

Most of the animals were anesthetized with "Nembutal". This was found to be far superior to chloretone, urethane, chloroform, or ether. All of the last mentioned drugs, in concentrations sufficient to produce anesthesia, cause such great variation in heart rate and vascular tone that it was impossible to work with them. Chlorotone anesthesia very frequently stops the flow of blood in the gills almost completely; this is not due to the constriction of gill vessels but due to a very low heart rate. With "Nembutal", however, the circulation was always very good in all parts examined, and the heart rate varied only with variations

in temperature. After some experimentation with the dosage, it was found possible to regulate this according to the size of the animal and the length of the anesthetic period desired. It was possible with "Nembutal" anesthesia, to make observations on the branchial vessels for as long a period as ten to fifteen hours. The animals usually recovered within twenty-four hours. A few curarized animals were used, and in order to avoid the possible criticism that the effect observed was due to the anesthesia, a number of experiments were performed on unanesthetized animals. These were immobilized by the method described previously. The incisions necessary were made under local anesthesia (cocaine).

After the animal was anesthetized it was pinned down in a wax-bottomed dish partly filled with salamander Locke-Ringer solution. An incision was then made in the abdominal wall just lateral to the estimated position of the tip of the lung. The tip of the lung was then pulled out and cannulated. The lung was then pushed back into the abdominal cavity to near the normal position. The cannula was kept in position by a holder that rested on the wax in the dish. It was arranged so that either a stream of oxygen or carbon dioxide or any desired mixture of these gases might by passed through the cannula. The gas which passed through the lungs was driven out, either through the glottis or through a hole in the tip of the other lung.

The humidification of the gases tested was effected by bubbling it through distilled water. Another bottle with a longer inlet tube and a higher column of water served as a pressure regulator, so that it was not possible for the pressure in the cannula to go above 6 cm. of water.

The effect of the variation in the oxygen and carbon dioxide tension in the blood was determined by direct observation on the gill vessels as described previously.

Results.

The effect of oxygenated blood on the branchial vessels was tested thirty-five times on ten different animals. The result was, in all cases, a decrease in the amount of blood passing through the gill. If the oxygen treatment was continued long enough, a complete cessation of the flow of blood in the gills resulted. This was not due to a decreased heart rate since this was taken before and during the administration of oxygen. Usually the heart rate was the same during the treatment with oxygen, occasionally less, and sometimes even higher. Moreover, it was noted that even though no blood flowed through the gill capillaries, it was flowing very rapidly through the capillaries in the gill blade, operculum, and the circulation in the lung was very good. The cessation of the flow of blood in a gill cannot, therefore, be due to a decrease in the heart rate or general blood pressure.

The direct observation of these vessels shows that the arteriole of the filament dilates in both limbs and

especially at the tip. One is also able to observe an active constriction of the capillaries. Both of these reactions are difficult to describe quantitatively. In addition to the observations on the change in diameter of the capillaries, there are other observations that may be taken as evidence of active constriction. If the capillaries were merely passive, and had no way of increasing or decreasing their tone, the only factor that would cause a cessation of the flow of blood in them, would be a decrease in the blood pressure on the afferent side, or an equalization of pressures at both ends of the capillary. That this is not the only factor operating to stop the flow of blood in the capillaries may be seen from these facts: (See Fig. 18)

1. The pressures on both ends of the capillaries are never equal as long as blood flows through the arteriole G, H, I, J, to K. For if the pressure at G were exactly equal to that at K, no blood would flow from one point to the other. The capillaries were frequently closed off while blood flowed through the arteriole loop from G to K.

2. If it were merely a matter of equalization of pressures at both ends of a capillary, we would expect all of them in one filament to close off at once. But this did not happen. In most cases, the capillaries at the base or middle of the filament closed off first and later the capillaries at the tip of the filament. Sometimes, however, it was just the opposite. At any rate, blood did not stop flowing in all of them at once. When carbon dioxide was given and blood again began to flow through the capillaries in the filament,

the last capillaries to close down were the first to open up.

We may conclude from this that there is an active constriction of the capillaries in the filament. When the filament is supplied with oxygenated blood.

A portion of a protocol of one of these experiments follows:

Animal XXVI B 6, Colorado Axolotl, curarized. January 13, 1934.

Condition: Larval, normal. Respiratory reflexes (as evidenced by occasional gill movements) intact during the entire experiment.

3.40-4.20 p.m. Operation: An incision made in right side, tip of lung pulled out and cannulated. Left lung was likewise pulled out and cannulated to allow the gas to escape from the tip of that lung.

4.25 p.m. Pulse rate - 57 beats in 60 sec.

4.28 Oxygen started through lungs. Bubbles coming out of animal's mouth and incision in left lung.

4.33 Many of the capillaries in gill filaments closing off. Heart beating as hard and fast as ever. Respiratory movement stopped. Filaments seem to be decreasing in size. Heart rate - 45 beats in 47 seconds.

4.35 p.m. Gill capillaries practically shut off, even at the base of the filaments.

4.40 Stopped oxygen and began ventilating animal's lungs with expired air.

4.45 Blood begins to flow through capillaries at tip of filament (evidence of capillary constriction at base of filament). Respiratory movements coming back. Blood in fifth aortic arch becoming darker.

4.50 Gill circulation opening up. Filaments seem to be larger and more fluffy. Pulse rate - 41 beats in 43 seconds.

5.35 p.m. Started oxygen through lungs which are distended in a few seconds. Circulation in gill capillaries normal. Distension of lung with oxygen seems to dilate lung capillaries.

5.37 Blood in aortic arches has become bright light red.

5.44 Pulse rate - 62 beats per 60 seconds. Filaments becoming pale.

5.45 Blood still flowing through capillaries at tip of filament.

5.49 Even the blood in the sixth aortic arch is light red.

6.00 Capillaries in gill completely closed off. Blood flows through the arteriole loop.

6.10 Same as above. Heart rate - 62 beats per 60 seconds.

6.20 Condition the same.

In general, it was observed that the capillaries in the first gill closed first, then those in the second, and the last ones to react were those in the third gill. This is related to the action of the spiral valve which sends most of the blood from the lungs to the first gill, a slight portion of it to the second gill, and very little, if any, to the last gill and sixth aortic arch. This observation will also be related to the morphology of the anastomosis at the base of the gill and will be referred to later.

As it had been established that a change from venous (reduced) to oxygenated blood caused a cessation of blood flow in the gill capillaries, and that this was not due to a slow pulse, the following question arose: Was the cessation of the flow of blood through a gill due entirely to the constriction of the gill capillaries, or could this

be related in any way to the reactions of the anastomotic vessels at the base of the gill?

This question was answered by making direct observations on the anastomosis at the base of the gill during oxygen treatment. Before the blood was thus oxygenated in the lungs, no blood flowed through some of the anastomotic vessels; they were, therefore, not noticed until three minutes after the oxygen treatment was started. These dilated gradually and finally carried a wide stream of blood. Eventually most of the blood passed directly to the dorsal aorta through the anastomosis and very little passed through the gill. Ten percent carbon dixoide was then passed through the lungs. The blood in the aortic arches became darker and the arterioles in the anastomosis gradually constricted. Blood soon stopped flowing in many of the arterioles of the anastomosis. Eventually, most of the blood passed through the gill and only a reatlively small quantity passed through the anastomosis.

It was thought that the branchial vascular reactions might be due to a reflex caused by the distension of the lungs. That the reaction was not a reflex was evident from the speed of the reaction and the long latent period. The blood did not stop flowing in the branchial capillaries suddenly. On the contrary, only a few capillaries at a time closed off and the whole reaction took place so gradually that it sometimes extended over a period of twenty to thirty minutes. Meanwhile, the blood in the aortic arches, judging from the color, became more and more saturated with

oxygen. The reaction time was also related to the degree of ventilation of the lung and to the condition of the circulation in the lungs. In the second place, the reaction cannot be a reflex caused by the distension of the lungs, because if expired air, nitrogen, or nitrogen plus carbon dioxide, was used to distend the lungs, just the opposite effect was produced. Blood circulated through the gill capillaries at a tremendous rate.

In spite of all this evidence against the possibility of the reaction being due to a reflex, the following experiment was performed. An animal was prepared and cannulated in the usual manner. All the gill capillaries were closed off by passing oxygen through the lungs. Fifteen per cent carbon dioxide was then passed through the lungs to cause blood to circulate in the gill capillaries. All the nerves to the lungs and gills were cut on one side. This included sectioning the vagus and glossopharyngeal nerves close to the chondrocranium. The oxygen experiment was then repeated and observations made on the gill vessels on both the operated and unoperated side. The reaction occurred on both sides at the same rate and degree.

In another animal, the same procedure was followed and the same results obtained. Then the other lung and gill were also denervated, and the lungs inflated with oxygen. The gill capillaries again closed and reopened when the lungs were ventilated with carbon dioxide. This is additional evidence that the reaction cannot be dependent upon a reflex.

Discussion

It was stated in the discussion of the hydrodynamics of the gill filament, that a cessation of the flow of blood in the capillary bed, M, N, O, might be related to an active constriction in the capillaries themselves, a dilatation of the arteriole at the tip of the filament I, or a dilatation of the anastomotic vessels at the base of the gill. (See Fig. 18) All of these reactions occur when the gill arch is supplied with oxygenated blood. There can be no doubt, however, that the dilatation of the arterioles in the anastomosis at the base of the gill are largely responsible for the cessation of the flow of blood in the capillaries of the filaments. Any increase in the diameter of the arterioles in the anastomosis will tend to equalize the pressure in the afferent and efferent branchial arteries, and unless a difference in pressure exists, no blood will flow from one to the other through the gill vessels. In the case of the third gill arch, the anastomosis is not well developed; and even though the arterioles dilate to the extreme, they cannot shunt all the blood of this arch to the systemic circulation. This is probably why it is frequently impossible to completely stop the flow of blood through the third gill. The active constriction of the capillaries in the filament are, therefore, of secondary importance in stopping the flow of blood through the capillary bed.

The reaction of one set of vessels is just the reverse

of that of the other. The reactions, however, augment each other in stopping the flow of blood through the gill. In other words, a summation of the effects produced by the differential reactions of the branchial vessels prevents the flow of oxygenated blood through the gill. This differential reaction of the branchial vessels, i.e., a dilatation of the anastomotic arterioles and a constriction of the branchial capillaries when they are supplied with oxygenated blood, is a truly remarkable mechanism. It prevents the waste of the energy that would be necessary to force oxygenated blood through the gill. Instead of having to pass through the gill, the oxygenated blood is shunted directly to the systemic vessels through the anastomosis. It is this same mechanism that makes it possible to reduce the gills, when during a normal metamorphosis, more and more oxygen begins to enter the blood at other respiratory surfaces.

III. SURGICAL CONTROL OF GILL CIRCULATION.

The Effect of Stopping the Flow of Blood in a Gill.

It was found that oxygenated blood caused the cessation of the flow of blood in the gill capillaries. Before it could be concluded that when the gills are supplied with oxygenated blood they would be reduced, it was necessary to show that a cessation of the flow of blood through a gill would cause the reduction of that organ.

Fortunately, this had already been demonstrated by ligation long before the oxygen experiments were made. The significance of the ligation experiment was, however, not appreciated until after the oxygen experiment.

Method and Materials

The ventral portion of the third, fourth, or fifth aortic and visceral arches were ligated in two places by a technique described in earlier papers. (Figge, 1934) Two dental floss ligatures were passed in one gill slit and out the gill slit just cephalad to this. The ligatures thus passed around one aortic and its corresponding visceral arch. One ligature was tied near the ventral end of the visceral arch and the other near the dorsal end. The arch eventually fell out between the ligatures. The two ends of the arch never again touched, therefore, there was no possibility of regeneration. This method stopped the flow of blood through the gill as well as through the anastomosis.

Some blood may have reached this gill from the dorsal aorta, but this had passed through the other gills and was, therefore, relatively high in oxygen content. Moreover, it did not flow through the gill, but fluctuated in the gill vessels, a condition very much like that produced by dilating the anastomosis with oxygenated blood. (See Fig. 23) Twenty-one gill arches were ligated in this manner.

Results.

The filaments disappeared from the ligated gills within three or four weeks. The filaments on the other gills of the same animal seemed to grow longer and larger to compensate for the loss of the operated gill. Figure 23 is a photograph of corresponding gill arches of the same animal. The filaments are completely reduced in the operated gill in which the flow of blood was prevented by a ligature on the ventral portion of the aortic arch. The filaments are large and long on the corresponding gill from the same animal.

Conclusion.

The gill filaments are reduced when the flow of blood through a gill is prevented by a ligature on the ventral portion of the aortic arch.

GENERAL DISCUSSION

General Discussion.

The problem, as stated earlier, was: First, to discover the factors that caused the gill vessels to be obliterated and other vessels to take over the respiratory function. Second, to determine the relationship between the morphogenetic process of gill reduction and the vascular reactions. This paper is a contribution to the solution of these problems.

Of the substances tested, only a few of them are to be considered as possible factors in a normal gill reduction. The substances are listed with the reactions obtained:

Substances Tested	Action on:- Anastomotic arterioles	Gill Capillaries.
Adrenalin	constricts	dilates
Lactic Acid	constricts	dilates
Acetic Acid	constricts	dilates
Carbon Dioxide	constricts	dilates
Pitressin	constricts	constricts
Acetylcholine	dilates	dilates
Oxygen	dilates	constricts

Fron this, we see that there are four types of substances. In the first group are four substances having the same action. Adrenalin, lactic and acetic acids and carbon dioxide constrict the arterioles in the anastomosis at the base of the gill and dilate the gill capillaries. Pitressin constricts,

acetylcholine dilates both sets of vessels. The effect of oxygen is just the opposite to that of the first group, (adrenalin, acid group) in that it dilates the arterioles at the base of the gill and constricts the capillaries in the gill filaments.

It may be seen that any increase in carbon dioxide, adrenalin, or acid metabolites in the blood would increase the circulation through the gill. Therefore, these substances would not bring about gill reduction. In this connection, it is interesting that Helff (1932) measured the pH of the blood of Anuran larvae. He found that during metamorphosis, there is a drop from 7.50 to 7.20, or an approximate drop of 0.3. He attempted to relate many of the so-called "degenerative changes" that occur during larval involution to this change in the hydrogen ion concentration of the blood. In the first place, the reduction of gills in Urodeles is not a degenerative process. It is a morphogenetic process. This concept was developed from an histological study of gill reduction. In the second place, the reactions of the branchial vessels to acid substances in the blood are diametrically opposed to what happens in a normal gill reduction. The opinion of Helff is, therefore, not substantiated in this work, at least as far as gill reduction is concerned.

Oxygen, of all the substances tested, has an action that is most effective in stopping the flow of blood through a gill. This has a double action, because it constricts the gill

capillaries and, at the same time, dilates the anastomosis at the base of the gill. This supports the contention that oxygenated blood going to the gills is one of the factors causing gill reduction.

Acetylcholine might also assist in gill reduction, because its action resembles that of oxygen, in that it dilates the anastomosis at the base of the gill. At the same time, however, it also dilates the capillaries in the filament. It would, therefore, not be such an ideal substance as oxygen for stopping the flow of blood through a gill.

The hypothesis, that the gills are reduced because of the cessation of the flow of blood through the gill capillaries, is substantiated not only by observations made on gills during a normal gill reduction, but also by experimental evidence furnished by experiments on ligation of branchial vessels.

Probably the most interesting and fundamental fact that emerges from this work is the observation that in the same organ, one set of vessels dilates while another constricts, when treated with the same substance, (oxygen, carbon dioxide, adrenalin, acids). Before these physiological reactions can be completely understood, the morphology of these vessels must by studied in greater detail. The differential reaction may be related to morphological differences in the contractile elements on the walls of the vessels in question. The reaction of the Rouget cells (Krogh, 1929; Vimtrup, 1922), or pericytes (Zimmermann, 1922) may be decidedly different from that of the

muscular tunic on the arterioles. Krogh and his school (Vimtrup and Zimmermann) claim that there is a gradual transition from the muscle cells on an arteriole to the Rouget cells on the capillaries. In addition, these cells have been seen to contract, (Vimtrup). They, therefore, assume that Rouget cells are the same as the smooth muscle cells in any other part of the vascular system, except that they have been modified into branching cells. But even though it would be possible to demonstrate a morphological difference between the two sets of vessels and the two sets of contractile elements, it would not explain the differential reaction, because some substances do not induce this reaction. That is, certain drugs, such as pibressin, cause both sets of vessels to constrict, while acetylcholine causes both sets of vessets to dilate.

That histamine constricts the arterioles, while it dilates the capillaries, was shown by Dale and Richards in 1918. No work on the vascular system, since the time of Harvey, shows such a clever analysis of the physiological results. They concluded, "that the vasodilator effect of histamine, and probably that of adrenalin (very low concentrations) are due to relaxation of the tone of capillaries, while that of acetylcholine is due to its action on arterioles. Histamine has a constrictor effect on arteries; the better known vaso-constrictor effect of adrenalin probably involves both arteries and capillaries." They admit, "That no exact line can be drawn as to the point where the characteristic arteriole reaction gives way to the characteristic capillary reaction." Even before Krogh,

these investigators suggested" that the current conception of the peripheral resistance to blood flow, as determined almost exclusively by the tone of the arterioles, allows too little importance to capillary tone as a factor".

The results of Dale and Richards have been discussed at such length, not only because it is an example of beautiful physiological analysis, but also because these investigators demonstrated a differential reaction of blood vessels to histamine. That is, that histamine constricts arterioles and dilates capillaries. Their results have been confirmed over and over again; but it must not be forgotten that the conclusion was not the result of direct observations on the capillaries, but of physiological speculation. That they realized as much, may be gathered from an excerpt from their paper: "For the sake of brevity and emphasis we have throughout this paper spoken of the vasodilator effect of histamine (and by analogy that of adrenalin) as due, in our opinion, to action on capillary walls; of the constrictor effect of histamine, and the dilator effect of acetylcholine as due to action on arterial muscle. IT IS IMPORTANT, THEREFORE, TO MAKE IT CLEAR THAT NO SHARP DISTINCTION IS WARRANTED BY OUR EVIDENCE. WHAT WE HAVE ACTUALLY ON EVIDENCE IS THAT THE ANATOMICALLY SEPARABLE ARTERIAL BRANCHES ARE STIMULATED TO CONSTRICTION BY HISTAMINE AND ADRENALIN, TO DILATION BY ACETYLCHOLINE; THAT, THEREFORE, THE DILATOR EFFECT OF HISTAMINE, WHICH UNDER IDENTICAL CONDITIONS, PREDOMINATES IN THE WHOLE ORGAN, MUST BE AN EFFECT ON VESSELS MORE PERIPHERAL THAN THESE. (The italics are mine.)

The conditions necessary to produce this vasodilator effect support the view that it is mainly an effect on capillaries. The possibility is not directly excluded that it may spread on to the smaller arteries, overlapping with the vasoconstrictor action in such a way that there is a zone in which one or other action is the effective resultant according to the dosage. At the same time there is no evidence of such overlap in the case of histamine, and such a conception would renew our original difficulty of reconciling a relaxing effect on arterial muscle with the stimulant action of histamine on all kinds of plain muscle in any dose in which it is effective at all."

The method of direct observation and the object used for study in this work, leave little room for much physiological speculation as to which blood vessels react, or how they react with any given substance. Dale and Richards demonstrated the differential reaction of capillaries and arterioles to histamine indirectly. In this work, the differential vascular reaction has been demonstrated by direct observation for a number of substances, (adrenalin, lactic and acetic acids, oxygen, and carbon dioxide). When the mechanism of differential vascular response to the same substance in the same organ is fully appreciated, and if it becomes possible to demonstrate this in other forms, the physiological significance of this work will become increasingly apparent.

Whether a similar mechanism of differential vascular response will be found in other organs or other forms remains

to be seen. The anatomical basis for the possibility of such differential vascular responses have been known for some time. Anatomists have known for many years of the existence of direct communications between the arteries and veins that are distinctly larger than capillaries. These were called arterio-venous anastomoses. Between the year 1862 and the present, at least fifteen different investigators have described arterio-venous anastomoses. (For reviews of this field see Krogh, 1929; Clark and Clark, 1934; and Weidenreich, 1933). Physiologists have consistently disregarded these structures, with the result that our knowledge of their function has been largely based on anatomical speculation. Recently, however, direct observations have been made on the activity of these arterio-venous anastomoses in the living animal. Clark and Clark used the transparent chamber in the rabbit's ear. Grant (1930) and Grant and Bland (1931) made direct observations on the arterio-venous anastomoses in the rabbit's ear, bird's foot, and human skin. All of these investigators found that an arterio-venous anastomosis may vary greatly in diameter from time to time. Grant found, in addition to the great variations in the diameter produced by changes in temperature, that local mechanical stimulation, acetylcholine, and histamine dilate arterio-venous anastomoses; while adrenalin and sympathetic stimulation produce constriction in these vessels.

"Heimberger (1925) has also been able to observe the reaction of arterio-venous anastomoses in a considerable number of fingers of persons with a delicate skin. He

describes them as short connections between arterioles and venules, short circuiting the long capillary loops. He finds these channels normally closed to be opened up for a short period by weak mechanical stimulation. His findings are supported by observations of the blood flow in superficial venules and capillaries which are explicable only if arterio-venous anastomoses exist in close proximity to the vessels under observation. In a few cases Heimberger has seen pulsation of the blood in venules when the corresponding arterioles were closed and the blood in the capillaries quiescent. This is possible only when the venule in question possesses another connection with the arterial system, through which the pulse is admitted. It follows from the observations of Heimberger that his 'derivating channels' must by able to contract and expand and to close up entirely" (Krogh, 1930)

This brief review of the work on arterio-venous anastomoses is topoint out the possibility that blood may pass from the arteries to the veins, either by passing through the arterioles and capillaries, or it may be shunted directly from arteries to veins by arterio-venous anastomoses. The circulation in a gill arch is quite similar; blood may pass, either through the gill capillaries, or take a short cut through the arterioles at the base of the gill. The arterio-venous anastomoses may be taken as the anatomical basis for the possibility of demonstrating a mechanism of a differential vascular response in organs

other than urodele gills. The influence that such arterio-venous short circuits might have on the flow of blood through the capillary bed of other organs would be well worth investigating.

SUMMARY

and

CONCLUSIONS

SUMMARY AND CONCLUSION

1. Repeated branchio-vagotomy or complete vagotomy (including the glossopharyngeal) did not inhibit metamorphosis of A. tigrinum larvae.

2. Neither branchio-vagotomy nor complete vagotomy had any effect on the metamorphosis which was induced by thyreo-activator.

3. It may be concluded the vagal reflexes are not necessary for the morphogenetic process of gill reduction or any other metamorphic process.

4. Removal of the cervical sympathetic trunk and stellate ganglion did not inhibit the gill reduction or metamorphosis which was induced by thyreo-activator.

5. Stimulation of the peripheral end of the branchial branches of the vagus caused the cessation of the flow of blood through a gill. This was probably due to the action of the depressor branchii muscle. Stimulation of the central end of the branchial vagus trunks caused inhibition of the heart. This physiological evidence supports the morphological hypothesis that the branchial branches of the vagus are homologous to the aortic and carotid nerves in mammals.

6. The blood pressure of the larval salamander was measured and recorded. This was found to vary between 4 - 10 mm. of mercury. The limitations of this method of determining vascular reaction were pointed out.

7. By perfusion of individual parts of branchial arches and gills, it was shown that:

a. Adrenalin dilated the gill vessels, and constricted the arterioles making up the anastomosis at the base of the gill.

b. That pitressin constricted both gill vessels and the anastomotic arterioles.

8. The results obtained by perfusion were confirmed by the method of making direct observation on the branchial vessels.

9. By mechanical stimulation, it was shown that:

a. The capillaries in the gill filament had the power of independent constriction.

b. That, if the capillaries in a certain area of a gill filament are stimulated mechanically, these constricted and the reaction spread in all directions. This constricted circle of vessels sometimes included the arterioles at the margin of the filament.

c. The above reaction took place in spite of treatment of the filament with cocaine or butyn. Thus, there is no evidence that this reaction is related to the axon reflex hypothesis as postulated by Krogh and Woollard.

10. It was established by direct observation that adrenalin, lactic and acetic acid, histamine, acetylcholine, urethane, butyn, and carbon dioxide dilate the gill capillaries. Pitressin, cocaine, methylene blue, and oxygen constricted the gill capillaries.

11. The most significant part of this work, both for the explanation of gill reduction and physiology of blood vessels in general, is the discovery of the differential

reactions of blood vessels of a branchial arch. Adrenalin, carbon dioxide, lactic and acetic acid constrict the arterioles and dilate the branchial capillaries, while oxygen dilates the arterioles and constricts the branchial capillaries.

12. Reduction of a gill was brought about by stopping the flow of blood through it by means of ligatures on the afferent branchial artery.

Since it was shown that oxygenated blood prevents the flow of blood through a gill, it may be concluded that a change in the oxygen content of the blood sent to the gills is a factor in gill reduction.

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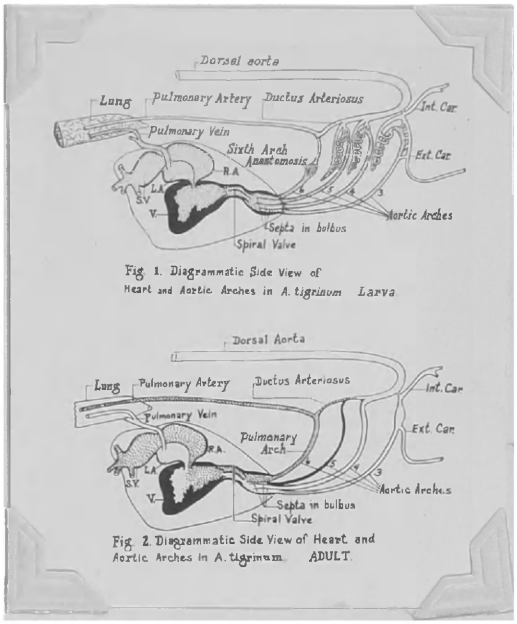


Figures 1 and 2

Diagrammatic Side Views of the Heart and Aortic Arches in Larval and Adult A. tigrinum

The vessels carrying blood of lowest oxygen content are stippled. These diagrams show how, during metamorphosis, the branchial circulation is eliminated, the fifth aortic arch is reduced, and the anastomosis on the sixth aortic arch is transformed into a large arterial trunk. Before metamorphosis the lung is supplied, for the most part, by oxygenated blood from the ductus arteriosus. After the anastomosis on the sixth arch is transformed into a large arterial trunk, the lung receives the blood of lowest oxygen content directly from the heart.

These diagrams are to emphasize the fact that in the larva, the branchial arches carry blood low in oxygen content; while in the adult, these same arches (if they persist), carry blood relatively high in oxygen content.

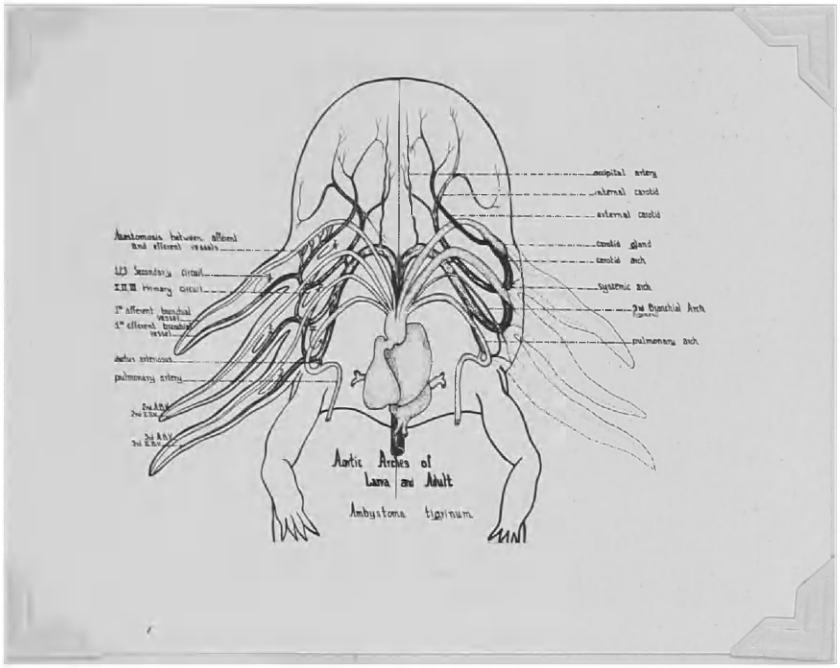


Figure 3.

A Semi-diagrammatic Ventral View of the Heart and Aortic Arches in A. tigrinum.

On the left, the aortic arches of the larva are shown. Arrows indicate the aortic arch or primary circuit, and the branchial or secondary circuit. The vessels in the filaments that connect the afferent and efferent branchial vessels are not shown.

On the right, the aortic arches of a recently metamorphosed adult are shown. The former position of the gills are indicated by broken lines. The third branchial or fifth aortic arch is greatly reduced. The remnants of the branchial vessels are attached to the ventral and dorsal ends of the part of the aortic arch that has formed in the place of the anastomosis.

This diagram is to emphasize the fact that during metamorphosis, the anastomotic or intermediate portion of a branchial arch becomes a large arterial trunk.

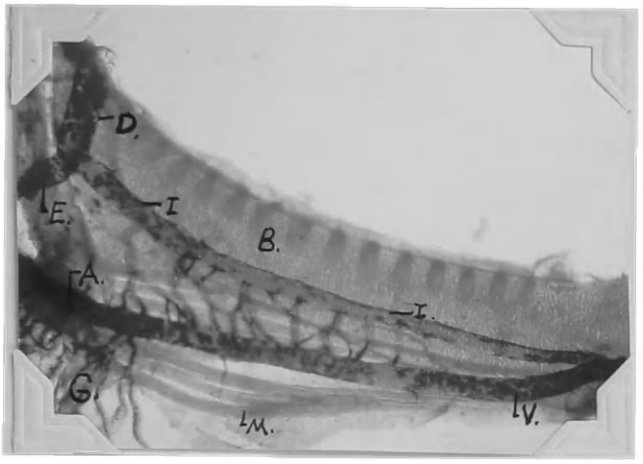


Figure 4.
The Anastomosis on the
Third Aortic Arch

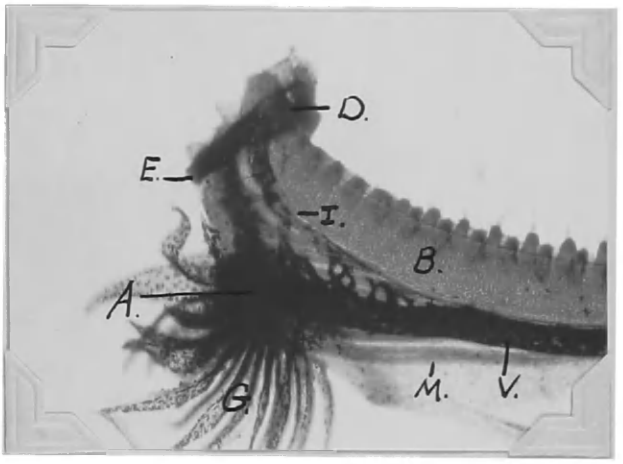


Figure 5.
The Anastomosis on the
Fourth Aortic Arch

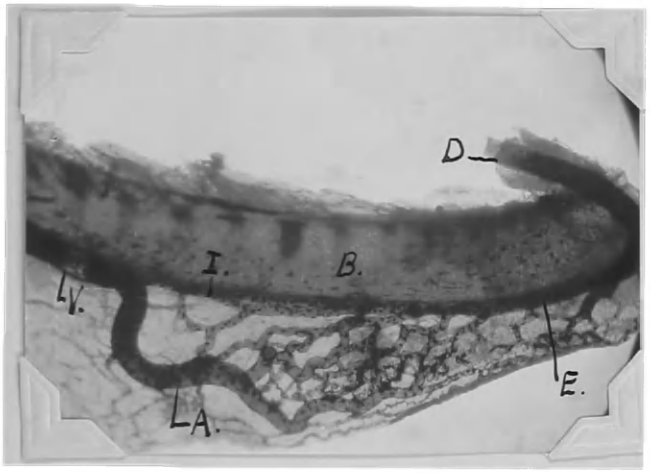


Figure 7.
The Anastomosis on the
Sixth Aortic Arch

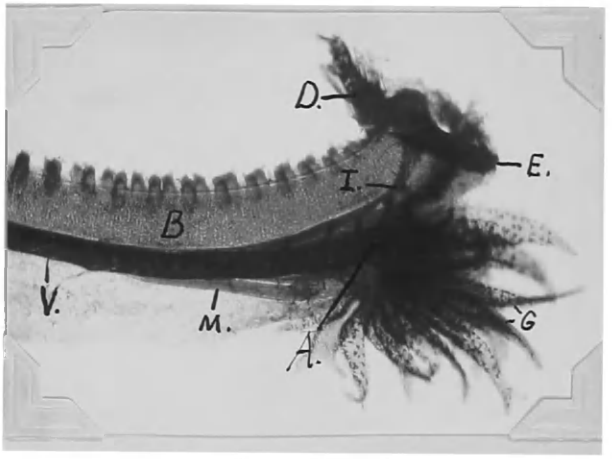


Figure 6.
The Anastomosis on the
Fifth Aortic Arch

A - Afferent Branchial Artery
B - Branchial Arch
D - Dorsal Portion of the Aortic Arch
E - Efferent Branchial Artery
G - Gill Filaments
I - Intermediate Anastomotic Vessel
M - Depressor Branchii Muscle
V - Ventral Portion of the Aortic Arch

Figure 8.

A Photograph of a Cross-section
of a Larval Gill.

A - Afferent Branchial Vessel
D - Depressor Branchii Muscle
E - Efferent Branchial Vessel
L - Lymphatic Branchial Vessel
M - Melanophores
N - Nerve, (Branchial Branch
of Vagus)

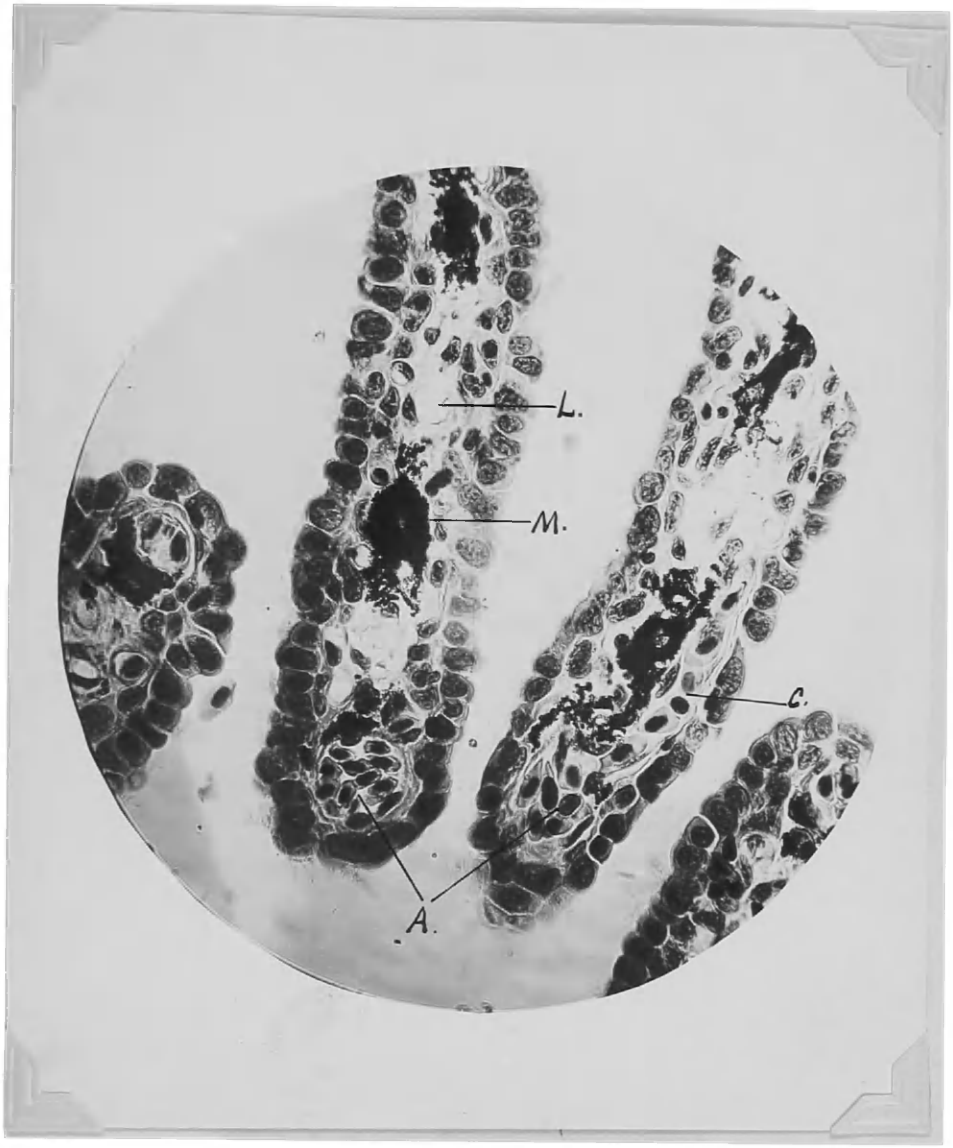


Figure 9.

A Photograph of a Cross-section through Several Gill Filaments.

A - Arteriole at Margin of Filament
C - Capillary with Blood Cells
L - Lymphatic
M - Melanophore

Figure 10.
A Photograph of the Blood Vessels
of a Gill Filament. x8.

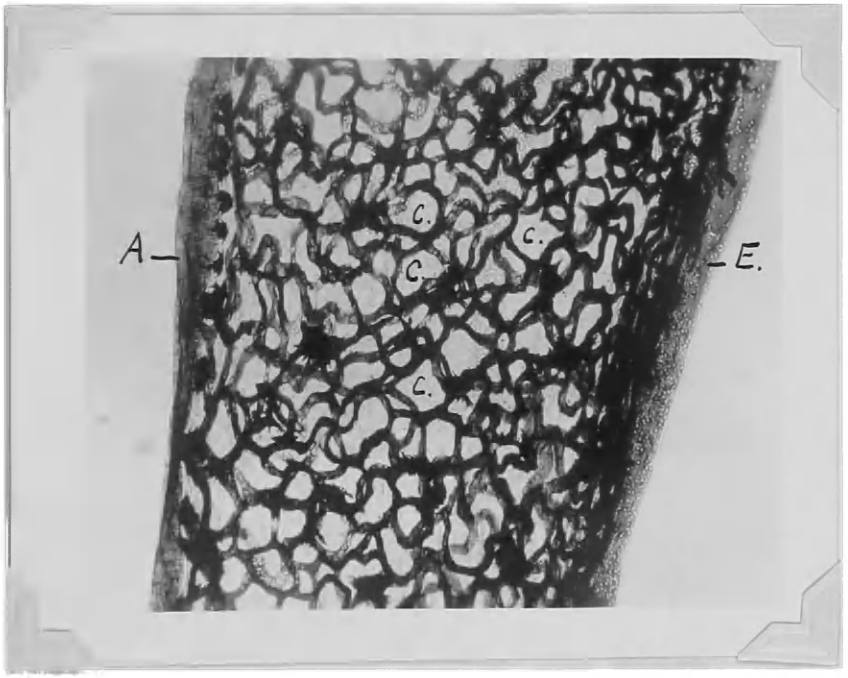


Figure 11.
Same as above. x35.

A - Afferent Limb of the Arteriole Loop
C - Capillary Bed
E - Efferent Limb of the Arteriole Loop

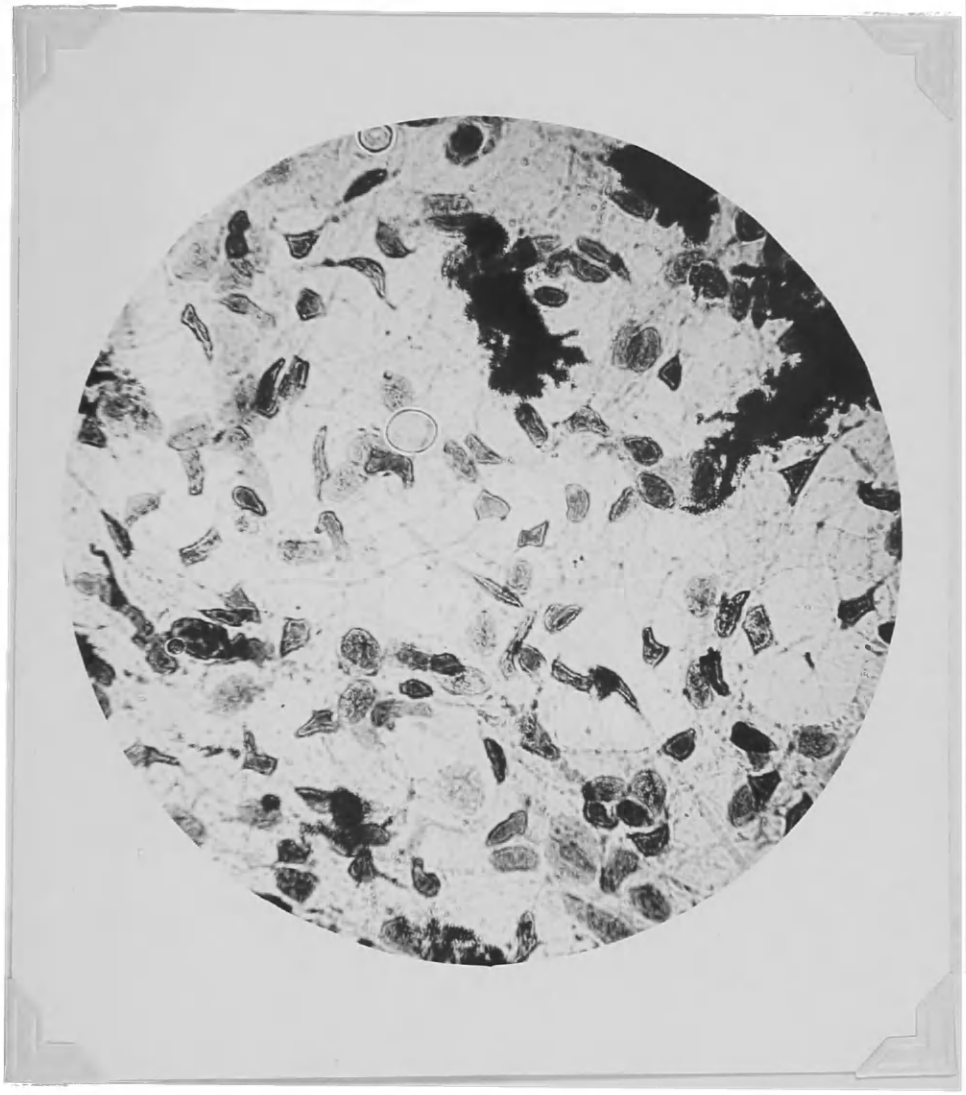


Figure 12.

A Photograph Showing Nerve Fibers in a Gill Filament. (Remak Fibers.)

A branching of the nerve fiber and two of the elongated spindle-shaped nuclei are shown. The capillaries are seen in outline.

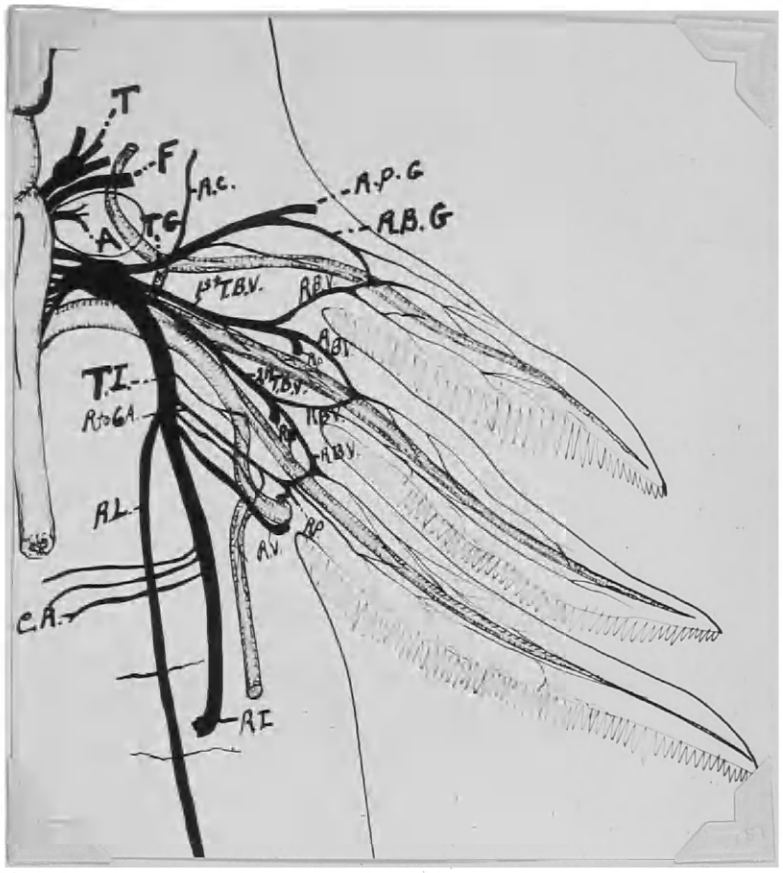


Figure 13.

A Diagram of a Dissection of the Vagus and Glossopharyngeal Nerves in A. tigrinum.

Only the main trunks and the branches to the gills are shown. The four main trunks originating from the ganglion from cephalad to caudad are: the truncus glossopharyngeus, first and second branchial vagus trunks, and the truncus intestinalis.

A - - - - Acoustic Nerve
CR - - - Cardiac Rami
F - - - - Facial Nerve
RBG - - - Ramus Branchialis Glossopharyngeus
RBV - - - Ramus Branchialis Vagus
RC - - - Ramus Communicans to F
RL - - - Ramus Lateralis
RP - - - Ramus Post-Trematicus Vagus
RPG - - - Ramus Post-Trematicus Glossopharyngeus
RV - - - Recurrent Vagus
R to 6A - Ramus to Sixth Aortic and Visceral Arch
T - - - - Trigeminal
1st TBV - First Truncus Branchialis Vagus
2nd TBV - Second Truncus Branchialis Vagus
TG - - - Truncus Glossopharyngeus
TI - - - Truncus Intestinalis

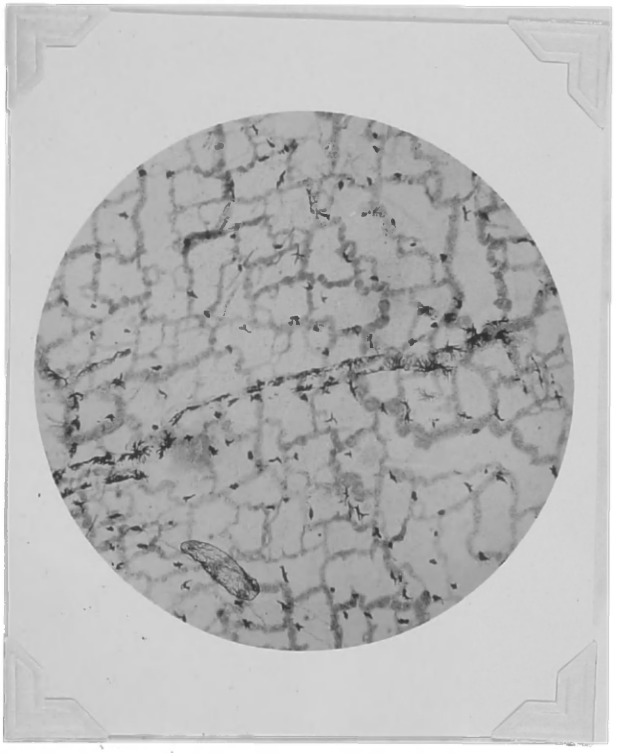


Figure 14.

A Photograph of the Skin
Vessels in the Operculum.

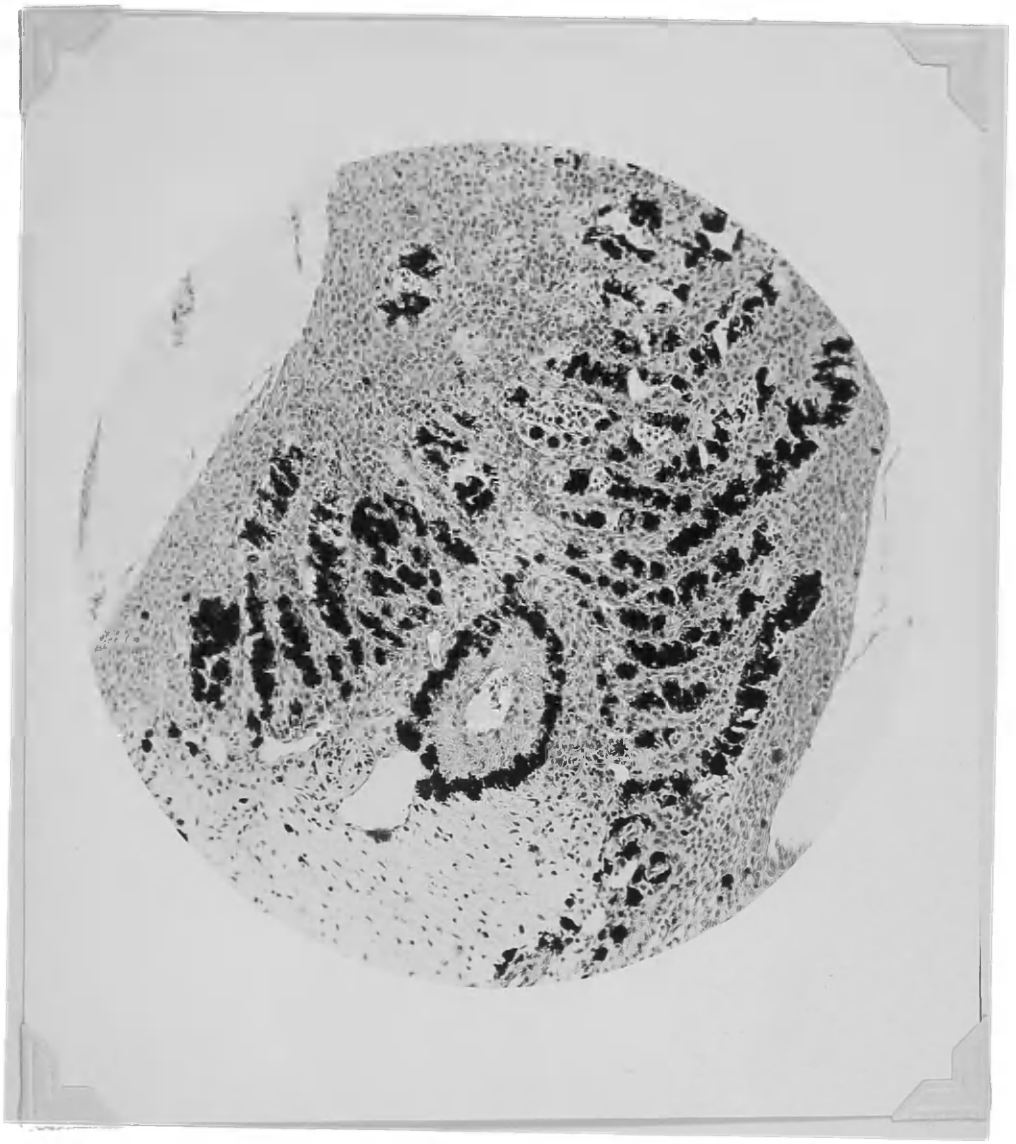


Figure 15.

A Photograph of a Metamorphosing Gill.

The melanophores are still well scattered and are arranged in rows. These rows of melanophores indicate the position of a filament. Between some of the rows of melanophores, two rows of fused epithelial cells are seen. At the margin of the melanophore area, one can see these epithelial cells taking a part in the formation of the capsule.

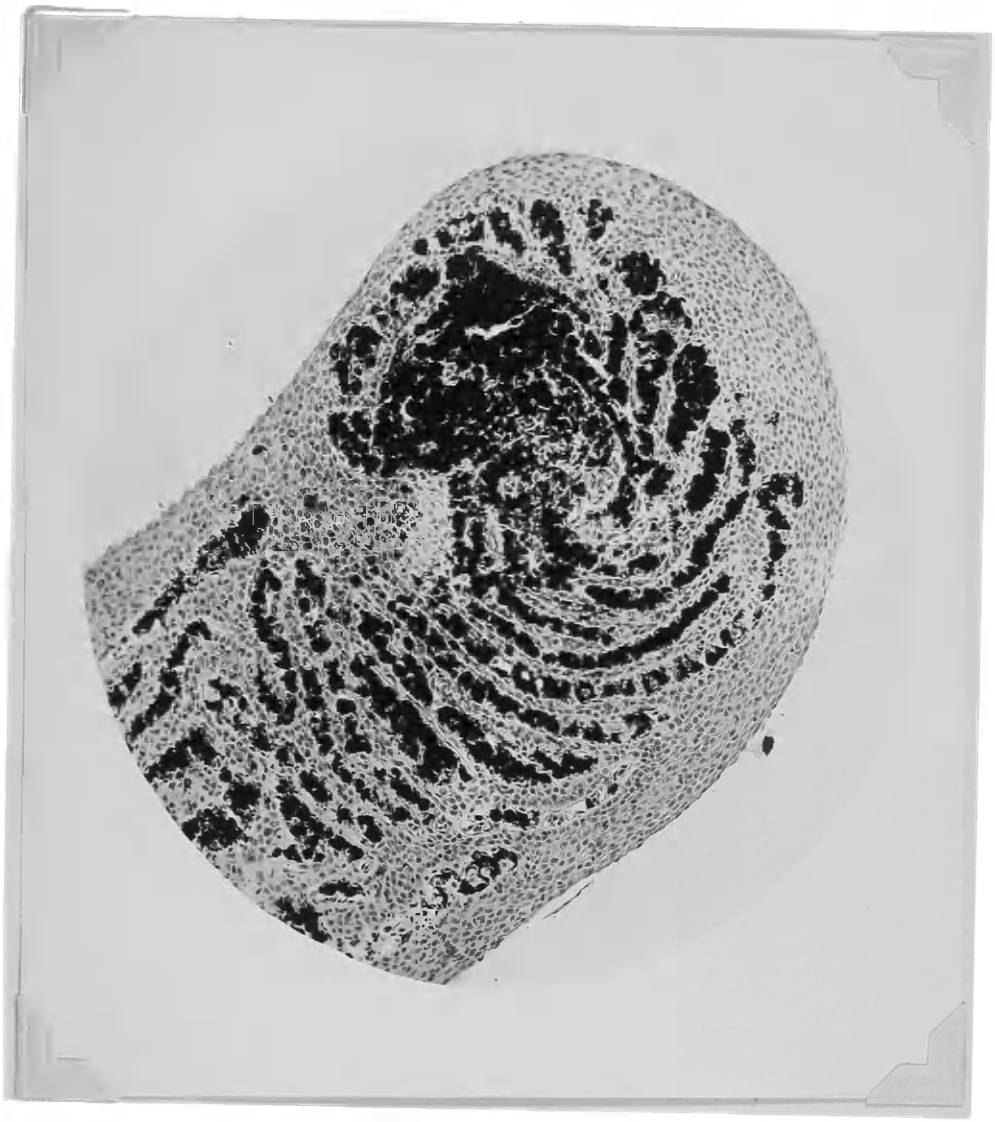


Figure 16.

A Photograph of a Metamorphosing Gill.

The melanophores at the tip of the gill stub are no longer arranged in rows, but form a conglomerate mass. Near the proximal end of the gill stub, the melanophores are still arranged in rows that are separated by rows of epithelial cells to form the capsule. No mitotic figures are seen during the formation of this capsule. The cells seem to become more and more flat as they approach the surface.

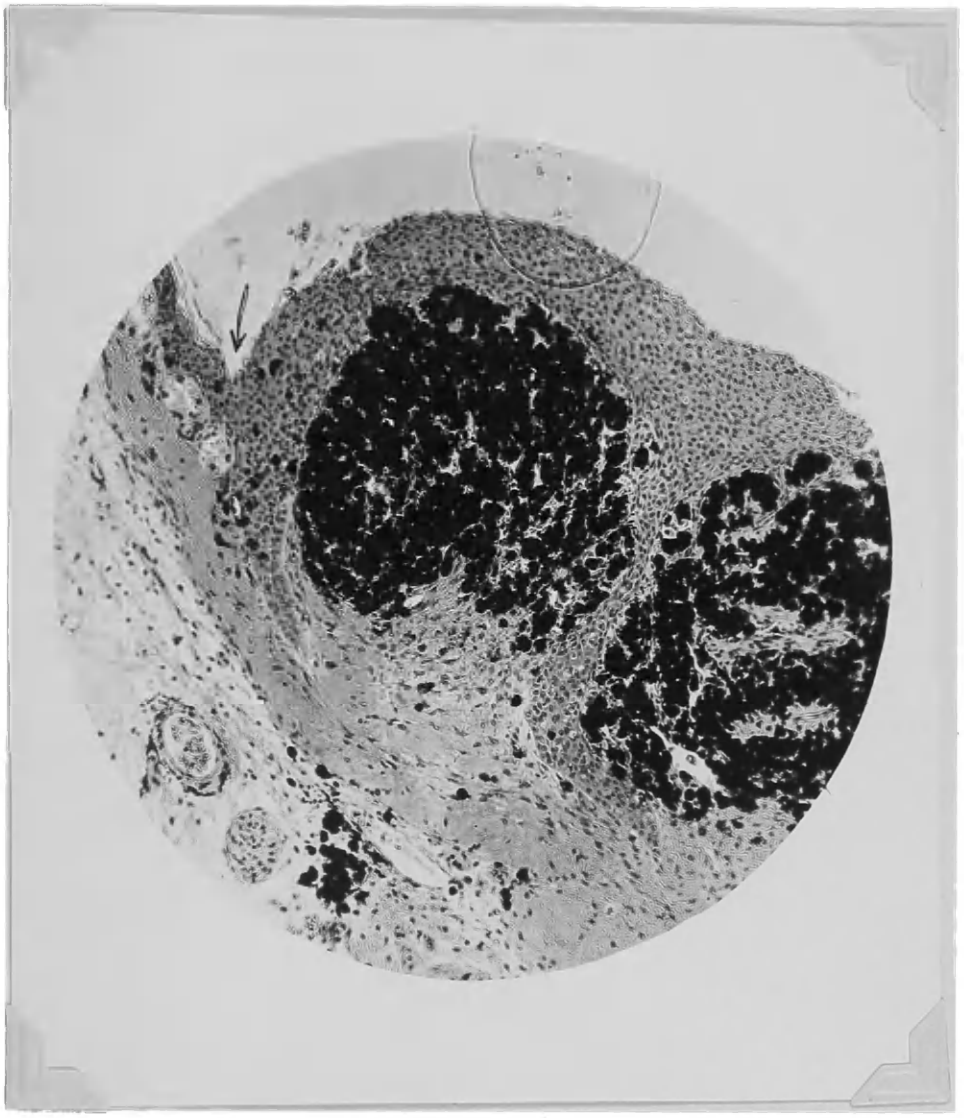


Figure 17.

A Photograph of a Metamorphosing Gill
in the Gill-Nodule Stage.

This shows the late stage of gill reduction. The melanophores are arranged in a conglomerate mass, separated by very few, if any, epithelial cells. The pigment mass for each gill is surrounded by a thick capsule. The continuity of the capsule and the skin is indicated by an arrow. The outer layers of skin and capsule cells show signs of desquamation.

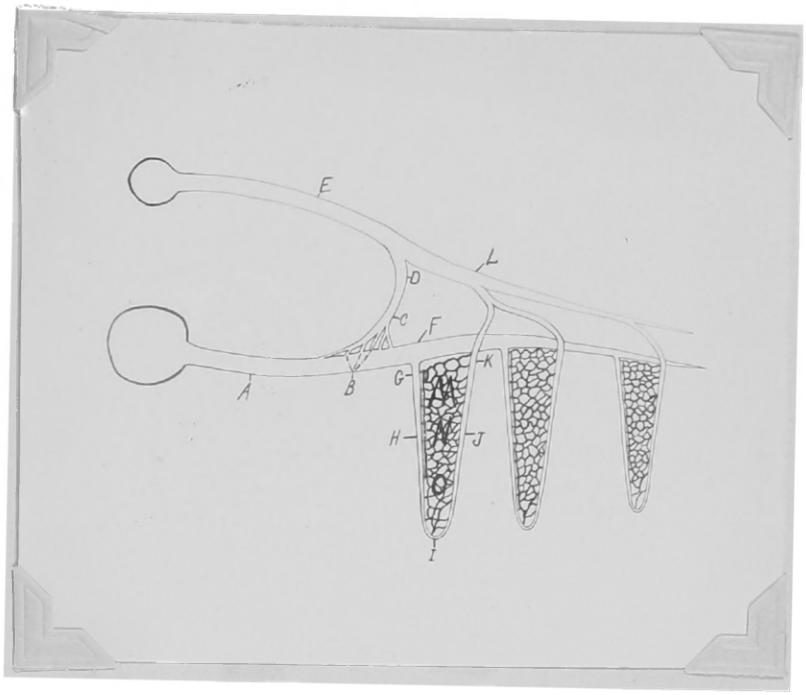


Figure 18.

A Diagram of the Blood Vessels
of a Branchial Arch.

A - - - Ventral Portion of the Aortic Arch
B - - - Anastomotic Arterioles
C,D - - Intermediate Anastomotic Vessel
E - - - Dorsal Portion of Aortic Arch
F - - - Afferent Branchial Artery
G,H, - Afferent Limb of the Arteriole Loop
J,K, - Efferent Limb of the Arteriole Loop
L - - - Efferent Branchial Artery
M,N,O - Capillary Bed of the Filament

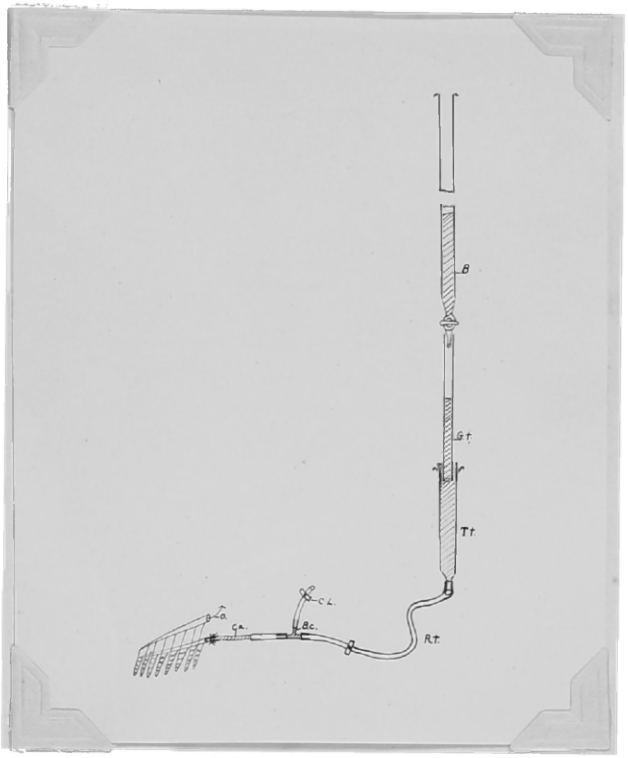


Figure 19.

A Diagram of the Perfusion Apparatus.

B - Burette
BC - Bubble Catcher (T-tube)
Ca - Cannula (with isolated gill attached)
Cl - Clamp
GT - Glass Tube (8 mm.)
O - Outflow Vessel
RT - Rubber Tubing
TT - Test Tube (open at both ends)

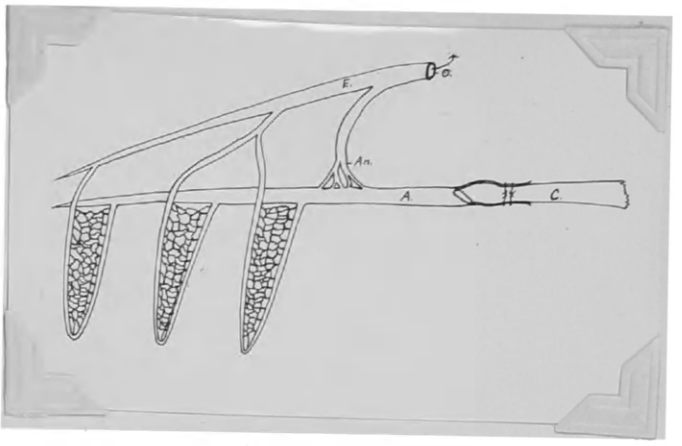


Figure 20.
A Diagram of the Cannulated Gill Arch

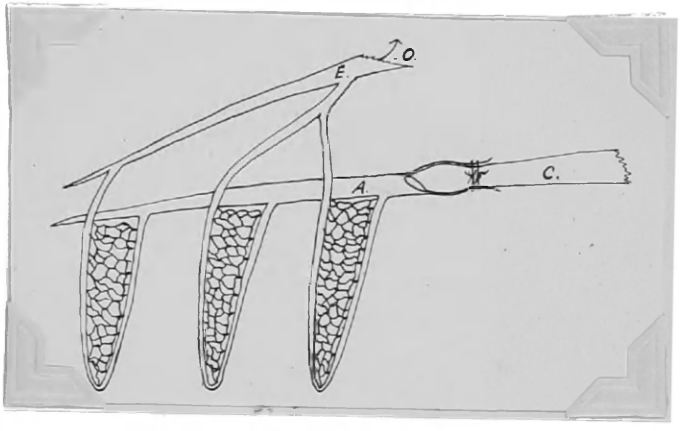


Figure 21.
A Diagram of the Cannulated Gill
(Without Anastomosis)

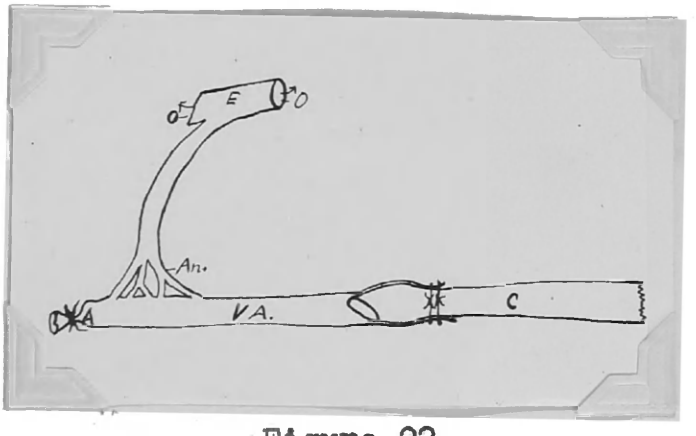


Figure 22.
A Diagram of the Cannulated Gill Arch Anastomosis

A - Afferent Branchial Artery
An - Anastomosis
C - Cannula
E - Efferent Branchial Artery
O - Outflow Vessel
VA - Ventral Portion of Aortic Arch

Figure 23.

A Photograph of the Third Gill Arches from the Same Animal.

The gill filaments have been reduced on the gill arch in which the ventral portion of the aortic arch was ligated at the point indicated by the arrow.

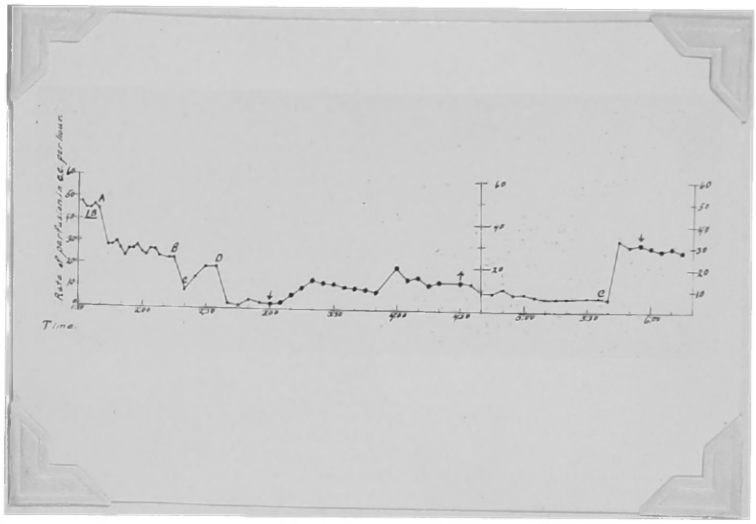


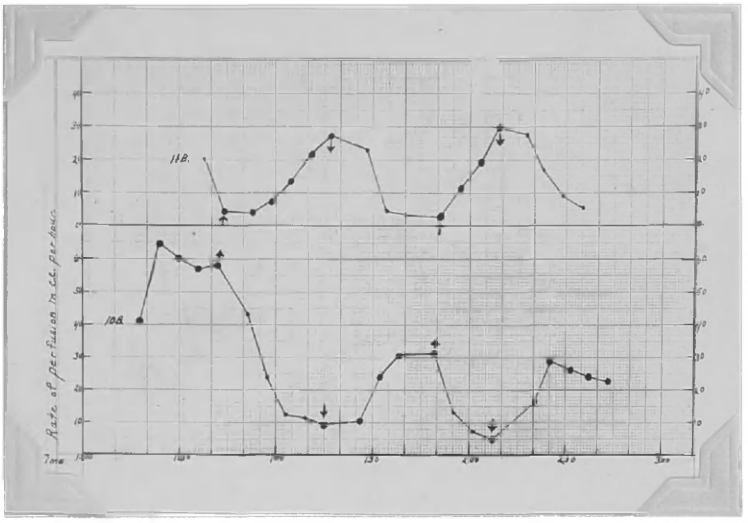
Figure 24.

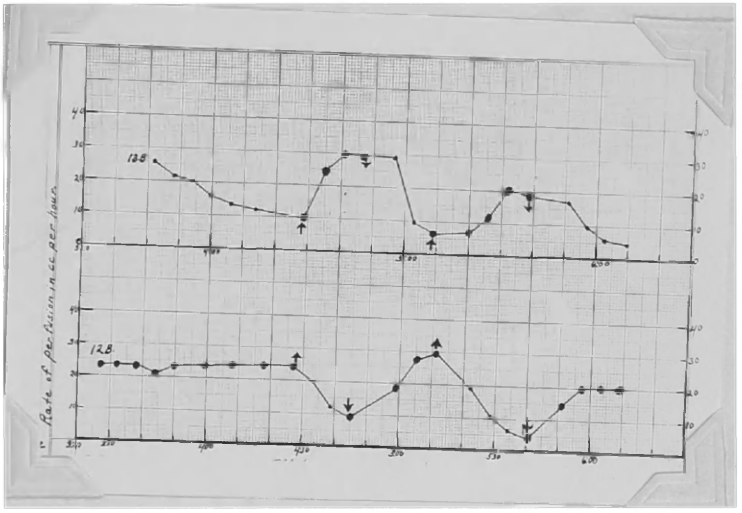
The Perfusion Rate in a Whole Gill Arch
Gill 1b Second Gill Arch

Perfusion with perfusion fluid alone •——•——•
Perfusion with perfusion fluid plus adrenalin, 1:100,000 ●——●——●
Arrows indicate the beginning and termination of periods during which the preparation was perfused with perfusion fluid plus adrenalin.

This graph shows that when the anastomotic vessel was ligated, adding adrenalin to the perfusion fluid increased the rate of perfusion. When the ligature on the anastomosis was released at C, and the whole gill arch was perfused, adding adrenalin to the perfusion fluid had no effect on the rate of perfusion.

A - The perfusion pressure was changed from 300 mm. to 200 mm. water.
B - The anastomotic vessel was ligated. (See Figs. 20 and 21)
C - The ligature on anastomotic vessel was released.
D - Ligature on anastomotic vessel was tightened and tied.





Figures 25 and 26.

The Quantitative Effect of Adrenalin
on the Perfusion Rate in Isolated Gills
(See Fig. 21)
10 b and 13 b - Right Third Gills
11 b and 12 b - Right Second Gills

Perfusion with perfusion fluid alone •——•——•
Perfusion with perfusion fluid plus adrenalin, 1:1,000,000 •——•——•——•
Arrows indicate the beginning and termination of periods during which the preparations were perfused with perfusion fluid plus adrenalin.

These graphs show, quantitatively, that the addition of adrenalin to the perfusion fluid during the perfusion of isolated external gills causes the perfusion rate to increase.

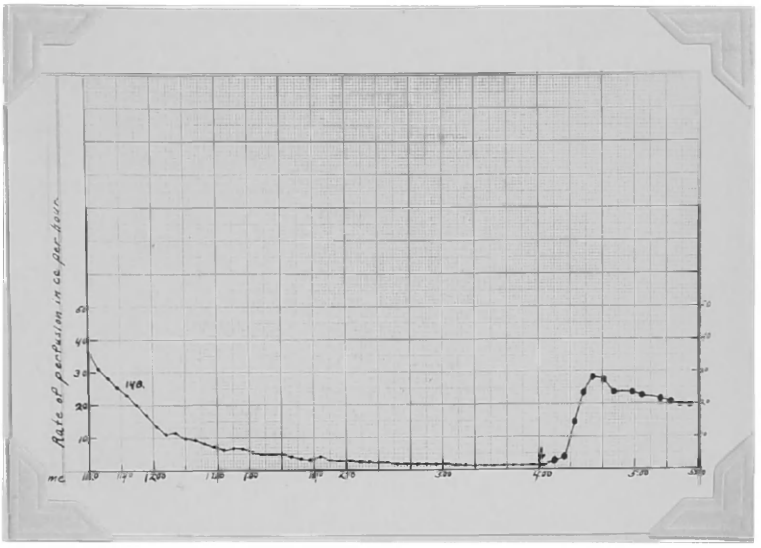


Figure 27.

The Perfusion Rate in an Isolated Left Second Gill.

Perfusion with perfusion fluid alone ——•——•——
Perfusion with perfusion fluid plus adrenalin, 1:500,000 ●——●——●
Perfusion pressure constant - 250 mm. water

The gill was perfused for five hours with perfusion fluid alone. At the arrow, the perfusion with perfusion fluid plus adrenalin was started. This graph shows that perfusion fluid alone gradually constricted the vessels of the isolated gill; but even after five hours of perfusion with perfusion fluid alone, perfusion with perfusion fluid plus adrenalin caused a great increase in the rate of perfusion.

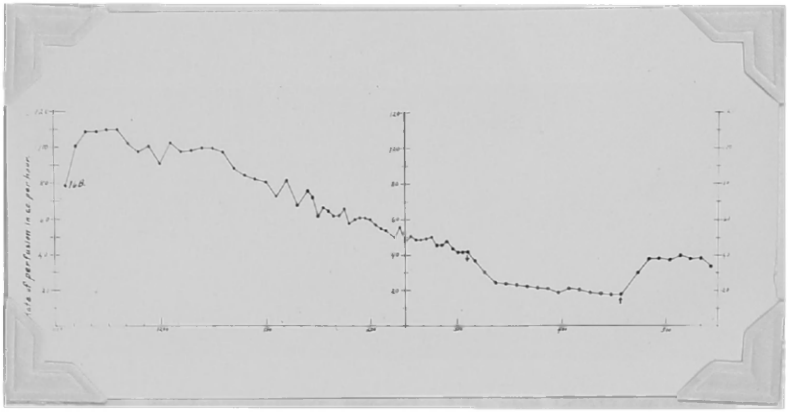


Figure 28.

The Perfusion Rate in an Isolated Left Second Gill.

The gill was perfused for four hours with perfusion fluid plus adrenalin, 1:500,000. At the end of this period (↓), the gill was perfused with perfusion fluid alone for over an hour. Perfusion fluid alone caused the rate of perfusion to decrease. About an hour later, the gill was again perfused with perfusion fluid plus adrenalin (↑). This greatly increased the rate of perfusion.

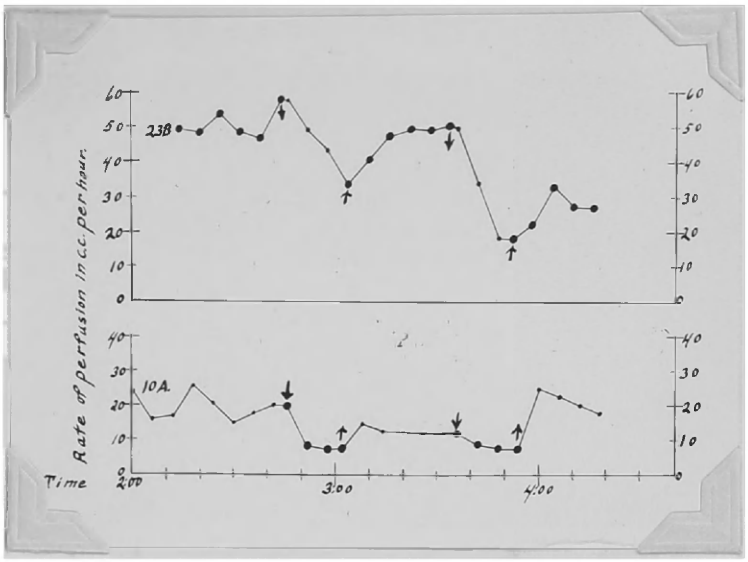


Figure 29.
Below - 10 a (1st Gill Arch)

The Effect of Adrenalin on
the Perfusion Rate in an Isolated
Gill Arch Anastomosis.
(See Fig. 22)

Above - 23 b (1st Gill Arch)

The Effect of Adrenalin on
the Perfusion Rate in an Isolated
Gill. (See Fig. 21)

Both of these preparations were taken from the same gill arch. Arrows indicate the beginning and the termination of periods during which the preparations were perfused with: Perfusion fluid alone •—•—• Perfusion fluid plus adrenalin, 1:50,000 ●—●—●

The isolated anastomosis was perfused at first with perfusion fluid alone while the isolated gill was perfused with perfusion fluid plus adrenalin. Then the tubes were exchanged on the cannulae. The anastomosis was now perfused with perfusion fluid plus adrenalin, which constricted the anastomotic vessels, as evidenced by the decrease in the rate of perfusion. Meanwhile, the gill was perfused with perfusion fluid alone. This (as has already been demonstrated) caused a decrease in the perfusion rate of the gill.

The two perfusion tubes were again exchanged on the cannulae. The gill was now perfused with adrenalin, while the anastomosis was perfused with perfusion fluid alone. The rate of perfusion increased in both preparations. Then the same procedure was repeated with similar results.

It is evident from this, that adrenalin constricts the anastomotic vessels at the base of the gill, while it dilates the vessels in the external gill taken from the same gill arch.

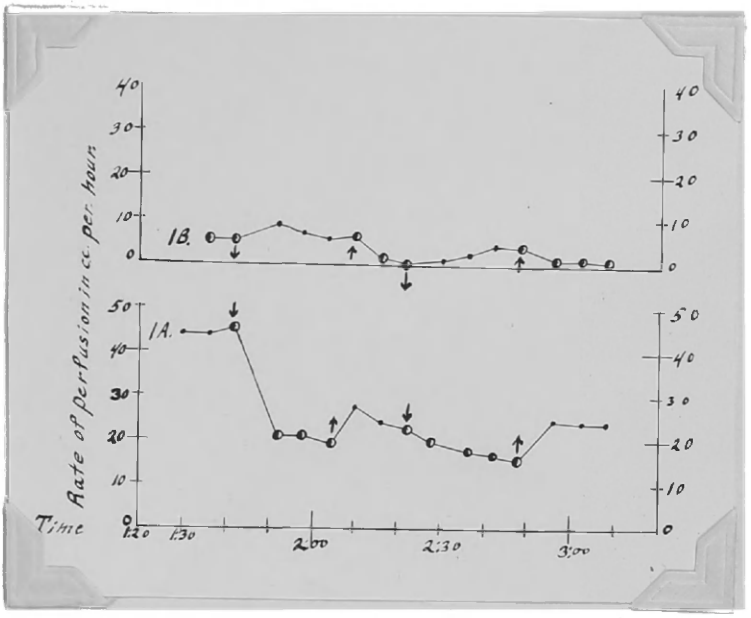


Figure 30.
Above - 1 b (2nd Gill)

The Effect of Pitressin on
The Perfusion Rate in an Isolated
Gill. (See Fig. 21)

Below - 1 a (5th arch)

The Effect of Pitressin on
the Perfusion Rate in an Isolated
Gill Arch Anastomosis.
(See Fig. 22)

Arrows indicate the beginning and the termination of periods during which the preparations were perfused with: Perfusion fluid alone -•-•-•-
Perfusion fluid plus pitressin (100 pressor units per liter) ●——●——●

Perfusion with perfusion fluid plus pitressin caused a decrease in the rate of perfusion in both preparations. The perfusion rate in the case of gill 1 b is quite low, because both pitressin and perfusion fluid have a tendency to constrict the gill vessels completely.