INVESTIGATIONS INTO THE DISTRIBUTION OF BLOOD IN THE HEART AND AORTIC ARCHES OF XENOPUS LAEVIS (Daud)

and

A NOTE ON AN ABNORMAL XENOPUS LAEVIS SPECIMEN WITHOUT HAEMOGLOBIN

and on some experiments performed as a consequence.

Thesis submitted for the Master of Science Degree

by

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BLOOD DISTRIBUTION IN *XENOPUS*

INVESTIGATIONS INTO THE DISTRIBUTION OF BLOOD IN THE HEART AND AORTIC ARCHES OF *XENOPUS LAEVIS* (Daud.)

In the blood vascular system of a typical fish, the blood is sent from the heart to the gills for oxygenation and thence to the rest of the tissues, before returning once more to the heart. This is a so-called "Single Circulation".

Where lungs make their appearance in the vertebrate series however, their blood supply is derived from the last of the aortic arches and is returned via the newly formed pulmonary veins, directly to the heart. A part of the blood leaving from the heart is sent to the lungs, and a part to the rest of the body tissues. Two circuits are formed, both of which have the heart as a central point, and a "Double Circulation" is thus established.

In post-embryonic birds and mammals, where metabolism is highest, these two circuits are separated by the development of a complete septal division in the heart, in such a way that all of the blood returning from the body tissues is first passed to the lungs for re-oxygenation. After this has occurred, it is returned to the heart so that it may receive sufficient propulsion for its journey to the body.

The intervening series of vertebrates, between these extremes (including the embryonic stages of birds and mammals, where the lungs are non-functional), have various degrees of anatomical division in the heart structure of the different classes. Up to some twenty years ago, it was generally accepted that, in spite of the imperfect separation of the two sides of the heart in these classes, some physiological "attempt" was made by the animal to approach the perfect double circulation. Relatively little experimental work has been done to test this idea.

The anuran Amphibia present a somewhat unusual case, in that the division of the heart is complete in the atrium, but incomplete elsewhere. The ventricle has no septal division at all and there is only a partial division of the bulbus cordis by a spiral valve. There appears to be less anatomical division therefore, in the Anura, than in the dipneumone Dipnoi or any of the reptiles. Even <u>Neoceratodus</u> possesses a septum in the ventricle. Another peculiar feature of the Amphibia as a whole, is an accentuated form of cutaneous respiration, which introduces an undetermined, but presumably large, proportion of oxygen into the blood stream returning from the body tissues. This seems an argument against the necessity of a division in the heart, at least if such a division is related to the distribution of oxygenation and de-oxygenated blood to the body and lungs respectively.

In view of the complication introduced by these peculiarities, the Anura do not seem to form logical subjects upon which to start an experimental investigation of the vertebrates with incompletely divided hearts. It appears to be these very idiosyncrasies however, which have stimulated a small number of physiologists. The problems involved, have been brought into prominence particularly by Vandervael (1933) and Foxon (1947, 1951, 1955), both of whom have presented evidence against the idea of a separation of blood streams in the anuran heart.

Nevertheless it is most surprising, considering the importance of frogs and toads as laboratory animals, that more attention has not been directed towards the functioning of their central circulatory apparatus. This "complacency", so to speak, has probably been the outcome of the superficially satisfying account, now generally known as the "classical theory".

The present investigations were carried out in the hope that they might add some information towards the understanding of the subject, as least in so far as one additional species, <u>Xenopus</u> <u>laevis</u>, is concerned.

The choice of this species rested not only on its ready availability, but also on another factor. The opinion has been expressed (Millard 1941, Charles 1931) that cutaneous respiration plays a relatively minor role in supplying the oxygen needs of the animal. If this is so, and if the separation of blood in the heart is related to its oxygen content, one would expect to find a more clear-cut picture than in genera such as <u>Rana</u>, where the cutaneous respiration is said to be of more importance (Krogh, 1904).

(I) STRUCTURE OF THE HEART OF XENOPUS.

Although very full and adequate descriptions of the blood vascular system of <u>Xenopus</u> and its development are available (Millard 1941, 1942, 1945, 1949), there has so far been no published account of the heart structure. A preliminary investigation of this seemed desirable, and was carried out by direct dissection of fresh and preserved material as well as by serial sections, cut at 15 to 25 μ and stained with haematoxylin/erythrosin or Mallory triple staih. The findings are in fairly close agreement with the descriptions of <u>Rana</u> <u>esculenta</u> and <u>Rana</u> <u>fusca</u> (Gaupp 1896), but a brief description follows, for the sake of completeness, and to serve as a revision of the structural features of the anuran heart. The main differences between <u>Rana</u> and <u>Xenopus</u> are also pointed out.

The sinus venosus is formed dorsally by the confluence of the anterior and posterior venae cavae and the large hepatic veins, and empties by way of a transversely oval aperture into the right atrium near to the mid-line (fig. 1). The sinu-atrial aperture is guarded by anterior and posterior thickenings of the atrial wall, of which the anterior is the more prominent. These presumably act as valves, preventing

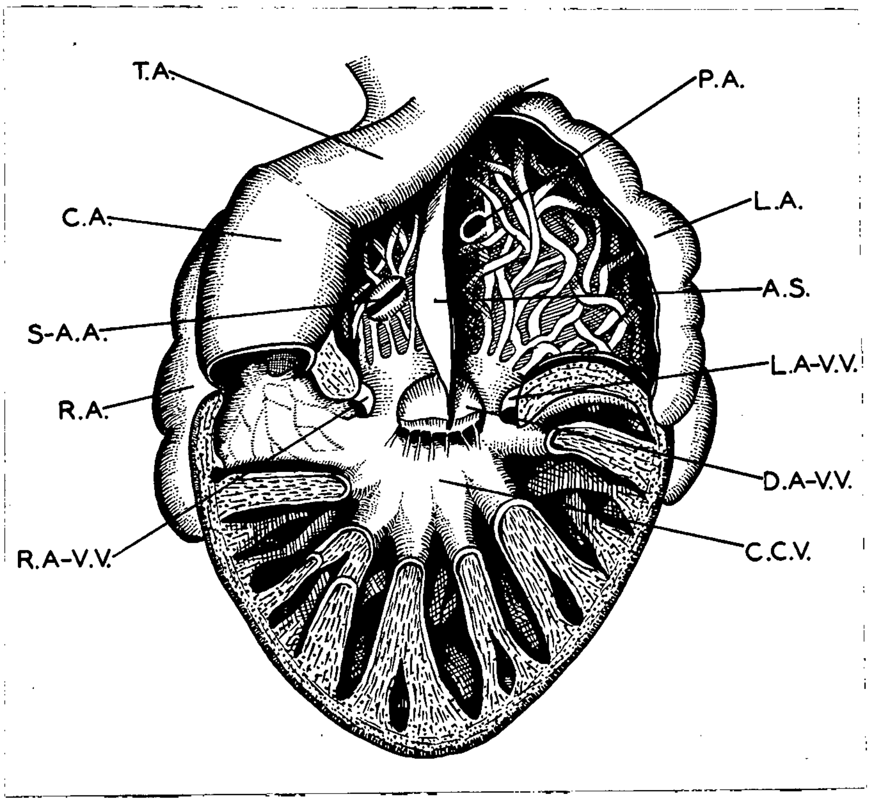

Figure 1 : HEART OF XENOPUS LAEVIS. Viewed from the ventral side. Ventral half of ventricle and ventral walls of atria, removed.

A.S. inter-atrial septum; C.A. conus arteriosus (bulbus cordis); C.C.V. central chamber of ventricle; D.A-V.V. dorsal atrio-ventricular valve: L.A. left atrium; L.A-V.V. left atrio-ventricular valve; P.A. pulmonary aperture; R.A. right atrium; R.A-V.V. right atrio-ventricular valve; S-A.A. sinu-atrial aperture.

reflux of blood into the sinus venosus on atrial contraction. The pulmonary veins run forwards dorsal to the sinus and closely bound to it. They unite to form a short common trunk, which then empties into the left atrium close to the inter-atrial septum, but slightly anterior to the sinu-atrial aperture (fig. 1). No valvular structures could be made out in the pulmonary aperture.

The two atria are completely separated by a thin, median, vertical septum and their interior surface, in the relaxed state, has a rugose appearance due to the projection of muscular bundles into the lumen. The position of the septum is not noticeable from the exterior, nor is there any external groove to indicate its line of attachment to the atrial wall. The margins of both atria, particularly

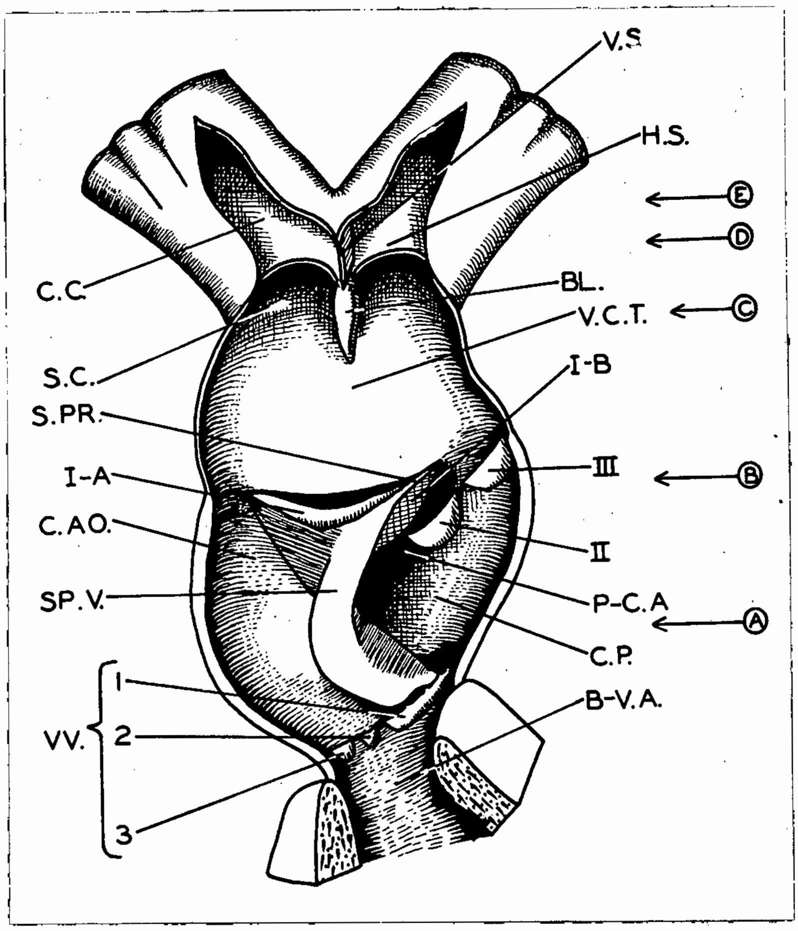

Figure 2 : BULBUS CORDIS AND TRUNCUS ARTERIOSUS OF XENOPUS LAEVIS. Viewed from ventral side. Ventral wall slit open and the two sides pulled apart.

BL. block of endothelial tissue; B-V.A. bulbo-ventricular orifice; C.AO. cavum aorticum; C.C. carotid canal; C.P. cavum pulmo-cutaneum; H.S. horizontal septum (oblique septum); P-C.A. aperture leading to dorsal chamber of truncus and to pulmo-cutaneous arches; S.C. systemic canal; S.PR. septum principale; SP.V. spiral valve; V.C.T. ventral chamber of truncus; V.S. vertical septum; VV. 1, 2, & 3 bulbo-ventricular valves; IA, IB, II, III valves at ant. end of bulbus
(A), (B)... (E) : Levels at which sections shown in fig. 3 were taken.

ventrally, where they overlap the ventricle, are partially subdivided into a series of pockets. These are more prominent than diagrams and descriptions of the hearts of <u>R. esculenta</u> and <u>R. fusca</u> indicate. Unlike the descriptions of <u>Rana</u>, no distinct difference in the anatomical size of the two atria is distinguishable.

Posteriorly, the atria lead into a large atrio-ventricular orifice guarded by two large, thick valves dorsally and ventrally. These valves are attached to the wall of the orifice anteriorly, but posteriorly have a number of chordae tendinae passing down and inserting into the ventricular musculature. Hardly to be described as semi-lunar, they are quite bulky in appearance, and have the same peculiar histological structure as the spiral valve of the bulbus cordis, which is presumably composed of hardened and modified endocardial tissue. On either side of the atrio-ventricular orifice is a weakly developed, semi-lunar valve (fig. 1.).

Posteriorly, the inter-atrial septum is fused with the middle of each of the large dorsal and ventral valves and projects for a short distance into the lumen of the ventricle.

The conical-shaped ventricle has a clear central chamber of relatively small size, into which the atrio-ventricular orifice leads, but the rest of its interior is broken up by the crossing of a large number of muscular trabeculae, which, on section, give the appearance of a thick spongy wall. The outside wall of the ventricle however, is a relatively thin layer. From the right, dorsal part of the central chamber, an aperture leads into the bulbus cordis (fig. 1).

The latter is provided at its base with a series of three semi-lunar valves, of which the largest is ventral and the other two smaller and dorso-lateral in position. Attached ventrally to the bulbus wall within the free margin of the ventral valve, is the base of the large spiral "valve", which then follows a spiral course round the wall of the bulbus in a clock-wise fashion when looked at from the posterior end, until at the anterior end it is on the right side, having turned through about 270° . Although at the posterior end it is only a comparatively small projection from the wall of the bulbus, it rapidly increases in size and, in dissections of fresh material, it would seem to almost fill the lumen of the bulbus. In serial sections its free margin extends about two-thirds across the bulbus through most of its length (fig. 3-A), but it is quite obvious that some shrinkage occurred during the preparation of the material.

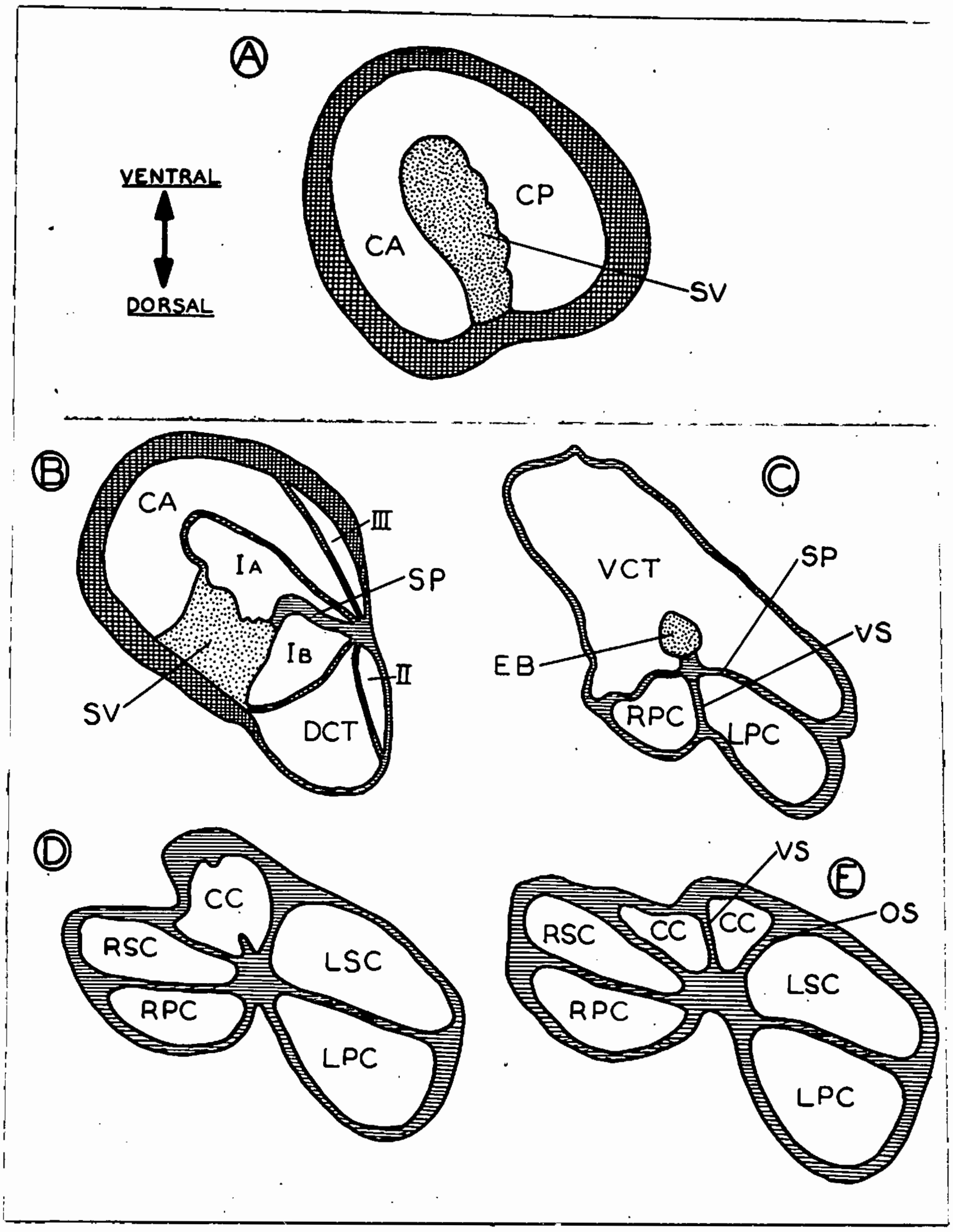

Figure 3 : SELECTED SECTIONS THROUGH THE BULBUS CORDIS AND TRUNCUS ARTERIOSUS OF XENOPUS LAEVIS.
(Semi-diagrammatic)

(A) Section through middle of bulbus.
(B) Section through junction of bulbus and truncus.
(C) Section through truncus before origin of carotid and systemic canals.
(D)(Sections through truncus in successively more
(E)(anterior planes
SEE FIGURE 2, FOR POSITIONS OF SECTIONS.

CA cavum aorticum; CC carotid canals; CP cav. pulmo-cutaneum; DCT dorsal chamber of truncus; EB endothelial block in ventral chamber of truncus; LPC left pulmo-cutaneous canal; LSC left systemic canal; OS oblique septum; RPC right pulmo-cutaneous canal; RSC right systemic canal; SV spiral valve; VCT ventral chamber of truncus; VS vertical septum; IA, IB, II & III valves at anterior end of bulbus.

The spiral valve then, partially divides the bulbus into two channels; a cavum aorticum, lying to the right side posteriorly and a cavum pulmo-cutaneum to the left side. Anteriorly however, due to the twisting of the spiral valve, these come to lie ventrally and dorsally, respectively.

Due to the way in which the spiral valve is twisted at the posterior end, the bulbo-ventricular orifice appears to lead into the cavum aorticum only, and this junction is guarded by the three valves already mentioned. The cavum pulmo-cutaneum, judging from dissection, seems to have no direct communication with the ventricle, although there is some doubt about this. The question will be discussed later.

Anteriorly, the bulbus is followed by a thinner-walled tube, the truncus arteriosus, which is completely divided by a horizontal septum (septum principale) into dorsal and ventral chambers, corresponding to the cavum aorticum and cavum pulmo-cutaneum of the bulbus. This septum is continuous behind, with the spiral valve (fig. 3-B, 3-C). At the anterior end of the bulbus are a series of semi-lunar valves, guarding its exit to the truncus. Two of these (valves IA & IB of Gaupp, 1896) are attached to the spiral valve and have their free margins projecting into the cavum aorticum and cavum pulmo-cutaneum respectively, while the others (valves II & III), are attached to the bulbus wall and lie opposite the first two (fig. 2 & 3-B).

Slightly anterior to the bulbus, the dorsal chamber of the truncus is split into right and left pulmo-cutaneous canals by a vertical septum (fig. 3-C). Still further forward oblique and vertical septa almost simultaneously divide the ventral chamber of the truncus into four canals, which run out to become continuous with the systemic and carotid arches (fig. 2, 3-D & 3-E). This is somewhat different from the arrangement in Rana, where both carotid canals arise from the right side of a main vertical septum, but resembles the urodelan structure, and the condition in Microhyla and Ramanella, described by Rau and Ramanna (1925).

Projecting posteriorly from the vertical septum into the ventral chamber of the truncus and attached to the ventral surface of the septum principale, is a block of tissue similar in composition to the spiral valve. This partially divides the chamber at its anterior end into two divisions, leading to the systemico-carotid canals on each side (fig. 2 & 3-C).

No valvulae paradoxae (Gaupp 1896) could be found in the systemic canals of the truncus or in the systemic arches themselves.

A coronary artery arises from the base of one of the carotid canals of the truncus and runs backward across the surface of the bulbus, but reaches no further. Venous drainage from the bulbus passes into a coronary vein, which runs down laterally between the ventricle and right atrium to join the sinus venosus. There is no coronary supply to the rest of the heart.

The outer diameters of the blood vessels issuing from the heart (measured in a number of living specimens, under a microscope with a graduated ocular lens), have an average ratio as follows :- carotid arch - 6; systemic arch - 12; pulmo-cutaneous arch - 11; pulmonary artery - 9; cutaneous artery - 3.

(II) THE PROBLEM.

(A) THE "CLASSICAL THEORY" OF BLOOD DISTRIBUTION.

The first reasoned account of the circulation of blood through the anuran heart is attributed by text-books (e.g. Sedgwick 1932, Ihle 1947, Bolk 1933) to Brücke and Sabatier. Brücke's ideas of the basic mechanism were modified and extended by Sabatier towards the end of the last century, and have now become known as the "classical theory", to be found in apparently unmodified form in most standard text-books of Zoology.

This "classical theory" states that there is a separation of "oxygenated" blood coming from the lungs and entering the heart through the left atrium, and "de-oxygenated" blood comihg from the rest of the body and entering the heart through the right atrium, and that this occurs in spite of the absence of a ventricular septum and the existence of only a partial separation of the bulbus cordis. On atrial contraction, it is supposed that the blood from each atrium would have very little opportunity to mix in the central chamber of the ventricle considering firstly, the propulsion it would have gained from the atrial contraction; secondly, the projection of the atrial septum down through the atrio-ventricular orifice; and thirdly, the simultaneous dilation of the ventricle, which draws the blood into its trabecular mesh-work.

During the ventricular contraction, the first blood to leave the ventricle would be the "deoxygenated" blood lying on the right side and this would then be passed into the cavum aorticum of the bulbus at pressure sufficient to distend the bulbus wall. The spiral valve would now not touch the opposite wall of the bulbus and the blood could have free access over the margin of the spiral valve into the cavum pulmo-cutaneum. It is assumed that the peripheral resistance of the pulmo-cutaneous blood circuit is less than that of the carotid or systemic circuits, a situation which would result in an easier flow of blood and a quicker reduction of pressure in the pulmo-cutaneous arches. Thus, at the beginning of ventricular systole, the "de-oxygenated" blood would tend to pass into the pulmo-cutaneous arches.

The resistance to the flow of blood into the other two arches, is supposed to be increased particularly by the presence of the carotid labyrinths, and in <u>Rana</u>, of the valvulae paradoxae, a pair of semi-lunar valves which are situated at the anterior end of the systemic canals of the truncus, and which open towards the heart.

Towards the end of ventricular systole, the bulbus begins to contract, bringing the free margin of the spiral valve into contact with the opposite wall, and completely separating the two cava of the bulbus. The cavum aorticum remaining in connection with the ventricle now receives the more oxygenated blood. This is the last to leave, since it comes from the left side of the ventricle, and is chanelled solely into the carotid and systemic arches.

Further elaborations have been added to this description of the basic mechanism, The cavum aorticum, for example, would still contain some "de-oxygenated" blood after the spiral valve had closed off the entrance to the pulmo-cutaneous arches, and this would be the first to enter the ventral chamber of the truncus. Since the left systemic arch is lying more in line with the truncus, it is presumed that it would receive the majority of this "de-oxygenated" blood, before enough pressure could be built up to "turn the corner" into the right division of the truncus, which in Rana, gives rise to the two carotid arches. The carotid arches would moreover be compressed by the distension of the systemic arches due to their greater peripheral resistance (just how this could happen is not at all clear), all of which would regiment against the carotid arches receiving any blood until the last phases of systole. By this time, only "oxygenated" blood would be passing through the ventral chamber of the truncus.

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Unfortunately it has not been possible to consult the original descriptions by Brücke or Sabatier, but from other sources (e.g. Foxon 1947), it appears that their "classical theory" has been derived only from anatomical considerations, and from direct visual observation of the beating heart, both of which methods could hardly reveal the delicate mechanisms proposed. Apparently the only concrete evidence is the observation that, in a pithed frog subjected to artificial respiration, the blood entering the left atrium is bright red in colour, that entering the right atrium is darker, and that this distinction is maintained in the ventricle and the arterial arches, so that the pulmo-cutaneous arches receive the darker blood and the carotid and systemic arches the lighter.

It will be appreciated that the validity of the "classical theory" depends upon the verification of a number of features :-

(A) Distribution of blood :

It must be determined whether there is in fact a selective distribution, and to what extent it may be termed selective; whether there are any differences between species; and whether selective distribution may be operative at some times but not at others, and if so, under what circumstances and by what mechanism.

(B) Mode of action of the heart :

The details of the mechanism proposed by the "classical theory" must be verified, and particularly whether the blood from the atria is actually kept separate in the ventricle; whether the blood from the left side of the ventricle is the first to leave during systole; whether the cavum aorticum only, is in direct communication with the ventricle, and whether the cavum pulmo-cutaneum actually has a physiologically blind posterior end; whether the spiral valve is in such a position as to give free access between the two cava during one phase of the heart beat, but during another is able to divide the bulbus into two distinct channels; whether the pressure in the pulmo-cutaneous arches does in fact drop to a lower level than that of the systemic or carotid arches; and finally, whether the carotid arches do receive the very last portions of blood to leave from the heart.

(C) Indirect evidence :

To this, might be added consideration~~s~~s as to whether the blood from the right atrium is actually less oxygenated than the blood from the left atrium and whether there is any physiological significance in maintaining a selective distribution; whether abnormalities in the structure of the heart occur, which make a selective distribution impossible in those cases and which deny the necessity of a a selective distribution; and whether accessory evidence may be gained from the study of the movements and volumes of blood in the various parts of the circulatory system.

It is proposed therefore, to survey the literature on the subject in order to determine what the present state of knowledge is about these problems.

(B) PREVIOUS EXPERIMENTAL WORK.

(1) EVIDENCE FOR AN AGAINST SELECTIVE DISTRIBUTION.

Initial criticism by Fritsch (1869) that the difference in colouration of the two sides of the heart and the different arches is solely due to difference in the thickness of the wall of the ventricle in various places and the difference in thickness or transparency of the arterial arches, has been met by Gompertz (1884), He found that during artificial respiration in *Rana*, this division could be seen, whereas it promptly disappeared when respiration was suspended. Since that time, however, the only recorded observation of a similar nature has been by Ozorio de Almeida (1923), who also found a distinct line of division in the ventricle between light and dark blood, when he applied artificial respiration to a giant South American species, *Leptodactylus ocellatus*. This distinction was moreover maintained in the arterial arches. He does not record any distinction in colour between the carotid and systemic arches.

Another testing method has been used by a number of experimenters. This consists basically of the introduction into the large veins, of some substance which can be traced through the heart visually or by photographic means, or which can be recovered from the arches leaving the heart.

Noble (1925) simply injected an indian ink suspension into the pulmonary veins of a series of American amphibians, including both Anura and Urodela, and reports that in all species having well developed and frequently used lungs and possessing a functionally complete atrial septum and a spiral valve, none of the suspension was passed into the posterior arches (pulmo-ctuaneous arches of the Anura). These included the four Anuran species on which he experimented, - Scaphiopus holbrooki (1), Hyla crucifer (3), Acris gryllus (2), and Rana clamitans (1). Rather similar techniques have been used by Vandervael (1933) and Foxon (1947), both of whom trans-illuminated the blood vessels for more accurate observation of the distribution of an ink suspension. As material, Vandervael used Rana temporaria and R. esculenta and Foxon, both R. temporaria and Bufo bufo. Their observations quite contradict the earlier work of Noble, in that the distribution is reported to be random.

Acolat (1931 b) replaced the blood of the central circulatory apparatus of "the" frog (R. esculenta ?) with Ringer solution. He then tapped into the posterior vena cava Ringer solution stained with methylene blue, and into the pulmonary veins, Ringer solution stained with eosine, both from reservoirs, and was able to observe a clear separation in the ventricle. He does not state what the colours of the fluids in the arches were, during the course of the experiment. From theoretical consideration however, Acolat concludes that some mixture must take place. He bases this on the fact that the right atrium is anatomically some three times larger than the left and that all of the blood which it contains, could not possibly be passed into the pulmo-cutaneous arches. He derives a theoretical "balance sheet" for the receipt and distribution of blood from the heart, in which one third of the blood from the right atrium is sent into the ventral chamber of the truncus, together with all of the blood from the left atrium, while the rest of the blood from the right atrium is passed into the pulmo-cutaneous arches (see fig. 4).

This, he attempted to verify in the following way. The blood in the central circulatory apparatus was again replaced by Ringer solution, and a solution of sodium ferrocyanide was tapped in through the posterior vena cava, while pure Ringer solution was introduced into the pulmonary veins,

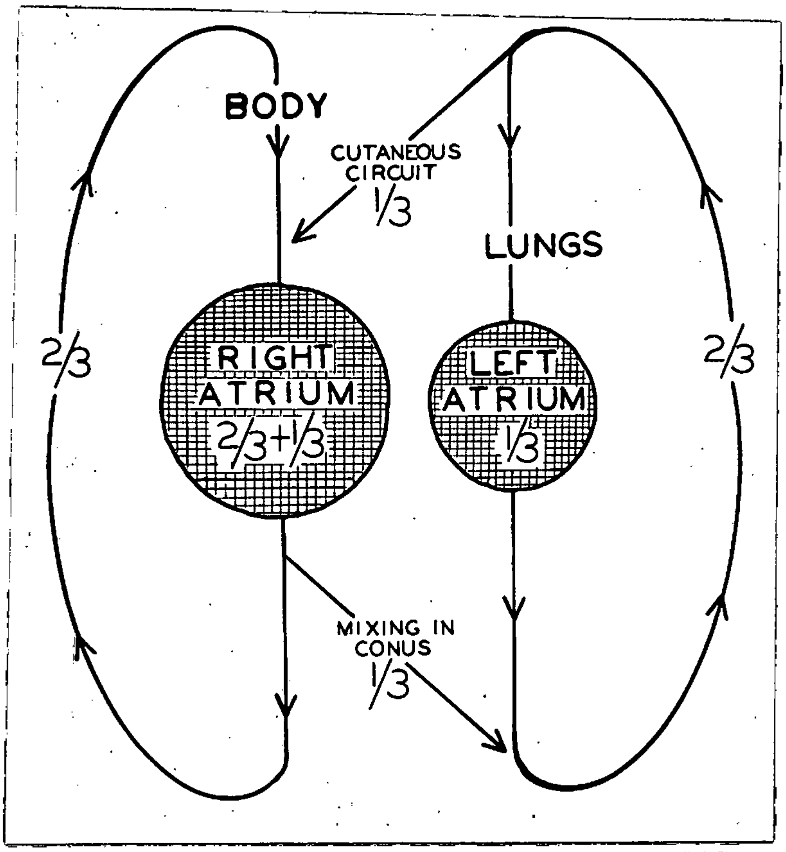

Figure 4 : BLOOD DISTRIBUTION OF THE FROG. (According to Acolat (1931 b), somewhat modified.)

The systemic and pulmo-cutaneous arches had previously been cut and cannulated, finer cannulae being placed in the pulmo-cutaneous arches to simulate the natural resistances at the ends of the two circuits. The fluids were then collected from the cannulae and a prussian blue precipitate produced by the addition of ferric chloride. The intensity of the blue colour in the sample collected from the pulmo-cutaneous arch, he states, was twice as great as that collected from the systemic arch. In order to satisfy his "balance sheet" for the return to the heart, it is necessary for Acolat to suppose that one half of the blood passing through the pulmo-cutaneous arches is sent through the cutaneous arteries and half through the pulmonary arteries. No consideration is given to the carotid circulation.

The obvious artificiality of such experimentation, and the highly theoretical nature of the resulting distribution scheme, needs no comment.

A much more satisfying quantitative method of studying the blood distribution was followed out by Savolin (1949). In this method, small quantities of about 0.2 cc. of a suspension of starch grains or uni-cellular green algae were slowly injected into the hepatic veins of *Bufo bufo*. After allowing a short time for passage through the heart, the bulbus was suddenly ligatured, thus stopping the blood flow through the arterial trunks, and allowing blood to be removed from these to be examined for the quantity of suspended medium which each contained. The results indicate an almost random distribution, except for a slightly higher ratio of blood from the left atrium entering the carotid arches.

Apart from the last quantitative method, the techniques mentioned so far, are largely dependent upon the accuracy of observation by the experimenter. Foxon (1951) was apparently concerned with this point and developed a radiographic technique, involving the injection of a radio-opaque dye ("thorotrast") into one of the veins leading to the heart and tracing it through the heart by serial X-ray photography. With this method, he claims to have confirmed that his earlier conclusions were correct, in so far as a random distribution was shown in *R. temporaria* and *B. bufo*.

Yet another injection method was employed by Simons and Michaelis (1953), in which a fluorescein-Ringer solution was injected, and its course traced by taking cinematographic records under ultra-violet illumination. They report that they found clear evidence that a selective distribution may occur under some circumstances in *Hyla caerulea*. What the circumstances are, they did not determine.

A discussion of the general principles of the "injection" methods will be postponed, but at the moment it must be pointed out that extremely conflicting results have been obtained.

As can be seen, a relatively small number of species have been used for experimental work, and considering the disagreement about the distribution even in these, no conclusions may be drawn as to whether there are any specific differences or not.

However, this disagreement may indicate that the distribution changes from time to time in the same species. Vandervael (1933) found that by disturbing the arches slightly during his indian ink injection experiments, the distribution could be changed radically. Ozorio de Almeida (1923) found that a disturbance of the ventricular action (by uni-lateral application of ice) also led to a changed distribution pattern, but there is no evidence that similar disturbances occur naturally. More illuminating is the statement by Simons and Michaelis (1953) that out of eight cinematographic records of fluorescein injections, six showed random distribution, while two gave clear evidence of selective distribution. No experimental work has yet been carried out to clarify this point..

(2) THE MODE OF ACTION OF THE HEART.

The work of Gompertz (1884), Ozorio de Almeida (1923) and Acolat (1931 b) would seem to indicate quite clearly that a physiological division of the ventricle can occur, although Ozorio de Almeida's observation that the bright oxygenated blood and the darker blood leave the ventricle and appear in the bulbus simultaneously, contradicts the idea of a "di-phasic" evacuation of the ventricle, envisaged in the "classical theory".

Ozorio de Almeida (1923), Vandervael (1933) and Foxon (1947) all observed that from the beginning of ventricular systele, a current of blood was seen to pass simultaneously up either side of the bulbus, in spite of the anatomical position of the spiral valve, which would seem to block off any direct connection between the cavum pulmo-cutaneum and the ventricle. Vandervael (1933) moreover, saw that blood continued to pass up the cavum pulmo-cutaneum, after he had diverted the blood in the cavum aorticum by cutting a hole in the bulbus wall. This throws doubt upon another tenet of the "classical theory".

Gompertz (1884) experimented with an excised heart in saline solution, after a piece of the bulbus wall had been removed. The resulting observations could hardly be reliable, but it should be placed on record, that in his opinion the spiral valve was at no time pressed against the opposite wall of the bulbus, so as to separate the two cava completely. No other opinion, based on experimental work, has been expressed on the subject.

Only two instances of simultaneous pressure recordings in the pulmo-cutaneous and systemic circuits have been found in the literature. Those by Gompertz (1884) were carried out under conditions which were again far from natural, and no weight can be placed on his findings that the pressure pulses in the two arches were synchronous, contrary to the demands of the "classical theory". Acolat (1938 a) found a small, constant difference in pressure in the pulmo-cutaneous and systemic arches of <u>Rana ridibunda</u> and <u>Bufo vulgaris</u>, but the method employed could give neither an accurate idea of the size of the pressure pulses, nor their time relations. The details of pressure measurement methods will be discussed in a later section.

Finally, there is only the evidence of Savolin (1949) to indicate that the carotid arches receive a slightly greater proportion of left atrial blood than either the systemic or pulmo-cutaneous arches.

The mode of action of the anuran heart in relation to blood distribution therefore, remains highly contentious.

(3) INDIRECT EVIDENCE

Vandervael (1933) was the first to stress the significance of the cutaneous respiration of the anura in relation to the distribution of blood. Prior to this, it had been assumed that the lungs were the more important respiratory organs and that the left atrium would be receiving the more highly oxygenated blood, while the return supply of blood from the skin, via the anterior vena cava, would

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add but little to the oxygenation of the blood in the right atrium. On this basis it would seem logical to suppose that it would be physiologically desirable for the latter to be selectively distributed to the lungs.

Vandervael (1933) however, points out that during a part of the year (hibernation periods), the lungs are not in use. He states that, under these circumstances, the left atrium would contain considerably less oxygenated blood than the right. A selective mechanism as postulated by the "classical theory", would then mean that the carotid and systemic arches would receive the less oxygenated blood, while the respiratory organs would have returned to them the more highly oxygenated blood, a rather peculiar state of affairs. This argument is re-examined later.

Other indirect evidence takes the form of reports of abnormalities in the blood vascular system of frogs (e.g. Baxter 1941), which cannot in any way, be reconciled with a selective distribution of blood.

By carrying out experiments with an artificial circulation system, which remains undescribed, and by making allowance for the viscosity of the blood and the pressure in the various arches, Acolat (1938 b) has claimed that he was able to calculate the "efficient capacities" of the atria (i.e. their discharge volumes, or the differences between diastolic and systolic capacities) in various reptiles and amphibians. The two anuran cases he cites with any confidence are a female <u>R. ridibunda</u> and a female <u>B. superciliaris</u>, and these show volumetric ratios of the left and right atria nearer to 1 : 2 than the proportion 1 : 3, given by anatomists. It would appear that Acolat's earlier "balance sheet" for the distribution of blood would therefore require some revision ! The interesting point which arises from this however, is not so much the actual capacities of the atria, but that any arguments based on the relative anatomical sizes of the atria cannot be accepted with any confidence. The actual volumes of blood taken in, and discharged from, the atria may be quite different from their anatomical sizes.

No measurements of the volume or speed of movement of the blood in the vascular systems of the Anura have to my knowledge been made so far.

* * * * * *

This brief summary serves to show upon what uncertain physiological ground the "classical theory" is based. Nor can any definite conclusions be drawn about the contradictory Random Distribution hypothesis. A much wider and more comprehensive investigation into the problem is required.

(III) PHYSIOLOGICAL INVESTIGATIONS.

(A) DISTRIBUTION OF BLOOD -- INTRODUCTION.

The basic question of whether or not there is a selective distribution, would seem to be the one most sorely in need of clarification. Most attention has therefore been given to this aspect of the problem.

Of the methods available for this study, that of measuring the oxygen content of the blood arriving at, and departing from the heart by various channels, has not yet been employed. The great difficulty here, is to find a method which will give sufficiently accurate results with the very small amounts of blood which can be removed, without risking a disturbance in the function of the heart. Considering the difficulty of ensuring that the conditions of respiration are kept constant in successive experiments, it would also be necessary to make simultaneous extractions from a number of blood vessels, a procedure of some delicacy. Nor does it seem possible to extract blood from the beating heart itself, without seriously interfering with its normal action. It is probably because of these obvious difficulties that this method has not been attempted.

Direct visual observations depend in too large a measure on the accuracy of observation and personal interpretation of the experimenter. This criticism is of especial importance when one considers the rapidity of the cycle of events in even a slowly beating heart. It would therefore seem desirable to have a recording method, which enables a careful analysis of the sequence, step by step.

Injection of radio-active substances and their tracing, is an obvious possibility. Simons (personal communication) however, has attempted the method without success. The main difficulty apparently, is to obtain a great enough concentration in the arterial arches to give significant measurements.

Other possibilities include the method of Foxon (1951) using radiographic techniques, and that of Simons and Michaelis (1953) involving a cinematographic tracing of injected fluorescein. The radiographic method is limited by a comparatively slow frequency of photographic exposure (3 per second), due to the necessity of moving large masses of sensitive material and of using lead shutters. In addition to this, there are difficulties, which prevented Foxon (1951) from obtaining more than about 20% "satisfactory" results. The fluorescein technique does not suffer from the same disadvantages, and has other attractions which will appear in the following discussion. Both techniques allow one to trace the injected material through the heart itself.

Savolin's (1949) method of injection and recovery of some identifiable substance does not permit the tracing of substances through the ventricle and bulbus, although it is possible, in principle, to obtain quantitative results.

Apart from these considerations, the value of any injection technique may be judged on the following criteria :-

(1) The animal should be subjected to a minimum disturbance from normal conditions, or should be subjected only to disturbances which may be determined and taken into consideration : For most injection techniques, it is necessary to expose the heart. Insufficient attention has been paid to the abnormalities which may result from this procedure, but in any case, the fluorescein technique can attract no more criticism than any other.

(2) The amount of material injected should not cause an appreciable rise in pressure in the injected blood vessel : Foxon (1951) states that for a "thorotrast" solution to be discernable in the systemic arch by the radiographic means, which he was then using, it had to be in a concentration of at least 25 %. Considering the dilution of the medium in its passage through the heart, it would be necessary to inject undiluted "thorotrast" into one of the pulmonary veins in quantities at least equal to the volume of blood normally flowing through this blood vessel ! Fluorescein however, is detectable at very low concentrations. In spite of this, Simons and Michaelis (1953) appeared to find it necessary to inject volumes of about 1 cc. The use of such quantities, apart from changing the viscosity of the blood, would even in a large frog, serve to increase the blood volume quite considerably, with unpredictable results.

(3) The injected substance should be of such a nature as to mix easily with the blood and not form a separate viscous mass. Foxon's (1951) work shows quite clearly how this may affect the blood circulation. One of the reasons for the failure of some of his experiments, he states, was that the "thorotrast" interfered with the action of the atrio-ventricular valves and on contraction of the ventricle, it was regurgitated into the atrium, -- this process continuing for the whole course of the experiment. One wonders whether the same effect was not being exerted to a lesser degree in those examples which he uses to illustrate the normal course

of the blood flow. A solution of fluorescein can have no such effect.

(4) The injected substance should exert no chemical effect on the heart. Fluorescein, as is well known, is physiologically neutral and has been used for a range of physiological experiments, even on man.

In principle therefore, the use of fluorescein dissolved in Ringer solution, as a tracer substances, seems to be beyond reproach, as long as attention is paid to the first two criteria mentioned above. This was therefore taken as the line of first approach.

Certain disadvantages in the technique appeared during the course of experimentation, the main one being in regard to quantitative analysis of the results. This will be discussed in due course.

(1) DETAILS OF FLUORESCEIN TECHNIQUE.

Preparation of toads :

In most cases, a preliminary subcutaneous injection of about 0.6 cc. of 20% ethyl urethane was given as an anaesthetic. As is well known, this has the effect of dilating the blood vessels, which would cause a general lowering of the pressure in the system. In other cases therefore, unanaesthetised toads were used as controls. It was deemed inadvisable to pith the animals, since this would result in both blood loss and possible disturbing effect on the heart due to the destruction of the centres of nervous control in the medulla. These are scruples, which do not seem to have bothered other workers. Vandervael (1933) in fact, considers the blood loss and slowing down of the heart resulting from pithing as advantageous, in that the action of the heart could more readily be observed.

The toad was then fixed to a cork mat and after a median longitudinal and a transverse posterior incision, the skin of the ventral surface was folded back. The abdominal cavity was opened, taking care to avoid the abdominal vein, and a median incision made through the pectoral girdle. The two sides of the pectoral girdle were then loosely pinned back to expose the heart and arterial arches. When it was necessary to make injections into the pulmonary vein or to observe the circulation through a lung, the latter was cleared of the mesenteries attaching it to the liver and to the dorsal abdominal wall, and the lung gently moved to one side. When carefully done, the blood loss resulting from these operations was insignificant. All exposed parts were kept moist during the course of the experiment with drops of Ringer solution, if necessary.

Female <u>Xenopus</u> were found to be unsatisfactory subjects because of the arrangement of the arterial arches. The pulmo-cutaneous arch was usually hidden, and the carotid arch was also frequently in an unsuitable position for photography. Dissecting these out and fixing them in an exposed position was found to lead to the possibility of mechanical interference with their blood flow, and so medium sized male toads with an average weight of about 45 gms. were used in almost all cases.

In the preliminary experiments, it was found most convenient for photography to fix the cork table in a vertical position. Although no definite indications of a disturbed heart action were observed, the possibility that too much tension was being placed on the anterior venae cavae made a re-arrangement advisable, so that the experiments could be carried out with the toads lying horizontally. Before the experiments were carried out, care was taken to verify that there was no interference with the normal free flow of blood in the vessels leading to and from the heart, and that the heart was not beating in an obviously abnormal fashion.

Injection method :

An adapted Agla micrometer syringe was used as the injection unit. By fixing a length of thin, lead tubing into the end of the glass barrel of the syringe and a very fine cannula (Record no. 21) on to the end of this, the syringe was found easy to manipulate. The lead tubing was sufficiently flexible for adjustment of the cannula position and yet remained at any angle at which it was placed. This appeared to eliminate difficulties encountered by Foxon (1951) with his injection technique, in which air would frequently be introduced into the blood vessel at the same time as his contrast medium. The cannulae used, were found to be small enough to allow insertion even into the pulmonary veins of the smaller specimens, without blood loss and without materially obstructing the flow of blood.

Various concentrations of fluorescein-Ringer solution were tried, the most satisfactory being a 1% solution. At concentrations higher than this, the fluorescein was found to diffuse into the blood before the actual injection was started, and this led to a preliminary generalised fluorescence of the heart and aortic arches.

The amounts injected varied between 0.15 cc. and 0.004 cc., the average figure being 0.046cc. Conservatively estimating the blood volume of a 40 gm. toad to be 3 cc. it cannot be supposed that the injection of such small volumes could have any significantly adverse effect on the blood circulation, either through raising the pressure or by decreasing the viscosity of the blood.

The solution was injected slowly and continuously during each experiment.

Illuminant :

Ultra-violet illumination was provided by three, or sometimes four 125 W Phillips mercury-discharge lamps, Type 57202E/70, mounted in polished aluminium reflectors. At a distance of about 1.5 ft. these reflectors were found to focus the light into an intense bluish patch of about 2 ins. diameter. Any unevenness of illumination was counterbalanced by the superimposition of the other lamps' brightest areas. Since the fluorescein solution was strongly fluorescent to the eye, both in the brightest patches of focussed light and immediately outside them, it seemed unlikely that the passage of any fluorescein would not be recorded by the camera due to inadequate ultra-violet irradiation in some areas.

The lamps were allowed to warm-up for a period of three or four minutes before the actual experiment, in order to reach their maximum ultra-violet output.

Ultra-violet penetration of the aortic arches :

To test for any possible differences of penetration of the ultra-violet light through the walls of the different arterial arches, the following experiment was carried out. Small sections of the arches were removed from a toad, washed out with Ringer solution and slipped over the end of a glass tube of bore approximately equal to that of the arch in its normal state. Various concentrations of fluorescein-Ringer solution were then drawn up into the tube and exposed to the camera under ultra-violet light. That the section did not transmit the fluorescence with equal intensity, as shown by the photographs, can probably be explained by the difficulty of ensuring that each of the sections was stretched to the same extent. It is interesting to note however, that the fluorescein solution still showed a clearly visible result on the negative down to a concentration of 1 : 10,000.

In order to overcome the difficulty of stretching the arches to their normal extent, an alternative method of testing for relative pentrability was tried. Small successive amounts of fluorescein solution were injected into the living toad, and after allowing sufficient time for an even distribution over the whole body, photographs were taken after each injection. This method however, was unsuccesful due to the diffusion of the fluorescein into the blood vessel walls themselves and due to interference by the reflected light from adjacent tissues, which after a short while were strongly fluorescent.

Histological examination of the walls of the arches showed no significant differences in structure or thickness, and it was considered that since such low concentrations of the fluorescein gave a distinctly recordable fluorescence, the relative penetrability of the blood vessel walls need not be considered in the qualitative or rough quantitative evalutaion of results.

Filming :

For photographic recording, a Paillard Bolex 16 mm. camera, with a parallax adjusting mechanism, was employed. Using a 75 mm. lens, with a short extension of 7 mm. and a 2 dioptre accessory lens, a field of about 1 X 1½ ins. could be photographed, with sufficient depth of focus at an aperture of f 3.5 for clarity. In order to eliminate any reflected ultra-violet light from the surface of the tissues or elsewhere, a Kodak Wratten K-2 gelatine filter was used, and in addition, a Voigtlander G-2 yellow filter to eliminate the fairly strongly visible, bluish light emitted by the ultra-violet lamps.

Preliminary experimentation with Ansco colour film proved to be unsuccessful, even with the maximum intensity of ultra-violet light which was available, but the use of Kodak Super XX panchromatic film was found to be eminently successful. With this, it was possible to photograph at a speed of up to 24 frames per second. No higher speed was attempted, but there is no reason why it should not be possible. The film was developed in the laboratory in short strips, using Ilford ID-11 M.Q.Borax Fine Grain developer and extending the development time more than is recomended by the makers. By this means it was possible to obtain negative results of quite satisfactory contrast, although, it must be admitted, of somewhat grainy texture. However, the necessity of obtaining and examining results quickly, prevented the film from being sent to the Kodak laboratories, where the development could have received more expert attention.

The field covered by the negative included the heart, main arterial arches and some surrounding tissue, and so, naturally enough, much of the fine detail was lost, especially movements of the bulbus. Attempts were made to obtain a more detailed idea of the sequence of events, but it was found that the depth of focus was so much restricted by narrowing the field, that the results were un-interpretable. As will appear, the main features exposed by the technique were the filling of the atria and ventricle, and the distribution of the fluorescein to the arterial arches.

Quantitative estimation of results :

In order to estimate the distribution of blood quantitatively in a technique such as this, the following factors must be determined :

(a) The ratio between concentration of the fluorescein and the resulting fluorescence; There is not necessarily a direct relationship and there might well be an optimum concentration, as with other fluorescent dyes (Radley and Grant 1939).

(b) The effect of the dilution of fluorescein solution with blood, to a varying degree, upon the resulting fluorescence. The nature of the solvent may affect the strength of fluorescence (Radley and Grant 1939).

(c) The effect of shielding of the fluorescence by blood cells at various concentration levels. (Rough tests for the shielding effect of the vessel walls were carried out -- see p. 26).

In addition to these, the photographic method must be such as to permit a calculation of the relative intensity of fluorescence in different regions. It is well known that changes in the development of a negative may vary the relative intensities of different areas of the photographic image ("contrast" of the negative). Extremely careful control of the development would therefore be necessary.

Furthermore, some accurate method of determining the density of various areas of the negative must be devised, a process of some difficulty with cinematographic film.

In view of these obstacles, no quantitative estimation was attempted, apart from visual examination. Most of the results were dealt with from a qualitative point of view only.

Number of Records :

As a result of the length of time required for the development of the technique, only thirty-seven records were made, and after critical examination, eight of these have had to be discarded. In two, the heart stopped beating for a period during the experiment, but the others were discarded for technical reasons only, such as the spilling of fluorescein solution, or the stoppage of the camera in the middle of a recording. Of the remaining twenty-nine records, eleven were of injections into an anterior vena cava or hepatic vein (i.e. via the right atrium), and eighteen of injections into a pulmonary vein (i.e. via the left atrium).

An analysis follows.

(2) RESULTS OF FLUORESCEIN EXPERIMENTS.

INJECTIONS VIA THE RIGHT ATRIUM :

Injections were made into the left anterior vena cava or into one of the hepatic veins. A typical recording is reproduced in Plates I & II.

The injection site and the proximal portion of the vein was always marked by a dark mass, due to the accumulation of fluorescein solution as a result of the slow movement of blood in these large vessels.

The subsequent course of events was somewhat variable. After one or two heart beats, the fluorescein might become visible first in either the right atrium or the ventricle, or both simultaneously. In all cases, the brilliance of the fluorescence took some time to build up in the atrium, particularly in the marginal pockets, which never obtained a full colouration until a few heart beats had occurred.

Passage of the fluorescein into the sinus venosus or between the atrio-ventricular valves was obviously not recorded, due to the thickness of the heart wall and the blood which it contained.

In most cases (including three films in which the development had been spoilt, so that further details were not visible), the appearance of fluorescein in the ventricle was only on the right side, with a more or less sharp line of division between this and the clear left side (Plate I). This colouration is interpretable as being due to the penetration of the fluorescein-laden blood into the intertrabecular spaces near to the surface of the ventricle. In four cases the line of demarkation between the two sides was not sharp, only a small portion of the ventricle on its extreme right or at its apex becoming coloured.

During contraction of the ventricle, the fluorescence remained, presumably since some of the fluoresceinated blood had remained in the muscular mesh-work (Plates I - IV). Foxon (1951) has proposed that this remnant, which he also found during his experiments on Rana and Bufo, might be blood concerned with the supply of oxygen to the ventricular musculature.

The division of the ventricle broke down some three or four beats after its first appearance, when the left side also became coloured. Sometimes the break-down occurred quite suddenly in the space of one or two ventricular diastoles (Plate II), or sometimes more gradually, and, as is to become apparent shortly, is associated with the recirculation of fluorescein through the pulmonary circuit, into the left atrium.

In the following descriptions, the numbering of the heart beats is commenced from the first appearance of fluorescein in the ventricle.

On the first or second heart beat, but not later, the fluorescein was quite distinctly detectable in the pulmo-cutaneous arches (Plate I). No sign of it however, appeared either in the ventral chamber of the truncus, carotid or systemic arches at the same time, except in one case (Plate V-B). In all the other cases (ten) the distinct appearance of fluorescein in the ventral chamber of the truncus was delayed until the third, fourth, or later heart beat, at which time there was a colouration of the left side of the ventricle, or at least there was the possibility of recirculation of fluorescein through the pulmonary circuit.

In most cases, the pulmo-cutaneous arches remained distihctly more fluorescent throughout the length of the record. In two cases the systemic or carotid arches attained a similar density at the fifth or sixth heart beat. In all cases, the maximum density of fluorescence in the pulmo-cutaneous arches was reached by the second or third heart beat. It must be recorded however, that the pulmo-cutaneous arches did not always reach the same density on both sides, a phenomenon which was repeated sometimes in the colouration of the systemic and carotid arches, but not always on the same side. Possibly some of the arches were shaded from ultra-violet light, due to the position of the heart. As in Plate I, the fluorescein sometimes appeared first in the form of streaks in the arterial arches.

The speed of flow of blood through the pulmo-ctuaneous arches and the pulmonary artery was obviously very rapid, for the fluorescein appeared in the lungs at the first (4 cases), second (5 cases), or third beat (1 case). Distinct appearance of the fluorescein in the pulmonary vein occurred at the second (2 cases), third (3 cases) or fourth beat (2 cases). In other instances, the lungs or pulmonary veins were obscured by surrounding structures. In no case was there any recordable fluorescence in the tissues supplied by the carotid or systemic arches, before the seventh or eighth heart beat.

As has already been stated, the left side of the ventricle usually became coloured at the third or fourth beat, and this could be seen to coincide with the return of fluorescein through the pulmonary vein. As was the case with the right atrium, the colouration of the left atrium was often delayed until after fluorescein was visible on the left side of the ventricle, and then only built up to a rather faint image. This was due to the time required for it to penetrate into the marginal pockets of the atrium.

A diagram of the bulbus in its normal relationship to the ventricle and ventral surface of the heart, is given in fig. 5, and it can be seen from this that the main part of the bulbus visible from the ventral surface, is that part which corresponds to the cavum aorticum. At most, only a small section of the cavum pulmo-cutaneum would be visible at the base and on the left side of the bulbus, from this angle. This would be separated from the cavum aorticum by the free margin of the spiral valve, which follows a diagonal line from the bottom centre to the top

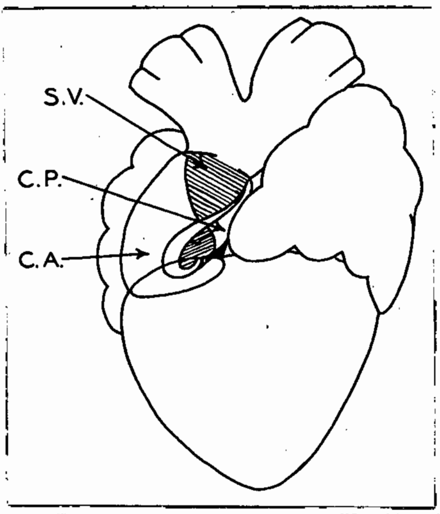

Figure 5 : DIAGRAM, SHOWING NORMAL POSITION OF SPIRAL VALVE IN BULBUS CORDIS. (Ventral view).

CA cavum aorticum; CP cavum pulmo-cutaneum; SV spiral valve.

left part of the visible region of the bulbus. The rest of the cavum pulmo-cutaneum would be hidden beneath the spiral valve. With these structural arrangements in mind, the following observations are of interest.

In four records no colouration of the bulbus was visible until the third beat, when the sudden appearance of fluorescein there, coincided with its appearance in the ventral chamber of the truncus, and with the probable re-circulation of blood through the lungs. In another two cases, the anterior part was clear, while a dark basal part was discernable, with a diagonal border, which may well be interpreted as the free margin of the spiral valve. This state of affairs is consistent with the supposition that the fluorescein-laden blood from the right side of the ventricle was either passing directly into the cavum pulmo-cutaneum, or was traversing only the lower part of the cavum aorticum, before passing over the spiral valve into the cavum pulmo-cutaneum. In four other cases however, a "pulse" of fluorescein was visible, which in one case clearly passed over the bulbus from right to left (Plate I, frames 16 - 20). During this time, no great fluorescence appeared in the ventral chamber of the truncus or in the four anterior arches. It would therefore appear that blood may be admitted to the cavum pulmo-cutaneum by first passing up the cavum aorticum. These observations are placed on record as an indication that the statements by Ozorio de Almeida (1923), Vandervael (1933) and Foxon (1947) that the blood passes up as two <u>separate</u> streams in the cava, do not always apply in <u>Xenopus</u>, since blood can pass over the spiral valve from the cavum aorticum to the cavum pulmo-cutaneum.

The use of ethyl urethane as an anaesthetic did not seem to have any effect on the type of result obtained.

INJECTIONS VIA THE LEFT ATRIUM.

Injections were made into the left or right pulmonary vein. A typical recording is reproduced in Plate III.

Certain significant differences emerged from the results of injections into the pulmonary veins. Where no differences are remarked upon in what follows, the picture was the same as before. This applies particularly to the gradual filling of the marginal pockets of the atria, which again occurred over the space of several heart beats. This confirms observations by both Foxon (1951) and Acolat (1938 b) that the discharge volumes of the atria are less than their anatomical structure leads one to expect. The main bulk of blood is taken in and expelled from the central parts of the atria, but a certain reserve is held in the marginal pockets. The atria are in fact, acting as reservoirs, which are not emptied completely at each beat, and whose degree of emptying may possibly vary from time to time.

In addition to change in discharge volume in this way, there is also the possibility of the thin inter-atrial septum bulging into one or the other atrium, thus changing the relative capacities of the latter. There is however, no evidence to support this.

In the records, the pulmonary vein injection site was not always clearly visible, either because this was not included in the area filmed or because it was hidden by the heart. In seven cases however, the site of the injection could be seen and showed that the injected solution was rapidly washed away by a wave of non-fluoresceinated blood at each atrial diastole. This indicates that the speed of flow through these vessels was considerably faster than that through the anterior vena cava or hepatic veins.

The initial filling of the ventricle was variable, but the following grouping has been adopted :-

(a) The whole ventricle appeared coloured at the first beat (9 cases) (Plate III)

(b) Spots appeared on both sides at the first beat, although those on the left side were denser (4 cases).

(c) Only the left side of the ventricle was coloured at the first beat (4 cases) (Plate IV). Two of these cases showed a line of demarkation between right and left sides, which lay well tothe right of the median line (Pl. V).

In one exceptional case, only the <u>right</u> side of the ventricle was coloured at the first beat, - a most surprising result (Plate V-E).

Where a division occurred, this tended to break down after a number of beats, but unlike the hepatic vein injections, this was not to be correlated with a recirculation of fluorescein. Here, it is probably due to the retention of a small quantity of fluorescein in the central chamber of the ventricle, which subsequently gets drawn into the trabecular mesh-work of the right side, when the ventricle dilates. The same effect would not be exerted when the initial colouration of the ventricle occurs on the right side, since this fluorescein would only traverse the right side of the central chamber and would not readily mingle with the blood flowing to the left side of the ventricle.

After the first one or two heart beats, the retention of fluorescein in the ventricle and itsspread to the right side, might make the resulting distribution scheme in the arterial arches, unreliable, because of "contamination2 of the right side of the ventricle. It might be argued that the same sort of mixing would occur naturally, but the photographic records possibly exaggerate its extent. This must therefore be kept in mind in following the appearance of fluorescein in the arterial arches.

The pulmo-ctuaneous arches showed colouration at the first beat in fifteen cases (Plates III, IV), and at the second beat in three cases. In twelve cases colouration of the bases of the carotid and systemic arches was simultaneous with that of the pulmo-cutaneous arches; in five cases after; and in one case before the pulmo-cutaneous arch colouration. In fifteen experiments the pulmo-cutaneous arches were more fluoreseent than the others for at least the first four beats. (Plate III).

In four records, they even remained darker throughout the length of the film strip (six to ten heart beats). This indicates that the pulmo-cutaneous arches were receiving a considerable proportion of the fluorescein injected into the pulmonary vein.

Fluorescence of the lungs and pulmonary veins occurred at about the same time as in the hepatic vein injections, but colouration of other tissues, such as the distributional area of the external carotid artery, tended to occur sooner.

At no time was any fluorescein detectable in the right atrium during the course of the experimental record (6 to 18 heart beats).

The cavum aorticum of the bulbus was usually fluorescent from the first beat, or at least from the time of the first appearance of dye in the ventral chamber of the truncus.

Again, the administration of urethane seemed to make no difference in the results.

(3) DISCUSSION OF RESULTS OF FLUORESCEIN EXPERIMENTS.

What, then, is the "average picture" of the blood distribution that is obtained from these results ?

Upon injection via the right atrium, there tends to be a division of the ventricle, so that only the right side is fluorescent, until the possibility of recirculation enters into consideration. No significant colouration of the systemic and carotid arches occurs before that time, while the pulmo-cutaneous arches fluoresce distinctly.

Upon injection via the left atrium, the fluorescein tends to spread all over the ventricle, or where division of the ventricle occurs, the line of demarkation tends to be towards the right side. The appearance of dye in the carotid and systemic arches now tends to coincide with its appearance in the pulmo-cutaneous arches, although the latter remain darker for at least the first few beats.

As far as can be seen, this situation is consistent with the following circumstances :-

(1) Almost all the blood expelled from the right atrium is sent to the pulmo-cutaneous arches :

This statement is a direct outcome of examination of the film records of injections via the right atrium, in which in only one case out of eleven, did the fluorescein appear distinctly in the carotid or systemic arches or the ventral chamber of the truncus, before recirculation of the fluorescein through the pulmonary circuit.

In a heart which has no complete anatomical separation of the two sides, it is patently impossible to propose that absolutely no mixing occurs. In view of this fact and the occurrence of the exceptional case, the films were re-examined and it was seen that there was a faint colouration of the systemic and/or carotid arches in some experiments before it could be said with certainty that recirculation had occurred. It is for this reason that the statement has been phrased to read "ALMOST all the blood.." to imply that at any rate, by far the larger quantity does so.

(2) A form of division of the blood from the left and right atria occurs in the ventricle, in so far as right atrial blood is absorbed only into the trabecular meshwork on the right side of the ventricle :

A selective distribution of the kind mentioned above would be impossible, unless the blood from the right atrium were being kept separate in the ventricle and bulbus in some way. Now in the fluorescein technique, it is obvious that a picture of the whole ventricle is not obtained, since both the ultra-violet light and the resulting fluorescence can only penetrate a short distance through the ventricular trabeculae, and the blood in between them. The line of

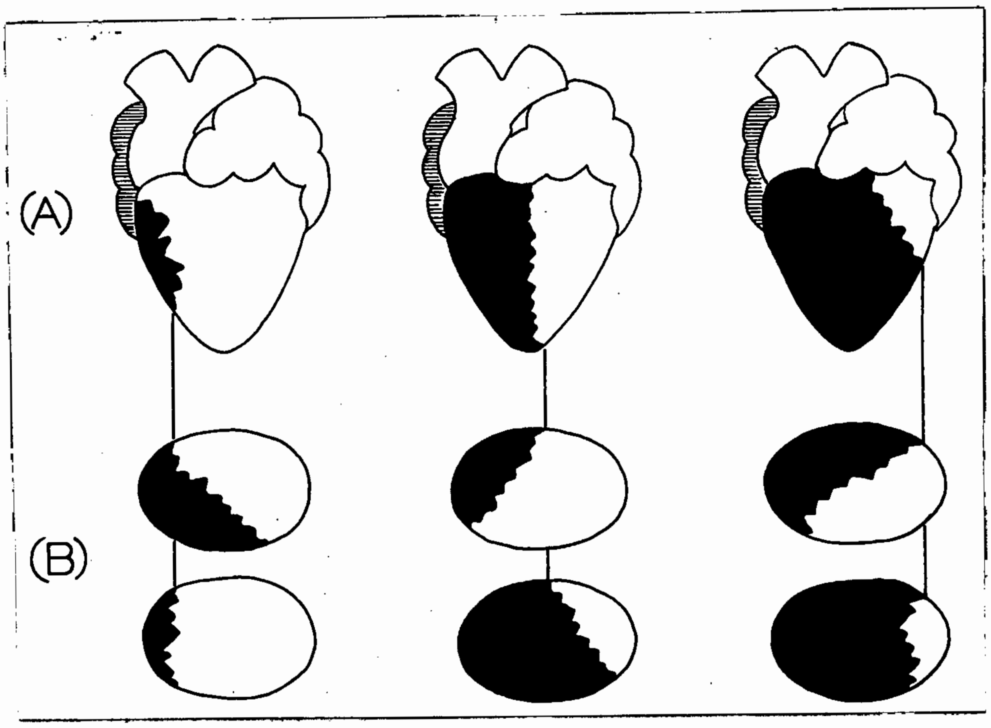

Figure 6 : WAYS IN WHICH THE VENTRICLE MIGHT BE FILLED FROM THE RIGHT ATRIUM.
(A) Apparent filling of the ventricle, as seen in the fluorescein injections via the right atrium.
(B) Possible actual modes of filling in each case, as seen in transverse section through ventricle.

demarkation between fluoresceinated and non-fluoresceinated blood, through the depth of the ventricle, might either be in a vertical plane or might incline in a diagonal fashion (fig. 6). The latter possibility is indicated where only a small area on the extreme right side of the ventricle became coloured at the first beat (not figured in a Plate, but see fig. 6 above). The bulk of the fluorescein in these cases, might have been lying more dorsally in position, where it would not have been recorded.

Yet it is not to be expected that the line of division would differ much from the vertical plane in which the inter-atrial septum projects into the central chamber of the ventricle. The sharp, median line of demarkation between light and dark sides of the ventricle, appearing in many of the records (Plate I), is therefore interpreted as being due to absorption of fluoresceinated blood only into the trabecular mesh-work on the right side.

This second statement supports descriptions by the authors of the "classical theory", Gompertz (1884), Ozorio de Almeida (1923) and Acolat (1931 b). Injections via the left atrium, which were followed by fluorescence of the whole ventricular surface, will be considered later.

(3) The blood from the left atrium is distributed to all the arterial arches :

This again is a statement made from direct examination of the records, where fluorescein tended to appear simultaneously in the bases of all the arches at the first beat, after injections via the left atrium. Following this, in nearly all cases, the pulmo-cutaneous arch(es) were more darkly coloured than the others for at least the first four beats. This indicates that the pulmo-cutaneous arches were receiving quite a large proportion of the blood from the left atrium.

(4) More blood is flowing through the pulmo-cutaneous arches at each beat, than is being sent through the carotid and systemic arches together :

In the absence of accurate quantitative measurements there can be no final proof of the above statement from the fluorescein technique. Nevertheless, interpretation of the films leads to the conviction that it is so.

The amount of blood flowing through the pulmo-cutaneous arches depends upon the discharge volumes of the two atria, and the proportions of each of these volumes which pass into the pulmo-cutaneous arches.

By a comparison of the films (See Plates I & III), it seems that the pulmo-cutaneous arches receive at the very least three-quarters of the right atrial blood, and one-third of the left atrial blood. Under these circumstances, the pulmo-cutaneous arches must receive more blood than the systemic and carotid arches together, if the right atrium has either a larger discharge volume, or one equal to that of the left atrium.

There is of course a third alternative, - that the left atrium has a greater discharge volume than the right, but this automatically implies that the pulmo-cutaneous arches receive more than half of the blood from the heart, since the left atrium is supplied by the pulmonary veins, and hence by the pulmo-cutaneous arches.

This argument is made more convincing by the discussion of statement (5) below, and on the following pages.

(5) In most cases, more blood is being returned to the heart via the left atrium than via the right atrium, and the left atrium has a greater discharge volume than the right atrium.

If more blood is passing into the pulmo-cutaneous arches, it does not necessarily follow that the left atrium must receive more than the right, since a certain part of this blood is diverted into the cutaneous artery, and returns to the heart via the anterior venae cavae. The cutaneous arteries (aa. cutanea magna) however, are very small in Xenopus, having about 1/9 the cross-sectional area of the pulmonary veins, and the film records do seem to indicate that the right atrium actually has a larger discharge volume.

In nine cases out of eighteen injections via the left atrium, the ventricle was seen to become fluorescent all over at the first beat. In another four cases, spots of colour appeared on the right side, even though the left side was more darkly coloured. The same caution is required in interpreting these results as was the case with the filling of the ventricle from the right atrium (pp. 37 & 38), but again it could hardly be supposed that the line of division through the depth of the ventricle could vary very much from the median plane in which the inter-atrial septum projects into the central chamber of the ventricle. The results are therefore consistent with the idea that the left atrium in most cases, fills a larger proportion of the ventricle than does the right. This would mean that a part of the left atrial blood must pass to the right side of the ventricle, to mingle with the right atrial blood there.

* * * * * *

One of the "corollaries" of the "classical theory", which has been emphasized by Acolat (1931 b), is that the lungs of the Anura are not able to deal with the same quantity of blood in unit time, as the systemic circuit. The same idea has been stressed in relation to the vertebrate series as a whole. It has been suggested that the lack of a complete division of the heart, encountered in all forms before the stage of the post-embryonic bird or mammal, may be looked upon as a short circuiting mechanism to relieve the load upon the lungs. The indications gained from the fluorescein experiments sharply contradict these suggestions.

In order to confirm the results, the most obvious need is to devise some method of measuring the volume of flow per unit time, in the arterial arches. A modification of Ludwig's "Stromuhr" technique was attempted, but the operation proved too delicate to be performed on the size of animal available.

Another method could be based on the principle of the "Thermostromuhr", but there has not been time to develop this.

Nevertheless, the supply of information to be gained from the present series of experiments, has still not been exhausted. This information relates to the speed of flow of the blood in the arterial arches.

Before starting on the experiments, it was hoped that it might be possible to calculate the speed of blood flow from the rate at which the fluorescein passed up the arches, and by taking into account the relative cross-sectional areas of the blood vessels, to derive figures for the volume-flow. In practice, this has not proved so simple, since there was no distinct "wave front" during the passage of the fluorescein. Some approximation however, may be gained from the following analysis.

In all cases, the fluorescein passed up the whole visible part of the pulmo-cutaneous arch during the same beat at which it appeared at the base of the arch. In eight cases it showed up in the lungs during the first beat, in fourteen cases during the second beat, and in three cases during the third beat. In three cases it was seen in the pulmonary vein at the second beat, in seven cases during the third beat, and in two cases during the fourth beat. In four cases of injections via the right atrium, the fluorescein was visible in the left atrium at the third beat, in four cases at the fourth beat, and in three cases at the sixth or seventh beats.

This indicates a very rapid rate of flow through the pulmonary circuit. By comparison, the impressions gained of the speed of flow in the systemic and carotid arches are as follows :-

In no case was fluorescein visible at the ends of the exposed portions of the carotid or systemic arches before the second beat after its first appearance at the base of those arches (The lengths of the exposed portions of all the arches were roughly equal.). More usually, where any details could be made out, it took some three or four beats for the dye to reach this distance. In some instances, the dye could be seen to progress a short distance up the arch with eadh beat, although no clear wave front was visible.

The appearance of fluorescein in the peripheral distributional area of the carotid or systemic arches occurred later than in the lungs.

In no case was any recirculation of fluorescein visible in the right atrium during the course of pulmohary vein injection experiments, even when these lasted for seventeen or eighteen heart beats. In fact, from direct visual observation, return of fluorescein through the anterior venae cavae was not detectable before about the twenty-fifth beat. Of course, the fluorescein would have been absorbed into the tissues to some extent, but the impressions of a much slower flow through the carotid and systemic vessels are supported by direct observation.

The movements of the blood corpuscles could be seen in the blood vessels of an un-injected specimen under a dissecting microscope and under strong, side illumination. In the carotid and systemic arches, these movements took place in a jerky fashion, which could be resolved into three phases :

(a) A sudden rapid movement over a short distance, which corresponded with ventricular systole, and during which the definition of the corpuscles was lost due to their rapid movement,

(b) A brief, complete halt, or a considerable reduction in speed, and

(c) An increasing speed of flow, which appeared to correspond with contraction of the bulbus, and during which the corpuscles were moving at a slow enough pace to be clearly seen.

The churning of the blood which must result from these movements is probably the reason that no "wave front" of fluorescein appeared, but it is interesting to compare these direct observations with the jerky progression of fluorescein mentioned above.

It is significant that by using the same method, the movement of corpuscles in the pulmo-cutaneous arches could not be observed, until some obstruction was applied, --- the blood was moving too fast all the time, for the corpuscles to be visible !

Calculation of the cross-sectional areas of the arterial arches from the average diameters, given on page 9, gives a ratio : pulmo-cutaneous arch - 3.8; carotid plus systemic arch - 5.6. Moreover, it can be seen from comparative measurements of the relative lengths of the capillary networks of the lungs and skin in those Anura which have been investigated (Czopek 1955), that the vascular bed of the bodily tissues must be far longer than that of the lungs. There is therefore no theoretical reason why the pulmo-cutaneous circuit could not deal with more blood per beat than the bodily circuit, if the blood is mov~~e~~ing rapidly enough.

* * * * * *

What has been discussed so far, is the "average picture". How much reliance may be placed on this ?

As far as possible, all the experiments were carried out under the same circumstances, except for the administration of anaesthetic in some cases, but not in others. It has already been noted that the urethane did not seem to have any effect on the results, but this does not rule out the possibility of individual variations for other reasons. (A consideration of the disturbances which may be introduced by the operative procedure is more conveniently deferred until a later section -- page 64). It is therefore necessary to examine the exceptional cases and if possible, to account for these.

The questions which should be asked here, are

(a) Is there any case of injection via the right atrium, which is not consistent with the statement that almost all of the right atrial blood is sent to the pulmo-cutaneous arch ?

(b) Is there any case of injection via the left atrium which is not consistent with the statement that a large proportion of the blood from this atrium is sent to the pulmo-cutaneous arch ?

To the first question, the answer is that, apart from some faint tingeing, the simultaneous appearance of fluorescein in all the arches took place in only one case out of eleven. It is interesting to note that this case corresponded to a division of the ventricle, which was well to the left of the median line (Plate V-B).

The answer to the second question is that there were only three cases out of eighteen injections into the pulmonary vein, in which the pulmo-cutaneous arches were not distinctly darker than the others for at least the first four beats. These exceptional cases showed a division in the ventricle, so that only the left side was coloured by the dye (Plate IV).

These cases can be explained by a change in the ratio of the discharge volumes of the atria. If the right atrium has an increased delivery, the blood from it would tend to occupy a greater part of the ventricle, and this would explain the type of ventricular division obtained, as well as the resulting distribution pattern.

The change in the distribution pattern is particularly interesting, in that it shows a physiological connection between the right side of the ventricle and the pulmo-cutaneous arch, and the left side of the ventricle and the carotid and systemic arches.

Finally, there was the experiment in which only the right side of the ventricle became coloured after an injection via the left atrium, explicable on the assumption that there was a restricted expansion of the left side of the ventricle.

The general conclusion to be drawn from these cases is that since they are exceptional, the "average picture" is a fair reflection of the situation in most cases. It is not known whether the exceptional cases occurred as a result of some undisclosed fault in the technique for those experiments, or whether there are naturally occurring individual variations, or variations from time to time in the same individual.

* * * * * * *

(B) PRESSURE MEASUREMENTS IN THE ARTERIAL ARCHES. - INTRODUCTION.

Other important evidence which is required for an understanding of the blood distribution, and the mechanisms by which it is brought about, concerns the pressure in the arterial arches, and how and when it fluctuates with each heart beat. This is a key-stone in the "classical theory", but it is also brought forward in arguments against the "classical theory", by Foxon (1951) and Vandervael(1933). What exactly is the evidence ?

Previous experimental work :

Only two references have been found to previous experimental work on this problem. Other investigations have of course been carried out on blood pressure in the Anura (e.g. Schultz 1906), but these do not reflect simultaneous measurements in the pulmo-cutaneous and systemic circuits in the same animal, and there is apparently sufficient variation between individuals to make this necessary. The relevant papers are those of Gompertz (1884) and Acolat(1938 a).

It might be of interest to quote here a section from Gompertz's paper, to illustrate the method he used :

> "Grossen Fröschen, deren Gehirn und Rückenmark zerstört waren, wurden die oberen Hohlvenen und die linke A. carotis abgebunden, eine Canüle in die linke Aorta, eine zweite in die linke A. pulmonalis nach abbinden der linken A. cutanea magna, eine dritte in die Vena cava inferior und eine vierte in den rechtsseitigen Canalis aorticus unter gleichzeitiger Unterbindung des rechtsseitigen C. pulmo-cutaneus und caritoco-lingualis eingebunden. Nachdem das Herz mit Kochsalzlösung sorgsam ausgespült war um jedes Gerinnsel zu entfernen, wurden die beiden Canülen der linksseitigen Aorta und Pulmonalis met zwei Manometern in Verbindung gebracht, deren Schwimmer senkrecht über einander auf eine rotirende Trommel schrieben. Durch die Canüle der V. cava inferior strömte verdünntes Kaninchenblut in das Herz ein und durch die Canüle des rechten Canalis aorticus bei jeder Contraction des Ventrikels stossweise in ein untergestelltes Gefäss aus. "

By using these methods, he demonstrated an almost identical pulse pressure wave in the pulmo-cutaneous and systemic arches, but his results are quite obviously not significant, because of the extremely unnatural conditions of the experiments.

Yet this evidence is quoted by Vanderval (1933) in argument against the "classical theory". He adds the remark that since the blood was seen to move simultaneously up the two sides of the bulbus, the pressures in the pulmo-cutaneous and systemic circuits must be the same. This is not necessarily the case at all.

Acolat (1938 a) apparently took more care than did Gompertz, in his handling of the heart. As far as he describes it, his method consisted of connecting two small identical, mercury manometers to the systemic and pulmo-cutaneous arches by fine cannulae, at as nearly as possible equal distances from the heart. He gives readings for two anuran specimens only, a female R. ridibunda and a female B. vulgaris, and these reflect a small pressure difference, the pulmo-cutaneous arch being between 1.25 and 3.5 mm. Hg. lower than the systemic arch. Foxon (1951) states that such small differences in pressure can hardly be regarded as significant, but it will become apparent shortly that because of the inertia of the mercury, it is impossible to obtain accurate measurements of the variations in pressure during a single heart beat (i.e. of the pulse pressure), by using a mercury manometer, however small this may be. Acolat's (1938 a) experimental method is therefore unsatisfactory, in that it can only give an idea of what the average pressures are.

Methods available :

What was required for the present experimental work, was a form of apparatus which would :

(a) create as little disturbance in the animal as possible,
(b) record pressures accurately, and
(c) respond rapidly and faithfully to sudden pressure changes.

The optical manometer seems to be just such an instrument. This operates by transmitting the pressure from the blood vessel through a fluid filled tube to an elastic membrane. Pressure causes a distortion of this membrane, which is proportional to the pressure applied, and the distortion is measured by an optical lever system, operating off a small mirror attached to the membrane.

A summary of the highly mathematical principles involved in optical manometer systems is given by Hamilton (1946) and Wiggers (1928), from which the following main points may be extracted. The inertia of the fluid system in the manometer depends upon the amount of fluid which must enter it, in order to move the mirror. When fluid enters the system, it must move all the fluid in the tube through a certain distance, and the longer and thinner the tube, the more difficult it is to accelerate the particles of the fluid, -- in other words, the "effective mass" of the manometer varies directly with its length and inversely with its bore. This means that in order to have a quickly responding instrument, the inertia must be cut down by having as rigid an elastic membrane and as low an effective mass, as possible. The speed of response is reflected by the "natural frequency" of the instrument, when thrown into oscillation. However, the speed of response is limited by the necessity of creating sufficient distortion of the membrane to give accurately measurable results (the sensitivity).

The Wiggers Universal Manometer has the disadvantage of requiring the use of a very wide cannula, which is obviously out of the question with animals as small as frogs. Hamilton <u>et al</u> (1934) and Gregg <u>et al</u> (1937) have described instruments which overcome this difficulty. In these, the increase in effective mass, created by the use of a small hypodermic cannula, is counterbalanced by the use of a more rigid elastic membrane of small area, thus maintaining a high natural frequency.

By comparison with instruments such as these, in which there is an extremely small movement of fluid, the mercury manometer used by Acolat (1938 a) is a crude mechanism.

Having the same operative principles as optical manometers are "pressure transducer units", in which the distortion of a membrane is measured by electrical means through a Wheatstone bridge and amplification system. One form of pressure transducer produced by the Statham Laboratories, California, was tried, but was found to have unsatisfactory frequency characteristics, since it was not really designed for use with very small cannulae, or with such low pressure ranges.

An optical manometer system was therefore used.

(1) DETAILS OF PRESSURE MEASUREMENT METHOD.

Construction of the manometers :

The design adopted was essentially the same as that described by Hamilton *et al* (1934), but with a few modifications. (fig. 7). Two manometers were constructed, as nearly as possible identical. A few brief comments follow on structural features not apparent in the diagram.

The taps were connected by rubber tubing to a reservoir containing a 5% sodium citrate solution. After the system had been filled completely, it was found that this arrangement was most convenient for cleaning out the cannulae without the danger of admitting air bubbles to the manometer.

After trying various types of membrane, it was found that rubber sheeting of 0.7 mm. thickness was most satisfactory. The advantages of using heavy rubber in place of shimm brass as used by Hamilton (1934), are pointed out by Gregg (1937), in that the excursion of the optical lever is more nearly a linear function of the pressure applied. The rubber sheeting was tied firmly over the end of the manometer tube with brass wire, exercising care to stretch it equally in all directions and to an equal degree in the two manometers. On this, was mounted a small triangle of the same rubber, glued with two points on the rim of the manometer tube, and one point in the centre, and the mirror was then mounted on this (fig. 7-B). This allowed the mirror to move on the segment principle (see Wiggers 1928) in the horizontal axis only.

The mirrors were made from 0.75 dioptre, plano-convex, wafer lenses silvered on the flat side and cut down to about 5 mm. squares. These concentrated the light beam sufficiently to enable the optical lever to be extended to fifteen feet, without excessively losing its sharpness.

The cannulae used were ½ in., No. 20, Record-fitting hypodermic needles, which were of small enough diameter to enable insertion into the arterial arches, without disturbing the normal flow of blood significantly. The needles made by the Everett Co. were found to be most satisfactory, because they were of thinner material and thus gave a smaller over-all diameter for the same effective internal bore. These needles are somewhat smaller than those used by either Hamilton or Gregg and their associates, and the results of this will be shown below.

Mounting of the manometers :

The manometers were held firmly in their adjustable outer housings and these in turn fixed in heavy metal clamps so as to lie horizontally beside each other. As a precaution against the mis-interpretation of accidental jolting of the equipment, base-line mirrors were attached to the outer housing.

The horizontal position of the manometers was necessary, to avoid parallax errors in recording on a vertically moving film-strip. The principles of parallax adjustment in optical manometer systems have been clearly explained by Wiggers (1928).

Filling of manometers :

The manometers were carefully filled with a boiled 5% sodium citrate solution, taking as much care as possible to eliminate all bubbles. The importance of this has been stressed by Wiggers and others, since bubbles have the effect of decreasing the frequency of the instrument. To make quite sure that they were eliminated, at the beginning of each series of experiments the whole manometer and lead connecting tube was placed in a vacuum chamber and the pressure reduced until the citrate solution boiled. It was found that a number of bubbles, which would not respond to tapping and jerking, could be eliminated in this way.

Illumination :

Illumination was provided by a microscope projection lamp with a Phillips 8V, 6A globe, which could be overloaded to 10 volts. An adjustable vertical slit, made of aluminium sheeting, was placed immediately in front of the lamp's lens. By this arrangement a series of beams were cast out from the vertical filaments of the lamp, and these could be trained upon all four base-line and moveable mirrors of the manometers at the same time.

The lamp- manometer distance could then be adjusted to bring these beams into focus at the distance of the recording camera.

Recording :

Recordings were taken with a photo-kymograph, especially constructed to a design by Prof. R. Goetz of the Surgical Research department, by the Trigonometrical Survey Department. This could run at a number of speeds, of which the most convenient was found to be 10mm. per second. The film used was Kodak R.P. 30, high-speed recording paper.

Frequency tests :

Frequency tests were carried out at intervals (see Plate VII-A), using the following method.

The cannula of the manometer was forced through a rubber bung in a thin glass tube and the tube filled with citrate solution to a level just above the needle tip

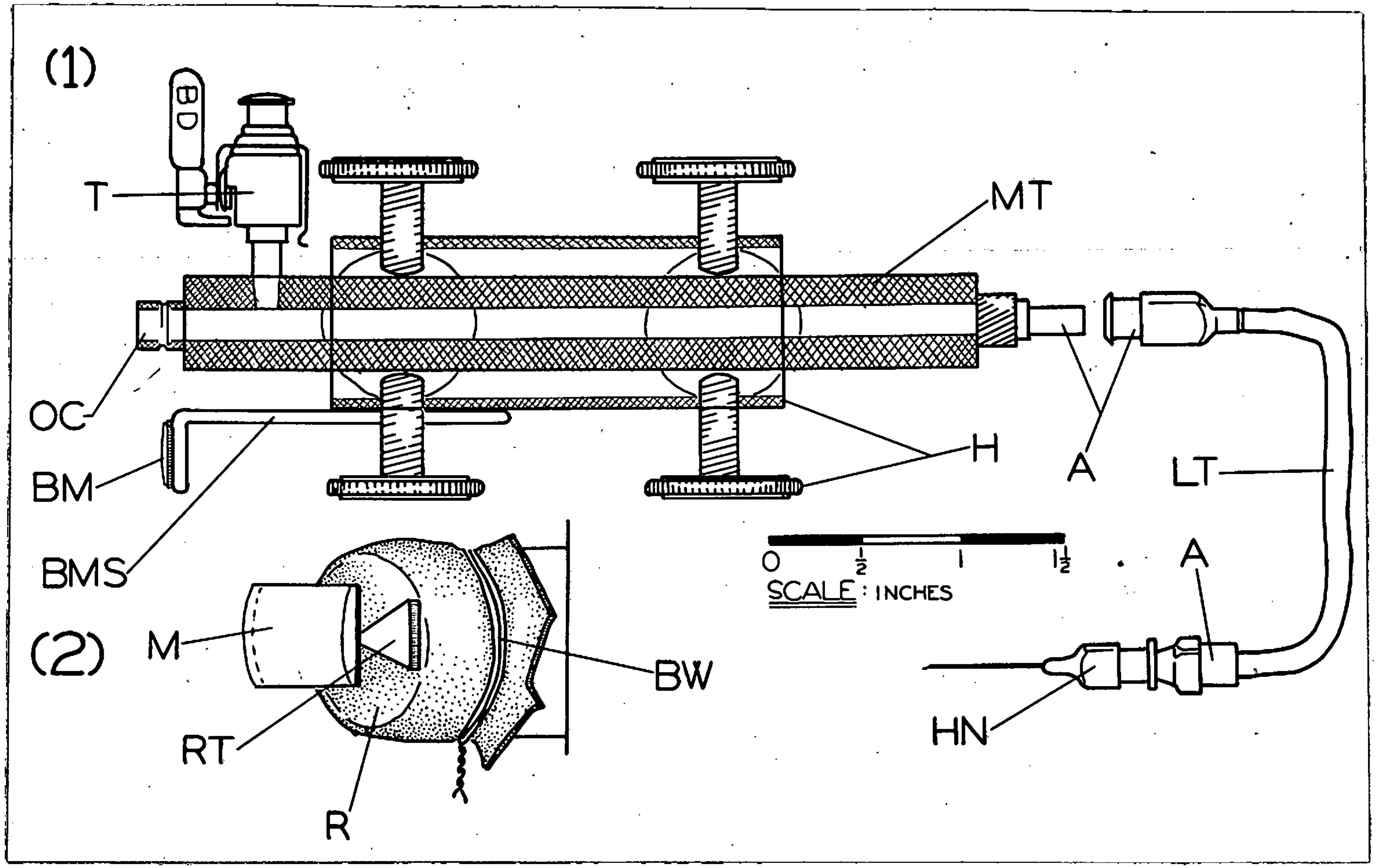

Figure 7 : CONSTRUCTION OF OPTICAL MANOMETER EMPLOYED.
(1) Whole manometer (2) Detail of optical capsule.

A. adaptors soldered to manometer tube and lead tubing; B.M. base-line mirror; BM.S. base-line mirror support; B.W. brass wire; H. adjustable housing; H.N. hypodermic needle; L.T. lead tubing M.T. manometer tube; M. mirror; O.C. position of optical capsule; R. rubber membrane; R.T. rubber triangle mounted on membrane; T. tap.

The glass tubing was clamped firmly in position and a rubber tube fitted to it. Air was blown into the rubber tubing and the tubing stopped with the tip of the tongue. The camera was then started at its fastest speed, and the tip of the tongue drawn suddenly away from the end of the tube.

Theoretically, this should have set the manometer into oscillation1 In practice however, it was found that, although the recording followed the rapid fall in pressure almost instantaneously, there were no oscillations at the end of the tracing. What appears to be oscillation in one place in Plate VII-A, was actually due to accidental shaking of the rubber tubing.

This aperiodicity may be put down to the damping effect of the extremely fine cannulae. Various ways of overcoming this were tried. Increasing the size of the cannulae to No. 18 had no appreciable effect and in any case, these were felt to be too large to use, without disturbing the blood flow. Decreasing the effective mass by increasing the thickness of the rubber membrane, served to cut down the sensitivity to such a degree that an optical lever of 15 ft. was no longer sufficient. Extending the lever for a greater distance, not only involved difficulties in obtaining a sharp record of sufficient intensity, but also increased the risk of erroneous recordings due to slight movements of the manometers in their housings. Stretching the membrane more tightly had no appreciable effect.

However, it was felt that, considering the quick response which the manometers showed to the sudden rise in pressure in the frequency tests, the results obtained would be significant for present purposes. It was expected that, although a recording would not be obtained of all the minute pressure changes that might occur, at least a good idea would be obtained of the pulse pressure, the timing relationship between the arches, and the general form of the pulse curves. It must also be remembered that the experiments were performed in order to find out what <u>differences</u> there were in the arterial arches, and the apparatus showed promise of giving significant comparative readings.

<u>Reversal of manometers</u> :

In order to be quite sure that differences between the pressures recorded were not due to a lack of absolute uniformity in the two manometers used, the manometers were often reversed in successive tests, so that readings in the systemic arch for example, were not all taken with the same manometer (See Plate VII B & C).

<u>Calibration</u> :

Following each experiment, the manometers were simultaneously calibrated.

A wide rubber tube, filled with citrate solution, was fitted to the bottom of a calibrated glass tube and was fitted with a screw clamp, so that the level of the solution could be raised or lowered in the glass tubing. After withdrawing the toad from the cannulae without changing the position of the latter, at the end of each experiment, the cannulae were gently pressed through the rubber tubing at the base of the calibrated tube. The manometer taps were then opened to see whether the solution could flow easily through the cannulae. This was a necessary precaution, because the cannulae occasionally became blocked with clotted blood, in spite of the use of a strong citrate solution. The solution in the tube was then raised to various heights and the camera allowed to run for a short while at each level (Plate VI).

Finer adjustment could be made with an aqueous solution in this way, instead of by direct calibration against mercury.

Each manometer was identified with its own base-line by briefly cutting off the light supply to one manometer installation during the recording.

It was found by a series of tests, that the accuracy of measurement was about ± 0.25 cm. citrate solution pressure (0.19 mm. Hg.) and so all measurements were taken to the nearest 0.5 cm. cit. soln. ((0.38 mm. Hg.).

Procedure for each experiment.

The toads were prepared in exactly the same way as had been done for the fluorescein experiments, but in addition, the arterial arches were cleared of membranes connecting them. No un-anaesthetised toads were used, because of the difficulty of preventing any struggling during the course of the experiments. Most of the toads used, were again males of about 45 gms. weight, so as to correspond as closely as possible with the fluorescein experiments.

After moving the lead tubes and cannulae to a suitable position, and also the toad, which was fixed to an adjustable cork table, the arterial arches were gently picked up with fine forceps and drawn onto the hypodermic needles. Before proceeding further, the position of the needles was adjusted so that they were in corresponding positions in the blood vessels, lying well to the side to prevent any blockage.

It was thought at first that the angle at which the tip of the cannula was lying might make some difference in the recordings, due to the direction of flow of the blood. However, tests with the cannulae facing in opposite directions in the same arterial arch (Plate VIII-C), made no appreciable difference. The effects of slight differences in angle of the cannulae therefore, are entirely negligible.

The recordings were taken as quickly as possible, because of the danger of the needles becoming blocked with clotted blood.

Number of measurements made :

Sixteen recordings were made with the manometers connected to the systemic and pulmo-cutaneous arches simultaneously; seven to the carotid and pulmo-cutaneous arches simultaneously; and six to the carotid and systemic arches simultaneously. In addition, a number of other tracings were taken to examine other aspects of pressure relationships.

(2) RESULTS OF PRESSURE MEASUREMENT EXPERIMENTS.

The basic results, and those upon which most attention is to be focussed, were those of comparative readings in the arterial arches. These are more conveniently summarised in tabular form (Table 1).

After conversion to mm. Hg., the readings are considerably smaller than those obtained by other workers (e.g. Schultz 1906). The highest measurements obtained in any experiment, including trials which were unsatisfactory or later experiments in which the subjects were very lightly anaesthetised, were of the order of 45 mm. Hg. So it may quite well be that the normal average blood pressure of Xenopus is lower than in Rana or other Anura.

Another immediately striking point, is the rather wide range of pressures in different experiments (See lines d, e & f in Table 1). There appeared to be no relation between the size of animal used and the blood pressure. The explanation for the variations on the contrary, may lie in the extent to which the animals were narcotised. Other effects of anaesthesia will be discussed shortly.

The main features which are brought out by these results are that the pulmo-cutaneous arch pressures are consistently lower than either the carotid or systemic pressures, and that the pressures in the carotid and systemic arches are remarkably similar. The pulmo-cutaneous arches for example, have a pressure at systole about 1 mm. Hg. lower and at diastole about 7 mm. Hg. lower than the other

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Table 1 : SUMMARY OF PRESSURE MEASUREMENTS IN ARTERIAL ARCHES **

(A) Simultaneous measurements in systemic and pulmo-cutaneous arches (16 records).

	Systolic	Diastolic
(a) Average systemic press.	32.5	26.0
(b) Average pulmo-cutaneous pressures	31.0	17.5
(c) Differences between averages	1.5	8.5
(d) Range of systemic press.	22.5 - 47.5	16.0 - 38.0
(e) Range of pulmo-cutaneous pressures	21.0 - 43.5	10.5 - 28.5
(f) Range of differences between arches	0 .5 - 4.0	4.5 - 12.5

(B) Simultaneous measurements in systemic and carotid arches (6 records).

	Systolic	Diastolic
(a) Average carotid press.	34.5	29.0
(b) Average systemic press.	34.5	29.5
(c) Differences between averages	0	systemic + 0.5
(d) Range of carotid press.	30.0 - 37.0	25.0 - 32.0
(e) Range of systemic press.	30.5 - 37.0	26.0 - 32.0
(f) Range of differences between arches	systemic -0.5 - +1.5	systemic -0.5 - +2.0
(Standard deveiation	0.29	0.39)

(C) Simultaneous measurements in pulmo-cutaneous and carotid arches (7 records).

	Systolic	Diastolic
(a) Average carotid press.	31.0	25.5
(b) Average pulmo-cutaneous press.	30.0	18.0
(c) Differences between averages	1.0	7.5
(d) Range of carotid press.	26.5 - 36.0	23.0 - 29.5
(e) Range of pulmo-cutaneous pressures	25.5 - 35.5	16.0 - 21.0
(f) Range of differences between arches	0.5 - 1.5	6.5 - 8.5

** NOTE : All measurements are here given in cml citrate soln. Conversion to mm. Hg. by dividing by 1.3

Experimental error ± 0.25 cm. cit. soln.

two arches and the diastolic pressure differences amount to about one-third of the systemic pressure, - quite a considerable amount. These figures are higher than those obtained by Acolat (1938 a).

Although the average figures, and the range of readings, seem to show that the carotid arches have a slightly lower pressure than the systemics, there is only one experiment that gives a statistically significant difference. The readings in this experiment may be due to an unobserved disturbance of the apparatus.

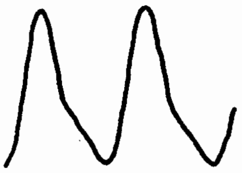
Shape of Pulse curves.

There was also some variation in the shape of the pulse curves. These have been grouped into four types as shown in Table 2, although in reality, there is probably no sharp dividing line between the groups. There was no difference between the curves of the carotid and systemic arches and so these have been grouped together.

In the pulse curves, the first wave is interpretable as being due to the main propulsive force of the ventricular contraction, while the secondary curve (minor in most cases), is due to the contraction of the bulbus. It is to be noted particularly that this bulbus contraction does not as a rule play an important part in raising the blood pressure in the arches. The main pressure rise is solely due to the ventricular contraction.

The gradually increasing role of the bulbus contraction in the series, as can be seen from the pressure readings in Table 2, and as was also noted from direct observation, is accounted for by a weaker action of the heart. In some cases, this weaker heart action was clearly associated with a rather heavy anaesthesia, or was in one or two cases due to some loss of blood while the cannulae were being inserted, but no definite correlations could be drawn.

SYSTEMIC AND CAROTID ARCHES :

Type	Pulse curve shape	Nol of cases	Average press.		Illustr. in Plate.
			Syst.	Diast.	
I		6	41.5	32.0	VIII-A
II		25	32.0	26.5	VII-C
III		3	27.0	22.5	IX-B
IV		1	23.0	20.5	IX-C

PULMO-CUTANEOUS ARCHES :

I		17	33.5	11.5	VI
II		4	23.0	13.5	VIII-B
III		1	25.0	14.0	IX-B
IV		1	21.5	15.0	IX-C

** NOTE : All pressures given in cms. citrate soln.

In general, the bulbus contraction was less marked in the pulmo-cutaneous arches than in either the systemic or carotid arches, but in one case the bulbus wave dominated the picture in both (Table 2, type IV). The reason for this could be seen quite clearly during direct visual observations. In those cases where the ventricular contraction was weak, the bulbus was seen to contract quite markedly, so that the cavum aorticum became almost empty, while in a strongly beating heart, it did not empty itself before the following ventricular contraction, and appeared to act more as a pressure relaying mechanism. Since the cavum aorticum lies more in line with the ventricle, this became distended to a greater extent than the cavum pulmo-cutaneum during ventricular contraction. The effect of the bulbar contraction would therefore naturally be felt in the systemic and carotid arches more than the the pulmo-cutaneous arches.

In one or two cases (Plate X-A), the systemic curve was seen to take on the same shape as figured for the pulmo-cutaneous arches (Table 2, type I), in which no bulbus contraction was evident. These were in very lightly anaesthetised toads, with a very strong heart action.

Considering these changes which occurred with weakening heart action, it is likely that the curves of type I in Table 2, are the more normal.

The question arises as to whether there is actually a <u>contraction</u> of the muscular wall of the bulbus, or whether it is merely an elastic recoil which produces the secondary wave in the pulse curve. Histological examination of the bulbus wall reveals no marked elastic fibres, as is the case in the arterial arches, and this would seem to indicate that an actual muscular contraction does occur. Besides this, one would not expect to have an increase in the effect of the bulbus contraction, with weakening heart action, if this were merely an elastic chamber. It would appear that the anaesthetic has less effect on the bulbar, than on the ventricular contraction.

Detailed shape of pulse curves :

By projecting the records through an epidiascope and tracing out the curves on paper, it was possible to obtain a more detailed idea of the sequence of events. The curves shown in fig. 8, have been chosen because they exhibit typical characteristics, and also represent successive experiments in which the manometers were reversed.

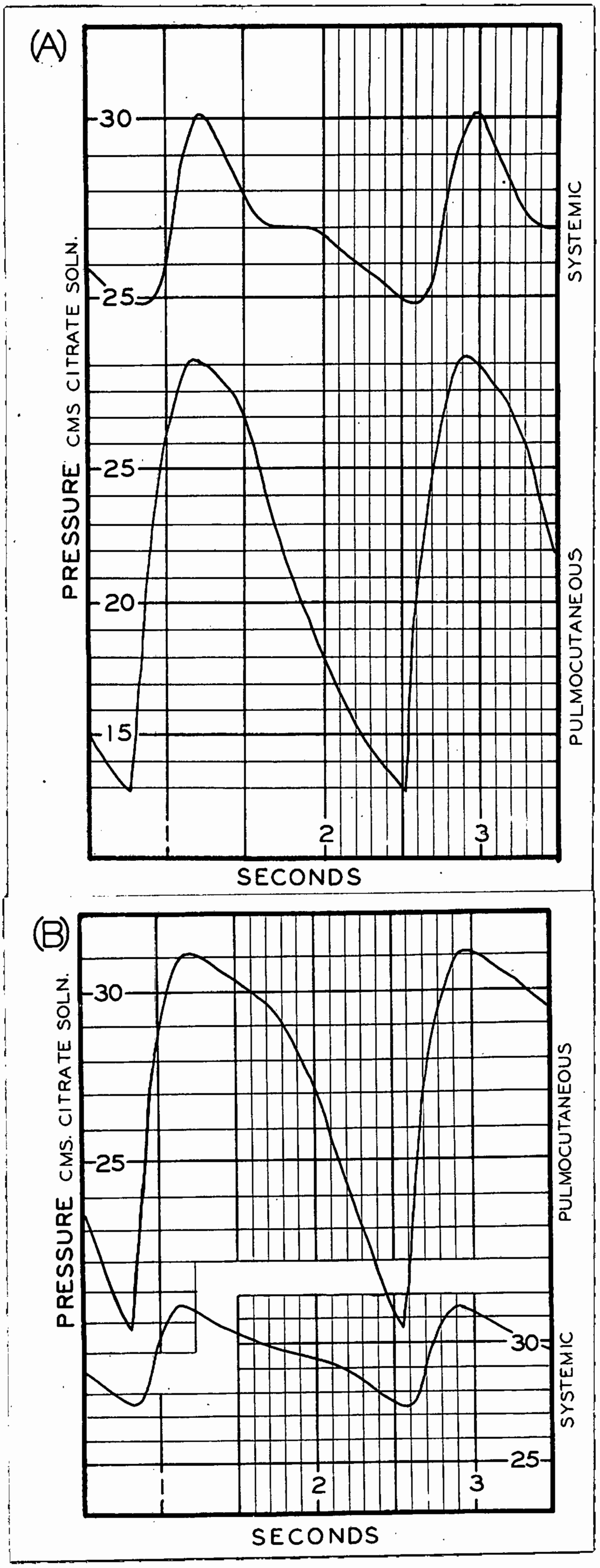
It is evident from a comparison of these curves that there is a slight lag in the time of response of the manometer in the systemic arch in fig. 8(A) and of the manometer in the pulmo-cutaneous arch in fig. 8(B). Since the manometers were reversed, this is the same manometer in each case. Nevertheless, the curves show up quite clearly what happens.

The pulmo-cutaneous arch pressure rises very abruptly and rapidly, whereas there is a slight delay in the beginning of the rise in the systemic arch (about 0.1 sec.). When the latter pressure does increase, it does so more gradually. Both curves (allowing for the time lag in one manometer) reach their maxima at about the same time, although the pulmo-cutaneous pressure at no time exceeds that in the systemic arch. The subsequent course of events varies, as has been explained, but the fall in pressure in the pulmo-cutaneous arch is invariably more rapid and is more suddenly interrupted by the succeeding ventricular systole.

More interesting data on the sequence of events might be obtained by simultaneous measurement of pulse curves in the ventricle itself, and in the two cava of the bulbus. This was impossible with the present technique and with the size of animal available, because of disturbance of the heart contraction, caused by inserting the hypodermic needles.

Venous pressure measurements.

Due to lack of time, only a few venous pressure



(A) Systemic and pulmo-cutaneous arches, taken from Plate VII-C.

(B) Pulmo-cutaneous and systemic arches, taken from Plate VII-B.

<u>Figure 8</u> ; <u>DETAILS OF PULSE CURVES</u>.

measurements were taken.

These indicated that the pressure in the pulmonary veins was about 3.5 mm. Hg. in a lightly anaesthetised toad, while the hepatic vein pressure was only about 1.5 mm. Hg. (Plate XI-B).

A small but distinct pulse was visible in the pulmonary vein. By connecting up the manometers simultaneously to the pulmo-cutaneous arch and pulmonary vein (Plate XI-c), it was possible to interpret the main wave of the pulmonary vein pulse as being due to pressure transmitted through the lung capillary bed. The other pressure fluctuations are probably related to the filling and emptying of the left atrium.

Simultaneous measurements in systemic arches of opposite sides.

Some recordings taken of pressures in the systemic arches of opposite sides, showed no significant difference in amplitude, shape, or timing of the pulse curves. (Plate IX-A).

(3) DISCUSSION OF RESULTS OF PRESSURE MEASUREMENTS.

These results partly explain how the pulmo-cutaneous arches come to receive more of the blood which leaves the ventricle, than do the carotid and systemic arches together.

Since there is a pressure in the pulmo-cutaneous arches some 7 mm. Hg. less than in the systemic and carotid arches at the beginning of systole, there is a natural tendency for the blood to be passed into the former vessels. The position of the opening of the dorsal chamber of the truncus might regiment against this, but it is quite evident from the pulse curves that this opening cannot become blocked off until late in the systolic sequence. If it did, one would expect the pulmo-cutaneous pulse curve to reach its maximum before the maxima of the carotid or systemic arches.

The position of the spiral valve and the two cava of the bulbus might also tend to counteract the lower pressure in the pulmo-cutaneous arches, but this will be discussed with the mechanism behind the blood distribution, in a later section (page 67).

That no significant differences appeared between the systemic and carotid pulse curves, is hardly surprising, considering the way in which these canals branch off from the ventral chamber of the truncus. The lack of difference between the pulse curves of opposite systemic arches is also not surprising. It is true that the right systemic canal branches off from the truncus at a sharper angle, but if the blood flow is not very rapid (as indicated by the fluorescein experiments), this anatomical arrangement could hardly be expected to cause a difference in the pressures of the two sides.

Both the higher pressure and the larger pulse found in the pulmonary veins, confirms the idea that there is less peripheral resistance in the pulmo-cutaneous circuit, and therefore an easier flow of blood. If carried further, the venous pressure readings might reveal more interesting data. If it could be shown for example, that the sinus contraction does not have a great effect in raising the pressure of the blood flowing through it to the right atrium, it would mean that the left atrium has a greater filling pressure than the right. This would account for the greater discharge volume of the left atrium, as indicated by the fluorescein experiments.

(C) DISTURBANCES CAUSED BY THE OPERATIVE PROCEDURE.

Many of the disturbances which might have been caused by the experimental method have been discussed already, and these have either been eliminated by modifying the technique, or allowance has been made for them in interpreting the results. Two important factors remain to be discussed. These are the effects of the anaesthetic, and the effect of opening the abdominal cavity.

Effect of anaesthetic.

As has already been observed, if the anaesthetic was administered in larger quantities or was allowed to act for a longer time, it seemed to decrease the propulsive power of the ventricle and alter the resultant pulse curve in the arterial arches.

It might be argued that urethane, by vaso-dilation might cause a greater reduction in the peripheral resistance of the pulmonary circuit than of the systemic or carotid circuits. This however, is not borne out by the pulse curve shapes, for if the peripheral resistance were greatly reduced, the drop in pressure after systole would be more rapid. This did not occur, as can be seen by a comparison of the curve shapes in Table 2. It might also be noted that there was still a large pulse in the pulmo-cutaneous arches in animals which were very lightly anaesthetised with ether (Plate XI-C).

So, even if it is proposed that the pulse curves obtained from animals narcotised with urethane, are not absolutely normal, it may be assumed that this has no direct effect on the distribution of the blood. It is not the exact shape of the pulse curve that is important, but the relationship between pulmo-cutaneous and systemic - carotid pressures.

Effect of opening the abdominal cavity.

Operative shock was kept down to a minimum by the use of anaesthetic in most cases; the destruction of nerves controlling the action of the heart was avoided in the dissection; and blood loss was not very great in any of the experiments. Apart from these possible disturbances, the main effect of opening the abdominal cavity would be the removal of pressure exerted on the internal organs by the abdominal musculature. As far as this is concerned, the main feature which might alter the blood distribution scheme would be a decrease in the peripheral resistance of the pulmonary circuit, since, under normal conditions, air pressure in the lungs might compress and partially block the lung capillaries.

A preliminary experiment (Plate X), in which the lungs were blown up through a tube in the glottis, suggested that this would have to be considered. The pulse curves of the systemic and pulmo-cutaneous arches in this case, became almost identical after distension of the lungs.

Measurement of the pressure normally present in the lung cavities was not entirely successful, since the insertion of a tube down the glottis prevented normal respiratory movements. Insertion of a cannula through the abdominal wall into the lung cavity was also not very satisfactory because the lung septa tended to block the tip of the cannula, besides which, there were the awkward and variable effects of surface tension at the air/manometer-fluid interface. However, it was evident that the pressure normally exerted by the abdominal muscles on the lungs, when the toad was out of water, was not more than about 5 cm. water pressure. When the lungs were distended by applying this small pressure, no appreciable effect on the pulse curves could be observed. The effects recorded in Plate X, therefore, were due to the application of abnormally high pressures.

Distending the lungs by applying a pressure of 5 cm. water, again does not correspond to the normal conditions under which the animals live. In nature, Xenopus may be found at depths of more than six feet, and the pressure of the water might have some effect. The water pressure is of course applied in all directions and to all the body tissues, and must be counteracted by an equal force in these tissues. It is not to be expected therefore, that any change in the relationship between pulmonary and bodily blood pressures would result from submerging the animal.

To make quite sure however, that there were no effects from unforseen circumstances, another series of experiments were performed, in which the toad was placed in a chamber, the pressure inside which could be raised by using a beau-manometer. Although the effect of increasing the pressure in the chamber was, quite expectedly, to raise the general level of the pressure in the blood vascular system, there was no change in the relative shapes or amplitudes of the pulse curves. The pressures applied were equivalent to a water depth of some six to seven feet (Plate XI-A).

(D) MODE OF ACTION OF THE HEART.

A brief statement has been given of the problems which confront the experimenter in determining exactly how a separation of the blood streams from the two atria may be achieved in the anuran heart (page 12). Although Xenopus presents a different type of selective distribution than was envisaged by the authors of the "classical theory", these problems still remain to be answered. Many of them must eventually be answered by more exact methods of determining the function of the bulbus and spiral valve, than have yet been devised, but some conclusions can be drawn from the present investigations :

(1) That a form of separation of the blood from the right and left atria takes place in the ventricle is clear. The blood from the left atrium tends to pass into both sides of the ventricle, while that from the right atrium partly helps to fill the right side. There is usually a mingling of left and right atrial blood in the right side of the ventricle only.

(2) Another conclusion that may be drawn is that the blood from the right side of the ventricle must be the first to leave during systole. There is no other way of explaining the passage of the fluorescein in the right atrial blood into the pulmo-cutaneous arch (in spite of what Foxon (1951) and Ozorio de Almeida (1923) have said). Whether this passes

up the cavum aorticum and then crosses over to the cavum pulmo-cutaneum, or whether it gains entrance to the cavum pulmo-cutaneum from the beginning cannot be decided, although the fluorescein technique shows that the former mechanism operates in at least some cases (page 32 and Plate I).

(3) Foxon (1951) and Vandervael (1933) state that a simultaneous movement of blood in all the arterial arches is inconsistent with the demands of the "classical theory". This may be, but it is certainly not in conflict with a selective distribution, as shown by the present experiments. Even if most of the blood which leaves the ventricle first is derived from the right atrium, any blood which may move simultaneously in the systemic and carotid arches, is not necessarily derived from the same source, since there must still be some reserve of left atrial blood in the cavum aorticum and ventral chamber of the truncus, from the last phases of the previous heart beat. I suggest that it would be this blood which would be forced into the systemic and carotid arches in front of the oncoming wave of right atrial blood, during the first phases of ventricular systole.

(4) In <u>Xenopus</u> at least, there cannot be any question of the spiral valve blocking off the entrance to the pulmo-cutaneous arches, before the major propulsive force of the ventricular contraction is expended. This is shown by the pulse curves of the pulmo-cutaneous and carotid-systemic arches, which reach their maxima at about the same time. The timing of the bulbus contraction is later, and it can be only then, that the spiral valve may come into contact with the opposite wall of the bulbus. Under these circumstances, the necessity of the spiral valve is not clear, but it might well be that the significant function of the spiral valve is not to bring about a separation of the blood streams in the way in which the "classical theory" implies, but to ensure that the carotid and systemic arches

receive at least some portion of the blood, in spite of their greater peripheral resistance. On might regard it as a ramp, which deflects some blood into the ventral chamber of the truncus.

(5) Experiments with Xenopus show that the carotid arches do not have a higher blood pressure than the systemics (page 58). These results may lead to the necessity of revising the ordinarily accepted opinion of the function of the carotid labyrinths, i.e. that they function to keep the pressure in the carotid arches at a high level. From direct observations of the blood vessels, it appeared that the movement of blood in the external and internal carotid arteries was much more regular than in the carotid arch itself. There is thus a possibility that the mechanical effect of the carotid labyrinths is merely to make for a smoother flow of blood towards the head regions.

(IV) GENERAL DISCUSSION AND CONCLUSION.

In fig. 9-A, a diagrammatic representation is given of the blood distribution scheme in Xenopus, as the present experiments have indicated it to be. A comparison of this with a similar diagrammatic representation of the "classical theory" (fig. 9-B) shows that the main difference is in which side of the heart and in which peripheral circuit, the greater volume-flow per unit time occurs. The difference is one of accent, but the accent alters the picture considerably.

If this scheme applies to other Anura as well, it may explain some of the contradictions in the results obtained by other workers.

Vandervael (1933) and Foxon (1951) injected only into the pulmonary vein. That they found an apparently random distribution would only be logical, if the distribution were as proposed here. On the other hand, Simons and Michaelis

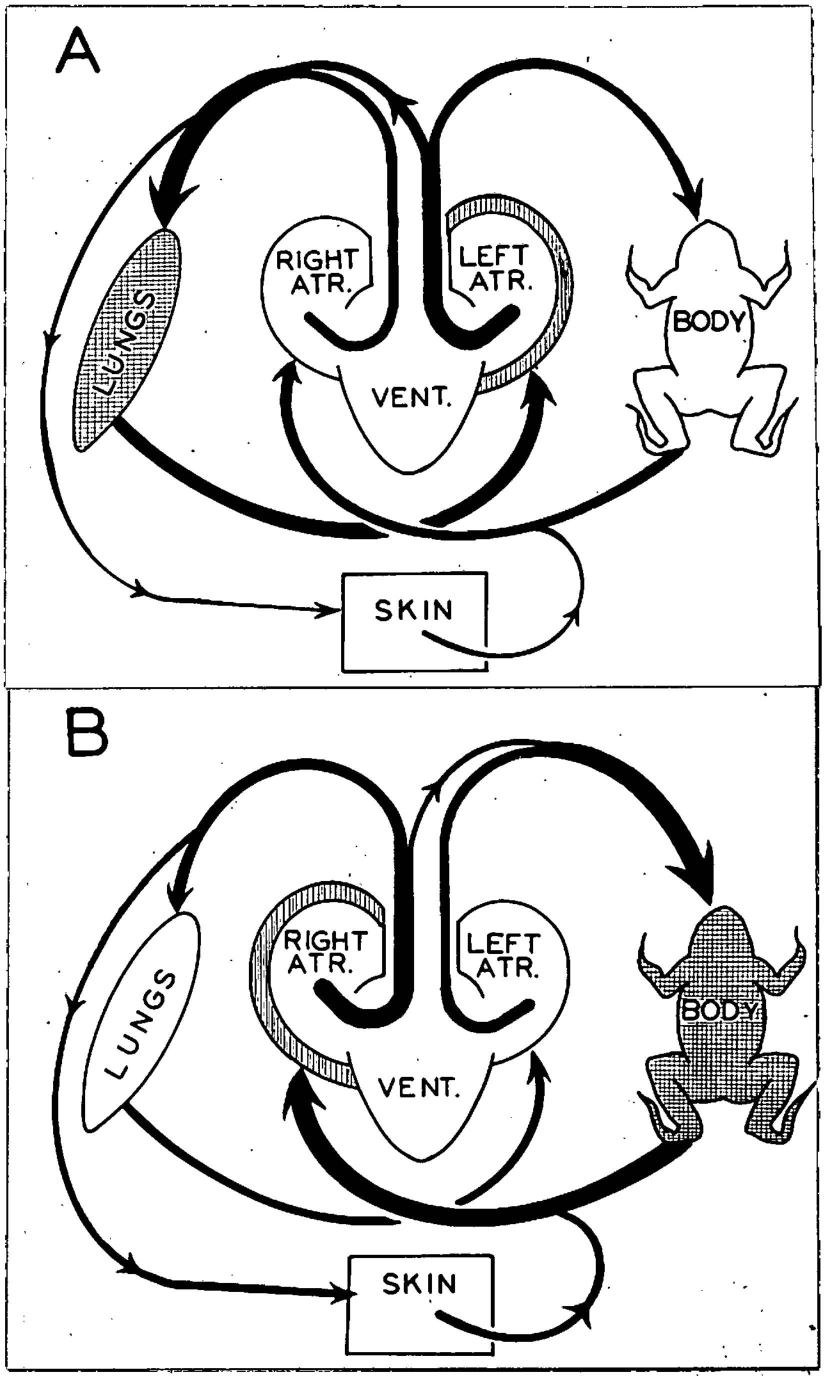


Figure 9 : DIAGRAMMATIC REPRESENTATIONS OF BLOOD DISTRIBUTION SCHEMES.

(A) XENOPUS
(B) ACCORDING TO THE "CLASSICAL THEORY".

(1953) injected into veins leading to the right atrium and reported a selective distribution in some cases, -- a result again to be expected according to the present proposals. The scheme may also explain the observations of the authors of the "classical theory", Gompertz (1884), Ozorio de Almeida (1923), Acolat (1931 b) and Foxon (1951), with regard to a physiological division of the ventricle. It is interesting to note that in spite of his disagreement with the "classical theory", Foxon (1951) does report signs of a physiological division of the ventricle.

Foxon (1947) and Foxon & Walls (1947) however, report having made injections via the anterior vena cava, with a resulting distribution not unlike the pulmonary vein injections. The scheme will also not explain the results of Noble (1925) or of Savolin (1949).

The light in which the "classical theory" was considered to be a satisfactory account, was largely in the consideration of the oxygen needs of the tissues. By the same sort of consideration, Vandervael (1933) draws the conclusion that the "classical theory" is far from satisfactory. We should therefore re-examine Vandervael's theoretical objections.

His argument consists mainly of a deduction of the oxygen distribution in an animal with non-functioning lungs, if the blood were distributed according to the "classical theory". If this applies, he argues, there would be a tendency for the body tissues to receive the de-oxygenated blood, while the lungs and skin would have the oxygenated blood returned to them. The fallacy in the argument lies in the fact that he has not carried it far enough.

Under these circumstances it is hardly to be expected that the lung tissues would extract for their own metabolism, much of the oxygen from the blood sent to the lungs. The large supply of blood in proportion to the bulk of the lung tissues denies such a supposition. If the circulation is taken a step further, therefore, it is seen that the left atrium would not be receiving deoxygenated blood, but blood with a considerable proportion of oxygen. This would then be distributed to the body. If one considers in addition that the "classical theory" proposes that some of the right atrial blood (more highly oxygenated in this case) must pass into the systemic arch in order to balance the differences in the blood capacities of the lungs and the tissues of the rest of the body; and in addition to this the probability that the metabolism of hibernating frogs is very much reduced and that they therefore have a much decreased oxygen requirement, -- the argument can be seen to be quite incorrect. There would be the seemingly undesirable physiological arrangement, whereby the main respiratory organ, the skin under these conditions, would be receiving the more highly oxygenated blood through the cutaneous artery, but this does not affect the argument about the oxygen needs of the body.

All of this of course, does not make the "classical theory" correct, and a random distribution of blood might suffice equally well for the transportation of oxygen to the tissues. Yet a form of selective distribution seems to be present in Xenopus. What is its significance in the life of the animal ?

It has been shown (see separate paper, page 80), that the oxygen requirements of Xenopus may at times be extremely low. They are able to survive, for example, with most of the haemoglobin of their blood blocked by carbon monoxide. They are also able to survive by cutaneous respiration alone, in spite of the very small size of their aa. cutanea magna. It would appear therefore, that when their lungs are functional, the blood contains more oxygen than is required for their survival. What the oxygen needs of a normally active individual are, is not known, but there is a strong possibility that a selective distribution need not be related to the oxygen needs of the body tissues.

At least one of the hydrodynamic features responsible for the selective distribution has been demonstrated, -- that is the smaller peripheral resistance of the pulmonary circuit. The significance of a hydrodynamic arrangement whereby there is a slow and volumetrically smaller circulation through the general body tissues, while a rapid and volumentrically greater flow occurs in the pulmonary circuit, escapes explanation at the moment.

One of the implications of the proposed distribution scheme is that a proportion of the blood makes two or more circuits through the lungs, before being sent to the rest of the body. Is this to ensure proper oxygenation by a number of rapid circuits, rather than one slower circuit ? It seems hardly likely, since the corollary of this state of affairs is that a slower flow takes place through the body tissues. With a slower flow, there is the opportuhity for more oxygen to be given up by the blood, but the same effect could be obtained by a rapid flow of less oxygenated blood. The total amount of oxygen transported to the tissues in either of these cases could be the same. Besides this, there is the liklihood that the blood contains a large reserve of oxygen, in an inactive toad.

Is it purely an accident arising from the design of the blood vascular system ? This also seems unlikely. Pure accidents in physiological design are seldom, if ever, encountered in nature.

Further research must undoubtedly take a wider form than has been applied so far. It should involve investigation not only into the actual distributional patterns observed, but also into the mechanism whereby it is achieved, into the volume-flow relationships in the different blood vessels and vascular beds, into the oxygen requirements of the tissues, and into the metabolism of the creature.

If the outcome of the present investigation is substantiated and especially if it is not proved by further work to be merely an eccentricity of <u>Xenopus laevis</u>, it will require not only a revision of the generally accepted principles of the functioning and physiological design of the anuran blood vascular system, but also a re-examination of the position in other vertebrates with imperfectly divided hearts.

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(V) SUMMARY.

(1) The structure of the anuran heart is revised, and the maih differences between *Xenopus laevis* and *Rana* pointed out.

(2) The problems concerned with blood distribution in the Anura are outlined, and a summary of previous experimental work given.

(3) A fluorescein-cinematographic technique (modified from Simons and Michaelis 1953) of tracing blood through the heart, is described.

(4) The use of this technique in *Xenopus*, leads to the following conclusions :-

(a) Blood from the right atrium is sent almost exclusively to the pulmo-cutaneous arches.

(b) A form of division of left and right atrial blood occurs in the ventricle, in so far as right atrial blood is absorbed only into the trabecular mesh-work of the right side of the ventricle, while left atrial blood is usually absorbed by the trabecular mesh-work on both sides of the ventricle, and mingles with the right atrial blood on the right side of the ventricle only.

(c) Blood from the left atrium is distributed to all the arterial arches.

(d) The pulmo-cutaneous circuit gives passage to more blood per heart beat, than the bodily circuit.

(e) The left atrium usually has a larger discharge volume than the right atrium.

(f) A physiological connection between the right side of the ventricle and the pulmo-cutaneous arches, and the left side of the ventricle and the systemic and carotid arches, is demonstrated by some exceptional results.

(5) It is not considered impossible that variations from this distribution scheme may occur in different individuals or in the same individual from time to time.

(6) The requirements of pressure measurement techniques are examined, and an optical manometer method, similar to Hamilton _et al_ (1934), is described.

(7) Simultaneous measurements of pressure pulses in the arterial arches show that :-

   (a) The pulmo-cutaneous arches have a larger pulse and their pressure remains consistently lower than that of the systemic or carotid arches.

   (b) The carotid and systemic arch pressures and pulse curves are similar.

(8) The significance of this in relation to the blood distribution is discussed.

(9) Disturbances produced by the operative procedure are examined.

(10) The mode of action of the heart in relation to blood distribution is discussed.

(11) The type of distribution indicated by the present experimental work, may explain some of the contradictions in the literature, if it applies to other Anura as well.

(12) The significance of the distribution scheme is discussed, but no conclusions are reached.

* * * * * * * *

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A NOTE ON A XENOPUS LAEVIS WITHOUT HAEMOGLOBIN and on some experiments performed as a consequence.

During the course of experimentation on the blood distribution of Xenopus laevis, an abnormal specimen was encountered, which appeared to have no red blood pigment at all. The specimen was one of a regular supply kept in the Department of Zoology, as students' class material, and had probably been kept in captivity for some months.

It has been injected with 0.6 cc. 20% ethyl urethane as an anaesthetic, and the abdominal cavity had been opened for the experiment, before anything untoward was noticed. Unfortunately it was no longer possible to keep the animal alive after the operation, but the following observations were made.

External features appeared quite normal, except for a slightly more bluish tinge to the skin than usual. The toad struggled in quite a normal fashion while the anaesthetic was being injected. Usually the urethane has the effect of dilating the blood vessels of the skin and abdominal musculature near the site of the injection, but this was not evident, when the skin of the ventral abdominal wall was removed.

After opening the abdomen, it was noticed that the muscles had a bluish tinge. The blood vessels were colourless, apart from the usual occurrence of chromatophores in their walls. The ventricle was whitish in colour; the atria, pale and translucent; and the main arterial arches, also pale and somewhat translucent. The spleen was white with a very slight yellowish tinge; the lungs a blue-grey colour; the liver, a dark brown; the gall bladder, pale-green; the ovaries very pale yellow, and partially mature; the kidneys, pale brown-grey; and the fat bodies, shrunken and dark olive-brown in colour.

The general anatomical structure was quite normal, although the spleen was somewhat smaller than usual.

About 1 cc. of blood was removed, and oxalated. It was quite colourless to the eye. Fresh blood smears revealed no cellular elements, apart from some bodies which had the appearance of spindle cells. Unfortunately it has not yet been possible either to have a spectroscopic examination or an iron estimation performed on the blood sample.

The toad was preserved in 5% formalin for some days, and the spleen was subsequently removed, sectioned at 5 μ and stained with Turnbull's Jenner stain. Comparison of the sections with those of a normal Xenopus spleen prepared in the same way, revealed that there were apparently no erythrocytes present, although the other blood elements and the reticular structure of the spleen looked normal. Mr. Jacobsen, of the Medical School, has kindly examined the preparations, and has assured me that there are in fact erythrocytes present, in the form of normoblasts, although these do not show a cytoplasmic staining similar to mature erythrocytes.

Possible causes of the abnormality.

Wolvekamp and Lodewijks (1934) noted that blood counts in Rana species tended to drop markedly when they were kept in captivity. They accounted for this by the greatly diminished activity and metabolism of the frogs. No reports have been found however, of the blood count decreasing to as low a level as seen in the abnormal Xenopus specimen.

The abnormality remains unaccounted for, so far.

Discussion.

The occurrence, even as an abnormality, of a vertebrate without haemoglobin is almost unprecedented, except for the Leptocephalus larvae of eels, and Petromyzon which has a blood pigment slightly different from that of other vertebrates. Ruud (1954) has also called to attention, the existence of certain sluggish South Georgian fish species, which do not possess either erythrocytes or blood pigments.

The absence of haemoglobin in an otherwise apparently normal toad therefore, leads to interesting speculation as to the significance of haemoglobin in cold-blood animals. Barcroft (1924) has said that as one passes from warm-blooded to cold-blooded vertebrates, quite a number of factors tend to make the demand for oxygen more nearly commensurate with the supply which could be maintained by a blood devoid of pigment. Amongst these factors, he includes the fact that the demand for oxygen is less, that the oxygen is more soluble in plasma at lower temperatures, and that the blood is exposed in the lungs of a frog, to higher oxygen tensions than in mammalian lungs. The last situation, he says, is due to a smaller partial pressure of both water vapour and carbon dioxide than in mammalian lungs.

Barcroft estimates that, from the position of both supply and demand of oxygen, a frog without haemoglobin would be about two hundred times better off than a mouse in the same circumstances.

Wolvekamp (1932) and Wolvekamp and Lodewijks (1934) have determined the oxygen dissociation curves of the blood of Rana esculenta. These are reproduced in figure A. Wolvekamp (1932) quotes evidence that the oxygen tension in the tissues of Rana temporaria and Bufo is not usually below 28 mm. Hg. and the carbon dioxide tension not usually above 14 mm. Hg. due to the easy diffusion of the latter through the skin. From a study of the dissociation curves, it can be

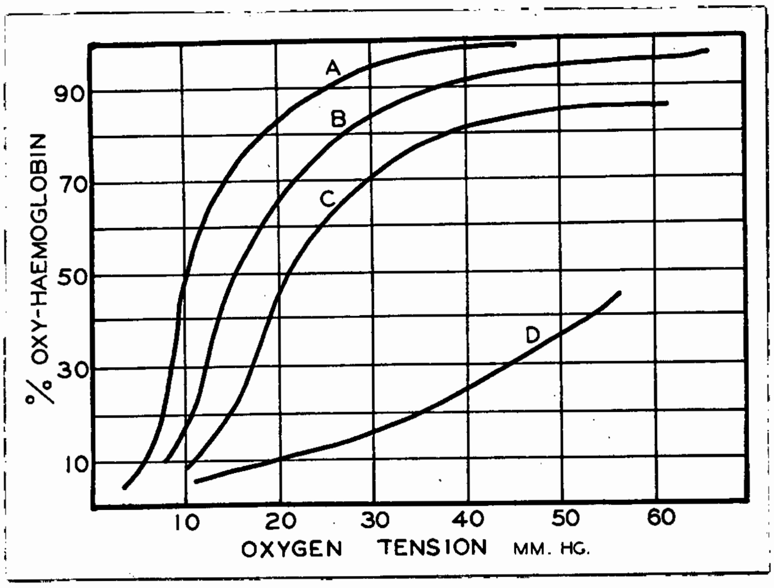

Figure A : DISSOCIATION CURVES OF BLOOD OF RANA ESCULENTA (Redrawn from Wolvekamp & Lodewijks)
Curve A: CO_2 tension, o mm. Hg.
Curve B: do , 8 - 9 mm. Hg.
Curve C: do , 15-17 mm. Hg.
Curve D: do , 48 mm. Hg.

seen that neither a Bohr-effect nor the tension of oxygen in the tissues under these circumstances, would tend to cause much dissociation of the oxy-haemoglobin. It would appear therefore, that in an inactive animal at least, the haemoglobin does not play a very important role in delivering oxygen to the tissues. It might act mainly as an emergency mechanism for times of greater activity, and increased metabolism.

EXPERIMENTS WITH XENOPUS.

To test this hypothesis, experiments were carried out with *Xenopus*, using the following method.

A number of animals were placed in a glass jar, and illuminating gas tapped in for five minutes. A short period of one or two minutes was then allowed for air to circulate, and some toads were removed and placed in separate containers. The procedure was then repeated a number of times with the remaining toads in the gas chamber.

It was found that specimens survived after a period of about thirty minutes in the gas, although it took them some hours to revive after the treatment. No longer period in the gas atmosphere was tried.

Immediately after the experiment, blood was removed from one of the specimens and tested for carbon monoxide content by the colorimetric, Pyrogallic-tannic acid method, described by Kolmer and Boerner (1945). This showed that after a thirty minute exposure to gas, the toads' haemoglobin was at least 80% blocked by carbon monoxide.

Cutaneous respiration.

Charles (1931) performed experiments which indicated that the cutaneous respiration of *Xenopus* accounted for only about 1/4 to 1/3 of the total absorption of oxygen, in richly oxygenated surroundings. Similar estimations by Krogh (1904) show a greater cutaneous respiration (1/3 to 1/2) in *Rana esculenta* and *Rana fusca*. This state of affairs is paralleled by the relative sizes of the aa. cutanea magna, those of Rana being much larger.

It is known that Rana species hibernate for long periods at low temperatures, under conditions where only cutaneous respiration is possible, and it has been shown by Serfaty and Gueutal (1943) that Rana esculenta is able to survive for two to three weeks when totally submerged in well oxygenated water at a temperature as high as 14 - 15°C.

In view of the indications by the carbon monoxide experiment that the oxygen requirements of Xenopus may be extremely low at times, a test of survival on cutaneous respiration alone, seemed desirable.

A number of small males were placed in a container and excluded from the surface of the water by wire gauze. Tap water at an average temperature of 15°C was then kept flowing through the container at a slow rate. No food was given to the animals. It was found that the Xenopus were still alive without suffering any obvious discomfort, after a period of four weeks, after which time the experiment was discontinued.

It appears therefore, that in spite of the very small size of their aa. cutanea magna (cross-sectional area about 1/9 of that of the pulmonary artery), the cutaneous respiration is well able to support small starving animals, which are in an inactive condition most of the time.

This again supports the contention that the oxygen requirements of the tissues may fall at times to a very low level, although it is not claimed that such conditions often occur in nature.

RELATION OF THESE FINDINGS TO THE DISTRIBUTION OF BLOOD.

At the time of the discovery of the abnormal Xenopus specimen, experiments were being carried out to determine to what extent a selective distribution of the blood from the two sides of the heart, occurred. The problems concerned with the distribution of blood have been dealt with elsewhere, and will not be revised here, but it should be noted that the distribution of the blood has been considered in the past largely from the point of view of the oxygen needs of the body.

The interesting point which arises from the investigations mentioned here, is that if a selective distribution is present in the Anura as a whole, it is not necessarily related to the oxygen supply and demand. If the oxygen demands are often as low as found here, a random distribution of the blood might equally well suffice.

The crux of the matter will lie in a determination of the oxygen needs of frogs and toads under their normal conditions of existence, and of the normal way in which these are supplied by lung and cutaneous respiration respectively. No experiments have to my knowledge been performed on Anura, in which the conditions have been carefully controlled, so as to correspond to the natural state of existence of the animals.

* * * * * * * * *

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Plates I & II : TYPICAL RESULT OF INJECTION INTO AN HEPATIC VEIN.

Key to labelled photograph :

C carotid arches; CA bulbus cordis; HV hepatic vein; L lung; LA left atrium; PC pulmo-cutaneous arch; PV pulmonary vein; RA right atrium; S systemic arch; V ventricle; VC ventral chamber of truncus; Vv, valves Ia and III at distal end of bulbus.

Experimental details :

Male *Xenopus*. No anaesthetic administered. No blood loss during course of experiment. Heart rate about 40/min. 0.025 cc. 1% fluorescein-Ringer solution injected. Filming at 16 f/s at f 3.5.

Every second frame printed in these plates, i.e. 8 f/s.

Explanation of plates :

4½ Heart beats are shown.
Ventricular systole starts at frames : 6, 18, 27, 48
Ventricular diastole starts at frames : 1, 13, 22, 33, 43.

The following points are noticeable :

(1) The injection site shows as a solid blotch of fluorescein.

(2) The right atrium does not fill completely with fluorescein at its first diastole (Cf frames 1, 6, and 18).

(3) A distinct division in the ventricle is seen (frames 4 to 30).

(4) Fluorescein appears very darkly in the pulmo-cutaneous arches only, at the first ventricular systole (frames 6 to 18).

(5) The bulbus becomes permanently darker from frame 21 onwards, only after a distinct colouration has been ~~fi~~ visible in the pulmo-cutaneous arches for some time, although. . . . .

(6) A "pulse" of fluorescein is visible in the cavum aorticum of the bulbus, passing over from right to left, in frames 6 to 8, and again in frames 16 to 20 (see arrow in frame 17).

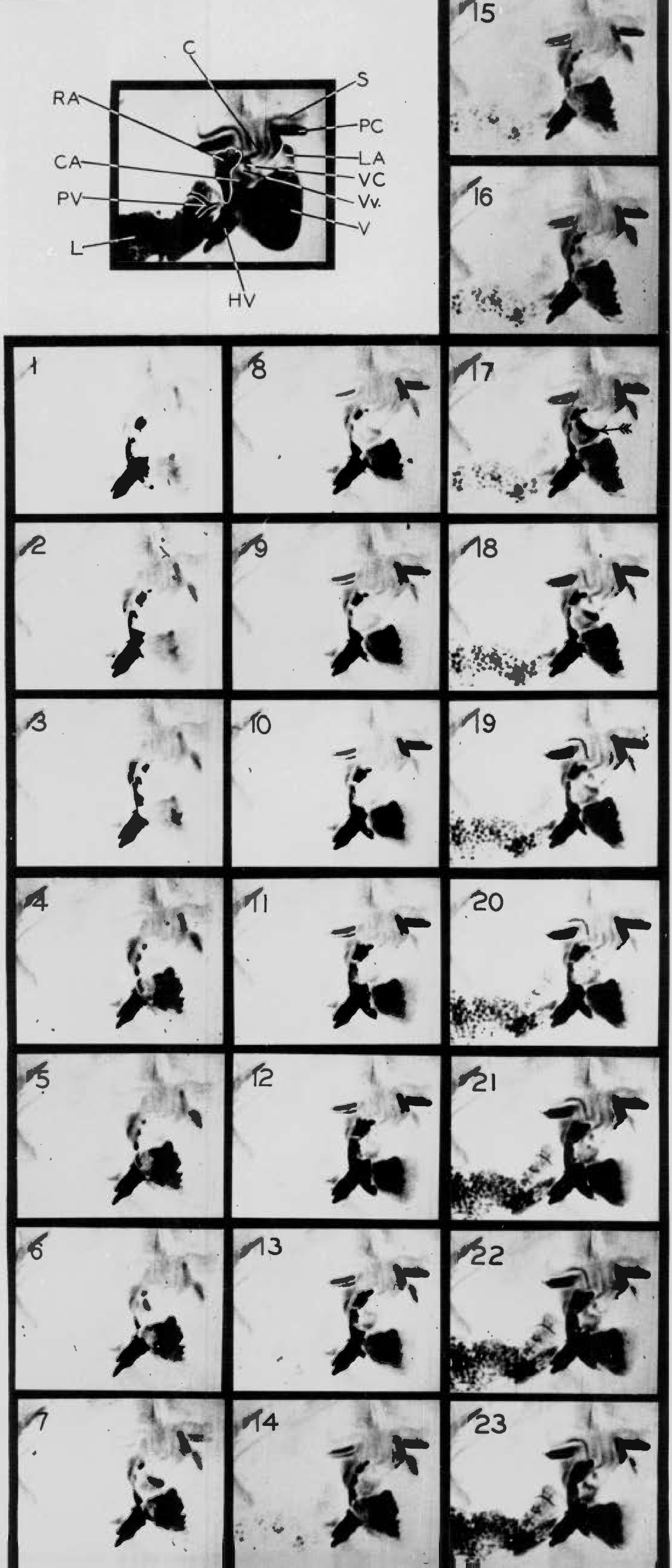
(7) The lungs start to receive fluorescein at frame 13 or 14, i.e. at the end of the first ventricular systole.

(8) The pulmonary vein starts to fluoresce at about frame 21, i.e. at the end of the second ventricular systole.

(9) The left atrium starts to fluoresce at about frame 28, i.e. at the third ventricular systole.

/Contd. with Plate II.....

PLATE I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Plates I & II : TYPICAL RESULT OF INJECTION INTO AN HEPATIC VEIN (Contd.)

Explanation of plates (Contd.) :

(10) The ventricular division breaks down from frame 33 onwards, i.e. at the fourth ventricular diastole, although the division is not clear towards the end of the previous ventricular systole. (frame 30 onwards). It is likely therefore, that recirculation of fluorescein from the lungs started before frame 33, i.e. in the third heart beat.

(11) The systemic and carotid arches only receive an appreciable quantity of fluorescein after the bulbus has become dark, after break-down of ventricular division, and after recirculation through the pulmonary circuit (frames 27 on). Some fluorescein is visible, in the form of a streak in the right systemic arch and in the bases of the other systemic and carotid arches from frame 18 onwards, but this has been accentuated in the plates, due to over-printing.

(12) No fluorescence of the distributional areas of the carotid or systemic arches is visible during the course of the record.

(13) The light streak at the anterior end of the bulbus up to about frame 40, is interpreted as being due to un-fluoresceinated blood retained in valves Ia and III of the distal end of the bulbus (see labelled photograph).

(14) It is noticeable that the fluorescein in the right systemic arch only reaches about half way up the fisible part of that arch during the second ventricular systole (frames 18 to 22), but progresses the rest of the way in the third ventricular systole (frames 27 to 33). This should be compared with the complete filling of the visible part of the pulmo-cutaneous arch at the first ventricular systole (frames 6 to 13).


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Plate III : TYPICAL RESULT OF INJECTION INTO A PULMONARY VEIN.

Key to labelled photograph :

C carotid arch; CA bulbus cordis; LA left atrium; L lung; PC pulmo-cutaneous arch; PV pulmonary vein; RA right atrium; TA truncus arteriosus; S systemic arch; SF spilled fluorescein; V ventricle.

Experimental details :

Male *Xenopus*. 0.6 cc. ethyl urethane as anaesthetic. No blood loss during experiment. Heart rate about 45/min. 0.12 cc. fluorescein-Ringer solution injected. Filming at 8 f/s at f 4.0.

Every frame shown on plate, up to frame 23.

Explanation of plate :

2 Heart beats are shown.
Ventricular systole starts at frames : 5, 19 .
Ventricular diastole starts at frames : 1, 15.

The following points are noticeable :

(1) At the first ventricular diastole, the whole ventricle fills (frames 1 to 4).

(2) At the first ventricular systole, the fluorescein appears in the cavum aorticum of the bulbus (frame 5 or 6), ventral chamber of the truncus (frame 6), and in the pulmo-cutaneous arches (frames 9, 10, 11), at the same ventricular systole (Cf plates I & II).

(3) The whole visible part of the pulmo-cutaneous arches becomes fluorescent at the first ventricular systole, while only a small part of the systemic and carotid arches does so.

(4) Fluorescein appears in the lungs in frame 11, i.e. at the first ventricular systole.

(5) The systemic and carotid arches are not completely blackened by frame 23, i.e. at the middle of the second ventricular systole.

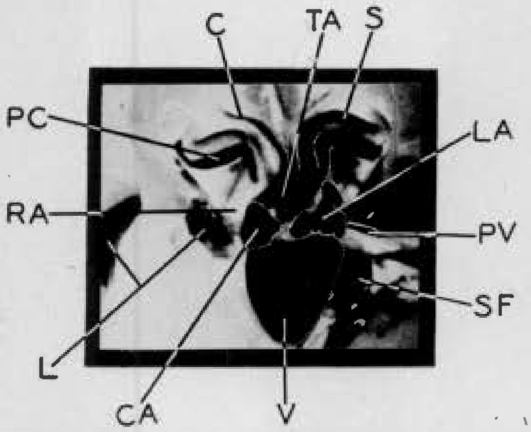
(6) No fluorescence is seen in the right atrium, nor was it visible in the rest of the film record not shown here (7 heart beats).

(7) The ventricle remains fluorescent during systole.

(8) A light streak at the anterior end of the bulbus is again interpreted as being due to retention of non-fluoresceinated blood in valves Ia and III of the bulbus (see arrow in frame 10).

(9) Fluorescein appears in the ventricle (frame 2) before it is visible in the marginal pockets of the left atrium (frame 5). The fluorescence of the left atrium gradually builds up during its second diastole (frames 5 to 15).

PLATE III

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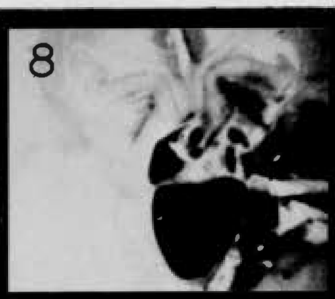
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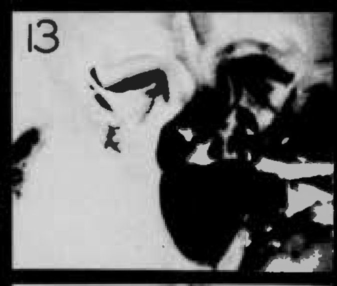
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Plates IV and V - A : AN EXCEPTIONAL CASE OF INJECTION INTO A PULMONARY VEIN.

Key to labelled photograph :

see Plate III.

Experimental Details :

Male *Xenopus*. No anaesthetic administered. No blood loss during experiment. Heart rate about 46/min. 0.03 cc. fluorescein-Ringer solution injected. Filming at 16 f/s at f 3.5.

Every second frame shown in plate, i.e. 8 f/s.

Explanation of plates :

3 Heart beats shown.
Ventricular systole starts at frames : 6, 17, 27.
Ventricular diastole starts at frames: 1, 12, 23.

The following points are noticeable :

(1) Only the left side of the ventricle darkens at the first diastole.

(2) Fluorescein appears in the ventricle before it is visible in the marginal pockets of the left atrium.

(3) Ventral chamber of the truncus and pulmo-cutaneous arch (left side) darken at the first ventricular systole (see frame 7).

(4) Fluorescein only passes part of the way up the systemic arches at the first ventricular systole (see frame 12).

(5) Fluorescein passes down the whole visible part of the left pulmo-cutaneous arch at the first ventricular systole, in the form of a "pulse", following after which is a clear wave (frames 6 to 11). A similar "pulse" is also visible in frames 30 to 32.

(6) No distinct colouration of the bulbus is visible during the course of the record shown on this plate. No explanation can be given for this, except for possible shielding from ultra-violet light.

(7) The external carotid arteries and the carotid labyrinth have no distinct fluorescence, even at the end of the recording, but some slight colouration of the lungs is visible towards the end of the record (see arrow in frame 23).

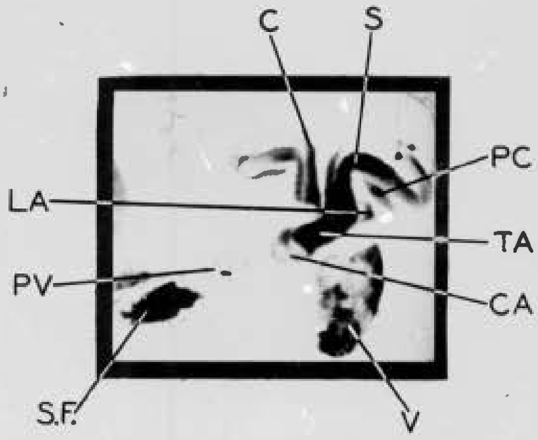
(8) A partial break-down of the ventricular division is visible in frames 30 to 32.

(9) The systemic and carotid arches are darker than the pulmo-cutaneous arches throughout the record.

(10) No recirculation of fluorescein into the right atrium is visible.

(11) Fluorescence remains in the ventricle during systole.

PLATE IV



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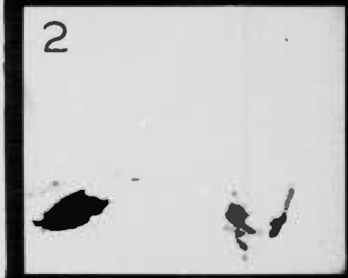

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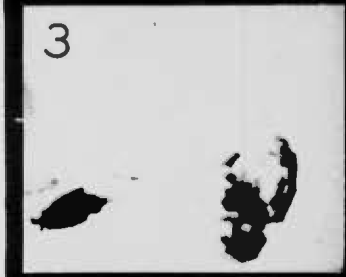

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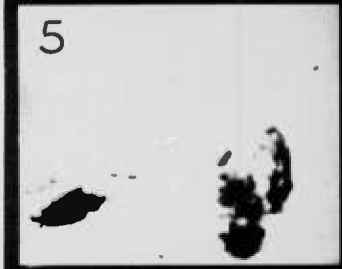

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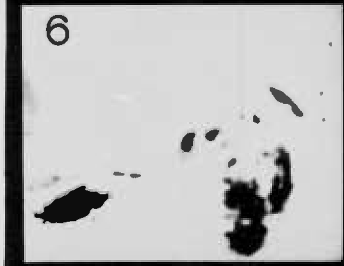

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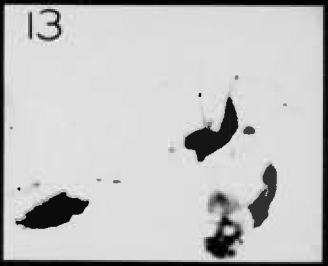

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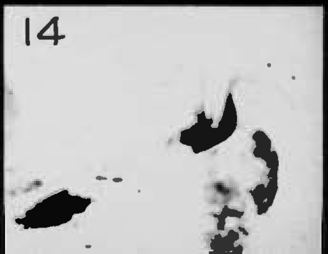

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Plate V - A : Continuation of Plate IV.

Plate V - B : AN EXCEPTIONAL CASE OF INJECTION INTO AN ANTERIOR VENA CAVA.

Explanation of plate :

Odd frames have been taken to show the course of the experiment.

The following points are noticeable :

(1) The injection site is shown by the arrow in frame 1.

(2) The division in the ventricle lies well to the left side.

(3) Fluorescein appears in the bases of the systemic and pulmo-cutaneous arches at the same time (see arrow in frame 2).

(4) The pulmo-cutaneous arch (right side) remained darker for some beats (see frame 6).

Plates V - C, V - D, V - F : VARIOUS EXPERIMENTS SHOWING DIFFERENT TYPES OF FILLING OF THE VENTRICLE FROM THE LEFT ATRIUM.

Plate V - E : EXCEPTIONAL CASE, IN WHICH ONLY THE RIGHT SIDE OF THE VENTRICLE FILLED, AFTER INJECTION INTO A PULMONARY VEIN.

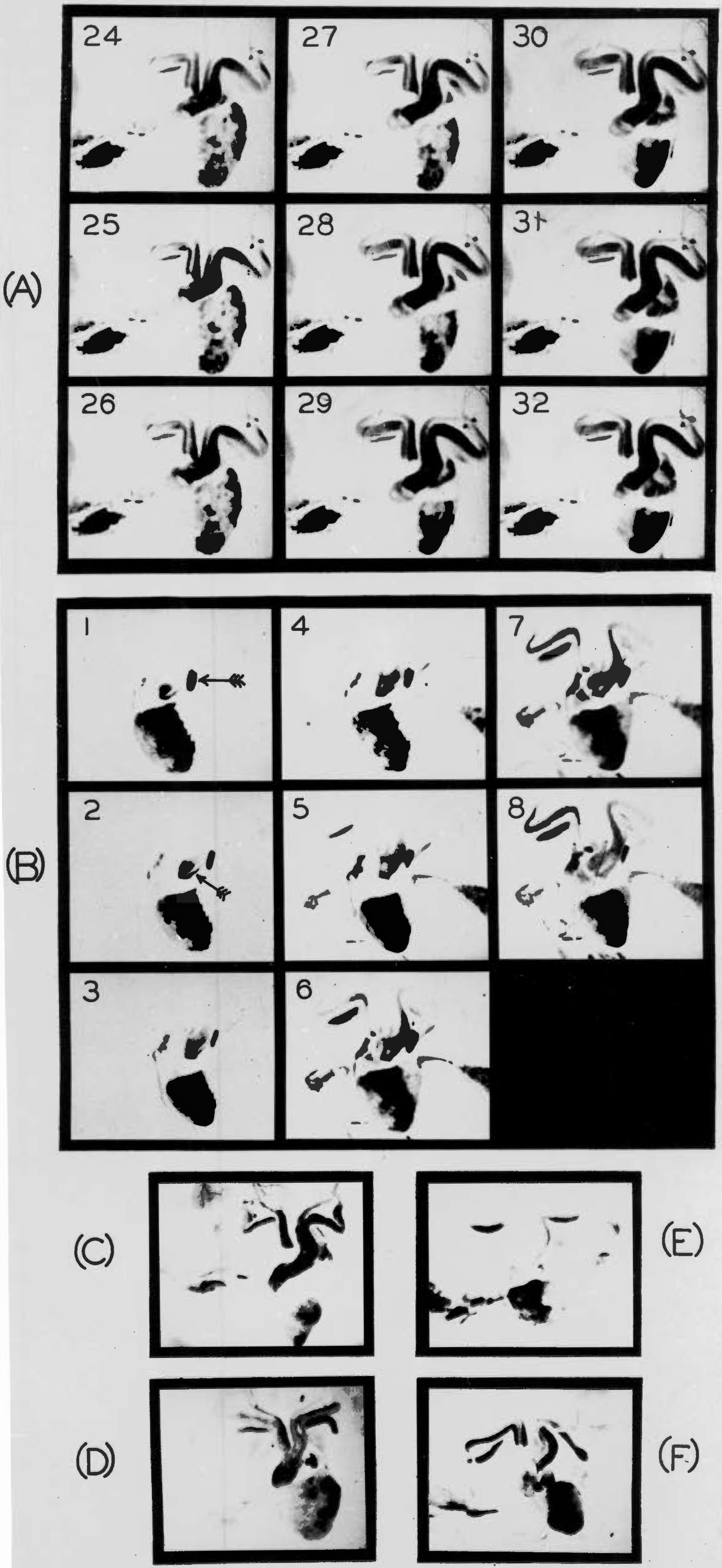
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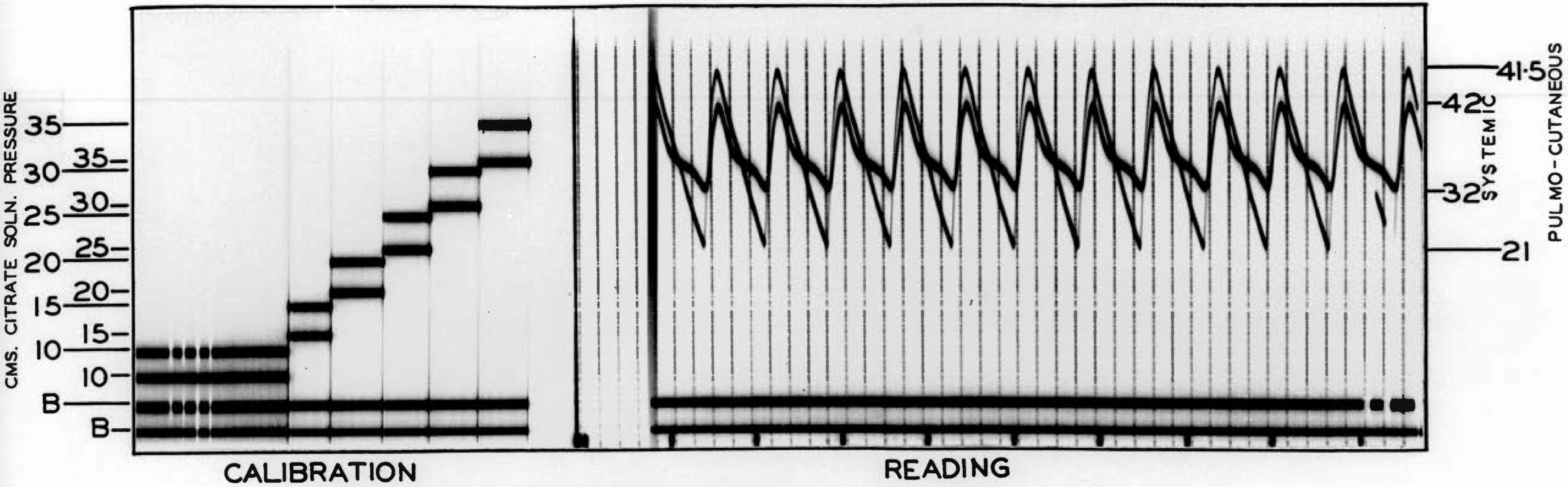
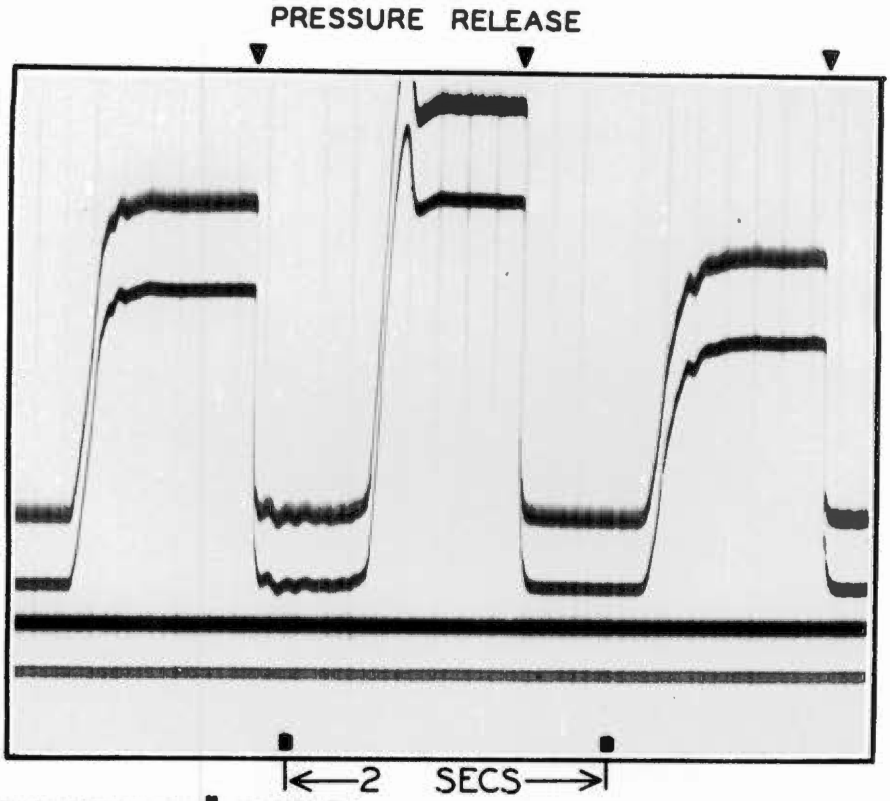
PLATE VI

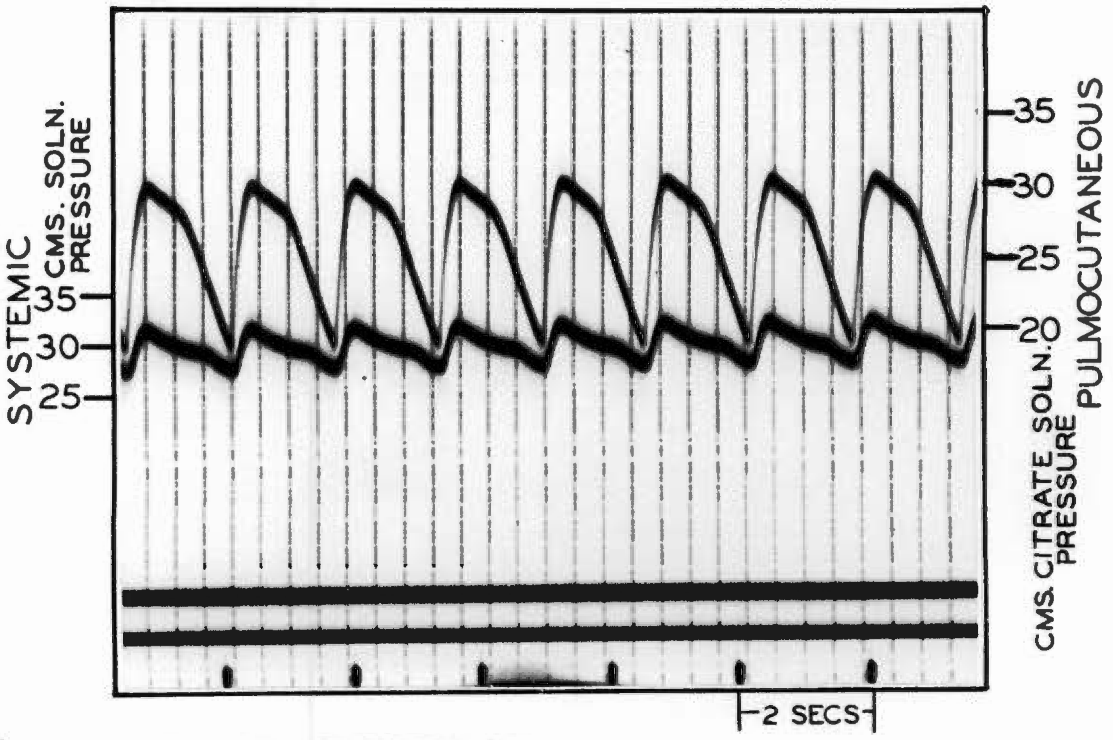


UPPER TRACING: PULMOCUTANEOUS ARCH.
LOWER TRACING: SYSTEMIC ARCH.

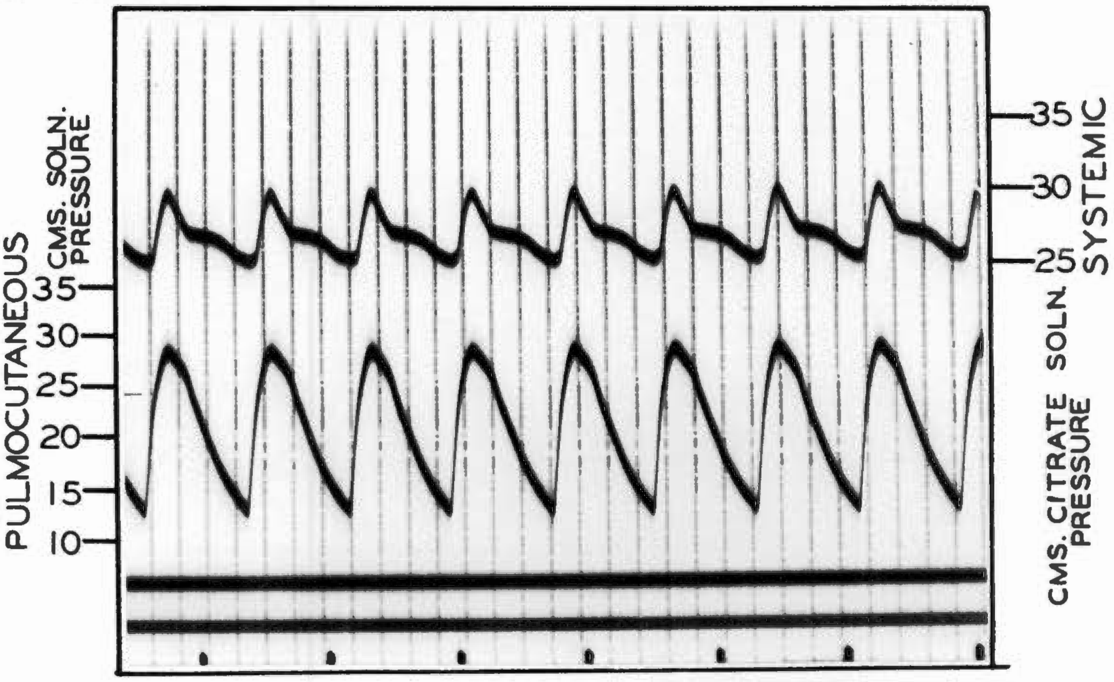
PLATE VII



(A) "FREQUENCY" TEST.

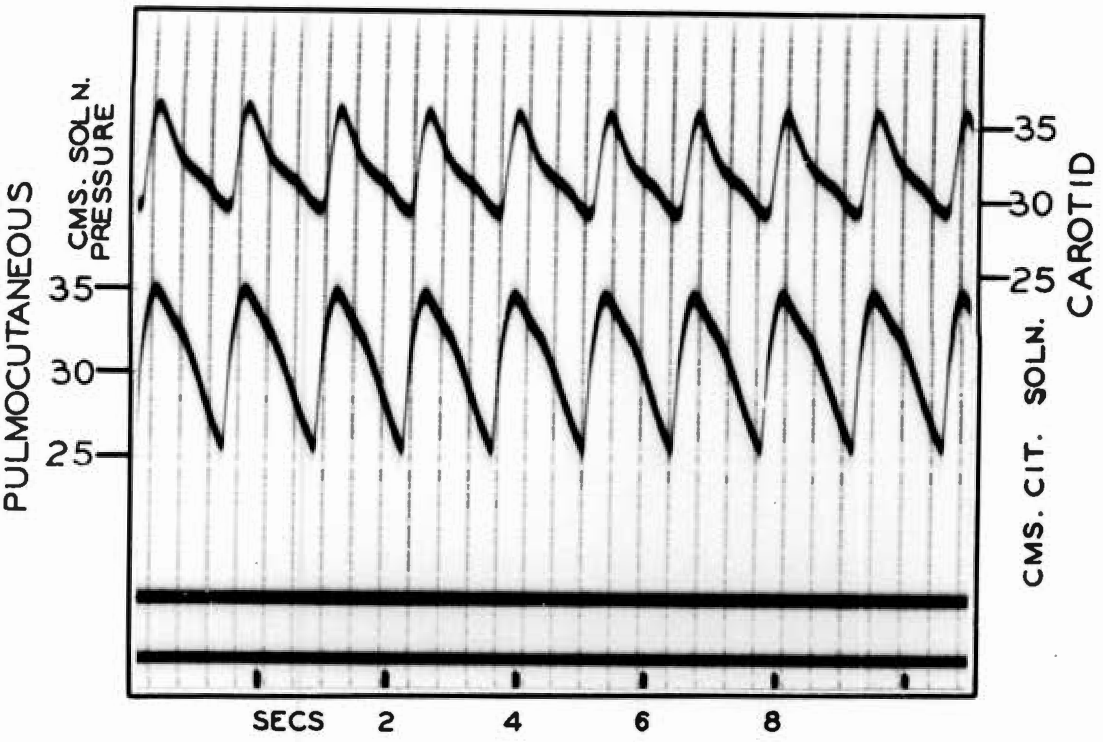


(B) PULMOCUT.-SYSTEMIC.

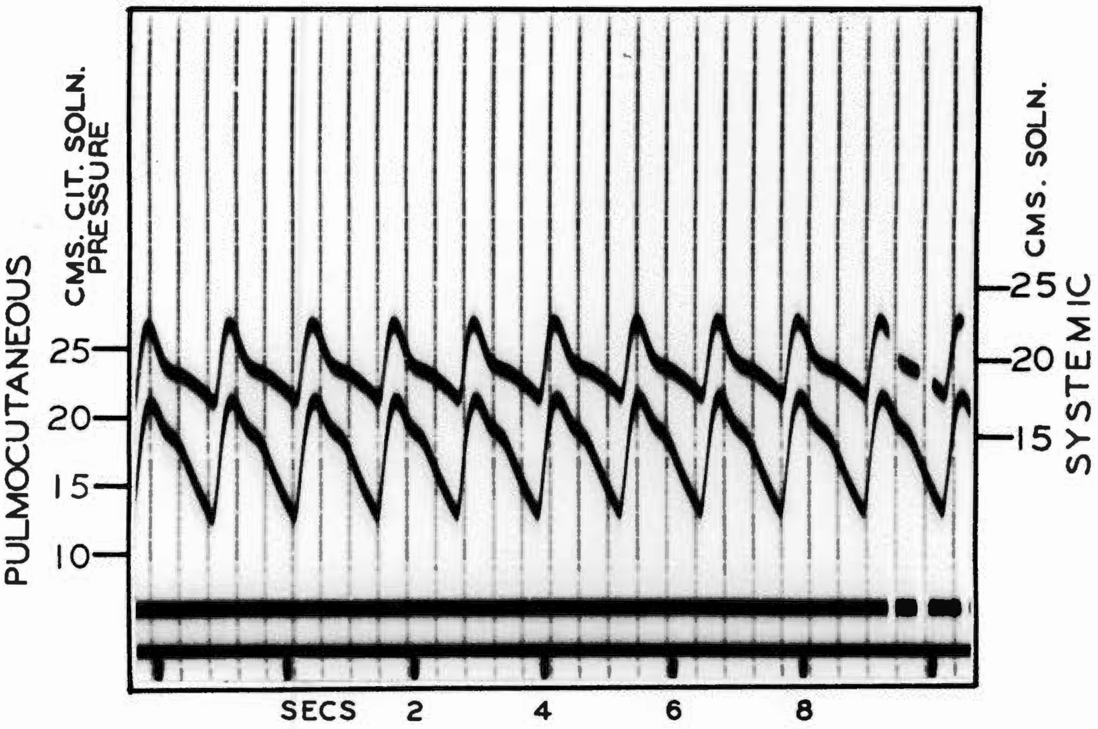


(C) SYSTEMIC - PULMOCUT. (MANOMETERS REVERSED)

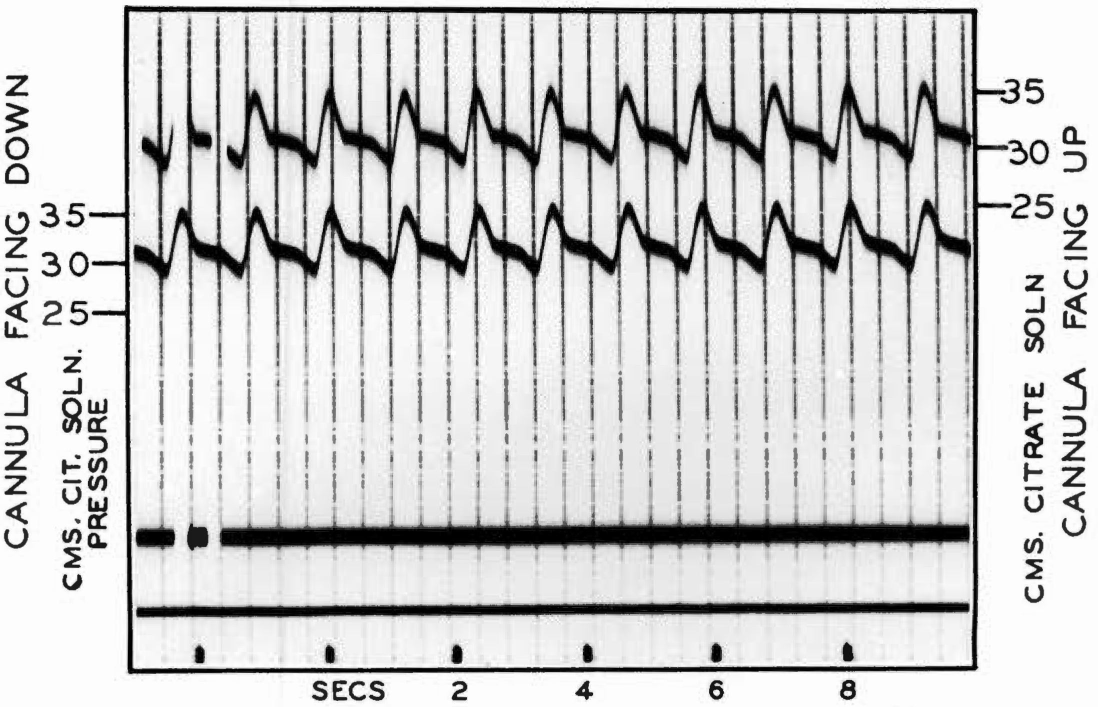
PLATE VIII



(A) CAROTID-PULMOCUT.

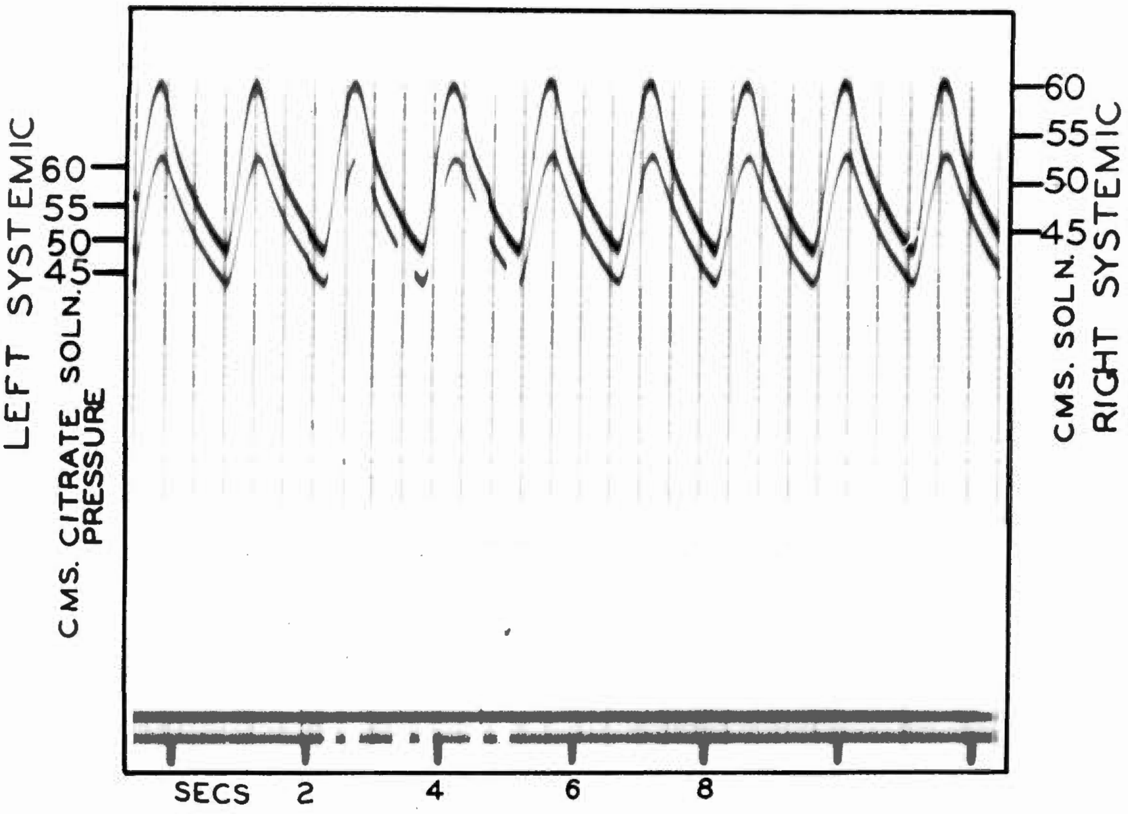


(B) SYSTEMIC-PULMOCUT.

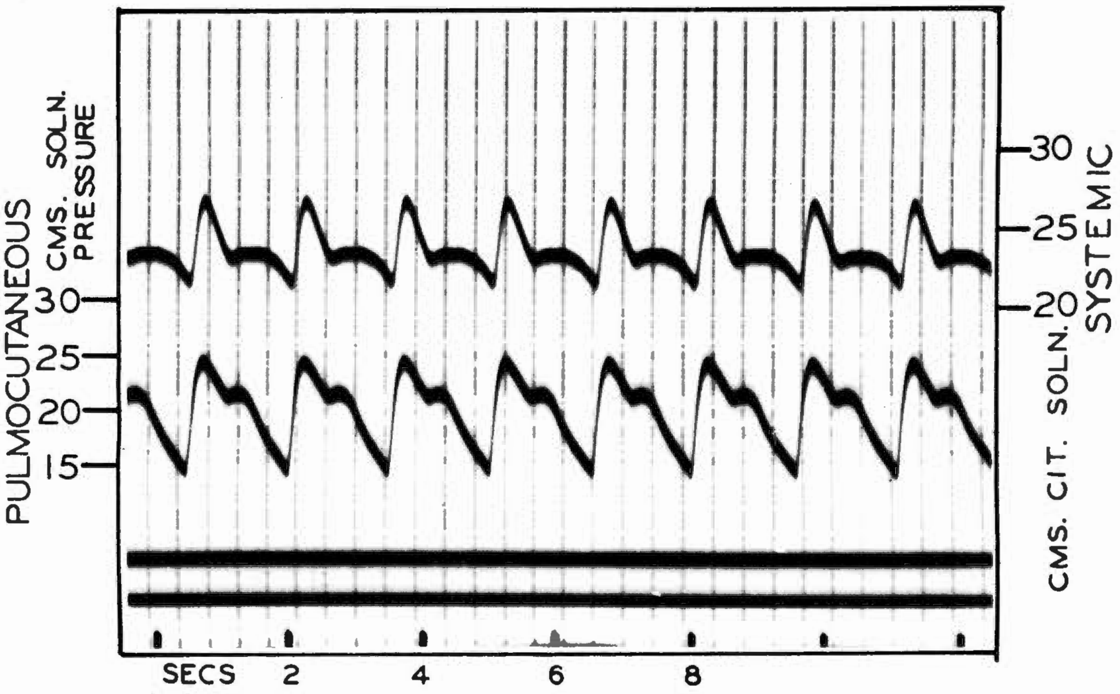


(C) SYSTEMIC (MANOMETER CANNULAE IN OPPOSITE DIRECTIONS IN SAME ARCH)

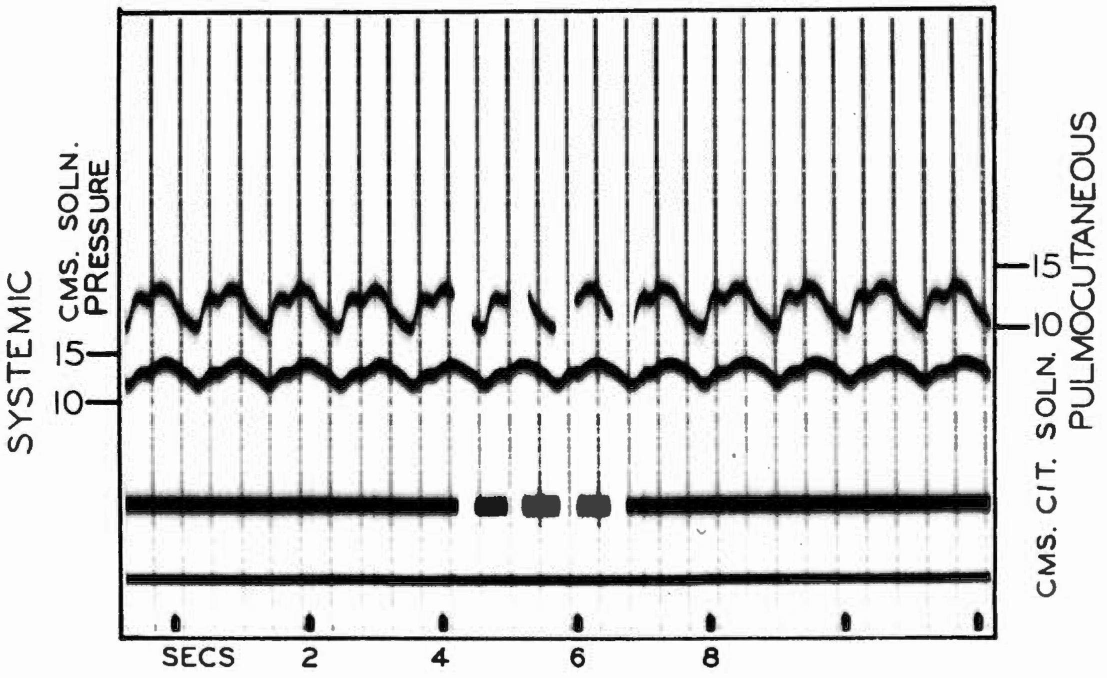
PLATE IX



(A) LEFT+RIGHT SYSTEMIC

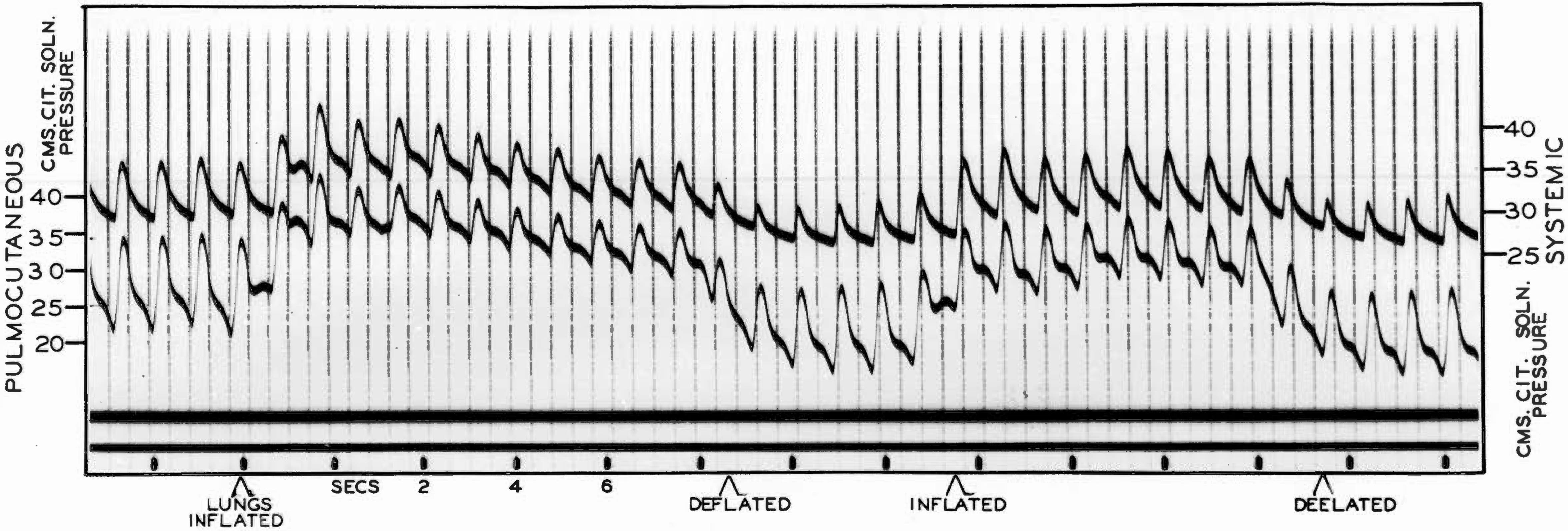


(B) SYSTEMIC-PULMOCUT.



(C) PULMOCUT.-SYSTEMIC

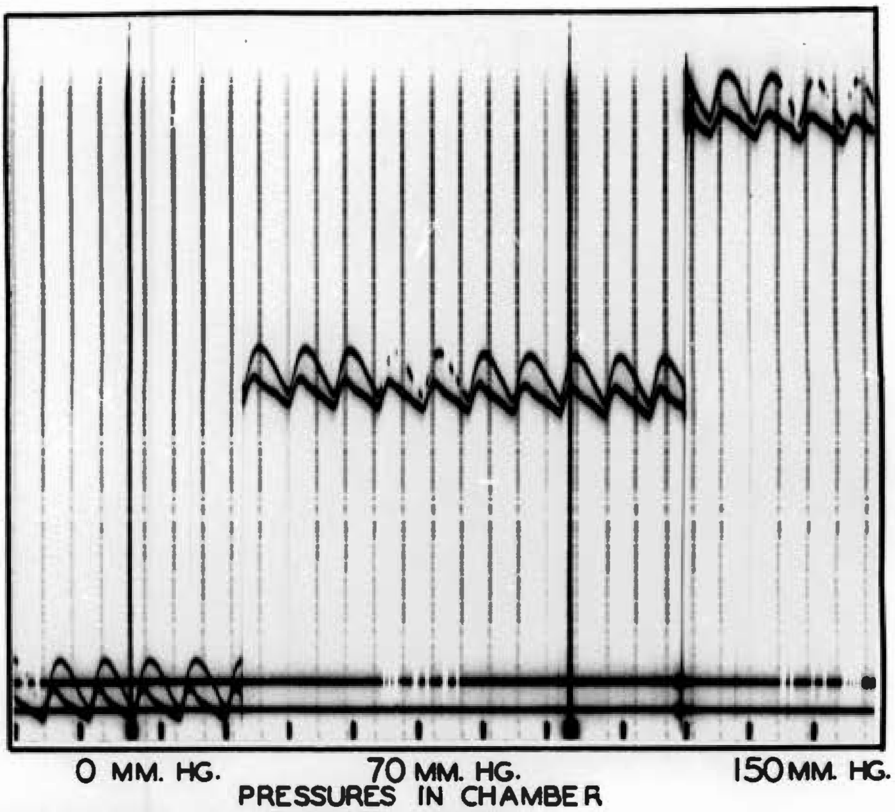
PLATE X



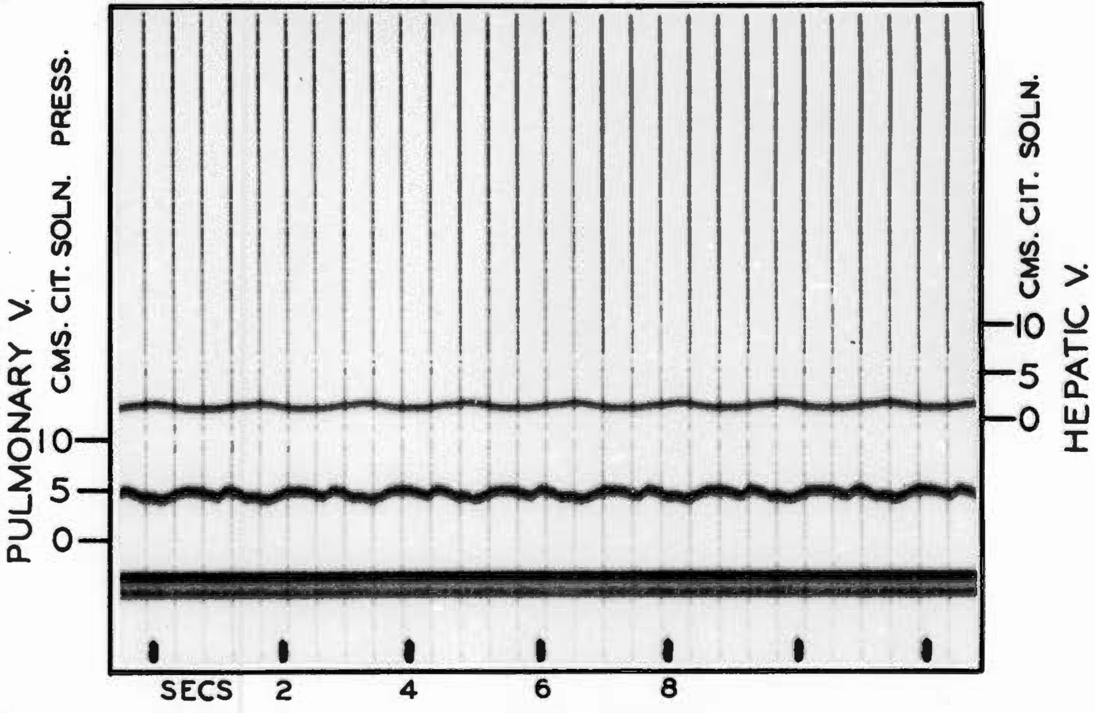
EFFECT OF INFLATING LUNGS

UPPER TRACING: SYSTEMIC ARCH
LOWER TRACING: PULMOCUTANEOUS ARCH

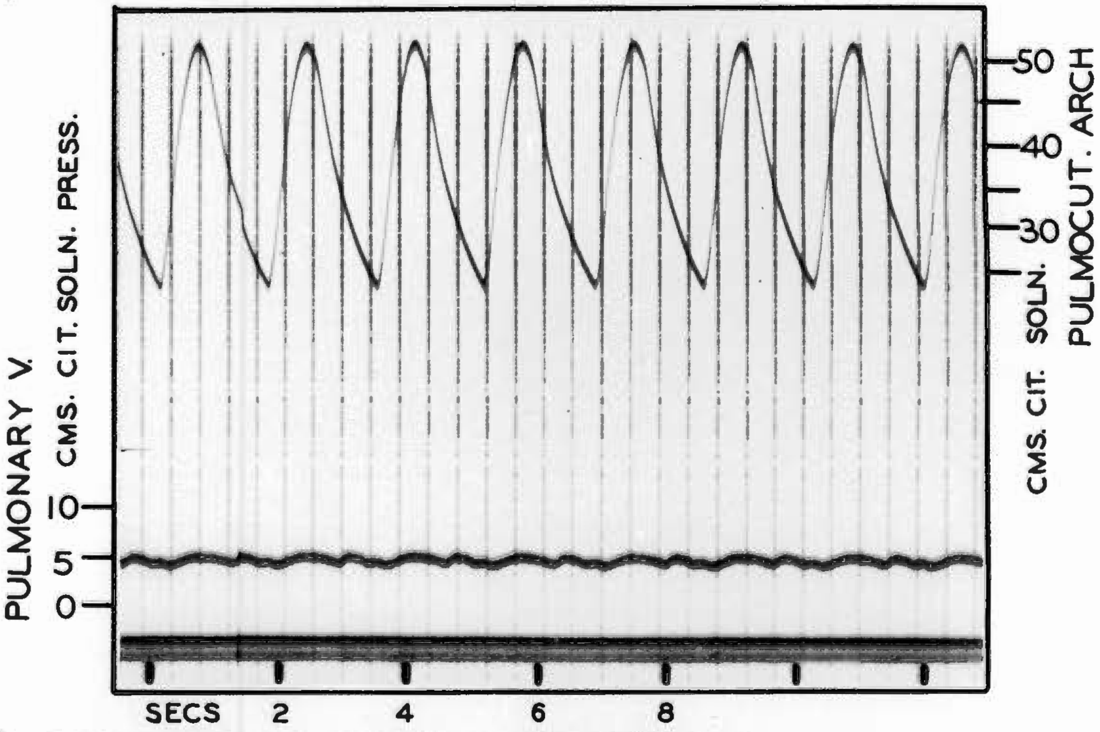
PLATE XI



(A) PRESSURE CHAMBER EXP.



(B) HEPATIC + PULMONARY VEINS



(C) PULMOCUT. ARCH + PULMON. V.