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THE STRUCTURE AND MODE OF INNERVATION of CAPILLARY BLOOD-VESSELS: THE DEVELOPMENT OF THE CARDIAC INNERVATION IN MAN.

(Thesis for the Degree of M.D.)

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INTRODUCTION.

Each of the researches embodied in this Thesis originated in an effort to extend the conclusions stated in a paper on the intra-muscular nerve elements of the ventricular myocardium published in the "Journal of Anatomy" for 1927. The work cited gave evidence for the presence throughout the myocardial syncytium of an intra-protoplasmic system of amyelinate nerves terminating in the neighbourhood of the nuclei of the cardiac muscle. In this situation, the entrant nerve was shown to become arranged in a geometrical pattern with longitudinal and transverse elements, beaded at their points of intersection. The appearance was like that of the muscle itself as revealed by certain methods of staining (e.g., intra-vital methylene blue, gold chloride and various silver methods.) The dimensions of its mesh were, however larger. At the margins of the terminal mechanism, nevertheless, the same form was repeated on a smaller scale -- the scale of the muscle structure itself — continuity being uninterrupted. This demonstration, at a time when the direct innervation of the heart muscle was not generally accepted, drew attention anew to the close association of nerve and muscle, and revived interest in the aphorism of Gerlach that the muscle fibres were the "contractile endings of the nerves".

Having demonstrated in its entirety the neuro-muscular connexion in the heart, the author sought to apply the technique which had so proved itself in this case (intra-vital methylene blue) to other unsolved problems of innervation, particularly cardiac and vascular innervation. The first of the papers now submitted for examination, that on the Structure and Mode of Innervation of the Capillary Blood-vessels, was the outcome of these earlier investigations. The results stated in the paper strengthened the writer's belief that most, if not all cells, and non-cellular tissues (the central nervous system excepted), were innervated; and definite reasons are adduced for the frequent failure of workers using even the most fruitful methods to demonstrate the whole of the neuro-muscular mechanism specifically. What may be termed the 'intensity' of innervation --- i.e., the sufficiency of effective communication between the nervous system and the cellular constituents of the tissues --- which was revealed, as well as the rarity of the situations in which perseverance failed to demonstrate the presence of nerves, made more doubtful in the writer's mind the possibility that the prevailing views of nervous development correctly represented the facts, and with increasing technical knowledge he began the investigation of embryological material with particular regard to situations in which he had already a fair knowledge of the conditions in regard to innervation in the adult.

Thus an introduction to the study of the appearance and development of the conducting mechanisms of the vertebrate heart was published in 1932, in the Transactions of the Royal Society of Edinburgh, based upon an embryological investigation of Lepidosiren paradoxa. The method here used was Bielschowsky's silver method applied to whole embryos, which were subsequently sectioned. The success of the method was such that it was possible to represent, on models reconstructed in wax, the whole nervous system of the heart and its connexions with the central nervous system at various stages of development. By this means it was shown to be a closed net in the larger meshes of which, as it were, the heart tube expanded to assume its adult form. In view of the interest which the heart of Lepidosiren has for comparative anatomists, in conjunction with the completeness of the demonstration of its innervation, efforts were made to repeat the investigation on human material. Several embryos from 3.5mm to 24mm were successfully treated in this manner, as well as a whole foetus of about 52mm. Of the preparations which resulted, those arising from the 24mm embryo were the most interesting, since they revealed a mechanism strictly comparable in its morphological and histological features with the structures demonstrated in Lepidosiren. A full description of these features concludes this Thesis. Two may be mentioned here. They

are the demonstration of a connexion between the sinu-auricular node and the auriculo-ventricular node; and a communication with the phrenic, as well as the vagus nerve and the sympathetic chain. This multiple connexion between the nervous system of the heart and the central nervous system so resembled the conditions in Lepidosiren that it was thought it might represent a transient condition of the developing nervous system, and efforts were made to discover whether the whole of the nervous communications evident in the human embryo were present in the foetus at full term and in the adult; and whether, by vital staining of the adult of other species, light could be thrown on the meaning of various connexions which it had not been expected to find. This inquiry led to the establishment of several facts concerning the sub-diaphragmatic connexions of the phrenic nerve, the connexions of the sinu-auricular node and the innervation of the derivatives of the embryological sinus venosus which must have significance in medicine as well as in comparative anatomy. On account of their general character, these results are presented here before the description of the embryological conditions instead of in the order in which the research was conducted.

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THE STRUCTURE AND MODE OF INNERVATION OF CAPILLARY BLOOD VESSELS

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ONE TEXT FIGURE AND THREE PLATES (SIXTEEN FIGURES)

Current descriptions of the innervation of blood capillaries —or rather, descriptions of the nerves which accompany such vessels—omit to describe the mode of termination of the nerves. On the other hand, if Krogh's view ('22) may be regarded as the accepted view of the mechanism subserving capillary function, the description of the effective apparatus of constriction pays little if any regard to the nervous system. The existence of this hiatus would be sufficient justification for reopening the question of capillary structure even in the absence of Bayliss's reminder ('23) that a capillary blood vessel as a distinct anatomical structure is still awaiting definition. The present investigation was undertaken to clear up these matters, if that might be done, for the ogan of definitive circulation is not of secondary importance even to those larger and better known structures which furnish it with its reserves of blood.

PREVIOUS WORK

Physiologically the capillaries form a network into which the afferent blood is forced before commencing its return to the heart. This network has been called the 'capillary bed' (fig. 2). To furnish the capillary bed with blood of the requisite quality at the appropriate rate is the object of the circulatory system. The performance of the functions of the blood in relation to the tissues of the body is dependent upon

the activities and upon the functions generally of the capillary bed. The network of vessels answering to the general description 'capillaries' has not the same appearance in all parts of the body of any mammal. In man, the network in the muscles varies in pattern and, to some degree, in structure from that in other organs. In the retina, the difference is so great that the organ is said not to contain any capillaries. Yet these differences, although well known, have not led to precise definition of the features distinguishing a capillary from other vessels. Usually what is meant by the term 'capillary' is the smaller of the vessels of the arterial system. The fact that the blood in very small vessels can be seen in the living frog to move forward spasmodically under the influence of the heart beat and the arterial pulse has led to the assumption that the capillaries are subject merely to pulse variations of pressure; but there is evidence also that the capillary circulation is controlled independently of the arterial and, possibly of the venous circulation. Thus Kuntz ('29) says that "*although the capillaries have no musculature,* they undergo changes in caliber which, at least in some instances, can hardly be accounted for as the result of changes in the caliber of arteries or veins with the capillaries playing only a passive role." In recent years many observations have been made of the movements of the blood within small vessels; but it must not be inferred that what is seen in all such cases is the blood flowing through capillaries. In most of these experiments, the blood cells are the moving objects actually observed. The vascular walls are either not seen at all or are imperfectly visible, and there arises some uncertainty concerning the precise meaning of the evidence collected in relation to the structures alleged to be involved.

The type of vessel chosen for description under the heading 'capillary vessel' is usually a vessel of the smallest size. As briefly as possible, with due respect to certain points which it may be necessary to consider later, such a vessel is a thin-walled tube, lined by an endothelium (Macalister, 1889) which is all that remains of a vaso-formative tissue resulting from

the confluence of the blood islands of Wolff and Pander, or from secondary budding from the walls of previously existing vessels. The new vessel is then actually or potentially a cellular tube from its first appearance, or vacuolation converts a series of cells attached end to end into a tube, as Sharpey-Schafer believes ('29). Earlier histologists were at pains to demonstrate the existence of openings at more or less regular intervals in the vessel wall. (The walls of capillaries were understood to be included.) Through these 'stomata,' cellular or fluid elements might pass. Doan, Cunningham and Sabin ('25), as well as Van der Stricht (1892) have thrown doubt upon the existence of such openings, which, indeed, appear to be unnecessary, in the light of modern knowledge of the physical conditions present in the tissues. If the endothelium forms, as here represented, a continuous lining for the little vessels, the 'wall' is held to be a mere reticulum, or a structureless 'perithelium' reinforced in special cases by adherent, branched connective-tissue cells, which 'anchor' the vessel to its surroundings (e.g., neuroglial elements of special type). So much for the vessels themselves.

More attention has been paid during recent years to various structures alleged to be involved in producing alterations in the vascular lumen. Evidence that these vessels were supplied with nerves was sought early, and very convincing pictures representing the vessels invested by a network of nerves resulting from Dogiel's investigation of the subject (Dogiel, 1895). Not all of these portray the conditions to be found in what the writer would call a capillary vessel.

It is necessary to refer to Rouget's very remarkable observations (1874) in greater detail, in order that the significance of discussions which arose later may be apparent. In 1873, Rouget described certain cells on the outer side of the endothelial capillary tubes. These cells, he said, "are arranged in the direction of the tubes, and surrounded by a zone of protoplasm with branched elongations which embrace the capillaries in a number of places like so many loops." As early as the tenth day, Rouget distinguished in Amblystoma

'branched contractile cells' and 'unbranched, fusiform fibre-cells.' He said:

the first contractile cells to appear on a vessel of recent formation, arise neither from the surrounding connective-tissue cells, with which they show no connexion at any period of their development, nor from the primitive vascular (endothelial) cells. (Op cit. Rouget, 1874, p. 562.)

His observations on batrachians, Rouget later extended to mammals (1879). He said:

I believe myself, then, to be in the right in concluding that, throughout the vertebrates, a similar contractile tunic, modified only in the form of its elements, in conformity with the changes from region to region in the manifestations of contractility, envelops the whole system of blood-vessels right from the heart to the capillaries, including both these, and is essential to every part of the blood-vascular system.

Krogh ('22) in relating the subsequent phases of the investigation refers to the rediscovery of Rouget's cells by Sigmund Mayer in 1902, and remarks that,

it did not inspire confidence. . . .that these interesting structures could be made visible only by staining with methylene blue, and even then only occasionally, and the further statement that a system of branched cells similar to that on the capillaries could occasionally be found where no capillaries could be detected could not fail to arouse the suspicion that they had no real physiological connexion with capillaries.

The appearance of Mayer's paper stimulated inquiry into the behavior of the capillary itself, and within a year Steinach and Kahn ('03) announced that the effect of contraction upon the capillary wall did not produce constriction of the lumen but threw the wall into longitudinal folds. To be associated with this observation is the very significant statement made by Clark and Clark ('25) that while in tadpoles the smaller capillaries become waved on contraction, in the case of the larger a different mode of action is evident, the cross section of the vessel being reduced. Clearly, it is of little use to study the structures associated with capillaries without knowing what they do.

With the development of physiological interest in the blood stream, search commenced for appearances suggestive of a differential control by the vagus and sympathetic nerves, and Glaser ('24) figured a 'capillary vessel' accompanied by fine nerves running in its direction, while surrounding the vessel, within the nerve network arising from the parallel nerves, is a much thicker 'nerve' arranged in a spiral formation '. . . .dickeren nerven spiralförmig.' (The appearance, more highly magnified than Glaser's specimen, is shown in figs. 1 and 1 A.) This demonstration is the only histological evidence known to me to support the view that capillaries receive a 'double innervation'—(the long and the coiled fibres). The vessel figured by Glaser has, together with the subscription referred to, with various emendations and in several languages, been very widely reproduced. Later in the course of this communication reasons will be given for the view that the vessel figured is not a capillary and the thick spiral investment is not a nervous but a muscular structure, being merely a succession of smooth muscle cells whose nuclei are clearly visible in suitable preparations.

It is important to notice that Rouget, Mayer and later Vimtrup ('22) described in relation to capillaries two and only two kinds of cells. One of these elements is distinguishable from the other:

Element one	*Element two*
Difficult to demonstrate	Easily detected in preparations stained by ordinary methods or not stained at all
Occasionally demonstrable unaccompanied by a capillary	An integral part of the vessel
Branched	Unbranched
Peri-vascular or adventitial in position	
Often seen where vessel forks	
Moves in relation to the vessel on contraction	

To these lists of distinguishing features other items will be added later. It will be convenient to refer to the first of these tissue elements as Rouget's branched element and to the second as Rouget's unbranched element. Bensley and Vim-

trup ('28) assert that the system of elements which I have identified as the branched elements or cells is in series with the circular muscle fibres of the pre-capillary arterioles. A great deal hangs upon this assertion. If it were true there would be reason for believing, in conformity with Krogh and his collaborators that the capillary is uniquely constructed and that the branched cells are contractile in spite of the sharp contrast they present in comparison with muscle cells of one form or another, which are the prevailing contractile elements in vertebrates.

MATERIAL AND METHODS

The material prepared by the vital methylene blue staining method over a period of years by the writer has been exhaustively examined for evidence bearing upon the present problem. This material covers most of the tissues of animals ranging from fishes to the usual laboratory animals, while preparations have been made additionally of embryonic tissues in birds, fishes and mammals. Bielschowsky preparations sectioned or prepared by some suitable process of dissection have been used also to elicit general information or to clear up special points. Ranvier's gold chloride method has been used for the same purposes. Further, an extensive series of preparations of the embryonic somatopleure and splanchnopleure of fishes was made by injecting the coelomic cavity of trout and salmon larvae before and after hatching—indeed from the 'eyed' stage until the disappearance of a noticeably pendent yolk sac.

Concerning the precise technique adapted to the study of capillaries and their nerves in adult mammals, little can be said. The most useful preparations from the point of view of the purposes of this paper came from the iris of an albino rabbit. The fact that quite different staining effects have resulted in different parts of this single organ, stained by injection of the whole animal, removed whole and originally prepared as a whole, shows how impossible it is to define the conditions necessary to produce a particular result. More-

over, as will be seen, variation from a standard result may provide material evidence especially, as in the present case, when such variation cannot be due to anything but technical causes. Since we cannot believe that there are capillary vessels of one kind in one area of a single layer of the iris and capillary vessels of quite a different kind in another comparable area, the appearance of such differences, if they occur, merits special study, and can be turned to great use. The specimen contained the whole of the pupillary and ciliary zones with some fragments of the ciliary body and the whole extent of the circulus iridis major. For convenience it was divided by a meridional incision, mounted and examined. Critical examination was not possible, and a general survey having been made, the preparations were placed first in benzol and later in benzyl benzoate, the pars iridica downward, for dissection. The radiating, flange-like laminae of the stroma were detached, some being mounted separately. There remained intact the sphincter and dilator muscles, the membrane of Bruch and the retinal layer. The two halves were then remounted, one with the retinal side uppermost, the other the reverse. With unaided vision, each may be recognized as one-half of an iris, stained a greenish hue by the methylene blue and transparent enough to read through.

Contained within each preparation is a layer which may be regarded as the iridial extension of the choroid coat. It is vascular. Near the periphery, bundles of nerves (twenty-six bundles, containing about 260 nerve fibres nearly all of the small medullated type) form two series of arches, one open peripherally, the other centrally. The region in which these arcades meet is roughly circular and corresponds to the major vascular circle. A similar system of nerve bundles corresponds to the minor circle. On the pupillary side of this lesser circle lies the whole of the sphincter pupillae. Not all the nerve fibres are stained, and while it is not easy to count the unstained fibres, they are probably as numerous as the stained fibres. A great increase in the number of fibres takes place in the direction of the pupil owing to the origin of collaterals

from the nodes of Ranvier of the larger fibres. Many of the fibres undergo transition from myelinate to non-myelinate during their course in the same direction, a phenomenon with which Langley ('21) was acquainted. The change occurs through a sudden great increase in the frequency and length of the nodes of Ranvier, so that within the distance of 20 to 40 μ what first appear as a series of long internodes with short nodes become a series of long nodes with short internodes represented by mere varicosities. Comparison with material stained by Weigert's method suggests that these varicosities are really droplets of myelin. In the iris, no nerve appears to proceed to its termination as a medullated fibre.

A band of smooth muscle fibres, varying from one to seven fibres in depth, fringing the pupillary margin is densely stained. The rest of the sphincter is unstained, although in several situations its nerves are stained clearly and in large numbers.

Over one area in the sphincteric region, extending into the zone between the major and minor vascular circles, and over some smaller isolated areas elsewhere, the plexus of capillary blood vessels with its nerves is well stained (fig. 2). Erythrocytes are to be seen in some of the vessels. A few of the larger vessels (arterioles) (fig.1 A) are also stained; and in many places the nerves which accompany vessels can be identified from their formation, the vascular elements themselves being invisible (fig. 4). Over the greater part of the iris, the dilator muscle is visible as a system of radiating pillars, or cylinders, speckled by fine granules. Viewed between Nicol prisms, each of these pillars appears as a series of rather broad bars. This effect is physical, and is due to the presence of minute fissures in the muscle columns. Within several of the 'pillars' may be seen an arborization of very minute neuro-fibrillae of much finer exture than the fibres in relation to ordinary smooth muscle.

Traversing the whole iris is a fine nerve network arising from the larger nerves (figs. 4 to 11). The meshes of this net are gathered together near the pupillary margin to form

arches, whence branches return between the fibres of the sphincter, and, turning abruptly at right angles, enter the muscle fibres and ramify within them. The nerves supplying the capillary vessels arise from the common network, and in several cases it has been possible to trace them back to their source in the same fibre that ultimately supplies one of the muscles. Indeed, close study of the nerves suggests the conclusion that there exists a single terminal innervation of the iris as a whole. If this is so, the means whereby the functional segregation of impulses may be brought about can only be conjectured. There are no nerve cells in the iris, and the nearest cell stations are those embedded in the substance of the sclerotic coat. Both Bayliss and Müller have advanced views concerning the mode of action of the peripheral nervous system which seem to call for such an anatomical relationship as here described (Müller, '1873).

STAINING OF CAPILLARIES

In both the preparations under review there are:

a) Capillaries stained showing their structure in various stages of staining, and the structure and arrangement of the accompanying nerves (figs. 2, 3, 6, 7).

b) Nerves stained continuing the course and assuming the same patterned arrangement of the capillary vessels, the presence of the later being unrevealed by any staining of their structure (fig. 4).

c) Capillaries stained to varying degrees, indicating their structure but not disclosing the presence of nerves.

d) Nerves stained revealing their structure and connections within the loops formed by the capillaries and forming a network independent of them.

e) Smooth muscle fibres with and without their nerve supply (figs. 12 and 13).

Thus all the possible modes of association of nerves with effector organs may be studied synthetically or analytically in the same preparations. The conditions are therefore ideal for the study of the structure and mode of innervation of

capillary vessels, in consideration of the symmetry and known anatomy of the regions of the iris under review.

The results obtained from the examination of this material had best be given before the embryology of the system is discussed.

STRUCTURE OF THE IRIDIAL CAPILLARIES

Proceeding systematically, the nervous parts of the capillary complex should be easy of description throughout their extent. If one could define these, what is left over must be the capillary, and what has to be determined is the place properly to be accorded to the apparatus described and redescribed as the Rouget cell. The nerve, then, is not merely a branched, tortuous thread, beaded irregularly in its course. There accompany such threads a series of cell nuclei of characteristic appearance and staining properties. Each of these is invested by a thin envelop of cytoplasm, which in many cases may be seen to extend along the adjacent nerve (fig. 11). In the angles between these nuclei and the nerve are minute polar ramifications which have an even closer relationship to the nuclei and seem to touch the rounded ends of these bodies through the instrumentality of a rather larger varicosity than most which are to be seen along the nerve. From this varicosity may be seen in many cases minute branches investing the nucleus, or departing at a tangent from its surface. Occasionally a number of such branches are gathered together again to form a branch of the nerve itself. The nucleus then assumes a more triangular form, and appears to give rise to the nerves at its angles. This appearance is responsible for the statement that small nerve cells occur in large numbers in the iris. If these nuclei are measured and otherwise compared with the nuclei (fig. 9) of the neurilemma of large medullated fibres, they will be found to be similar in every respect, and the peculiarity which these bodies possess of sometimes staining with methylene blue and sometimes not, and further of staining a pale color, tinged with purple, noticeably different from the blue of other cell nuclei are additional

means of identification. The bodies are the nuclei of the neurilemma of the small non-medullated nerve fibres. In relation to the arterioles, the perivascular or adventitial position of the nerves and their neurilemma is easy to observe (fig. 1). In such situations, too, the pale, purplish nuclei may be seen frequently overlying the smooth muscle cells, arranged spirally around the lining endothelium (fig. 6, R). Their identification in relation to the capillaries proper is, however, a very different matter. Again it must be emphasized that the staining of capillaries by methylene blue is fickle. When the capillary vessel is unstained, the relationship of the nerve to it is, of course, impossible of observation. In cases where a short length of the vessel is stained, a curious appearance is often produced, as though the stain had picked out isolated units in the tissue, and in some cases these may be seen scattered over the field at more or less regular intervals (fig. 10, A and B). Upon close inspection of such units, they can usually be made out to consist not of a single cell but of two elements (fig. 10, B, N and M), one of these possessing a minute filament at each of its two ends. Examination of such filaments shows that many of them may be traced for considerable distances, and even to the nerve net already described. The second element is fusiform. Its nucleus is bluer, and its cytoplasm very finely granular. Frequently, too, the nervous element present may be seen to give off minute branches. Here and there these double cells are linked together in rows which soon become a continuous length of recognizable vessel. There cannot thus be any doubt remaining that the nervous mechanism of the true capillaries is of the same nature as that of the larger vessels. In relation to the capillaries, whenever the neurilemma cells are well stained, their nuclei can be observed to stand out from the wall of the vessel (fig. 8) and even to lie directly in relation to the nucleus of the second element present (fig. 6) and on its outer side in relation to the axis of the vessel.

It is evident, therefore, that one of the two elements associated with capillaries can be identified with certainty. It is

nerve, and if reference be made to Rouget's description of the two elements present the description of the nervous element here given accords in every particular with what I have called element one. It is: a) difficult to demonstrate, b) occasionally demonstrable unaccompanied by a capillary, c) branched, d) peri-vascular or adventitial in position, e) often seen over the forking of a vessel. Whether it moves in relation to the vessel or not may be inferred from its relative independence of that structure. In addition, it has a relatively large pale nucleus, which may be quite unstained and if stained is of a purplish color. Rouget's cell, then is a unit of the neurilemma together with the accompanying nervous filament and its branches as these appear in an exceptional position. It is presumably not contractile, but is motor to the other element present, whatever that is, or to a third element for the existence of which we have no evidence at all. *Entia non sunt multiplicanda praeter necessitatem.*

THE SECOND ELEMENT

It will be apparent, now, why there is much more to be found in the literature bearing upon capillary structure about innervation and about the Rouget cell as a special mechanism than about the essential element or elements composing the wall of the capillary itself. The early descriptions of the capillary, in its adult form and in development, present the structure as that of a simple endothelial tube. The numerous instances in which cellular structure is absent, or incomplete, the contents of the vessel being directed in its movements only by a reticulum, have been collected over a relatively long period of investigation (Bouin, '29), and their bearing upon the question of capillary structure in general has passed unnoticed.

Evidence relating to the acquisition of cell structure by the capillaries will be given later in discussing the embryology of the innervation of blood vessels in fish larvae. In dealing with the adult structure it may be emphasized that the capillary is small, often much smaller than the erythrocyte which

passes so freely through it, and the expanse of pavement epithelium described by earlier workers who used silver salts to effect the staining of intercellular substance, so revealing the cell outlines, cannot be accepted as wholly defining the nature of the lining membrane of very small vessels. Indeed, this method is open to particular objection when used to elicit evidence of the structure of such vessels, for, contrary to general opinion, the silver, even when used crudely on fresh tissues afterward exposed to light, stains other things besides the cement substance. It stains the smaller nerves, and in preparations of the cat's mesentery in the writer's possession, although the larger nerve bundles are unstained, the serial capillaries running in their midst, between the nerve fibres, are well mapped out together with the small non-medullated nerves accompanying them. In the small vessels, then, there is much danger of confusing such structures with the cell outlines, and as the cells become fewer and more elongated this danger is increased. Within the mesenteric nerves, stained as indicated, the cellular elements composing the capillaries can quite easily be made out to be fusiform, separated from one another, sometimes by a greater distance than the width of the cells themselves, which present a twined, or elongated spiral formation.

This is precisely their appearance and arrangement in the iris stained by methylene blue. The question arises, what are the elements?

For the elucidation of this all-important point, it is necessary to refer to the behavior of larger vessels when stained intra-vitally with methylene blue. In veins and arteries, then, the circular and longitudinal muscle cells may stain in large numbers, or not at all, or a cell here and there may be picked out. Their form and size may therefore be ascertained.

It is remarkable that as the arteries become reduced in size, they cease to stain in their continuity, and only short lengths can be see as a number of rings encircling a common axis (figs. 1, 1 A and 5). These rings are smooth muscle fibres, the remains of the circular coat, the longitudinal layer having

disappeared. As the vessel becomes still further reduced in size, a further change occurs in the disposition of these muscular loops. They open out (fig. 1 A), and may be seen to be joined together by a thin reticulum, or stroma (fig. 1 A, S), which cannot be very different from the stroma of any smooth muscular organ—e.g., the bladder—which unites the individual elements and renders their contractions effective. In gold preparations, as well as in those stained by methylene blue, this stroma may be seen folded in the line of the vessel, and slightly collapsed toward the axis of the lumen. Its appearance is characteristic.

Just as the arterioles do not appear in their continuity in methylene blue preparations, neither do the capillaries; and it is very unusual to see the mode of transition from one to the other. But in one or two cases in the preparations here described, this transition may be observed as a still greater elongation of the spirally arranged muscle, and, further, the end of the arteriole can be identified from the characteristic appearance of the stroma referred to (fig. 7, S; fig. 2, A). Why this transition cannot be observed commonly is due, I suggest, to the essentially physiological nature of the method used. The distribution of the staining fluid is dependent upon the normal function of the vessels, and in any case it seems that some function resident in them determines the ultimate distribution of the fluid unevenly in small loculi. Considering the tendency to stain short, even very short lengths of the capillaries from this point of view, it is clear that if the complete emptying of the capillaries were avoided in the normal constriction of these channels, great economy of energy would result, since the volume of blood in the capillary bed is great, and the total replacement of it must be a proceeding involving the expenditure of considerable energy. What is true of the system as a whole is true of the parts. When it comes to rest, broken up into small beads, the blood must be rapidly inactivated. When movement is resumed, on the dilatation of the vessel, fresh blood is supplied in an active condition, without the necessity for the complete replenishment of the volume of blood equal to the capacity of the dilated vessel.

To return to the structural problem, at the site of the transition, the stainable elements of the arteriole are continued directly into the capillary. Only their arrangement changes. They assume, as already stated, a more spiral formation, and intertwine with one another loosely, the end of one fibre overlapping the commencement of the next, and never more than two smooth muscle fibres can be seen at any level in the capillary system (figs. 3, M; 7, M; 12).

In the arterioles, these muscular elements can be seen to be innervated. The appearance presented has been widely de-

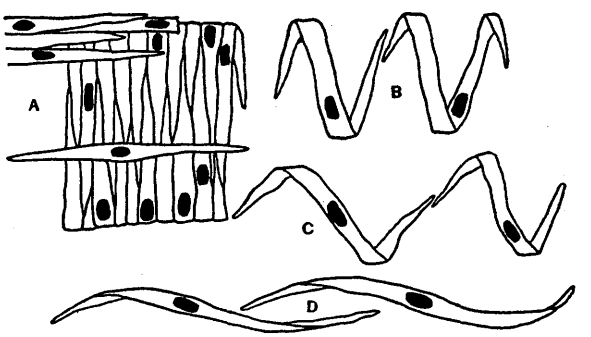

Fig. A Muscular elements of small vessels. A, small artery, showing longitudinal and circular muscles fibres. B, arteriole. C, transitional arrangement. D, capillary. A stroma unites the margins of neighboring muscle fibres to form the capillary tube.

scribed (i.e., the appearance of innervation of a smooth muscle fibre). Hill ('27) states that the arrangement of the nerves is external to the muscle cell. The matter should not be one of opinion, and while the accuracy of the observations cited cannot be questioned, sight must not be lost of the fact that the cell wall is a boundary of some significance, separating two regions of widely different constitution. It is, therefore, not impossible that the staining might stop short at such a membrane, and indeed it is the writer's experience that staining by methylene blue does so terminate suddenly in many characteristic situations, of which the muscle boundary is one. Failure to reveal the ultimate destination of the nerve in this way may

lead easily to wrong conclusions. In consideration of the material here described, the opposite conclusion to Hill's cannot be avoided: that the nerves enter the muscle fibres and ramify within them, coming into the closest relationship with the nucleus itself.

There is thus no need to invoke any exceptional apparatus in order to explain capillary constriction. The vessels are compounded, with the greatest economy, of those neuromuscular elements upon which so many of the vital functions rest. They are nothing but innervated smooth muscle arranged in a manner adapted to the maintenance of the special physiological function of the part.

DEVELOPMENT OF CAPILLARY INNERVATION

The yolk sac of Salmonidae is an expansive region where the ~~condition of the~~ tissues are embryonic, and is bounded by the developing larval body. Between the ectoderm and the yolk membrane lies a cavity, complete in later stages but incomplete in early stages, lined by mesoderm, the splanchnic layer being the site of the vitelline vessels. Toward the axis of the embryo, the character of the somatic mesoderm changes suddenly along a line which represents the extreme edge of the myotome which is advancing to cover the anterior body wall. This region of the yolk sac is ideal for the study of blood-vascular development because of the sharp distinction between the embryonal and differentiated tissues. Also, the area is large but very thin, and both the deep and superficial boundaries can be explored with moderately high powers of the microscope. If the somatic layer alone is considered, it is merely a lamina of the ectoderm lined by a non-cellular, amorphous 'mesoglia,' which is without optical depth. Nothing visible within this lamina can leave it unseen. This is important, because there are nerves embedded in the 'mesoglial' lining—the layer which used to be called 'neurogenic' by embryologists. Now, if the vitelline membrane (shell) be scratched with a piece of broken cover slip, or other very sharp piece of glass, the larva may, with a little practice, be

hatched at very early stages, living, undamaged and with its yolk sac intact. The ectoderm covering it is tough, and may be seized by forceps at the abapical pole in such a way as not to grasp the yolk membrane. A small incision, again with a spicule of cover slip, facilitates the injection of the coelomic cavity with methylene blue solution, and, if the operation is successful, the hole made in the ectoderm will immediately be occluded by herniation of the yolk membrane. The ectoderm is now slightly distended by the blue solution, which rapidly decolorizes through reduction to methylene white. (Even the small vessels may be injected if a piece of glass tubing drawn to a fine point is used as a syringe.) It is essential that the dye should be reduced and imprisoned in the way described, otherwise the nervous elements do not stain but the wall of the cavity does, presenting a rough, dirty appearance. (Older larvae are better anaesthetized. Touching the surface of the water in the Petri dish containing the larva with a finger moistened with chloroform effects this.) The time taken for nerves to appear is very short—often less than 2 minutes. The images fade again quickly, and, once fading has commenced, it is useless, in my experience to try to fix the dye by immersion in any of Bethe's fixatives.

Staining shows, in larvae just before and after the time of hatching, an intricate plexus of nerves along the edge of the myotomes (figs. 14 and 15). Held ('09) has figured the corresponding plexus in Siredon pisciformis. He does not, however, describe the nerves leading off from it, nor does he deal with its relation to the myotomes, being chiefly concerned, as is Haidenhain ('11) with its nuclear-free character.

The full description of the nerves lateral to the edge of the muscle plates belongs to the general problem of neurogenesis rather than to the subject of this paper; but one or two facts must be given to make clear the fact that the embryonal tissues contain these elements before the process of differentiation spreads to reach the region from the embryonic axis. From the stage represented by a larva 6 mm. long about twelve nerves (the number cannot be fixed with greater cer-

tainty because adhesion of the somatopleure and splanchnopleure prevents free distribution of the stain in early stages) on each side of the embryo, pass from the edge of the myotomes to the apex of the ectodermal yolk sac. This is at the time, or before it, that the transient dorsal ganglionic cells of Rohon and Beard are beginning to appear, and definitely before the permanent nervous elements are commonly believed to be differentiated. If we derive these twelve nerves from the Rohon-Beard cells, they are considerably in defect of the number of these elements present. If we derive them from the neural axis of a period of development during which the body segments correspond with them in number, we shall have to refer them to the 2-mm. stage; and if we try to relate them to the segmental nerves developing at any time between the eyed stage and the disappearance of the yolk sac we shall find that they are constantly fewer in number than the segmental nerves to which they are directly related through the medium of the lateral plexus. Anatomically, then, this series of nerves belongs to the somatopleure and to the embryonic axis; but morphologically it is anomalous. The plexus which affords continuity between the two sets of nerves is borne progressively in a ventral direction by the edge of the advancing myotomes, or rather by an unsegmented sheet of mesenchyme, partially differentiated, which precedes the myotomes. In all cases anastomosis occurs between the nerves deep to the myotomes and those which lie superficially, the union occurring along the abaxial border of the mesenchyme (fig. 15, M–M). By this process the nerves beyond the plexus, still in their very early, non-nucleated condition, are reduced to the status of fine twigs directed from each side toward the ventral midline. Not only is the histology associated with this process unexplored; but the process itself, which must possess great embryological significance, has not hitherto been described.

What is material to the subject of this paper is the apparent lack of neural organization within the region of this lateral system. The intercommunicating processes are, as it were,

awaiting organization in a neurological sense, and are awaiting the allocation of function to them fortuitously as the complex developmental changes within the tissues provide opportunity. One of these opportunities is the appearance of the slender channels for the blood extending within the mesenchyme of the somatopleure. Although here and there one of these early vessels extends beyond the advancing edge of the muscle plate, they are not commonly found far out on the yolk sac. It is clear that those farthest out are the latest formed. Their appearance is very distinctive when compared with the much more obvious vessels of the splanchnopleure. The latter are irregular in size and through them passes a vigorous circulation. They are resistive to violence, and relatively difficult to tear open with a splinter of glass. The former, on the other hand, are straighter, of uniform diameter, or for limited distances in their course dilated to form distinctive lacunae. Moreover, they are of the greatest delicacy, and the slightest bruising suffices to rupture their walls.

Vital staining, while not incapable of revealing the walls of minute vessels, rarely does so. But if a small area of the somatopleure is detached and placed on a slide, the details of its structure can be made out by staining with Haidenhain's haematoxylin, or with phosphomolybdic-acid haematoxylin. Cleared specimens of the region under discussion show an ectodermal covering of two cell layers, and (if an appropriate area is chosen) parallel nucleated muscle fibres arranged segmentally and represented more peripherally by an unsegmented, narrow zone of undifferentiated mesoderm with an abrupt edge. But neither in the septa between the muscle segments, where methylene blue, by staining the contained erythrocytes, shows the vessels constantly to be present, nor in the clear mesenchyme beyond is there to be found the slightest trace of a cellular vascular wall.

Now, before it is possible to make clear the relationship of the nervous apparatus to the developing blood vessels, it is necessary to draw attention to some points which are not

usually presented with great emphasis by writers dealing with the early vascular system.

The embryonic vessels of the entodermal yolk sac are non-cellular. When the dead somatopleure is detached and stained with a nuclear stain, the mesoderm can often be stripped off and examined. Near the muscle fibres it is thicker and strewn with nuclei. Farther away, it is thin, non-nucleated, and finely reticular in character. The earliest vascular channels are spaces in this reticulum. That the wall of a vessel may in some cases be of this character is admitted by Bouin ('29) and other histologists. Bouin says:

> Tous les capillaires ne possèdent pas, nous l'avons déjà vu, la structure qui vient d'être indiquée. Il existe des régions du système circulatoire où les capillaries ne sont pas formés de cellules endothéliales, mais sont composés par un simple tube protoplasmique semé de noyaux. Ils ont conservé la *structure embryonnaire* primitive. . . . Bien plus, dans les *lacunes* la paroi endothéliale fait entièrement défaut.

Bouin is referring to the blood spaces of the ectoplacenta in the guinea pig, where the place of the endothelium may be regarded as being taken by the ectoplacental cells. But the matter is not to be settled by tranferring to one epithelium the functions of another, and the primitive embryonic structure is not, as I have shown, in all cases "un simple tube protoplasmique semé de noyaux."

Graham Kerr ('19) draws attention to the 'remarkable feature' of the development of the dorsal aorta of Polypterus, which requires further investigation both in that genus and in other vertebrates in which it may be found to occur. In *Polypterus,* in the stage immediatly preceding that in which the aortic cells collect together, the position of the future aorta is distinctly marked out by the arrangement of the *delicate reticulum that is visible connecting up the various* organ rudiments of the larva.

Preparations made by Bielschowsky's method for nerves and mounted whole reveal this reticulum where it exists. It is seen easily through one of the numerous large oil globules

which separate the densely staining yolk from the surface of the yolk sac in the salmon. The stained filaments of the reticulum form a spongy matrix, and only in relation to the larger vessels does this matrix contain nuclei in its meshes. Where it does so, the fibrillar network appears in many cases to interpentrate the cytoplasm surrounding these nuclei. After all, no reason can be assigned to explain why only the central cells of the islets of Wolff and Pander should detach themselves, float away in the plasma and continue to multiply themselves, while in the cells more peripherally placed all these activities are completely inhibited. It is certain, however, that many of the vascular channels of the area under consideration in the Salmonidae possess no more wall than those of the ectoplacenta of the guinea pig figured by Bouin (op. cit. '20, p. 300). "Les lacunes ne sont revêtues d'aucun élément vasothélial." In another reference to minute vessels which are devoid of the usual structural elements, Bouin cites as examples of those which have retained the primitive embryonic form—a syncytium—the choroidal vessels and those of the liver, the intestinal villi, and the renal glomeruli. He points out that this 'morphological imperfection' reaches a maximum in sinusoidal capillaries (of the heart, endocrinous glands, parathyroid and suprarenal glands) where it is of advantage because it facilitates exchanges. "Ici l'endothélium est discontinu, fenêtré, et n'interpose plus sa mince frontière protoplasmique entre le sang qui les baigne de façon directe."

To return now to the young blood vessels as they develop at the edge of the myotome in the salmon. In this region, they appear in the septa between the myotomes, and, crossing the myotomes obliquely and externally, form loops. These loops do not correspond in position to the loops of the nervous plexus, but are independent of them. The former are nearer the embryonic axis than the nerves, and are often accompanied by protecting pigment cells. Evidently the vascular loops are in continuity with the segmental vessels. Whether or not the larger vessels are cellular at this stage, the terminal loops are not.

Now, although there is a complete absence of cellular wall, it is a remarkable fact that there is no lack of evidence of the existence in the neighborhood of the vessels of a developing nervous mechanism. The filaments composing this mechanism are the finest I have been able to observe in relation to the somatopleure. Fixed preparations which show them are difficult to obtain; but it is possible to preserve the delicate processes permanently. For a short time during vital staining, however, these nerves are clearly to be seen as a continuity of exceedingly minute rods and granules forming an extensive and delicate tracery around the lumina of the vascular channels at a small distance from them (fig. 16). The connections between these encircling ramifications and the branches of the nerves are more easily to be made out and are in some cases nucleated. These vascular nerves, therefore, do not accompany the vessels from their source; but are to be seen in relation to short and variable lengths of these vessels, forming loops the ends of which arise from the nerve trunks or return to them. This is, indeed, not dissimilar from the arrangement in adult vessels small enough for the facts to be observed (fig. 16). Thus it is clear that innervation is previous not only to the appearance of a contractile mechanism but to the acquisition of a cellular vascular wall.

It has been mentioned that the cellular character is acquired by a process which involves the appearance of nuclei within the meshes of the reticulum which is the first wall of the vascular tube. While my preparations throw no light upon the process whereby the early nervous mechanism acquires a contractile end (shown in the earlier part of this paper to be an ordinary smooth muscle cell), it is not necessary to infer that more is involved in the completion of the effective apparatus than a redistribution of the nuclei of the region. The fact that the primary reticulum can be seen in some cases to interpenetrate the cytoplasm surrounding the nuclei in relation to larger vessels is an indication that we are dealing with a syncytial formation within which nuclei are free to move from one place to another. We know that this will happen in relation to the

young nerves, giving rise to what is called the neurilemma sheath. The constitution of the contractile ending and the acquisition of the characteristic appearance in the adult is doubtless a similar and probably closely related developmental event.

CONCLUSION

There is nothing exceptional in the histiological constitution of the capillary blood vessels. They consist essentially in a neuromuscular mechanism of the same nature as the walls of larger vessels and contractile viscera generally. Rouget's cells belong to the nervous, not to the contractile element in this complex. The contractile elements are ordinary smooth muscle cells. In circumstances favorable to uncomplicated observation, the early vessels may be seen to be in the first instance non-cellular, and the first sign of effective physiological investment is the extension of elements in continuity with the nervous system in their neighborhood. The difficulties associated with precise description of capillary structure arise from the unusual arrangement of the smooth muscle fibres which form the more massive element in their constitution.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

1 Arterioles in the retina (albino rabbit) stained intra-vitally with methylene blue, showing slightly extended circular muscle fibres and nerve bundles, M and N. V, indicates the situation of a venule, marked out by the accompanying nerves and a few nuclei. × 250.

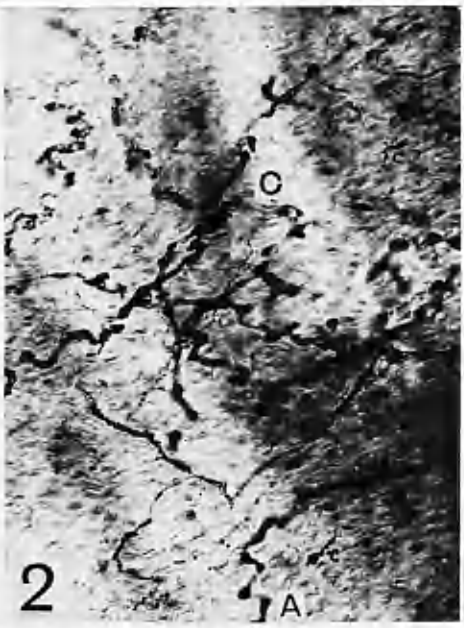
1 A Arteriole from iris, showing transitional arrangement of muscle fibres at T. The stroma uniting the edges of the muscle cells is visible at the ends of the radiating white lines. × 250.

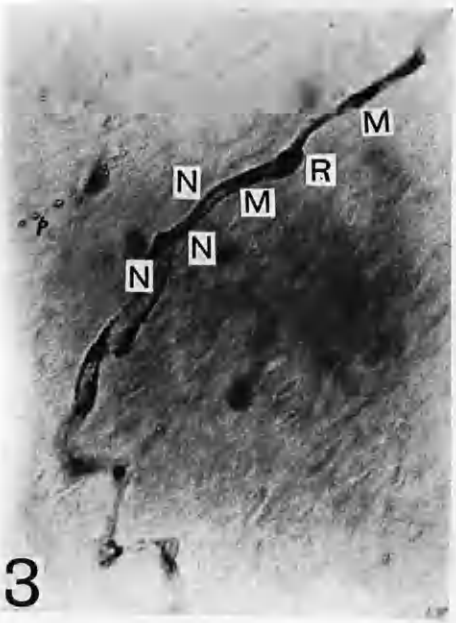
2 The capillary bed of the iris (albino rabbit). A, indicates the transition from arteriole to capillary. At C, the nerves accompanying the capillary are visible. × 250.

3 Capillary at higher magnification (× 700) showing alternating muscle cell nuclei and neurilemma neuclei. The beaded nerve fibre can be seen in the neighborhood of N. M, is a muscle cell nucleus and R, a neurilemma nucleus.

4 The nerve nework in the iris. Two short lengths of capillary vessel can be made out on the left. Otherwise the capillaries are unstained. × 250. Figures 1 A, 3 and 4 are from the same preparation of intact material, unsectioned.


M
N
N
N
V
1


C
2
A


M
N
R
M
N
N
3


T
1
A

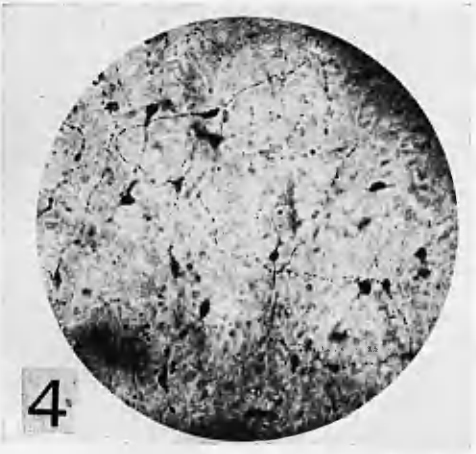

4

PLATE 2

EXPLANATION OF FIGURES

5 Camera lucida drawing of part of the vessels shown in figure 1.

6 Neurilemma cell nucleus overriding a muscle cell nucleus at the forking of a capillary vessel.

7 Camera lucida drawing of the details of an arteriole undergoing transition into a capillary. N, nervous; M, muscular element; S, stroma.

8 The middle nucleus is that of a neurilemma cell. Observe its peripheral situation.

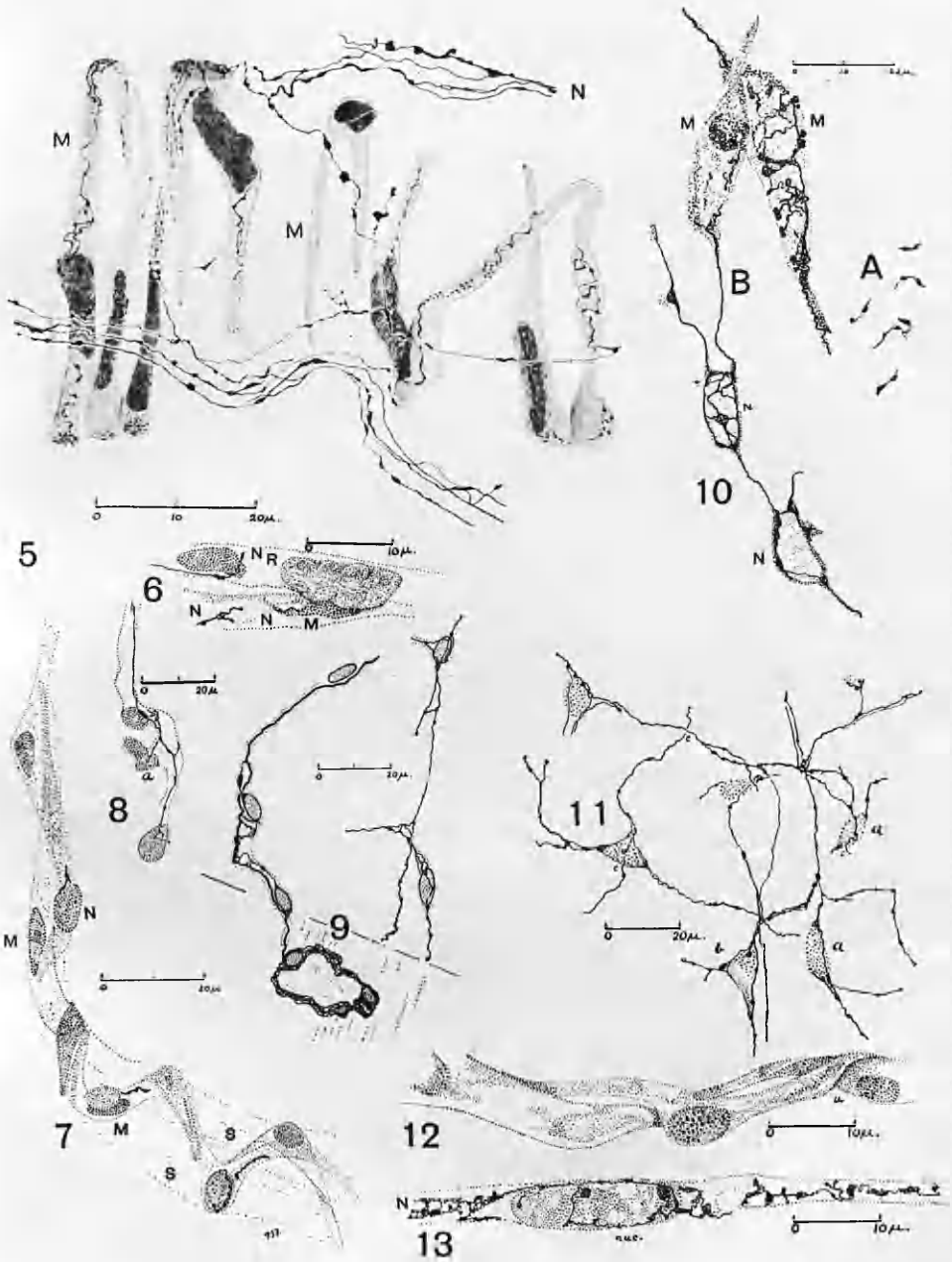
9 Neurilemma cell nuclei and nerves, myelinated and non-myelinated for comparison with figure 11, which is of the iridical network.

10 A, low power view of tissue fragments in lungs of Testudo referred to in text. The fragments are compound and consist of nervous and muscular components, shown more clearly in B. The appearance is identical with that found in the iris and generally in mammals, and is the result of the breaking up of the column of staining fluid in the capillaries. The process reveals the dual character of capillary structure.

11 Iridical nerve plexus from the iris. No capillaries are stained in this area (see fig. 4).

12 Parts of three smooth muscle cells from a capillary in the iris, showing spiral arrangement. Nerves unstained.

13 Part of a smooth muscle cell from the sphincter iridis, showing intracellular nerve network and its relation to the nucleus.


M
N
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0 10 20μ.
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N R
0 10μ.
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0 20μ.
a
8
0 20μ.
9
N
M
0 20μ.
7
M
S
S
11
a
0 20μ.
b
a
12
a
0 10μ.
N
0 10μ.
13

34.

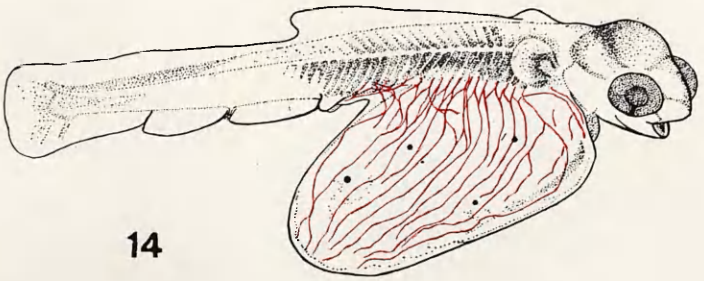
PLATE 3

EXPLANATION OF FIGURES

14 Young salmon larva showing plexus of nerves along the edge of the myotomes and yolk-sac extensions.

15 Details of the plexus.

16 A yolk sac branch of the plexus, showing minute granular extensions around the young blood vessels before these have acquired a cellular wall.



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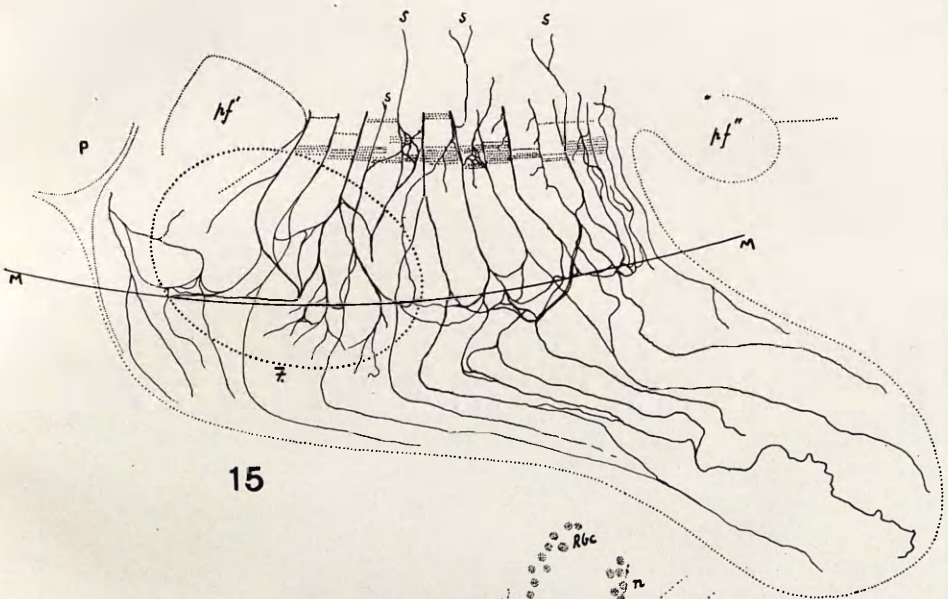

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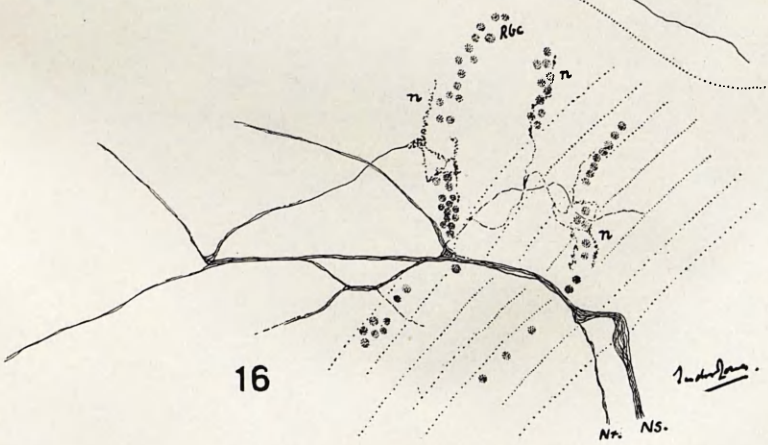

16

The ANSA of WRISBERG.

The English accounts of the connexion, on both sides of the body, between the Phrenic and Splanchnic nerves, through the medium of the semi-lunar (coeliac) ganglion, referred to as the "anse mémorable" by Testut, have descended, practically unaltered, from Sharpey. The following from the seventh edition (1867) of Quain's Anatomy is typical:-

> Semilunar ganglion. --- The solar plexus contains .... several ganglia; and by the presence of these bodies, and their size, it is distinguished from the other prevertebral plexuses. The two principal ganglionic masses, named semilunar, though they have often little of the form the name implies, occupy the upper and outer part of the plexus, one on each side, and are placed close to the suprarenal bodies by the side of the coeliac and the superior mesenteric arteries. At the upper end, which is expanded, each ganglion receives the great splanchnic nerve.
>
> Diaphragmatic plexus. --- The nerves (inferior diaphragmatic) composing the plexus are derived from the upper part of the semilunar ganglion, and are larger on the right than on the left side. Accompanying the arteries along the lower surface of the diaphragm, the nerves sink into the substance of the muscle. They furnish some filaments to the suprarenal body, and join with the spinal phrenic nerves.
>
> At the right side, on the under surface of the diaphragm, and near the suprarenal body, there is a small ganglion, ganglion diaphragmaticum, which marks the junction between the phrenic nerves of the spinal and sympathetic systems. From this small ganglion filaments are distributed to the vena cava, the suprarenal body, and the hepatic plexus. On the left

side the ganglion is wanting, but some filaments are prolonged to the hepatic plexus. (1).

Thane's drawing (2) is not completely accordant with this description; but it embodies interesting features not mentioned in the description, for example, the curious mode of communication between the lower end of the phrenic nerve and the medial half of the semilunar ganglion, arched over by the roots of a large nerve supplying the suprarenal gland. A short length of porcupine quill (the lowermost) passes behind this communication in the photograph (Figure 1.) The intricacy of the ansa of Wrisberg is perfectly illustrated in the remarkable drawing which Léveillé made for Hirschfeld. (3).

Macroscopically, an ansa of this kind forms, with the spinal cord, a complete circle, of which there are many examples. That under discussion is, however, excepting the two sympathetic chains, united in front of the coccyx, the largest in the body in respect of its radius. Macroscopically, it presents an appearance more or less consistent with the neurone theory in the adult, but not in the embryo, in which it is complete, a neuroreticulum for the most part without nerve cells or even cell-nuclei of any description. Its composite character is secondary, and it is therefore not remarkable that components ascribed to one end of the ansa may in reality belong to the other. If the nerves

of the body grow outwards from a common axis like the branches and twigs of a tree, it is easy to understand that their terminations should be separated from one another. The arrangement of the constituent parts of an ansa such as has been described is altogether different, and would be imitated if we should take, without breaking them, two twigs and bind them together so that their ends faced in different directions. This is to regard the formation as it is understood to exist in the adult. In the course of development it is different. It is to its development, therefore, that we must look for an explanation of its closed form. In the adult, no part of the loop can be finally disposed of without reference to the other parts.

To return then to established facts, that part of the total formation which is known as the phrenic nerve was shown by Ferguson (4) to contain sensory fibres to the proportion of one in three in man and in the cat. In the neck, sympathetic fibres are now said to reach the phrenic nerve through the middle and inferior cervical ganglia from the seventh and eighth cervical and the first thoracic nerves. (5). Luschka (6), whose great monograph is the basis for most descriptions since it was written, included in its distribution the costal and mediastinal pleura; the diaphragmatic pleura; the inferior vena cava and, on the right side, the right auricle of the heart; the diaphragm;

the anterior abdominal peritoneum from the sternum to the umbilicus; the ligaments of the liver; the sub-diaphragmatic inferior vena cava and the suprarenal body. Pansini (7) attributed to a plexiform, ganglionated communication between the two phrenic nerves with each other and with the last three intercostal nerves the function of securing a measure of automatism in the movements of the diaphragm.

The occasion for undertaking the present investigation was the discovery in a Bielschowsky-stained human embryo of 24 mm. of a connexion between the right phrenic nerve and the sino-auricular node (8). This passed between the pleura and the pericardium the very short distance to the base of the right venous valve, which becomes later the valves of the inferior vena cava and the coronary sinus.

Material.

The material chosen for investigation was a post-mortem body, the thorax and upper part of the abdomen of which were completely eviscerated after securing the splanchnic nerves, the sympathetic chain and its connexions and the phrenic nerves on both sides. The mass of tissue, comprising the heart, lungs, liver, oesophagus, the aorta below the level of the fifth thoracic vertabra, the right kidney (the left was missing), the inferior vena cava to the level of the renal veins and the right suprarenal gland, the diaphragm

having been dissected from its attachments, was placed in as natural a position as possible in formalin. The appearance of the dissected ansa of Wrisberg and its connexions is shown in the photograph (Figure 1). (Right side).

Special attention is directed to the following features illustrated in the photograph and the diagram (Figure 2.):-

(1) Luschka mentions a branch from the phrenic nerve to the right auricle. This is shown in front of a quill near the upper border of the photograph (marked "1" in the diagram). The nerve was traced upwards to the level of the branch of the right coronary artery which supplies the sinus node. Here it entered the crista terminalis and was lost. The photograph clearly shows the nerve originating from the lower part of the posterior (called by Testut 'inferior') division of the phrenic. It leaves the parent nerve in an UPWARD direction, passing behind the anterior division (called by Testut the superior), with which it communicates.

(2) Buried in the muscle of the diaphragm, the posterior division of the phrenic nerve meets with branches of the inferior phrenic artery and vein, enlarges to several times its original size, divides into separate strands, which intercommunicate and reunite, to expand again in the form of the triangular phrenic ganglion. This ganglion communicates with the aortic sympathetic, with the right vagus nerve,

the coeliac plexus, the suprarenal plexus and a small nodular ganglionic mass at the inner end of the semilunar ganglion. This also communicates with the coeliac plexus, the suprarenal plexus and the semilunar ganglion.

(3) Between its separation from the anterior division and the phrenic ganglion, the expanded nerve mass gives off three flat, ribbon-like straps of nerve, as well as some smaller branches, two passing upwards and forwards above the inferior phrenic artery in to the substance of the diaphragm, and one inwards to the back of the inferior vena cava. In this situation communications are established with the aortic sympathetic. Twigs to the vessel itself ascend in its wall. (Inferior vena cava).

Histology.

The following parts of the nervous formation described were removed, mordanted and cut in celloidin for staining by Weigert's method:-

(1) The phrenic nerve in relation to the superior vena cava.

(2) The nerve immediately above its division into anterior and posterior (Testut: inferior and superior) divisions.

(3) The posterior division.

(4) The anterior division.

(5) The posterior division at four levels, the lowermost being low down in the infra-diaphragmatic

expansion.

(6) The strap-like band passing to the back of the inferior vena cava.

(7) The lower of the two strap-like bands passing in the substance of the diaphragm above the inferior phrenic artery. The upper of these two bands was included in the lowermost of the four pieces enumerated under (5).

In addition, the two splanchnic nerves, the semi-lunar ganglion and the phrenic ganglion were prepared by Bielschowsky's method and sectioned in paraffin.

Results.

The results gained from this procedure are set out in a comprehensive form in Figure 3., which has been prepared by tracing actual photographs of the Weigert-stained sections side by side with a diagram of the whole nervous formation. The parts in black represent bundles of myelinate fibres, stained dark blue by Weigert's method, the parts in white represent amyelinate fibres, the stippled parts represent mixed nerves and the areas containing stellate markings are ganglionic. The striped parts at the foot of the diagram represent nerves in which some amyelinate fibres are scattered. The levels are numbered in order from 1 to 11, and each section is referred to its

correct position in the formation as a whole.

The following major points concerning the formation may be referred to before presenting an account of its detailed structure:-

(1) The ganglion not hitherto described, found embedded in the musculature of the diaphragm, is much larger than the phrenic ganglion (described by most writers and editors as a "small" ganglion). The larger ganglion is composed of three elongated fusiform masses which unite with one another above and below. Like the phrenic ganglion, it is confined to the right side of the body. The total length, including the rather swollen upper end, of the phrenic ganglion in the specimen was 15mm. From the lower border of the phrenic ganglion to the upper end of the fusiform mass was 58mm. From the lower end of the mass itself to the same point above was 40mm.; but it was difficult to distinguish precisely the points of termination above as well as below.

(2) About half of the lead onto the back of the inferior vena cava (i.e. the lower lead) is ganglionic.

(3) The connexion between the phrenic ganglion proper and the tripartite mass is itself ganglionic.

(4) The connexion between the phrenic ganglion and the nodular ganglionic mass at the inner end of the semilunar

ganglion is ganglionic.

(5) The total cross section of the lower end of the phrenic nerve in the thorax ("2" in the diagram) is considerably less than in the upper part of the thorax.

(6) The sum of the total cross sections of the anterior and posterior divisions (superior and inferior divisions) is much greater than the section of the nerve from which these nerves arise.

(7) The posterior division increases greatly in size as it passes downwards, until it is about equal to the phrenic in the upper part of the thorax.

(8) Both the anterior and the posterior divisions are divided by fibrous septa into numerous bundles of nerve fibres. Some of these are myelinate, some not, the two kinds of fibres keeping together.

(9) No bundles of amyelinate fibres could be detected in the Weigert-stained sections of the upper phrenic.

(10) In the posterior division, the bundles of amyelinate fibres coalesce progressivley from above downwards.

(11) In the fusiform mass myelinate fibre-bundles are smaller and fewer above than below.

Reference to the diagram will reveal other points of interest, such as the curiously symmetrical arrangement of the composite parts of the fusiform mass, of which one half (taken by cutting the middle component in two) is the rotated image of the other half. Detailed consider-

ation will be given to this feature and to the appearance of the section represented at level 7. This appearance is shown in the photograph (Figure 4.)

ARRANGEMENT OF NERVE FIBRES IN THE ANSA.

It will be recognised from what has been stated that a continuous mass of nerve cells and their processes connects the semilunar and coeliac ganglia with the upper end of the fusiform mass, (much larger than the 'small' phrenic ganglion,) which the writer proposes to call the SINUS GANGLION, for reasons which will appear later.

The ganglion (Sinus ganglion) may be said to terminate above in a large amyelinate band which enters the phrenic nerve obliquely from below, crosses it in a postero-medial direction, breaks up into smaller bundles, and is thus distributed in an UPWARD direction through the phrenic nerve. The stages of this redistribution can be seen in the diagram (constructed from actual photographs of the nerve) representing the levels numbered 7, 6, 5, 4, 3 and 2. No bundles of amyelinate fibres could be seen in Weigert preparations of the phrenic nerve in the upper part of the thorax. The bundles in the lower part come from below.

At its lower end, the ganglion forks, equally large

parts passing one to the nodular upper end of the phrenic ganglion, the other to the back of the inferior vena cava in company with an amyelinate nerve as large in cross section as the ganglion and some very small bundles of mixed, myelinate and amyelinate nerve fibres. Between the two ends, the ganglion divides into three large parts, united together in several places by amyelinate bundles. Small flat bundles of myelinate fibres intersect these ganglionic columns, while larger bundles of myelinate fibres flank the formation. Amyelinate contributions from the middle ganglionic parts are made at intervals to these small nerves, which, diminishing as they reach lower levels, are terminal twigs of the phrenic nerve itself. No myelinate fibres can be seen intermixed with the cells and amyelinate fibres in the extensive lower parts of the ganglion, and it must be inferred that the preganglionic fibres are themselves amyelinate. The myelinate branches of the phrenic nerve are dorsal in position to the ganglion and spread around it, like the fingers of a hand partially enclosing it. The curiously symmetrical arrangement is shown in Figure 5.

The method used does not lead to certain information concerning the preganglionic fibres of the ganglion. The general arrangement, as ascertained by histological study of the formation, and the gross appearance of the strands gathered together suggest that we are dealing with two

contributory systems, a myelinate system projected downwards and an amyelinate system projected upwards. This upward-proceeding system supplies structures within the diaphragm, probably blood-vessels, and a voluminous stream of fibres to the hepatic veins, the inferior vena cava and the region of the sulcus terminalis of the right atrium. These, it is to be noticed, are all derivatives of the embryonic sinus venosus, and those at higher levels have been displaced upwards in the course of development. The phrenic nerve, therefore, is in a sense the expression of two opposite movements in development, the downward displacement from cervical levels of the diaphragm, and the upward displacement from a lumbar level of the sinus venosus, particularly that part of the sinus incorporated in the heart. While it would be consistent with this developmental complex to find that the preganglionic fibres of the great ganglionic mass described descended in the phrenic nerve to a level corresponding with the earlier situation of the sinus, which received its nerve supply at that level or below it, there is no positive evidence to support this view.

(Evidence of the destination of some of these sinus fibres will be stated later in this paper in describing an investigation of the conditions in the guinea pig by the intra-vital methylene blue method.)

Positive evidence has been forthcoming that, in Cavia, the ascending nerve fibres of the inferior vena cava (which has a long intra-thoracic course as in other rodents) end in the muscle fibres of the tunica media and the atrium in the neighbourhood of the sinu-auricular node.

The writer is indebted to Professor Walmsley (9) for the following note, recording the absence of effect upon an electrocardiographic tracing taken during operation on the phrenic nerve:-

> "Avulsion of right phrenic: adult ♀, aet. 27: local anaesthetic and operation at root of neck: electrocardiograms taken while (1) nerve was exposed and pinced with forceps: (2) nerve was sectioned: (3) nerve was avulsed (lead 2 only). Patient made no complaint during operation: electrocardiograms show no disturbance of heart function at any time."

It appears certain, therefore, that, anaesthetic considerations apart (and the anaesthetic did not prevent contraction of the diaphragm), if the nerve found ascending to the sinu-auricular node in the human subject (and a communication between the phrenic nerve and the node is present in the 24 mm. embryo) is motor and akin to the nerves seen in Cavia, its preganglionic fibres do not descend in the phrenic nerve. *(Tracings: Figure 10)*

The infra-diaphragmatic connexions of the phrenic nerve assume greater importance in the light of this fact, and attention is focussed upon the possibility that the splanchnic nerves contain the preganglionic fibres to the large ganglion described, although the vagus and aortic

sympathetic systems must not be excluded from consideration. In the course of the present investigation, the semi-lunar ganglion, the greater and lesser splanchnic nerves and the communication with the phrenic ganglion were prepared by Bielschowsky's method and studied after cutting; but the evidence gained did not suffice to determine the source of the fibres concerned with certainty. Parts of the splanchnic nerves stained by Mallory's method showed these nerves to contain only a minority of myelinate fibres.

Similar general arrangements and connexions were found in three newly-born infants by Hurst (10).

ASCENDING MOTOR AND SENSORY NERVE FIBRES IN THE GUINEA-PIG.

Figures 6 and 7 are photomicrographs of the phrenic nerve of the guinea pig stained by the intra-vital methylene blue method, the darker portion to the left of each photograph being the inferior vena cava. At "x" in Figure 6 a branch of the phrenic nerve dividing into the small bundles marked "A" and "B" in both photographs is seen leaving the descending phrenic nerve and ascending towards the vein. Figure 7 leaves no doubt that such nerves ascend the vena cava. Figure 8 shows a photomicrograph of the entry of the vein into the pericardium, and is additional evidence of the generally upward direction of

the nerves. The wealth of innervation of this great vessel is no less than that of any other vein, and presents a picture which suggests a functional control far in excess of the rather meagre physiological knowledge concerning it. That one of the functions concerned is active contraction of the wall of the vessel is demonstrated in figure 9, a low power photograph of the guinea-pig's inferior vena cava, showing the adventitia (lighter right-hand part) stripped from the media (darker left-hand part) and held by a dissecting needle. At "x" are visible the individual muscle fibres of the media. In performing the dissection under a binocular microscope, the nerves could be seen in large numbers passing to the media, and, in many cases, terminating in relation to muscle cells within it.

CONCLUSION.

The Ansa of Wrisberg consists principally of a chain of ganglia, intervening between the semi-lunar ganglion and the phrenic nerve. Three expansions were clearly and sharply demarkated in foetuses examined and in the human adult, viz., (1) a globular enlargement at the medial end of the semilunar ganglion, the inferior phrenic (or phrenic) ganglion of anatomical textbooks, and a much larger ganglion embedded in the muscular substance of the diaphragm. This ganglion supplies amyelinate nerves in an upward direction to the phrenic nerve proper, which distributes them with its subphrenic, pericardial and atrial branches.

The amyelinate nerves in these bundles occupy characteristic positions in groups, and do not come from the phrenic nerve in the upper part of the thorax, where there is no such arrangement. Some of the ascending fibres reach the crista terminalis. In the guinea-pig, such ascending fibres have been shown conclusively to innervate muscle fibres of the inferior vena cava, and have been traced to the auricular myocardium in the immediate neighbourhood of the sino-auricular node. The methods of investigation employed do not conclusively demonstrate the source of the preganglionic fibres of the infra-diaphragmatic ganglia associated with the phrenic nerve. Possible sources are the greater and lesser splanchnic nerves, the vagus nerves of one or both sides, and the aortic sympathetic.

Since the conditions described are not found on the left side, where there is nevertheless a slender communication between the phrenic nerve and the semi-lunar ganglion, and in view of the extensive distribution from the ansa to structures derived from the right side of the sinus venosus, there being no left inferior vena cava, the conclusion is drawn that the formation is particularly related to the sinus.

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(1) Quain's Elements of Anatomy, London, 1867. Vol.2. p699.
(2) Quain's Elements of Anatomy, London, 1895. Vol. 3, pt. 2. p374.
(3) Hirschfeld, L. Systeme Nerveux, Paris 1866.
(4) Ferguson, J. The Phrenic Nerve. Brain. Vol. 14. 1891. p282.
(5) Müller, L.R. Die Lebensnerven. Berlin 1924 p. 452.
(6) Luschka . Der Nervus phrenicus des Menschen, Tübingen, 1862.
(7) Pansini. Il Progresso Medico. 1888.
(8) Jones, Tudor. The Lancet
(9) Walmsley, T. (personal communication to the writer).
(10) Hurst, M.M. (personal communication to the writer).

Description of Figures.

Fig.1. Dissection of the inferior diaphragmatic region in a human adult. OES = Oesophagus. D = Diaphragm. V = Inferior vena cava. SP = Great splanchnic nerve. SR = Supra-renal gland. PG = Phrenic ganglion. The semilunar ganglion is at the foot of the photograph. The uppermost horizontal quill is placed behind the branch ascending to the sulcus terminalis, and in front of the phrenic nerve just before its division into anterior and posterior (superior and inferior) branches. The lowermost horizontal quill is placed behind one of the communications between the phrenic ganglion and the globular ganglion at the medial end of the semilunar ganglion.

Fig.2. Diagrammatic reproduction of the previous figure. (Figure 2a. = diagrammatic representation of the conditions in the guinea-pig.) ipa = Inferior phrenic artery. 3 = branches of the aortic sympathetic plexus above the coeliac ganglion. V = Right vagus nerve.

Fig.3. Some of the levels of the ansa submitted to microscopical examination. The sections were drawn from photomicrographs at a constant magnification, and are set to the same scale.

Fig.4. Photomicrograph of the phrenic nerve at level 7 in the diagram (x80). The mass to the left is composed of amyelinate fibres entering the nerve from below.

Fig.5. Photomicrograph of the appearance at level 8. (x80).

Figures 6 and 7. Photomicrographs of the methylene-blue-stained phrenic nerve in the guinea-pig, showing recurrence of the nerves on the inferior vena cava. A and B = recurrent branches shown descending from the nerve at x.

Fig.8. Methylene blue preparation of the inferior vena cava of the guinea-pig at the diaphragmatic level, showing nerves ascending. Photomicrograph (x30).

Fig.9. As above but a higher level. The dark shadow is that of a dissecting needle holding down the adventitia. The darker part of the photograph is the vein. Nerves are visible on both the adventitia and the media, and at x individual muscle fibres are visible. Nerves were traced to these.

Fig. 10 Electrographic tracings.

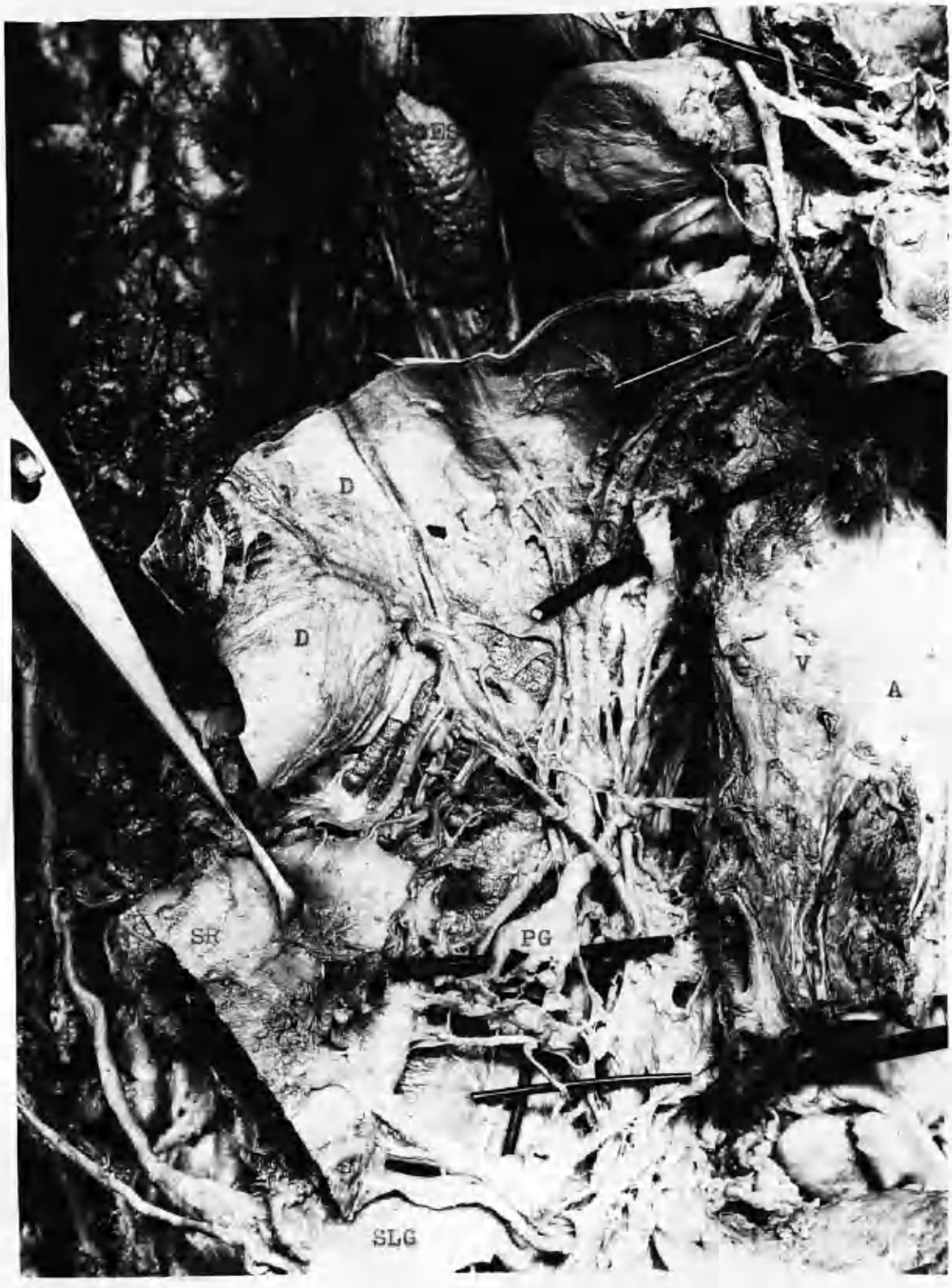


FIGURE 1.

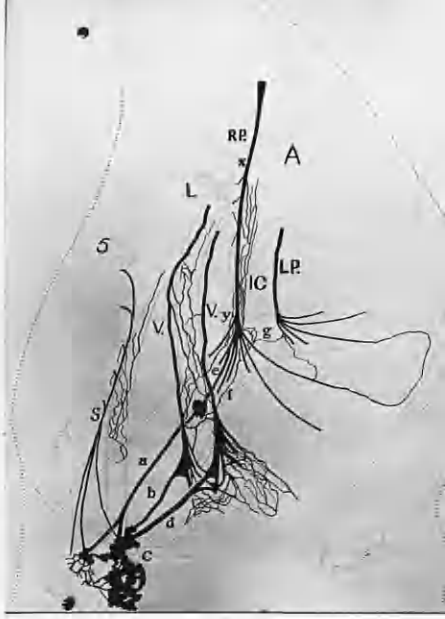


Figure 2a.

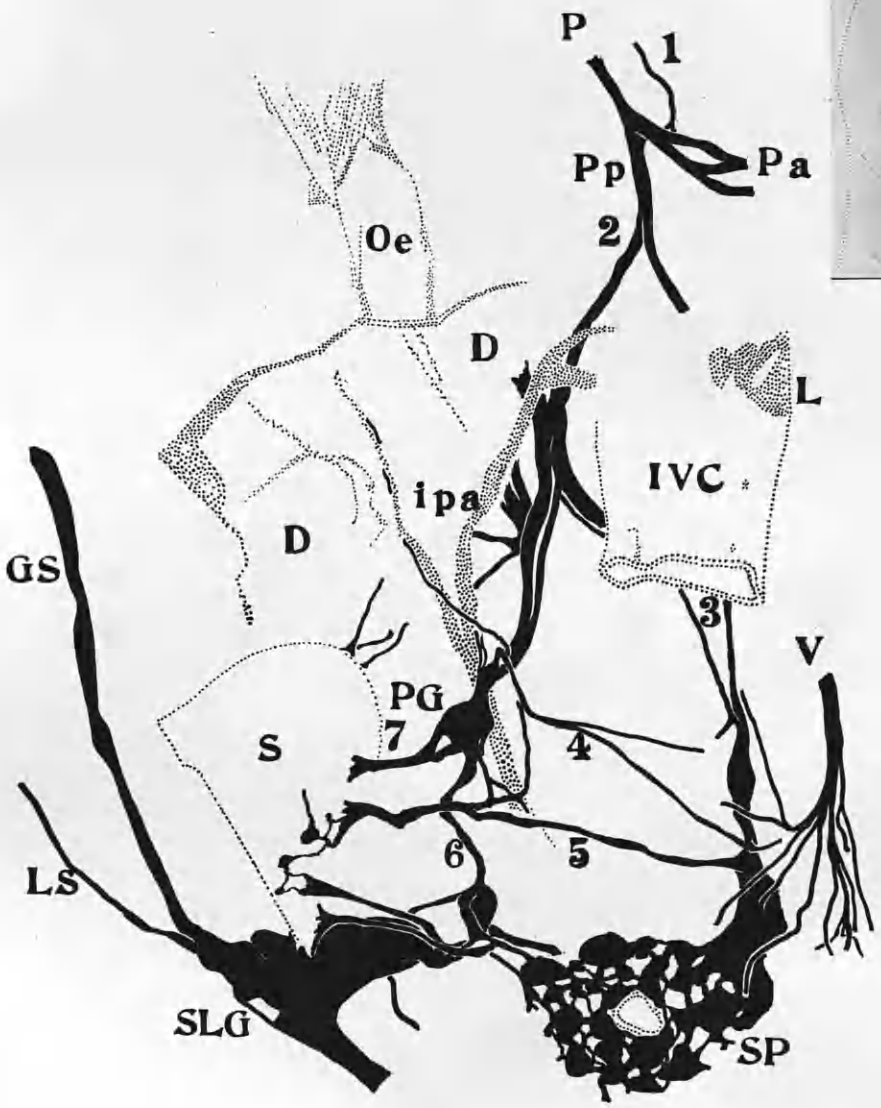


Figure 2.

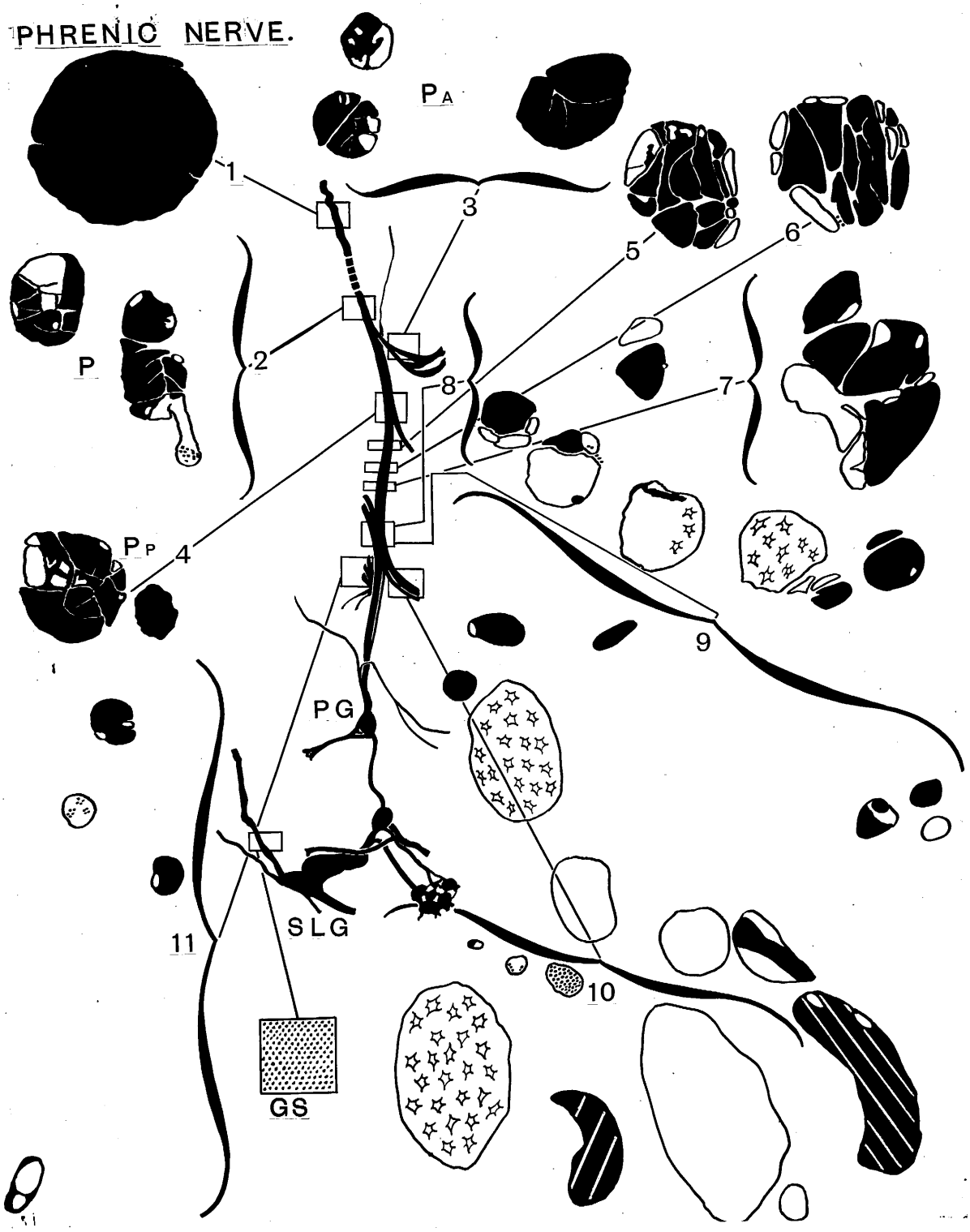


Figure 3.

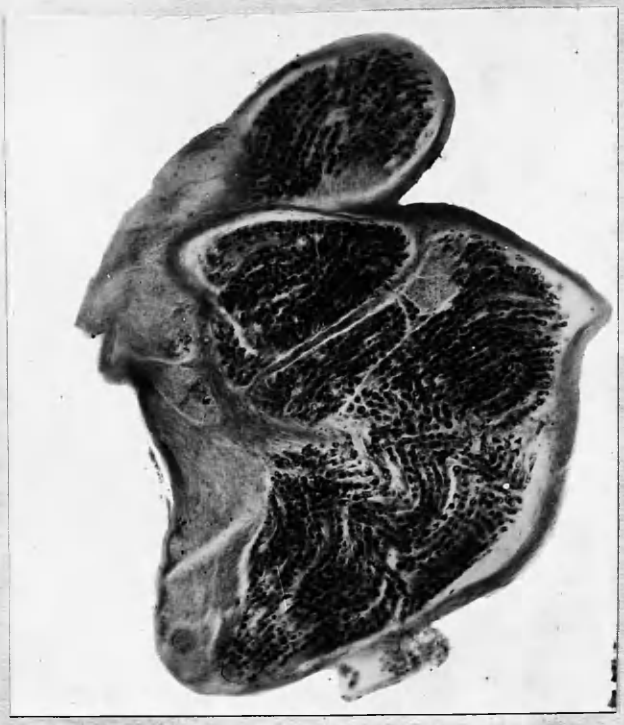


Figure 4.

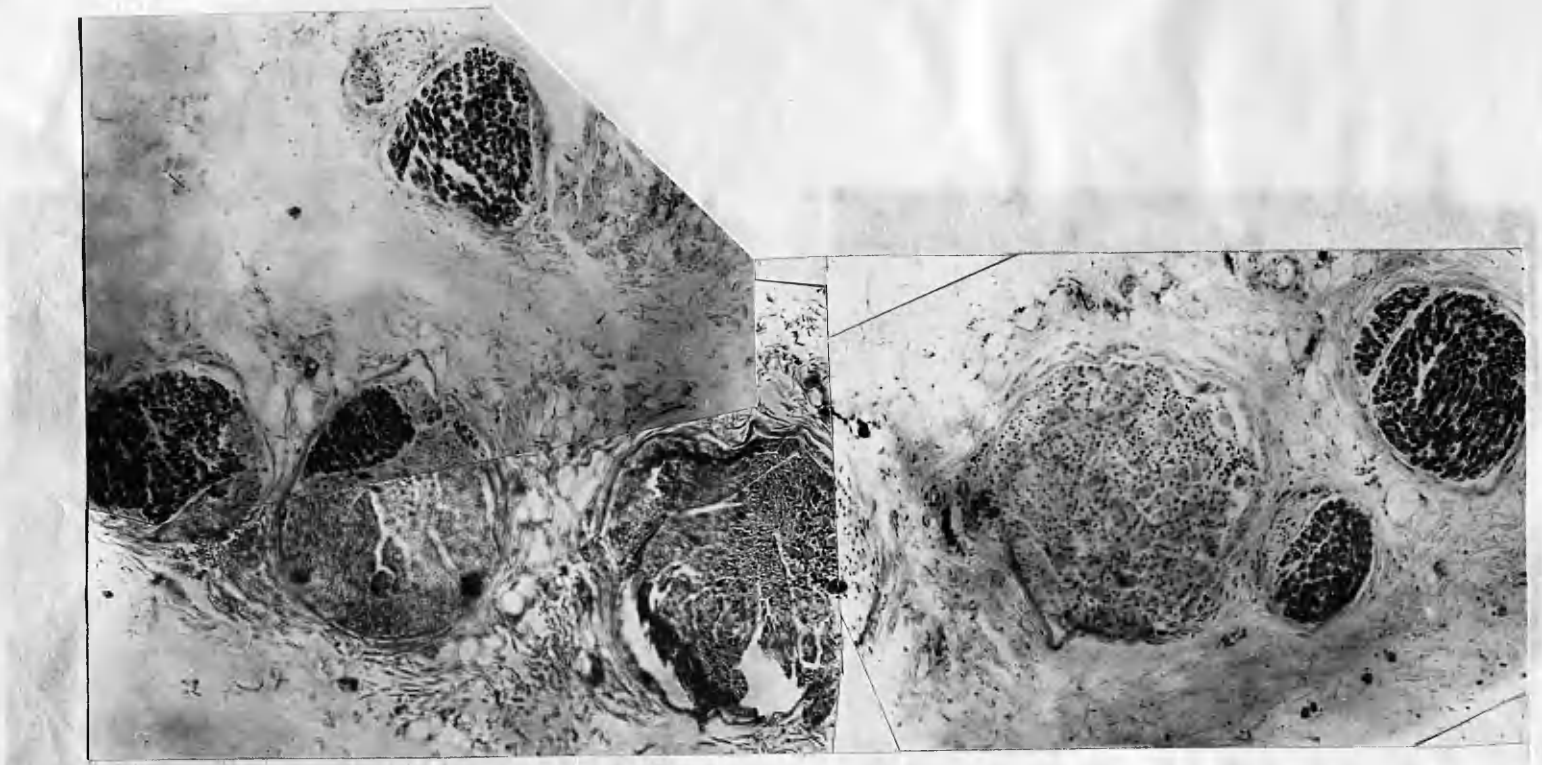


Figure 5.

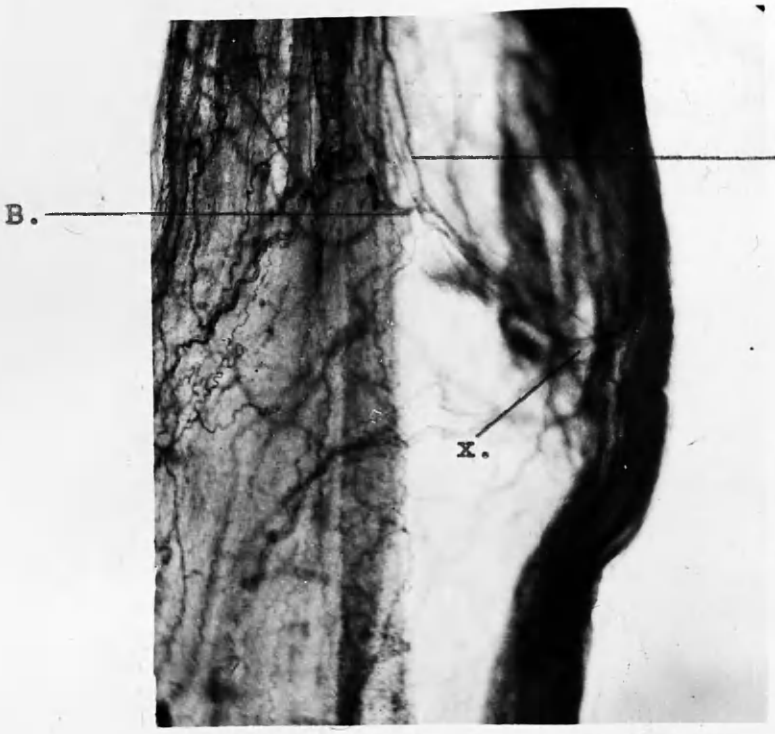


FIGURE 6. x 30.

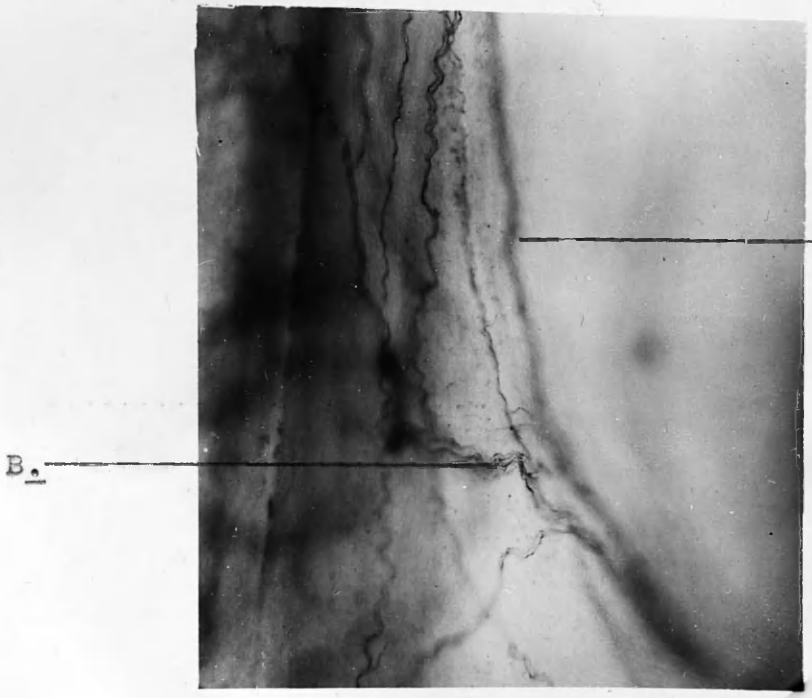


FIGURE 7. x 90.

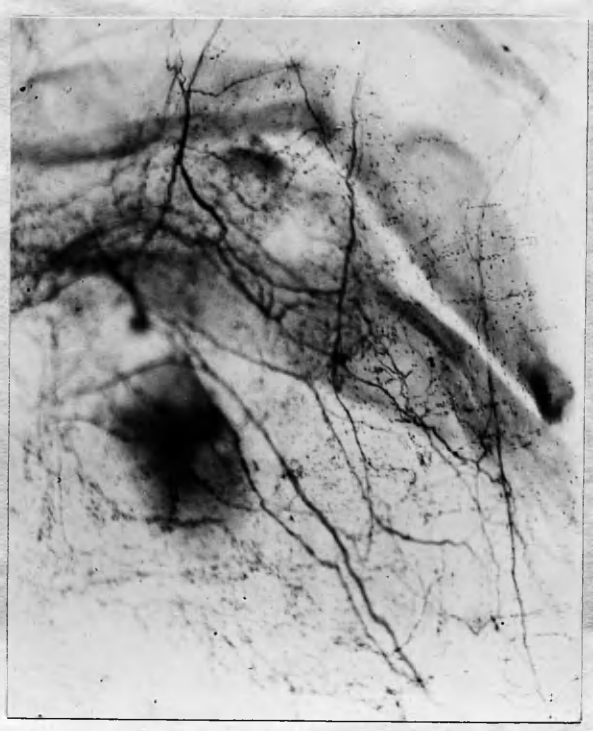
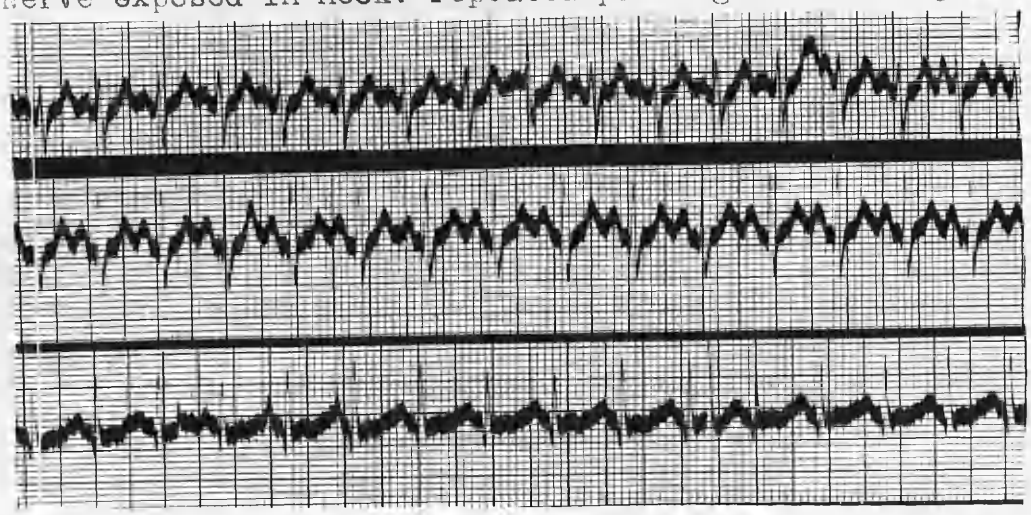


Figure 8 x30.

Figure 9 x30.

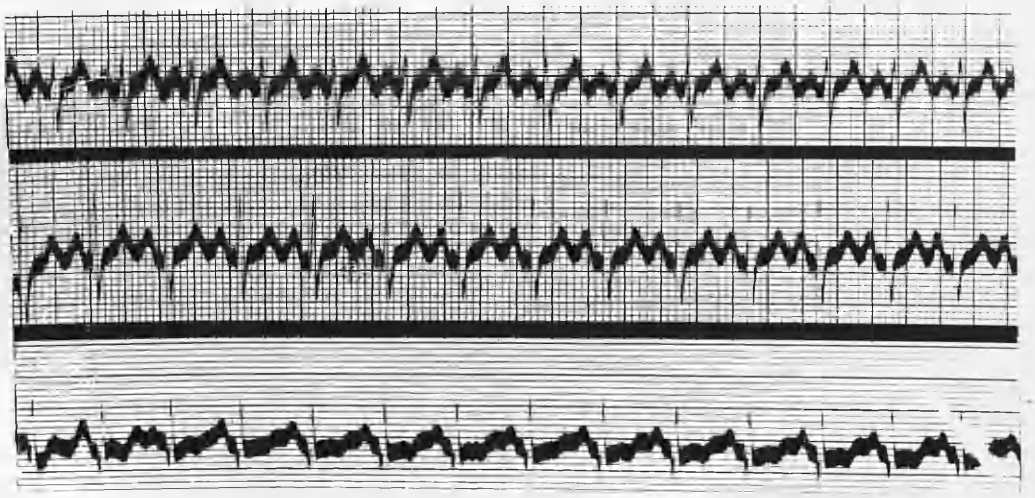
CASE REPORTED BY PROFESSOR WALMSLEY.

Nerve exposed in neck: repeated pinching with forceps.



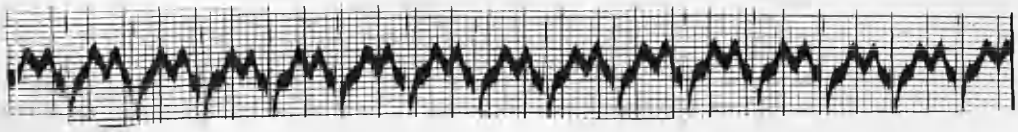
(1).

Nerve divided.



(2).

Nerve avulsed.



(3).

Electrocardiographic tracings.

Figure 10.

THE CARDIAC INNERVATION in a Human Embryo of 24 mm.

The relationship between the present section and the work previously recorded is stated briefly in the introduction:

> The "multiple connexion between the nervous system of the heart and the central nervous system so resembled the conditions in Lepidosiren that it was thought it might represent a transient condition of the developing nervous system, and efforts were made to discover whether the whole of the nervous communications evident in the human embryo were present in the foetus at full term and in the adult."

Two points of developmental importance are involved:-

(1) The presence in the embryo at comparatively early stages (earlier than are here described) of minute connexions which subsequently are merely displaced by the development of the characteristic adult features, which do not interrupt their continuity.

(2) The absence at such stages of the histological features (discrete cell-bodies, dendrites, axons); i.e., the conventionally recognised parts of the neurone.

There is a peripheral 'anlage' of neuroreticulum, topographically representative of the adult central nervous system and all its peripheral connexions and intercommunications, however complex, which has not yet acquired those

histological features which are alleged to be primary.

In Lepidosiren, this reticulum (1) was found at several stages, and its continuity with the nervous system was established as well as its form in relation to the developing heart. This was found to cover all the chief conducting mechanisms, whether nervous or constituted of some 'specialised' tissue, of other vertebrates --- that is to say, practically all the structures which had previously been described under the heading of 'nodes', atypical muscle fibres, accompanied or unaccompanied by nerves, plexuses and ganglia could be referred to some part of the 'preneural reticulum mapped out. Human embryos which, on the ground of their history and state of preservation were thought likely to reward the difficulty and trouble involved in submitting them to Bielschowsky's laborious method for pieces were prepared, each whole embryo being treated as a 'piece', in order to see whether the observations made on Lepidosiren paradoxa could be repeated, with particular reference to the heart.

The method was found to yield its characteristic results in embryos as small as 4.2 mm. (crown-rump length.) Since, however, the characters of the neurofibrillar reticulum remain comparatively unchanged for long after this stage, the embyro now described, in regard to the nervous features of its heart, was chosen for special study. In this embryo, stained by Bielschowsky's method and cut, the neuro-

reticulum has the following visible characteristics, situation for situation:-

(1) THE MANTLE LAYER of the brain and spinal cord: Very slender, discrete, branched filaments of heavily-stained neurofibrillar substance extend in all directions, but chiefly radially, through the thickness of the layer. (Figure 8.) The neuroblastic nuclei of both basal and alar laminae have clear outlines, but although the protoplasmic reticulum can be seen connecting the nuclei with each other, and is, in places, consolidated into a fusiform 'axon cap; the stainable neurofibrillar material forms nowhere more than a thin crust on the surface of scattered nuclei. This is in marked contrast to the conditions in Lepidosiren paradoxa at stages 32 (Keibel) to 37, when the nuclei bordering upon the marginal layer (but not the deeper-situated nuclei) are enveloped in a dense interlacing network of neurofibrillae, similar to those of the dorsal root ganglia. (Figures 11 and 12.)

(2) The MARGINAL LAYER:

(a) Brain: has the general appearance of this layer in the spinal cord. It is a reticulum of intercommunicating protoplasmic processes. The texture is finer than in the cord, and the colour is yellow to brownish red. There is no characteristic reaction to Bielschowsky technique (a black compound with the neural protoplasm).

(b) Cord: as under (a). The characteristic reaction of Bielschowsky is, however, very marked, and can be detected even with the naked eye. In both transverse and longitudinal sections of the spinal nerves, the arrangement of this darkly stained reticulum is similar, the elements fusing with one another to form a network. The filaments of the mantle layer are continuous with this reticulum, and so are the neurofibrillae of the nerve roots; but the individual neurofibrillae are not discrete even for relatively short distances, e.g. 20-40 μ.

(3) The DORSAL GANGLIA: Figures 6 and 7 (the latter taken with infra-red rays) give an impression of the conditions here which is lacking only in that, of necessity, only one plane is photographed, and a proper understanding of the structure can be gained only by focussing, and following the neurofibrillae in three dimensions with the eye. Figures 11 and 12 reproduce the corresponding

appearances in Lepidosiren paradoxa, in which the large size of the elements and the clear-cut differentiation of the neurofibrillae place some of the facts beyond question; in particular, the fact that the neurofibrillar network of one nuclear territory crosses the boundary between it and other nuclear territories, and that there is clear evidence that, whatever occasions it, the tendency to the deposition of the neurofibrillar material in a densely-staining reticulum is one which spreads from nuclear territory (the protoplasm immediately surrounding one nucleus, but continuous with that immediately surrounding neighbouring nuclei) to other nuclear territories. In the case of both the dorsal ganglia and the mantle layer of the cord, the situation in which this process is initiated is one on the periphery of the nuclear group concerned. Unaided by such positive evidence as that provided by Lepidosiren, it is doubtful whether similar appearances would have been detected in the course of mammalian development, for, not only are the elements concerned much smaller in mammals, but the neurofibrillar substance itself appears to be more finely divided and thus tends to form diffuse clouds. Nevertheless, if figures 6 and 7 are carefully compared with figures 11 and 12, a close correspondence can be discerned, and in any case, if the features to which attention has been drawn are

painstakingly sought, abundant instances can be found in which the continuity of the neurofibrillar network from nuclear territory to nuclear territory is clearly apparent. It is surely no reflection upon the existance of objective appearances that observation of some of them is proportional to close and patient attention.

At least the evidence is conclusive, in the writer's mind, that the nervous organisation of the 24 mm. embryo has not reached that stage in which the neurones of histology have been constituted. In the case of the grey matter of the spinal cord, the process, so far from its having terminated, has scarcely begun.

(4) NERVE ROOTS: These are not distinguished from one another by any observable difference. The grey and white rami are not separated, and the common ramus of communication contains finer neurofibrillae than the spinal roots. The nerve twigs in the digits are as well developed as the limb plexuses, and in both these peripheral situations the neurofibrillae are heavier and more massive than in immediate relation to the central nervous system. In the vagus nerve and in the hypoglossal the fibrillation is finer than in the spinal nerves. The photomicrograph of the phrenic nerve in the diaphragm (Figure 9) and that of the same nerve at the place of origin of its cardiac communication

(which terminates in the sinu-auricular node) (Figure 10) give an excellent impression of the appearance of the cross-section of the peripheral nerves generally. It will be seen how prominent a feature is the transverse anastomosis of the neurofibrillae --- a feature which is as evident in sections of nerves cut longitudinally. The sharp differentiation of the neurofibrillae from the surrounding tissue elements is well shown in Figure 9, which was taken with a red screen and has not been retouched in any way. It will be appreciated that by following such nerves as are here illustrated to their finest branches conclusive evidence can be gained concerning the appearance of the smallest subdivisions of the nervous system, and that such small elements can be studied in the light of certain knowledge, not dependent upon staining reactions, that they are nervous structures. In this way errors of interpretation have been practically eliminated in the present work.

(5) SYMPATHETIC GANGLIA of the SYMPATHETIC CHAIN: of the synaptic regions of the nervous system, these are less well-formed than the dorsal and ventral horns. Here and there a small 'polar cap' can be seen in relation to a nucleus, forming an irregular brown-black 'crown', like the masses shown in figures 6 and 7 (of the dorsal root ganglia); but they are much less well-developed.

'Flecks' of neurofibrillar material are scattered throughout the field covered by the ganglia, and these, although often 'gnarled' and branched, have a common direction.

(6) PERIPHERAL GANGLIA other than the Sympathetic chain: The study of the situations in which these should appear introduces a difficult problem. These situations are such formations as the Ansa of Wrisberg, already described in the adult, the heart, the neurofibrillar plexuses at the root of the lung (which are expansive in section), the gut, and so on. In the heart of Lepidosiren, it was found that the neuroreticulum formed a closed system which was capable of being modelled (1). This formation contained no cell nuclei, and therefore no nerve-cells. Yet, in its anatomical relationships, it represented the whole of the conducting mechanisms. Modified in accordance with the evolution of the human heart, the latter contains a like formation, shown in the diagram on the following page. It is, however, more diffuse than in the case of Lepidosiren, and the strands which compose it are merely thin 'streams' of neurofibrillae in close contact with the surrounding myocardium. One cannot say of it, therefore, that it is non-nucleated, although, since even the great nerves are in this condition, it is unlikely that any 'sheath' elements are present. It is,

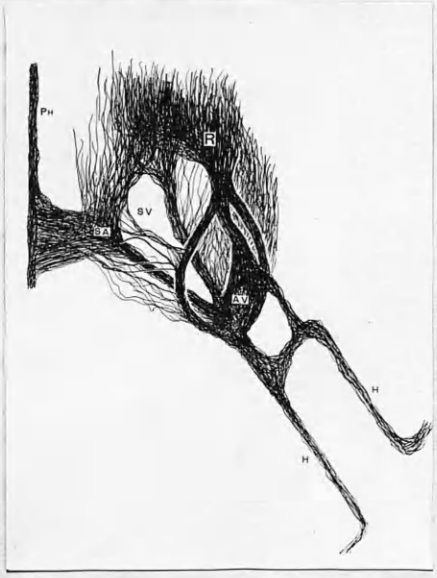


DIAGRAM representing the neurofibrillae in the heart of the 24 mm. human embryo.

Ph = phrenic nerve.
SA = sinu-auricular node.
AV = auriculo-ventricular node.
SV = opening from sinus venosus.
R = fibrillae in the atrial roof and on the dorsum of the heart.

nevertheless, significant that in the aortic plexuses (which in section are large masses of neurofibrillae), in the bridge of neural tissue joining the phrenic nerve with the sinu-auricular node, and in the masses at the roots of the lungs, as well as in the nerves in relation to the gut, there are no cell-nuclei of any kind, and it seems reasonable to infer that the peripheral ganglia are not yet constituted, although all their connexions anatomically are represented. This is in line with Ross Harrison's view that the sheath nuclei (sheath cells) travel along the nerves (neuroreticulum) (2). There is no reason why nuclei perhaps more closely related to neural function should not do the same.

Anatomy of the Heart in the 24 mm. Embryo.

The form of the heart, modelled in wax, is shown in Figure 1. Figure 4 shows the more anterior parts, with the aorta, pulmonary artery and ductus arteriosus. The azygos vein entering the superior vena cava is also shown. The nerves are the right and left vagus, the right recurrent laryngeal, the right and left phrenic and, below the parts of the heart represented, the nerves descending in relation to the sinus venosus and left atrium to participate in the formation of the cardiac nervous mechanisms.

Figures 2 and 3 are representations of the "dissected" wax model, the "steps" in Figure 3 indicating additions to the part shown in Figure 2. "A", Figure 3, and "C" are respectively the aorta and the left posterior cusp of the aortic valve. The ridge between the figures "7" and "8" (Figure 3) is the edge of the right venous valve, "7" in Figure 2 marking its base extending dorsally to the sulcus terminalis ("6"). "9" is the left atrium, and "9'" the pulmonary venous outgrowth. The bridge between "8" and "9" (Figure 2) is the septum primum, and the ledge to the left of "8")in the diagram) is the dorsal end of the septum secundum. Thus, the sinus venosus is still a tube-like cavity interposed between the two atrial expansions. This is well shown in the diagram which follows this page, representing a still more caudad level that Figure 2. In this diagram, the left atrium is represented only by the right and left lower pulmonary veins.

The heavy black lines of Figures 2 and 3 represent the distribution of neurofibrillae in the parts modelled. A photomicrograph of a section at the level of the visible surface of the model shown in Figure 2 is given in Figure 16; and a part of this section at a higher magnification is shown in Figure 15. In the latter, the neurofibrillae are visible at "N". The details of the neural histology within the heart are shown in Figures 5, 13 and 14.

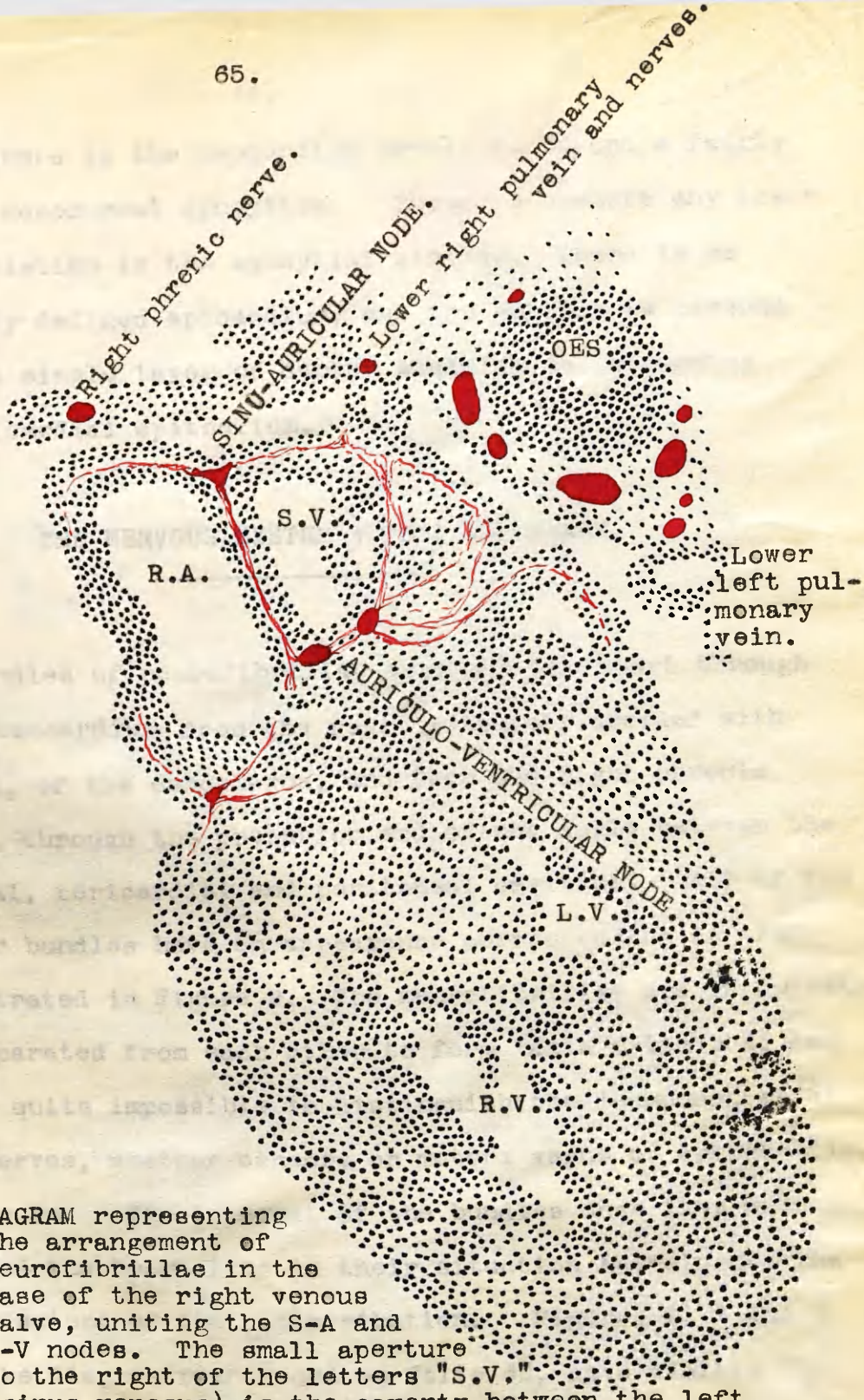


DIAGRAM representing the arrangement of neurofibrillae in the base of the right venous valve, uniting the S-A and A-V nodes. The small aperture to the right of the letters "S.V." (sinus venosus) is the caverty between the left venous valve and its base.

Nowhere is the myocardium developed beyond a fairly dense mesodermal syncytium. There is nowhere any trace of striation in the syncytial strands. There is no clearly defined epicardium, and the surface is covered with a single layer of darker staining cells forming a pericardial epithelium.

THE NERVOUS SYSTEM WITHIN THE HEART.

Bundles of neurofibrillae approach the heart through the mesocardium from the region, densely crowded with nerves, of the oesophagus, and from the right phrenic nerve, through the posterior end of the angle between the pleural, pericardial and peritoneal cavities. All of the larger bundles have an appearance corresponding to that illustrated in Figure 9. The neurofibrillae are incompletely separated from each other to form 'axis cylinders,' and it is quite impossible to distinguish the 'components' of the nerves, whether sensory or motor; vagus or sympathetic, and so on. The interest of the bundles once they have reached the heart lies in their situation therein and the great extent of their distribution. Figures 2, 3 and 4, and the diagram reproduced on folio 62, give visible expression to these facts. The diagram is a drawing of a model in copper wire, and does not reproduce the various streams of neurofibrillae otherwise than diagrammatically.

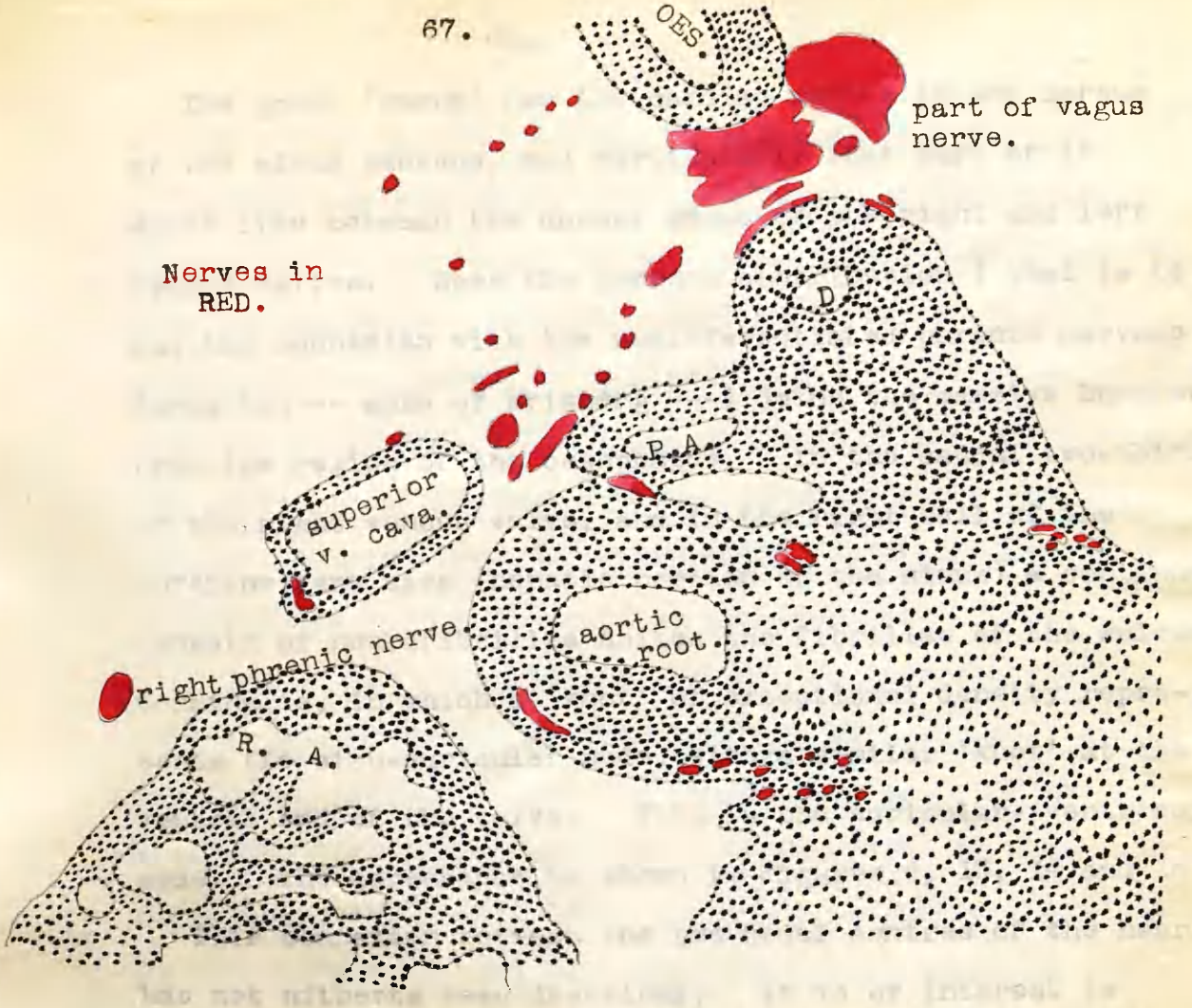


DIAGRAM: RELATION OF NEUROFIBRILLAE. Arterial level.

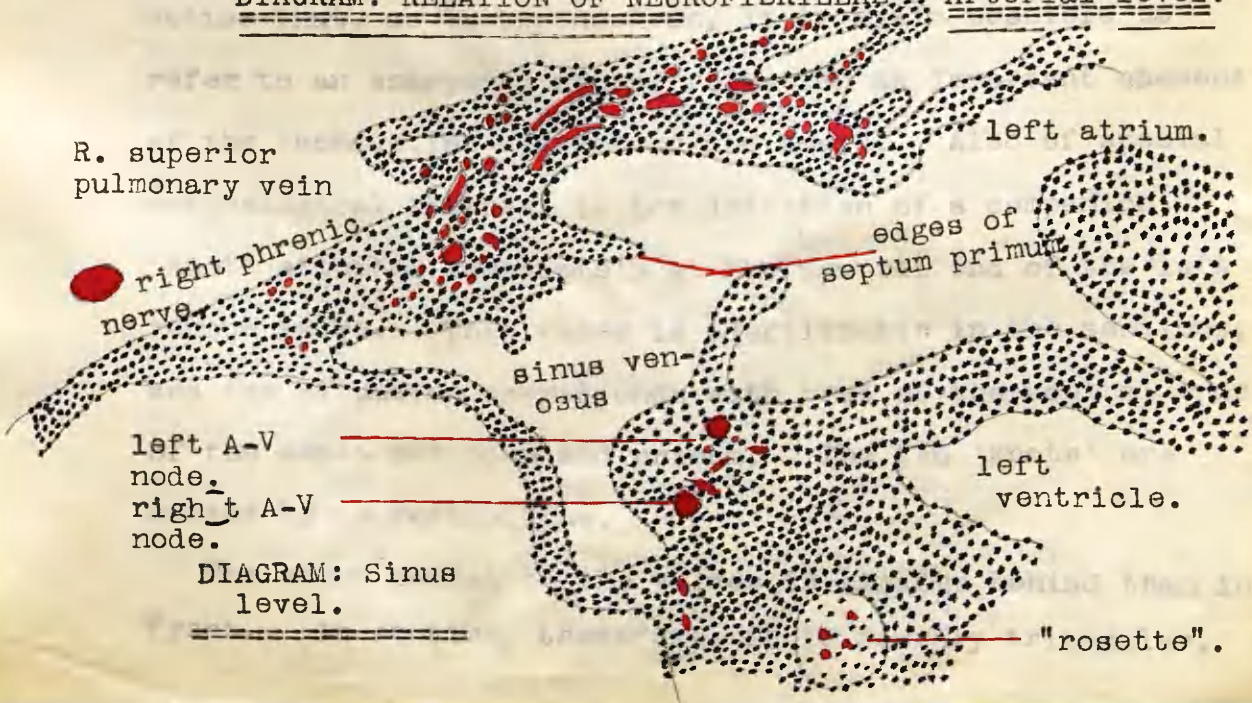


DIAGRAM: Sinus level.

The great 'focus' for the cardiac nerves in the dorsum of the sinus venosus, and particularly that part of it which lies between the dorsal edges of the right and left venous valves. Here the phrenic contribution (that is to say the connexion with the undifferentiated phrenic nervous formation -- ansa of Wrisberg ---) joins the massive bundles from the region of the oesophagus. In the caudal two-thirds of the right venous valve, and in the right wall of the inferior vena cava (hepatic portion of the sinus) a continuous 'sheet' of neurofibrillae unites the fibrillae of the sulcus terminalis, in which a 'knot' of exceptional density represents the sinu-auricular node, with a similar 'knot' at the ventral end of the valve. This is the auricular- ventricular node. The appearance is shown in Figures 5, 13, 14 and 16.

This connexion between the two nodal centres of the heart has not hitherto been described. It is of interest to notice that, as in Lepidosiren, it is again possible to refer to an embryonic neural formation an important element of the 'conducting System' of the heart. Also of special morphological interest is the detection of a companion 'knot' situated immediately at the ventral end of the left venous valve. This valve is identifiable in the sections, and the situation corresponds with that of the ventral ends of the septa secundum and primum. The two 'knots' are united by neurofibrillae.

The sinus caudal to the valves is broader behind than in front. In section, therefore, it is roughly triangular,

and each side of the triangle is occupied by a part of the cardiac nervous mechanism. The rest of the system is best described as an extension of this triangular formation, about which it is symmetrical anatomically, and would be geometrically symmetrical if the right and left sides of the heart were of equal size. The orifices of the superior vena cava, inferior vena cava and coronary sinus are all within the triangle. In other words, this part of the mechanism is a true ring at the sinu-auricular junction. The apex of the triangle lies behind the interventricular septum, and is formed by the right and left auriculo-ventricular nodes joined together by neurofibrillae. Of the two nodes the left is, presumably, that recognised by Keith and Flack following Tawara (3). From this situation, lateral extensions embrace the auriculoventricular canal to unite at the ventral end of the interatrial septum, which, with the atrial roof, forms a means of reunion of the formation with that part which lies behind the heart. The lateral walls of the two atria is another mode of effecting this reunion of what may be termed the post-sinus and post- ventricular parts of the formation. As described up to the present point, the formation, although more diffuse than in Lepidosiren, is exactly comparable. In the dipnoan, however, there is merely a septal plug instead of a complete ventricular septum, and the two limbs of the Bundle of His extend ventrally from the situation of the A-V node; while

in the mammalian heart a complex process of development unites the interatrial, interventricular and interbulbar septa, the pars membranacea septi of man representing a potential failure in the region of union. In the writer's preparations, it is clear that the ventral end of the septum primum affords one means of transmitting neurofibrillae to the site of the dorsal end of the interventricular septum, and, in addition to to situation where the Bundle of His of the left side arises from a common communication with the A-V node. Particular attention should be given to the fact that, at the dorsal end of the interventricular septum, the atrial canal is exceedingly short, and the walls of the sinus and ventricles are practically continuous with one another. If this point of union be taken as a centre, it will readily be appreciated that the septal mechanisms of the atria and ventricles may be regarded as sickle-shaped extensions from it, one cranially, arching over the inside of the atrial roof, while the other passes first caudally then cranially to reach the bulbus. Although differently expressed, this description accords very closely with Frazer's (4). The 'hole' in front of the A-V. node in the diagram on folio 62 corresponds with the pars membranacea septi, and it will be clear, if this account has been followed, that there is no necessity for any interruption, or migration of the nervous parts in order that the complete formation described should

be constituted. It is possible that the same developmental processes which divide the atrio-ventricular canal merely bring the nervous parts together, or, alternatively, that the tubular heart merely expands within the meshes of its own neural primordium. Additional force is given to this suggestion in regard to the statement of Keith and Mackenzie, (5): "We were able to verify that fact that the part of the left auricle (vestibule) in which the pulmonary veins end is a derivative of the sinus venosus. The nodal tissue of the frog occurs at the sino-auricular junction, but instead of being continuous round the whole extent of that junction, as in the eel, it is concentrated in one part. As one ascends the scale of animals the concentration becomes more marked." The present investigation shows that the concentration is a developmental acquirement, and, in regard to the sinus origin of the pulmonary vein, the relationship of the atria to the sides of the sinus 'triangle' is identical on the two sides of the heart. The right posterior angle of the 'triangle' is displaced to a situation anatomically in front of the sulcus terminalis (S-A. node). The left posterior angle is not: the left atrium, with the pulmonary vein, is still farther to the left. The point is probably of more fundamental significance than the observation of tissue changes such as are to be expected as one passes to a structure so different from the atrium as is the pulmonary vein.

Before the Bundle of His finally separates into a right

and left part, there is a curious appearance which the writer is unable to explain. According to Walmsley, (6), the A.-V. bundle is recognisable in the 19.35 mm. stage as "not unlike that of the anlage of a sympathetic ganglion, only it is poorer in the number of cells." The point of Walmsley's arrow, indicating the "ventricular septum" marks a circular tissue-irregulatity matched by another like it in the figure (Figure 63, op. cit.) slightly to the (anatomically) left side. These appearances may be incidental to focus (the figure represents a photomicrograph); but the situation is, in any case, that of the appearances referred to. Walmsley does not draw attention to the point. In the writer's preparations, the situation is occupied by a mass of darkly staining nuclei and branched, syncytial cytoplasm containing neurofibrillae, which are also seen in the interspaces. Such appearances extend from the bulb to the diaphragmatic wall of the ventricles, and have in some situations a "rosette-like" form, the whole formation being continuous with the bundle of His on the one hand, and with the A.-V. nodes on the other. It is partially divided into two parts, a left and a right, which is more caudal in its extent, the left being the larger. The axis of the formation is the chief site for neurofibrillae which, in some places form quite large tufts, as seen in section. Is it possible that the more massive parts of this formation are such a centre of growth of the bundle and, arising from it, of the muscular myocardium, as

Retzer (7) has suggested? There is commencing striation in skeletal muscle, but none in the cardiac muscle in these preparations.

CONCLUSIONS.

The writer can find no record of the application of a highly selective technique for the staining of nervous structures to human embryos so small as those here studied, which range from 3.5 mm. to 24 mm. In view of the facts here brought to light, e.g., the non-cellular character of the neuroblasts as late as the 24 mm. stage, and the absence of discrete 'axons' and sheath nuclei, and well as the continuity of the cardiac nervous system and its definable relation to the structures specifically referred to as 'conducting mechanisms', the greatest interest centres in this stage, at which, while the embryological characteristics of special interest are still retained, the heart as a gross structure is well developed. In regard to the development of the form of the heart, few additions can be made to the findings of those who have laid the foundations of our knowledge of cardiac embryology and amatomy. Among these, however, is the considerable reason for regarding, in the light of its nervous relationships, the dorsal aspect of the sequence sinus-atrium-ventricle as being so short as to be practically non-existent, there being thus a common 'focus,' coinciding with the dorsal nervous pathway to

the heart, about which the cardiac tube expands to attain its characteristic form. While this is more strikingly apparent in Lepidosiren, close study reveals it to be equally a feature of the human heart. The writer suggests that if the expansion of the heart is studied from the point of view of the nervous structures now shown to be present, the 'natural relativity of the tissues' may be admitted to be sufficient explanation for an otherwise profoundly obscure embryological event. A simpler instance of this process is the way in which the lateral line of the vagus in Lepidosiren, while retaining all its connexions, literally 'cuts through' the lateral muscle mass to reach its deep situation near the notocord, and, by means of its connexions, dividing at the same time the dorsal part of the mass into myotomes.

The left venous valve in the preparations now under review is a well-formed semi-lunar cusp at the same level as the base of the right valve, which is, however, very much broader, as will be seen on estimating the distance between the upper figure "7" in Figure 3 and the figure "5" in the same illustration. On the same scale, the height of the left valve is no more than one seventh or one sixth of this distance. The writer can find no reason in his preparations for referring the origin of the conducting system to the left valve, as Retzer does (7). This,

however, raises the question of what is meant by this functionally significant term and the similar 'connecting' system? There is a richly innervated "core" of the heart, constant in its morphological relationships and possessing some atypical adjuncts which are as constant. Of this system, the first elements to be recognised were the atypical adjuncts, and the latest the continuous innervation. In development the order is the reverse, and the present writer would point out that recognition has already been given to the fact that the earliest appearance of the 'anlagen' of the adjuncts (nodal tissue) is in a form in which differentiation from the surrounding tissues is not marked by the special features seen in the adult. As Walmsley says, "in a human embryo of 19.75 mm., length, the bundle of His can be recognised,... the nuclei being very dark and the protoplasm poorly staining,... not unlike that of the 'anlage' of a sympathetic ganglion."

If anything is deserving of the special appellation 'conducting' or 'connecting' system surely it is the whole, not the parts in order of their discovery. The whole was there before the parts were discovered. Again, as in the dipnoan so far removed from Man, not merely some but all of the conducting mechanisms, whether they are described under the heading 'nodes' and atypical muscle fibres or nervous, ganglia or plexuses, are referable to a continuous system of neurofibrillae present in the human embryo.

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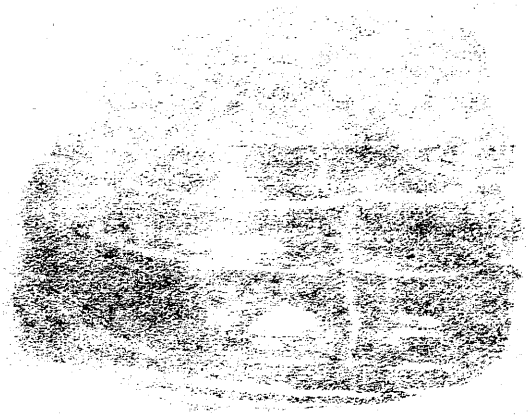
1. Tudor Jones. Trans. R.S.E., Vol. LVII. Pt.1. No.6. p.225, 1932.
2. Ross G. Harrison. J. Comp. Neurology. Vol.37.1.123.
3. Keith and Flack. J. Anatomy and Physiology, Vol XLI. 11. 173. 1907.
4. Frazer. J. Anat. Vol.LI. 1916.
5. Keith and Mackenzie. Lancet, 1910. Vol.1. p.101.
6. Walmsley. Quain's Anatomy. Eleventh Edn. Vol.IV. Pt.III. 1929. p.97.
7. Retzer. Contributions to Embryology, No.272. Washington, 1920.

Description of Figures:

Figure 1. Wax model of the Heart of the 24.mm. embryo.
2. Part of the model at the level of the right venous valve. The heavy dark lines in the 'dissected' spaces indicate the situation of neurofibrillae.
3. The same as previous figure with 'steps' added to the level of the aortic valve.
4. Diagrammatic representation of the cranial part of the model:
RV, LV.= Right and left ventricles.
RA, LA.= Right and left auricles.
PA. = Pulmonary artery.
D. = Ductus arteriosus.
A. = Aorta.
AzV. = Azygos vein entering superior vena cava.
P. = Phrenic nerves of right and left sides.
VV. = Vagus nerves.
R. = Recurrent laryngeal nerve.
6. = Sinu-auricular node.
5. = Communication with A.-V. node (2).
2. = A.-V. node.
3 and4 = Right and left parts of bundle of His.
5. Photomicrograph (x720) of the connexion between the sinu-atrial and atrio-ventricular nodes. "S" = sinus venosus.
6. Dorsal root ganglion. The red lines indicate situations in which neurofibrillar continuity between adjacent nuclear territories is visible; while the figures indicate the number of such territories concerned.
7. As 6. The photograph was taken with infra-red rays.
8. Basal lamina.
9. Phrenic nerve of right side in the diaphragm.
10. As 10, showing branch to sulcus terminalis, and sinu-atrial node.
11. The conditions in Lepidosiren corresponding to those illustrated in Figures 6 and 7.
12. As 11. "x" indicates a part of the neuroreticulum less dense than on the surface of the ganglion. It involves at least four nuclear territories.
13. Neurofibrillae in the A.-V. node.
14. As 13.

Figure 15. Neurofibrillae in the left wall of the left atrium (n).

16. Low-power photomicrograph of the embryo at the level represented in previous figure.



Wax Model of the Heart.
24mm. Embryo
(Human).

FIGURE 1.

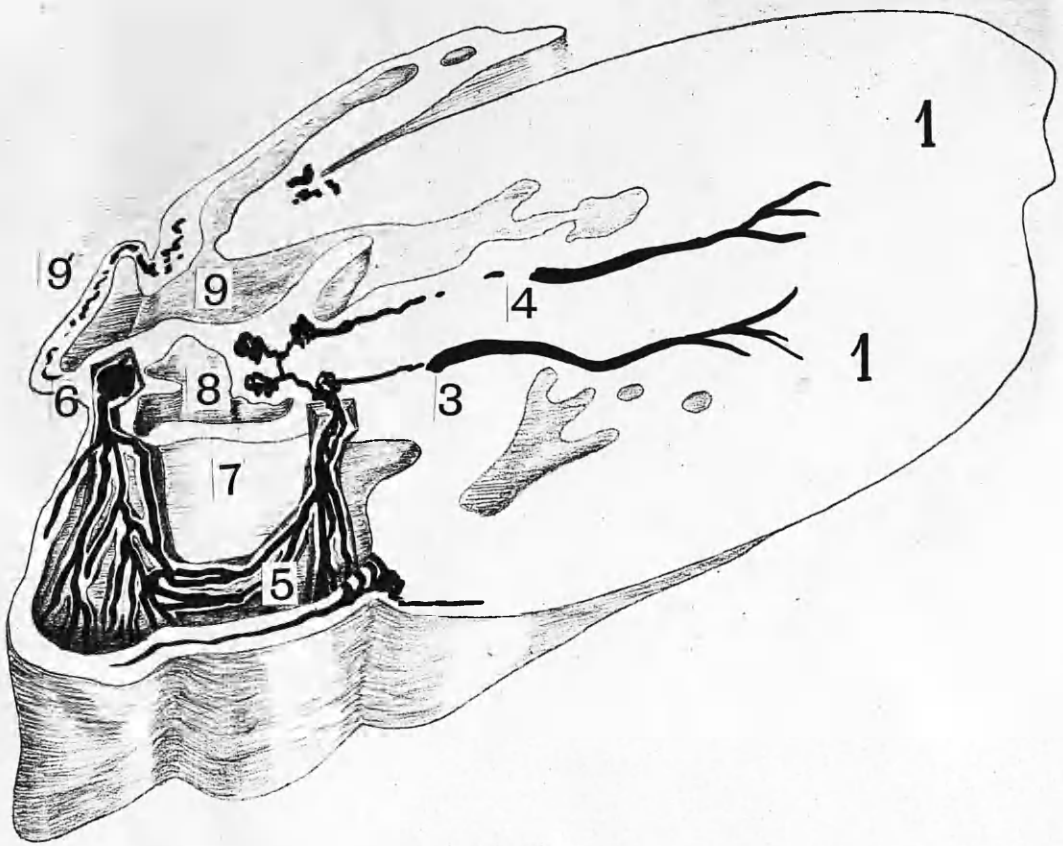


FIGURE 2.

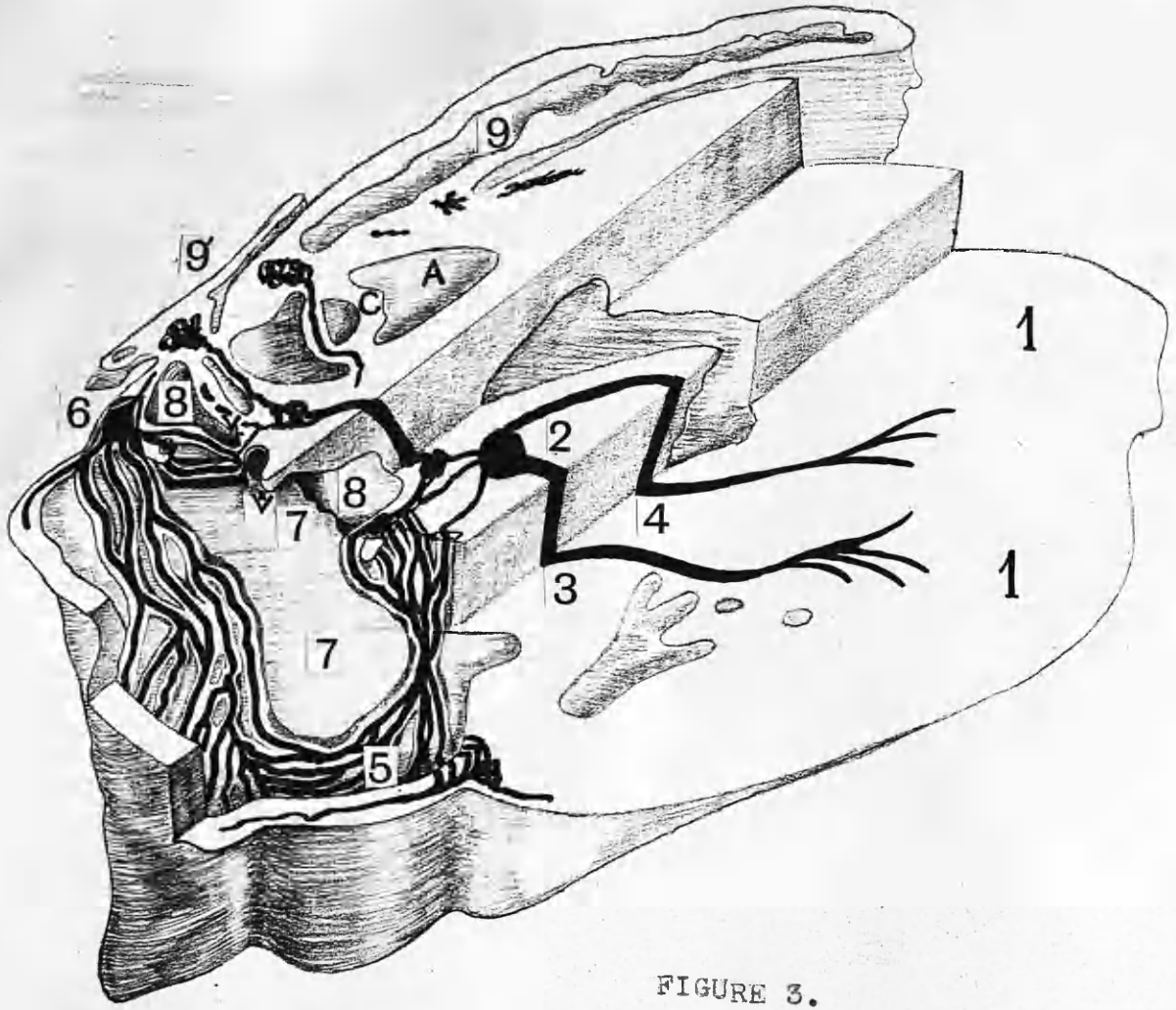


FIGURE 3.

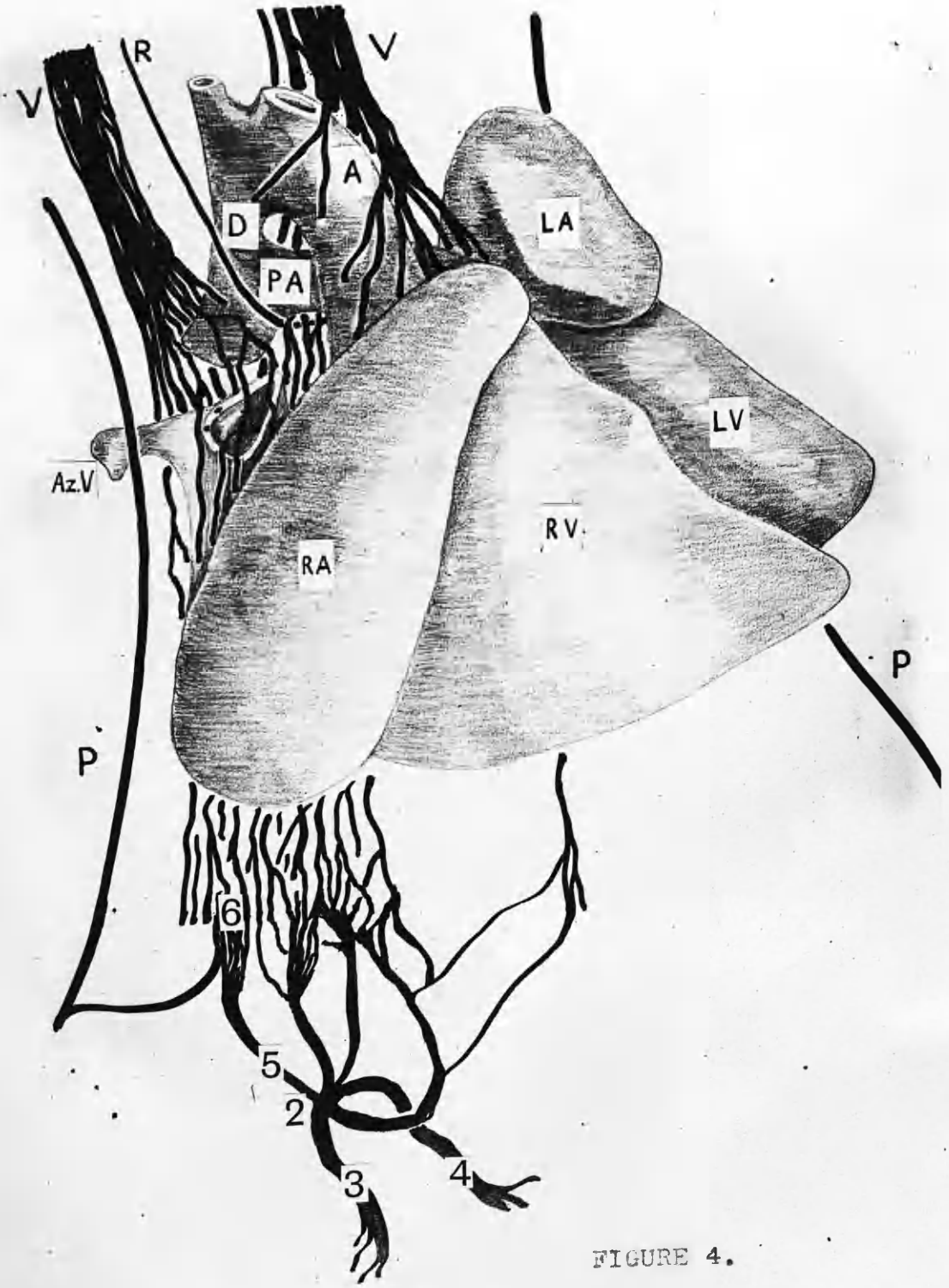


FIGURE 4.

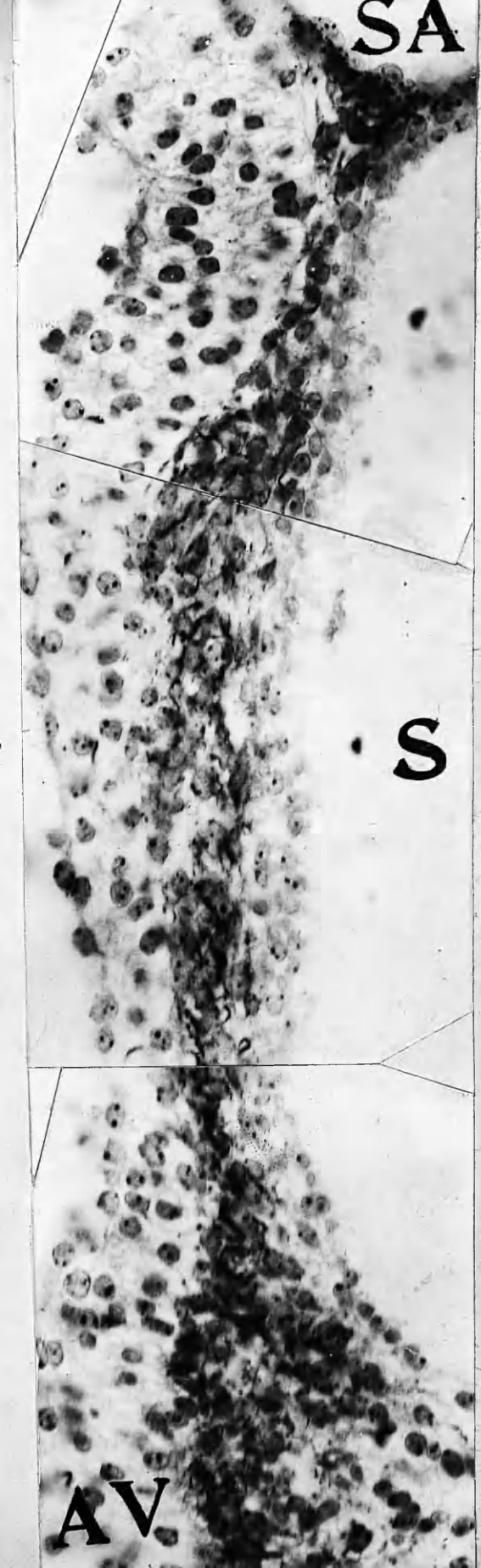
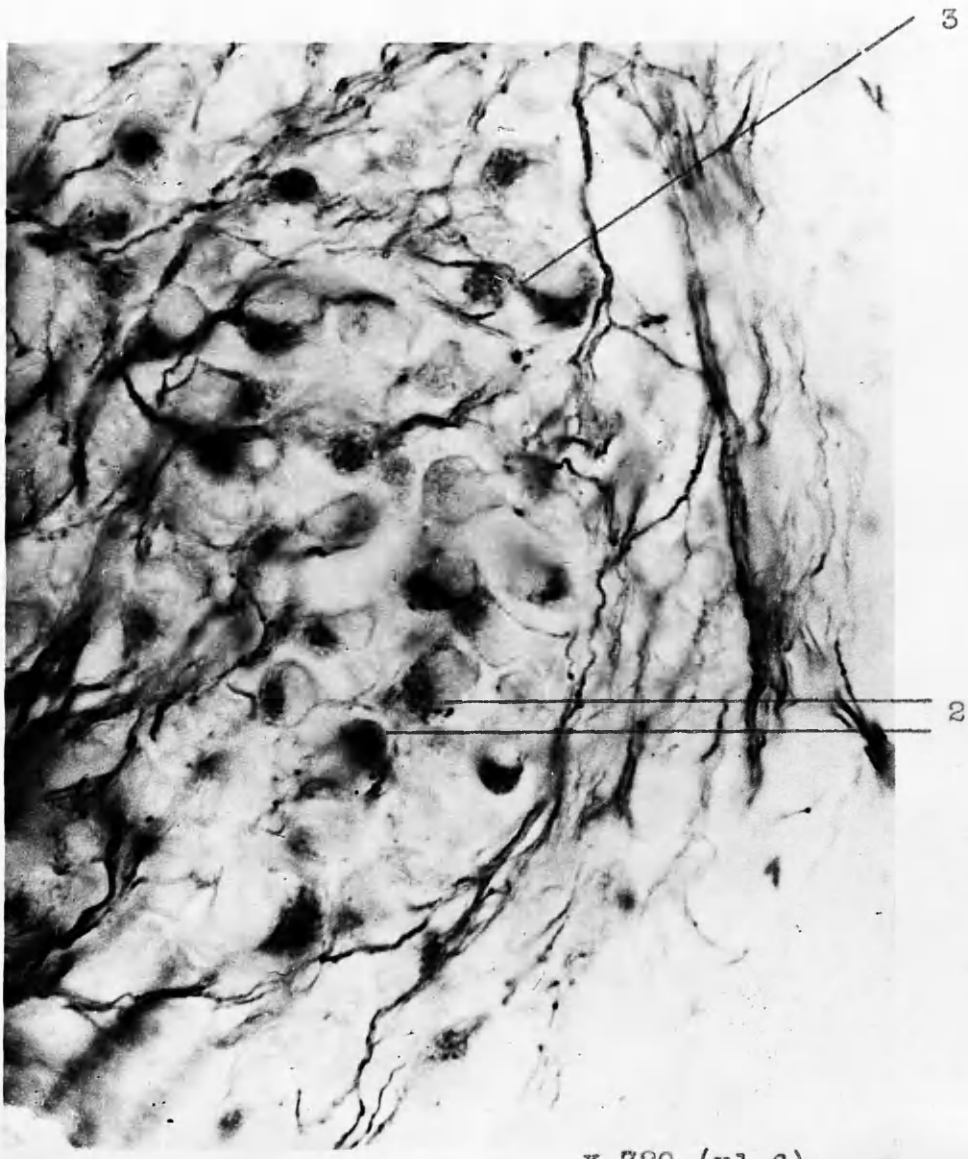


FIGURE 5.

x 720 (x1.6)

Human 24 mm. embryo.
Dorsal root ganglion. (Bielschowsky.)

Figure 6.



Human 24 mm. embryo.
Dorsal root ganglion (Bielschowsky.)

FIGURE 7.

x 720 (x1.6)

Human 24 mm. embryo.
Basal lamina (Bielschowsky.)

FIGURE 8.

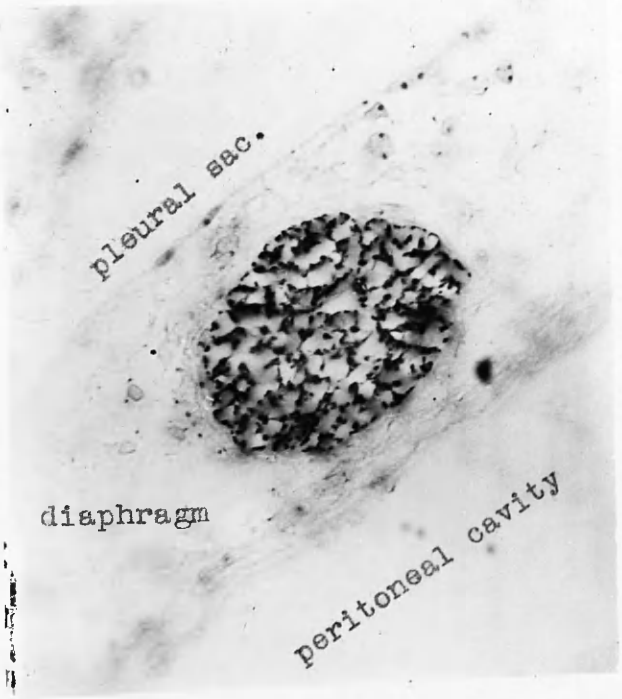


FIGURE 9. x 320.

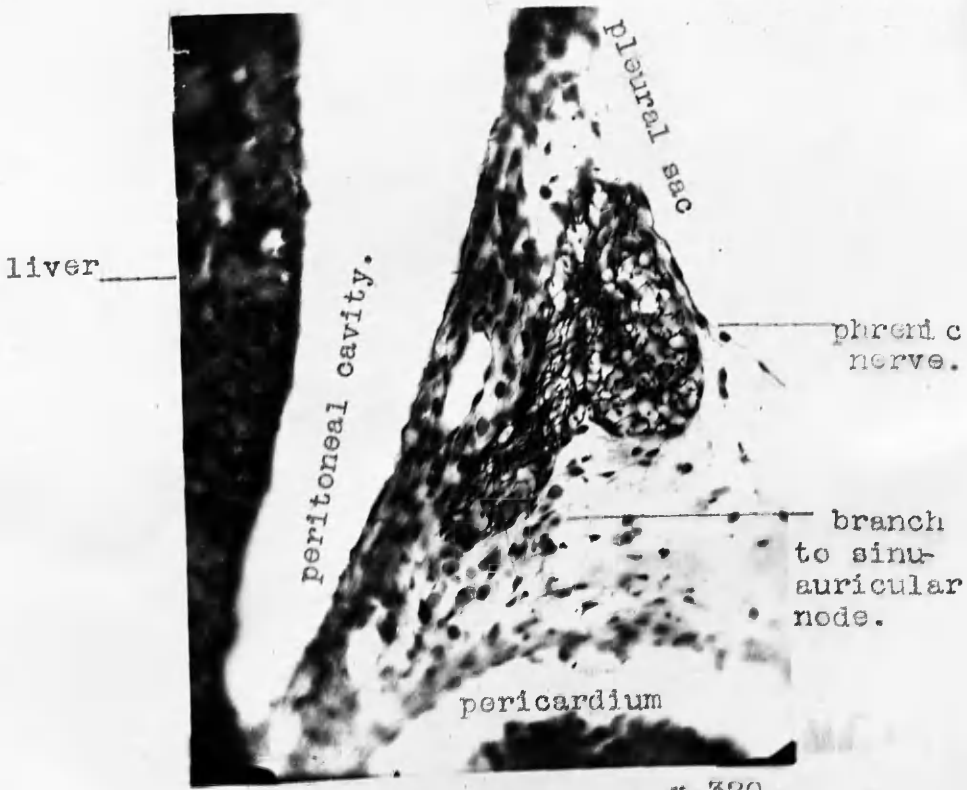


FIGURE 10. x 320.

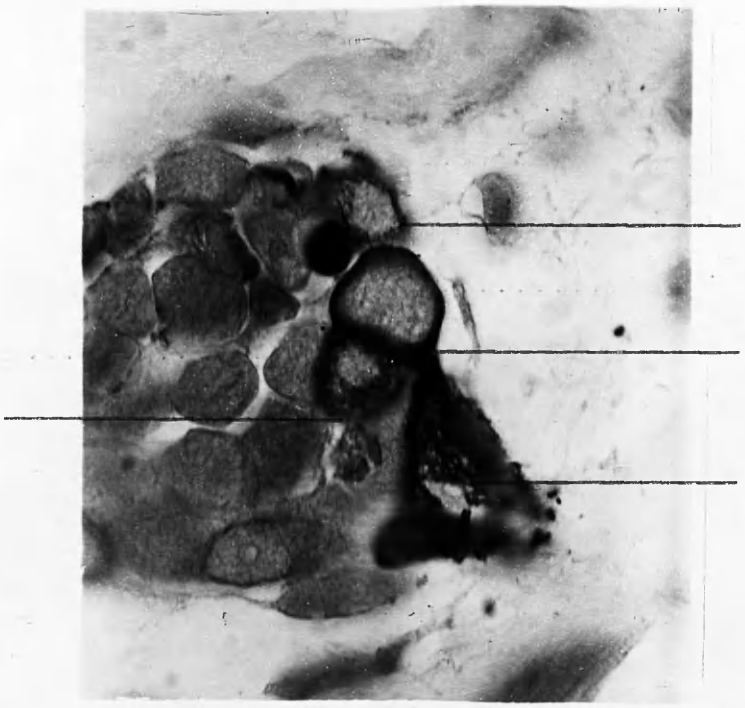


FIGURE 11. x 720.

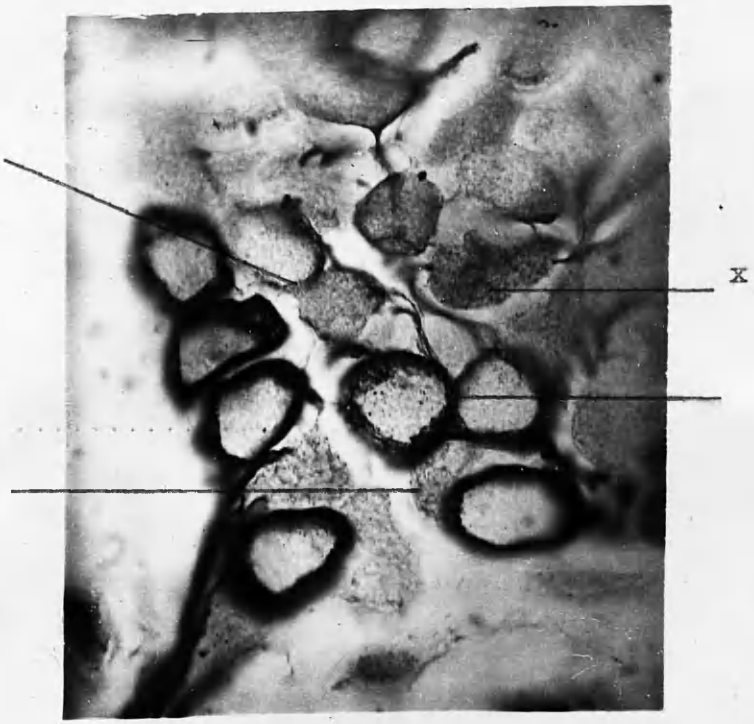


FIGURE 12. x 720.

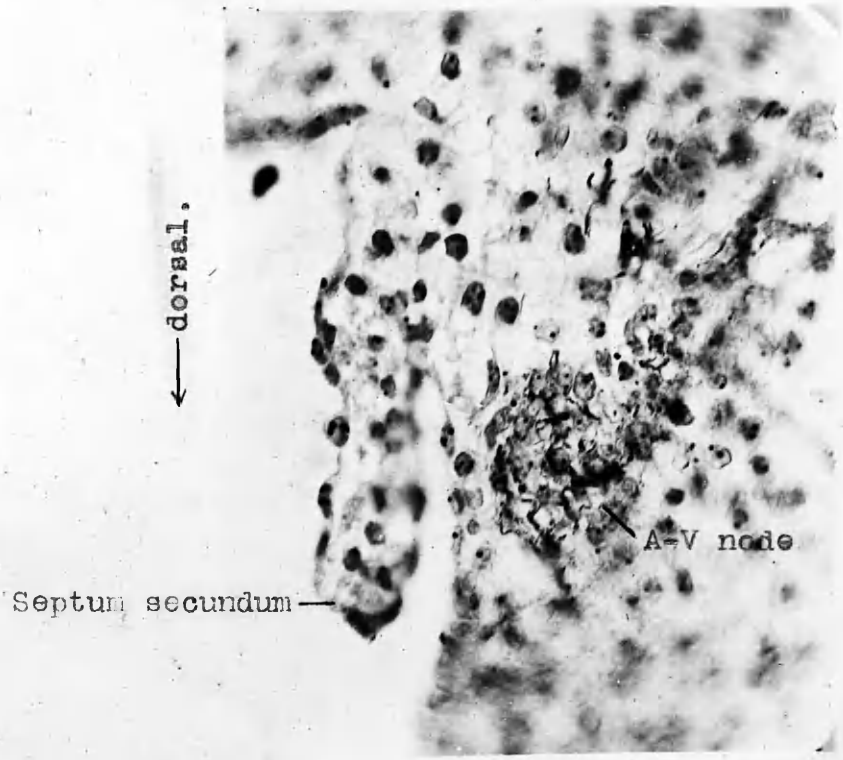


FIGURE 13. x 720.

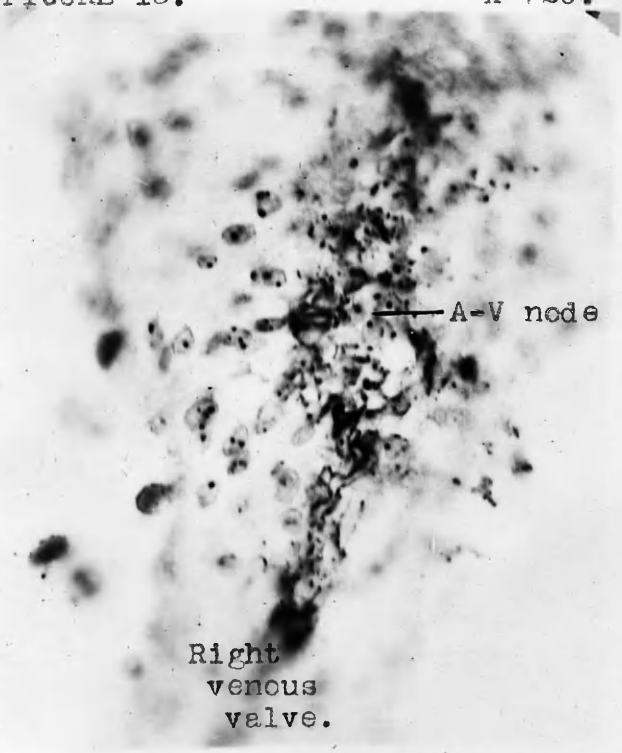


FIGURE 14. x 720.

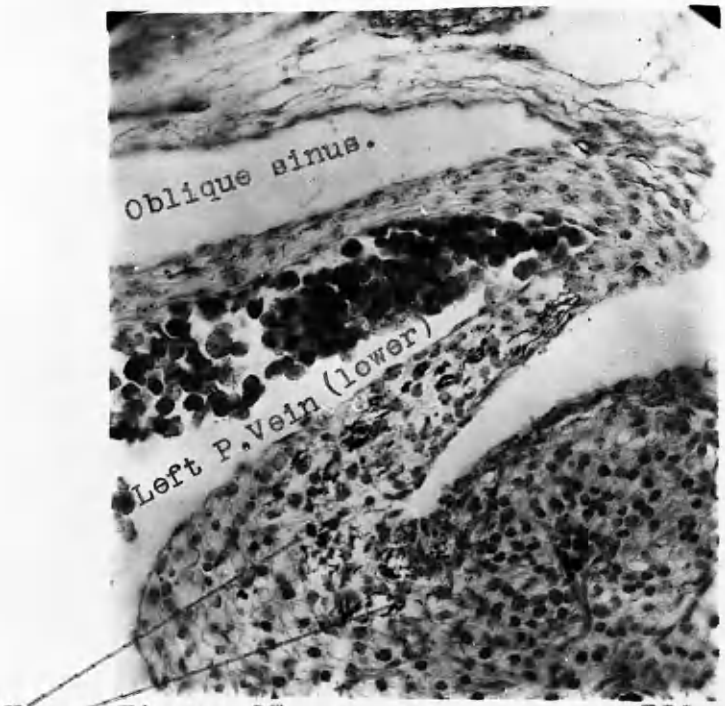


Figure 15. x 320.

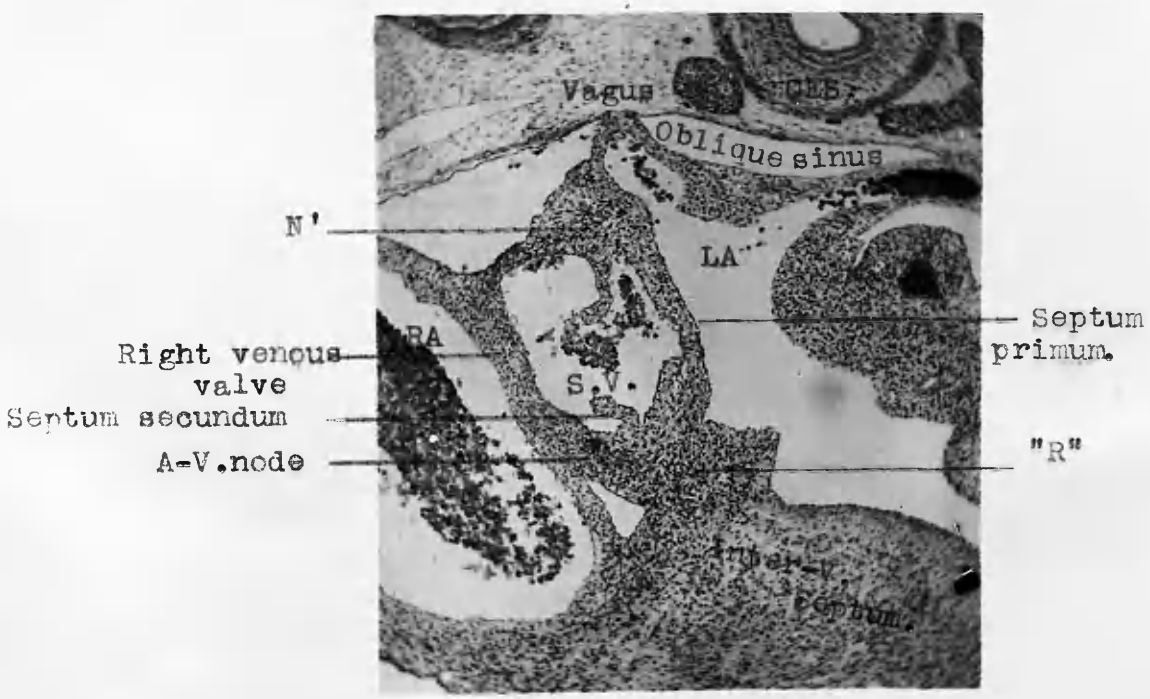


FIGURE 16 x 80.
HUMAN 24 mm. embryo.