VI. *The Chromaffine System of Annelids and the Relation of this System to the Contractile Vascular System in the Leech*, Hirudo medicinalis.

A Contribution to the Comparative Physiology of the Contractile Vascular System and its Regulators, the Adrenalin Secreting System and the Sympathetic Nervous System.

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[Plates 14–17.]

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I. Introduction.

The Distribution of the Chromaffine System in the Annelid Kingdom.

The possession of a chromaffine system, consisting of cells which take a yellow stain with chrome salts, is a common property of almost all the members of the vertebrate kingdom. The presence of this reaction is coincident with, and probably dependent upon, the secretion by these cells of the substance adrenalin. The investigations of Lewandowski (30), Langley (29), Elliott (11), and others have established that the physiological actions of this latter substance are the same as those which result from the stimulation of the sympathetic nervous system. The latest researches of Elliott (12), Von Anrep (43) and others, have shown in addition that the adjuvant action of adrenalin is essential for the efficient performance of the functions of the sympathetic nervous system. This is supported by pathological considerations, for it has long been recognised that many of the symptoms of Addison's disease, in which the chief lesion is the destruction of the medullary chromaffine tissue of the supra-renal glands, are those of failure of the sympathetic nervous system. From a physiological standpoint, it is, therefore, necessary that the two systems should co-exist, and a close morphological relationship between them is rendered probable.

The researches of KOHN (26) on the embryological origin of the chromaffine system in the mammalia have established that the ganglion cells of the sympathetic system and the cells of the chromaffine system, which are in the embryo widely distributed through the body, arise from a common group of mother cells; KOHN therefore names the chromaffine system the Paraganglion system. These researches are in agreement with the original statement of BALFOUR (2) that the paired suprarenal bodies of Elasmobranchs, which consist of chromaffine cells, are developed in the sympathetic ganglion masses; KOHN has also made similar investigations in these fishes and confirmed the observations of BALFOUR.

The evidence of embryology therefore also points to an intimate relationship between the chromaffine system and the sympathetic system, and to a possible common origin for both. In any investigation of either system the other cannot be left out of account, and the investigation of the origin of the chromaffine system must necessarily have bearing upon the origin of the sympathetic system. Physiological considerations also make it necessary that in any such investigation still another system must be considered, namely the contractile vascular system, for throughout the vertebrate kingdom the chief function of both the sympathetic system and adrenalin system is the regulation of the cardio-vascular apparatus. The presence of any system or systems physiologically corresponding to the two former, therefore necessarily implies the presence of the latter, on which they may operate.

The investigation of the vertebrate kingdom is not alone sufficient to solve the problem of the origin of these systems; for, even in the lowest fishes, the Cyclostomata, a well-developed system of chromaffine cells is present, which is in every way comparable to that of other vertebrates. The knowledge of the chromaffine system in this group we owe to GIACOMINI (17), who has also thoroughly investigated the other groups of fishes, in which a chromaffine system was previously unknown, namely, the Teleostei (18), the Ganoidei (19), and the Dipnoi (20), and has demonstrated a similar system in these groups also. The occurrence of chromaffine tissue in the Elasmobranchs has been long known, having been first described by LEYDIG (31), and the secretion of adrenalin by this tissue has been shown by SWALE VINCENT (42) and others. In the case of the cyclostome, *Petromyzon fluviatilis* (14), I have been able to show that a substance of the nature of adrenalin is present in the chromaffine tissue. The presence of a chromaffine system, which secretes adrenalin, is therefore established in all members of the vertebrate kingdom, in which a contractile vascular system exists. In the case of Amphioxus, however, in which the heart is merely represented by a non-muscular blood space, I have been entirely unable to discover a chromaffine system. Prof. GIACOMINI also informs me that he has likewise been unsuccessful in a search for such a system in this animal.

The condition in Amphioxus thus supports the view that a chromaffine system cannot exist where there is no contractile vascular system, on which it can act.

The presence of a sympathetic nervous system is well recognised throughout the

greater part of the vertebrate kingdom, including, among fishes, the Elasmobranch group. In other groups of fishes, however, its presence is not so clear; and, although the researches of GIACOMINI, following those of CHEVREL (9) and others, have established the presence of an irregular sympathetic system distributed along the cardinal veins in the Teleostei (18) and the Ganoidei (19), and of a definite double sympathetic chain in the Dipnoi (20) comparable to those in higher vertebrates; in the cyclostomes the presence of a true sympathetic system is still doubtful.

GIACOMINI, however, describes in Petromyzon (17) certain nerve cells which occasionally occur around the cardinal veins, and which may be the representatives of the sympathetic system in this animal. In Petromyzon, as is also the case to a lesser degree in the Teleostei, Ganoidei, and Dipnoi, the chromaffine tissue is extremely diffusely, though regularly, distributed, lying in the walls of the cardinal and segmental veins and their tributaries. The scattered sympathetic nerve cells have an identical distribution, and occasionally lie in contact with a group of chromaffine cells. If the function of the chromaffine system is to regulate the vascular mechanism, its extremely diffuse distribution may enable local control to be brought about by a local discharge of adrenalin without the necessity of any action of a true sympathetic nerve. It is, therefore, a possible hypothesis that in this animal the chromaffine system to a great extent replaces the sympathetic system, the wide distribution of the chromaffine tissue accounting for the extreme scantiness of the sympathetic nerve cells present. If it be allowed that the two types of cell arise from an indifferent mother cell, it is quite possible that in certain cases one system may be widely developed at the expense of the other. This question will be further discussed at the end of this paper.

A chromaffine system is, therefore, present in all vertebrates with the exception of Amphioxus, and is very well developed even in the most primitive group of fishes, the Cyclostomata.

The investigation of its origin must be carried further down in the scale of evolution, and evidence for the existence of such a system must be sought for in the invertebrate kingdom. POLL and SOMMER (37), in 1903, recorded the discovery that certain nerve cells in the abdominal ganglia of certain worms, Hirudo, Aulostoma, and Pontobdella, gave a chromaffine reaction with chrome salts, similar to that obtained in the cells of the suprarenal medulla of mammals. These observations have been recently supplemented by BIEDL (3), who claims to have obtained the biological tests for adrenalin from these cells.* The present investigation has therefore been directed to the annelid group.

The only other observations on the presence of a body resembling adrenalin in an invertebrate are those of ROAF and NIERENSTEIN (40) in *Purpura lapillus;* but this tissue lies in the wall of the branchial chamber and apparently secretes externally

* During the printing of this paper I have been able to confirm BIEDL'S statement by obtaining an inhibition of the virgin uterus of the cat with an extract of ganglia of *Hirudo medicinalis.*

into this cavity : it has, therefore, little analogy to the adrenalin tissue of vertebrates. It is, however, possible that its function is similar, for the blood brought to the heart by the pulmonary vessel, which lies in the wall of the branchial chamber, freely interchanges with the contents of the cavity certain of its constituents, such as oxygen and carbon dioxide. It is possible that adrenalin present in the branchial chamber may be absorbed in a similar way into the circulation. As this question has not been personally investigated, it will not be further referred to, more especially as the Mollusca are not held to be on the direct line of vertebrate descent.

The investigation of the annelid group was begun during a stay at the Zoological Station at Naples in the summer of 1911, attention being directed to the Polychæte group. A representative collection of these was made and examined systematically both at Naples and after returning to England. The material was treated as follows :—It was fixed in two ways : part in a solution formed by adding 10 per cent. formalin to Müller's fluid and part in a solution of 10 per cent. formalin in sea-water ; the material fixed in formol-Müller solution was transferred after 48 hours to Müller's solution. This formol-Müller fixation is very efficient for the demonstration of chromaffine tissue and the formalin-fixed material is then an efficient control. An examination of controls is essential in such an investigation, as many systems of cells in annelids are normally pigmented with yellow or brown pigments, and are therefore easily confused with the true chrome reaction. For instance, this mistake has been made by Apàthy in discussing the statement of Biedl (3), already referred to, that adrenalin is present in the nerve cells of Hirudo, for he there calls the chlorogogen cells, which are thickly distributed in the sheath of the sinus in which the nerve cord lies, chromaffine cells ; in reality, the two types of cell have no relation whatsoever to one another.

The material, when fixed, has been embedded in gelatine and cut on a freezing microtome (15). By this method the chromaffine reaction is more characteristic and easier to recognise than if paraffin methods are used ; shrinkage of tissues is also almost eliminated. The colour obtained in chrome-reacting cells is a bright canary yellow, not the brownish colour which is usually seen in paraffin preparations.

In the further investigations that were subsequently made in certain members of the non-marine annelids, belonging to the Hirudinea and Oligochætæ, the procedure was throughout the same, with the exception that the control tissues were fixed in a 10-per-cent. solution of formalin in physiological saline solution.

In the search for chromaffine tissues the sections were either examined unstained or lightly stained with Mayer's hæmalum ; they are identifiable in sections stained with such stains as hæmalum and watery eosin, and hæmalum and Sudan III, but the yellow colour of the chrome reaction is considerably obscured. The search was not confined to the central nervous system only, but in a good many animals complete transverse sections of various parts of the body have been examined. The search outside the central nervous system was, however, in all entirely negative. No less

than six different systems of pigmented cells are found generally distributed in the annelid group, which have no relationship to chromaffine tissue, namely :—

(1) Yellow or brown cells situated in the skin.

(2) Yellow or brown cells situated in the wall of the intestine.

(3) A brown pigment in the red blood cells, when such are present, or a diffuse yellow pigment in the blood when its colouring matter is in solution ; in this case the endothelium of the vessels is often deeply pigmented.

(4) The dark brown pigment in the cells of botryoidal tissue, when present.

(5) Yellow or brown chlorogogen cells round the walls of the blood vessels, often associated with fat ; and other pigmented cells of the interstitial connective tissue.

(6) Certain pigmented cells in the central nervous system.

The most important of these systems with regard to the present investigation is that met with in the central nervous system, as such cells are liable to be confused with the true chromaffine cells. They have been noted by many observers, and the view has been advanced by LEYDIG and others that they might be related to the suprarenal medullary cells. A very complete description of them in *Halla parthenopeia* has lately been given by ASHWORTH (1), and my investigations were first turned to the Polychæte worms on the supposition that this pigmentation had relationship to the chromaffine reaction. It was soon clear, however, that no such relationship existed ; on the contrary, the nerve cells, which give the chrome reaction, are usually entirely free from permanent pigmentation.

These pigmented systems have, therefore, no relation to any chromaffine system, being equally well marked in control material.

The search for the chromaffine cells in the central nervous system has been carried out in the following way :—Serial frozen sections cut 15 μ thick have been cut through a block of tissue containing the nerve cord, and have been collected in groups of 20 to 30 in separate dishes of water. The dish containing the sections of the nerve cord is then found by examining a sample section, and the whole of its contents are then stained and mounted ; all sections containing nerve cord have thus been examined. The sections, as a rule, have been cut in a dorso-ventral longitudinal plane so as to include several ganglia.

In the Polychæte group the great majority of species examined have been found not to contain nerve cells giving the chromaffine reaction. The following is a list of those examined which are negative in this respect, and may be claimed to fairly represent the group :—

Alciope.
Aricia fœtida.
Diopatra neapolitana.
Halla parthenopeia.
Hesione sicula.
Dasybranchus caducus.
Lumbriconereis impatiens.
Cirratulus filigera.

Glycera siphonostoma.
Glycera convoluta.
Nephthys hombergi.
Nereis violacea.
Polynoë elegans.
Spirographis spallanzani.
Psammolyce arenosa.

In two members of the Polychæte group, however, small chromaffine cells have been found to be present, six being situated in each ganglion, namely, in *Aphrodite aculeata* and *Eunice gigantea.*

Among the Hirudinea, *Hirudo medicinale*, *Aulostoma gulo*, and *Pontobdella verrucosa* have been examined, and have all been found to contain six chromaffine nerve cells in each ganglion.

Among the Oligochætæ, *Lumbricus herculeus* has been investigated, and has been found to contain a similar arrangement of six chromaffine cells in each ganglion.

This list corresponds to the latest list given by POLL (36) in HERTWIG'S text-book; he has, however, in addition found that certain other members of the Hirudinea, Nephelis and Clepsine, also contain the chromaffine cells. Examples of these I have not myself had the opportunity of examining.

The number and situation of these cells is remarkably constant in whatever animal they are found; in each case there are six cells in every ganglion, two lying mid-ventrally and two on each side lying laterally in the region where the lateral nerves are given off. They, however, vary greatly in size, being small in Aphrodite and Eunice, somewhat larger in Lumbricus, and attaining their greatest size in the Hirudinea, being especially large in Hirudo and Aulostoma. The ventral pair of cells are always larger than the lateral pairs; in Hirudo they have been designated by RETZIUS (39) colossal cells, owing to their enormous size.

Further investigations of this chromaffine nerve-cell system have therefore been carried out in *Hirudo medicinalis*, which is easily obtained in quantity. The detailed description of the position and relations of these cells will be given in the chapter on the central nervous system in Hirudo, a description which also holds in the case of the other annelids in which these cells occur. The preliminary enquiry thus points to Hirudo as the animal to be studied, as in this animal the systems under consideration obtain their highest development as far as the annelid group is concerned. Further investigations have therefore been confined to Hirudo alone, but it is probable that the similar arrangement of the chromaffine cells in the other members of the annelid group, which contain them, implies that similar physiological relationships obtain here also. It has been found necessary during the research to re-investigate the vascular system and the central and peripheral nervous systems of Hirudo, as no sufficiently complete anatomical descriptions of them, with their relationships to one another, is known to me. A detailed description of each of these systems will first be given, and then their physiological properties and relationships will be discussed, and also the probable meaning of the occurrence of the chromaffine reaction in the six nerve cells.

Investigation of the vascular system of Hirudo shows that a certain portion of it is well clothed with muscles which have a contractile rhythm of considerable power. This rhythm is unaffected by the action of curare, and can, therefore, be easily studied in the curarised animal. It is, however, affected by adrenalin and is also under the control of the central nervous system.

Hirudo, therefore, possesses a contractile vascular system, which will be shown to be comparable both in these and other ways to the vertebrate vascular system, on which the secretion of the chromaffine cells can act. When, however, we turn to the Polychæte group, in which, speaking generally, a chromaffine system is absent, we find that a muscular coat to the wall of any part of the vascular system is also absent. On the contrary, in the other animals which contain a chromaffine system, a contractile vascular system is found. The hearts in the anterior portion of the body of Lumbricus are well known, and Aulostoma has a contractile system which is practically identical with that of Hirudo. Pontobdella also has a perfectly definite muscular sheath to its dorsal and lateral longitudinal vessels, though this is not so highly developed as in Hirudo; according to Bourne (5) a similar muscular wall is present in Clepsine and the other members of the Hirudinea, in which chromaffine nerve cells are present.

The condition in *Eunice gigantea* is of interest, for although the main vessels do not appear to contain muscle fibres in their walls or to be contractile, at the base of the well developed branchiæ there are certain portions of the vessels supplying blood to them which are described in Huxley's text-book (25) as being contractile. I have been able to identify these "hearts" histologically and find that the contractile portion of the vessel which forms them is slung on a mesentery in the body cavity, so as to allow free rhythmical contraction to take place, and contains muscle fibres in its wall. *Eunice gigantea*, therefore, possesses a contractile vascular apparatus which is branchial in function.

With regard to Aphrodite, I have only obtained a single specimen for examination, and have not been able to study its circulatory system, or to obtain a control of the chromaffine reaction in a formalin-fixed animal. I, therefore, mainly rely on Poll's (36) statement that the diffusely yellow staining nerve cells observed are true chromaffine cells.

In the annelid group, therefore, wherever chromaffine cells exist in the central nervous system, a contractile vascular system is also present.

The purpose of this paper will be to show, as far as possible, the relationships of this contractile vascular system to the nervous system and especially to the chromaffine cells in it; the hypothesis will also be advanced that the study of these annelids shows that the three systems, the rhythmically contractile vascular system and its regulators, the adrenalin system, and the sympathetic nervous system, have all arisen together, their representatives in this group being in many ways closely comparable with the more highly developed systems found throughout the vertebrate kingdom.

Certain other observations with regard to the physiological properties of various parts of the muscular and nervous systems of Hirudo, which have been established during the course of these investigations, have also been thought to be worthy of record, though they bear only indirectly upon the main problem.

This preliminary investigation in the annelid kingdom confirms the original investigations of Poll and Sommer (37 and 38), and establishes the presence in certain annelids of a group of nerve cells lying symmetrically in each of the segmental ganglia, which are not only nervous in nature but also secrete a substance of the nature of adrenalin, a combination of properties which the embryological researches of Kohn (26) demand for the common mother cells of the sympathetic system and the chromaffine adrenalin secreting system.

As will be further discussed in the final chapter, these cells may therefore be the ancestors of both the sympathetic system and the chromaffine adrenalin secreting system. They only occur in an animal having a contractile vascular system, and become larger and more important with the greater development of such a system. It can, therefore, be argued that their processes have to do with the innervation of the contractile vessels in a manner comparable to the innervation of the vascular system by the sympathetic system in vertebrates.

II. The Vascular System.

1. *Anatomy.*

The most complete description of the vascular system hitherto given is that by Goodrich (21). By means of careful injections, and by examination of serial sections, he was able to show that there are in the leech three separate systems, which contain blood and communicate with one another only by means of finely divided capillaries. These systems are similar in each segment, the description of one segment is therefore sufficient. The modifications at the anterior and posterior extremities do not concern the present paper. Goodrich also gives full references to the literature of the subject and comprehensive drawings of the connections of the three systems.

Goodrich made use of camera lucida drawings and an elaborate reconstruction method. I have used Edinger's projection apparatus, by means of which the magnified image of the section is thrown directly on to a sheet of paper and can be traced on this. Serial sections can be thrown on to the same field one after the other, and any structure can be traced with accuracy through any number of sections. I have thus been able to make projections in all three planes, following out the course of the vessels through every section. A most accurate and complete drawing can thus be made, and every connection and branch traced throughout its course. Serial sections of a complete segment were cut in paraffin, after fixation in formol-Müller, and stained with Mayer's acid hæmalum and eosin in the ordinary

way. The fact that the hæmoglobin of the leech's blood is in solution, and that the blood therefore takes the eosin stain very strongly, makes the tracing of vessels, when containing blood, a matter of no difficulty.

The finer details of the structure of the vessel walls, the valves, etc., have all been taken from serial sections, primarily made for tracing nerves, which had been fixed in formol-Müller solution, embedded in gelatine (15), cut frozen and stained with hæmalum and Sudan III.

This method eliminates all shrinkage from the preparations and enables the structure of the muscle fibres to be more accurately observed. The results obtained from paraffin sections have also been confirmed in this series.

The arrangement of the larger vessels has also been studied in life by dissections of the curarised leech. The circulation can be readily observed by such dissections, as curare has no effect on the rhythmically contractile muscle of the vessel wall, though it completely paralyses the longitudinal and circular muscles. This action of curare will be further described and discussed in the chapter on the action of drugs.

The results of my investigations have been to confirm the observations of GOODRICH in the main, though there are one or two points of considerable physiological importance which he does not record, such as the existence between each segment of a valve on the main course of the lateral contractile vessel.

The three separate systems which contain blood consist of:—

(1) The contractile longitudinal lateral vessels and their branches;
(2) The dorsal sinus system;
(3) The ventral sinus system;

and are shown in detail in fig. 1. As has been already mentioned, these three systems only intercommunicate by means of capillaries, though this capillary communication is a very extensive one. The question of the origin of these spaces, which in their entirety form a continuous closed vascular system, is a matter of comparatively small physiological importance and will not be touched on in this paper.

(1) The two contractile longitudinal vessels and their branches lie symmetrically on each side of the body, and freely communicate from side to side both dorsally and ventrally in each segment. Each longitudinal vessel consists of a thick-walled tube lying laterally in the body slightly to the ventral side (fig. 1). It can be readily exposed for studying its circulation and position by cutting through the ventral edge of the yellow line which runs along the lateral aspect of the body of the leech, the incision being just deep enough to pass through the longitudinal muscles. The vessel will then be exposed along the whole length of the incision (Plate 14, fig. 2). In the living state it comes conspicuously forward in the wound, where it bulges round the distended nephridial vesicles or bladders. The wall of the vessel is composed of two layers of large muscle cells, an inner longitudinal layer of single cells, and an outer

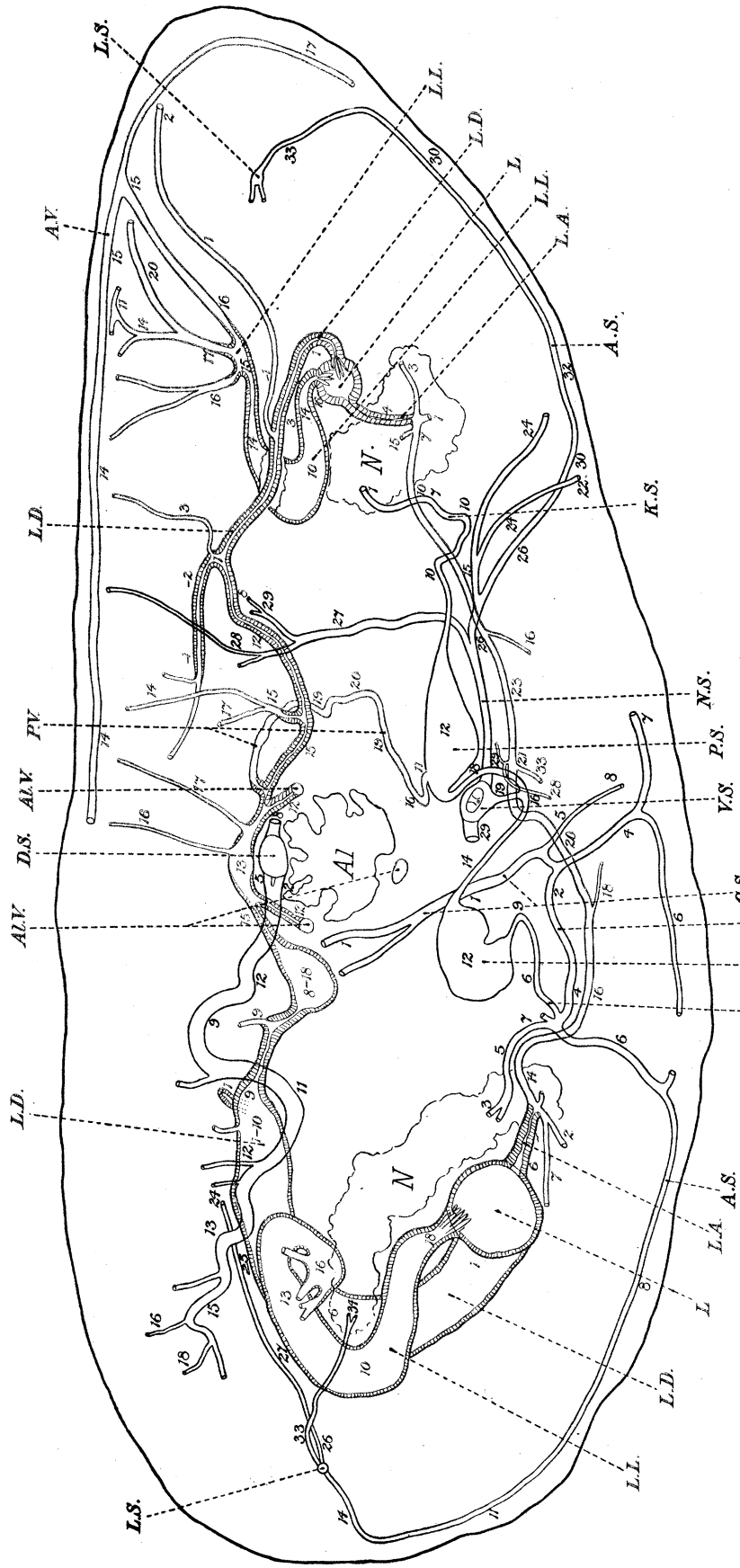

FIG. 1.—Projection on a transverse plane of the vessels of the leech. The contractile system is shown in red, and also the branches which arise from it. The contractile portion is cross-hatched and is shown fully distended on the right and contracted on the left. The dorsal and ventral sinus systems are shown in black.

Al., alimentary canal; N., nephridium. L., lateral longitudinal vessel giving off three branches. L.L., latero-lateral vessel soon breaking up into many branches connecting with A.V., the annular vessels. L.D., the latero-dorsal vessel dividing into two branches, the anterior of which connects with its fellow of the opposite side across the mid-dorsal line; this anterior branch also connects with the lateral alimentary vessel, Al.V., it also occasionally connects with the perinephrostomial sinus by means of P.V., the ascending vessel of Gratiolet. L.A., the latero-abdominal vessel, dividing into two branches, which connect across the ventral side, so as to form a diamond-shaped figure (only one of these connections is shown); it also supplies branches to the nephridium. D.S., the dorsal sinus lying just above the alimentary canal; one of its more tortuous branches only is shown. V.S., the ventral sinus containing the nerve cord; it gives off a pair of sinuses, one of which, N.S., is shown on the right, which accompany the posterior nerves and their branches and may therefore be called neural sinuses; various ventral branches of these form annular sinuses, A.S.; the ventral sinus also connects with the perinephrostomial sinus, P.S.; from the latter a further sinus, K.S., runs out to the nephridium and may or may not connect with the arched sinus, R.S., as is shown on the left; the arched sinus gives rise to an annular sinus, A.S., and also connects with the connecting sinus, C.S., which connects together the ventral and dorsal sinus systems. L.S., the small lateral sinus lying in the lateral longitudinal muscle giving rise to a sinus connecting with the nephridium and a sinus connecting with the dorsal intermuscular plexus, the annular sinuses all terminate in this lateral sinus. The distribution of a muscular wall in the contractile system is indicated by a double outline and cross hatching. The numbers marked on the diagram indicate the slides in which these portions of the vessels occurred; the highest numbers are anterior, the lowest posterior. The segment traced is in the region of the straight hind gut. (Drawn with Edinger's projectoscope.)

circular layer of the same (fig. 3). These muscle cells histologically bear a very close resemblance to the other muscle cells of the leech; but, as will be seen later, they have very different physiological properties and reactions. The vessel is lined by an endothelial layer of flattened cells similar in all respects to that lining the non-contractile vessels (fig. 3); it lies embedded in the extremely loose connective tissue that is characteristic of the animal. It also contains segmentally arranged a great thickening of its wall which acts as a valve, completely occluding the lumen when the vessel is contracted (fig. 4). This valve lies equidistant from two consecutive ganglia at the junction of the first and fifth annuli, that is to say, between two segments; the origin of the lateral abdominal vessel is just posterior to it (fig. 2). It is composed of a thickening of the wall of the vessel so arranged that the lumen through it is inclined at an angle to the main axis of the vessel, the thickening at the anterior end being greatest on one side of the wall, at the posterior end being greatest on the opposite side. The greater part of this valvular thickening is composed of a clear hyaline material somewhat similar in appearance to the contractile external portion of the muscle fibre; there is also, however, at this point an increase of muscle fibres which apparently belong to the internal longitudinal muscle layer. It is probable that the hyaline thickening is a portion of the cell structure of these longitudinal muscle fibres, though it may also be developed to some extent from the endothelial lining cells. This point will be further discussed in describing the valves at the origin of the branch vessels.

The three branches of the main vessel in each segment can be readily seen in the dissection already described (fig. 2). Two are given off dorsally—(*a*) the latero-lateral vessel of Gratiolet; (*b*) the latero-dorsal vessel of Dugès; these two are occasionally peripherally connected together at some little distance from the main vessel: one is given off ventrally—(*c*) the latero-abdominal vessel of Dugès.

(*a*) The latero-lateral vessel is a short trunk arising in the second annulus anteriorly to the bladder and nephridium (fig. 2), passing almost vertically upwards and then curving round slightly so as to lie just under the botryoidal tissue. It soon breaks up into numerous branches which run out through the muscle layers to reach the subepithelial region. Three or four of these branches join annular vessels running across the dorsal region and round the lateral region towards the ventral side of the body, lying just subepithelially. The musculature of the latero-lateral vessels ceases abruptly at the point of division into branches; the circulation through these vessels beyond this point must therefore be dependent to some extent on the condition of the longitudinal and circular muscles through which the thin-walled channels run. The muscle wall of the branch vessel is similar in every respect to that of the main vessel. At the junction of the branch and the main vessel there lies a valve (fig. 5) first discovered by Goodrich. It consists of a series of finger-like hyaline masses of protoplasm which take origin from the inner wall of the lateral vessel just where it joins the main vessel, and hang down through the opening of the

former into the lumen of the latter. These processes, when the muscle wall around them contracts, completely occlude the lumen of the branch vessel at its origin. They appear to be directly continuous with the layer of the internal longitudinal muscles of the branch vessel. Their protoplasm is hyaline, being similar to that which mainly forms the valve on the main vessel. It is probable, therefore, that they are modified cells belonging to the muscle group, which forms the internal muscle layer of the branch vessel; their similarity in many respects to the contractile portion of the muscle fibre makes it possible that they are also contractile. By their contraction they would be drawn into the mouth of the branch vessel, and would therefore assist in more efficiently occluding this. There are indications in these valves of the branch vessels that the endothelial lining of the main vessel covers them over, which also supports the view that they are derived from the internal muscle layer.

Similarly the contraction of the hyaline material, of which the valve of the main vessel is chiefly composed, would tend to shorten and thicken the valve, and to increase the angle of deflection of its potential lumen to the axis of the main vessel, thus increasing its efficiency as an obstruction to the blood flow.

(*b*) The latero-dorsal branch arises in the fourth annulus behind the bladder (fig. 2). It runs at first dorsally, but soon turns towards the middle line, lying just under the botryoidal tissue; it then runs directly inwards to meet its fellow of the opposite side above the dorsal sinus (fig. 1). Just before reaching the middle line a branch is given off to the alimentary canal, which in the region of the anterior sacculated gut breaks up into branches on its wall. In the region of the straight hind gut these branches join two longitudinal non-contractile vessels which run along it in a dorso-lateral position (fig. 6). These vessels terminate in the pair of latero-dorsal vessels which lie just anterior to the hind gut; the two alimentary vessels also connect with a further longitudinal alimentary vessel lying ventral to the alimentary canal. These three vessels form a system supplying blood to the intestinal wall, which is in free communication with the contractile system.

The wall of the latero-dorsal vessel is in all probability muscular throughout, as contractions can be seen to pass right across the middle line. However, the presence of muscles in the median part of the cross connection has not been histologically demonstrated. The two branches joining the alimentary vessels have a muscular wall.

The latero-dorsal vessel gives off, about half-way along its course, a large branch which is muscular and runs in towards the middle line, lying in a more posterior plane than the main vessel, and terminating in two branches without musculature which run out to the subepithelial region; this is the posterior branch of GOODRICH. The sudden loss of musculature, which has already been mentioned, is conspicuous in this branch vessel (fig. 7). There is also a branch given off early in the course of the vessel which runs out laterally, and gives rise to an annular vessel lying in the second

annulus; it loses its musculature just after its origin and passes through the muscles laterally so as to reach the subepithelial region. Another branch is occasionally present, arising just before the branch to the lateral alimentary vessel, which quickly loses its musculature and then turns downwards between the central and lateral portions of the alimentary canal to ultimately join the perinephrostomial sinus, belonging to the ventral sinus system. This is the only direct connection which has been found between the contractile system and either sinus system. It corresponds with an ascending vessel described by GRATIOLET (22); it is only occasionally present. Other branches are given off dorsally along the course of the latero-dorsal vessel running out to the dorsal subcutaneous region through the muscles. No direct connections between the branches of either the latero-dorsal or the latero-lateral vessels and the botryoidal sinuses have been observed.

(*c*) The latero-abdominal vessel arises in the first annulus just posterior to the main valve (fig. 2), and runs for a short distance directly ventrally; it then divides into two main branches, one running forwards and ventrally, and the other backwards and ventrally, to join corresponding vessels from the opposite side in the mid-ventral line; they thus form a diamond-shaped arrangement of vessels lying just ventrally to the level of the nervous system, and also form a free cross connection from side to side. The wall of this vessel is muscular only to its point of division, and there is apparently no valve at the actual opening into the main lateral vessel. At the point of division, and in the early part of the course of the branch vessels, numerous branches are given off to the nephridium, with which these vessels lie in close contact. Smaller branches are also given off through the ventral muscles to connect with the capillaries of the ventral subcutaneous plexus. This ventral system of vessels often connects in the mid-ventral line from segment to segment by a short channel running from the point of junction of the two posterior branches from each side in one segment, to the point of junction of the two anterior branches in the next segment.

(2) The dorsal sinus system (fig. 1) consists of a large longitudinal sinus lying in the mid-dorsal line just above the intestine. It gives off two lateral branches on each side in every segment (fig. 6). These branches run out laterally under the botryoidal tissue; one loops round the latero-dorsal vessel in a characteristic manner, and gives off many branches, which communicate with the plexuses of the dorsal longitudinal muscles and with the dorsal sub-epithelial capillaries; the other runs out directly laterally and breaks up into similar branches. They both freely communicate by fairly large branches with the blood sinuses of the botryoidal tissue, and through these sinuses with the plexus of the dorsal longitudinal muscles, with which they have direct communication. The dorsal sinus also gives branches off to the wall of the intestine, which communicate by means of a capillary plexus with the similar branches arising from the lateral and ventral alimentary vessels.

(3) The ventral sinus system (fig. 1) consists of a large longitudinal mid-ventral

sinus, in which the nerve cord lies, and various other large sinuses connecting with this either separately or in conjunction with the lateral nerves. The nerve cord lies freely in this sinus, being only held in place by the lateral segmental nerves. The main sinus gives off, in connection with the posterior segmental nerve, a large branch on each side, which divides shortly after its origin at the point where the posterior nerve also divides, so that each branch accompanies a division of the nerve.

The dorsal branch runs directly dorsally in company with the dorsal branch of the posterior nerve, on reaching the level of the upper boundary of the alimentary canal it breaks up into numerous branches, which run out to join the plexus of the dorsal longitudinal muscles and the subepithelial plexus. None of these branches communicate with either the dorsal sinus system or the contractile system.

The lateral branch follows the lateral branch of the posterior nerve for some little distance, giving off in the earlier part of its course a branch which runs anteriorly and ventrally so as to reach the subepithelial region in the second annulus; this latter branch runs round towards the dorsal side, forming an annular sinus. Soon afterwards the lateral branch divides into two other branches which also run out and reach the subepithelial layer on the ventral side about half way between the mid-ventral and lateral regions. One runs directly outwards so as to get to the periphery in the third annulus, the other runs out more posteriorly so as to get into the fourth annulus. They then run round just under the epithelial layer so as to form annular sinuses.

This lateral branch therefore gives rise to three annular sinuses.

The ventral sinus also communicates with a pair of large perinephrostomial spaces which lie symmetrically arranged in each segment in the fifth annulus. This communication may be direct or in the manner shown in fig. 1. At the opposite side of each perinephrostomial sinus, another sinus runs out which has intimate connection with the nephridium throughout its course, finally connecting with the nephridial branches of the latero-abdominal vessel through a nephridial capillary plexus. Another connection with the perinephrostomial sinus is occasionally present, namely, that with the latero-dorsal contractile vessel already described. The sinus in connection with the nephridium may also have a connection with another ventral sinus, as is shown on the left hand side of fig. 1. This latter sinus arches up from the subepithelial layer, at a point near the mid-ventral line, to the inner side of the longitudinal muscles; it runs for some little distance in this region and then passes out again through the muscles to the subendothelial layer, and finally forms an annular sinus which runs round in the fifth annulus. This arched sinus also connects at the inner upper limb of the arch with a sinus corresponding in position to that running up in the third annulus with the dorsal branch of the posterior nerve; this latter sinus lies in the first annulus of the next segment so as to be intermediate between two of the posterior dorsal nerve sinuses, it has free communications with the subendothelial and intermuscular plexuses of the dorsal and ventral surfaces, the

inner limb of the arched sinus giving the ventral communication; it has, therefore, been called the connecting sinus by GOODRICH. The two arched sinuses communicate freely together across the ventral surface, so that a connection with the nephridial sinus on one side only is sufficient to communicate with the whole of the arched sinus system.

The annular sinuses, therefore, all arise from this ventral sinus system, and form the main subepithelial vessels in the ventral and lateral regions; they all communicate freely across the ventral region. They number, as a rule, four or five, and roughly lie each in one annulus of the segment. In the lateral region they interdigitate with the annular vessels, which arise from the contractile system and run round towards the ventral surface. The dorsal surface therefore has annular vessels, the ventral surface annular sinuses, while in the lateral region both are present.

The annular sinuses, on reaching the extreme lateral position, turn inwards and connect with a small longitudinal sinus, lying in the lateral longitudinal muscle which runs the whole length of the body; passing through this, they continue inwards and dorsally so as to get to the region of the dorsal botryoidal tissue, and break up into branches to this and to the intermuscular plexus. Another sinus running out from the extreme dorso-lateral portion of the nephridium also connects with the longitudinal lateral sinus.

The whole ventral sinus system therefore consists of an extremely complicated series of blood spaces intercommunicating with one another, and having mainly to do with the nervous system, the nephridium, and the ventral surface of the body.

The actual capillary connections between the three systems have been thoroughly worked out by GOODRICH (21). The contractile system and the dorsal sinus system connect through the capillaries of the dorsal subepithelial plexus, and also by a system of capillaries in the wall of the gut, which GRATIOLET (22) first described. The dorsal subepithelial plexus is connected with the contractile system through the annular vessels, its connection with the dorsal sinus system is direct through many small branches.

The ventral sinus system and the contractile system connect through the capillaries of the ventral and lateral subepithelial plexuses, also through the nephridial plexus, and thirdly by means of the connecting sinuses through the dorsal subepithelial plexus. The ventral sinus system therefore only obtains blood, either cleansed by passage through the nephridial excretory system, or oxygenated in the subepithelial plexuses. The nervous system is thus supplied with the best blood and does not come into contact with the blood of the dorsal sinus, which contains the products of digestion.

The botryoidal tissue directly connects only with the dorsal sinus system, in the opposite direction its vascular spaces are directly continuous with the intermuscular plexus, which lies amongst the fibres of the dorsal longitudinal muscles. This

position, between the rest of the circulation and the collecting system of the alimentary canal, is comparable to the position of the liver in the vertebrate; the cells of the tissue may therefore deal with the products of digestion in a similar manner. The view which has been advanced that these cells are excretory in nature is difficult to understand, as they have no connection with any excretory duct.

2. *The Circulation.*

The circulation can best be studied by making use of the curarised leech. If about 0·2 c.c. of a 1-per-cent. solution of curare is injected into the connective tissue, the animal becomes completely paralysed in about half an hour, and can then be dissected and the circulation studied. Care must be taken that the injection needle does not penetrate into the alimentary canal, which occupies a considerable portion of the internal volume, especially if the animal has been comparatively recently fed. Such an injection into the alimentary canal has absolutely no paralysing effect. It has been found in practice most satisfactory to make use of leeches which have been curarised on the previous day, as the circulation has then steadied down.

All observations on the circulation must be made in animals dissected and kept in Ringer's solution. The most satisfactory strength for this has been found to be mammalian Ringer's solution diluted with one-third of its volume of distilled water. An exposed vessel has been kept beating under these conditions for 24 hours. The circulation is due to the active rhythmical contraction of the muscle walls of the lateral contractile system, and is normally in two directions: (1) along the main lateral vessel from behind forwards; (2) from side to side in each segment along the latero-dorsal and latero-ventral connecting vessels. This latter circulation is the more powerful and important.

The contraction in any segment starts at the valve separating it from the segment posterior to it, and flows along the vessel, causing blood to be sent up the various branches (fig. 2). The latero-dorsal vessel is therefore first filled, and then the latero-lateral and the latero-abdominal. Meanwhile the valve separating the segment from the next anterior to it keeps closed, but finally opens when the chief wave of contraction reaches it, and allows the blood to also flow into the segment in front.

The rate of beat usually seen in a leech, curarised the day before, and opened laterally, is about six beats per minute; this is probably the normal rhythm. If the vessel is exposed in an animal which has only just become paralysed by curare, or has not been treated by curare at all, the beat varies greatly in rate in each individual instance, and, especially in the latter case, is extremely irregular; in an animal which has been curarised the day before, however, the beat is remarkably regular.

The chief wave of contraction is preceded by a much smaller wave, which has the effect of putting the muscle wall into a state of tone. This preliminary toning up

also causes the valve on the main vessel to close, so that, when the main contraction wave passes along the vessel, the valves of the lateral vessels first allow blood to flow through; and it is not till these lateral vessels are well filled with blood, that the pressure becomes sufficiently great to open the valve on the main vessel, and let the blood flow through to the next segment. The action of this valve is therefore to insure the efficiency of the cross circulation in each segment. There is no evidence that the valve has any special innervation or any power of contraction apart from, or antagonistic to, the main vessel contraction.

The contraction wave from the main trunk normally passes up the branch vessels, flowing along them to the extreme limit of their musculature; the blood is thus driven along the latero-lateral vessel so as to fill the annular vessels on the dorsal surface, and along the latero-dorsal vessel to fill the lateral alimentary vessel, and to cross over and distend the latero-dorsal vessel of the opposite side, and finally the main vessel of that side. The blood is also driven by the contraction of the main vessel and the very short muscular trunk of the latero-abdominal vessel, which contracts with it, into the vessels which connect with the ventral subepithelial plexus and also into the vessels of the nephridium. The blood driven through the latero-abdominal vessels also gets across to the opposite side and assists in filling its main vessel.

The valves at the origin of the latero-dorsal and latero-lateral vessels close with the general wave of contraction and so prevent back flow from the branch vessels into the main vessel. Under normal circumstances the two main contractile vessels have an alternate rhythm, the left side contracting when the right side is relaxed, and *vice versâ*. The cross flow in the latero-dorsal vessel and the latero-ventral vessel thus set up is mainly responsible for the filling of the relaxed vessel on the opposite side. There is therefore in each segment a to-and-fro circulation which is of as great or even greater importance than the caudo-cranial flow along the main vessel; this cross circulation was first observed in the Hirudinea by Johannes Müller (34).

The filling of the main contractile vessel is also assisted to some extent by a return flow from the large thin-walled vessels with which the muscular branches are in continuity, when these latter become relaxed.

Under conditions of injury this contractile mechanism is profoundly modified. For instance, if a segment has been badly damaged, the wave in the segment posterior to it frequently takes up a contraction rhythm passing in the opposite direction to the normal, the most anterior portion of the contractile segment contracting first, and the contraction then travelling in the posterior direction. The branch vessels also often assume a rhythm independent of that of the main trunk, being either faster or slower according to the conditions of injury. The alternate rhythm of the two main trunks also is easily interfered with, and they become independent, often contracting at approximately the same moment for a certain number of beats. The result of injury to the circulation of a segment, if severe, is very striking in another way, the

segment in which the injury takes place emptying its vessels of blood, and the contractile walls going into a condition of tonic contraction, the filling from the neighbouring segments also ceasing. This mechanism prevents further loss of blood, so that the neighbouring segments are still able to carry on an efficient circulation. In this respect again the circulation acts as a segmental one.

It seems therefore that, though as a rule there is an orderly sequence in the passage of the contraction wave over the contractile system, and that the two sides alternate with one another, this sequence is easily disturbed, and each segment of vessel, either the main lateral vessel or its latero-dorsal and latero-lateral branches, has the power of taking up an independent rhythm, the direction of the wave in the case of the main lateral vessel in some instances becoming reversed; that is to say, each vessel is capable of acting as an entirely independent unit. Evidence will be given in Chapter IV that each of these units is innervated by a separate nerve, therefore any control which the central nervous system can have on their rhythm may also act independently.

The strength of the contraction is dependent, among other factors, on the adequate filling of the vessel when relaxed. This filling is again dependent mainly on the efficiency of the cross circulation of the segment; it is therefore important, in studying the circulation, to cause as little damage as possible to this by making use of the lateral incision already described, the only vessels damaged by this incision being the annular ones.

The function of the valves both on the main vessel and at the orifices of the branch vessels is in no way to determine the direction of flow—when relaxed they are equally permeable in either direction; they merely act as a means of causing a local occlusion of the lumen, and are therefore more of the nature of sphincters than of true valves.

The circulation as regards any particular segment may be summed up as follows:—

(1) A flow along the main lateral vessel towards the anterior end;

(2) A flow from side to side through the latero-dorsal and latero-abdominal vessels;

(3) A flow through the latero-dorsal and alimentary vessels to the alimentary canal, returning from this to the dorsal sinus, and thence to the botryoidal tissue and dorsal intermuscular plexus;

(4) A flow through the latero-lateral and latero-dorsal vessels to the annular vessels and dorsal subepithelial plexus, and through the latter to the dorsal intermuscular plexus;

(5) A flow through the latero-abdominal vessel to the ventral subepithelial plexus, and thence to the ventral sinus system;

(6) A flow through the latero-abdominal vessel to the nephridium, and thence to the ventral sinus system;

(7) The ventral sinus system is also connected with the dorsal subepithelial plexus

by the connecting sinuses, and the sinuses accompanying the dorsal branches of the posterior nerves;

(8) A flow through a vessel, occasionally present, from the contractile latero-dorsal vessel to the perinephrostomial sinus, which forms a part of the ventral sinus system.

These arrangements are symmetrical on either side, the normal rhythm being an alternate one with regard to the two sides.

The contraction of the longitudinal muscles must also have an influence on the thin-walled vessels and sinuses which lie between their fibres, causing irregular movements of the blood fluid in such spaces.

The functions of many of the capillary systems have already been referred to, that of the alimentary canal being digestive, that of the botryoidal tissue probably being to deal further with the products of digestion, somewhat after the fashion of the vertebrate liver, and that of the nephridium being excretory. The functions of the remaining extensive dorsal and ventral subepithelial plexuses is almost certainly respiratory. These plexuses lie right against the outermost epidermal cell layer, and according to LANKESTER (27) fine capillaries extend between the cells themselves; even the large annular vessels and sinuses may lie only just under the epidermis. Respiratory interchange between the blood and surrounding water or air can thus easily take place.

It is easy to keep leeches alive for a week or more when fully under the influence of curare, until they completely recover from the effects of the drug; they are best kept in shallow glass dishes for this purpose in water changed once or twice daily. Respiration, therefore, depends on an efficient circulation only, curare having no effect on the circulatory muscles, though paralysing the voluntary musculature completely.

III. THE CENTRAL NERVOUS SYSTEM.

The methods employed for the study of the central nervous system have been the following:—

(1) Serial sections have been cut from material embedded in gelatine (15) and stained by Mayer's hæmalum and Sudan III or by Nile blue sulphate A.

(2) Serial sections have been cut in paraffin and examined both stained and unstained from material fixed with osmic acid, either applied as a vapour or in solution, either alone or in the form of Flemming's mixture.

(3) Methylene blue methods, the original method used by RETZIUS (39) being found the most satisfactory. The combination of this latter method with bichromate has been found to give a most characteristic differentiation of the chrome-staining nerve cells from all others.

The central nervous system consists of a large ganglion lying in the centre of each segment in the mid-ventral line internal to the muscle layers and ventral to the alimentary canal. These ganglia are joined together by paired connectives, and in

addition by a small bundle of fibres lying ventrally between these but quite separate from them, the whole being bound together in one connective tissue sheath and lying freely in the ventral blood sinus. Each ganglion gives off laterally an anterior and posterior nerve on each side, by which the ganglion is suspended in the ventral sinus (fig. 8).

The ganglion consists of a central mass in direct continuity with the connectives; in the anterior and posterior extremities of the ganglion the latter still continue as two separate bundles, but in the central portion of the ganglion they fuse to form a single mass. On this central core are arranged six groups of ganglion cells, similar to those described in other Hirudinea (fig. 8), for example, in Nephilis by BRISTOL (6), in Clepsine by WHITMAN (45), etc., but larger and therefore in closer contact with one another, and forming a more complete covering to the central mass (fig. 8).

Each group is separated from the others by septa of fibrous tissue, and from the surrounding blood sinus by the main fibrous sheath of the ganglion. The six groups consist of two lying on the mid-ventral aspect of the ganglion, and four lying laterally around the roots of the lateral nerves, arranged as follows:—

(1) An anterior ventral group covering the anterior ventral aspect of the ganglion mass.

(2) A posterior ventral group covering the posterior ventral aspect.

(3 and 4) An anterior lateral group on each side, lying in front of the anterior nerve and extending over the ventro-lateral and dorso-lateral aspects of the ganglion.

(5 and 6) A posterior lateral group on each side, lying anterior to the posterior nerve and extending round dorsally and ventrally, but lying as a whole more dorsally than the anterior lateral group; this group in its more dorsal and ventral portions extends posteriorly so as to form a kind of horseshoe surrounding the posterior nerve. A portion of it therefore appears, when the ganglion is viewed as a whole, to lie posterior to the posterior nerve.

These lateral groups lie close against one another and on their internal ventral aspect against the ventral groups, so as to form one continuous ventral and lateral covering to the central ganglion mass. It is only on its dorsal aspect that this mass is not covered with ganglion cells.

The nerve cells themselves are all unipolar, and each group hangs practically free in its own compartment, lying in a nutrient fluid; the latter with certain fixations gives a granular precipitate, and therefore probably contains an albuminous constituent. There are also present in certain groups one or two cells which have round clear nuclei and a much branched cell body which is not connected with any axon process; these are probably of the nature of supporting cells and lie, as a rule, in that part of the compartment where the nerve cell processes are leaving it; they therefore do not prevent the bodies of the nerve cells from hanging freely in the nutrient fluid. The actual position in a group of any particular cell is thus not of much importance,

for instance, the colossal cells can be easily moved about in their compartment by altering the pressure on the cover-slip of a methylene blue preparation; again, if a compartment be ruptured the nerve cells are freely extruded from the ganglion. The nerve cells of the ganglion vary greatly in size; two are extremely conspicuous, as they are very much larger than any of the others; they have been named the colossal cells by RETZIUS (39). The six chrome-staining cells lie in pairs in three of the cell groups (fig. 8). The most conspicuous are the two colossal cells, which lie usually more or less centrally placed in the anterior ventral group one behind the other (Plate 15, figs. 9 and 10). Each lateral pair, which are of the size of medium sized nerve cells, lie in a posterior lateral group (fig. 12).

In a preparation stained by fat-staining dyes the ganglion is seen to contain fat in very small globules scattered diffusely through the fibrillar substance and in the cell groups (figs. 12 and 13). In the latter situation fat is seen in osmic preparations to lie dotted round the periphery of the nerve cell. Sudan and Nile blue preparations also show this fat, and in addition a considerable amount of fat in the nerve cells themselves, either in an extremely finely divided state or in fairly large globules. These globules may be considerably increased as the result of injury, such, for instance, as the experimental section of the lateral nerves some weeks previously (fig. 13). There are indications that the nutrient medium in which the cells lie contains fat; the globules round the periphery of the nerve cell, therefore, may be an indication of the absorption of this fat by the ganglion cell.

The nuclei of the cells take basic stains such as hæmalum and Nile blue deeply; their protoplasm also takes these stains to a very considerable extent, especially in the region round the nucleus (figs. 10 and 13). This marked staining of the protoplasm by hæmalum is characteristic for nerve cells wherever situated throughout the animal, differentiating them most conspicuously from all other cells. The analogy of staining reactions with the Nissl granules in the nerve cells of vertebrates, which also are conspicuously stained by hæmalum as well as other basic dyes in frozen sections, makes it probable that the nerve cells of the leech also contain a similar substance to that forming the Nissl granules.

The central fibrillar mass of each ganglion, formed by the fused connectives, contains also two strands of fibres, running from side to side at right angles to the main axis, and forming an anterior and posterior commissure connecting the two anterior lateral ganglion groups and nerves and the two posterior lateral groups and nerves respectively (figs. 12 and 13). These two commissures are not very clearly defined, but tend to occupy the whole of the central part of the ganglion.

The general course of the various fibres running out from the cells of the ganglion and running into the ganglion from the periphery has been studied by RETZIUS (39) by means of methylene blue preparations. I have been able to confirm his statements by similar means, and have in addition been able to identify the chrome-staining cells by means of a combined methylene blue and bichromate reaction.

Various methods of methylene blue staining were tried, but the best method was found to be that of RETZIUS, by which remarkably clear pictures of individual fibres can be obtained. The paralysis of the voluntary muscles by a preliminary injection of curare greatly facilitates the application of the method. The leech is curarised by 0·2 c.c. of a 1-per-cent. solution of curare, and then at the end of half an hour or so, when completely paralysed, is pinned out on cork and opened from the ventral surface by an incision in the middle line. The vascular sheath of the central nervous system is opened by means of needles, the blood is washed away with Ringer's solution, and a few drops of $\frac{1}{4}$-per-cent. solution of methylene blue in distilled water are put on the ganglion with a pipette. The preparation is then put into a Petri dish with moist blotting paper beneath it, forming a moist chamber. Methylene blue solution is added as required, and staining is allowed to continue for two to three hours. The ganglion is then excised, washed in distilled water, mounted in this under a cover-slip, immediately examined under the microscope, and a diagram of the stained cells and fibres made. If the preparation is now irrigated with a potassium bichromate solution, such as Müller's fluid, the stained cells and fibres will gradually fade, either completely or to a light reddish colour. The chrome-staining cells are, however, unaltered by this procedure, and retain their blue stain in a most conspicuous manner, being thus differentiated from all other ganglion cells. This is probably due to a protective action on the blue stain by the chromaffine substance in the cell body; the differentiation obtained is remarkably permanent, being retained for months in specimens allowed to dry under the cover-slip. If, however, the preparation is put in a moist chamber, or the bichromate is applied to a preparation without a cover-slip over it, the blue colour soon fades from these cells also; the protection, therefore, is only relative, an excess of bichromate solution being able to overcome the protection afforded by the chromaffine substance in the nerve cell, and therefore bleaching the methylene blue stain. This reaction is a further proof that the six cells contain a substance which combines with chrome salts in the manner of the vertebrate adrenalin-containing chromaffine cells.

For the purpose of examining cell processes in the ganglion, when such are seen to have been stained, it is best to transfer the preparation into glycerine, thus rendering it more transparent; it can then be readily examined with a high power objective.

If it is desired to make a preparation permanent, it can be fixed with a 10-per-cent. solution of ammonium molybdate at a temperature of about 0° C. The preparation is left in this for 18 to 24 hours, and then transferred to distilled water at the same temperature for a further 24 hours. It is then dehydrated in absolute alcohol at the same temperature, cleared in xylol and mounted in Canada balsam. It is extremely important that the whole process should take place at 0° C., as otherwise a diffusion of the stain through the whole ganglion takes place. This process of fixation is conveniently carried out in a refrigerating chamber.

The directions of the processes of the various cells have also been studied to some

extent by means of serial sections of ganglia embedded in gelatine; such preparations confirm those obtained by methylene blue methods.

The processes of the cells lying in the various cell groups will now be described.

The anterior lateral cell group is composed of cells of varying size, in which larger cells chiefly predominate, in contrast to the other groups, where smaller cells are commonest. No chromaffine cells are present in this group. The cells may be grouped as follows, according to the direction of their processes.

1. Cells whose processes cross in the anterior commissure to the anterior nerve of the opposite side. These are fairly numerous.
2. Cells whose processes run out in the anterior nerve of the same side.
3. Cells whose processes run out in the posterior nerve of the same side.
4. Cells whose processes split into two in the ganglion, sending a thick branch to the anterior nerve, and a thin branch to the posterior nerve of the same side.
5. A cell has occasionally been observed with a process which splits in the ganglion into a large branch running into the anterior nerve of the same side and a fine branch running towards the anterior connective and probably continued into this.
6. A cell whose process runs directly into the anterior connective.
7. A similar cell whose process runs into the posterior connective.

It is possible that the cells of groups 2, 3, and 4, are all really similar, and have processes which split in the ganglion and supply a branch to both the anterior and posterior nerve of the same side, for it is only in very favourable preparations that such a splitting can be made out with certainty. Methylene blue preparations leave no doubt that such splitting fibres occur; and RETZIUS also has described them. The significance of this splitting and its possible bearing on the question of reciprocal innervation will be discussed in the final chapter. The existence of cells whose processes run directly into the connectives is not recorded by RETZIUS; such cells are of interest as their processes probably serve to link up ganglion with ganglion, they would thus occupy a position physiologically similar to the intermediate cells of the spinal cord of vertebrates.

The posterior-lateral cell group contains :—

1. A number of cells whose processes cross in the posterior commissure to the posterior nerve of the opposite side.
2. Cells whose processes run out in the posterior nerve of the same side.
3. One or two cells whose processes go to the anterior nerve of the same side. It is possible that these processes split in the ganglion into two in a manner similar to the cells of the anterior group which have been already described. The evidence obtained of this is, however, not clear, though some specimens might be interpreted in this manner. If it occurs, the second fibre runs in the posterior nerve.
4. A pair of cells occasionally seen whose processes loop on themselves in the ganglion and then run into the posterior connective.

The cells of this posterior lateral group have processes whose distribution in the posterior nerves is very similar to that of the anterior lateral group to the anterior nerves. Most of the cells of this group are fairly small. It contains the two lateral chromaffine cells, which lie usually round the root of the posterior nerve. It has not been possible to identify the processes of these cells with certainty, as, if they stain at all, they stain extremely late, when large numbers of other fibres are already stained.

The anterior ventral group contains the two chrome-staining colossal cells and also other smaller cells. The colossal cells have processes which first run dorsally and then turn outwards and divide into two in the fibrillar substance; one branch going to each lateral nerve. The various complicated paths which RETZIUS has described for these processes are merely due to the freedom of movement which these cells possess in their compartment, they can be easily moved about by pressure on the cover-slip of the preparation. The processes of the small cells in this group are difficult to stain, some of them apparently join the internal median bundles. Two fairly large cells at the most anterior extremity have processes running straight through the ganglion into the posterior connective; another cell occasionally is seen to send a fibre crossing in the anterior commissure to the anterior nerve of the opposite side; this cell may, however, really belong to the anterior lateral group.

The posterior ventral group consists mainly of small cells; but two fairly large ones are situated in its most posterior extremity, whose processes run forward and then cross in the posterior commissure to the posterior nerve of the opposite side. Another pair of cells of somewhat smaller size lie close to these, the processes of which run straight forward to the level of the anterior commissure, and probably into the anterior connectives; they have not, however, been traced farther forward than the anterior commissure.

These ventral groups, therefore, appear to supply a certain number of the fibres of the connectives; they probably are also of the nature of intermediate cells.

The greater number of the cells whose fibres run out in the lateral nerves are probably motor in function; the lateral groups are considerably the larger and supply the main bulk of such motor fibres.

The processes of all these cells which lie in the central nervous system are small, whatever the size of the cell. Large fibres are also found in the lateral nerves, which are processes of certain nerve cells to be described in the next chapter, which lie peripherally in the intermuscular layer. In the ganglion they do not connect directly with nerve cells but break up into numerous branches which ramify in the fibrillar substance. They are the only large fibres in the peripheral nerves.

The anterior nerve contains five of these, two very large ones and three moderately large ones. One of the very large ones breaks up into branches which ramify in the fibrillar substance of its own side; the other divides into two, one branch going to the fibrillar substance of its own side, the other crossing in the anterior commissure,

to make connections with the fibrillar substance of the opposite side. One of the three smaller fibres crosses in the anterior commissure to the fibrillar substance of the opposite side, the other two ramify in that of their own side.

In the posterior nerve also there are five large fibres whose distribution in the ganglion is very similar, one of the two largest ramifying in the fibrillar substance of its own side, the other bifurcating and going to both sides, its crossing fibre lying in the posterior commissure. A smaller fibre has not been seen to cross in the posterior commissure, it is probable therefore that all three of the small fibres ramify in the fibrillar substance of their own side. As has already been stated all the other fibres in the lateral nerves are small, the processes of the colossal cells must therefore also be small.

The relations of the connectives with the fibrillar substance of the ganglion remain to be described; the tracks of the various bundles of fibres are shown most clearly in the gelatine sections stained with hæmalum and Sudan III, as a differentiation is obtained between bundles which are passing through the ganglion, which stain blue, and those which break up and make connections in the main fibrillar mass, which take a more pink stain. The single median bundle of the connective, known as Faivre's nerve, will first be traced and then the path of the various bundles forming together the main lateral connectives. This median bundle is composed of one very large fibre, which lies on its dorsal side, and a few smaller ones, it runs right through the ganglion, keeping a median position throughout. After entering the anterior end of the ganglion (fig. 11) it becomes situated more dorsally, so that at the level of the fusion of the two lateral bundles it is lying in the centre of the ganglion (fig. 13). The main bundle of smaller fibres continues backwards in the same position, lying just dorsally to the large fat cells which will shortly be described (fig. 12). At the posterior margin of the fused mass it emerges slightly to the dorsal side of the centre of the ganglion, then, gradually assuming a more ventral position, finally leaves the ganglion in the original median ventral position between the two lateral connectives. The large fibre takes a somewhat different course. When the bundle fuses with the main fibrillar mass of the ganglion, the large fibre separates from it and runs more dorsally, fusing with the dorsal bundles of the lateral connectives where these join across the middle line (fig. 12). At the point where these dorsal bundles again separate it leaves them and turns ventrally to rejoin its proper bundle.

The fibres of the lateral bundles, just as they enter the ganglion, become divided into two groups, one external, the other internal (fig. 11). The internal group consists of three bundles which persist through the ganglion in a more or less median position, but become separated from each other by the crossing fibres of the ganglion. The dorsal internal bundle fuses with the bundle of the opposite side and spreads dorsally over the fibrillar mass, being separated from the median internal bundle and Faivre's nerve by the anterior and posterior commissures (figs. 12 and 13). The median internal bundles do not fuse but retain their position right through

the ganglion. With Faivre's nerve they form three striking bundles running through the main mass (figs. 12 and 13). The processes of the colossal cells hook round the median bundles on each side. The ventral internal bundles (fig. 11) also maintain their position through the ganglion but spread out ventrally over the fibrillar mass. In the centre of the ganglion their internal extremity lies very close to the main bundle of Faivre's nerve but does not fuse with it (fig. 13). Anterior and posterior to this the large fat cell separates them from the other median bundles and from each other (fig. 12). On emerging at the posterior end of the ganglion these bundles again assume their original relations.

The external group does not separate into any definite sub-grouping, but becomes fused with the outer part of the fibrillar mass of the ganglion. Dorsally and ventrally, however, a certain number of fibres seem to keep to some extent separate, and join with the more lateral fibres of the dorsal and ventral internal bundles in forming a dorsal and ventral covering to the main fibrillar mass. These fibres and the fibres of all the internal bundles take a blue colour with the hæmalum and Sudan III stain, while the main fibrillar mass and the greater portion of the external bundles, which fuse with it, take a pink colour. The internal group, therefore, is composed in the main of fibres which run through the ganglion, while the fibres of the external group freely connect with the various processes of the fibrillar substance. The fibres of these internal bundles often show up conspicuously in the methylene blue preparations, and in these also appear to pass straight through the ganglion without making connections in it. These internal bundles compare closely with those described by HARDY (23) in Astacus.

The two large fat-containing cells, which have been referred to, lie approximately in the middle line on the extreme ventral side of the fused ganglion mass, one lying anteriorly, and the other posteriorly (fig. 10). They are cells with a round clear nucleus of considerable size and stout branching processes (fig. 12). Their conspicuous characteristic is their very great fat content; this fat is in the form of small globules of fairly uniform size distributed throughout the cell protoplasm and lying especially thickly at the periphery of the cell. The fat content of these cells is out of all proportion to that in the nerve cells, so that in fat-stained specimens they form extremely conspicuous objects. They have no long processes and probably are supporting cells and not nerve cells, though they are clearly identical with the "median nerve cell" described by BRISTOL (6) in Nephilis. Other supporting cells with similar nuclei and processes, but containing no fat, are found, as has already been described, with the ganglion cell groups. Large branched supporting cells are also found at intervals along the connectives.

With regard to the nature of the fat in the fat cells it stains black with osmic acid, bright red with Sudan III (fig. 12), a deep purplish with Nile blue sulphate A (fig. 10) and is not doubly refractile; it is easily soluble in alcohol and ether. It is probably therefore a mixture of neutral fat and fatty acid. It is unlikely that any

cholesterin fats are present, as the globules are quite large enough to show the characteristic fluid crystal appearance under polarised light. No comparison can, therefore, be drawn between these cells and the cells of the suprarenal cortex.

Two other cells lie on the outside of the ganglion between the origins of the lateral nerves, which have been described by many authors, and are known as Leydig's cells (fig. 8). They are large cells, each of which curls round in the gap between the origins of the lateral nerves and extends for some distance along the sheath of each nerve; they are also in all probability supporting cells.

The connective tissue sheath of the ganglion and the sheath of the connectives contain small muscle fibres which lie longitudinally embedded in them (fig. 13). A pair of these is always present on the dorsal side of the ganglion, lying one on each side of the middle line. Their action must be to shorten the longitudinal axis of the ganglion and the connectives; and they are probably brought into play when the animal contracts longitudinally, so as to prevent any looping of the nerve cord.

IV. The Peripheral Nervous System.

The peripheral distribution of the segmental nerves has been chiefly studied in sections fixed in formol-Müller solution, embedded in gelatine (15) and cut serially, the sections being stained with hæmalum and Sudan III. The nerve fibres are differentiated by these stains from muscle fibres and fibrous tissue, as they take a faint but definite pink colour with Sudan III, indicating that they contain a certain amount of fatty substance in their composition.

The absence of shrinkage by this method enables the identification of fine nerve fibres to be much more easily made, than if paraffin sections are made use of.

Complete series of paraffin sections have, however, also been examined, and, as far as the nerves can be traced, confirm the results obtained from the gelatine-embedded preparations; the finest nerve fibres, such as those which innervate the vascular system, were not, however, identifiable in the paraffin ones. The gelatine sections were cut 15 μ thick, and a complete series covering a whole segment of the animal, and consisting of 150 sections, has been used. Such a series leaves an ample margin, as 100 of these sections cover a complete segment. The nerves have been traced by means of the projectoscope in the manner described in the chapter on the Vascular System.

The more peripheral ramifications of the segmental nerves lie in one particular layer, namely, the surface of junction of the longitudinal and circular muscles; this layer will be referred to in the description which follows as the intermuscular layer. These nerves thus lie on a cylindrical surface, and frequently various branches cross one another on this surface and appear to become, for a short distance, incorporated (fig. 15), though ultimately they again separate into their original constituents; the exchange of any considerable number of fibres at these crossings certainly does not occur.

From each ganglion two nerves are given off on each side, the anterior and posterior nerves; they at first run out directly laterally, gradually separating from one another. The anterior nerve, on leaving the ganglion, consists of five large fibres, two being very large and three smaller, and many uniformly small ones. As will be shortly described, all these large fibres have been traced to peripheral cells, of which they are the main processes; their final much-branched terminations ramify in the ganglion in the manner already described in the previous chapter. The small fibres are all processes of cells situated in the ganglion itself. If, therefore, the colossal cells have processes running in the anterior nerve, these processes are no larger than those of cells of ordinary size.

This anterior segmental nerve runs out directly laterally (fig. 14), lying between the viscera and the longitudinal muscle layer, and passing across the nephridial duct posteriorly. It finally, in the extreme lateral region, passes through the longitudinal muscle, and gets to the intermuscular layer; just before reaching this it divides into two large branches, one of which runs round in the fourth annulus (fig. 14) to the dorsal side, the other runs forward into the second annulus and there divides into two branches which run dorsally in the first and second annuli. The final branches of these nerves supply the whole of the circular musculature, that in the fifth annulus being supplied from the branch in the fourth, that in the third from the ventral branch of the main nerve about to be described. The physiological proof of this supply will be given in the next chapter. In the more central portion of its course, before it reaches the intermuscular layer, the anterior nerve gives off various branches. The largest of these is a large ventral branch (fig. 14), arising about one-quarter of the way from the ganglion to the lateral region, soon passing down through the longitudinal muscles and reaching the ventral intermuscular layer just on the inner side of the nephridiopore. This ventral branch contains all the larger fibres of the anterior nerve, together with a very few small ones; the main nerve peripheral to the origin of the branch consists of small fibres only. The branch passes over the duct of the nephridiopore anteriorly and then runs right round on to the dorsal side in the third annulus (fig. 14). Just as it crosses the duct two bipolar nerve cells are seen lying incorporated in the nerve—one very large and one smaller one; these cells are continuous with a large fibre and a smaller fibre respectively, which disappear from the more peripheral part of the nerve. Where the cells lie a special blood sinus comes in contact with the nerve, so as to form a small blood space round them. A group of two smaller cells is found at the point where the nerve is in an extreme lateral position; they are continuous with two more of the smaller fibres and also have a blood space round them. The remaining large fibre finally ends in a large cell lying dorsally over the more lateral part of the alimentary-canal. The large fibres of the anterior nerve are, therefore, all continuous with large cells situated at the periphery in the intermuscular layer, which are possibly sensory in function; their processes terminate entirely within the segmental ganglion.

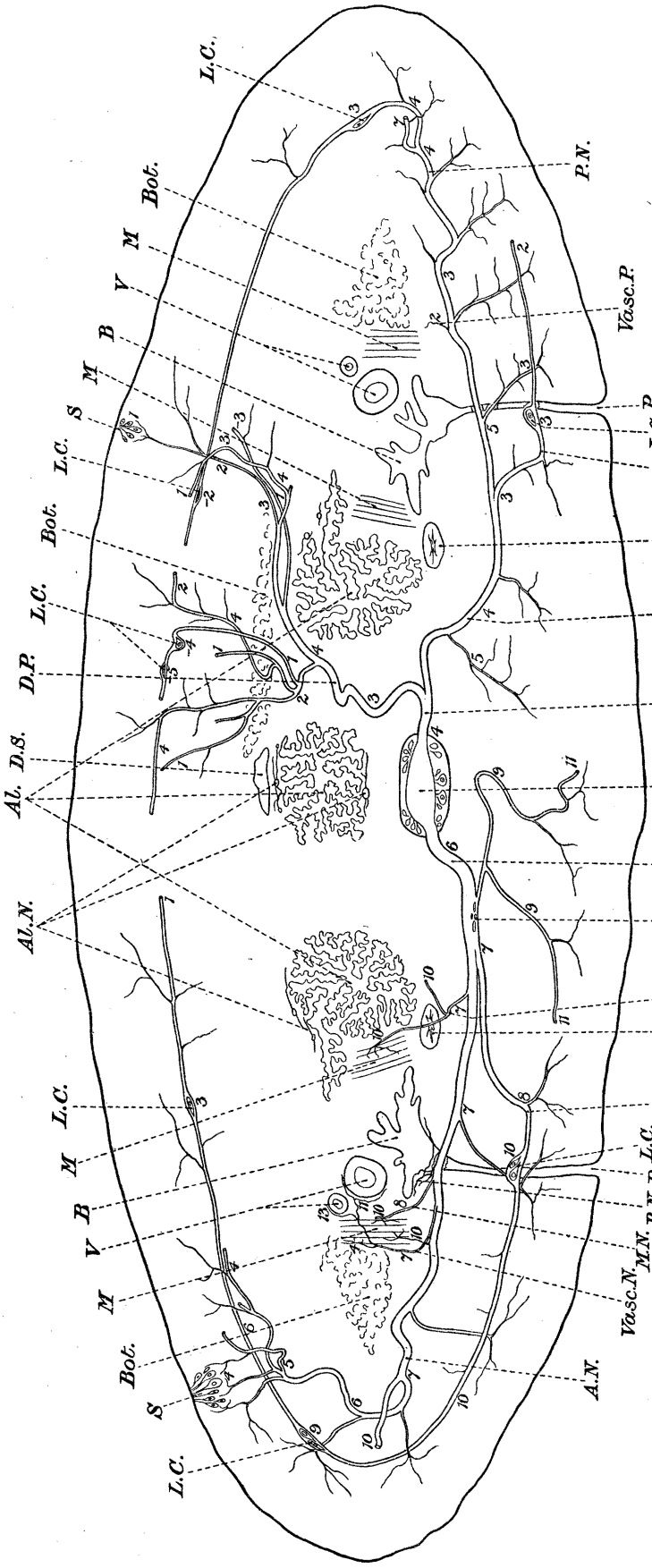

FIG. 14.—Projection on a transverse plane of the peripheral nervous system of the leech. The posterior nerve and its branches are shown on the right, the anterior nerve on the left.

Al., the sacculated alimentary canal. B., the bladder or nephridial vesicle. P., the nephridial pore; S.D., the segmental duct. D.S., the dorsal sinus. V., the main lateral contractile vessel with its latero-dorsal branch. M., the groups of dorso-ventral muscles. Bot., the botryoidal tissue. G., the nerve ganglion. P.N., the posterior nerve, soon dividing into its two divisions, the dorsal D.P. and the lateral L.P., which again divide peripherally into two main branches; V.P., the ventral branch of the lateral division; Vasc.P., the small branch which may join the vascular nerve. A.N., the anterior nerve dividing into two in the extreme lateral position. C., a group of small nerve cells early in its course. V.A., its ventral branch containing all the large fibres. Vasc.N., its vascular branch supplying the vascular muscle. M.N., branches to the dorso-ventral muscles. B.N., nerves to bladder and nephridium. S., sense organs or sensillæ; two only of these are shown. L.C., the large nerve cells lying in the intermuscular zone in which the main peripheral nerves ramify. Al.N., the alimentary nervous system. The segment shown lies in the anterior gut region. (Drawn with Edinger's projectoscope from serial gelatine sections.)

The anterior nerve as it passes ventrally beneath the viscera gives off fine branches supplying the various groups of dorso-ventral muscles, which lie between the various organs. These nerves may be called dorso-ventral nerves. At the point where the anterior nerve crosses the duct to the nephridiopore, fine fibres are given off to the segmental bladder and possibly to the general nephridial system; these have, however, not been followed out in detail.

Just beyond this point another small branch is given off, part of which supplies the vascular system. It may therefore be called the vascular nerve; as it also supplies dorso-ventral muscles, it may be considered to be one of the general group of dorso-ventral nerves. This vascular nerve runs outwards for a short distance, and then turns dorsally through a bundle of longitudinal muscle fibres, so as to get to the lateral aspect of the group of dorso-ventral muscles, which lie to the outer side of the main lateral vessel (fig. 14). It here divides into two branches, one turning anteriorly and the other posteriorly, and running directly forwards and backwards respectively. These branches lie approximately in the same relative position in all transverse sections examined. Some evidence has been obtained that the anterior branch of the vascular nerve of one segment may be directly continuous with the posterior branch of the corresponding nerve belonging to the next segment in front, that is to say, that these branches may form a longitudinal nerve chain on each side, running the whole length of the animal.

The posterior branch of the vascular nerve runs backwards for a short distance, then gives off a branch supplying a group of dorso-ventral muscle fibres, lying laterally to the lateral vessel. After giving off this branch it passes through the muscle group, so as to get to the median side of it, and then continues backwards, finally reaching the wall of the lateral vessel just anterior to its segmental valve (fig. 16); it then ramifies on the wall of the vessel supplying the muscle fibres, of which the latter is composed. These branches not only supply the wall of the vessel on the proximal side, but also cross over the valve to the anterior end of the vessel wall belonging to the next segment. The supply is therefore not entirely confined to that segment of the vessel which belongs to the segment of the animal under consideration. A separate branch is also given off from the posterior division of the vascular nerve, which turns forwards again and supplies the latero-dorsal artery; before reaching this it gives a branch to supply certain fibres of the dorso-ventral muscle group. Another fine branch continues backward in the region lateral to the dorso-ventral muscles, and may be continuous with the anterior branch of the segment behind. A special branch to the short contractile latero-lateral vessel has not been definitely identified, but probably exists.

The question whether fibres from the posterior segmental nerve pass into the posterior branch of the vascular nerve has not been settled with certainty. The main posterior segmental nerve trunk passes ventrally to the posterior branch of the vascular nerve, in close proximity to it, and gives off fine fibres just at the point of

approximation, one or two of which may become incorporated in the vascular nerve ; this continuity, however, could not be definitely traced in any of the specimens examined.

This nerve supply to the contractile vessels has been traced three times in the series of sections examined ; in none of these examples was there any sign of a nerve cell on the course of the fibres, either before they reached the vessel or on the wall of the vessel itself. The nerve cells of the fibres supplying the vascular system must therefore lie in the main segmental ganglion. It is probable that in each segment the main lateral vessel and its two contractile branches, the latero-dorsal and latero-lateral vessels, have each a separate nerve supply ; as has been described in the chapter on the vascular system, each can certainly maintain a contractile rhythm independent of the others, it is therefore a reasonable supposition that each has a separate nerve supply.

The main anterior nerve also gives rise to other ventral branches besides the main one, which run out and supply the circular muscles on the ventral side of the segment. Stimulation experiments show that the innervation of the circular muscles is exactly confined to the five annuli comprising a segment, in the centre of which the segmental ganglion lies. The anterior nerve also contains a small group of small cells, apparently nervous in nature, which lie incorporated in its ventral aspect, just before the main ventral branch is given off (fig. 14). It is possible that these cells are merely supporting cells ; they stain, however, like nerve cells. If nervous, no indication of their function has been obtained ; for experimental purposes they can be considered to group with the central ganglion cells.

The posterior segmental nerve, on leaving the ganglion, contains two very large fibres and three smaller ones, the rest of the fibres being uniformly small (fig. 11). It runs out directly laterally at first, then, shortly after its origin, it divides into two branches, a dorsal branch running directly upwards and a lateral branch running outwards (fig. 14), the dorsal branch being considerably the larger of the two. The lateral branch runs directly laterally, following a similar course to that pursued by the main anterior segmental nerve, but in a more posterior transverse plane. Both main lateral nerves, the anterior and this branch of the posterior, therefore lie posterior to the nephridial duct (fig. 14). On reaching the extreme lateral region, this lateral branch divides into two branches, one running round in the fourth annulus in the intermuscular layer, with the branch of the anterior nerve, the other crossing the third annulus and the ventral branch of the anterior nerve, and running round in the second annulus. The former branch contains two larger fibres, which connect with two nerve cells lying in the branch running round the fourth annulus, one lying in the lateral region corresponding to the lateral cells on the ventral branch of the anterior nerve, the other lying dorsally over the most lateral part of the alimentary canal, corresponding again in position to the cell on the ventral anterior nerve (fig. 14). The lateral branch of the posterior nerve also gives off a ventral branch, similarly situated to the ventral branch of the anterior nerve ; this

branch carries one of the largest fibres, all its other fibres being small. It reaches the intermuscular layer just to the inner side of the nephridial duct, passes across this posteriorly, and then runs round in the fourth annulus towards the dorsal side. As it passes behind the duct it contains a large nerve cell, which is continuous with the large fibre, the latter disappearing peripheral to this point.

Other branches arise from the main trunk, running out to supply the longitudinal muscles of the ventral side; no branches have been observed running dorsally from it, except some small ones to supply portions of longitudinal muscle, ventral to which it passes in the more lateral part of its course, before reaching the intermuscular layer. One of the innermost of these small branches (fig. 14) may join the vascular branch of the anterior nerve in the manner already described, and so take part in the innervation of the vascular muscles.

The dorsal branch of the posterior segmental nerve turns directly dorsally, being accompanied by a large sinus in connection with the ventral sinus, in which the nerve cord lies. It contains the remaining large fibre of the posterior nerve and sometimes also a smaller one. It runs up between the lateral diverticulum of the alimentary canal and its central portion, but gives off no branches in this portion of its course. On reaching the upper level of the canal it breaks up into a great number of branches supplying the longitudinal muscles of the dorsal region. Its one or two larger fibres run in a comparatively small branch, which runs posteriorly and dorsally, finally reaching the intermuscular layer (fig. 14). At this point are found one or two cells with which the larger fibres are directly continuous, the largest fibre being continuous with a large nerve cell, the smaller, if present, connecting with a smaller cell. A large branch passes out laterally over the lateral alimentary canal diverticulum, and then divides into various branches running out towards the intermuscular layer through the longitudinal muscles, which it supplies; another runs anteriorly and somewhat towards the middle line, to break up also into similar branches. Though carefully searched for, no communications were found between these nerves or any other branches of the posterior or anterior segmental nerves and the nervous system of the alimentary canal, soon to be described.

The branches of the posterior segmental nerve are mainly concerned with the innervation of the longitudinal muscle fibres, the lateral branch supplying the ventral longitudinal group, the dorsal branch the dorsal longitudinal group. The evidence of stimulation experiments confirms this. It is possible that a small branch is also given off to join the vascular nerve, and supply the vascular musculature.

Although the main supply of the anterior and posterior nerves to the various groups of muscles is as has been described, fine branches of the anterior nerve have been observed to run into the longitudinal muscles, and fine branches of the posterior nerve to end in the region of the circular muscles. These branches are of interest when considered in conjunction with experimental results. This matter will be further discussed in the next chapter.

The system on the alimentary canal consists of a network of fibres connected with numerous small ganglion cells, scattered over the wall of the alimentary canal; the largest nerve runs under its mid-ventral aspect. No connection between this system and any branch from the segmental nerves has been observed, though repeatedly searched for.

My observations are, therefore, in agreement on this point with those of BRISTOL (6) in Nephelis, and of others, that the nervous system of the alimentary canal is separate from the segmental ganglionic system, its only connections being with certain ganglia on the circumoral ring.

To sum up the possible functions and connections of the different varieties of nerve fibres, all the larger fibres have been found to be continuous with peripheral nerve cells lying in the intermuscular layer; their terminal ramifications all lie in the segmental ganglion and have been described in the previous chapter on the central nervous system.

All the large fibres being thus accounted for, it follows that any processes of the colossal cells which run in the peripheral nerves must be small, and indistinguishable from the main mass of small motor fibres forming the axons of the other cells in the segmental ganglion.

An arrangement of nervous rings with longitudinal connections, such as BRISTOL (6) has described in Nephelis, has not been observed: his description may, perhaps, be explained as due to a misinterpretation of the peripheral nerve distribution in the intermuscular layer. The evidence from stimulation experiments, about to be described, is conclusive that all the longitudinal and circular muscles of each segment are innervated directly by the axons of nerve cells situated in the corresponding segmental ganglion; the anterior nerve being the motor nerve to the circular and dorso-ventral muscle systems, the posterior nerve being the motor nerve to the longitudinal muscles.

Various collections of peculiar large cells are present just beneath the epithelium at various points in the segment.

These may be of a sensory nature and have been called sensillæ by some authors: they are also identical with some of the epithelial structures which HAVET (24) has described as consisting of nerve cells. In gelatine preparations they bear no similarity to nerve cells proper, however stained. Their innervation by numerous nerves, in some cases belonging to the anterior segmental nerve, in some cases to the posterior, renders it probable that they have a sensory function. This innervation implies that they are connected with neurons, whose cell bodies are placed in the central ganglion, and that such nerve cells are sensory in function. The central ganglion is therefore not alone composed of motor-cells, but contains also some sensory cells. A similar conclusion, that sensory cells are present in the central ganglia, has been arrived at by HARDY (23) in his investigation of the nervous system of Crustacea.

V. The Physiological Reactions of the Voluntary and Vascular Muscles.

(1) *The Control by the Central Nervous System.*

The study of the control of the central nervous system upon the voluntary and vascular muscles of the leech is hampered by the small size of the peripheral nerves. The anterior and posterior segmental nerves can only be identified with certainty under a dissecting microscope, and are too small to be isolated along a length sufficient to apply even the finest bipolar electrodes.

The method of stimulation has therefore been to dissect out a length of the central nerve cord cranial to the ganglion of the segment to be observed, by opening the animal along the mid-ventral line, exposing the nerve cord by slitting up the ventral sinus, and dividing the lateral segmental nerves. The electrodes are then applied to the nerve cord as near as possible to the ganglion. The cord has usually been divided caudal to the ganglion under observation, and may or may not be divided above the electrodes at the extreme cranial end of the incision; this latter proceeding is apparently without influence on the results obtained.

The electrodes used have been either a pair of very fine wires mounted on a movable stand, or the electrode invented by Keith Lucas (32), modified so that it can be applied extremely closely to the ganglion under consideration. While the former method had to be applied to a nerve cord lifted out of the Ringer's solution in which most experiments must be conducted, the latter is used under the fluid, which is a greatly preferable procedure. It also has the additional advantage that the ganglion can be made the point of maximum stimulation, by being applied sufficiently closely to the electrode. All experiments and observations on the vascular system must be made, as has already been mentioned, under Ringer's solution; a solution three-quarters the strength of mammalian Ringer being the most satisfactory.

The effect of stimulation of either anterior or posterior nerve was studied by dividing all the other nerves from the ganglion. A method of direct unipolar stimulation, to be described in detail when discussing the action of curare, was also used, and confirmed the results obtained by the other methods.

The majority of the experiments on the vascular system have been performed on animals curarised the day before; those on the voluntary muscle system and a few on the vascular system, in which curare interferes with the proper nervous action, have been performed on the decapitated animal.

(a) *The Innervation of the Voluntary Muscles.*—A length of nerve cord of a decapitated leech was isolated as described and enclosed in Lucas' electrode (32), so that the ganglion of the segment to be observed lay close against the latter; the nerve cord was cut through below the ganglion and dissected out and removed for some segments. The anterior nerves were then divided so that the two posterior nerves only were left. Stimulation then caused a strong contraction of all the longitudinal muscles of the segment, the dorsal, lateral, and ventral groups all

contracting so as to cause a marked diminution in the width of the five annuli composing the segment. This contraction was strictly confined to the one segment only, in the centre of which the stimulated ganglion was placed. The edges of the mid-ventral wound made to expose the nerve cord were not drawn apart, showing that no contraction of the circular muscles took place.

The posterior segmental nerve therefore is the motor nerve of the longitudinal muscles, both dorsal and ventral.

A similar preparation was also used, in which the posterior nerves were cut and the anterior left intact.

Stimulation applied as before caused no contraction of the longitudinal muscles, for the annuli did not decrease in width; but the sides of the ventral wound were drawn away from the middle line so as to increase the width of the opening, showing that a contraction of the circular muscles had taken place. The animal also became flattened dorso-ventrally owing to contraction of the dorso-ventral muscles. The contraction of the circular muscles was more clearly shown in further experiments, in which the ventral skin and muscle layers of one side of the segment were isolated by cuts in a transverse direction along its boundaries; such a procedure does not interfere with the course of the segmental nerves. On stimulation of the ganglion with posterior nerves cut, the edge of the original incision was seen to move a considerable distance away from the middle line.

The anterior nerve is therefore the motor nerve to the circular muscles of the segment, and to the dorso-ventral muscles also.

The transverse cuts made in these experiments caused a marked contraction of the longitudinal muscles; when, however, the anterior nerve was stimulated, the isolated tissue distinctly lengthened in a longitudinal direction. There is therefore a possibility that the stimulation of the anterior nerve causes relaxation of the longitudinal muscles; that is to say, the anterior nerve is not only motor to the circular and dorso-ventral muscles, but also carries inhibitor fibres to the longitudinal muscles, a condition of things which would harmonise with present views of the antagonistic innervation of voluntary muscles in the vertebrate.

It is possible also that the posterior nerve similarly contains inhibitor fibres to the circular and dorso-ventral muscles.

We may conclude therefore:—

(1) That the segmental innervation is exactly confined to each particular segment;
(2) That the anterior nerve is motor to the circular muscles and the dorso-ventral muscles, and possibly inhibitor to the longitudinal muscles;
(3) That the posterior nerve is motor to the longitudinal muscles and possibly inhibitor to the circular and dorso-ventral muscles.

These results agree with the anatomical description of the peripheral innervation, given in the previous chapter, for it is there shown that the innervation of the

longitudinal muscles is not entirely confined to the posterior nerve, but also includes some fibres of the anterior, which may well be the inhibitor fibres to these muscles. Similarly, certain fibres from the posterior nerve, which have been shown to run to the circular muscles, may be their inhibitor fibres.

There is thus both physiological and anatomical evidence for the existence of an inhibitor nerve supply to the voluntary muscles in addition to a motor supply.

(b) *The Innervation of the Vascular Muscles.*—The effect on the contractile rhythm of the vascular system of experiments similar to those just described may be divided into two stages: (1) the effect of cutting a nerve; (2) the effect of stimulation with that nerve cut.

In these and subsequent experiments on the contractile vascular system, the chief difficulty has been to obtain a reliable recording method. The vessel is too small and delicate for any ordinary mechanical method to be used. An attempt was made to obtain records with a tripod recorder, one foot of which was cut to a fine point and rested on the vessel, while the other two rested on the dissecting tray, but this failed owing to the impossibility of eliminating the movements of the underlying alimentary canal. Recourse was therefore had to direct observation under a dissecting microscope, the moment of contraction of the vessel being recorded by an ordinary signal on a revolving drum, and the signal key being kept down till relaxation again occurred. The key was either manipulated by an assistant or worked directly by the observer. The beginning of the contraction is sharply defined, and therefore has been recorded fairly accurately, but the end is often ill-defined, so no reliance has been placed on this in interpreting the curves. All measurements have been made from the beginning of one contraction to the beginning of the next.

The effect of cutting a posterior nerve is shown in Plate 16, figs. 17 and 18. A marked increase in the rate of rhythm occurs whether the beat is slow or fast. In fig. 17 a very slow rhythm, with intervals between the beats of 28 seconds, is increased to one with an interval of only 17·7 seconds; in fig. 18 a rapid rhythm with intervals of 10·5 seconds is increased to one of 8·5 seconds. In this series of experiments, besides the quickening of rate, an increased strength of contraction was frequently observed, and in one experiment a beat which had been entirely stopped by the cutting of the anterior nerve was renewed on cutting the posterior nerve.

The effect of cutting the posterior nerve therefore is to cause an increased rate of beat and increased contraction.

These experiments were all performed on curarised animals, so that the rhythm in every case was fairly regular and alterations in it were easy to determine.

The effect of cutting the anterior nerve is not so clear as that of cutting the posterior nerve; but the tendency is, however, always in one direction, towards the slowing of the beat and a relaxation of the vessel. This effect is shown in fig. 19, in which an interval between beats of 11 seconds has been increased to one of

13·5 seconds; similar results of an equal or less degree were obtained in other experiments, while in two experiments the cutting of the nerve caused total inhibition. These experiments again were performed on the curarised animal. In the non-curarised animal the beat is so irregular, owing to the dissections necessary for the experiment, that no reliable records of the effect on the vascular rhythm of this cutting could be made.

The cutting of the anterior nerve, therefore, has an effect which is directly opposite to that of cutting the posterior nerve; it causes decrease of rate of beat and diminished contraction.

A further series of experiments were made by stimulation of the ganglion, using the methods already described, with one nerve cut and the other left intact. Stimulation with the posterior nerve cut and the anterior left intact caused in all experiments a marked acceleration of rhythm; an example of this is shown in fig. 20, in which an interval of 14·4 seconds between beats has been diminished to an interval of 9·8 seconds. This experiment was performed on a curarised leech, as were most experiments of this series. A similar experiment performed on a decapitated leech gave a similar result, which is shown in fig. 21. This curve illustrates the difficulty of working on a non-curarised animal. In contrast to the regular rhythm shown in fig. 20, the beats occur very irregularly, a small change of rhythm is, therefore, not easy to identify. In fig. 21, however, the acceleration is well marked, an average interval between beats of 18·4 seconds diminishing to one of 10·7 seconds. After being allowed to rest for about 15 minutes, this same preparation was made use of to investigate the effect of ergotoxin, a drug which in the vertebrate abolishes motor effects on the heart and vascular muscles. In order to see whether in the leech also it would remove the motor effect of the anterior nerve on the vascular muscle, 0·05 c.c. of a 1-per-cent. solution of ergotoxin phosphate was injected by the method which will be described when considering the action of drugs, and a few minutes later the curve shown in fig. 22 was taken. In consequence of the injection of the drug the beats had become much slower and weaker, and were progressively slowing in rate. Stimulation of the anterior nerve is seen to have had no accelerating effect. Ergotoxin therefore destroys the motor action of the anterior nerve.

The effect of stimulation of the anterior nerve with the posterior nerve cut is therefore to accelerate the rate of beat, an effect which is destroyed by ergotoxin.

Another series of experiments was made with the anterior nerve cut, the posterior nerve left intact, and the ganglion stimulated. When performed on curarised animals these experiments were entirely inconclusive, both increase and diminution in rate of beat being obtained, whilst in a few no change of rate occurred. Experiments were therefore tried on the decapitated leech, and then showed a marked slowing of rate and diminution of contraction in every case. Fig. 23, Plate 17, is an example of this, for though the beat is extremely irregular, there is no question that a marked slowing

has occurred, the average interval between beats increasing from 14 seconds to 20·4 seconds.

The conclusion may therefore be drawn, that the effect of stimulating a posterior nerve with the anterior nerve cut is to cause a marked slowing in the rate of beat and a diminution in the strength of contractions. The action of curare masks this inhibitory effect, therefore experiments on curarised animals are entirely inconclusive.

Further experiments were also made in which the ganglion was stimulated with both nerves left intact. In the case of curarised animals such stimulation as a rule caused acceleration. Fig. 24 is an example of this, an interval of 12 seconds between beats being diminished to one of 10·5 seconds. In the non-curarised decapitated animal, however, stimulation caused slowing of beat, as is illustrated in fig. 25, an average interval of 16·6 seconds increasing to one of 20·5 seconds. In these experiments, therefore, curare reverses the result obtained. Normally stimulation of both anterior and posterior nerves brings out an effect similar to that of stimulation of the posterior nerve only, giving rise to an inhibition; the inhibitory effect of the posterior nerve therefore overcomes the motor effect of the anterior nerve. Curare, as has been seen when the posterior nerve alone is stimulated, removes its inhibitory effect, so that when both nerves are stimulated under curare, the motor effect of the anterior nerve becomes evident and an acceleration of beat takes place.

To sum up the results of these experiments :—

Both the anterior and posterior segmental nerves regulate the rate and power of the contractions of the vascular muscles. The anterior nerve is the motor nerve, stimulation of it causing increase, and section of it causing decrease, in the rate and power of contraction. The posterior nerve is the inhibitor nerve, stimulation of it causing decrease, and section causing increase, in the rate and power of contraction. When both nerves are left intact, normally the inhibitory effect of the posterior nerve overcomes the motor effect of the anterior nerve; this inhibitory effect can, however, be removed by curare and then the motor effect of the anterior nerve becomes evident.

The action of these two nerves is comparable to the action of the sympathetic and the vagus on the vertebrate heart, the anterior nerve corresponding to the sympathetic, and posterior nerve to the vagus. The action of curare on the posterior nerve and on the vagus is another point of comparison, curare entirely masking their effect in each case.

The anatomical confirmation of such an innervation of the vascular muscles has already been given and, as far as it has been ascertained, is in harmony with the above experimental results. The motor nerve, running in the anterior segmental nerve, has been traced from the central ganglion to its peripheral ending on the vascular muscle, and the existence of an innervation through the posterior nerve has been shown to be probable, though not as yet definitely proved. Evidence has also been given that these vascular nerves are the processes of cells situated in the central

ganglion, and the hypothesis has been advanced that the motor nerves are the processes of the chromaffine cells. On this supposition these cells not only have the function of secreting adrenalin, which, as will be shown, has a distinct physiological effect on the vascular muscle, but also are at the same time the motor cells for that muscle. They, therefore, control the vascular muscles in two ways in virtue of their combined secretory and nervous properties, being comparable, with respect to each property, to the suprarenal medulla and the sympathetic nervous system of vertebrates respectively.

(2) *The Action of Drugs.*

The chief difficulty met with in attempting to study the action of drugs on the circulation of the leech is, that the animal must be kept entirely under Ringer's solution, in order to obtain a regular and continuous rhythm in an exposed vessel. As has been already mentioned, the best Ringer's solution in the case of a leech is one made up of three-quarters the strength of mammalian Ringer, with the various salts in the same proportion. For this reason a direct application of the drug was not possible, and the extremely small size of the vessel made an intravascular injection impracticable; the injury to the vessel by attempts at the latter method also caused too much disturbance of the rhythm. The best method to obtain a local effect was found to be the following: a leech curarised the day before was taken and a length of vessel was exposed by cutting through the ventral edge of the lateral yellow line which runs down the side of the animal. When the cut is just sufficiently deep the vessel protrudes through it and can be easily observed.

A fine injection needle was then inserted through the skin and muscle layers two or three segments above the region under observation, and passed downwards, so that the point of the needle could be seen to lie in the loose tissue around the vessel exposed. The solution to be tested is then injected, filling the tissue spaces round it. In order to eliminate any possible effect due to increased tension of the surrounding tissue, Ringer's solution was injected in this manner, and was found to have no effect on the beat. Owing to the rapid diffusion of the drug away from the point of application, as is shown by the emptying of the spaces round the vessel, fairly strong solutions of drugs were employed, though it is also probable that in the leech relatively larger doses are necessary to obtain a visible effect than is the case with the more highly differentiated tissues of vertebrates. The actions of drugs on other muscular systems of the leech have also been noted in so far as they have bearing on the relationships of these systems to those of vertebrates.

Curare.—When introduced subcutaneously into the intact animal, curare has an action similar to that in vertebrates, for it differentiates the voluntary somatic muscles from the involuntary muscles, by causing complete paralysis of the former, but not affecting the latter in any way.

The voluntary muscles consist of the circular and longitudinal muscles and the dorso-ventral muscles, all of which are completely paralysed by curare, the modified

muscles of the suckers at the anterior and posterior extremities are also paralysed. The dose required for an ordinary leech in order to obtain complete loss of movement for purposes of operation is about 0·2 c.c. of a 1-per-cent. solution. A dose of half this amount causes complete relaxation but does not paralyse completely all reflexes. This dose is large in comparison with those required for vertebrates, in fact so large that some observers, for instance STRAUB (41), have denied that curare has any effect on invertebrates comparable to that in vertebrates. STRAUB, in his experiments on the earthworm, hardly reached with his maximum dose the minimum dose required to give any effect.

I have made use of this action on the leech for the purpose of examining the potency of a considerable number of samples of curare. Owing to the large size of the dose required in the leech the relative worth of the various samples has been easy to determine; 0·1 c.c. of a 1-per-cent. solution of a good sample is just sufficient to cause complete relaxation.

The involuntary musculature, which is unaffected by any doses of curare of this type, consists of the following systems: the two muscle layers of the vessel walls, the scattered muscles over the intestine, and the muscles belonging to the nephridial and the generative systems.

The animal can remain alive when completely under the influence of curare, and can ultimately completely recover from its effects. If a dose of 0·2 c.c. of a 1-per-cent. solution be given, the leech remains completely paralysed for about a week, and then gradual recovery begins to take place; the power of swimming is partly regained in about a fortnight, but the suckers cannot yet be used. Complete recovery occurs in about a month. Arguing from the sequence of events during recovery, it is probable that the circular and dorso-ventral muscles, which are those used in swimming, recover sooner than the longitudinal muscles.

For the purpose of studying the circulation the method has been to inject the animal on the previous day with 0·2 c.c. of a 1-per-cent. solution of curare, and to leave the animal in a shallow dish of water till required for investigation.

The ease with which recovery takes place from the drug shows that respiration is not interfered with, so long as circulation is intact. Respiration therefore, which takes place in the subepithelial capillary plexuses, is not in any way dependent upon the action of voluntary muscles.

The point of action of curare is the neuro-muscular junction, for the following reasons: the muscle is still easily made to contract by direct electrical or mechanical stimulation; stimulation of the peripheral nerve, however, causes no contraction. This latter point was experimentally proved by unipolar stimulation. One pole was formed by wrapping a fine wire completely round the animal, two or three segments away from the exposed nerve; the other by a very fine wire, bent in the form of a hook, by which the peripheral nerve could be hooked up from the tissue in which it lay. If the nerve is thus completely isolated in the air and care is taken that the

hook does not touch any other portion of the animal or the slime which is secreted into the wound, stimulation causes no contraction of the muscle. This experiment must be conducted in air; if attempted under Ringer's solution or with the nerve and hook not completely isolated, diffusion of current causes a muscular contraction.

The drug only takes effect if introduced into the tissues of the animal, if introduced into the fluid in which the animals lie or injected into the alimentary canal no paralysis takes place.

Injection into the alimentary canal is often difficult to avoid, for if the animal has been fairly recently fed, the alimentary canal, distended with blood, forms a large part of its bulk.

The action of curare in the leech with regard to the various muscular systems is therefore identical with its action in vertebrates.

Carbon Dioxide.—This gas has a marked anæsthetic effect upon the leech. It can be most conveniently applied in the form of ordinary soda water. The animal must be entirely immersed, by being placed in a vessel completely filled with soda water and then securely stoppered. Under these conditions complete anæsthesia is obtained in about three to five minutes; and the animal can then be removed and operated on under Ringer's solution. Such an anæsthesia lasts about 15 minutes, during which time an operation, such as cutting a peripheral nerve, can be easily accomplished, and the operation wound closed with sutures. If then replaced in water the animal in a short time completely recovers, being able to swim and move as usual.

The drug presumably acts by absorption through the cutaneous capillary respiratory system; sometimes actual bubbles of a gas, presumably CO_2, have been observed in the blood vessels of anæsthetised animals. The CO_2 thus absorbed is carried by the circulation to the central nervous system, and causes complete anæsthesia by acting on the ganglion in each segment.

Adrenalin.—In order to study the action of this drug, it is necessary to obtain a neutral solution of it. The ordinary preparations of the drug in the form of the chloride are strongly acid, and this acid has a marked effect on invertebrate muscle, causing contraction. For example, a solution of hydrochloric acid, of equivalent acidity to Parke Davis' preparation of adrenalin diluted to 1 in 100,000, will cause a marked contraction in a strip of muscle from either the longitudinal or circular muscle coat of *Lumbricus herculeus*, which has previously been suspended and allowed to relax in Ringer's solution. The base adrenalin is insoluble in neutral solutions, it must therefore be used in the form of a neutral salt. The preparation used has been synthetic adrenalin borate, as supplied by Meister, Lucius and Brunning; this has a reaction practically neutral to litmus. A fresh solution must always be made up immediately before using, as the salt oxidises with great rapidity.

The action on the voluntary muscles has been studied in the earth worm, *Lumbricus herculeus*, as it is easier to make strip preparations of the longitudinal and circular muscles from this animal, and the reactions are essentially the same as

those in the leech. Circular and longitudinal strips of the muscular body wall were cut out and suspended in recording troughs in Ringer's solution. They were then left for an hour or two to relax in this solution, as the cutting out of the strips at first caused strong tonic contraction. A small amount of adrenalin borate powder was then sprinkled into the trough, so as to make a weak solution of a strength between 1 in 10,000 and 1 in 100,000. This procedure did not give rise to any action on the muscle. If, however, a solution of similar strength, made from Parke Davis' adrenalin chloride solution, was substituted, the muscle strip was thrown into strong contraction. This contraction, as has already been stated, is due to the acid and can be imitated exactly by an equivalent hydrochloric solution. Adrenalin has therefore no effect on the voluntary longitudinal and circular muscle systems.

The action on the vascular muscles has been studied in the leech. A solution of about 1 in 10,000 of adrenalin borate, applied by injection by the method already described, causes a distinct quickening of the beat of the vessel. Such an experiment is shown in fig. 26, in which an average interval between beats of 14·5 seconds is diminished to one of 9·4 seconds; it also causes a distinct increase of contraction, the vessel not relaxing to the full extent between the beats, and in addition to this, some irregularity of rhythm, which can also be observed in fig. 26. Another form of experiment also illustrates the effect of adrenalin. Two separate lengths of vessel were exposed on the same side; if left undisturbed, the beat was seen to travel from the more caudal portion to the more cranial, the two lengths contracting in this order. A solution of adrenalin borate was then injected into the perivascular tissue of the more cranial portion; the rate of beat of this gradually increased so that after a time the cranial portion contracted before the caudal, the order of contraction being thus reversed owing to the acceleration of rhythm caused by the adrenalin solution. The cranial portion also relaxed less and was sometimes observed to constrict right up while the caudal was still beating. Adrenalin borate therefore causes acceleration of the rate of beat and also constriction of the vascular muscle, effects which are comparable to those on the vertebrate heart and vascular system.

The absence of effect of adrenalin upon the voluntary muscles of the leech and earthworm further confirms the evidence, obtained from the action of curare, that these muscles are in many respects comparable to the voluntary muscles of vertebrates.

Atropine.—The action of atropine, injected in the manner already described, causes an increase of rate and efficiency of beat, both contraction and relaxation being maximal. An example of this is shown in fig. 27; this experiment was performed on an animal, curarised the day before, in which the beat was already rapid, the interval between beats averaging 5·5 seconds; the injection of one drop of 1-per-cent. atropine solution increased the rhythm to one with an interval of 4·2 seconds, the fastest rhythm which has been observed to take place. Atropine therefore causes an increase of rate and efficiency of beat as in the vertebrate heart.

Muscarine.—An alcoholic extract of this drug, for which I am indebted to Prof. DIXON, was evaporated to dryness, and redissolved in Ringer's solution. When injected in strong solution, it caused complete cessation of beat and stasis in diastole, fig. 28. In very weak solution it caused slowing and weakening of the beat. The complete cessation of the beat caused by muscarine can be removed by a subsequent injection of atropine, if this atropine injection is made soon after the cessation of the beat. If, however, the muscarine is allowed to act for some time before the atropine is injected, the latter will no longer bring back the beat.

The action of muscarine and atropine on the vascular muscles of the leech is therefore similar to their action on the vertebrate heart, atropine causing stimulation of rate of beat and size of contraction, while muscarine causes diminution of these in weak doses, and complete stasis in diastole in strong doses. Stasis in diastole with the vessel filled with blood is a very unusual condition in the lateral vessels of the leech, this action of muscarine is therefore the more striking. The antagonistic action of atropine to muscarine has also been demonstrated, though it will only act if the atropine is administered soon after the muscarine effect has been established.

The action of these various drugs, therefore, increases the comparison between this contractile vascular system of the leech and the circulatory system of vertebrates.

To sum up the physiological relations and reactions of this system; it is rhythmically contractile, this rhythm being an intrinsic property of the muscle itself, but being normally under the control of nerves whose centres lie in the segmental ganglia. These nerves are two in number, one being accelerator in function and the other inhibitor. The muscle also reacts in a similar way to the heart muscle of vertebrates towards the following drugs—curare, adrenalin, atropine and muscarine.

The action of adrenalin is of especial interest, as it is extremely probable that this substance is normally supplied to the circulation by the secretion of the chromaffine nerve cells, which lie in the central ganglia. The method of carriage of the secreted adrenalin to its point of application at the periphery is probably the same as is found in the vertebrate, namely, by the circulation. It is, however, just possible that it travels down the nerve fibre itself, as the early part of the axon of a giant cell has occasionally been observed to be coloured with chrome salts, and to remain blue by the methylene-blue chrome salt method. This coloration has, however, not been traced further than the entrance of the nerve fibre into the fibrillar mass of the ganglion, and may therefore have little significance.

VI. DISCUSSION AND CONCLUSIONS.

The investigations detailed in previous chapters support to a considerable extent the hypothesis advanced in the introductory chapter, that the homologues of the three systems in vertebrates, the adrenalin system, the sympathetic system and the contractile vascular system, are present in a primitive state in certain annelids. The

points of comparison will now be discussed, and starting from this hypothesis the possible evolution of the three systems will be traced, and its close agreement with the indications afforded by the embryology of the higher vertebrates will be shown.

As has already been stated, only comparatively few members of the annelid kingdom contain the chromaffine nerve cells, which have been advanced as the common ancestors of both the sympathetic and adrenalin systems. The presence of such cells is always accompanied by the existence in the animal of a contractile vascular system, which is under the control of the central nervous system, and whose motor nerves are very possibly processes of the chromaffine cells. The particular annelids in which these systems are most highly developed are the Hirudinea, and especially those members of the group which can exist with the greatest ease in varied surroundings. The two possessing a maximum development in these respects, Hirudo and Aulostoma, may be truly looked upon as amphibious in nature, being able to exist for prolonged periods in air. The Oligochæte Lumbricus also, living in moist earth, leads a similar intermediate existence. For such a mode of life a true circulation becomes necessary. In the great Polychæte group on the other hand, which is essentially composed of sea-living animals, the necessity for an independent circulation has not arisen, the circulating fluid being practically the same as that in which the animal is immersed. Although many of the Polychæte group are much more highly developed in many ways, and in particular with regard to their supra-œsophageal and infra-œsophageal ganglia, nevertheless there is no development of a contractile vascular system and chromaffine nerve cells are not present.

Assuming that these cells are the common ancestors of both the chromaffine and sympathetic systems, their relations to these systems, as they exist in vertebrates, may now be discussed; the justifications for looking upon the two systems as morphologically one will first be stated. The embryological evidence afforded by the work of Kohn (26), who has extended and amplified the original conceptions of Balfour (2), has already been mentioned. His researches in the Mammalia show that, in the early stages of development, a most diffuse mass of tissue exists, lying around the abdominal aorta, from which both chromaffine cells and sympathetic ganglion cells originate; these two types of cell arise quite irregularly throughout the mass, often lying intimately intermingled.

A remarkable parallel to this is seen in the conditions found in the lower vertebrates, the accurate description of which we owe to Giacomini. In the introductory chapter his researches in various groups of fishes have been referred to, and the intimate relations which exist with regard to distribution between the chromaffine cells and the cells of the sympathetic system have been emphasised. In the Cyclostomata (17), Ganoidei (19), Teleostei (18), and Dipnoi (20), both systems lie in close relationship to the cardinal and segmental veins, and the relationship between individual cells of the two systems is often extremely intimate, a ganglion cell lying in close contact with a group of chromaffine cells. In Elasmobranchs the intimate

juxtaposition of the chromaffine cell masses and the sympathetic ganglia, and their origin from a common mass, has long been known, having first been described by BALFOUR (2). In the Amphibia, with the increased development of the sympathetic system, the intimate mingling of chromaffine cells and sympathetic ganglion cells to form one mass is still more remarkable, as the researches of GIACOMINI (16) and others clearly show.

In all these lower vertebrates the diffuse distribution of the chromaffine system is very marked, being the more conspicuous the lower the descent in the vertebrate scale, whereas the sympathetic system becomes less evident. According to GIACOMINI, the sympathetic system in the Dipnoi is present chiefly in the form of two very fine lateral chains, comparable to those of the higher vertebrates, but in the Teleostei, Ganoidei and Cyclostomata it is not yet aggregated into regular chains, but is scattered diffusely in groups of various sizes along the course of the veins in company with the chromaffine cells; its component cells are still fairly numerous in the Teleostei, less numerous in the Ganoidei, and extremely few in the Cyclostomata.

This intimate relationship of the two types of cell agrees with their embryological development described by KOHN (26). GIACOMINI is strongly opposed to the view that the chromaffine cells have any relationship to the sympathetic ganglion cells; he, however, admits that the distributional relationship of the two systems is close. It is not here maintained that the chromaffine cells of vertebrates have ever possessed the nervous properties of the sympathetic cell, but only that the two cells were originally derived from a common ancestor. I agree with GIACOMINI that the cells of the chromaffine system in the vertebrate have never possessed any other function besides that of secretion. This does not, however, exclude the possibility that they are derived from what may be termed an indifferent cell, having potential power of development in one of two directions, either becoming secretory or nervous in function. The actual existence of a cell possessing both these properties in annelids is a strong argument in favour of such a possibility. One of the factors of evolution has always been the multiplication of cells of any type whose function is becoming increasingly important. In the course of the multiplication of the chromaffine nerve-cells it is easy to conceive of the separation of their two functions so as to become the separate property of two different types of descendant cells.

In the preliminary chapter the suggestion has been made that the scarcity of sympathetic cells in the Cyclostomata may be compensated for by the extremely diffusely distributed chromaffine tissue, the control of the vessels being mainly brought about directly by the agency of adrenalin. The latest work of ELLIOTT (12) has shown that adrenalin is being constantly secreted into the vessels, and VON ANREP (44) has demonstrated that quite localised vascular reactions are dependent upon this supply. It is conceivable, therefore, that in the case of the Cyclostomata an actual local secretion of adrenalin may be the main method of control of the vascular system. If we consider the chromaffine system and the

sympathetic system as forming together one morphological entity, this will be extremely adequately represented in the Cyclostomata, though the chromaffine system preponderates greatly over the sympathetic system.

Another relationship of the chromaffine system of Petromyzon is of great importance, that with the posterior root ganglia; it was described by GIACOMINI (17), but no great importance was attached to it by him; my own observations (14) have clearly shown this relation. Chromaffine cells run up in each segment with the segmental vessels, lying in the wall of the vein; they also lie in intimate contact with the ganglion cells of the posterior root ganglion; at the point where the vein passes by it the two types of cell touch one another, and the amount of chromaffine tissue here aggregated is considerable. The relation of the chromaffine cells and the posterior root is not alone confined to the ganglion, but is continued in the peripheral prolongation of the nerve, which lies in intimate contact with the segmental vein and the chromaffine cells in the wall of it. The peculiar distribution (14) of the chromaffine tissue in the wall of the vein is probably determined by the position of the posterior nerve, rather than by that of the artery, for, as I have already described in my previous paper, the chromaffine cells do not lie between the artery and vein, where they are most closely in contact, but a little to one side of this position. The chromaffine cells may therefore be considered to lie along the course of the posterior nerve. In the other groups of fishes in which GIACOMINI has described the chromaffine system, he frequently mentions the chromaffine cells as seen lying along the course of nerve filaments. It can therefore be maintained that these diffuse chromaffine systems are arranged along the posterior segmental nerves.

The work of ONODI (35) has demonstrated that in the Mammalia the sympathetic ganglia arise as lateral outgrowths from the spinal cord in company with the posterior root ganglia, and only become separated from these at a later stage. In certain of the Amphibia, for example in Bufo and Bombinator, according to GIACOMINI'S (16) researches, this intimate relationship is maintained even in the adult. If the chromaffine and sympathetic systems may be regarded as a morphological entity, and therefore interchangeable, this intimate contact with the posterior root ganglia is again evident in Petromyzon, the only difference being that here chromaffine cells are in contact, and not sympathetic ganglion cells. The embryological researches of later observers confirm this origin of the sympathetic ganglia from the central nervous system itself. According to the views advanced in this paper the origin of the chromaffine cells is the same as that of the sympathetic ganglion cells; chromaffine cells therefore have also migrated out from the central nervous system along the course of the posterior root nerve, and the common ancestor of both systems should be found actually in the central nervous system. The chromaffine nerve cells of the Hirudinea and some other annelids, which have been shown to possess the properties of both nerve cells and adrenalin secreting cells, are situated in the very region in which the consideration of the vertebrate would lead us to expect to find

them. Additional evidence of the intimate relationship between the cells of the suprarenal medulla and sympathetic nerve cells has lately been given by MACALLUM (33). He finds that nerve cells in a fresh state become blackened by silver nitrate solutions, and further that this reaction is a specific for nerve cells, the only exception amongst all other tissues being the cells of the suprarenal medulla. These cells blacken extremely deeply in a manner comparable to the cells of the sympathetic system, which also blacken more deeply than other nerve cells.

To summarise the views advanced in this paper with respect to the chromaffine and sympathetic systems, it is held that the properties of the two systems were originally resident in a common ancestor of both. This ancestral chromaffine nerve cell was first formed in the annelid group arising in connection with the development of a contractile vascular system, to which it acted as regulator in two ways, both by the secretion of adrenalin and by nervous action. This common ancestor was originally situated in the central nervous system, but in the course of the evolution of the vertebrate, its descendants have migrated out in connection with the posterior root ganglia. In the case of the lowest vertebrate, Petromyzon, this intimate relationship still exists between chromaffine cells and the posterior root ganglion, but the migration has spread further, so that chromaffine cells are distributed, in addition, diffusely along the ramifications of the posterior nerve, which lie around the large veins. The other type of cell, the sympathetic nerve cell, is also present, lying sparsely scattered among the chromaffine cells. Advancing higher in the vertebrate kingdom the sympathetic element becomes more evident, more and more replacing the chromaffine tissue, though at first, for instance in Ganoids, it has only a diffuse segmental arrangement, similar to the chromaffine cells of Petromyzon. In the Dipnoi, however, a definite pair of lateral chains have become formed, while in the Amphibia the sympathetic system has practically reached the development which is found throughout vertebrates to the highest Mammalia, though chromaffine cells and sympathetic nerve cells are still most intimately mixed. It is only in adult Mammalia that the two types of cell become definitely separated off, the chromaffine tissue completely disappearing from the sympathetic ganglia, and becoming aggregated entirely in the mass of the suprarenal medulla. In the course of evolution, the preponderance of the chromaffine cell element, which is so marked in Petromyzon, has gradually given way to an increasing development and complexity of the sympathetic system, so that ultimately the only surviving remnant of the originally diffuse chromaffine system is the tissue of the suprarenal medulla. Such a view of the general evolution of the two systems compares very closely with their known embryological development. A mass of cells arises in the lateral portion of the spinal cord, which migrates out and gives rise to two types of cells which ultimately become the cells of the chromaffine system and of the sympathetic system. In the embryo these are at first intimately mixed together, and extend over a large number of segments of the body; gradually, however, portions of the main mass get

separated off and become more and more composed of sympathetic nerve cells only, while other portions are separated off, which show an increasing preponderance of chromaffine cells, though for a long time a few chromaffine cells persist in the sympathetic cell masses, and sympathetic cells in the chromaffine masses. Of the latter two only finally survive, one in the region of the cœliac vessels, corresponding therefore to the semilunar ganglion, and one in the region of the inferior mesenteric vessels, corresponding to the inferior mesenteric ganglion. The upper mass finally becomes enclosed by the inter-renal body, so as to form the compound suprarenal organ, while the lower forms the organ of Zuckerkandl, which disappears soon after birth.

The two systems have been concerned throughout their existence primarily with the regulation of the vascular apparatus. Such an intimate relationship between an internal secretion and a nerve-cell complex, and the presence of the internal secretion actually in the nerve cell in the most primitive form, may not be confined alone to the sympathetic system and its associate the chromaffine system, but may also be a combination which is present in connection with many different systems of nerve-cells.

The relationship of the primitive contractile vascular system, which is found in the Hirudinea, to that of the vertebrate may now be discussed. This system has been shown to consist of certain vessels clothed with muscle-fibres, which have many properties analogous to the heart muscle of vertebrates, both with regard to their reactions to various drugs and to the possession of a rhythm which is intrinsic to the muscle, but is normally regulated by nerves which come from the central nervous system.

Embryology may again be referred to in endeavouring to explain the relationship of such a contractile system to that which exists in vertebrates. The vertebrate heart is formed by the fusion of two longitudinal vessels, which run parallel to one another along the ventral side of the body. These vessels reach the mid-ventral position by the growing round of the two lateral body folds; before this occurs they therefore lie in a very lateral position in the embryo, in fact, in the very position in which the two longitudinal contractile vessels lie in Hirudo. In 'The Origin of Vertebrates,' W. H. Gaskell (13) has shown that an extremely close comparison can be drawn between these two longitudinal vessels of the vertebrate embryo and the two longitudinal venous sinuses of Limulus. The latter function as branchial vessels supplying the blood to the branchiæ for aeration. The chief function of the longitudinal contractile vessels of the leech, as has been explained in the second chapter, is to supply the annular vessels, and through them the cutaneous capillary system in which respiration takes place. Both in position and function the longitudinal vessels of the leech are comparable to the longitudinal venous sinuses in Limulus; and the comparison between the longitudinal vessels of the vertebrate embryo, which later form the heart, and the longitudinal venous sinuses of Limulus

will hold equally well with the main contractile vessels of the leech, which in addition have physiological properties closely comparable with the vertebrate heart.

Further, with respect to the position of the longitudinal lateral vessels of Hirudo, when the animal swims its body is flattened by the contraction of the dorso-ventral muscles; the yellow line under which the lateral vessel lies is then seen to form the extreme lateral angle on each side. This lateral portion of the body is even more constricted dorso-ventrally than the main portion, and thus forms a kind of lateral fold running along the whole length of each side of the body, and containing in it the lateral vessel, in a manner similar to the primitive folds of the vertebrate embryo which contain the vessels later forming the heart.

The contractile system of Hirudo is, however, not merely composed of the longitudinal lateral vessels, but there also lies in each segment a contractile branch, the latero-dorsal vessel which meets its fellow of the opposite side across the middle line dorsal to the alimentary canal, and connects with a pair of longitudinal alimentary vessels in the posterior half of the body.

According to the descriptions of BOURNE (5) in other leeches, for example Clepsine, Pontobdella and Branchellion, a dorsal longitudinal vessel with muscular walls lies in this mid-dorsal region, being often enclosed by the dorsal sinus. BOURNE holds that in the Gnathobdellidæ, this dorsal vessel has vanished, the primitive arrangement in Hirudinea being four vessels, a dorsal, a ventral, and two lateral, each lying in a sinus and freely intercommunicating. In Branchellion lateral vessels similar to those in Hirudo exist, and communications between these lateral vessels and the dorsal vessel have been observed by DE QUATREFAGES (10), which can be compared with the latero-dorsal vessels of Hirudo; the latter may therefore be looked upon as the representatives of the connections between primitive lateral and dorsal vessels.

The dorsal longitudinal vessel lying in its sinus is in the position of the dorsal heart of arthropods, such as Limulus, in which also the longitudinal venous sinus is comparable to the lateral vessels of Hirudo. The latero-dorsal vessels of Hirudo would then be analogous to the remarkable veno-pericardial muscles of Limulus and the Scorpions, which are attached at one end to the pericardium and at the other to the longitudinal venous sinuses. These veno-pericardial muscles, according to BLANCHARD (4), LANKESTER (28), and others, are formed of muscle fibres arranged in a cone, the centre of which is filled with blood and connects with the lumen of the longitudinal venous sinus. In the scorpion, according to BLANCHARD, they contract synchronously with the heart. In Limulus, according to CARLSON (8), they contract with the respiratory muscles, but at the same time the motor nerves to them are also the motor nerves to the heart in each segment. Their relation to the vascular apparatus is therefore a very close one, and a derivation from the dorso-lateral vessels of the leech would give an explanation both of their peculiar blood-filled lumen connecting with the longitudinal venous sinus, and also of their connection with the heart, which has been formed from the median dorsal vessel of the Hirudinea.

Such a theory derives both the systemic dorsal heart of the arthropod and the branchial ventral heart of the vertebrate from the primitive contractile system which is found in the Hirudinea, the lateral vessels of this system forming the vertebrate heart, and the dorsal vessel the arthropod heart.

The innervation of the contractile vessels of Hirudo is also comparable to that of the vertebrate heart. The motor nerve to their musculature, which runs in the anterior segmental nerve, and is in all probability formed of the processes of the chromaffine cells, corresponds to the sympathetic innervation of the heart in the vertebrate, which is also entirely motor in function. The inhibitor nerve which runs in the posterior segmental nerve must then correspond with the cardiac vagus of the vertebrate. The possibility of the evolution of the sympathetic system from the chromaffine nerve cells of annelids has already been advanced; the derivation of the vagus system from the segmental inhibitor nerves is, however, not so clear. Such a derivation demands the concentration of a segmentally distributed nerve to the anterior end of the nervous system. On the lines of the origin of vertebrates advanced by W. H. GASKELL (13), this can be conceived to have occurred with the concentration of segmental ganglia, to form the ganglia of the medullary region of the brain. The concentration demanded for the formation of the inhibitory mechanism to the ventrally disposed vertebrate heart is also required in the case of the dorsally disposed arthropod heart.

CARLSON (7) has shown that in Limulus the inhibitor nerves of the heart arise from the fused infra-œsophageal ganglion, although its motor nerves arise from the segmental ganglia in a manner precisely similar to those of Hirudo.

The infra-œsophageal ganglion has been formed by the fusion of many segmental ganglia, and the inhibitory mechanism originally segmentally distributed has become concentrated in these fused ganglia, while the motor mechanism is still left segmentally arranged. The differentiation of these centres of nervous control of the dorsal arthropod heart derived from the dorsal vessel of Hirudinea thus affords evidence of the differentiation also required for the different type of heart found in vertebrates, derived from the lateral vessels of the Hirudinea. It is not suggested that the cardiac vagus of vertebrates can be derived from the annelid arrangement by way of the arthropod group, but only that in these two different types of heart a similar differentiation of the nervous mechanism has taken place.

The course of evolution of the contractile vascular mechanism advanced in this paper is therefore as follows: The primitive contractile vascular system originally arose in the annelid kingdom, contemporaneously with the origin of its regulators, the adrenalin and sympathetic systems. In annelids it consists of two longitudinal lateral vessels from which the vertebrate heart has been derived; it consists in addition of a longitudinal dorsal contractile vessel which has given origin to the arthropod heart. Even in the primitive form found in Hirudo, the muscle of the contractile system possesses many of the properties found in higher animals, being

rhythmically contractile, reacting to many drugs in the same way as the similar muscle of arthropods and vertebrates, and being controlled in a similar way by the central nervous system. The derivation from a common origin in annelids, which already shows similar properties, would explain the close physiological relationships which CARLSON (8) has shown to exist between the dorsal heart of the invertebrate and the ventral heart of the vertebrate.

The arrangement of the contractile vascular system and its regulating nerves in Hirudo has bearing upon the question of the nature of the rhythmical contractile wave in the vertebrate heart. In Hirudo processes of cells situated in the central nervous system run directly to the vascular muscles, no peripheral nerve cells are found in the course of these fibres. The contractile rhythm of the vessel has been observed to continue for over an hour after complete severance of the peripheral nerves. The contractile rhythm is therefore independent of nervous action, and is an intrinsic property of the vascular muscle itself. If the heart of the vertebrate can be derived from such a system, the condition in Hirudo strongly supports the myogenic theory of the heart beat, which has of late been rendered doubtful by CARLSON'S (8) work on Limulus. The theory advanced in this paper would, moreover, make the conditions in Limulus not strictly comparable to those in the vertebrate, but would rather emphasise the relationship of the latter to those in Hirudo.

The descriptive portions of this paper, dealing with the vascular and nervous systems of Hirudo, have been summarised at the end of each chapter, they, therefore, require little mention here.

The chief points of interest with respect to the circulation are the following: The principal function of the contractile vascular system is to send blood to be aërated in the cutaneous capillaries, though it also drives blood to some extent through the alimentary and nephridial systems; its chief function is, therefore, to act as a branchial heart. It is only connected with the dorsal and ventral sinus systems by these capillary systems. The dorsal sinus system acts as a collecting system from the alimentary canal and is connected on the opposite side with the botryoidal tissue and the intramuscular plexus; the function of the botryoidal tissue may therefore be digestive in nature. The ventral sinus system only obtains blood after it has been purified by passage either through the excretory system or the cutaneous capillary system; the nerve cord which lies in its principal sinus therefore only comes into contact with purified blood.

The segmental nervous system consists of nerve cells arranged in groups in the central ganglion, which are mainly motor in function, and of large cells situated peripherally in the intermuscular zone whose function is probably sensory; a few sensory cells are also present in the central ganglion. Many of the motor cells have processes which cross in the ganglion to innervate the opposite side of the body, others send processes in one or both of the lateral nerves to their own side. The sensory cells situated in the ganglion supply sensory organs in the skin. The large

peripheral sensory cells have processes of larger size than other nerve cells, which run directly to the central ganglion and make connections there. Other cells are also present in the ganglion whose processes run in the connectives; these are comparable to the intermediate cells of the vertebrate central nervous system.

The innervation of the voluntary muscles is of interest. The anterior nerve is motor to the circular and dorso-ventral muscles, and the posterior nerve is motor to the longitudinal muscles, the innervation being strictly confined in both cases to the segment of five annuli in the centre of which the ganglion lies. The anterior nerve possibly also contains inhibitor fibres to the longitudinal muscles, and the posterior nerve inhibitor fibres to the circular muscles. A reciprocal innervation of antagonistic muscles may therefore be already developed in Hirudo.

The nervous supply of the alimentary canal is entirely separate, and has no connection with the segmental system.

Conclusions.

1. Certain nerve cells exist in each segmental ganglion of certain annelids, in particular the Hirudinea, which give a chromaffine reaction and most probably secrete adrenalin.

2. In these annelids a contractile vascular system exists which reacts to adrenalin, and which has other properties similar to those of the vascular system of vertebrates.

3. The development of this vascular system in each animal is proportional to the development of the chromaffine nerve-cells.

4. This vascular system is under the control of the central nervous system, the anterior segmental nerve containing motor fibres to it, and the posterior segmental nerve inhibitor fibres in each segment. These fibres are the processes of cells situated in the segmental ganglia, the motor nerves being in all probability the processes of the chromaffine cells.

5. These chromaffine nerve cells are the common ancestors of both the chromaffine and sympathetic systems of vertebrates; in the course of evolution they have emigrated out from the central nervous system, and have become differentiated into the two different types of cell. Throughout their further development the two systems still give evidence of their common origin by intimate physiological and anatomical relationships.

6. In the most primitive vertebrate, Petromyzon, the chromaffine sympathetic complex is chiefly represented by the chromaffine portion, which is so arranged as to be able to carry out efficiently the vascular control. With the advance in the vertebrate scale, the sympathetic portion becomes more and more prominent at the expense of the chromaffine element, which ultimately becomes confined to the medulla of the suprarenal glands.

7. The presence of secretory power as well as nervous activity in a particular type

of nerve cell may not be confined only to the ancestors of the sympathetic and chromaffine systems but may also apply to other nerve-cell groups.

8. The primitive vascular system of annelids is suggested to be the common ancestor of the ventral vascular system of the vertebrate, in which the heart is branchial in function, and the dorsal vascular system of the arthropod, in which the heart is systemic; the lateral vessels giving rise to the vertebrate heart and the dorsal vessel to the arthropod heart.

9. The chief function of the lateral contractile vessels of Hirudo is to fill the cutaneous capillary systems in which respiration takes place; it therefore acts as a branchial heart.

10. In Hirudo the contractile muscular rhythm is an intrinsic property of the vascular muscle itself; it therefore supports the myogenic theory of the heart beat.

11. The segmental nervous system of Hirudo consists of six groups of cells, which are arranged round the fused connectives, the cells composing these groups are mainly motor in function and have small peripheral processes which frequently cross to the opposite side and run out in the lateral nerves.

Certain large fibres are also found in these nerves which are the processes of large cells lying in the intermuscular layer, and which terminate in many arborisations in the central ganglion; they are possibly sensory in function.

12. The lateral nerves consist of two on each side. The anterior nerve contains the motor fibres to the circular and dorso-ventral muscles, and possibly inhibitor fibres to the longitudinal muscles; the posterior nerve contains motor fibres to the longitudinal muscles, and may also have inhibitor fibres to the circular muscles.

13. The inner bundles of the fibres of the connectives form tract fibres running through the ganglia.

14. The voluntary and involuntary muscles have similar reactions to those of the vertebrate with respect to drugs. They are, however, less sensitive and larger doses must be employed. For instance, curare given in large doses completely paralyses the voluntary systems, leaving the involuntary in most respects unaffected.

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8. DESCRIPTION OF PLATES.

Plate 14.

Fig. 2.—Diagram showing the branches of the longitudinal lateral vessel and their positions in each segment. The numbers refer to the five annuli which form a segment, being numbered from the most anterior.

B, the bladder or nephridial vesicle; G., the nerve ganglion; T., the testis; L., the lateral longitudinal vessel; L.D., the latero-dorsal vessel; L.L., the latero-lateral vessel; L.A., the latero-abdominal vessel; V., the valve on the main vessel.

The arrows indicate the normal direction of the contraction wave.

Fig. 3.—Wall of main artery. × 200. Hæmalum and Sudan III.

E.M., external circular muscle layer; I.M., internal longitudinal muscle layer; L., lumen; E, endothelium; N., nuclei of muscle cells; C., outer contractile portion of muscle cell.

Fig. 4.—The valve of the main vessel. × 50. Hæmalum and Sudan III.

M., the two layers of muscle fibres of the vessel wall; V., the valve; E., endothelial lining of the lumen.

Fig. 5.—Valve at the opening of a branch vessel into the main vessel. × 50. Hæmalum and Sudan III.

L., lumen of main longitudinal vessel; M., its muscular coats; E., its endothelial lining; B., lumen of branch vessel; V., the valve.

Fig. 6.—Diagram of the vessels lying over the dorsal side of the anterior end of the straight hind gut. The contractile system and its connections are shown in red, the dorsal sinus system in black. The thicker red lines represent the portions of the contractile system which have muscular walls. Drawn from a dorsal dissection.

D.S., the dorsal sinus with two pairs of branches in each segment.

L.D., the latero-dorsal vessels joining together over the mid line above the dorsal vessel, and giving branches to the two lateral alimentary vessels Al. V. These latter terminate in the pair of latero-dorsal vessels which meet just in front of the hind gut. The latero-dorsal vessels connect across in a similar manner above the sacculated anterior gut, but the two lateral alimentary vessels are absent.

Fig. 7.—Termination of the muscle sheath of a branch vessel. × 250. Hæmalum and Sudan III.

M., muscle fibres. E., Endothelium. L., lumen, which is tightly constricted where the wall is muscular, but is patent where the wall contains no muscle.

Fig. 8.—Diagram of a ganglion showing the groups of nerve cells, the two fat cells, and the chrome-staining cells. The various cell groups are outlined in red, their ventral limits being shown on the left, their dorsal limits on the right. The outline of the fibrillar substance is shown in black. The small diagram shows the relationships through the dotted line of the large diagram.

C., the connectives. F., Faivre's nerve. P., the posterior lateral nerve. A., the anterior lateral nerve. L., Leydig's cell. F.C., fat cells. G., the chrome-staining giant cells. Lat., the two lateral chrome-staining cells; the two on the opposite side are not shown. V.P., the ventral posterior cell group. V.A., the ventral anterior cell group. L.P., the lateral posterior cell group. L.A., the lateral anterior cell group.

Plate 15.

Fig. 9.—Longitudinal section through a ganglion showing the two chrome-staining giant cells. × 60. Unstained.

G., the giant cells stained bright canary yellow with chrome salt; F., fibrillar substance; C., connective; V.S., ventral sinus showing a pale yellow colour due to included blood.

Fig. 10.—Longitudinal section of a ganglion showing fat cells and giant cells and their relationships to one another. × 60. Nile blue sulphate A.

G., giant cells showing chrome staining; F.C., fat cells; the globules of fat stain purple or deep blue; F., fibrillar substance; C., connective.

Fig. 11.—Cross-section through the posterior end of a ganglion, showing bundles of tract fibres. A cross-section of a posterior nerve is also shown. × 60. Hæmalum and Sudan III.

D.I., dorsal internal bundle; M.I., median internal bundle; V.I., ventral internal bundle; F., Faivre's nerve, with its dorsally placed large fibre; V.P., beginning of ventral posterior cell group; L.P., beginning of lateral posterior cell group; P., posterior nerve; M., muscle fibre lying in its sheath; L., the five large nerve fibres; V.S., ventral sinus.

Fig. 12.—Cross section through a ganglion showing a fat cell, lateral chrome-staining cells, and the bundles of tract fibres. × 60. Hæmalum and Sudan III.

F.C., fat cell; C.C., lateral chrome-staining cell lying in the lateral posterior cell group L.P.; V.P., ventral posterior cell group; P., posterior nerve; Fib., fibrillar substance; D.I., dorsal internal bundle; M.I., median internal bundle; V.I., ventral internal bundle; F., Faivre's nerve; L., its large fibre separated from it and lying between the two dorsal internal bundles; V.S., ventral sinus; P.C., posterior commissure.

Fig. 13.—Cross section through a ganglion showing the giant cells and bundles of tract fibres. × 60. Hæmalum and Sudan III.

G., the giant cells lying in the ventral anterior cell group. The chrome reaction is masked by the staining by Sudan III; L.A., lateral anterior cell group; A., root of anterior nerve; D.I., dorsal internal bundle; M.I., median internal bundle; V.I., ventral internal bundle; F., Faivre's nerve; Fib., fibrillar substance; M., small muscle fibres of the ganglion sheath; V.S., ventral sinus; A.C., anterior commissure.

The conspicuous globules of fat in the nerve cells, though present in a normal ganglion in less amount, may have been increased in this instance, owing to previous experimental section of peripheral nerves.

Fig. 15.—The crossing of two nerves in the inter-muscular zone. After fusing the two are again separating into their original constituents. × 200. Paraffin. Hæmalum and eosin.

V.A., ventral branch of anterior nerve; A., branch of main anterior nerve; C., cells incorporated in the nerves at their junction, probably supporting connective tissue cells; F., large nerve fibre.

Fig. 16.—Shows the termination of the vascular nerve on the vessel wall. × 60. Hæmalum and Sudan III.

The two portions of the lateral vessels shown connect together in neighbouring sections, the main valve lying in this connection.

M, the muscle wall; E., endothelium; L.A., latero-abdominal vessel; N., the vascular nerve. In the figure this is shown too thick.

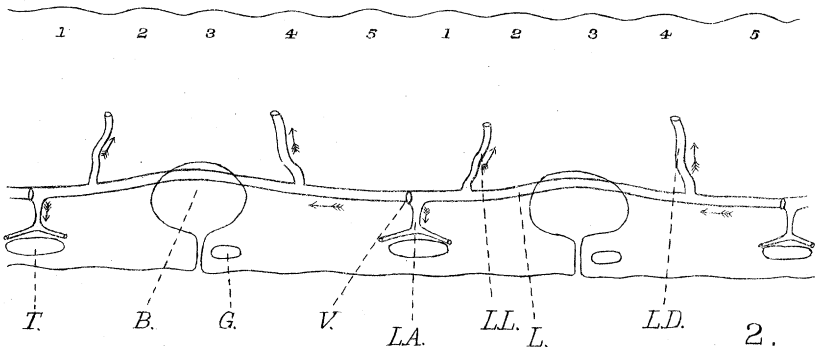
Plate 16.

Fig. 17.—Shows the effect of cutting the posterior nerve in the curarised leech. Beat very slow, average interval between beats, 28 seconds. At P.N. posterior nerve cut; the rate quickens, the average interval between beats being reduced to 17·7 seconds. This quickening lasted for some time.

Fig. 18.—Shows the effect of cutting the posterior nerve in the curarised leech. Beat fairly rapid. Average interval between beats 10·5 seconds. At P.N. posterior nerve cut, causing quickening, average interval between beats now being 8·5 seconds. The strength of beat also increased.

Fig. 19.—Shows the effect of cutting the anterior nerve in the curarised leech. Beat moderately fast, the interval being 11 seconds. At *an.* anterior nerve cut, causing slowing of rate, the interval becoming 13·5 seconds.

Fig. 20.—Shows the effect of stimulation with the posterior nerve cut in the curarised leech. Coil at 4. Beat rather slow, average interval of 14·4 seconds. At "on" stimulation of the ganglion causing acceleration, diminishing the average interval to 9·8 seconds. At "off" stimulus removed, and the rate again slows, the average interval becoming 12·5 seconds, not returning entirely to the original rate.



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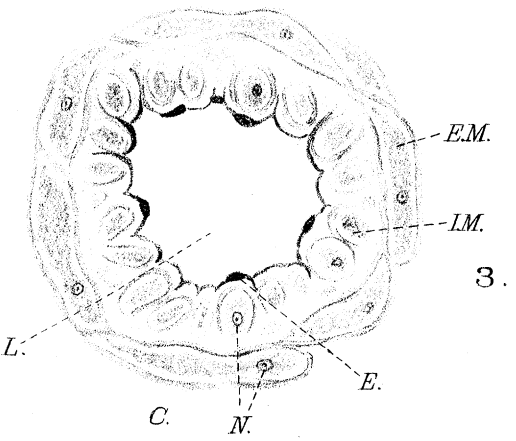

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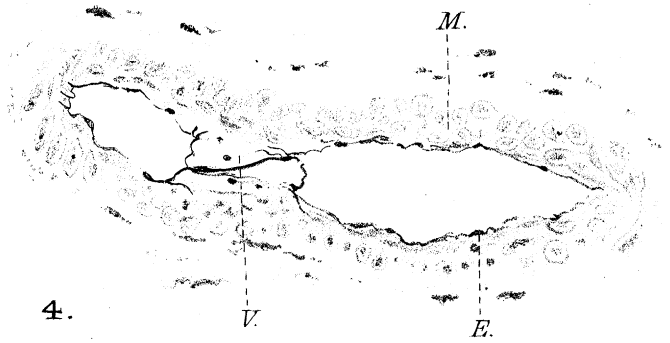

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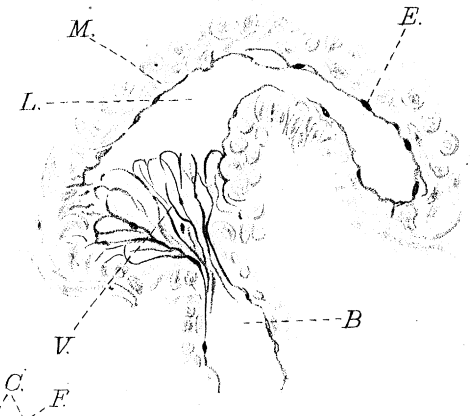

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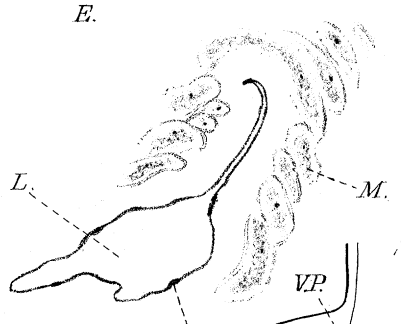

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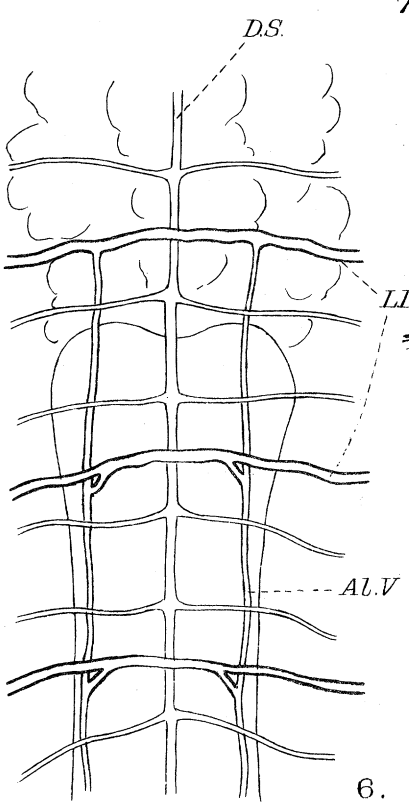

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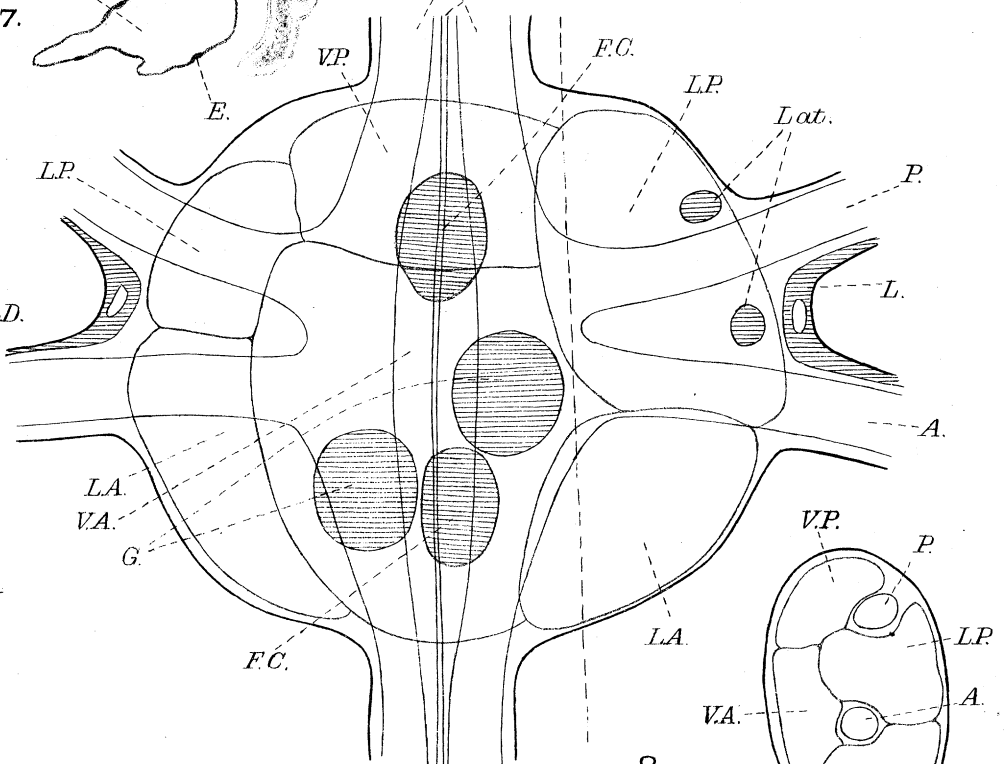

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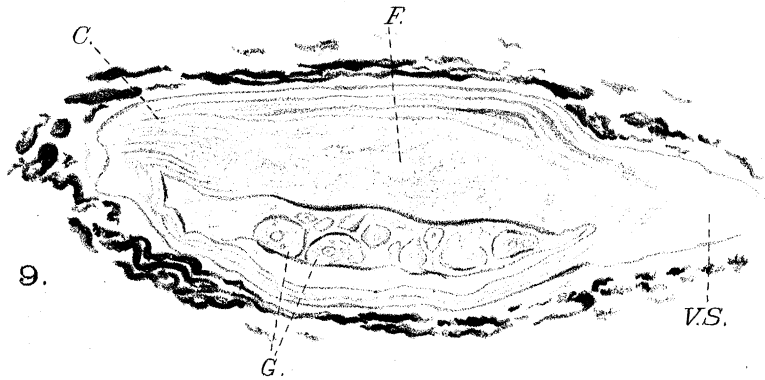

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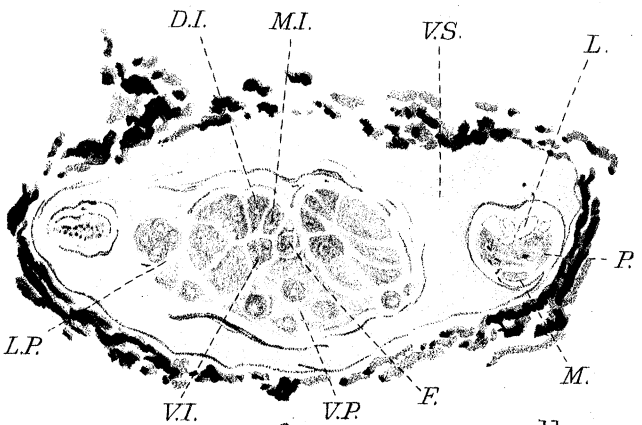

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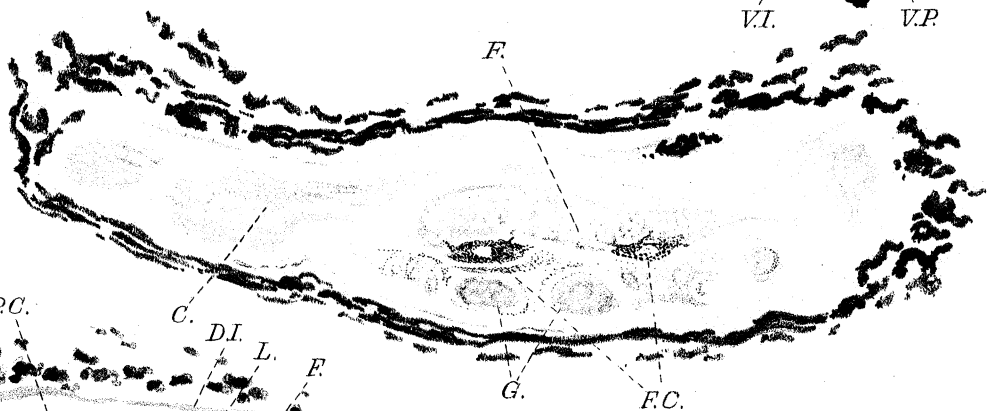

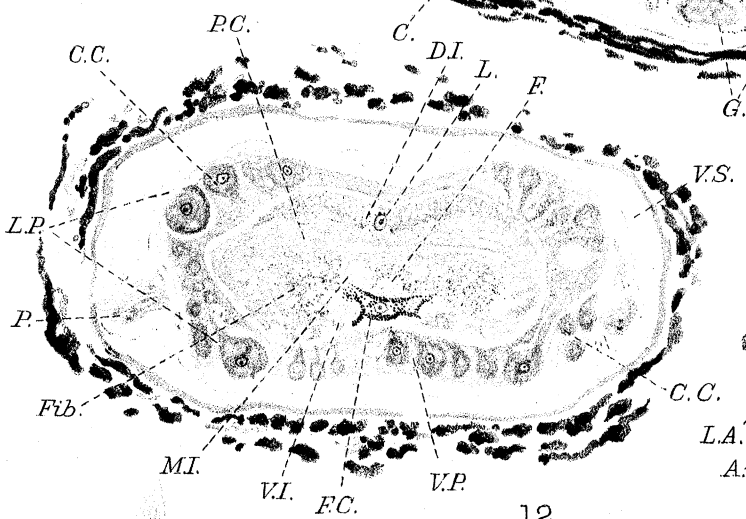

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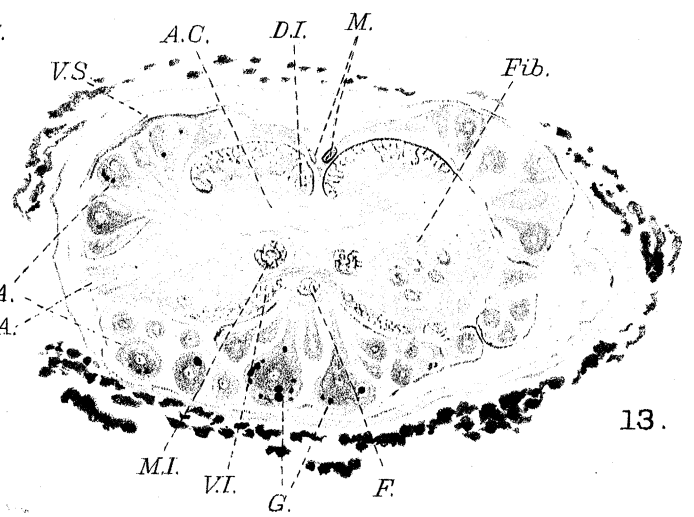

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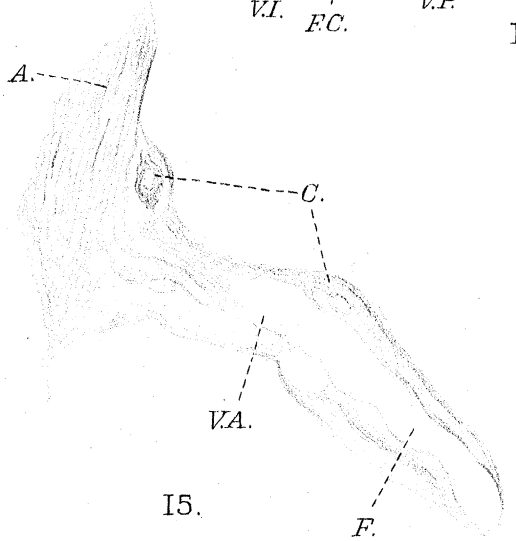

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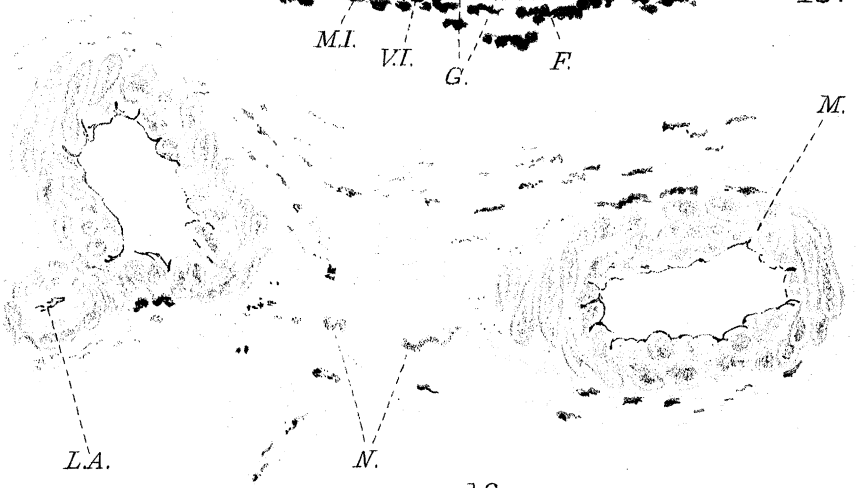

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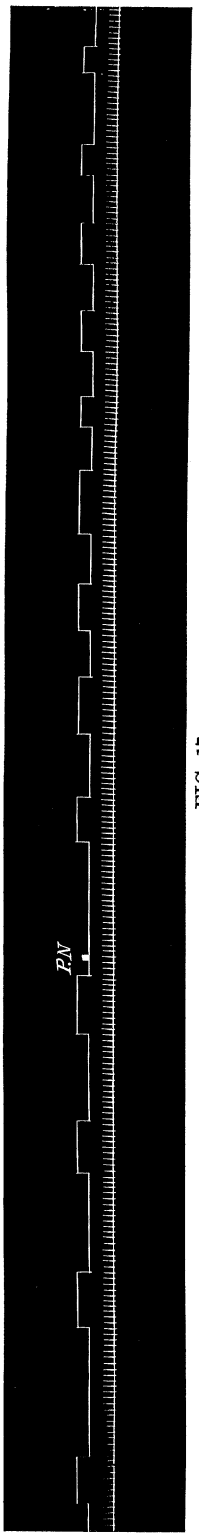
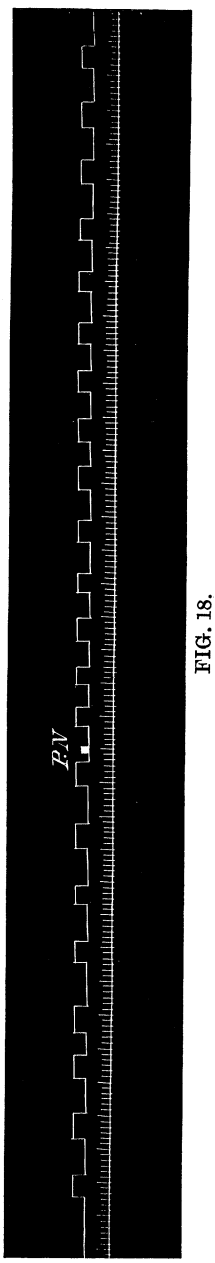


FIG. 17.



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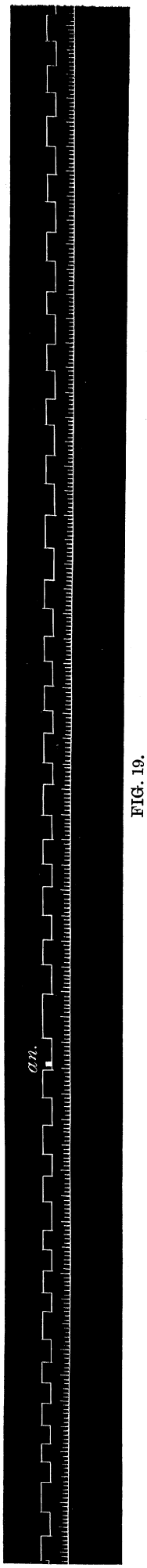

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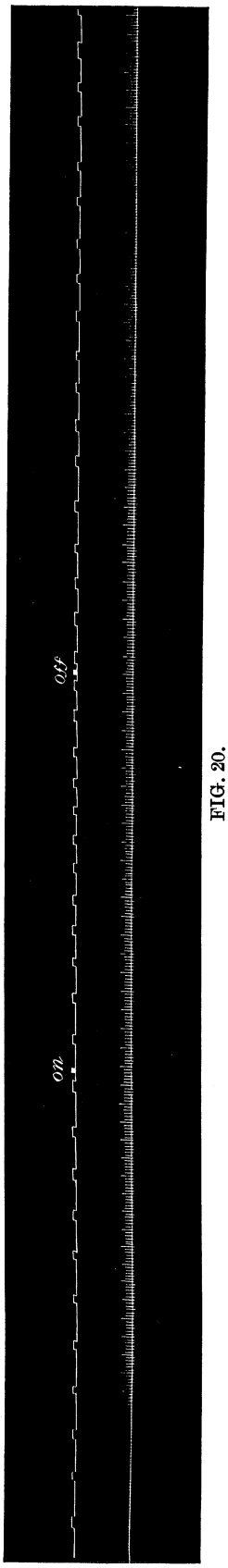

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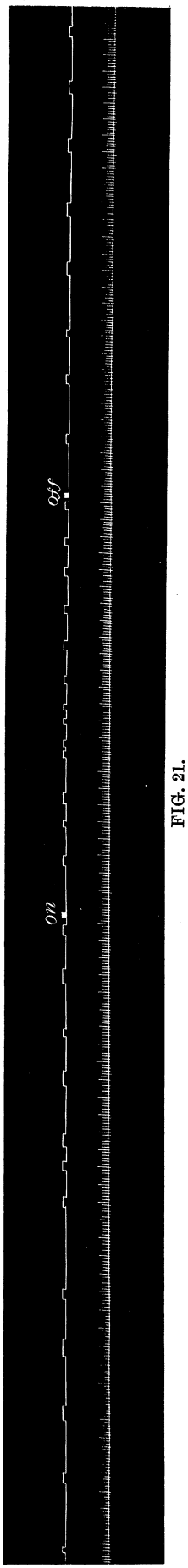

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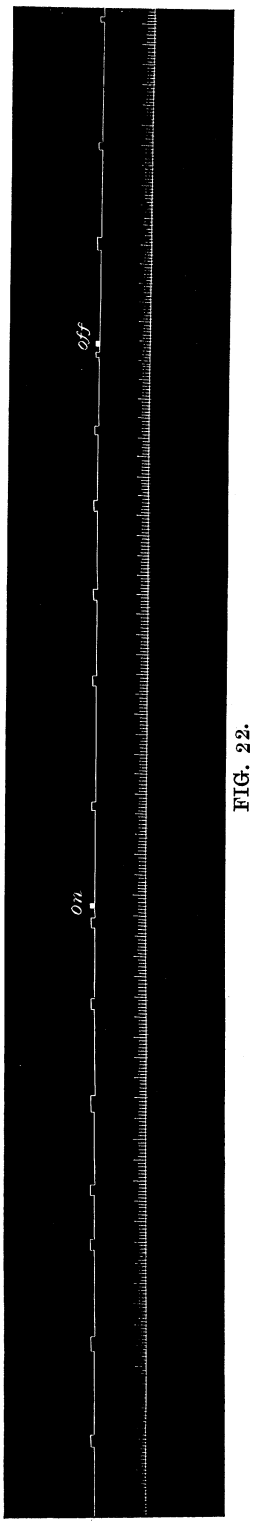

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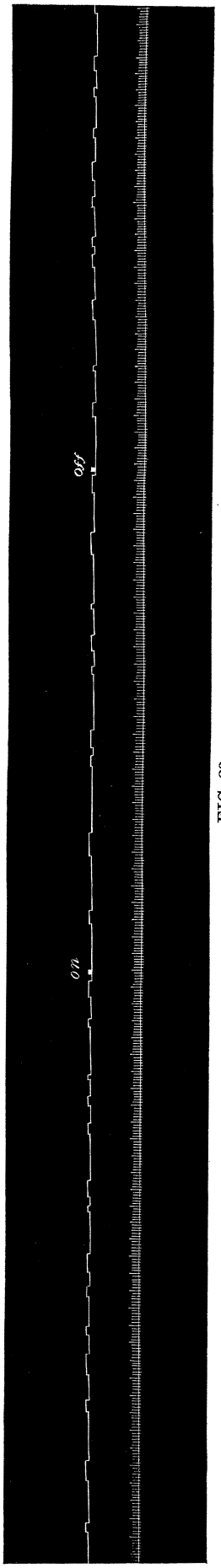

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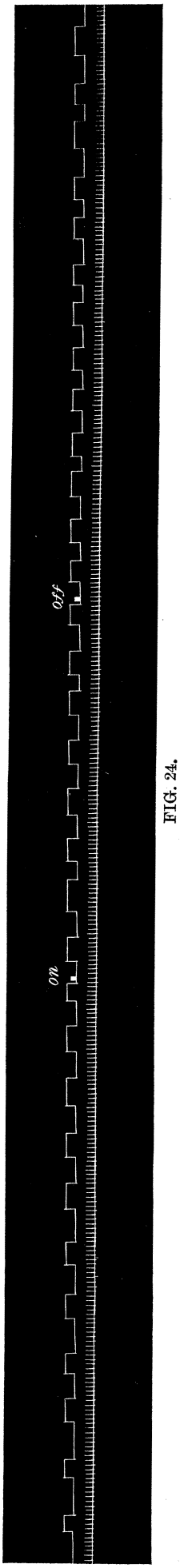

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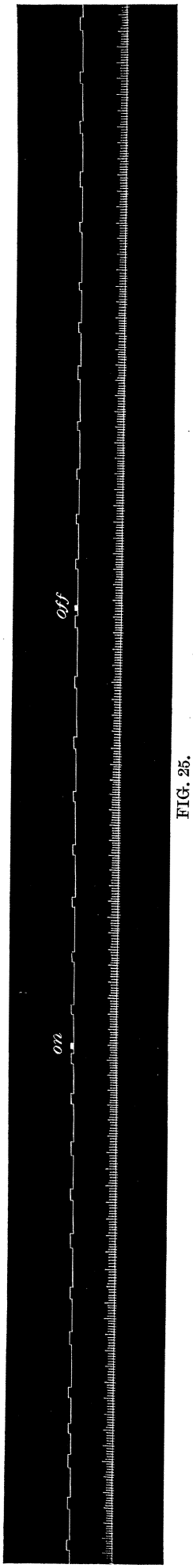

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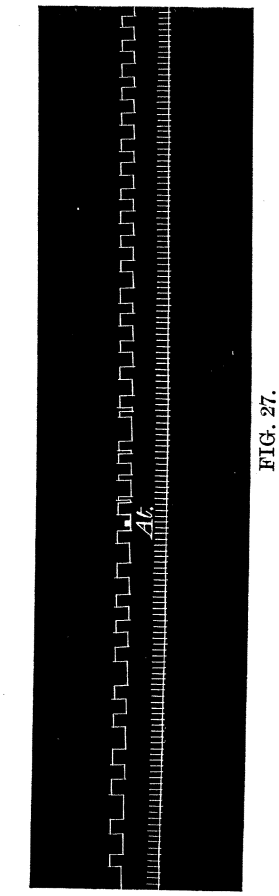

FIG. 27.

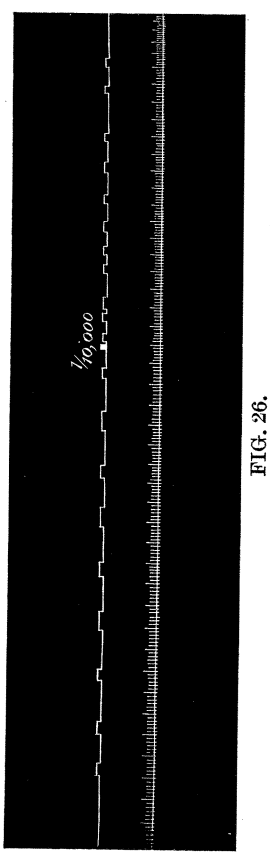

FIG. 26.

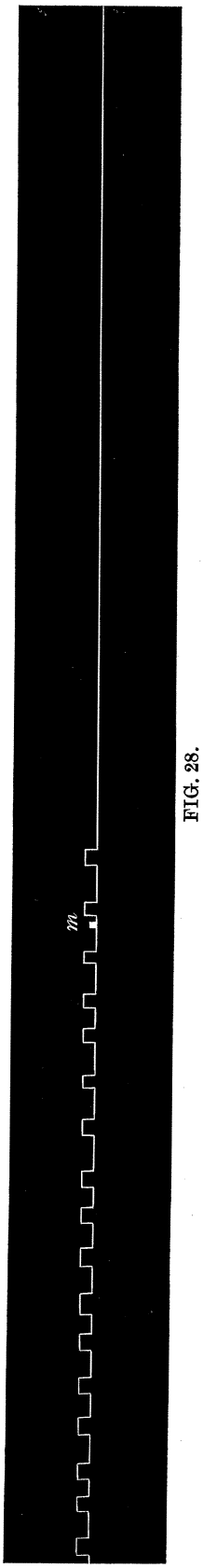

FIG. 28.

Fig. 21.—Shows effect of stimulation with the posterior nerve cut in the decapitated leech. Coil at 4. An irregular beat with an average interval of 18·4 seconds is increased to one with an average interval of 10·7 seconds by stimulation at "on." On removal of the stimulus at "off" the rate again slows to one with an average interval of 20 seconds.

Fig. 22.—The same animal a few minutes after the injection of 0·05 c.c. of a 1-per-cent. solution of ergotoxin phosphate. The average interval is now 25 seconds. Stimulation at "on" causes no acceleration, the average interval during stimulation being 28 seconds. The beat is decreasing both in rate and strength.

PLATE 17.

Fig. 23.—Shows the effect of stimulation with the anterior nerve cut in the decapitated leech. Coil at 4. An irregular beat, with an average interval of 14 seconds, is diminished in rate to one with an average interval of 20·4 seconds by stimulating at "on." On removal of the stimulus at "off" the rate returns to one with an interval of 14·6 seconds.

Fig. 24.—Shows the effect of stimulation with both nerves intact in the curarised leech. Coil at 6. A rate of beat with an average interval of 12 seconds is increased by stimulation at "on" to one with an interval of 10·5 seconds. This acceleration persists after the stimulus is removed at "off."

Fig. 25.—Shows the effect of stimulation with both nerves intact in the decapitated leech. Coil at 4. A beat with an average interval of 16·6 seconds is diminished in rate to one with an average interval of 20·5 seconds by stimulation at "on." The removal of this stimulus at "off" does not cause complete return of the beat to its original rate, the average interval remaining 18·5 seconds.

Fig. 26.—Shows the effect of adrenalin borate. A beat with an average interval of 14·5 seconds is accelerated by an injection of one drop of a 1 in 10,000 solution to a beat with an interval of 9·4 seconds. Later this particular segment stopped in systole and at the same time the neighbouring segments increased their rate of beat.

Fig. 27.—Shows the effect of an injection of one drop of a 1-per-cent. solution of atropine, a rapid rate of beat with an interval of 5·5 seconds is increased to one with an average interval of 4·2 seconds. Both contraction and relaxation became maximal. Atropine injected at "At." The beats became so rapid that at first they were imperfectly registered.

Fig. 28.—Shows the effect of injection of muscarine. After the injection at *m*, the vessel gave two more beats, and then ceased to beat entirely, remaining distended with blood in diastole.

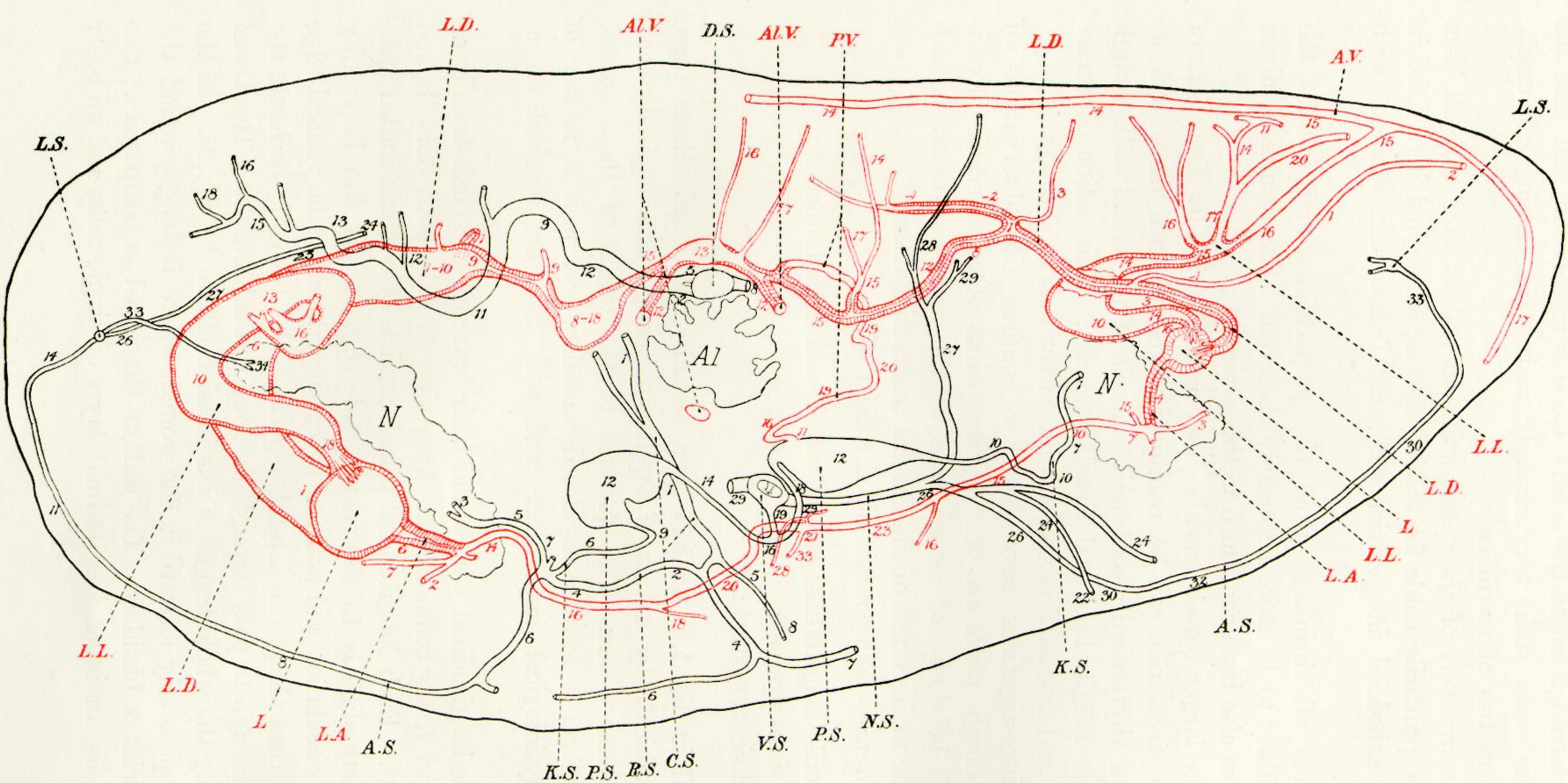

FIG. 1.—Projection on a transverse plane of the vessels of the leech. The contractile system is shown in red, and also the branches which arise from it. The contractile portion is cross-hatched and is shown fully distended on the right and contracted on the left. The dorsal and ventral sinus systems are shown in black.

Al., alimentary canal; N., nephridium. L., lateral longitudinal vessel giving off three branches. L.L., latero-lateral vessel soon breaking up into many branches connecting with A.V., the annular vessels. L.D., the latero-dorsal vessel dividing into two branches, the anterior of which connects with its fellow of the opposite side across the mid-dorsal line; this anterior branch also connects with the lateral alimentary vessel, Al.V., it also occasionally connects with the perinephrostomial sinus by means of P.V., the ascending vessel of Gratiolet. L.A., the latero-abdominal vessel, dividing into two branches, which connect across the ventral side, so as to form a diamond-shaped figure (only one of these connections is shown); it also supplies branches to the nephridium. D.S., the dorsal sinus lying just above the alimentary canal; one of its more tortuous branches only is shown. V.S., the ventral sinus containing the nerve cord; it gives off a pair of sinuses, one of which, N.S., is shown on the right, which accompany the posterior nerves and their branches and may therefore be called neural sinuses; various ventral branches of these form annular sinuses, A.S.; the ventral sinus also connects with the perinephrostomial sinus, P.S.; from the latter a further sinus, K.S., runs out to the nephridium and may or may not connect with the arched sinus, R.S., as is shown on the left; the arched sinus gives rise to an annular sinus, A.S., and also connects with the connecting sinus, C.S., which connects together the ventral and dorsal sinus systems. L.S., the small lateral sinus lying in the lateral longitudinal muscle giving rise to a sinus connecting with the nephridium and a sinus connecting with the dorsal intermuscular plexus, the annular sinuses all terminate in this lateral sinus. The distribution of a muscular wall in the contractile system is indicated by a double outline and cross hatching. The numbers marked on the diagram indicate the slides in which these portions of the vessels occurred; the highest numbers are anterior, the lowest posterior. The segment traced is in the region of the straight hind gut. (Drawn with Edinger's projectoscope.)

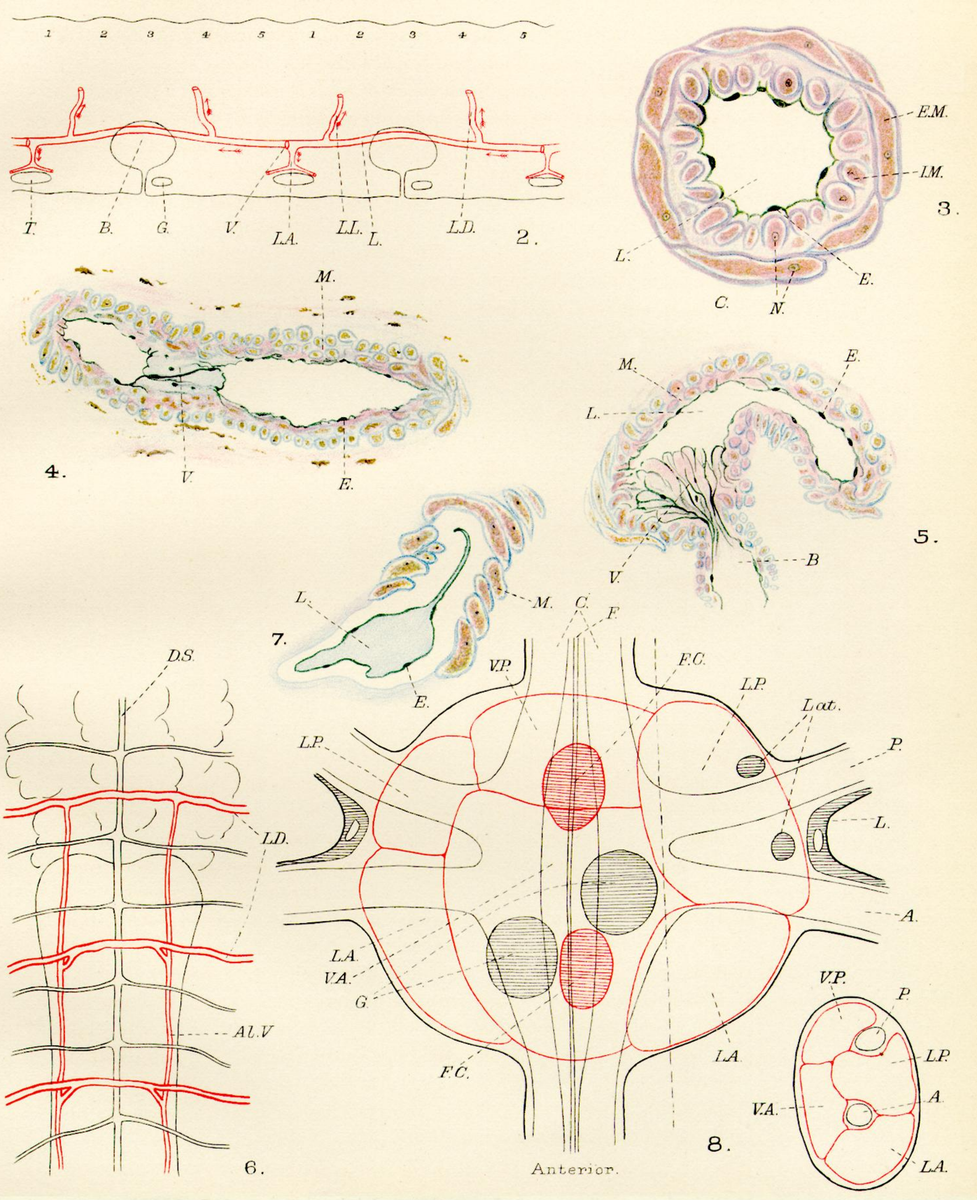

PLATE 14.

Fig. 2.—Diagram showing the branches of the longitudinal lateral vessel and their positions in each segment. The numbers refer to the five annuli which form a segment, being numbered from the most anterior.

B, the bladder or nephridial vesicle; G., the nerve ganglion; T., the testis; L., the lateral longitudinal vessel; L.D., the latero-dorsal vessel; L.L., the latero-lateral vessel; L.A., the latero-abdominal vessel; V., the valve on the main vessel.

The arrows indicate the normal direction of the contraction wave.

Fig. 3.—Wall of main artery. × 200. Hæmalum and Sudan III.

E.M., external circular muscle layer; I.M., internal longitudinal muscle layer; L., lumen; E, endothelium; N., nuclei of muscle cells; C., outer contractile portion of muscle cell.

Fig. 4.—The valve of the main vessel. × 50. Hæmalum and Sudan III.

M., the two layers of muscle fibres of the vessel wall; V., the valve; E., endothelial lining of the lumen.

Fig. 5.—Valve at the opening of a branch vessel into the main vessel. × 50. Hæmalum and Sudan III.

L., lumen of main longitudinal vessel; M., its muscular coats; E., its endothelial lining; B., lumen of branch vessel; V., the valve.

Fig. 6.—Diagram of the vessels lying over the dorsal side of the anterior end of the straight hind gut. The contractile system and its connections are shown in red, the dorsal sinus system in black. The thicker red lines represent the portions of the contractile system which have muscular walls. Drawn from a dorsal dissection.

D.S., the dorsal sinus with two pairs of branches in each segment.

L.D., the latero-dorsal vessels joining together over the mid line above the dorsal vessel, and giving branches to the two lateral alimentary vessels Al. V. These latter terminate in the pair of latero-dorsal vessels which meet just in front of the hind gut. The latero-dorsal vessels connect across in a similar manner above the sacculated anterior gut, but the two lateral alimentary vessels are absent.

Fig. 7.—Termination of the muscle sheath of a branch vessel. × 250. Hæmalum and Sudan III.

M., muscle fibres. E., Endothelium. L., lumen, which is tightly constricted where the wall is muscular, but is patent where the wall contains no muscle.

Fig. 8.—Diagram of a ganglion showing the groups of nerve cells, the two fat cells, and the chrome-staining cells. The various cell groups are outlined in red, their ventral limits being shown on the left, their dorsal limits on the right. The outline of the fibrillar substance is shown in black. The small diagram shows the relationships through the dotted line of the large diagram.

C., the connectives. F., Faivre's nerve. P., the posterior lateral nerve. A., the anterior lateral nerve. L., Leydig's cell. F.C., fat cells. G., the chrome-staining giant cells. Lat, the two lateral chrome-staining cells; the two on the opposite side are not shown. V.P., the ventral posterior cell group. V.A., the ventral anterior cell group. L.P., the lateral posterior cell group. L.A., the lateral anterior cell group.

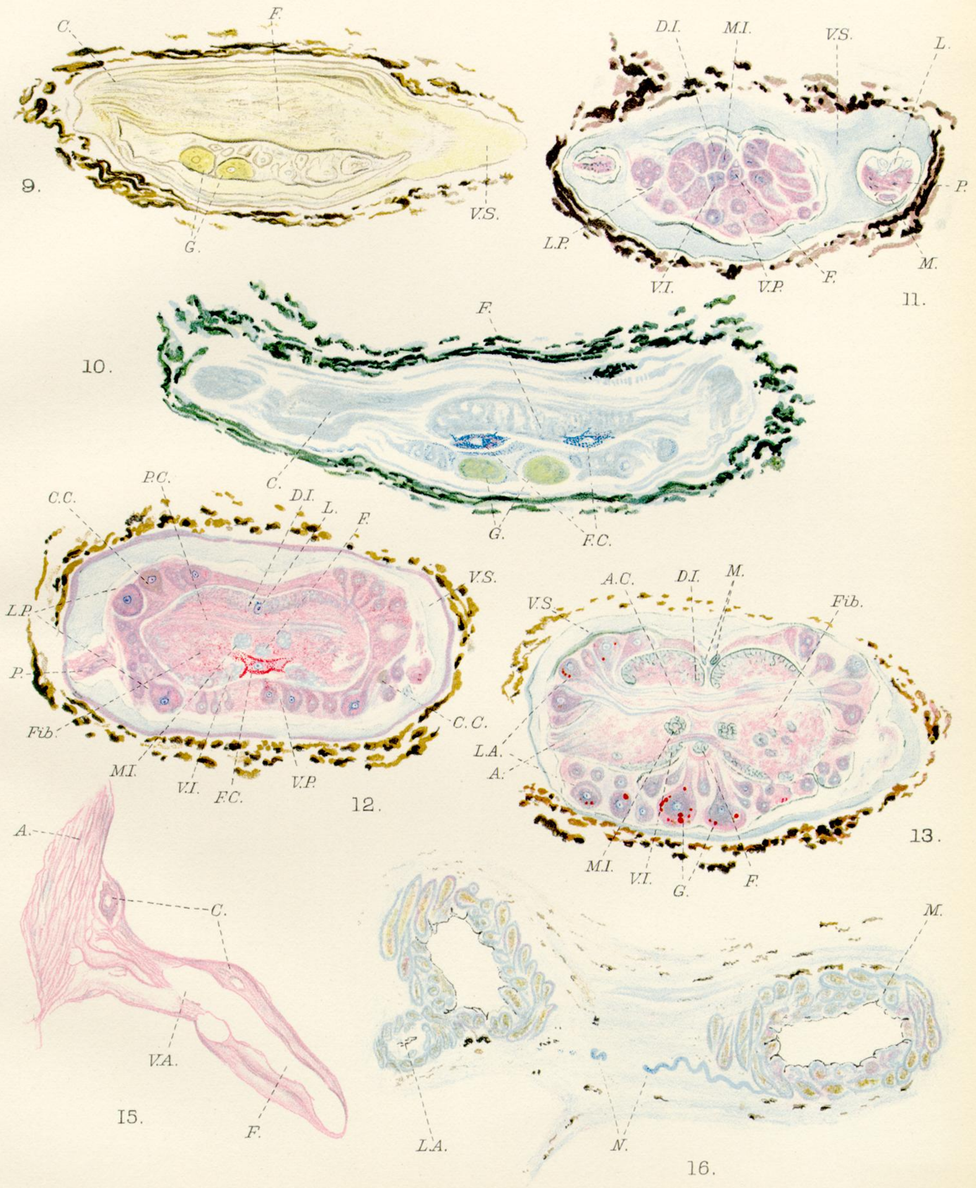

PLATE 15.

Fig. 9.—Longitudinal section through a ganglion showing the two chrome-staining giant cells. × 60. Unstained.

G., the giant cells stained bright canary yellow with chrome salt; F., fibrillar substance; C., connective; V.S., ventral sinus showing a pale yellow colour due to included blood.

Fig. 10.—Longitudinal section of a ganglion showing fat cells and giant cells and their relationships to one another. × 60. Nile blue sulphate A.

G., giant cells showing chrome staining; F.C., fat cells; the globules of fat stain purple or deep blue; F., fibrillar substance; C., connective.

Fig. 11.—Cross-section through the posterior end of a ganglion, showing bundles of tract fibres. A cross-section of a posterior nerve is also shown. × 60. Hæmalum and Sudan III.

D.I., dorsal internal bundle; M.I., median internal bundle; V.I., ventral internal bundle; F., Faivre's nerve, with its dorsally placed large fibre; V.P., beginning of ventral posterior cell group; L.P., beginning of lateral posterior cell group; P., posterior nerve; M., muscle fibre lying in its sheath; L., the five large nerve fibres; V.S., ventral sinus.

Fig. 12.—Cross section through a ganglion showing a fat cell, lateral chrome-staining cells, and the bundles of tract fibres. × 60. Hæmalum and Sudan III.

F.C., fat cell; C.C., lateral chrome-staining cell lying in the lateral posterior cell group L.P.; V.P., ventral posterior cell group; P., posterior nerve; Fib., fibrillar substance; D.I., dorsal internal bundle; M.I., median internal bundle; V.I., ventral internal bundle; F., Faivre's nerve; L., its large fibre separated from it and lying between the two dorsal internal bundles; V.S., ventral sinus; P.C., posterior commissure.

Fig. 13.—Cross section through a ganglion showing the giant cells and bundles of tract fibres. × 60. Hæmalum and Sudan III.

G., the giant cells lying in the ventral anterior cell group. The chrome reaction is masked by the staining by Sudan III; L.A., lateral anterior cell group; A., root of anterior nerve; D.I., dorsal internal bundle; M.I., median internal bundle; V.I., ventral internal bundle; F., Faivre's nerve; Fib., fibrillar substance; M., small muscle fibres of the ganglion sheath; V.S., ventral sinus; A.C., anterior commissure.

The conspicuous globules of fat in the nerve cells, though present in a normal ganglion in less amount, may have been increased in this instance, owing to previous experimental section of peripheral nerves.

Fig. 15.—The crossing of two nerves in the inter-muscular zone. After fusing the two are again separating into their original constituents. × 200. Paraffin. Hæmalum and eosin.

V.A., ventral branch of anterior nerve; A., branch of main anterior nerve; C., cells incorporated in the nerves at their junction, probably supporting connective tissue cells; F., large nerve fibre.

Fig. 16.—Shows the termination of the vascular nerve on the vessel wall. × 60. Hæmalum and Sudan III.

The two portions of the lateral vessels shown connect together in neighbouring sections, the main valve lying in this connection.

M, the muscle wall; E., endothelium; L.A., latero-abdominal vessel; N., the vascular nerve. In the figure this is shown too thick.