The Nervous System of a South Australian Giant Earthworm of the genus Megascolex; a Study of its Anatomy and Physiology.

Submitted as a thesis for the degree of Doctor of Medicine in the University of Adelaide by William Ross Adey.

This work was aided by a grant from the Commonwealth Research Fund.

It is my belief that this thesis advances scientific knowledge and practice in the following respects:--

(1) It is the first detailed account of the connections of the ganglion cells of the cerebral ganglia and ventral nerve cord of an oligochaet worm.

(2) Significant structural differences have been shown to exist between the nervous system of the worm studied and the closely allied Megascolides australis.

(3) The giant fibres of the ventral nerve cord have been investigated for the first time in an Australian worm and shown to possess an intra-neuronal system of fibrils. The presence of such a system has been disputed in oligochaets examined by other observers. The special relation of this system to the macrosynapses of the giant fibres has been described.

(4) Interconnections between the giant fibres have been demonstrated histologically.

(5) The portions of the worm body innervated by the giant fibres have been shown physiologically to differ significantly from any oligochaet previously examined.

(6) An accurate mathematical relationship has been achieved between conduction velocity and nerve fibre size for the first time in any annelid giant nerve fibre.

(7) Variations in conduction velocity in the giant fibres have been shown to occur in different portions of the worm. There is a relationship between length of worm and conduction velocity in the median giant fibre.

(8) By continued electrical stimulation it has been possible to produce a type of polarity in the giant nerve fibres, which would appear to depend on the presence of macrosynapses within these fibres. This observation is of particular importance in view of the large body of work at present being conducted with a view to elucidating the functions of the synapse.

(9) The first accurate determination has been made for the annelid giant fibre of the relative proportions of nerve fibre sheath/axon diameter in stained and unstained material. The shrinkage of the giant fibres during formalin fixation and dehydration has been determined in the annelid giant fibre for the first time.

(10) The electronic apparatus used in this work has been designed and constructed by me and represents new advances in valve application. This applies particularly to the cathode-ray oscilloscope circuit in which it has been possible to use a single-beam and a double-beam cathode-ray tube in conjunction with direct-coupled sweep circuits while maintaining independence of focusing positioning and intensity controls.

INTRODUCTION.

The invertebrate nervous system has been the subject of close examination in recent years on account of its simplicity when compared with the relatively complex vertebrate nervous system (Prosser, 1946). Both physiologists and anatomists have found the invertebrate a fruitful source of information about the fundamental structural and functional units of the nervous system. The investigators in the field in the last fifty years have been legion, but chronologically their work falls into two distinct phases.

The first phase covers the last quarter of the last century, when the morphologist was in his hey-day, seeking ever fresh fields for comparison and contrast. During this period, a fairly complete examination of the nervous system of Lumbricus as a typical representative of the oligochaets was carried out by Friedlander (1888, 1889), Cerfontaine (1892), Nansen (1887) and Retzius (1892). At the same time Spencer (1888) described the anatomy of Megascolides australis, devoting a special section to the anatomy of the nervous system. This was the first account of the nervous system of an Australian earthworm.

While in all cases the observations were made most carefully and critically, the staining methods of the period were inferior to modern techniques, and were not adequate for the solution of some of the problems that arose. Thus it was only gradually that the giant fibres in the dorsal part of the nerve cord were accepted as nerve fibres. Leydig (1864) was the first to assign to them a nervous function, but in the ensuing years they were

described variously as nutritive tubes, blood vessels and supporting organs. Hansen (1887) asserted that they were nervous in function, but Spencer (1888) in his description of Megascolides australis still accepted the view that they were supporting structures, stating that "no connection exists between the fibres and any of the nervous elements, and it seems probable, more perhaps because it is difficult to suggest any other use for them, than because of any direct evidence in its favour, that the usually accepted idea of their possessing solely a supporting function is the correct one. They doubtless serve also, as suggested by Cunningham, 'to prevent the nerve cords being bent at a sharp angle, causing them always to remain in curves, and so to escape injury during the wriggling and burrowing of the worm'".

From this it is clear that the connections between the ganglion cells of the ventral nerve cord and the giant nerve fibres were not appreciated, largely because of inadequate histological technique. Nevertheless, at this time Friödlander (1888) suggested that they were true nerve fibres, having the function of bringing about the sudden contraction of the whole body. This is now known as the "startle reaction."

Very little subsequent investigation was carried out in the annelid nervous system for nearly thirty years until the demonstration by Stough (1926) of the presence of apparently complete segmental septa subdividing the giant fibres into a series of discontinuous portions of axoplasm. It was suggested

that structurally the junction of two portions of the giant fibre at the septum constituted a giant synapse. Stough observed differential staining of the axoplasm of the two sections of the giant fibre where they meet at the septum and suggested that the giant fibres are polarized to conduct in one direction only. He described a series of experiments (1930) designed to prove this hypothesis.

The development of electrical methods of recording nerve impulses brought a new and powerful weapon to the biologist's aid. Eccles, Granit and Young (1933) recorded impulses from the nerve cord of Lumbricus and showed that conduction in the giant fibres occurred equally well in either direction from the point of stimulation - that is, that the segmental synapses described by Stough are unpolarized. This work has been elaborated by Bullock (1945) and Rushton (1945a. and b., 1946) using more modern recording methods.

Whether the septa constitute a true cellular membrane and consequently are to be regarded as the physiological boundaries between adjacent neuronal units, has not yet been settled. Eccles's theory of synaptic conduction (1946) throws some doubt on the functioning of the septum as a true synaptic membrane. He has shown that a significant delay is theoretically necessary at any true junction where a membrane of the type around the axon interrupts the conduction pathway. However, according to Bullock (1947), not all neurophysiologists accept the view of Eccles that some delay is demanded by the membrane theory of synaptic transmission for all possible junctions.

In the intensive research into the physiological properties of the annelid giant fibre septum, there has been a tendency to divorce the giant fibres from the rest of the annelid nervous system, and to overlook the advantages of a combined histological and physiological examination. The connections of the giant fibres with the ganglion cells have been hitherto very incompletely investigated, and much conflicting evidence is presented concerning the finer structure of the giant fibres (Stough, 1926; Smallwood and Holmes, 1927). Especially has there been a tendency to study activity in the giant fibres of the various invertebrates without regard to the broader biological functions which the giant fibres serve in the animal's natural state as part of a relatively complicated nervous system.

It is the purpose of this study to examine histologically the structure of the cerebral ganglia and the ventral nerve cord by a modern histological technique in a local earthworm of the genus Megascolex; to investigate the connections and the finer internal structure of the giant fibres; and to correlate these findings as far as possible with the activity observed with electronic recording methods.

Bullock (1945) has shown that in Lumbricus the median giant fibre is activated by a tactile stimulus anterior to the clitellum, and that a stimulus behind the clitellum activates the lateral fibres. This problem has been studied in Megascolex, and the portions of the body innervated by the respective giant fibres found to be significantly different from the arrangement in Lumbricus. It is also proposed to show that

there is a correlation between the length of the worm and the maximum conduction velocity in the median giant fibre; that the velocity is a linear function of the giant fibre diameter and sheath thickness; and that the sheath dimensions as determined histologically agree well with the sheath dimensions measured in the fresh state by polarized light, in contrast to the findings of Bear, Schmitt and Young (1937) in the squid giant axon. The phenomenon of fatigue in the conduction of impulses in the giant fibres under electrical stimulation has been examined; in addition, there is evidence that the fatigued fibre shows some modification of the apparent lack of polarity.

MATERIAL AND METHODS.

Material.

The worms used belong to the genus Megascolex. I am indebted to Miss D. F. McCarthy of the Zoology Department of this University for this classification. She reports: "This earthworm belongs to the genus Megascolex and is a species hitherto undescribed. Like other Australian genera, this one has the conspicuous spermiducal glands peculiar to the Australian native earthworms (Sweet, 1900). These glands are lobate and situated in 18th. segment in the genera Cryptodrilus, Megascolex and Digaster. Distinction between the three genera can be made in that Digaster has two gizzards, the other two only one, and that Cryptodrilus has meganephridia and Megascolex plectonephridia. The characteristic features described above mark the worm as belonging to the genus Megascolex."

The worms occur in sandy soil in higher parts of the Mount Lofty Ranges, a few inches below the surface in winter but many feet deeper in the moister subsoil during the summer months. They are usually found in burrows with their tails towards the surface; the tail is withdrawn rapidly when touched, and the anterior end is then thickened and expanded, causing the worm to stick in the burrow and resist any attempt to withdraw it by pulling.

Pulling on the tail is rapidly followed by complete separation of the tail portion (autonomy). After capture the worms were kept in moist earth from their native surroundings until used, but they rarely survived for more than three weeks. The worms vary in length from about seven to seventeen inches, most being ten to twelve inches long and three-eighths of an inch in diameter. They are closely related to the giant earthworms of southeastern Australia which attain a length of four or five feet (Spencer, 1888). They are dark brown in colour with a creamy brown clitellum placed on the 13th.-17th. segments. This is in contrast to Lumbricus in which the clitellum is opposite segments 36-40.

In determining the transition point in the innervation of the giant fibres, 29 worms were examined. One hundred and forty six estimations of conduction velocity were carried out in 23 worms, and 155 estimations of fibre size made in this material. In the measurement of shrinkage, the fibre size was determined in 65 frozen sections, and 68 measurements were made of the same material after staining. The ratio: axon diameter/fibre diameter

in fresh material was measured by polarized light in 35 preparations.

Apparatus.

The conduction experiments were carried out in a small shielded cage in an unshielded room. Silver-silver chloride electrodes were used initially to pick up the impulses, but satisfactory results were subsequently obtained with fine silver wires placed on the surface of the nerve-cord, providing they were kept clean. The impulses were led to a five-stage push-pull amplifier.

The amplifier circuits employed are shown in Figs. 14 and 15. The signal voltage is fed to two potentiometers mounted in the shielded cage. This arrangement has a number of advantages. When dealing with very large signal voltages which could cause blocking in the amplifier, the amplitude of the signal can be fully controlled by the operator without moving away from the cage. Swinging the moving arm of one potentiometer to the earth position allows the amplifier to be operated with a single-ended input, rather than with the push-pull input outlined above.

The first three stages of the amplifier, each consisting of a pair of type 1603 valves in push-pull, are completely battery-operated. The over-all voltage gain of this part of the amplifier is 100,000. By the use of a very large cathode resistor in each stage, a considerable degree of degeneration is achieved. This assists in stabilizing the amplifier at very low frequencies. The negative grid bias developed across the resistor is to a large extent offset by a "bucking" voltage

applied from a battery in each stage.

The coupling condensers between all stages are 4 mfd. oil-filled paper condensers. In addition, a switching arrangement allows a choice of either 0.1 mfd. or 4 mfd. condensers between the second and third stages. The frequency response of the whole amplifier (both battery and A.C. operated) is uniform from 1 cycle/sec. to 2000 cycles/sec., using the 4 mfd. condensers between stages two and three. The high frequency response could probably be extended by the use of some low-impedance coupling device, such as cathode-followers, to connect the battery-operated amplifier to the A.C.-operated part. The shielded line used for this purpose has sufficient capacity to act as a considerable by-pass to frequencies higher than 2000 when connected directly into the plate circuit of a high-impedance penthode valve of the 1603 type.

The low frequency cut-off is at about 10 cycles/sec. with the 0.1 mfd. condensers between stages two and three. The over-all frequency may be regarded as adequate for the reproduction of impulses without distortion. As a quarter cycle of 2000 cycle/sec. sine wave occupies 0.125 millisec., the steep-fronted action potential will be reproduced by the amplifier with negligible distortion. This point is of considerable importance in velocity measurements, as pointed out by Pumphrey and Young (1938).

The long time-constant associated with the 4 mfd. coupling condensers was troublesome when the amplifier was first built. A sudden large impulse would charge the condensers for several seconds, during which the amplifier was inoperative. Very little "choking" is observed when using the smaller condensers available

with the switching arrangement outlined above.

The output of the battery-operated amplifier is fed to an amplifier operated from the A.C. mains. This amplifier has a pair of type 6SJ7 valves in push-pull, driving a pair of 6J5 valves acting as cathode-followers. The output of the cathode followers is fed to the cathode-ray oscilloscope, and also to a type 6V6GT valve which drives a monitor loud-speaker. (Fig. 15). The voltage gain of this section of the amplifier is about 90, giving an over-all voltage gain of about ten million. The whole amplifier has a high degree of mechanical and electrical stability, and shows only slight acoustic feed-back from the loudspeaker when operating at high gain.

The cathode-ray oscillograph consists of two cathode ray tubes, each with its own focus, positioning and intensity controls, but with their deflecting plates so coupled together that a trace on one is simultaneously produced on the other. One tube, a VCR 97, has a green fluorescent screen and is used for continuous monitoring. The other, a Cossor type 09J., has a blue fluorescent screen with a high actinic value and thus very suitable for photography of non-repetitive waveforms. This tube is used only during actual photography of traces. It is a double-beam tube, one beam carrying the signal voltage, the other a time marker of 50 cycles/sec. obtained from the A.C. mains.

Accelerating potential for the cathode-ray tubes is obtained from a half-wave 1500 volt transformer with type 2X2

rectifiers (Fig. 16). Provision is made for voltage doubling in this power-supply, which then delivers about 4000 volts. However, although this potential is quite suitable for the type VCR97 tube, it is somewhat higher than the maximum recommended for the type 09J. The supply was therefore usually run as a simple half-wave system, giving about 2000 volts to the two cathode-ray tubes.

The sweep-circuit (Fig. 16) is direct-coupled throughout. It uses a type 884 thyratron valve as a relaxation-oscillator, with capacitors of varying size in parallel with the valve to produce sweeps at rates from one every eight seconds to 5000/sec. The trace speed is fairly constant in all parts of the sweep over this wide range. Bias for the thyratron is obtained from the main cathode-ray power-supply bleeder network. A rectifier valve type 5V4G is connected between the plate of the 884 valve and a point on an external bleeder network maintained 120 volts positive with respect to the cathode of the 884. This rectifier tube can be switched in and out of circuit. When in circuit, the anode voltage of the 884 is limited to 120 volts; the grid bias on the 884 is adjusted so that at an anode potential of 120 volts it is just non-conducting. An external pulse applied to the grid of the 884 valve will now trigger the sweep once for each applied pulse. When the 5V4G valve is switched out of circuit, the 884 valve behaves as a free-running oscillator.

The sweep voltage generated in the 884 valve is amplified by a twin triode valve type 6SN7 operating in push-pull. Positioning of the horizontal trace is achieved by adjusting

the grid-bias on the first triode. The amplified voltage is applied directly to the horizontal deflecting plates of each cathode-ray tube. The deflecting plates are thus several hundred volts positive with respect to earth, in contrast to the usual arrangement, but no difficulty is caused by this procedure provided that the high tension supply to the sweep circuit is free from ripple.

The pulse generator for administering shocks to the nerve cord (Fig. 17) uses a pair of 807 valves in a type of multivibrator circuit. The wave form from the generator is rectangular, the duration of the pulse being controlled by one 807 and its associated resistance-capacity network, and the repetition rate of the pulse by a similar network in the other valve. Pulses can be produced varying in duration from 0.03 millisec. to 100 millisec. and in frequency from 1 to 50/sec. The pulse so produced can be applied either directly to the nerve or fed into a Harvard inductorium and thence to the stimulating electrodes. It is also used to trigger the cathode-ray tube sweep circuit.

In measuring conduction velocities, the time interval from the shock to the appearance of the spike was measured to within 0.1 mm. on the cathode-ray tracing. At the sweep speed used, a distance of 0.1 mm. corresponds to a time interval of 0.05 millisec. The method used is open to some criticism on the grounds that the cathode-ray trace may not begin at the instant that the shock is applied to the nerve, because of delays in the fly-back, and in triggering the thyratron sweep

oscillator. However, these delays have been measured and found not to exceed 0.1 millisec. The time interval was measured to the apex of a unipolar impulse, and to the centre of a biphasic wave. The shock duration was usually adjusted to 0.5 millisec.

Tactile stimulation of the body wall is also effective in producing impulses in the giant fibres. Tactile stimulation was usually by means of a dry glass rod. The impulses in the giant fibres varied in amplitude from 50-1500 microvolts.

The cathode-ray traces were photographed with a special camera using 35 mm. film, up to 200 ft, being accommodated in the magazine. The camera has an F/2 "bloomed" lens and may be used either as a "still" camera or as a strip-film camera - in the latter instance, film is propelled through the gate by an electric motor at speeds varying from 2-25 cms./sec. The latter method was used in recording impulses from tactile stimuli. In measuring conduction velocities shocks were applied to the cord about once per second, and the camera used as a "still" camera.

In the stimulation experiments, the nerve cord was exposed by a lateral incision and the body wall retracted by pinning the worm to a small wooden block. The nerve cord was constantly bathed in a pool of coelomic fluid. This appeared to keep the nerve cord sufficiently moist to allow conduction for two or three hours. No external fluid was applied to the worm or nerve cord. Care was taken to prevent rupture of the thin intestinal wall during exposure of the nerve cord, as the contents of the gut seemed to hasten the failure of conduction in the giant fibres.

At the conclusion of the experiments all worms were fixed in 10% formalin in normal saline. Portions for section were later embedded in paraffin and stained with lead haematoxylin according to the method of MacConnaill (1947). This stain has been found extremely useful in work with unmyelinated or poorly myelinated nerve fibres. The method is simple and reliable and has yielded results comparable with those from silver techniques, but without their uncertainty. These sections provide the basis for the macroscopic reconstruction as well as the histological detail.

GENERAL ANATOMY OF THE NERVOUS SYSTEM.

1. Gross Anatomy.

In general structure, the nervous system of this species of Megascolex follows the same broad pattern found in other oligochaets. The nervous system of Lumbricus has been described in considerable detail by Friedlander (1888, 1889), Cerfontaine (1892); Hansen (1887), and Retzius (1892). The only detailed account of the nervous system in an Australian earthworm is that by Spencer (1888) of the nervous system of Megascolides australis. Although that worm is closely allied to the one used in this study, there appear to be certain important differences in the respective arrangements.

The anterior end of the central nervous system in the local species of Megascolex has paired cerebral ganglia placed above the pharynx in the first segment. Radiating from the cerebral ganglia around the sides of the pharynx are two large bundles of

nerve fibres - the cerebral commissures. These commissures unite on the ventral aspect of the pharynx with the anterior end of the ventral nerve cord on the ventral aspect of the pharynx. At the point of union, a slight swelling is visible in the ventral nerve cord - the subpharyngeal ganglion, or "unterschlundganglion", of Friödlander.

A number of branches arise from the cerebral commissures. The first is a very small one which arises from the most lateral part of the cerebral ganglion itself, rather than from the commissure. Spencer (1888) describes this branch as arising from the commissure in Megascolides australis, but in the present material the branch gains attachment to the lateral part of the dorsal surface of the cerebral ganglion. It is a very fine branch and is distributed to the most anterior part of the dorsal and lateral body walls around the stoma.

About half way down the commissure, opposite the most lateral part of the pharynx, a large nerve is given off from the lateral side of the commissure. Its fibres end in the body wall of the anterior two segments. At the same level on the commissure, but from its postero-internal surface rather than the lateral, arises a compact mass of fibres which immediately breaks up into a number of branches of increasing size from above downwards (Fig. 1D). This bundle of nerve fibres was described briefly by Spencer in Megascolides australis and is known as the stomato-gastric system. In the local species of Megascolex, the majority of the fibres in the upper, smaller

branches of the stomato-gastric system pass dorsally around the wall of the pharynx to enter the typhlosole, supplying its covering epithelium. The larger lower branches pass forward into the walls of the pharynx.

In the lower part of the cerebral commissures another branch is given off and runs forward to supply the ventral part of the body wall in the vicinity of the prostomium. Two or three smaller branches also reach this region from the subpharyngeal ganglion.

The ventral nerve cord extends caudally from the suboesophageal ganglion through the whole length of the body of the worm. The nerve cord increases gradually in size as it is traced back from the suboesophageal ganglion, and attains a maximum diameter in the middle portion of the worm. It then tapers slightly towards the tail, and ends more or less abruptly in the terminal segment of the worm. In a fixed preparation the cord shows alternate swellings and constrictions, one swelling to each body segment. Each swelling is at the site of a collection of nerve cells, forming a segmental ganglion within the cord. The macroscopic arrangement of the ganglia is much more obvious in fixed than in unfixed preparations.

From each side of a segmental ganglion branches are given off to the body wall. Two branches arise from each ganglion, a smaller one at the extreme anterior end of the ganglion, and a larger one near the posterior end. The branches are very small and barely visible to the naked eye, but can be clearly displayed with a dissecting microscope. The arrangement is

very constant. Spencer (1888) found that in Megascolides australis each ganglion gives off three branches, one anterior and two posterior, the latter arising close together. A fixed specimen of Megascolides australis was examined and Spencer's finding confirmed. The three branches are roughly of equal size and it is possible that the two posterior branches in Megascolides australis are fused in the local species of Megascolex to produce the larger posterior branch. Stough (1926) states that in Lumbricus the posterior two branches are frequently fused at their bases to form a common root. In the local material the posterior nerve is single as far as it can be traced in the dissections and in the stained material. It was also noted in Megascolides australis that occasional additional branches are present between the two posterior branches and the anterior one.

At the junction between two body segments the peritoneal lining of the coelome is raised in a transverse fold or mesentery which acts as a support to the interganglionic part of the nerve cord. The peritoneal coat is very thin and glistening in Megascolex, and easily stripped from the cord. In Megascolides australis the peritoneum is much tougher and more fibrous in texture, producing a peritoneal septum through which the nerve cord appears to pass. In the region between these partitions, the peritoneum is also much more firmly adherent to the nerve cord than in the local species.

On the dorsal aspect of the cord, the giant fibres can be seen with the dissecting microscope, appearing as dark streaks

against the white nerve cord. The giant fibres are visible from the region of the clitellum to within 1 cm. of the tail, but beyond these points are usually more deeply placed within the substance of the nerve cord.

2. Histological Findings.

As the average length of the worms is 25-30 cms., it is not practicable to cut a complete set of serial sections. A continuous series of sections was therefore cut only as far back as the posterior end of the clitellum, a distance of 5 cms. A further serial examination for a distance of 2 cms. was made in the region of the 60th. segment to determine the changes in the innervation of the giant fibres. Sections were cut from the remainder of the worm at appropriate intervals but showed no significant alteration in the general pattern.

a). The Cerebral Ganglia. The most striking feature of the ganglia (Fig. 1A and B) is the relative absence of nerve cells in a region in which they are so abundantly concentrated in higher animals. The ventral nerve cord appears considerably more complicated in the arrangement of its ganglion cells than the cerebral ganglia. Apparently the dominance of the head end is not as well established, nor is there such pressing need for complete and immediate control of the rest of the body by an integrating organ of the calibre of the brain of higher animals. The giant fibres of the ventral cord, which are such an integral part of the animal's most effective protective mechanism - the withdrawal reflex - have no easy access to the cerebral ganglia. Indeed, they appear to become lost even before reaching the

cerebral commissures, so that whatever connections do exist, must be very fine unmyelinated (and hence slowly conducting) fibres.

The cerebral ganglia are ensheathed by a layer of coelomic epithelium. Unlike the ventral nerve cord, they have no smooth muscle sheath. Within the epithelial layer, there is an exceedingly well-developed hyaline membrane (Fig. 1B and C). This membrane stains a pale pink in the lead haematoxylin preparations, and appears devoid of any internal structure. It can be traced as a definite sheet into the cerebral commissures and thence into the subpharyngeal ganglion. Beyond this point it gradually fades away in the anterior part of the ventral nerve cord. Its gradual disappearance coincides with an ever-increasing development of the smooth muscle coat on the external surface of the ventral nerve cord. The most anterior part of the ventral nerve cord has a well-developed hyaline membrane but no layer of smooth muscle. The smooth muscle coat is very obvious in the remainder of the nerve cord. Spencer (1888) noted the presence of "a definite development of connective tissue, forming a sheath for the whole nervous system" within the coelomic epithelium in Megascolides australis, but did not mention any special development in relation to the cerebral ganglia.

The nerve cells within the cerebral ganglia are collected in two main groups, an antero-ventral and a dorsal group. Between the two groups is placed an extensive commissure of transverse fibres connecting the two cerebral ganglia (Fig. 1A and B). The cells of the antero-ventral group are concentrated close to

the ventral aspect of the ganglion. They are of medium size with exceedingly granular cytoplasm. Their arrangement is irregular but in general their axons are directed dorsally towards the overlying commissural fibres. The fibres immediately overlying the nucleus run transversely and are more densely grouped than in the rest of the fibre mass. Some of these overlying fibres turn ventrally to end adjacent to the cells of the nucleus. Laterally they are continuous with the fibre tracts of the cerebral commissure. It is reasonable to infer from their position and connections that these fibres are afferent in function.

The most medial cells of the anteroventral nucleus are larger than the rest and have their axons directed dorsally through the transverse fibre mass. The axons can be traced to the region of the large cells in the medial part of the dorsal nucleus.

The dorsal nucleus (Fig. 1A and B) can be divided into two clearly defined parts, a lateral and a medial. The lateral part is the larger and is formed of small cells which extend laterally, as a mantle to the ganglion, almost as far as the commencement of the cerebral commissure. Running to them from their ventral side is the bulk of the fibres of the cerebral commissure. These fibres are also probably afferent in function.

The medial part of the dorsal nucleus consists of much bigger cells with large nuclei and conical cell bodies. The apex of the cone is directed ventro-medially, and from it the axon of the cell runs downwards and medially in a smooth arc to

cross the midline. The decussation with the corresponding axons of the opposite side produces a definite commissure midway between the dorsal and ventral aspects of the cerebral ganglia. Having crossed the midline, the axons can be traced towards the commencement of the opposite cerebral commissure, and in one case could be seen to enter it. They are thus efferent from the cerebral ganglion.

At the posterior extremity of the cerebral ganglia, the large-celled medial part of the dorsal nucleus expands to occupy almost the whole extent of the ganglion from its dorsal to its ventral walls. The axons of these cells are short and cannot be traced far beyond their cells of origin. In the same part of the cerebral ganglia, the lateral small-celled portion of the dorsal nucleus extends laterally as a broad flare to the root of the cerebral commissure.

b). The Ventral Nerve Cord (Figs. 1F and 11A). The anterior end of the nerve cord is formed by the union of the two cerebral commissures. In his account of Megascolides australis, Spencer (1888) states categorically that "the commencement of the commissures is indicated by the absence of ganglion cells, none of which are found in the commissures, nor as far as could be ascertained by a long series of consecutive sections, in the stomato-gastric system." Examination of the commissures in Megascolex shows that at the lower ends, the ganglion cells are continued for some distance into the commissures (Fig. 1E). The cells are small and darkly staining and are most numerous where

the branches to the ventral body wall are given off from the basal part of the commissure. The fibres of the branch appear to be directed towards these cells.

In serial sections of the suboesophageal ganglion a succession of three to five branches leave the ganglion on each side and pass towards the ventral body wall. The interior of the nerve cord at this level is largely occupied by a mass of nerve fibres in the form of a dumb-bell placed on its side - the neuropil mass. At its periphery are grouped the ganglion cells, scantily distributed at the anterior end of the ganglion, but increasing rapidly in number as each successive nerve leaves the ganglion. The cells are radially arranged and their axons enter the neuropil. The axons of the more dorsally placed cells reach the midline and appear to join the median giant fibre which is exceedingly small and deeply placed in this region (Fig. 1F).

Typically there are three giant fibres in the ventral nerve cord, a median and two laterals. The median giant fibre is first visible in the suboesophageal ganglion opposite the point of exit of the second of the small somatic nerves. The fibres of this nerve are directed dorsally towards the ganglion cells at the edge of the upper part of the neuropil, and it is the axons of these cells which can be traced medially towards the anterior extremity of the median giant fibre. The lateral giant fibres are not visible until the sections reach a point considerably further caudally. At first they are very small and apparently discontinuous - appearing in a few sections and then thinning away to a condition in which they can no longer be recognized.

From their origins the giant fibres extend caudally to the posterior end of the nerve cord. The median giant fibre is at first a tiny thread placed in the centre of the neuropil mass, which intervenes between it and the dorsal surface of the cord. In a more caudal situation, approximately opposite the seventh segment, the neuropil mass becomes divided into two almost completely separate portions, only a small isthmus of neuropil connecting the two masses dorsally. The separation is effected by an increase in the number of ganglion cells and nerve fibres which appear on the ventro-medial side of the two masses. At the same time, the median giant fibre is carried dorsally and comes to lie immediately under the dorsal smooth muscle integument of the cord. It is wrapped in a very extensive connective tissue sheath, and has increased considerably in size. The lateral giant fibres, although still small, and apparently disappearing at intervals, occupy positions beside the median giant fibre and are similarly wrapped in connective tissue. There is, however, a very distinct septum separating the median giant fibre from the adjacent lateral giant fibre on each side.

The apparent discontinuity of the lateral giant fibres at the anterior end of the nerve cord is of interest in view of a similar discontinuity in the lateral giant fibres in the crustaceans. Johnson (1923) found in Cambarus that a single portion of the lateral giant fibre extends approximately two segments, ending anteriorly in a few short blunt branches. Each portion is arranged to overlap one immediately cranial or caudal

to itself. It is also physically in contact with those adjacent overlapping portions. Johnson remarks: "It is therefore a side to side association of fibres as contrasted with the absence of contact of axis cylinders in the typical synapse. It is a relationship either through very short lobed processes of one of the fibres involved, or without any subdivisions of any kind as opposed to the numerous fine processes of both of the fibres concerned in the synapse. And it is a condition in which only two fibres are involved, differing from the interminglings of the fibrillations of a number of neurons in the case of a synapse." He also noted that the lateral giant fibre may end in contact with the median giant fibre. The significance of a similar finding in Megascolex is discussed later.

The lateral giant fibres increase in size progressively towards the tail. The median giant fibre increases rapidly in size in the middle portion of the worm, and then decreases slightly in the posterior third. Throughout the cord the lateral fibres are subject to greater fluctuation in size than the median. The two lateral fibres are freely interconnected throughout the cord. The interconnections pass ventral to the median giant fibre from which they are separated by a small amount of neuropil. A similar interconnection was demonstrated histologically in Lumbricus by Stough (1926) and functionally by Eccles (1933) and later by Bullock (1945).

Within each ganglion of the ventral nerve cord the giant fibres are completely subdivided by an obliquely placed septum. This arrangement is identical with that found in Lumbricus by

Stough (1926). (Fig. 12C). Typically the septum in the median fibre is placed transversely and runs from the ventral to the dorsal wall of the fibre as it is traced caudally. The septum in the lateral fibre runs in the opposite direction. However, the septum frequently runs a spiral course, or is obliquely placed.

The precise arrangement of the ganglion cells within each ganglion of the ventral cord is dealt with later. The finer features of the giant fibres are also considered in the light of some of the physiological findings.

EXPERIMENTAL RESULTS.

1. Tactile Stimulation of the Giant Fibres.

Bullock (1945) has shown that in Lumbricus a tactile stimulus applied to the body in the anterior 35-40 segments will excite the median giant fibre and the impulse so produced is conducted equally well in both directions from the point of stimulation. Similarly, a stimulus applied behind the 35th.-40th. segment will excite the two lateral giant fibres.

The first experiments were carried out to see if a similar arrangement was present in Megascolex. The nerve cord was exposed in the posterior one-third of the worm and light touch stimuli applied in front and behind the pick-up electrodes. The nerve cord was then exposed towards the anterior end of the worm and the observation repeated. Stimulation began at the anterior end and was carried progressively in a caudal direction. The impulses were observed on the cathode ray screen and also reproduced by a loudspeaker. When the impulses are picked up

from the posterior end of the worm, stimulation of the anterior end produces short bursts of 3-10 impulses from each stimulus. They are small in amplitude (150-300 microvolts) and last about 1.5 milliseconds. To the ear they produce a sharp click, almost metallic in quality. As the stimulation is carried caudally these are suddenly replaced by bursts of impulses of much larger amplitude (500-1500 microvolts) and longer duration (about 3 milliseconds) with the quality of a dull thud (Fig. 2E and F). The change-over normally occurs abruptly, with very little overlap between the 55th. and 63rd. segments, and seems to bear no relation to the total number of segments in the worm (Table 1). Repeating the process with the pick-up electrodes near the anterior end of the worm (usually just behind the clitellum, which lies on the 13th.-17th. segments) a similar change in the nature of the impulses was observed when the stimulating rod crossed the dividing region in the vicinity of the 60th segment. Where the impulses were picked up from the nerve cord anterior to the clitellum stimulation of any part of the body produced impulses of uniform size and no point of demarcation could be found. The possible significance of this finding will be discussed later. The site of change-over was carefully marked and the worm preserved for section of this region.

Table 1.

Total segments in worm.	Site of change from small to large impulse.
153	58
166	61
189	63
192	61
191	55
216	63
192	61
171	67
161	60
169	50
196	60
194	30
167	65
191	58
148	57
158	55

In the light of Bullock's work it seemed reasonable to infer that the small fast spikes obtained from stimulation anterior to segment 60 are due to impulses travelling in the median giant fibre, and that the larger slower spikes from behind segment 60 are conducted by the lateral fibres. This view is supported by the following experiment, which is a modification of one carried out by Rushton (1945b).

In a worm of 197 segments, the nerve cord was exposed posteriorly, at segment 120 approximately. The line of demarcation between the smaller faster impulses and the larger slower ones was found at segment 65. The nerve cord was then exposed at segment 94, and a very finely sharpened needle was introduced into the median dorsal region of the cord under the dissecting microscope with the object of cutting the median

giant fibre. The pick-up electrodes were reapplied to the cord at segment 120 and it was found that there were no responses to touching the worm anterior to the line of demarcation. Behind this line, touch produced the larger slower impulses. The pick-up electrodes were then transferred to segment 68 and the worm restimulated. Now the smaller, faster impulses again appeared from the anterior end of the worm, and the larger, slower impulses could still be recorded from the posterior end, having traversed the damaged region (Fig. 3). Serial section of the damaged region showed that only the median fibre was injured (Fig. 2G and H).

Rushton (1946) found that in Lumbricus tactile stimulation produced most responses at each end of the worm, and that the middle portion was relatively inexcitable. In Megascolex, all parts of the worm body appeared to be uniformly excitable, with the tip of the tail and the tip of the proboscis slightly more sensitive than the remainder. The presence of the thick clitellum did not seem to influence the excitability in this region. It was noticed that in regions where the longitudinal muscle fibres were contracted, thus shortening and thickening the body, the worm became completely inexcitable and remained so until the contraction wave relaxed, when the responses to touch became normal. The response was rapidly adapting, a burst of three to ten impulses following each touch, but if the pressure was maintained, no further response ensued. Bullock (1945) has suggested that in Lumbricus vibration could prove an

adequate stimulus: in Megascolex such responses were difficult to produce, although in some preparations taoping the table proved effective.

2. Electrical Stimulation of the Giant Fibres.

Previous work on the giant fibres of annelids and cephalopods (Pumphrey and Young, 1938; Eccles et al., 1933; Bullock, 1945) has indicated that conduction occurs at 25-35 metres/sec. in the average case. This problem has been studied in Megascolex from four aspects: firstly, to determine the rates of conduction in various parts of the same worm; secondly, to see if any connection existed between the length of the worm and the rate at which impulses are conducted by its giant fibres; thirdly to correlate as far as possible the rate of conduction with fibre size; and finally to examine the relationship of sheath thickness to conduction velocity.

Estimations of conduction velocity are liable to considerable error where the nervous pathway is short and the time intervals consequently brief. In the earthworm the actual length of the pathway can alter considerably where two points on the body are fixed to a wooden block and the intervening region is free to lengthen or shorten. Bullock (1945) has shown that alterations in length of the worm are accompanied by appreciable alterations in the speed of transmission. Megascolex exhibited much muscular activity when first pinned to the wooden block, producing variations up to 25% in the distance between the fixed points. However, after a few minutes this movement ceased and the length became steady enough to measure to within 1 mm. A minimum path

distance of 4 cms. was used, and wherever possible this was increased to 7-10 cms., so that errors in measurement did not exceed 2-3%. At the conclusion of the experiment the worm was fixed in formol-saline, great care being taken not to stretch or modify its shape in any way.

Gradually increasing strength of stimulus fired first the median giant fibre, and then, at a higher threshold, the lateral giant fibres. The lateral giant fibres always fired together producing a more slowly conducted impulse than the median. All conduction experiments were carried out in both directions over the same path. Impulses travelled at equal speeds in both directions, and at frequencies up to 10/sec. there was no evidence of polarization in the fibres even when stimulated for many minutes (Fig. 4).

a). Conduction Rates in Different Parts of the Worm: In determining conduction rates in different parts of each worm the nerve cord was exposed near the tip of the tail, at the junction of the middle and posterior thirds, at the junction of the middle and anterior thirds, and at the anterior end of the worm, usually at, or just in front of the clitellum (Fig. 5). The speed was then measured over each portion of the path in both directions. It was found that regardless of worm size, the median giant fibre conducted most rapidly in the middle portion of its course, less rapidly in the posterior part of the worm, and still more slowly in the anterior third. The slowing at the anterior end seemed to increase very rapidly as the pick-up point approached the snout. Whereas in many cases conduction as far forward as

the clitellum was only slightly slower than in the middle of the worm , the impulses slowed very considerably in front of the clitellum. Quite a different distribution of velocities is seen in the lateral fibres. Here conduction occurred most rapidly in the posterior third, less rapidly in the middle third, and slowest in the anterior third (Fig. 5). These findings are correlated with fibre size as seen below.

It has been previously remarked that in their normal habitat these worms live with their heads at the deeper end of the burrow. Any external interference from the surface therefore would approach the tail end. Stimulation of the tail, by exciting the lateral giant fibres, elicits the startle reflex. It is therefore not surprising to find that the impulses in these fibres travel fastest in the part of the worm to be withdrawn from danger most rapidly. Moreover, stimulation of the tip of the tail produces a slightly more numerous burst of impulses than does stimulation of other parts of the tail region; the sensitiveness of the tail in this regard has been noted in other earthworms (Bullock, 1945).

b). Relationship between Conduction Velocity and Worm Length.

From a functional viewpoint it would seem desirable that long worms should be acquainted with happenings at remote points on the body surface as rapidly as short ones, otherwise mere length would be a very material disadvantage. In correlating conduction velocity with worm length a number of problems arose. Estimation of worm length is difficult because of its variability from moment to moment. A worm which has shortened in a startle

reaction is but two-thirds or less of its previous length. Therefore in measuring the length of the worm the following procedure was adopted. The worm was placed on a smooth table and allowed to recover from any startle reaction. Within half a minute its length increased considerably and normal progression began. After moving a short distance it was found that the worm length became reasonably constant, and that as the anterior end was elongated, the tail was drawn in by an equivalent amount. This length was measured as accurately as possible without interfering with the progress of the worm, but could not be estimated more accurately than to within 1 cm.

Since velocity varies considerably in each giant fibre in different parts of the same worm (see above), it was decided to compare the maximum observed velocities in each worm - namely in the median giant fibre in the middle part of its course. The mean rate of conduction in both directions in this part of the giant fibre was used. The resulting graph (Fig. 6) shows considerable scatter in its points but the inference can reasonably be drawn that there is an approximately linear relationship between median fibre conduction velocity and the length of the worm. When a similar comparison is made between worm length and velocity in the lateral fibres in the posterior part of the worm (the fastest part of their course) no such relationship seems to exist (Table 2). That the velocity measurements are reasonably correct is confirmed by measurements in other parts of the worm and by measuring the velocity in both directions in each case.

Table 2.

Worm length. (cms)	Velocity in Lateral Giant Fibres. (m/sec.)
17.8	5.8
21.6	13.5
23.5	9.1
23.5	9.5
26.7	5.1
27.9	9.4
29.8	12.0
30.4	6.3
31.6	9.9
32.9	8.0
36.7	14.4
39.4	8.6

c). Relationship between Conduction Velocity and Fibre Size.

A portion of the worm's body about 2.5 cms. in length was removed from each part of the worm used in the conduction experiments, after the whole worm had been fixed in formol-saline. This portion was embedded and sections cut and stained with lead haematoxylin. The sections were projected onto the ground-glass screen of a photo-micrographic apparatus at a magnification of 390 or 780 diameters. The measurements were then made directly from the ground-glass screen. Sections were cut at intervals from the block to check for sudden and significant alterations in fibre size but, apart from the regions of the segmental septa, they showed only a very gradual and uniform increase or decrease in size. The measurements were made about midway between the points of stimulation and pick-up. As in the majority of cases the fibres were oval in outline, the maximum diameter in each direction was measured and the mean diameter calculated. The

mean diameter has been used in the graphical representation (Fig. 7).

Hursh (1939) working with cat nerves found that random variations in size of the fibre constituted an appreciable source of error in correlating size with velocity. Thus in 100 serial sections from 0.6 mm. of nerve Hursh found the standard deviation of a single measurement of a 6.5 mu fibre to be $\pm$0.47 mu. Duncan (1934) found even larger variations in measurements of spinal root fibres from cows, cats and rats fixed in osmic acid. Portions of the nerve examined after teasing in glycerine varied in fibre diameter as much as 50%, the fibres being indented opposite the sheath cell nucleus. Taylor (1940) in measuring the giant fibres in Lumbricus in the fresh state, observes that a fibre having a diameter of .75 mu at a segmental partition measured only 52 mu between successive partitions. A large median fibre examined in situ had a diameter which varied from 109 to 163 mu. In Megascolex considerable increase in size occurs at the partitions but it was not usual to find alterations in fibre diameter in the regions between the partitions in excess of 10% over a distance of 1 cm. This distance usually involved several body segments in the fixed worm. All diameters were measured in parts of the cord between the segmental partitions. The giant fibres showed a gradual tapering in size over many centimetres rather than any sudden large increase or decrease in the course of a few millimetres.

It will be seen (Fig. 7) that the velocity/fibre size relationship is very nearly a linear one, and the same in both the median and lateral giant fibres. A large number of measurements both of velocity and fibre size in different worms have been used, yet the relationship holds very closely at rates varying from 5-45 m./sec.

Very little definite information is available to indicate the extent of shrinkage in the annelid giant fibre during sectioning and its effect on nerve fibres of varying size. Previous workers have tended to ignore this when correlating fibre size with conduction rate. Thus Gasser and Erlanger (1927) in their work on the frog, ignored the factor of shrinkage in the material fixed in 1% osmic acid. Douglas, Davenport, Heinbecker and Bishop (1934) used 10% formalin and 3% acetic acid as a fixative and alternate portions were either stained with osmic acid or embedded unstained in paraffin, but the degree of shrinkage was not determined. Pumphrey and Young (1938) examined the fibre diameter in the giant axons of the squid mantle nerve directly in the fresh state with an ocular micrometer and compared the measurements with preparations fixed in saturated picric acid in sea water and stained by Mallory's technique. While observing that "the giant fibre is likely to shrink proportionately more than the smaller fibres," no figures are given for the percentage of shrinkage. Gasser and Grundfest (1939) found that the conduction velocity in the rabbit nerve was approximately proportional to the axon diameter;

their material was fixed in osmic acid. While quoting the work of Hursh, Arnell and Duncan on nerve fibre shrinkage, they made no estimation of the degree of shrinkage in their own material.

Obviously shrinkage causes trouble only where it is differential - affecting large fibres to a greater or lesser degree than small fibres. Hursh (1939) examined the problem of shrinkage in cat nerve fibres whose conduction velocities were known. He concluded that it was not differential for fibres larger than 10 mu when fixed in either osmic acid or formalin, and embedded in paraffin. He considered that a large proportion of the shrinkage during dehydration of a nerve bundle is due to the connective tissue elements. Shrinkage averaged 10.1% ± 0.16%. There was no correlation between the percentage of shrinkage and the fibre size. He also found that "the best curve relating velocity and diameter is a straight line. It holds equally well in all parts of the range of velocities. Curves drawn in accord with the hypothesis that the velocity varies as the square or square root of the diameter vary widely from the observed points." Arnell (1934) also noted a shrinkage of 10% in formalin-fixed spinal nerves from men and dogs. Young (1948) using the nerve to the medial head of the gastrocnemius in the rabbit, remarks that his technique of fixation in Flemming's fluid "is estimated to produce a shrinkage of about 10% in all dimensions, but this figure is a guess. There is no reason to suppose that shrinkage affects the axon and the myelin differently, but it may well do so."

Thus it was necessary in our material to determine as

accurately as possible any error in measurement due to shrinkage and to ascertain whether the shrinkage was differential. A number of worms were therefore divided into portions about 2.5 cms. long, the gut removed, and frozen sections cut immediately. Particular care was taken to mount the nerve cord vertically on the microtome table. Six to ten sections were cut from each block at 20-25 mu, and examined and measured immediately. The remainder of the block was then fixed in formalin, embedded in paraffin, and stained and cut in the usual way. The stained sections were measured and the results compared with the frozen sections from the same block. The frozen-section method itself introduces some distortion of the fibres, which swell slightly in the freezing process and may not regain their original contours when placed in normal saline. However the distortion is far less than with any method involving fixation and dehydration. Frozen sections were used in preference to the direct examination of teased preparations, because the fragility of the teased and unsupported giant fibre makes it unsuitable for subsequent dehydration and paraffin-embedding.

It was found that the tissues showed an increased tendency to fragmentation when fixed and embedded after freezing, and measurement of the giant fibres was more difficult as a result. However, it will be seen that shrinkage in fibres ranging from 20-75 mu in diameter averaged 10% and was not differential (Fig. 8). In view of the inevitable slight variations in technique during paraffin embedding it was surprising to find such correspondence in the extent of shrinkage in different

blocks of tissue.

d). Relationship between Sheath Thickness and Fibre Diameter.

Friedländer (1889) showed that the annelid giant fibre sheath could be stained with osmium tetroxide and occupied about 5% of the axon diameter. In this regard the fibre lies intermediate in position between the cephalopod giant fibre having a sheath about 1% of the axon diameter, and the heavily myelinated vertebrate fibre with a sheath 25% of the axon diameter. While the myelin sheath of a vertebrate fibre can be measured in stained preparations and is easily distinguishable from the adjacent connective tissue envelopes, the more thinly sheathed invertebrate fibre has presented some difficulty in the assessment of true sheath thickness.

Examination of the sheath has been mainly conducted with polarized light and more recently by X-ray diffraction methods. The optical properties of nerve fibres - comprised on the one hand, of the heavily myelinated vertebrate fibre, and on the other, of the so-called unmyelinated fibres - vary widely. The unmyelinated fibre when viewed with polarized light exhibits a birefringence of the same sign as typical protein fibres, such as muscle, i.e. positive with respect to its length. The heavily myelinated fibre is negatively doubly refracting under the same conditions. Ambronn (1890) recognized that gradations between these two extremes existed, and that the negative birefringence of a well-myelinated nerve could be converted to the positive type by the removal of its myelin with lipoid solvents. The observed birefringence is the resultant of these two opposing

effects. Göthlin (1913) showed that when invertebrate nerves are immersed in a solution of high specific gravity, such as glycerine, the birefringence changes from the positive to the negative type typical of the more heavily myelinated nerve. He named the reaction metatropic, to indicate the reversal of birefringence, and showed that it did not occur if the fibre had first been exposed to some lipoid solvent. He erred in believing that the lipoid formed a cementing substance between the fibres, and that the metatropic reaction was an artefact produced during dehydration of the tissues by hypertonic solutions.

Bear and Schmitt (1937) reinvestigated the problem in the crayfish nerve using polarization optics. They employed the minimal concentration of solutions of metatropic reagents compatible with reversing power, thus reducing dehydration distortion in the individual fibres. The part of the fibre showing the characteristic lipoid birefringence was found to lie close to the axis cylinder and inside the surrounding connective tissue layers, in a similar position to the myelin sheath of myelotropic fibres. Bear and Schmitt showed that the positive birefringence, or, as they named it, "form" birefringence, results from the shape and orientation of the protein micelles, which are probably arranged in a circular fashion. The negative birefringence is due to the lipoid micelles which are radially arranged.

Bear, Schmitt and Young (1937) examined the sheath components in the giant fibres of the squid by polarized light,

and found that the metatropic sheath lay inside several wrappings of fibrous tissue from which it was histologically indistinguishable. In the earthworm the problem of differentiating the metatropic sheath by histological methods does not seem to have been satisfactorily settled hitherto. Thus Friedlander (1889) found that the sheath stained with osmic acid and that it occupied about 5% of the diameter of the axon. More recently Taylor (1938, 1940, 1942, 1943), using only polarized light studies, found that in Lumbricus the relative sheath thickness increases with decrease in fibre diameter below 40 mu. In fibres larger than 40 mu, the ratio axon diameter/fibre diameter approximated a constant K = 0.90.

Measurements were therefore made of the metatropic sheath in Megascolex as far as it could be discerned in the stained preparation and the results compared with sheath measurements made in fresh fibres under polarized light. In the sections stained with lead haematoxylin, the axis cylinder is seen to be surrounded by an exceedingly darkly staining sheath of fairly uniform thickness. This sheath is easily differentiated from the much looser areolar layers surrounding it. It was considered that this inner sheath constituted such a definite entity in the stained material that it would be worthwhile to compare its dimensions with the optically active sheath in the fresh state. This inner sheath was measured in the stained sections in a number of places in each fibre and its mean size plotted against fibre diameter (Fig. 9). The sheath thickness

is approximately a linear function of the diameter for fibres between 30 and 70 mu in diameter, and occupies approximately 5% of the fibre diameter. In the 20-30 mu fibres the sheath thickness diverges somewhat from this linear correlation, the sheath being somewhat thinner than would be expected to fulfil the above relationship. However, as the sheath is less than 1 mu thick in these fibres, very considerable errors must inevitably occur in the measurement, and it is considered that the results may be unreliable in these fibres.

The sheath dimensions were then determined with polarized light. A Watson photomicrographic apparatus was equipped with a Zeiss polarizer and analyzer, and the giant fibres were prepared by teasing a portion of the nerve cord in normal saline under the dissecting microscope. Particular care was taken to preserve the continuity of the giant fibres and torn fragments were rejected. The teased preparation was mounted in normal saline on a well-slide and quickly examined and measured in the photomicrographic apparatus (Fig. 12D). When plotted against fibre diameter, the sheath thickness is again a linear function for fibres between 30 and 70 mu in diameter (Fig. 10). Nor does the proportion of sheath to fibre diameter appear significantly different by this method from the results obtained from the stained preparations. Thus by the staining method, a 60 mu fibre would have an expected sheath thickness of 3.2 mu, or 5.3% of the fibre diameter. In the fresh state by polarized light, a 60 mu fibre would have a sheath thickness of 3.6 mu or 6% of the fibre diameter. It is unfortunate that the linear

relationship between sheath dimensions and fibre diameter precludes any conclusion being drawn concerning the relative importance of the sheath and the fibre diameter in determining the conduction velocity. Recently, Sanders and Whitteridge (1946) found in rabbits that "the conduction velocity depends in the first instance upon the myelin sheath thickness: fibres with a thick sheath conducting faster than fibres of a similar or even greater diameter but with thinner sheaths."

3. Fatigue Phenomena during Stimulation of the Giant Fibres.

When stimulated electrically, the giant fibres of Lumbricus will conduct an impulse in both directions from the point of stimulation without any apparent polarization (Eccles, Granit and Young, 1933; Bullock, 1945). Where the rate of stimulation is between 25-50/sec., the fibres will continue to conduct in both directions for many minutes. Bullock found that in Lumbricus, each shock produces one impulse (or two above the second threshold) up to a frequency of 250/sec. When fatigue occurs, the spike drops out, at first occasionally and then more often, but with a latency that increases up to 20% just before complete cessation of the response. In Megascolex, fatigue occurred after 3-4 minutes stimulation at 30-50 per second. The impulses arrived gradually later in time (but unaltered in appearance) in the case of the last five or six impulses before the cessation of the response (Fig. 13E and F).

A curious phenomenon was noticed when using the posterior half of the nerve cord of Megascolex in these fatigue

experiments. It was found that when the direction of conduction was antero-posterior, (i.e. stimulating electrodes placed anterior to the pick-up electrodes) the lateral giant fibres regularly fatigued earlier than the median giant fibre. Conversely when stimulated postero-anteriorly, the median giant fibre fatigued earlier than the laterals. Fatigue was produced in all cases by stimulation for several minutes at 30-50 stimuli per second. Bearing in mind that the lateral giant fibre system is probably intended primarily to subserve anterior conduction of stimuli from the tail, and the median fibre posterior conduction of impulses from the head end, it would appear that when called upon to conduct what might be regarded as anti-dromic impulses, they fatigue more readily.

This observation gives point to Stough's (1926) claim that in Lumbricus the axon material in the giant fibres shows differential staining on each side of the complete segmental partition. He found the material on one side distinctly darker in colour than that on the other. In the median fibre, the darker material occurs in the posterior part of the anterior element, while in the lateral fibres, the darker material occurs in the anterior part of the posterior element. This arrangement was constant throughout the cord in Lumbricus. Stough therefore inferred that the nerve fibres were polarized and that probably conduction occurred in only one direction within the fibre. It is now known that this inference is incorrect under normal physiological conditions, since the impulse travels equally well

in both directions. Possibly, however, the relative absence of polarity in the fibre is considerably modified under stress.

The results of fatigue experiments with the anterior half of the nerve cord were much less constant. Frequently both giant fibres fatigued simultaneously, or conflicting results occurred when impulses passed in the same direction over the same pathway at intervals of a few minutes.

It should be pointed out that the lead haematoxylin method does not show any differential staining in Megascolex at the segmental septa. Smallwood and Holmes (1927) have denied its existence in Lumbricus despite Stough's detection of darker staining in Lumbricus terrestris, L. rubellus, Eisenia foetida, Heliodrilus caliginosus and in the lateral giants of Nereis.

4. Microscopic Findings in the Nerve Cord in Relation to Certain Physiological Observations.

a). The Ganglion Cells. In view of the fact that the changeover from median fibre activity to lateral fibre activity from a tactile stimulus could be determined with considerable accuracy, it was decided to examine the region of transition by serial transverse section. The position of changeover was determined during life and accurately marked. The worm was subsequently fixed in formalin, stained with lead haematoxylin, and serial transverse sections cut for a distance of 1.5 cms. on each side of the transition point.

It was found in each ganglion of the ventral cord behind the transition point, comprising in all more than two thirds of

the total ganglia in the cord, that there are altogether four main groups of cells forming parts of the afferent neuron system to the lateral giant fibres (Fig. 11A-C, Groups I-IV). At the posterior end there are two very large cells placed ventrally in the cord near the midline, one lying slightly anterior to the other, so that they are seen successively in transverse sections. The cell bodies are vesicular and show a peculiar dappling with the lead haematoxylin stain. The nucleus is small, round and intensely staining. The axons of these cells appear to run at first in a dorso-lateral direction to enter the neuropil mass, then turning medially in a smooth arc, they pass ventral to the median giant fibre to enter the opposite lateral giant fibre from its ventral aspect (Fig. 11B and C). This accords well with the account by Smallwood and Holmes (1927) of paired cells similarly situated in Lumbricus and also connected with the opposite lateral giant fibre. These cells appear to receive afferent fibres directly from the body nerves. Fine fibres can be traced from the point of entry of the body nerve along the ventral aspect of the nerve cord to the region of the cell, and although small globules resembling boutons terminaux can be seen in contact with the cell in some sections, their continuity with the fine afferent fibres was not proven. The axon of the giant cell in the first part of its course appears to give off a number of fine branches which spray out in the neuropil mass and cannot be traced further.

Immediately anterior to these giant cells is a group of three or four smaller cells, with a more uniformly staining

cytoplasm. They are situated in a similar position to the giant cells and also send their axons dorso-laterally before turning medially to cross the midline and reach the opposite lateral giant fibre (Fig. 11C). A little further forward there is a group of laterally placed cells, four or five in number, arranged with their long axes resembling the spokes of a wheel, the axons converging on a point at the rim of the neuropil. The axons of these cells form a single bundle of fibres which pass in an unbroken course transversely through the neuropil and decussate ventral to the median giant fibre (Fig. 11E and F). Finally at the anterior end of the ganglion, the ventral and lateral aspects of the neuropil mass are fringed with a large number of small cells whose axons pass directly into the neuropil (Fig. 11G). Their axons can only be traced for a short distance in most cases but some of them appear to run dorsally to enter the lateral giant fibre of the same side.

The arrangement of the cell groups in the afferent neuron pathways in the part of the cord anterior to the transition point shows fundamentally the same pattern. At the posterior end of each ganglion is a pair of giant cells ventro-medially placed and with their axons discharging, not into the lateral fibres by a circuitous route, but into the median giant fibre (Fig. 11D). The axons run dorsally adjacent to one another, and appear to be connected with one another by a yoke of tissue some distance ventral to their termination in the median giant fibre. They enter this fibre separately. The giant cells in this part of the cord are not as large as those

behind the transition point, and their cytoplasm stains more deeply and uniformly (Fig. 11D). The next group of cells lies immediately in front of the giant cells. Their axons pass dorsally to reach the median giant fibre. The third and fourth groups of cells are very similar to the corresponding groups in the posterior part of the cord. The third group is made up of cells lying in the lateral part of the cord. Their long axes are radially arranged about a focal point at the edge of the neuropil. Their axons converge on this point and pass as a single bundle of fibres through the neuropil to enter the median giant fibre. The fourth group of cells lies against the neuropil and sends short axons into it. In all parts of the cord there is a variable amount of overlap between the cell groups.

The arrangement of the efferent or motor pathway is by no means as clear as the afferent system. Posterior to the paired giant cells and slightly more laterally is a large column of cells which extends for a short distance behind the rest of the ganglion. These cells are in some cases multipolar, but frequently are unipolar with an axon passing downwards and laterally towards the adjacent segmental nerve. Before reaching the body nerve, however, the axon divides into two branches, one of which runs dorsally around the neuropil to the region of the giant fibres, and the other enters the body nerve. It is conceivable that these cells are part at least of the efferent pathway to the musculature of the body wall, but it seems unlikely that it is the whole, or necessarily a major part.

There remains the enigma of the neuropil mass, composed mainly of longitudinal fibres, but whose connections are unknown. It occupies such a large proportion of the total volume of the nerve cord that to try to build a complete functional picture of the annelid nervous system while so little is known about it would be futile.

b). The Neurofibrillae within the Giant Fibre. Smallwood and Holmes (1927) described in Lumbricus neurofibrillae running from the point of entry of the afferent fibrils within the substance of the giant fibres towards the segmental septa. At the septum they break up into "an immensely complex branching of fibrils." The neurofibrillae were seen in Levadite sections and also in the material stained by the intravitam method of Krawany. The neurofibrillae occupy the central part of the nerve cord. While it is not absolutely clear from the text, it appears that in the Levadite preparations the fibril within the giant fibre was either single or composed of closely intertwined fibrillae. Smallwood and Holmes say however of the Krawany preparations that "in some regions there was more than one fibril present, but the multiple structure of the central strand was not uniformly clear. In his preparations the fibrils are confined in general to the central region of the giant fibre."

In Megascolex, using the lead haematoxylin preparations, the neurofibril within the giant fibre appears in many of the longitudinal sections. It is at all times single and appears as a very dark discrete thread against the paler background of the axoplasm. It runs a very tortuous course in the centre of

the giant fibre, and seems to be twisted on itself in an extraordinary way (Fig. 12A, B, C). At the septum it breaks into the peculiarly branched mass described by Smallwood and Holmes. There is certainly no continuity between it and the adjacent mass on the other side of the septum. The point of entry of the fibrillae into the giant fibre is seen in very few longitudinal sections of the nerve cord, but where it can be observed, the fibrillae, having entered the giant fibre divide to pass both anteriorly and posteriorly from the point of entry.

It is interesting to speculate on the possible role of such an arrangement. It may well be that the fibrillae are "triggers" which by their tiny activity adjacent to the septum are responsible for the much larger activity in the giant fibre as a whole. That the appearance may be an artefact cannot be excluded, but its presence in different worms with different staining methods makes this unlikely. Moreover the presence of but a single fibril within a very large fibre can hardly be explained by chemical changes in a large mass of axoplasm. Dehydration and fixation changes would surely produce a mass of such fibrils. The constancy of the branching near the septum is also difficult to ascribe to an artefact.

c). Interconnections between the Giant Fibres. Stough (1926) states that in Lumbricus "it is possible, as has been mentioned by a number of investigators, that there is a connection between the median and lateral giant fibres." More recent work concerned with the physiology of conduction in the giant fibres has thrown considerable doubt on the possibility of such a connection. In

stimulation experiments, the median giant fibre behaves as a discrete unit, and the lateral giants behave as an interlocked pair of fibres. Destruction of one unit in no way alters conduction in the other. However in examining serial transverse sections through the clitellum it was noted in one series that at one point the left lateral giant fibre approached the median giant fibre as it ran anteriorly, fused with it for a short distance and then regained its identity at a slightly more anterior position (Fig. 13A, B, C). If the lateral giant fibre actually joins the median giant fibre in this situation, the implications are numerous.

It means that the median giant fibre in this position must possess a certain polarity which will prevent a slowly conducted impulse coming from the posterior end of the worm in the lateral giant fibres from entering the median giant fibre and being conducted posteriorly again at high speed by the median giant fibre. Such a phenomenon was never observed during stimulation of the posterior end of the nerve cord. Conversely stimulation of the median giant fibre anteriorly would be expected to produce impulses in the lateral giant fibres at the posterior end of the nerve cord. This has certainly not been observed in these experiments in Megascolex, and suggests that the apparent junction, though convincing in appearance, may be an artefact. Stough (1926) reported one case in Lumbricus histologically showing "direct fusion between the median and a lateral giant fibre. These fibres remained completely fused over a short distance, then separated, and

both resumed their ordinary positions."

On the other hand, that the fusion may sometimes be a functional as well as a structural one is suggested by the similarity in shape and duration of the faster and slower impulses recorded from the nerve cord anterior to the clitellum on posterior stimulation of the nerve cord (Fig. 13D). Such a finding is relatively common in recording from the nerve cord anterior to the clitellum. When using tactile stimulation and recording in this region, it is often impossible to detect a transition point from median to lateral fibre conduction by an examination of the form and size of impulse.

DISCUSSION.

The general structure of the nervous system in this species of Megascolex follows the same general pattern found in other oligochaets. There are however important differences between the nervous system of this worm and that of the closely related Megascolides australis described by Spencer (1888). In the local material, the branches arising from each ganglion of the ventral nerve cord show a very constant arrangement. There is a small anterior branch and a large posterior one. The posterior branch remains undivided as far as its terminal distribution in the body wall. It is interesting to compare this arrangement with that in Megascolides australis and also in Lumbricus. Spencer's finding has been examined and confirmed that in Megascolides australis there are three branches of approximately equal size, one at the anterior end and two close together at the posterior

end of each ganglion. Stough (1926) states that in Lumbricus the posterior two branches are frequently fused at their bases to form a common root. It is therefore suggested that the two posterior branches in Megascolides australis are fused in the local species of Megascolex to produce the larger posterior branch.

Examination of the cerebral ganglia with a lead haematoxylin stain indicates a relative absence of nerve cells from a region in which they are so abundantly concentrated in higher animals. The giant fibres taper rapidly at the anterior end of the ventral nerve cord and appear to be connected with the cerebral ganglia only by the fine unmyelinated fibres of the cerebral commissures. Such a connection provides only a slow-conduction pathway to the cerebral ganglia. As the giant fibres are the mediators of the withdrawal reflex, the animal would appear to rely on the intrinsic reflex pathways of the ventral nerve cord when exposed to external danger. The cerebral ganglia would thus appear to play a relatively minor part as integrating organs at a dominant head-end.

The cerebral ganglia are ensheathed in a hyaline membrane which can be traced into the cerebral commissures, but which fades away in the anterior part of the ventral nerve cord as the outer smooth muscle coat on the cord becomes increasingly developed. This membrane does not appear to have been described previously as a special development around the cerebral ganglia, although Spencer (1888) noted a sheath "around the whole nervous system" in Megascolides australis.

The cells within the cerebral ganglia are clearly grouped into an antero-ventral nucleus and a dorsal nucleus. The two nuclei are separated by commissural bands of fibres. These fibres enter the cerebral ganglia from the cerebral commissure on each side and terminate in the cells of the antero-ventral nucleus and the lateral part of the dorsal nucleus. The main efferent pathway from the cerebral ganglia is composed of the axons of large cells in the medial part of the dorsal nucleus. The axons pass ventro-medially to cross the midline and leave the cerebral ganglia by the opposite cerebral commissure.

The cerebral commissures pass round the walls of the pharynx and unite on its ventral aspect to form the ventral nerve cord. Groups of ganglion cells are clearly visible in the basal parts of the cerebral commissures. These ganglion cells appear to receive fibres from the branches to the body wall arising from this part of each commissure. Spencer (1888) states that no cells are to be found in the cerebral commissures of Megascolides australis.

The giant fibres of the ventral cord have been closely studied at their anterior ends. The median giant fibre appears first in sections at the posterior end of the suboesophageal ganglion opposite the point of entry of the second small somatic nerve. It increases in size in a regular fashion as it is traced posteriorly. The lateral giant fibres first appear somewhat further caudally and are at first discontinuous. Johnson (1923) noted a similar discontinuity in the lateral giant fibres of Cambarus.

The giant fibres in this species of Megascolex have been shown to innervate portions of the body significantly different from those described by Bullock (1945) for Lumbricus. The median giant fibre innervates approximately the anterior 60 body segments. It might reasonably be inferred that the deep burrowing habits of this worm during the dry months of the Australian summer demand the use of a rapid conduction system over more of the anterior end of the body than in Lumbricus. The disposition of the clitellum and the subjacent sex organs does not appear to be a factor in determining the relative distributions of the median and lateral giant fibres. In Lumbricus the clitellum is situated opposite the 32nd.-36th. segments and according to Bullock the median fibre is innervated from the anterior 35-40 segments. In Megascolex, however, the clitellum is situated opposite the 13th.-17th segments, and a very considerable part of the body behind the clitellum is connected with the median giant fibre. No surface markings could be found to indicate the region of changeover, but it was noted that the worm seemed unable to autonomise that part of the body innervating the median giant fibre - the anterior 60 segments behaved as a unit which the worm was unable or unwilling to rupture when traction was exerted on the tail end.

Tactile stimulation produced bursts of impulses in this worm just as in Lumbricus. There did not appear to be any diminution in sensitivity to touch in the middle portion of the body. This work was done in unanaesthetized worms, and the findings differ from those of Rushton (1946) in Lumbricus.

Rushton used anaesthetized worms and this may have affected the excitability. Slightly more numerous bursts of impulses followed tactile stimulation of the tip of the nose and the tip of the tail. The response was rapidly adapting and disappeared in a fraction of a second even with continued pressure. Tapping and other vibration stimuli also evoked a response, but visual stimuli produced no effect. Visual stimuli will produce impulses in the segmental nerves of the earthworm (Prosser, 1935) but do not seem effective in firing the giant fibres. No response followed tactile stimulation of a part of the worm which was contracted.

In measuring the speed of conduction in various parts of the worm it was found that the median giant fibre conducts fastest in the middle part of the worm, more slowly at the posterior end and slowest at the anterior end of the worm. At all times conduction in the median giant fibre was 2-4 times as fast as in the lateral giant fibres in the same part of the cord. Conduction in the lateral giant fibres occurs most rapidly at the posterior end of the worm, more slowly in the middle, and slowest at the anterior end.

The correlation of conduction velocity with fibre size shows that the relationship is very nearly a linear one over a range of 5-45 metres/sec. Moreover, the same relationship holds for the lateral and median giant fibres, indicating that their mode of conduction is identical. In the past various relationships have been suggested between fibre diameter and conduction velocity. Douglas, Davenport, Heinbecker and Bishop

(1934) found that in the vertebrate nerve (cat, rabbit and turtle) the conduction rate varies as a function of size between the linear and the square of the diameter. Erlanger and Gasser (1937) found that the velocity of the impulse in a frog nerve fibre varied as the square of the diameter, to the nearest approximation. Pumphrey and Young (1938) found that in the cephalopod giant axon the velocity varied as the square root of the axon diameter. Offner, Weinberg and Young (1940) obtained a similar relationship for this type of fibre on mathematical grounds from a consideration of the core-conductor theory of nerve impulses. Gasser and Grundfest (1939) showed that in mammalian A fibres the velocity of conduction is approximately proportional to the axon diameter, "but a slight curvature in the graph connecting the two variables may change less rapidly than if the relationship were strictly linear." Hursh (1939) considered that in cat nerves the best curve relating velocity and diameter is a straight line.

Curves drawn in accordance with the hypothesis that the velocity varies as the square or square root of the diameter vary widely from the observed points in Nereocolex. The only previous attempt to achieve a correlation in the annelid giant fibre has been by Eccles, Granit and Young (1933) who stated that the rate of conduction is approximately proportional to the diameter of the fibre. They give no figures for the relationship, however. This relationship has been found to hold in Nereocolex within narrow limits. A careful examination of the amount of shrinkage in these giant fibres when formalin-fixed and paraffin-

embedded indicates that it is not differential in the range 20-75 mu and therefore cannot distort the velocity/fibre size relationship. The amount of shrinkage averages 10%.

The conduction velocity in any fibre would appear to be determined jointly by its diameter and its sheath thickness. Sanders and Whitteridge (1946) have shown that after lesions in the rabbit's peroneal nerves, the largest fibres proximal to the lesion conduct 11% faster than the corresponding fibres in normal nerves. Such fibres have thicker myelin sheaths than the corresponding normal fibres. In Megascolex the sheath is poor in lipoid which occupies 5% of the fibre diameter in stained preparations and slightly more when examined by polarized light. The sheath thickness is a linear function of the fibre diameter and the relative importance of sheath thickness and fibre diameter in determining velocity is impossible to assess in this case. Sanders and Whitteridge concluded that as there is an approximately linear relationship between myelin sheath thickness and axon diameter, there is reason to believe that the assumption V = K × myelin sheath thickness would also give an adequate reconstruction of the axon potential. It is also possible that sheaths of equal thickness may show different velocities for other reasons. Thus the largest fibres in the peroneal nerve in the cat and rabbit are of the same size (20 mu) and have the same sheath thickness (2.5 mu) yet the maximum velocity in the cat's nerve is 110 m/sec. but only 69 m/sec. in the rabbit.

The finding of a single neurofibril within the giant fibre is in agreement with the findings of Smallwood and Holmes (1927) in Lumbricus. It differs fundamentally, however, from Stough's (1926) description of the neurofibrillae. In his iron haematoxylin sections and vom Rath preparations, the appearance of the cross-sections suggests that the giant fibres consist of "a large number of slender, parallel axons, so closely appressed as to make their outlines hexagonal, and each one with a dark central point." He detected this in Lumbricus, in Eisenia, and in Nereis. Such fibrillae had been reported by previous workers, but Friedlander (1888) could not detect them in the earthworm. Hamaker (1898) found polygonal areas in Nereis which Ashworth (1909) declared to be artefacts. The appearance described by Stough resembles the findings of Richards, Steinbach and Anderson (1943) in squid giant nerve axoplasm using the electron microscope. In air-dried and frozen-dried smears of axoplasm they found that it had taken on the form of fibrillae 15-50 mu in diameter made up of smaller elements, not clearly visible, but probably composed of oblong particles in linear aggregation. Such an appearance is highly suggestive of a fixation artefact. In the lead haematoxylin sections the axoplasm of the Megascolex giant fibres appears a hyaline pale blue without structural detail apart from the single darkly-staining, centrally placed neurofibril. This neurofibril breaks up adjacent to the septum in the fashion described by Smallwood and Holmes.

ACKNOWLEDGEMENTS.

I am indebted to Professor A. A. Abbie who suggested this subject for investigation and has freely provided facilities and advice throughout. Mr. T. Canny has cut and stained most of the sections, while Miss G. Walsh has drawn some of the illustrations from my sketches. To all of these I wish to express my gratitude.

SUMMARY.

1. The general anatomy of the central nervous system of Megascolex is described.

2. The dorsal giant fibres of Megascolex are so arranged that the median giant fibre is excited by a tactile stimulus to the anterior 55-60 segments of the body. Stimulation behind this point excites the lateral giant fibres. The point of changeover is relatively constant and bears no relationship to the total number of segments in the body.

3. A tactile stimulus to any part of the worm's body is uniformly sufficient to produce a giant fibre response. A local contraction temporarily diminishes or abolishes the excitability by a tactile stimulus.

4. The conduction velocity in the giant fibres reaches a maximum of 45-55 m./sec. The velocity is a linear function of fibre size and sheath size.

5. The velocity varies in different parts of the worm. In the median fibre it is fastest in the middle of the worm, slower posteriorly, and slowest at the anterior end. The lateral fibres conduct progressively more slowly from behind forward.

6. The maximum velocity in the median giant fibre is approximately a linear function of the length of the worm.

7. Under physiological stimulation an impulse in the giant fibres is conducted equally well in both directions. When fatigued by continued electrical stimulation, a type of polarity appears in the giant fibres at the posterior part of the nerve cord. The lateral fibres cease to conduct posteriorly and the median fibre will not conduct anteriorly.

8. The sheath of the giant fibres has been estimated in stained sections and in fresh preparations under polarized light. It is a linear function of fibre diameter and approximates 5.3% in the stained sections and 6.0% by polarized light. Shrinkage during processing has also been estimated at 10% of the diameter in the fresh state and is not differential for fibres between 20 and 75 mu.

9. The finer structure of the giant fibres is discussed. Each giant fibre appears to possess a single centrally placed neurofibril. The possibility of interconnections between the giant fibres at the anterior end of the nerve cord is considered.

REFERENCES.

AMBRONN H. 1890, "Das optische Verhalten markhaltiger und markloser Nervenfasern,"
Ber. K. sachs. Ges. (Akad.) Wiss., Bd. 42., S. 419.

ARNELL N. 1936, "Untersuchung über die dicke des Achsenzylinders und Markscheide in nicht fixierten Spinalnerven des Menschen und des Hundes,"
Acta psychiat. et. neurol., Vol. XI, p. 5.

ASHWORTH J.H. 1909, "Giant Nerve Cells and Fibers of Halla parthenopeia,"
Philos. Trans., B. Vol. 200, p. 427.

BEAR R.S. and SCHMITT F.O. 1937, "Optical Properties of the Axon Sheaths of Crustacean Nerves,"
J. Cell. and Comp. Physiol., Vol. 9, p. 275.

BEAR R.S., SCHMITT F.O. and YOUNG J.Z. 1937, "The Sheath Components of the Giant Nerve Fibre of the Squid,"
Proc. Roy. Soc., B. Vol. 123, p. 496.

BULLOCK T.H. 1945, "The Functional Organization of the Giant Fibre System of Lumbricus,"
J. Neurophysiol., Vol. 8, p. 55.

BULLOCK T.H. 1947, "Problems in Invertebrate Physiology,"
Physiol. Rev., Vol. 27, p. 643.

CERFONTAINE P. 1892, "Contribution à l'étude du système nerveux du Lombric terrestre,"
Bull. Acad. Roy. Belgique, ser. 3, T. 23, p. 742.

DOUGLAS T.C., DAVENPORT H.A., HEINBECKER P. and BISHOP G.H. 1934, "Vertebrate Nerves: some Correlations between Fibre Size and Action Potential," Am. J. Physiol., Vol. 110, p. 165.

DUNCAN D. 1934, "A Relation between Axon Diameter and Myelination Determined by Measurement of Myelinated Spinal Root Fibres," J. Comp. Neurol., Vol. 60, p. 437.

ECCLES J.C. 1946, "An Electrical Hypothesis of Synaptic and Neuromuscular Transmission," Ann. N.Y. Acad. Sci., Vol. 47, p. 429.

ECCLES J.C., GRANIT R., and YOUNG J.Z. 1933, "Impulses in the Giant Fibres of Earthworms," J. Physiol., Vol. 77, p. 23.

ERLANGER J. and GASSER H.S. 1937, Electrical Signs of Nervous Activity, Univ. Penn. Pr., p. 31.

FRIEDLANDER B. 1888, "Beitrage zur Kenntis des Centralnervensystems von Lumbricus," Zeits. Wiss. Zool., Bd. 47, S. 47.

_____ 1889, "Uber die markhaltigen Nervenfasern und Neurochorde der Crustacean und Anneliden," Mitt. Zool. Sta. Neapel., Vol. 9, p. 205.

GASSER H.S. and ERLANGER J. 1927, "Size of Nerve Fibres and the Action Potential," Am. J. Physiol., Vol. 80, p. 522.

GASSER H.S. and GRUNDFEST H. 1939, "Axon Diameters in Relation to Spike Dimensions and the Conduction Velocity in Mammalian A Fibres," Am. J. Physiol., Vol. 127, p. 393.

GOTHLIN G.F. 1913, "Die doppelbrechenden Eigenschaften des Nervengewebes ihre Ursache und ihre biologischen Konsequenzen," Kungl. Svenska Vetenskap. Akad. Handl., Bd. 51, S. 1.

HAMAKER J.I. 1898, "The Nervous System of Nereis virens Sars. A Study in Comparative Neurology," Bull. Mus. Comp. Zool. Harvard College, Vol. 32, p. 87.

HURSH J.B. 1939, "Conduction Velocity and the Diameter of the Nerve Fibre," Am. J. Physiol., vol. 117, p. 131.

JOHNSON G.E. 1923, "The Giant Nerve Fibres in the Crustaceans," J. Comp. Neurol., Vol. 36, p. 323.

KRAWANY H. 1905, "Untersuchungen über des Zentralnervensystems des Regenwurms," Art. Zool. Inst. Wien, Bd. 15, S. 281.

LEYDIG F. 1864, "Vom Bau des thierischen Körpers," Handuch der vergleichenden Anatomie, Bd. 1, Tubingen.

MACCONNAILL M.A. 1947, "A Method of Staining Unmyelinated Nerve Fibres," J. Anat., Vol. 81, p. 371.

HANSEN F. 1887, "The Structure and Combination of the Histological Elements of the Central Nervous System," Bergens Museums Aarsberetning for 1886, p. 27.

OFFNER F., WEINBERG A. and YOUNG G. 1940, "Nerve Conduction Theory: Some Mathematical Consequences of Bernstein's Model," Bull. Math. Biophysics, Vol. 2, p. 89.

PROSSER C.L. 1935, "Impulses in the Segmental Nerves of the Earthworm," J. Exper. Biol., Vol. 12, p. 95.

RETZIUS G. 1892, "Das Nervensystem der Lumbricinen," Biol. Unters., N.F., Bd. 3, S. 1.

RICHARDS A.G., STEINBACH H.B. and ANDERSON J.F. 1943, "Electron Microscope Studies of Squid Giant Nerve Axoplasm," J. Cell. and Comp. Physiol., Vol. 21, p. 129.

RUSHTON W.A.H. 1945a, "Motor Responses from Giant Fibres in the Earthworm," Nature, Vol. 156, p. 109.

_____ 1945b, "Action Potentials from the Isolated Nerve Cord of the Earthworm," Proc. Roy. Soc. Lond., B, Vol. 133, p. 109.

1946, "Reflex Conduction in the Giant Fibres of the Earthworm," Proc. Roy. Soc. Lond., B, Vol. 133, p. 109.

SANDERS F.K. and WHITTERIDGE D. 1946, "The Conduction Velocity and Myelin Sheath Thickness in Regenerating Nerve Fibres," J. Physiol., Vol. 105, p. 152.

SCHMITT F.O. and BEAR R.S. 1939, "The Ultrastructure of the Nerve Axon Sheath," Biol. Rev., Vol. 14, p. 27.

SMALLWOOD W.M. and HOLMES M.T. 1927, "The Neurofibrillar Structure of the Giant Fibres in Lumbricus terrestris and Eisenia foetida," J. Comp. Neurol., Vol. 43, p. 327.

SPENCER W.B. 1888, "The Anatomy of Megascolides australis," Trans. Roy. Soc. Victoria, Vol. 1, p. 3.

STOUGH H.B. 1926, "The Giant Nerve Fibres of the Earthworm," J. Comp. Neurol., Vol. 40, p. 409.

_____ 1930, "Polarization of the Giant Nerve Fibres of the Earthworm," J. Comp. Neurol., Vol. 50, p. 217.

SWEET, G. 1900, "On the Structure of the Spermiducal Glands and Associated Parts in Australian Earthworms," Linn. Soc. J. Zool., Vol. 28, p. 109.

TAYLOR G.W. 1938, "The Birefringence of the Sheath of the Earthworm Giant Nerve Fibres," Anat. Rec., Vol. 72, Supp: p. 79.

TAYLOR G.W. 1940, "The Optical Properties of the Earthworm Giant Fibre Sheath as Related to Fibre Size,"
J. Cell. and Comp. Physiol., Vol. 15, p. 363.

_____ 1942, "The Correlation Between Sheath Birefringence and Conduction Velocity with Special Reference to Cat Nerve Fibres,"
J. Cell. and Comp. Physiol., Vol. 20, p. 359.

TAYLOR G.W. and WERNDLE L. 1943, "Sheath Birefringence as Related to Fibre Size and Conduction Velocity of Catfish, Mauthner, Muller and Peripheral Fibers,"
J. Cell. and Comp. Physiol., Vol. 21, p. 281

YOUNG J.Z. 1945, "The History of the Shape of the Nerve Fibre,"
Essays on Growth and Form, pp. 41-44, edited by W.E. Le Gros Clark and P. B. Medawar.

YOUNG J.Z. 1948, "Growth and Differentiation of Nerve Fibres,"
Growth in Relation to Differentiation and Morphogenesis, pp. 57-74, Symposia of the Society for Exp. Biol. No. 2.

Fig. 1.

A. The left cerebral ganglion, showing the dorsal and antero-ventral nuclei separated by transversely directed fibres. These fibres are more densely grouped in the ventral part of the ganglion. Magnification 50 diameters.

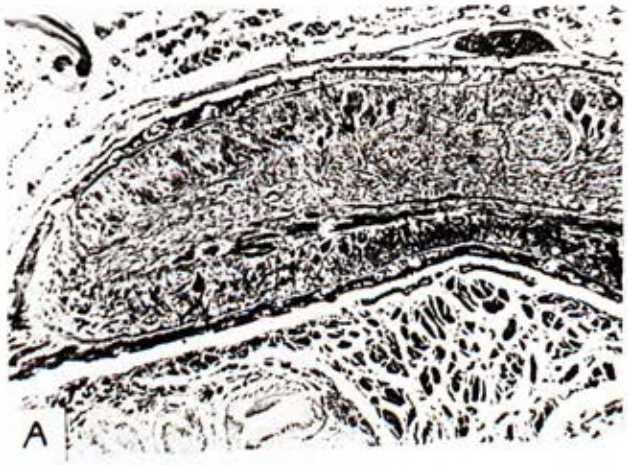
B. The left cerebral ganglion. The well-developed hyaline membrane within the coelomic epithelium is clearly visible. The cells of the dorsal nucleus are of two types - smaller laterally and larger medially. The axons of the larger medial cells can be seen passing ventrally and decussating in the midline. The axons of the medial cells of the antero-ventral nucleus pass dorsally towards the large medial cells of the dorsal nucleus. Magnification 75 diameters.

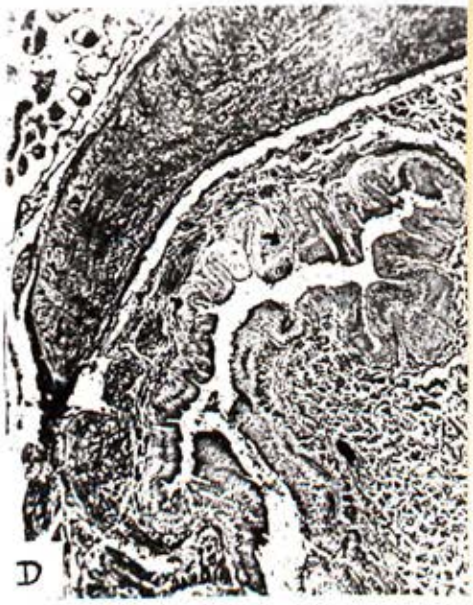
C. The left cerebral ganglion and the adjacent part of the cerebral commissure. The hyaline membrane around the ganglion is continued into the commissure. Magnification 50 diameters.

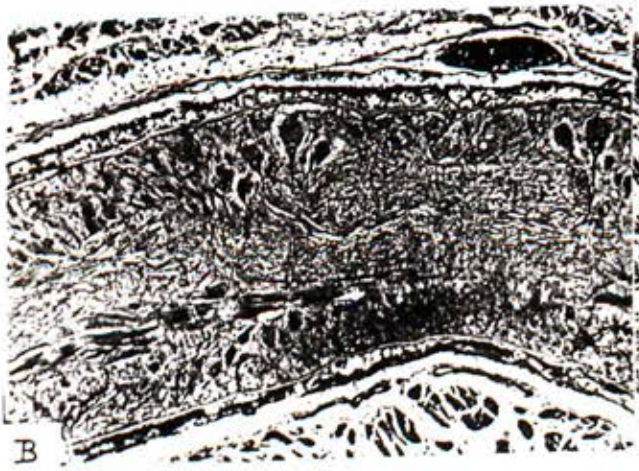
D. The left cerebral commissure and the larger branches from it forming the stomato-gastric system. The lower branches are larger than the upper. These branches lie close to the lining epithelium of the pharynx. Fibres from the upper branches can be seen running around the dorsal pharyngeal wall into the typhlosole. Magnification 50 diameters.

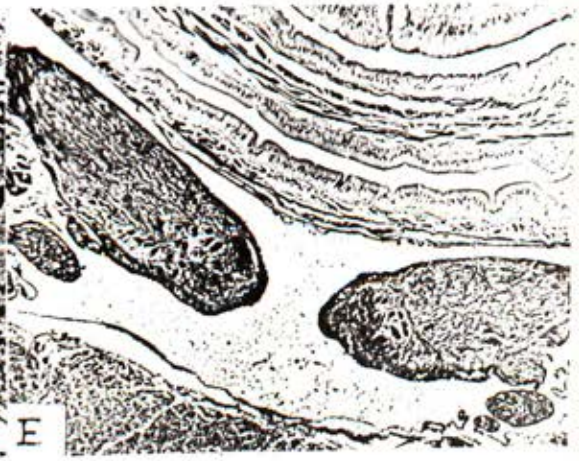
E. The lower ends of the cerebral commissures. Small ganglion cells are clearly visible in the medial part of each commissure. Branches are seen leaving the commissure at this level. Magnification 50 diameters.

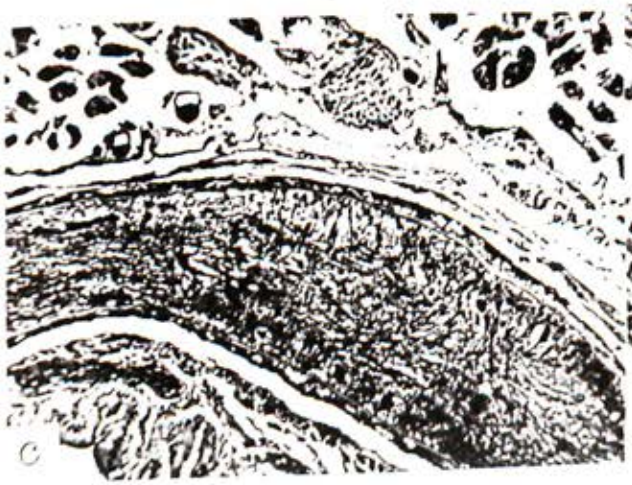
F. The ventral nerve cord at the site of commencement of the median giant fibre. On each side a branch leaves the nerve cord. The fibres in the branch end medially in the vicinity of the small ganglion cells in the dorsal part of the nerve cord. From these cells, fibres run medially through the neuropil to the deeply situated median giant fibre. Magnification 50 diameters.











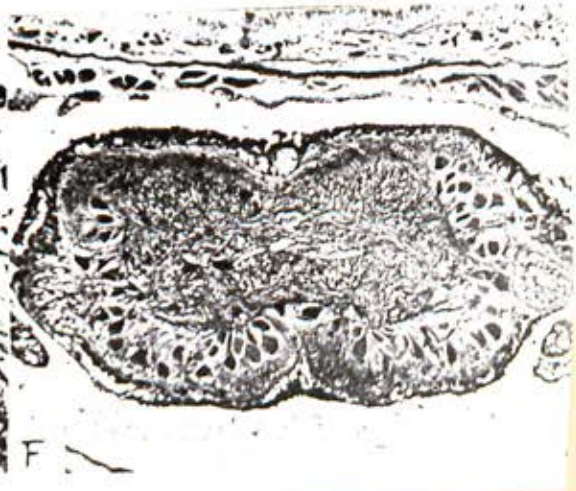


FIG. 1

Fig. 2.

A,B,C,D. Giant fibre responses to tactile stimulation. Time traces in these, and all subsequent records - 50 cycles/sec.

E,F. Giant fibre responses picked up from the posterior part of the cord. Fig. E. shows impulses of large amplitude and long duration conducted by the lateral giant fibres, and produced by touching the body behind segment 60. Fig. F. shows the smaller impulses in the median giant fibre from stimulation anterior to segment 60. Both median and lateral responses were picked up from the same point on the nerve cord, and were produced within a few seconds of one another without alteration in amplifier gain or electrode position.

G,H. Needling of the median giant fibre. In Fig. H. the median giant fibre has been damaged. The dorsal smooth-muscle integument of the nerve cord has been disrupted. The sheath of the median giant fibre is fragmented. Fig. G. shows a portion of the adjacent normal nerve cord.

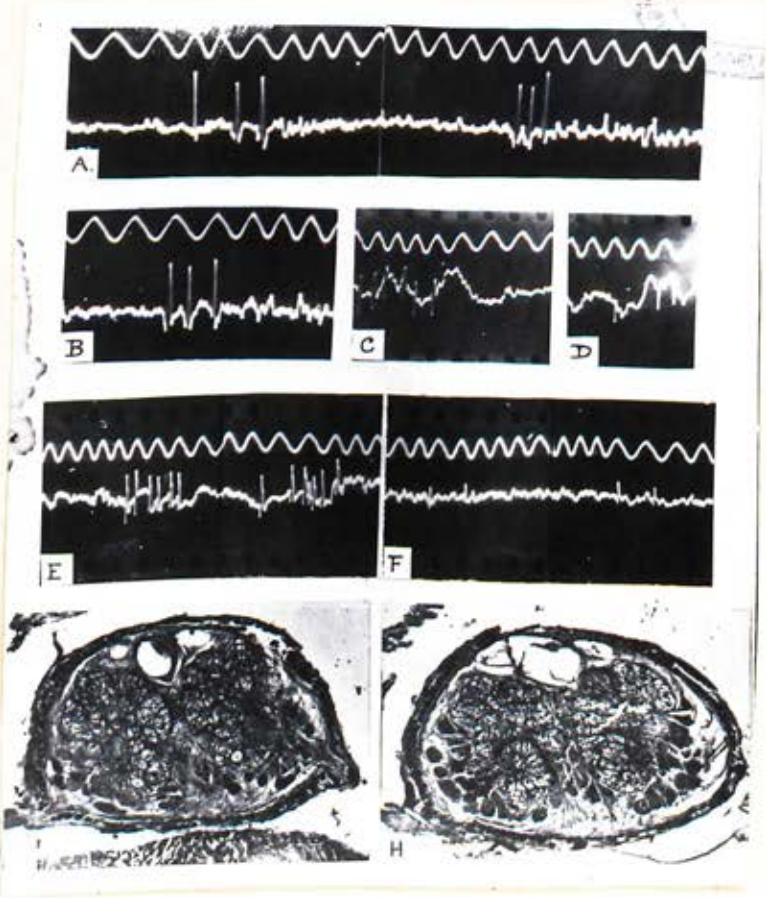


FIG. 2.

Fig. 3.

Needling of the median giant fibre. When this was damaged at segment 94, no impulses from the anterior 65 segments could be obtained behind the point of damage. At points immediately anterior to the damaged region, impulses could be recorded from both the anterior and posterior ends of the worm, those from behind have traversed the damaged region in the lateral giant fibres.

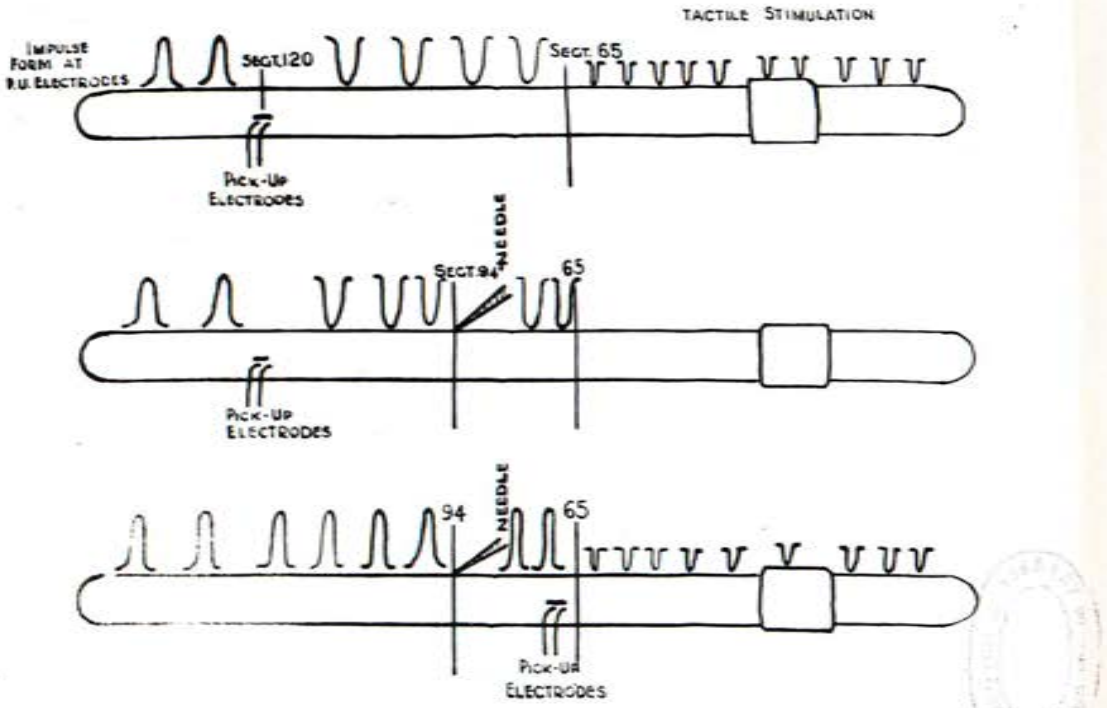

TACTILE STIMULATION
IMPULSE FORM AT P.U. ELECTRODES
SEGT.120
SEGT. 65
PICK-UP ELECTRODES
NEEDLE
SEGT.94
65
PICK-UP ELECTRODES
NEEDLE
94
65
PICK-UP ELECTRODES

FIG. 3.

Fig. 4.

Electrical stimulation of the nerve cord, using a triggered cathode ray sweep. The shock applied to the nerve cord also starts the trace moving from left to right. Two impulses are visible in each trace. The first is carried by the median, and the second by the two lateral giant fibres. The first impulse is frequently smaller than the second. Since the path length was known, velocity could be estimated. Time marker 50 cycles/sec.

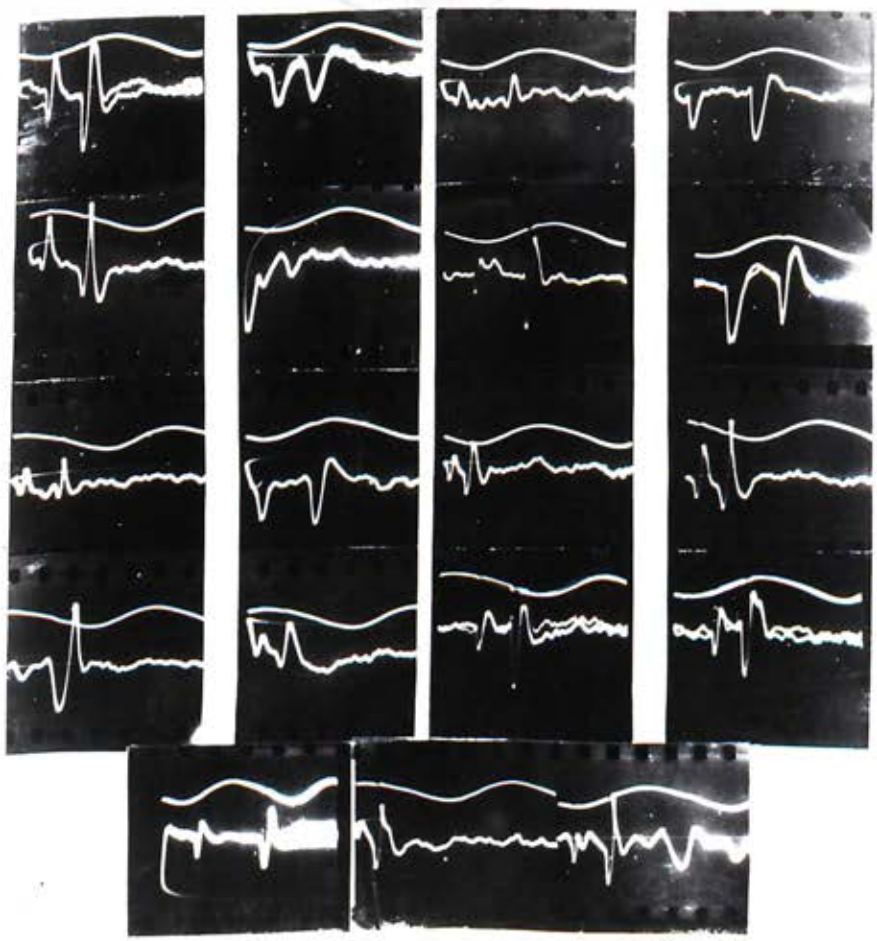


FIG. 4.

Fig. 5.

Relative conduction velocities in the giant fibres in different parts of the same worm. The median giant fibre conducts fastest in the middle, more slowly posteriorly, and slowest at the anterior end of the worm. The lateral fibres conduct progressively more slowly from the posterior end of the worm.

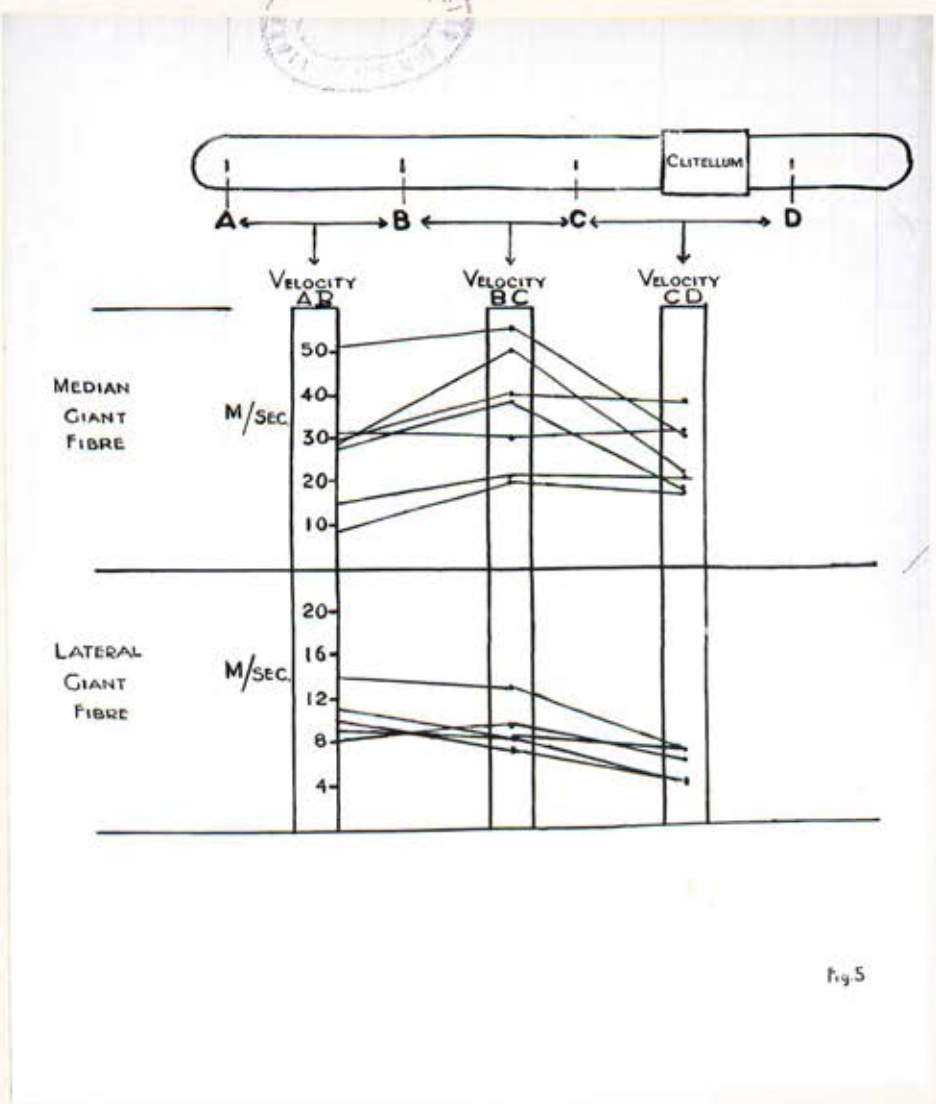

CLITELLUM
A
B
C
D
VELOCITY AB
VELOCITY BC
VELOCITY CD
MEDIAN GIANT FIBRE
M/SEC.
50
40
30
20
10
LATERAL GIANT FIBRE
M/SEC.
20
16
12
8
4

Fig. 5

FIG. 5.

Fig. 6.

Graph showing the relationship between conduction velocity in the median giant fibre and the length of the worm. Velocity in the middle part of the worm (i.e. the fastest part of its course) was used in this determination. Worm length was estimated while the worm was progressing steadily across a smooth table.

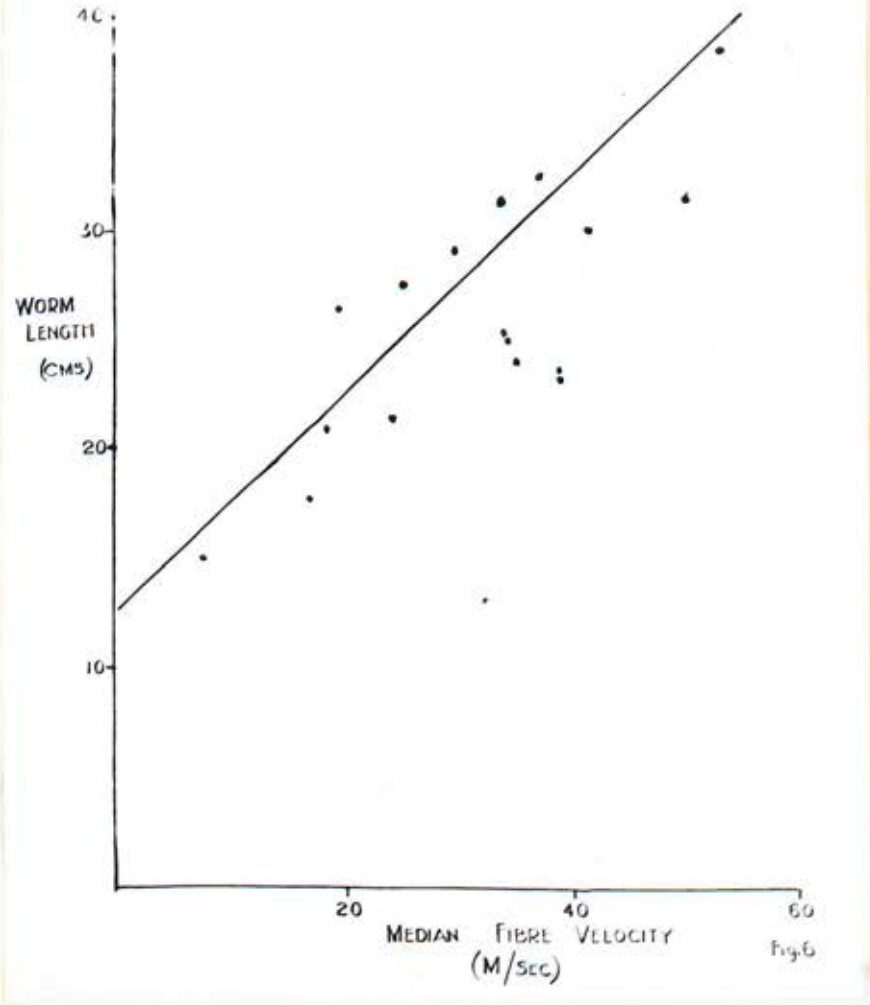

40
30
20
10
WORM LENGTH (CMS)
20
40
60
MEDIAN FIBRE VELOCITY (M/SEC)
Fig.6

FIG. 6.

Fig. 7.

The relationship between conduction velocity and fibre diameter. Fibre diameter was determined in stained preparations. The same relationship holds in both median and lateral giant fibres. The fibre diameter was estimated at intervals along the conduction path, and a mean figure obtained.

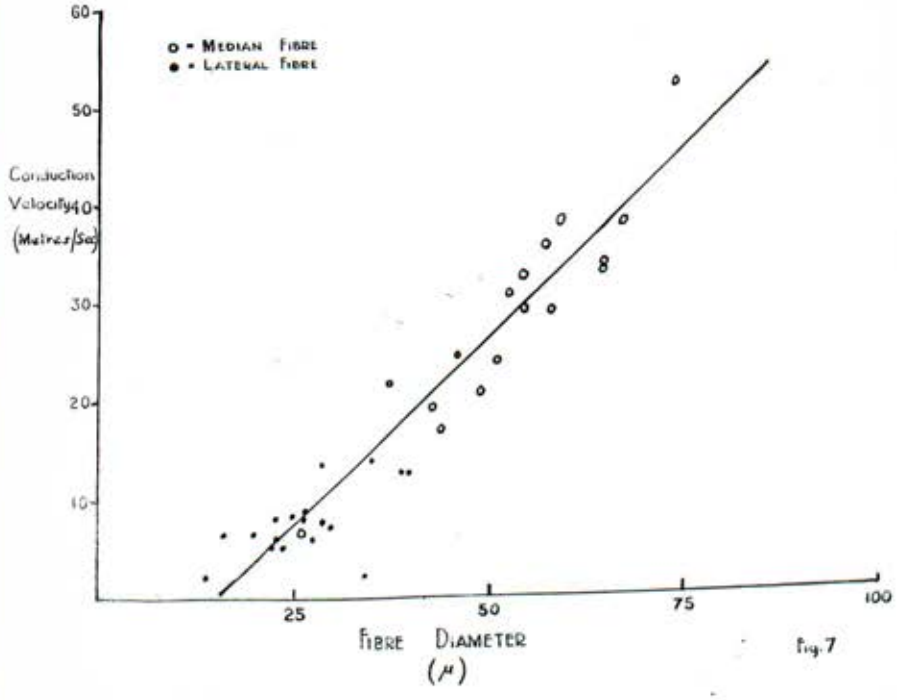

o = Median Fibre
• = Lateral Fibre
Conduction Velocity (Metres/Sec)
60
50
40
30
20
10
25
50
75
100
Fibre Diameter (μ)

Fig. 7

Fig. 7.

Fig. 8.

Estimation of degree of shrinkage in the stained preparations. A portion of the nerve cord was examined by frozen sections in the fresh state and the diameter of the giant fibres determined. The remainder of the same piece of nerve cord was then fixed and stained in the usual way, and the diameter estimated in the stained material. Shrinkage averaged 10% and was not differential between 20 and 75 mu.

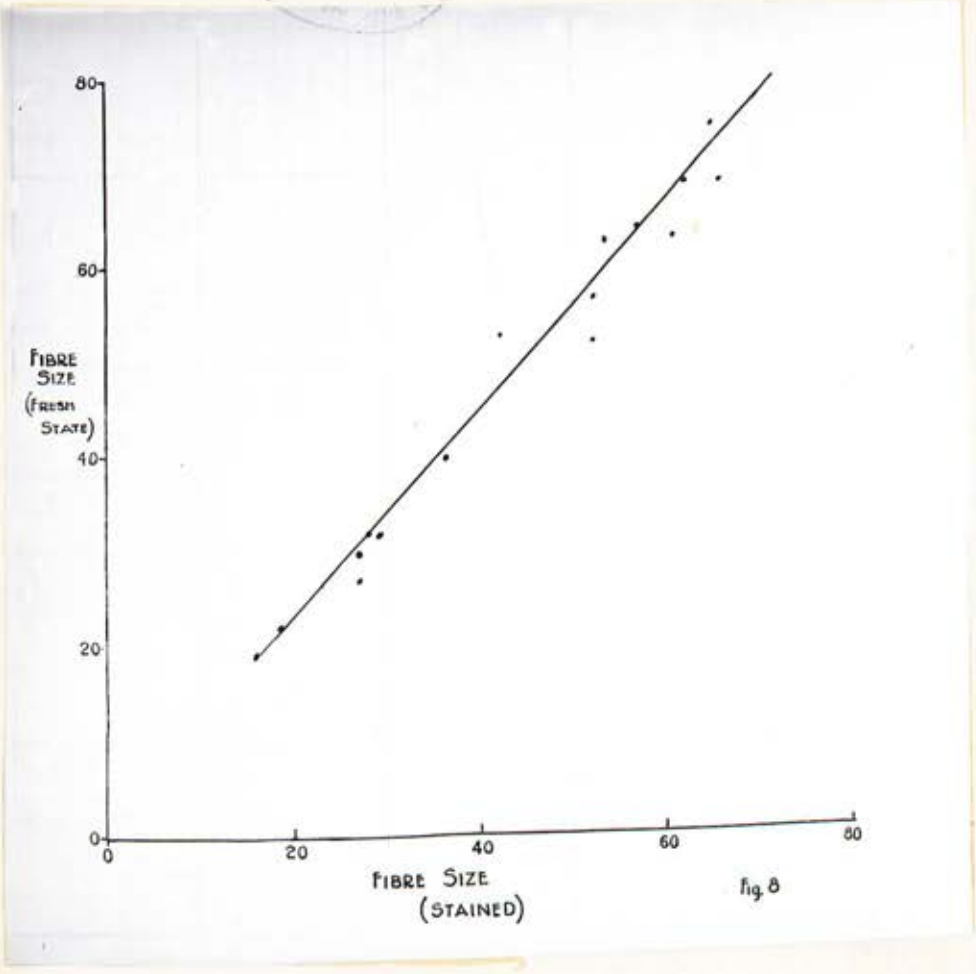

80
60
40
20
0
0
20
40
60
80
FIBRE SIZE (FRESH STATE)
FIBRE SIZE (STAINED)
fig. 8

FIG. 8.

Fig. 9.

The sheath thickness was estimated in stained sections and plotted against fibre diameter. The sheath is approximately a linear function of the diameter for fibres between 30 and 70 mu in diameter, and occupies approximately 5% of the fibre diameter. In the 20-30 mu fibres the sheath thickness diverges somewhat from this linear correlation, the sheath being somewhat thinner than would be expected to fulfil the above relationship. However, as the sheath is less than 1 mu thick in these fibres, very considerable errors inevitably occur in the measurement.

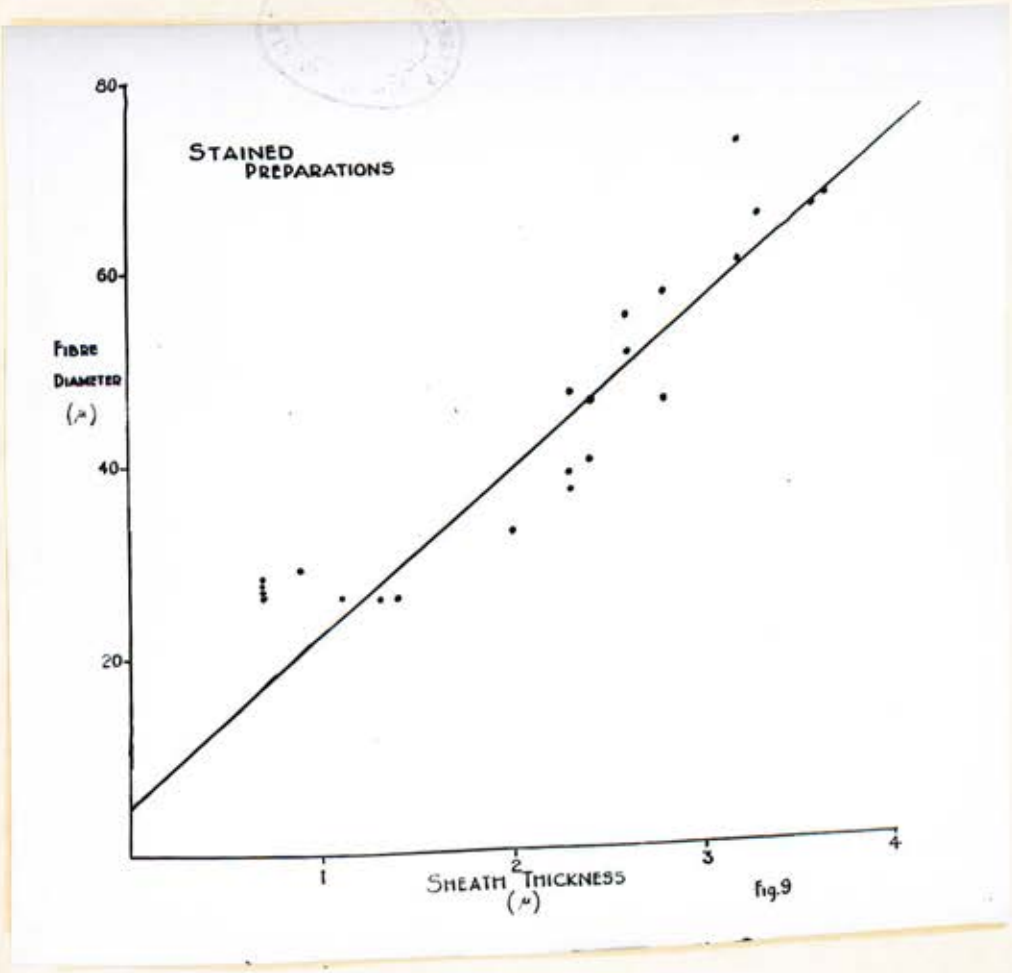

STAINED
PREPARATIONS
80
60
40
20
FIBRE
DIAMETER
(μ)
1
2
3
4
SHEATH THICKNESS
(μ)
Fig.9

FIG. 9.

Fig. 10.

The sheath dimensions were determined in the fresh state by suspending a single teased giant fibre in normal saline, and examining the fibre by polarized light (Fig. 12D). The sheath thickness is a linear function of fibre diameter and averages 6.0% of the fibre diameter.

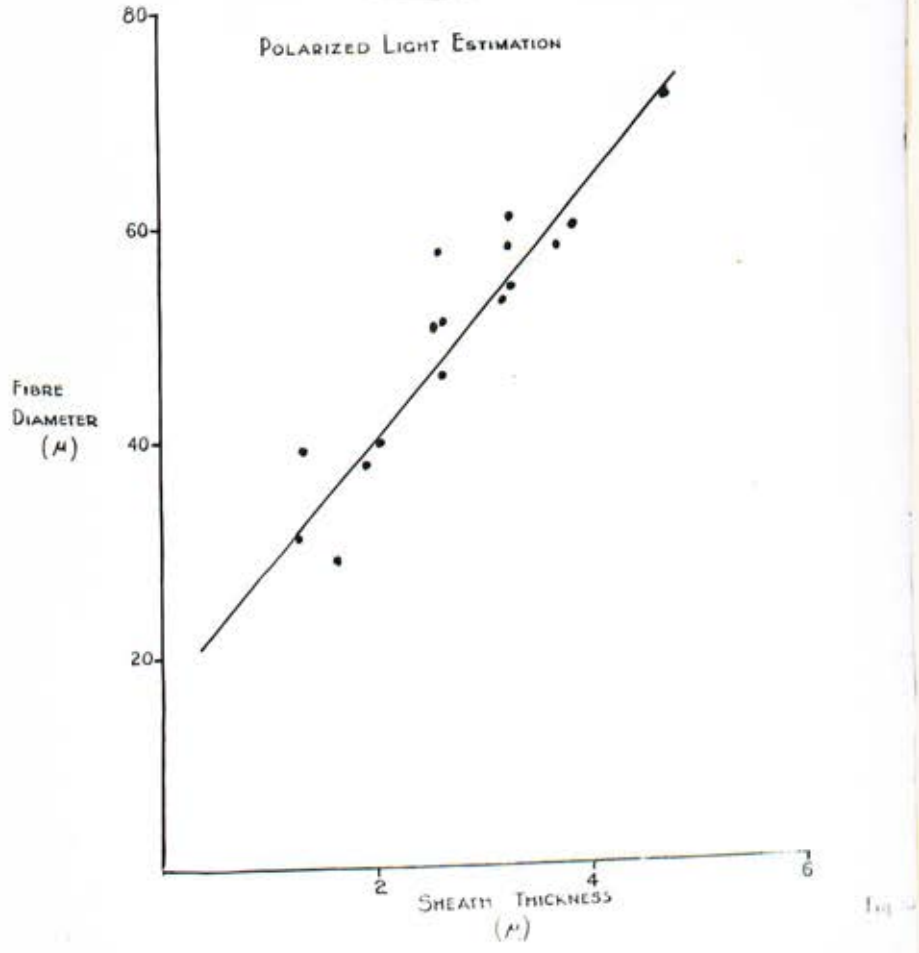

POLARIZED LIGHT ESTIMATION
80
60
40
20
FIBRE DIAMETER (μ)
2
4
6
SHEATH THICKNESS (μ)

FIG. 10.

Fig. 11.

Arrangement of ganglion cells.

A. Typical transverse section of the nerve cord. Magnification 75 diameters.

B. Ventral giant cells, with small nuclei and dappled cytoplasm, discharging towards the giant fibre. The axons of group III of the ganglion cells can be seen decussating and then running dorsally to the opposite lateral giant fibre. Magnification 225 diameters.

C. Ventrally and to the left of the midline, a giant cell can be seen with its axon running dorso-laterally into the neuropil. The axon then turns medially and crosses the midline to enter the opposite lateral giant fibre. To the right of the midline, ganglion cells of group II occupy a similar position to the giant cell on the left. The axons of these cells can be seen sweeping in an arc dorso-laterally and then medially to cross the midline. Magnification 150 diameters.

D. A ventral giant cell discharging dorsally in to median giant fibre. A transverse section anterior to the transition point. (Compare with Fig. C. which is of the cord behind the transition point). Magnification 87½ diameters.

E. Cells of group III disposed in a radial cluster at the edge of the neuropil sending their axons medially to decussate and enter the opposite lateral giant fibre. Ventrally and to the right of the midline, cells of group II can be seen with their axons sweeping out into the neuropil. Magnification 85 diameters.

F. Axons of cells of group III passing medially through the neuropil. Magnification 150 diameters.

G. The numerous cells of group IV at the edge of the neuropil. Their short axons enter the neuropil and in the majority cannot be traced further. In a few cases the axons appear the giant fibres. Magnification 75 diameters.

H. A longitudinal section of a ganglion near the median plane. To the left at the posterior end of the ganglion, a large ventral giant cell can be seen. Anterior to it, the slightly smaller cells of group II are visible. In front of them are the numerous small darkly staining cells of group IV. Dorsally is one of the lateral giant fibres. Magnification 85 diameters.

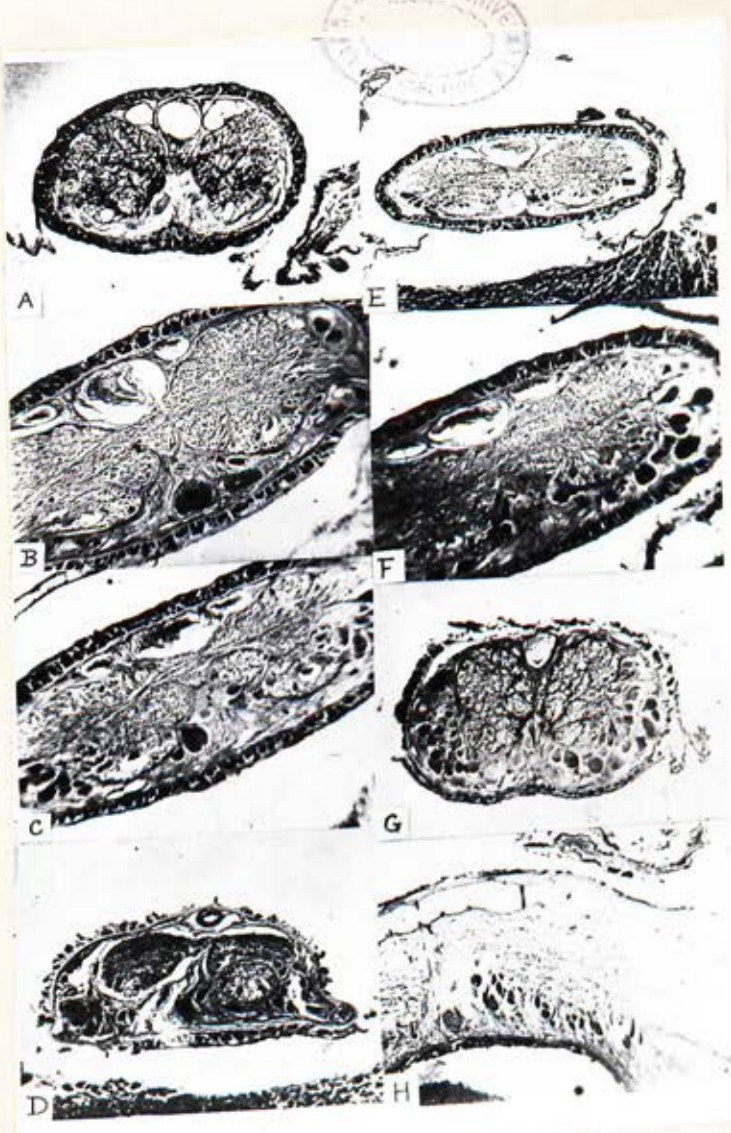


FIG. 11.

Fig. 12.

A,B. Neurofibrillae within the giant fibre. The fibril is central in position and runs a tortuous course. Magnification 730 diameters.

C. Portion of a giant fibre in the region of a segmental septum which appears as a dark line running almost transversely across the picture. Dorsal to the septum, a fibril can be seen approaching it and breaking up into a tangled skein against the septum. Magnification 730 diameters.

D. Teased preparation of a single giant fibre suspended in normal saline, showing sheath dimensions by polarized light.
Magnification 580 diameters.

FIG. 12.

Fig. 13.

A,B,C. Interconnections between the giant fibres. Successive sections of the nerve cord anterior to the clitellum. Fig. A. shows the lateral giant fibres normally disposed around the median giant fibres. In Fig. B. the left lateral giant fibre is approaching the median giant fibre. In Fig. C. it appears to have completely fused with it. Magnification 80 diameters.

D. Impulses in the giant fibres at points anterior to the clitellum produced by shock stimulation of the posterior part of the cord. The impulses are small (50-100 microvolts) and the spike form is very similar in the median and lateral giant responses. This close similarity is not seen at points behind the clitellum.

E. A comparison of a single shock to the nerve cord with the effects of repeated stimulation at 50/sec. for several minutes. The median giant fibre has ceased to conduct in the right-hand tracing. The lateral giant fibres are fatiguing, the impulse arriving gradually later in time, but unaltered in form.

F. (Left). Fatigue in the lateral giant fibres.
(Right). Fatigue in the median giant fibre.

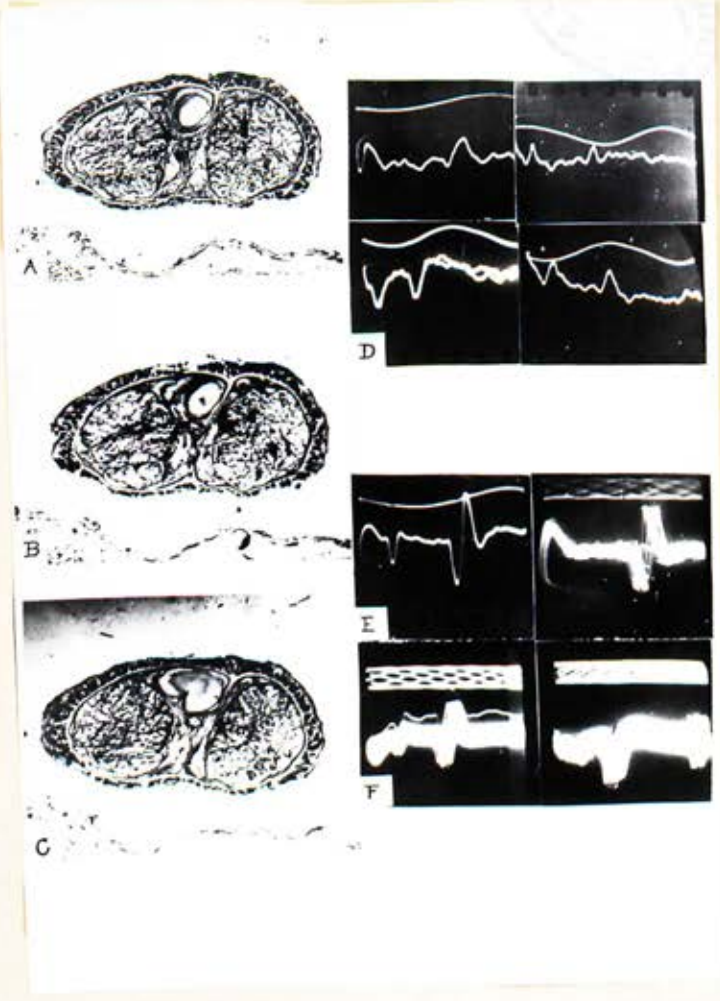
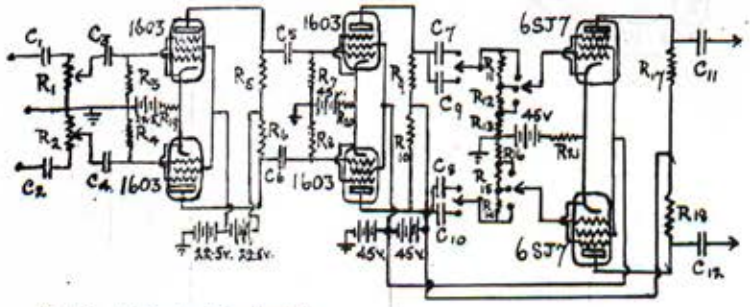


FIG. 13.

Fig. 14.

The battery-operated amplifier used as the initial amplifier of impulses from the nerve cord. This amplifier feeds the A.C.-operated amplifier and monitor speaker of Fig. 15. There is a choice of coupling condensers between the second and third stages. Sensitivity is controlled by the potentiometers at the input as well as by the attenuator between stages two and three.



R_1, R_2 : 1·0 Megohm potentiometers.

$R_3, R_4, R_5, R_6, R_7, R_8, R_9, R_{10}, R_{17}, R_{18}$: 0·5 Megohm carbon resistors, ½ watt.

R_{11}, R_{14} : 0·25 Megohm carbon resistors, 1 watt.

R_{12}, R_{15} : 0·1 Megohm carbon resistors, 1 watt.

R_{13}, R_{16} : 0·01 Megohm carbon resistors, 1 watt.

R_{19} : 0·1 Megohm carbon-resistor, 1 watt.

R_{20}, R_{21} : 0·2 Megohm carbon resistor, 1 watt.

C_1, C_2 : 0·1 or 1·0 mfd. paper condensers, 600v. D.C.W.

$C_3, C_4, C_5, C_6, C_7, C_8, C_{11}, C_{12}$: 4 mfd., 600 v. D.C.W, condensers (oil-filled)

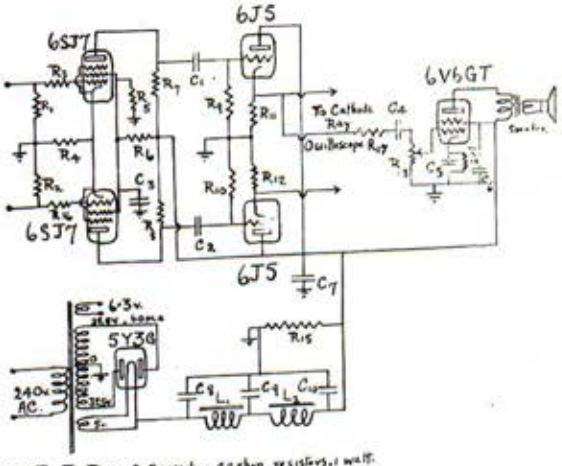
C_9, C_{10} : 0·1 mfd. paper condensers, 600 v. D.C.W.

BATTERY-OPERATED AMPLIFIER.

FIG. 14.

Fig. 15.

The final amplifier used to drive the monitor loudspeaker and the oscilloscope. The oscilloscope is fed from 6J5 cathode-followers, and the loud-speaker from a type 6V6GT valve.



R_1, R_2, R_9, R_{10} : 0·5 megohm carbon resistors, 1 watt.
R_3, R_{11}, R_{17} : 1·0 megohm carbon resistors, 1 watt.
R_4 : 2000 Ω, 1 watt wire-wound resistor.
R_7, R_8 : 200,000 Ω carbon resistors, 1 watt.
R_6 : 0·1 megohm carbon resistor, 1 watt.
R_5 : 0·01 megohm carbon resistor, 1 watt.
R_{13}, R_{16} : 3000 Ω 1 watt wire-wound resistors.
R_{18} : 1·0 megohm carbon potentiometer.
R_{14} : 400 Ω wire-wound resistor, 2 watt.
R_{15} : 25000 Ω voltage divider, 15 watt.
$C_3, C_4, C_7, C_8, C_{10}$: 8 mfd. electrolytic condensers, 600 v. peak.
C_1, C_2 : 2 mfd. 600 v. D.C.W. condensers (oil-filled)
C_4 : 0·5 mfd. paper condenser, 600 v. D.C.W.
C_5 : 50 mfd. electrolytic condensers 25 v. D.C.W.
L_1 : 15 henry, 75 ma. filter choke.
L_2 : 30 henry, 75 ma. filter choke.

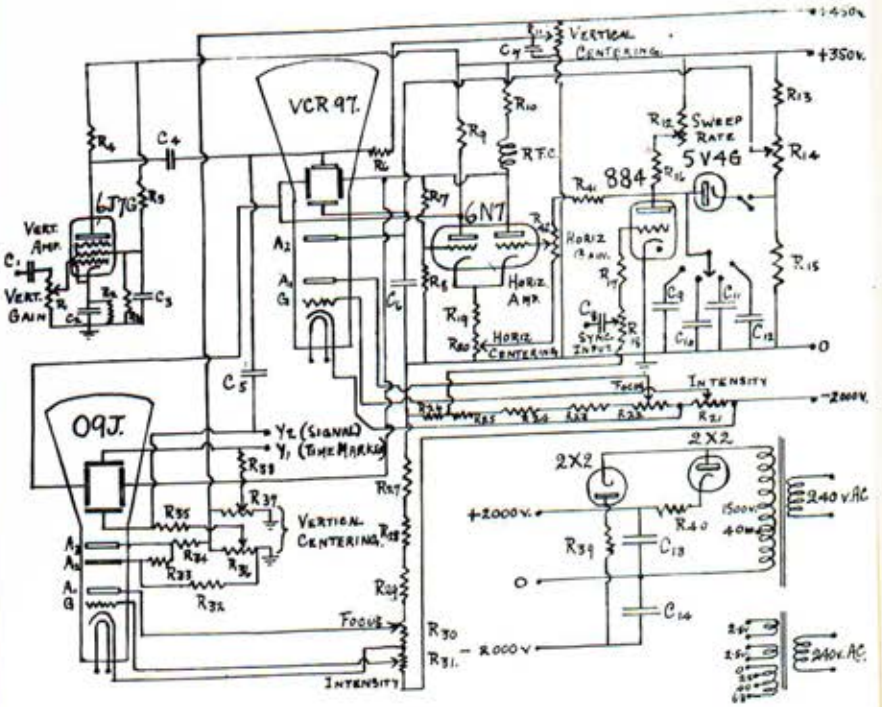
A.C.-Operated Amplifier.

FIG. 15.

R1.: 0.5 megohm potentiometer,
R2.: 1000 ohm wire- wound resistor.
R3.: 0.01 megohm resistor,1 watt.
R4.: 0.20 megohm resistor,1 watt.
R5.: 0.06 megohm resistor,2 watts.
R6.: 1.0 megohm resistor,1watt.
R7.: 2.0 megohm resistor,1 watt.
R8.: 0.06 megohm resistor,1 watt.
R9.: 0.08 megohm resistor,1 watt.
R10.: 0.08 megohm resistor,1 watt.
R11.: 2.0 megohm potentiometer.
R12.: 2.0 megohm potentiometer.
R13.: 0.1 megohm resistor, 1 watt.
R14.: 0.1 megohm potentiometer.
R15.: 0.1 megohm resistor, 1 watt.
R16.: 0.1 megohm resistor, 1 watt.
R17.: 0.01 megohm resistor,1 watt.
R18.: 0.1 megohm potentiometer.
R19.: 750 ohm wire wound resistor.
R20.: 5000 ohm wire wound potentiometer.
R21.: 0.05 megohm potentiometer.
R22.: 0.25 megohm potentiometer.
R23.: 0.25 megohm resistor, 1 watt.
R24.: 0.5 megohm resistor, 1 watt.
R25.: 0.5 megohm resistor, 1 watt.
R26.: 0.04 megohm resistor, 1 watt.
R27.: 0.50 megohm resistor, 1 watt.
R28.: 0.5 megohm resistor, 1 watt.
R29.: 0.5 megohm resistor, 1 watt.
R30.: 0.25 megohm potentiometer.
R31.: 0.05 megohm potentiometer.
R32.: 0.25 megohm resistor, 1 watt.
R33.: 0.04 megohm resistor, 1 watt.
R34.: 0.5 megohm resistor, 1 watt.
R35.: 1.0 megohm resistor, 1 watt.
R36.: 2.0 megohm potentiometer.
R37.: 2.0 megohm potentiometer.
R38.: 1.0 megohm resistor, 1 watt.
R39.: 0.05 megohm resistor, 1 watt.
R40.: 0.05 megohm resistor, 1 watt.
R41.: 6.0 megohm resistor, 1 watt.
R42.: 0.5 megohm potentiometer.

C1.: 0.5 mfd. paper condenser.
C2.: 50 mfd. electrolytic condenser, 25 volt working.
C3.: 8 mfd. electrolytic condenser, 525 volt peak.
C4.: 0.25 mfd. paper condenser.
C5.: 0.25 mfd. paper condenser.
C6.: 8 mfd. electrolytic condenser, 525 volt peak.
C7.: 0.25 mfd. condenser (paper).
C8.: 0.1 mfd. paper condenser.
C9.: 0.004 mfd. paper condenser.
C10.: 0.03 mfd. paper condenser.
C11.: 0.25 mfd. paper condenser.
C12.: 4.0 mfd. paper condenser.
C13.: 1.0 mfd. oil-filled condenser, 5000 volt D.C.W.
C14.: 1.0 mfd. oil-filled condenser, 5000 volt D.C.W.

All paper condensers are 600 volt D.C. working unless otherwise specified.

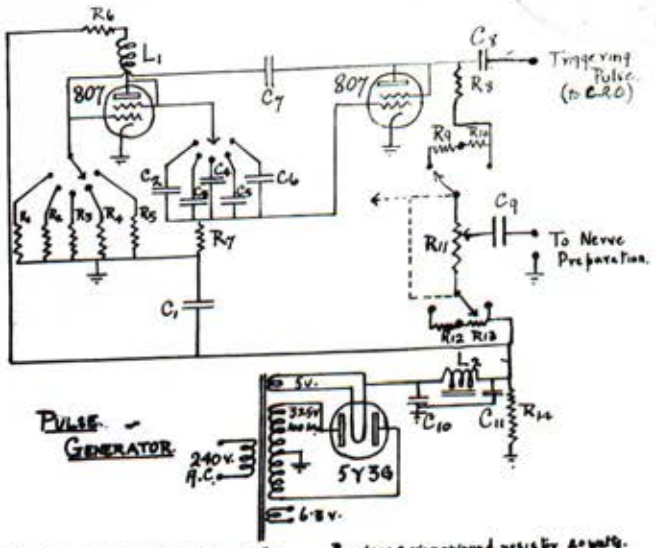


CATHODE-RAY OSCILLOSCOPE AND HIGH VOLTAGE POWER SUPPLY.

FIG. 16.

Fig. 17.

Pulse generator used in the velocity estimations and fatigue experiments. The wave form is rectangular and the pulse duration can be adjusted from 0.03 millisec. to 100 millisecs. The repetition rate can be varied from 50/sec. to 1/sec. The output is adjusted by means of an attenuator which incorporates a vernier potentiometer to adjust the stimulus strength over a narrow range. The output impedance is low and the alteration in pulse amplitude due to parallel loading by the nerve preparation is small.



R_1: 1·0 megohm carbon resistor, ½watt.
R_2: 0·1 megohm carbon resistor, ½watt.
R_3: 0·01 megohm carbon resistor, ½watt.
R_4: 1000Ω carbon resistor, 1 watt.
R_5: 250Ω carbon resistor, 1 watt.
R_6: 2500Ω Wire-wound, resistor, 20 watts.
R_7: 7·0 megohm carbon resistor, 1 watt.
L_1: V.H.F. R.F. choke. L_2: 20 henrys, 30 m/a.
R_8: 1000Ω wire-wound resistor 20 watts.
R_9, R_{10}, R_{12}, R_{13}: 50Ω W-W resistors, 5 watts.
R_{11}: 50Ω Wire-wound potentiometer.
R_{14}: 15000Ω voltage divider, 20 watts.
C_9, C_{10}, C_{11}: 8 mfd. electrolytic condensers, 500 volt peak.
C_2: 0·1 mfd. C_3: 0·02 mfd. C_4: 0·01 mfd.
C_5: 0·006 mfd. C_6: 0·001 mfd. C_8: 0·1 mfd.
C_9: 0·5 mfd. (All 600 v. D.C.W. paper)

FIG. 17.

Fig. 18.

General arrangement of the nervous system in Megascolex.

The anterior section at the level of the cerebral ganglia indicates the main cell groups in these ganglia. The two principal nuclei are the dorsal and the antero-ventral. The cells in each nucleus are larger in the medial parts of the nucleus. The main efferent pathway is from the medial cells of the dorsal nucleus whose axons cross the midline to leave by the opposite cerebral commissure. From the medial side of each commissure a number of branches arise, forming the stomatogastric system. The dorsal ones enter the typhlosole.

The middle section shows the typical arrangement of a ganglion in the ventral nerve cord in the anterior 60 segments. Here the ventral giant cells discharge dorsally into the median giant fibre. Group II cells also send their axons dorsally into the median fibre. They are joined near the median giant fibre by the axons of the laterally-placed cells of group III.

The posterior section shows the arrangement behind the 60th segment. The ventral giant cells and the cells of group II pass in an arching fashion to the opposite lateral giant fibre. Group III cells also send their axons to the opposite lateral giant fibre.

In both anterior and posterior sections the numerous cells of group IV are placed at the edge of the neuropil mass, into which they send their short axons.

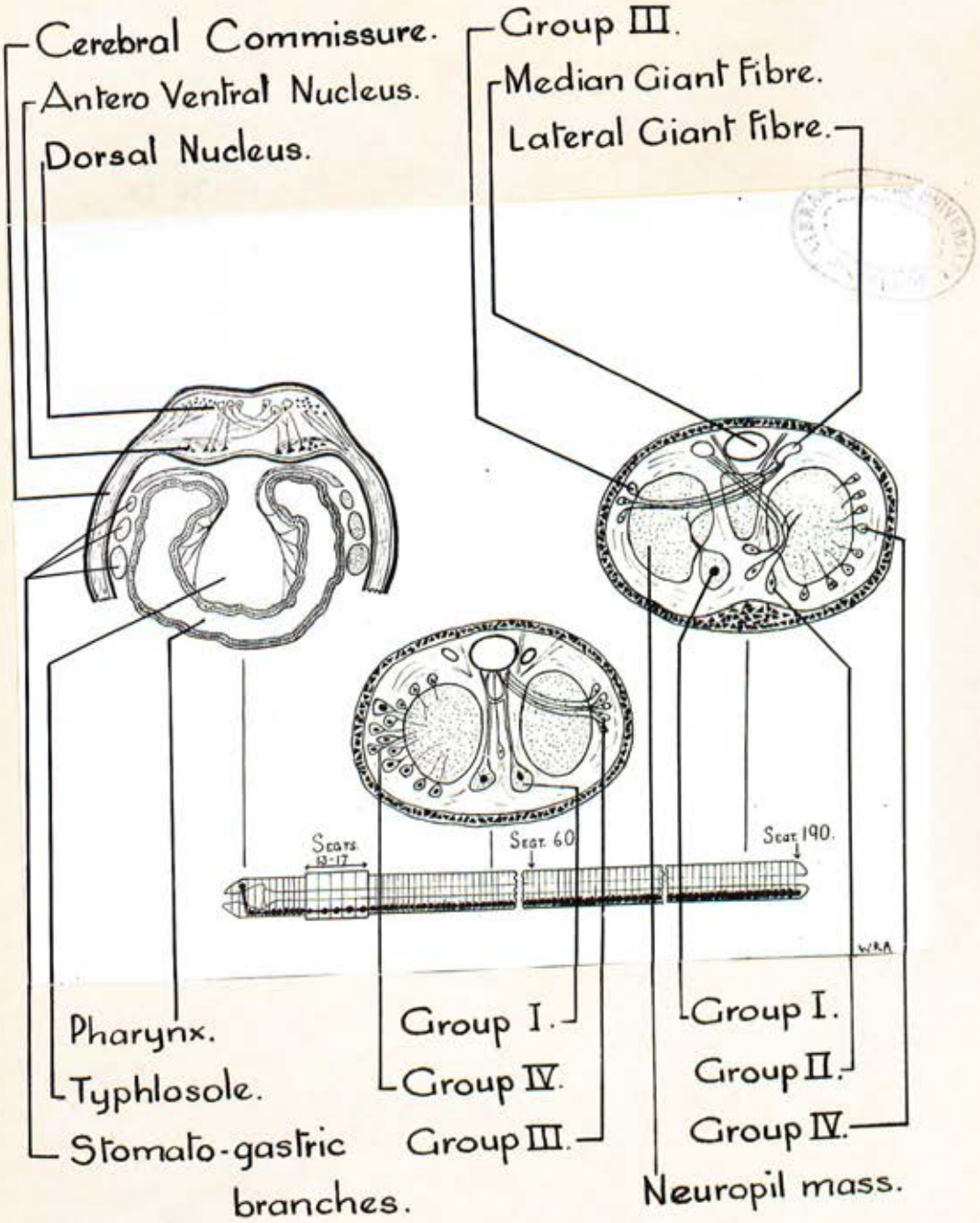

Cerebral Commissure.
Antero Ventral Nucleus.
Dorsal Nucleus.
Group III.
Median Giant Fibre.
Lateral Giant Fibre.
Segs. 13-17
Seg. 60
Seg. 190.
W.R.A.
Pharynx.
Typhlosole.
Stomato-gastric branches.
Group I.
Group IV.
Group III.
Group I.
Group II.
Group IV.
Neuropil mass.

FIG. 18.