VAGAL AFFERENT ACTIVITY IN THE NODOSE GANGLION.

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INTRODUCTION

The purpose of this series of experiments was to investigate the possibility of studying impulses from various single afferent fibres already described in the vagus nerve, by inserting micro-electrodes into the nodose ganglion.

The method used hitherto - (originally by Adrian - 1933,) was to dissect out strands of the nerve until a bundle containing only one active fibre was left, from which the recordings were made. This is laborious and it requires much care and patience to preserve the nerve fibres in a good condition. On the other hand, since the electrode is relatively far away from the active fibre, the tissues short-circuit most of the currents set up, and the spikes usually obtained are comparatively small (c. 50 microvolts).

Micro-electrodes have been much used in recent years to study the activity of nervous tissue (e.g. Lorente de No, 1939, 1947; Adrian and Moruzzi, 1939 Renshaw, Forbes and Morrison, 1940; O'Leary and Bishop, 1939; Therman, Forbes and Galambos, 1940; Parrack, 1942; Brooks and Eccles, 1947; Brookhart, Moruzzi and Snider, 1950). The value of micro-electrodes and the problem of interpreting results obtained by their means, are considered from the point of view of the potential theory by Lorente de No, 1939, 1947, and by Renshaw, Forbes and Morrison,

1940. Two of the conclusions reached by the latter are especially relevant:-

a) The difference in potential between any point "n" near an active cell and a distant point (which is at approximately average or zero potential) decreases very rapidly as the distance of "n" from the cell increases.

b) The potential at a point in a conducting medium due to activity in portions of a cell which is at a distant part of the medium, is small (approaching or actually zero as the distance increases). Therefore an electrode remote from a nerve centre may be considered indifferent with regard to the electrical activity in that centre. From this, it follows that, micro-electrodes may be able to record in a localized way, and that large potential differences may be observed.

The experiments of Renshaw et al., in which they used glass micro-electrodes (15-40 microns) to study activity in the isocortex and the hippocampus, confirmed their expectations. They point out that the signal to noise ratio is inversely related to the resistance of the electrodes. Their own electrodes often had a resistance of over 1 megohm. They required a correspondingly high input grid leak resistance (4-5 megohms).

As far as could be ascertained from the

available literature, the nodose ganglion has not been the object of any such investigations. Microelectrodes have been inserted into the superior cervical ganglion (Therman, Forbes and Galamboos, 1940). These were smaller glass electrodes than those previously mentioned, some having tips as fine as 5-10 μ, and they were well able to show the responses of single fibres. The spinal ganglia have been investigated by this technique (Parrack, 1942). In both cases, the experiments differed from the present series in that preganglionic electrical stimuli were employed to provide regular responses which could then be studied in detail.

A number of afferent impulses from single vagal fibres have been described. The best known are the pulmonary stretch impulses first recorded by Adrian, (1933). They are produced by stretching of the lung tissues during inspiration or artificial inflation, and the frequency of discharge is directly proportional to the volume of the lung for moderate degrees of inflation. They are characteristically slow adapting and so resistant to asphyxia that they may be heard for as long as one hour after the heart has stopped. Some may be firing more or less continually, and others have, in addition, a cardiac rhythm superimposed on the respiratory rhythm. It has been suggested recently (Weidmann et al., 1949) that most of the stretch receptors are actually in or very near the pleura.

For a long time there has been a great deal of controversy about the mechanism of respiration. Are the stretch fibres recorded by Adrian the only afferent system regulating respiration ? Adrian himself described other relatively slow adapting fibres which discharge in response to suction from the lungs, both in rabbits and cats. Some of these were ordinary stretch fibres, but others, he claimed, were distinct and only responded to deflation. However, he did not think they played any part in normal respiration. Hammouda and Wilson (1932) found that rapid forced deflation increased the rate of respiration, but not after bilateral vagotomy. Creed and Hertz (1933) stated that small deflation caused increase, and a large one decrease, of the rate. They suggested that different fibres may be involved. In this connection, it may be recalled that Head (1889) was rather astonished to find that, during gradual recovery from freezing of the vagus, for a certain period of time artificial inflation has an inspiratory effect.

Hammouda and Wilson (1935) showed that two sets of pulmonary afferent fibres are present in the vagus. One set inhibits respiration; it does not conduct at temperatures below 8°C. The other is accelerator, and its conductivity is only abolished near 0°C. Both are stimulated by positive inflation.

Their conclusions were challenged by Partridge,

(1939). She identified fibres in the vagus which discharged with respiratory and cardiac rhythms, and found that the former were silenced at 8°C., the latter only at 4°C. Acceleration of breathing was only obtained (with the vagus at 8°C.) if the cardiac and carotid sinus nerves were intact. She thought, therefore, that the stimulation of respiration is the result of activity of the carotid sinus - cardiac depressor mechanism.

In two further papers, Hammouda, Samaan and Wilson, (1943); Wilson and Samaan, (1947) reaffirmed their opinion, and gave more evidence, that the respiratory accelerator fibres of the vagus come from intra-pulmonary nerve endings.

Worzniak and Gesell (1939) found that suitably timed inflation during inspiration increases the activity of inspiratory muscles. More recently Knowlton and Larrabee, (1946) studied the electrical activity of single stretch fibres in the cervical vagus. They recorded, in addition to the well known slow adapting activity, discharges from stretch endings which were fast adapting, and had a rather higher threshold. Some fibres from both sets, but mostly from those fast adapting, also responded to deflation, but no fibres were found which discharged only on deflation. They suggest that slowly adapting endings inhibit inspiration while those that are fast adapting excite it. The main function of

the latter would be to reinforce inspiration during deep respiration.

Adrian (1933) had noticed the presence of fibres in the cervical vagus which discharged with a cardiac rhythm. The frequency of discharge was directly related to the blood pressure, and he concluded that they were aortic depressor fibres. He did not record any other impulses with a cardiac rhythm, (except for some stretch fibres). It was not until quite recently that a discharge related to pressure changes in the great veins or auricles was first described. (Amann and Schaefer, 1943; Walsh and Whitteridge, 1944). Further papers confirmed the original findings (Walsh, 1947; Whitteridge, 1948). Jarisch and Zottermann (1948), and Dickinson (1950) used a new type of condenser manometer to record the venous and intra auricular pressure more accurately while studying venous impulses. These discharges correspond to the phases of the venous or right auricular pressure recordings and are affected by a change of venous pressure. They are produced by mechanical stimulation of the right auricle and the orifices of the great veins. According to Whitteridge (1948) the venous fibres are blocked at a slightly lower temperature (8 - 12°C.) than the stretch fibres (12 - 16°C.).

Impulses from the left auricle or the pulmonary veins can be distinguished by their relative independence of the effective venous pressure

(Whitteridge, 1948) and by mechanical stimulation in the left auricle (Jarisch and Zottermann, 1948).

A discharge which originates in right intraventricular pressure receptors was reported by Whitteridge (1948) and Dickinson (1950). The impulses occur very early in systole, and one set was seen on one occasion when the aortic valves failed to open. Artificial inflation seems to increase their discharge.

The aortic depressor impulses recorded by Adrian (1933), Partridge (1939), and by most of the authors quoted above, are relatively late in systole, and closely related to the systemic blood pressure.

A final type of vagal afferent fibre has been described fully by Whitteridge and his co-workers (Walsh and Whitteridge, 1944; Walsh, 1947; Whitteridge, 1948). This is thought to arise from nerve endings in the pulmonary arterioles or capillaries. It discharges late in systole, is inhibited by artificial inflation of the lungs, but shows a great increase in activity after the lungs have been allowed to empty, at a time when the aortic pressure is still reduced. The impulses disappear at 3 - 4°C. and the duration of the spikes (0·58 msec.) is significantly greater than that of stretch impulses (0·36 msec.). Using a new method of recording pulmonary blood flow and pressure (Baxter and Pearce, 1950) it has been possible to show that activity of these fibres is directly related to changes in the

pulmonary blood pressure (Pearce, unpublished observations, 1951).

The pulmonary vascular fibres may solve the long-standing problem of respiratory reflexes from the lungs. It had been shown by Dunn (1919) that starch embolism in goats causes an increase in the rate of respiration, but not after bilateral vagotomy. Walsh, (1947), Torrance and Whitteridge (1947), and Whitteridge (1948) repeated the experiment on cats and found some evidence that embolism increased the activity of pulmonary vascular fibres. In a review of this subject, Whitteridge (1950) suggests that stimulation of these fibres may cause reflex vaso-constriction in the lungs. However, Daley et al (1951) claim that pulmonary hypertension following multiple embolism is caused entirely by mechanical obstruction.

METHOD

Anaesthesia

The cats were anaesthetized with ethyl chloride, ether and chloralose. The dose of chloralose was 80 mg./kg, enough to induce deep anaesthesia for over 12 hours. This keeps the blood pressure at a good level, but it is liable to cause trouble if the brand used is not a satisfactory one.

In several experiments 2 - 5c.c. 25% urethane were also given, to favour the dilation of small vessels supplying the ganglion. On two occasions,

however, this caused an immediate, but temporary, arrest of respiration.

The nine cats used varied in weight from 0·9 to 3·5 kg.

Dissection

The aim in dissection was, on the whole, to interfere as little as possible with the ganglion, the vagus, and their blood supply and venous drainage.

The arterial supply is maintained by a number of small branches of the internal carotid artery, which form a little plexus in relation to the superior cervical and nodose ganglia. This is also joined by some small branches of the external carotid artery. All these vessels are intermingled with fibrous connective tissue, and a venous plexus which is particularly evident deep to the ganglia. The venous drainage is through this system of veins, which is connected by a quite substantial vessel to vertebral veins. A large internal jugular vein was seen in one instance, on the right side.

There is much variation in the detailed arrangement of the blood vessels. Small vessels run over the surface of the nodose and sympathetic ganglia within the sheath of connective tissue which covers and binds them. One of these is usually more prominent than the others, and marks the line of separation between the two ganglia.

Much variation is also to be found in the

position and shape of the nodose ganglion. On the right side it is often more accessible as it is somewhat farther away from the mid-line, and at the medial side of the sympathetic ganglion, rather than deep (dorso-medial) to it, (- as is usual on the left side.). At this level, the two nerves turn medially and more deeply in the direction of the jugular foramen. They become separated from the artery and are twisted along their longitudinal axis. It is on the varying extent to which they are twisted that the accessibility of the nodose ganglion depends. It was found in one cat that the two ganglia on the left side were quite separate, and as much as an inch away from the base of the skull.

No satisfactory way was found of fixing the ganglion really firmly without undue traction upon the vagus and the blood vessels. The sheath was opened, before the insertion of micro-electrodes, to make penetration easier and prevent compression of the tissues.

On several occasions the vagus was cut or ligated proximally to the ganglion to reduce any efferent activity. To avoid damaging the nodose ganglion, or its blood supply, this was not done when it was found to be very near the base of the skull.

As soon as the dissection was completed the part was covered with liquid paraffin to prevent drying, and the cat was transferred to the special steam chamber.

Steam chamber

A box was designed and built, for these experiments, to provide a moist atmosphere and electrostatic shielding. It consisted of a brass framework with a Tufnol floor, and a covering of Windolite. The latter is a pliable, translucent material containing a wire mesh well insulated on both sides, but with a resistance equal to zero along its length, (as measured on a Taylor universal meter.). It is resistant to water and steam, is attacked by mineral acids, and is easily soluble in acetone. It can be soldered to brass fairly easily. The box was large enough to hold the head and the upper half of the trunk of a full grown cat. There was a glass window above, and a side opening to allow manipulation inside, as well as alternative apertures for the body of the animal.

A water trough and a controllable immersion heater generated steam as desired. The steam tended to accumulate in the upper reaches of the box, and so, for the parts to be kept moist, and at a temperature of approximately 37°C., a considerable amount of steam was required. The interior of the box was insulated from the framework and the wire mesh, which were earthed.

Head clamp. (see Fig. 1.)

It was essential that the head of the animal should be kept absolutely still to avoid mechanical

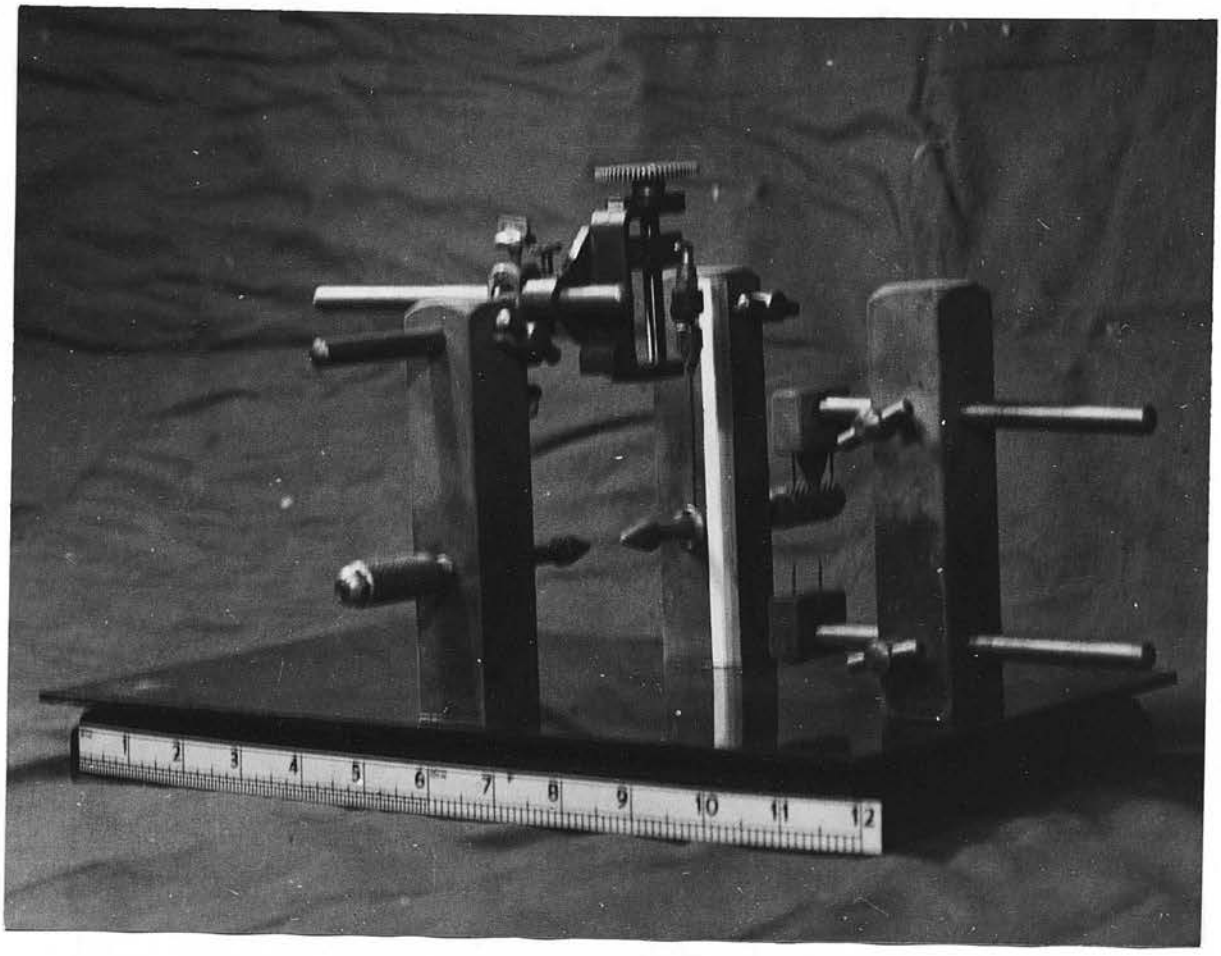

Fig.1. Photograph of head clamp, with micro manipulator, and a needle in position.

disturbances during recordings.

The special clamp was constructed on a Tufnol covered retort stand base, which, by its weight and rigidity, made for great stability. The main features of the clamp were as follows:-

a) Two lateral plugs which could be screwed into the cat's ears. They were mounted in stout bakelite pillars, and had a scale by which their movement was controlled.

b) A rostral bakelite pillar with two horizontal pieces firmly grasping the lower jaw and the nose respectively by means of gramophone needles.

c) Holes were made in the lateral pillars into which the micro-electrode holder was fixed. The electrode and the head were thus part of the same rigid system.

All parts made of bakelite were soaked in molten paraffin wax for several hours to ensure waterproofing.

Micro-manipulator

Micro-manipulation was only possible in the plane of penetration of the electrode. The moving part of the manipulator had a bakelite cone to fit the hypodermic needle bases on which the micro-electrodes were mounted.

One full turn of the screw gave a displacement of 600 microns. The wheel by which it was turned had 60 divisions, each of which was equivalent to 10 μ.

The path of a needle traced out on smoked paper

proved to be a straight line.

Micro-electrodes.

The method used in preparing the electrodes was derived from that described by Bishop and Collin (1950).

Steel embroidery needles were soldered on to the free bases of hypodermic syringe needles. Each needle was then sharpened by a process of electrolytic disintegration. It was connected to the positive terminal of a 2 volt battery, and dipped into a 10p.c. solution of concentrated hydrochloric acid. The anode consisted of a ring of thick copper wire. To enable the reaction to proceed more smoothly and more rapidly, the needle was washed under running tap water and wiped with a soft cloth after each dip. This removed the surface layer of chloride. The whole procedure was controlled by the use of a microscope with a suitable micrometer scale. It was quite easy to obtain sharp points of the order of 2 - 3 μ . The angle of taper behind the tip could be varied. A longer exposure to the action of the acid gave a very fine, long tip with a very slow taper. Such a tip, however, is extremely fragile. Before varnishing, the needles were degreased thoroughly by wiping them with cottonwool soaked in trichlorethylene.

Bakelite (L 3128) was found to be a sound insulating varnish. It can be used alone or with varying proportions of thinner (1/6 to 1/2 of thinner).

When it has been thinned down, the coating is easier to apply evenly without the formation of blobs, but more layers are necessary to give adequate insulation. The needles were dipped into the varnish and pulled out very slowly to get an even film all over. The very fine tip remains uncovered, and therefore un-insulated. The varnished needle was then baked in an oven at 180°C. for 4 - 5 minutes, depending upon the thickness of the coating solution. Three or four layers were applied in this way, according to the viscosity of the mixture. A final, hard outer coat was cured in paraffin oil at 200°C. for one minute. If the solution is rather thick, and the temperature too high, the varnish may boil, leaving a very rough surface which cannot be used. If the varnish is allowed to dry for a minute or two before curing, this accident is less likely to happen.

The impedance of the electrodes, and of the varnish, was tested by means of a sine wave oscillator, giving a note at about 1000 c.p.s., a pair of ear-phones and a Wheatstone bridge. The circuit was completed by the micro-electrode, a thin layer of saline on chloroform, and an indifferent electrode consisting of a similar needle, neither sharpened nor varnished. The mid-point of the bridge was earthed to increase the sensitivity, and capacity effects of the electrode could be balanced by a variable condenser connected across the corresponding arm of

the bridge. The resistance of the tip was found to be usually between 10,000 and 80,000 ohms, but occasionally it was as low as 5,000 - 6,000 ohms, or (once) as high as 120,000 ohms. The resistance of the varnish was usually over 4 megohms. At values over 5 megohms the bridge became very inefficient; in consequence no upper limit could be defined. It was clear, however, that a good insulation (over 2 megohms) could be expected over nearly the whole length of the needle (3 - 5 cm.). The insulation was often defective at the base where soldering made the surface irregular, but that was of no consequence in these experiments.

The extent to which the tip was left unvarnished was tested by mounting gold-leaf on paraffin wax, and driving the electrode with the micro-manipulator through it. The movement of the tip was observed under the microscope. Most of the needles tested only made electrical contact over a distance of much less than 10μ . Some, however, did so intermittently over 10 - 30μ , and a few were not evenly insulated over as much as 50μ or even more. There was no way of distinguishing the latter by microscopic examination.

The micro-electrodes were finally tested in the brains of frogs, a rabbit and a cat. In each of these, good recordings were made of injury discharges and spontaneous single unit activity. In addition,

definite axon-like spikes were picked up from afferent systems in the mid-brain of the cat. In the experiment (14.2.51) done on a cat, anaesthetized with chloralose, the micro-electrode was inserted into the mid-brain ($3\frac{1}{2}$ mm. from mid-line, 12 mm. behind bregma). Very good axon-like spikes were obtained at a depth of 1.92 cm. whenever the opposite hind limb was touched with a glass rod. As the electrode went in deeper (about 1 mm.) the responding region gradually passed up the trunk to the forelimb. The nervous discharge was only evoked by contralateral stimulation, and there was a sharp boundary along the median plane. Very sensitive responses to auditory, and also some to visual, stimuli were obtained in other parts of the mid-brain.

In the frog-brain (3.2.51.) strong nervous discharges in response to bilateral stimulation of the hind limbs were recorded from a depth of 1.8 mm. in the right optic tectum. At a slightly higher level, there was much activity elicited by any vibrations of the table upon which the frog was resting. Simple sound stimuli were not effective.

Two other methods of preparing micro-electrodes were tried also. The first, in which the steel needles were ground on a rotating grindstone, was found to be rather tedious and very much slower than the electrolytic method. It was difficult to get tips as small as 2 - 3 μ, and the angle of taper was

much greater.

The second is the preparation of silver-filled micro-capillaries (after Weale and Lander, 1950). A 2 mm. thick glass Pyrex tube (internal diameter 1 mm.), when heated by a few coils of resistance wire (at 6 v.), is drawn out by a weight suspended at its lower end. In this way extremely fine tips of less than 1 μ can be obtained. A fine silver wire, soldered to a platinum wire, is introduced into the wide end of the tube which is then sealed. The capillary is filled by boiling in silver nitrate solution, and silver crystals are deposited inside the capillary electrolytically (using a silver anode). The result is a very fine glass electrode with a relatively low resistance.

There were, however, many difficulties involved in making these electrodes. Such micro-capillaries are exceedingly fragile. The small size of the lumen made it very liable to be clogged up by minute particles of dust, etc., so that the capillary could not be filled evenly with the solution of silver nitrate. The electrolytic deposit of silver may take the form of only a very thin chain of crystals. Some of these difficulties may be avoided by very thorough cleaning of the tubing and the silver wire. As time was limited the only electrode that was at all successful could not be tested since it would have

been necessary to construct a special holder for it.

Other electrodes

In the experiments the indifferent and the earthing electrodes were steel needles exactly similar to those used in making the micro-electrodes. They were placed, whenever possible, in tissues which were not likely to give rise to any considerable electrical discharges - e.g. dead muscle, skin, and subcutaneous tissues. The indifferent electrode was kept as near the nodose ganglion as possible (bearing the above in mind). The oesophagus was often suitable at a point about 1 cm. away. On one occasion, however, this produced a definite discharge, in relation to expiration, whose origin was not appreciated for some time. This showed the importance of being extremely wary in interpreting nervous impulses with an unusual rhythm.

An E.C.G. could be superimposed on the recording by moving the earth electrode to the chest or one upper limb.

Recording apparatus

This consisted of a high gain biological amplifier with a cathode-ray tube and a loud speaker. The input grid leaks were through a resistance of about 1 megohms. The internal noise level was usually about 7 microvolts. At maximum gain the screen deflections were of the order of 10 microvolts/cm.

The camera used for permanent photographic records was not always available. It was employed in conjunction with a double beam commercial oscilloscope (Cossor) on which a time marker tracing could be projected.

Histological techniques

Preparations of the nodose ganglion were made to study the size, morphology and arrangement of cells and fibres, and also to find needle tracks, if possible. Specimens were fixed in 10 p.c. formalin in saline, or in ammoniated alcholhol (for 24 hours). Staining methods used were pyridine-silver (after Ranson 1912), Mallory's, pyrrol blue, and Weil's. Sections were mounted in paraffin, and were 10 μ in thickness.

RESULTS

Nine experiments were done. Recordings, however, were only made in eight of them. Most of the depth measurements made were not really satisfactory as the no-dose ganglion was liable to move with the electrode to an extent that was never predictable or measurable.

As the upper and lower ends are rather ill-defined, measurements on the surface of the ganglion were made in relation to an approximate mid-point. There was much variation, but the total length was usually of the order of 10 - 12 mm., and the thickness, at the middle, about 2 - 3 mm.

The first experiment was largely an exploratory one. The finer points of the dissection, the details of the blood supply and possible methods of fixing the ganglion were studied. In addition, the efficiency of the system originally devised to secure the micro-manipulator was tested. It proved inadequate, so that the electrode could not be inserted satisfactorily.

Experiment II

A doubtful respiratory rhythm was heard in the background for some 6 - 8 cycles during one electrode stab. What may have been a venous discharge was heard at one point, but it was not clear enough to allow a definite judgement. The discharge was quite high-pitched; it was inhibited by artificial

inflation of the lungs, and increased by deflation (suction). Many "injury" discharges were heard and seen.

These injury responses have been found in all the nervous tissues tested with micro-electrodes. They were especially prominent where groups of nerve cells were known to be present. They have a characteristic pattern of discharge. As the needle is pushed into the tissues very high-pitched bursts of impulses are heard. They sound like a sharp "ping", or sometimes like air suddenly escaping out of a small balloon. On the screen, they are seen as small groups of spikes very close together at first, gradually separating and also becoming distinctly smaller. In amplitude they vary considerably; they may be as large as one millivolt or more. The homogeneous appearance of each group clearly relates it to a single neurone. Quite frequently the discharge may continue at a high frequency for several minutes, remaining at a certain amplitude. Or again, it may slow down until the spikes occur only once a second or so, but persist for a long time. Occasionally, after the initial high frequency, the discharge goes on at a more moderate rate, to disappear very suddenly with a final "ping".

Such injury discharges could not be influenced in any way by pressure on the abdomen, or by inflation of the lungs. They are never related to either the

cardiac or respiratory rhythm. They were, therefore, supposed to be the result of mechanical injury to nerve cells and fibres.

Experiment III

In the left ganglion definite stretch impulses were recorded in six different stabs, at intervals of about ½mm., between the upper pole, and the middle of the ganglion. Very good single unit discharges could be studied. For instance, one set of spikes, 250 microvolts in amplitude, fired off for about 2 seconds during each inspiration at a rate of about 10 - 15/sec, slowing down at the onset of expiration, (a total of 20 - 30 spikes). With artificial inflation this rate became somewhat greater than 100/sec., but adaption was apparently negligible. This fibre possibly had a relatively high threshold. as background fibres could be heard to start firing some 1 - 2 seconds before and stop about ½ second after. The spike was partly above and partly below the line. To begin with, ¾ of it was below the base line, then, as the needle went deeper, more of it appeared above the line, until the two portions were equal. For this to happen, the total movement of the electrode was some 1.5 mm.; this shows the extent to which the ganglion was liable to move.

A rough method of measuring the spike duration was tried by allowing a certain amount of A.C. mains interference on to the screen. The wave obtained

was assumed to have a frequency of 50 c.p.s. The spike duration worked out at 0.4 msec.

The stretch afferent single units found in this ganglion numbered about a dozen, but they did not all give such large potentials as the one described above. Most of them were of the order of 20 - 50 microvolts. They were always associated with other similar fibres heard in the background, and sometimes as one fibre gradually became less prominent one or more of these would take the stage.

On artificial deflation (suction) a very clear, low pitched, rapidly adapting discharge was heard. When the electrode was recording a good single unit, it was evident that some other fibres were now firing. One of these on one occasion could be made out fairly clearly from the background. It behaved like a true deflation afferent fibre, discharging only on suction; it was fast adapting. Most of the other possible deflation impulses were less distinct; but the low pitched, very rapidly adapting discharge always seemed characteristic. It was sharply contrasted by the longer, comparatively high pitched stretch discharge, heard as the suction was released.

Two single units giving a cardiac rhythm were also picked up about 1 and 3 mm. above the middle of the ganglion respectively. The spikes were between 50 and 100 microvolts in height, and constant, The discharge consisted of about 7 - 12 spikes,

firing relatively fast initially. They were mostly over the base line, but gradually shifted down as the electrode moved deeper (over 1 mm.). When an E.C.G. was superimposed upon the tracing, the impulses coincided with the T-wave. The effect of strong artificial inflation was to stop the rhythm after about 3-5 seconds. On releasing the pressure it returned gradually over 4 - 5 beats, after a pause which depended upon the degree of inflation. Deflation, on the other hand, caused an acceleration of this rhythm. This behaviour is typical of aortic depressor fibres.

Experiment IV

Definite stretch impulses were heard at about the middle of the right ganglion. No single units were distinguished.

A discharge with cardiac rhythm was also heard for about ½ minute in the same ganglion, in the course of a stab about ½ mm. to the left of the previous one. It was very high pitched and sounded different from that described in the previous experiment. It disappeared very suddenly before it could be tested; the cell may have slipped away from the electrode point.

Experiment V

Several very good stretch afferent single units (about 100 - 250 microvolts) were found. They were recorded from the left ganglion, about 4 mm. below

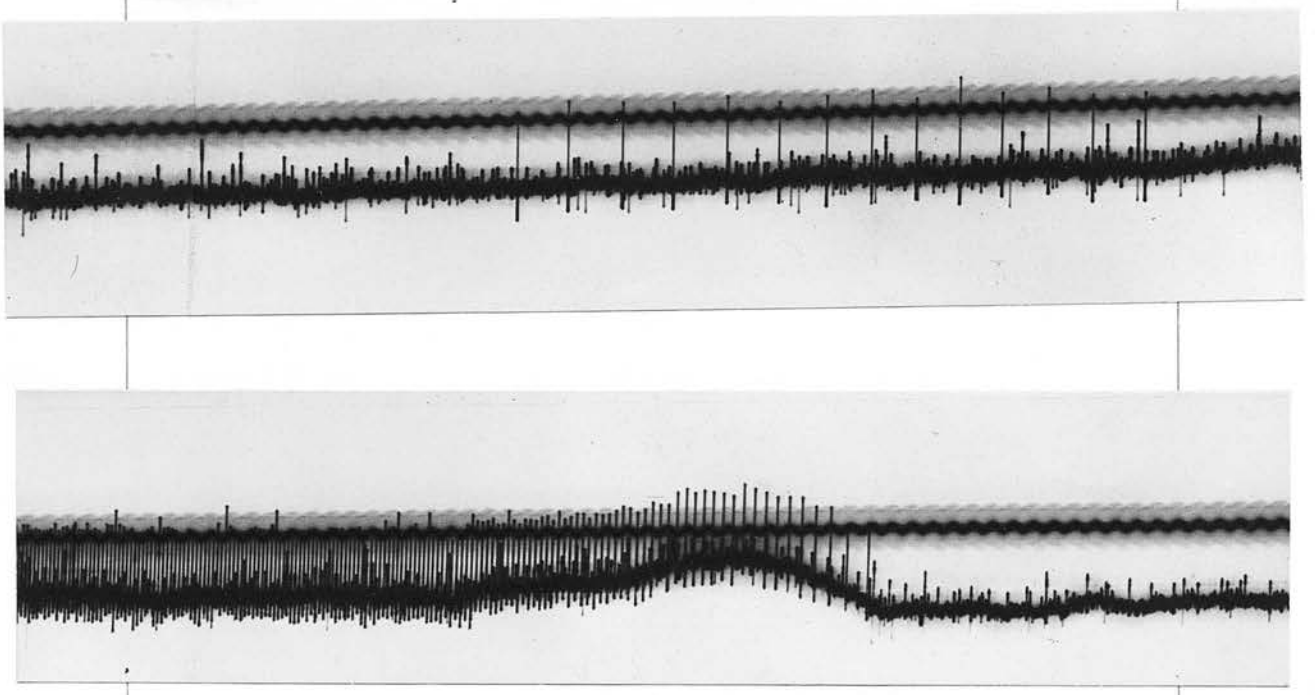

Fig. 2. Recordings from the nodose ganglion, (experiment V) showing, (a) upper tracing: a single stretch afferent unit firing off during inspiration (100 microvolts). (b) lower tracing: the same unit towards the end of artificial inflation. The shift of the base line was caused by mechanical disturbance.

Time marker tracing - 50 c.p.s.

the mid-point, at the surface and over a depth of 1mm. Units firing continually, but with a frequency varying with the phases of respiration, and others only firing during inspiration or inflation, were clearly heard and seen. Some of these were photographed, (see figs. 2). As the electrode travelled deeper their amplitude and shape gradually altered. When the tracheal tube was closed, only the continuous units still kept discharging, but with a diminished inspiratory acceleration. At a point about ½ mm. farther to the left many stretch impulses were heard from the surface to a depth of about 3 mm., but about 1 mm. higher up they were only found some distance below the surface. Several other stabs in the same region gave stretch impulses, but 2 others farther up were negative.

Two definite cardiac rhythms were picked up also. Both were in relation to the groups of stretch fibres. They were not prominent enough to be recorded satisfactorily, but they sounded, and on testing, behaved, like those described in experiment III, and were probably aortic depressor impulses. In addition to these, impulses coinciding with expiration, inhibited by suction, and stimulated by inflation, were recorded from several parts of the ganglion. They turned out to be artefacts produced by spread of potentials from muscles active during expiration.

Experiment VI

Two stretch afferent single units (about 30 - 40 microvolts) were found in the right ganglion; on the left side stretch signals were present, but no individual fibre could be made out. Fairly slow adapting discharges were heard in response to suction on the left side.

Experiment VII

On the left side, very good stretch afferent single units were recorded, varying in amplitude from 50 - 500 microvolts. They were found at a level about 1 mm. below the mid-point of the ganglion. All were within a relatively restricted range in any one stab (about 400 μ), and movement of about 60 μ was enough to alter their appearance significantly.

The fibres were of more than one type. Some were continually firing, while others apparently had a high threshold and only discharged a few impulses at the peak of inspiration. Suction quite clearly stimulated several stretch fibres, particularly those with a low threshold which then adapted much more rapidly. No independent deflation fibres could be identified.

Stretch signals could also be heard clearly on the right side, but there were no single units. A cardiac rhythm was heard on the same side for about a minute. The effect of inflation was tried, but in that short space of time no conclusive result could be had.

SUMMARY OF RECORDINGS.

Experiment	Stretch Units	Aortic depressor Units	Deflation Units	Other cardio-vascular Units	Miscellaneous	Injury response
II	+-	-	-	+?	-	++
III	+++	++	+	-	-	++
IV	+	-	-	+?	-	++
V	+++	+	-	-	-	++
VI	++	-	+?	-	-	++
VII	+++	-	-	+?	-	++
VIII	-	-	-	-	++	++
IX	+++	-	+?	+?	-	++

Experiment VIII

In this experiment no recognisable respiratory or cardiac rhythm was found anywhere in either of the two nodose ganglia. One large unit, which was firing apparently spontaneously, could be speeded up considerably by a slight pull on the trachea, (the micro-electrode was in the upper pole of the right ganglion).

An odd, variable, discharge made up of groups of irregular potentials, at intervals of 3 - 5 seconds, and lasting 1 - 3 seconds, was also found near the upper pole. After a variable number of cycles, a rather larger group would occur. At times the rhythm was in phase with respiration. Inflation, deflation and pressure on the abdomen had no obvious effect on this.

Experiment IX

On the right side good stretch afferent single units were obtained at about the middle of the ganglion. They varied from 20 to 500 microvolts in size. Some fired continually; they speeded up during inspiration but not to a very great extent. Others, higher-pitched, only discharged during inspiration or during artificial inflation. One stretch afferent fibre gave a fast and continual discharge during inspiration, but during expiration it fired off only slowly and intermittently, in small groups of 3 - 4 impulses, with an obvious cardiac rhythm. Suction

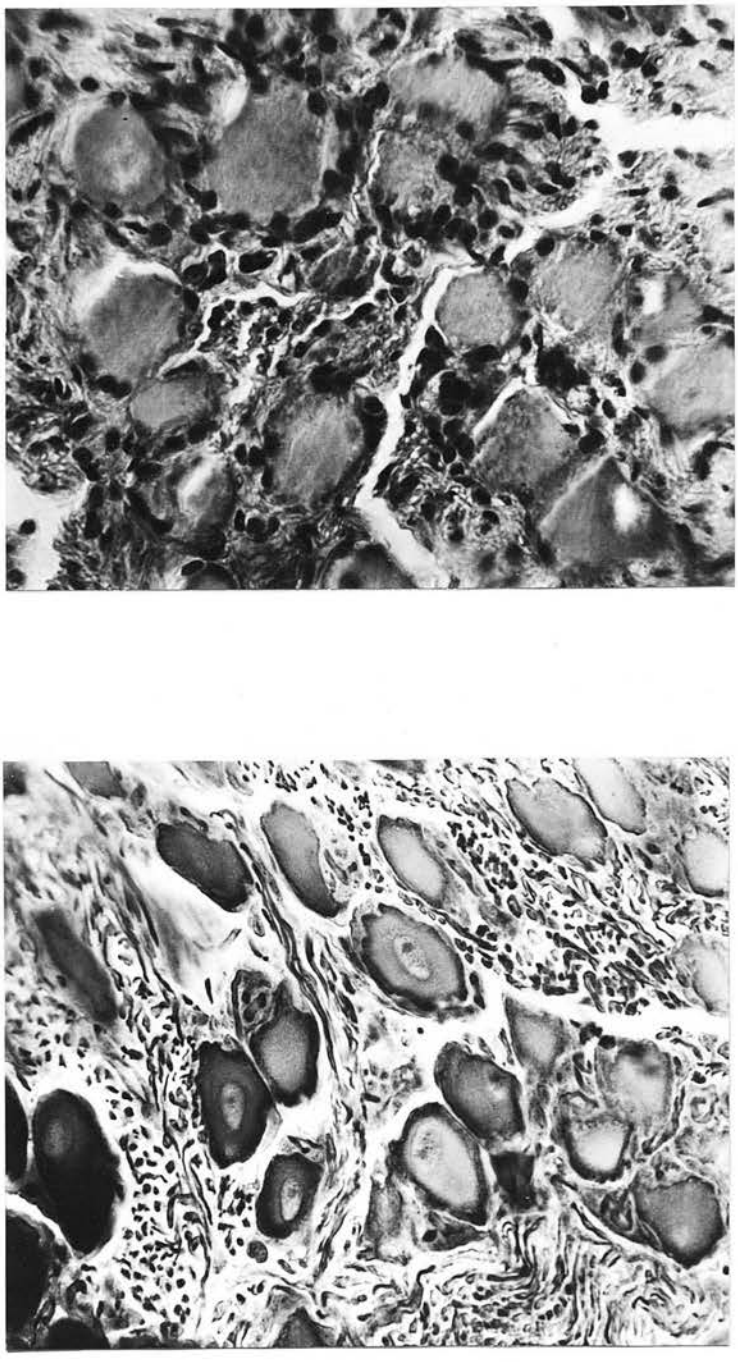

Fig. 3. Photomicrographs of sections of the nodose ganglion near the lower pole. (500 X)

Upper section was stained by Weil's method. The myelinated fibres are stained black.

Lower section was stained by the pyridine silver method. Both myelinated and unmyelinated fibres are shown. This gives an idea of the relative numbers of the two kinds of fibres. There is some distortion from shrinkage in the lower section.

silenced most of these fibres. The continually firing fibre first mentioned, would, at a particular degree of deflation, also adopt a cardiac rhythm. Stronger suction stopped the discharge completely. On the other hand, fast adapting, low pitched impulses, which seemed as different in size or shape from the others as could be assessed without a camera, fired off on suction. Closing the tracheal tube did not help much in distinguishing true deflation fibres, as obvious stretch fibres still discharged during inspiration, though to a lesser extent.

Stretch signals were heard in the background on inflation with the electrode in the middle of the left ganglion. A distinct cardiac rhythm was heard also for a few minutes. It was too weak and too ephemeral to allow any definite conclusions about its origin.

One rather peculiar feature of these experiments was that in all cases good single units were not obtained until some 5 - 9 hours after recordings had begun. Injury discharges, on the contrary, tended to be most numerous at the beginning, becoming less and less evident as time went on.

Histology

The large clear cells of the nodose ganglion were well shown in sections stained with pyrrol blue, pyridine silver and by Weil's method. (see fig. 3)

Distortion from shrinkage was comparatively severe with pyridine silver. Measurements of the diameter of cells in different sections were mostly of the order of 30 - 40 microns.

Nerve fibres were not stained satisfactorily by pyrrol blue except where they form a large bundle, as in the superior laryngeal nerve. Pyridine silver showed all fibres, unmyelinated and myelinated, but Weil's stain only those that are myelinated. These two stains therefore, give some idea of the relative numbers of the two kinds of fibres (see fig. 3)

No clear needle tracks could be found, probably because of the small size of the ganglion, and the difficulty of inserting the electrode exactly at a right angle to the surface.

DISCUSSION

On the whole the results of these experiments are disappointing. Distinct single unit activity was recorded, but not without much effort. Moreover, it was limited practically to stretch and aortic depressor fibres. It seems that the technique employed is not, after all, very suitable for the investigation of vagal afferent activity. The reasons for this failure could not be established definitely, but certain points may be significant

1. The pulmonary vascular fibres which are

probably the most numerous of those with a cardiac rhythm (Whitteridge, 1948) probably belong to the gamma or delta group (Whitteridge, 1948). According to Grundfest (1939) the slowest A fibres are almost as sensitive as B fibres to anoxia. This does not apply to the stretch fibres (Adrian, 1933), nor to the venous and aortic depressor fibres (Amann and Schaefer, 1943; Whitteridge, 1948).

The ganglion could not be fixed very well. It was very liable to move with the micro-electrode, and this might well cause kinking of its blood vessels. The resulting anoxia would put out of action the most numerous vascular fibres. No quantitative evaluation of the various fibres has ever been made, but it may be presumed that the venous fibres are relatively few since they were not described until fairly recently.

The very tough sheath covering the ganglion made matters even more difficult. If it was left intact, the electrode tended to push it into the ganglion - compressing the cells and the blood vessels, while to slit it open meant cutting a substantial number of vessels which supply the ganglion.

2. The possibility that the delicate needle tip or the insulating varnish might be damaged by remnants of the sheath was considered. All micro-electrodes were examined before and after use. There was no evidence that they were spoilt after one insertion.

It was usually only after several stabs that the tip was found to be damaged. Furthermore, the good records of single fibre activity obtained suggest that the electrodes were not at fault.

3. To what extent are the cells of the nodose ganglion likely to be receiving afferent messages from the lungs and the large vessels ? Cajal (1909) assumed that the unipolar ganglion cells with T - shaped axons were sensory, like those in the ~~original~~ SPINAL ganglia. However, as early as 1886, Gaskell had stated that large numbers of unmyelinated fibres appear in the vagus below the nodose ganglion. By degeneration experiments in crocodiles and alligators he showed that these were viscero-motor fibres and that they have cell-bodies in the ganglion. He claimed that fine medullated fibres from the medulla are connected to one or more cells in the ganglion from which unmyelinated fibres pass to the periphery. In 1889, after further experiments, he thought the nodose ganglion was mainly an afferent ganglion.

Langley (1900) who had done experiments on cats, believed the unmyelinated fibres were efferent; they had lost their sheaths, and were not connected to the ganglion cells. In his paper on the vagus and its ganglia, Molhant (1913) made no mention of the unmyelinated fibres. He quoted the results of experiments in which were studied chromatolytic changes in the nodose ganglion, after removal of a pulmonary lobe (Ikegami and Yagita, 1907) after

section of the depressor nerve (Kosaha and Yagita, 1905) and after section of cardiac branches of the vagus (Kosaka and Yagita, 1907). According to these the proportion of cells affected in the first two cases were about 1/19, (equivalent to 1/8 for the whole lung), and 1/12, of all the cells. In the last case, there were changes in only a small number of cells. Molhant's own experiments demonstrated a functional localisation of afferent cells in the ganglion. He claimed, for instance, that pulmonary cells were distributed diffusely in the middle and inferior portions, while cardiac and aortic depressor cells form a nucleus half way between the middle and the upper end.

Chase and Ranson (1914) showed the presence of large numbers of unmyelinated fibres in the vagal rootlets. They recognised the increase in the numbers of these fibres below the nodose ganglion but thought that Langley's explanation would account for this. Chase (1916) showed conclusively that, in the dog, sympathetic unmyelinated fibres do not join the lower vagus in any great number. Ranson and Mihalik, (1932) reached similar conclusions.

New light was thrown upon the subject by Jones (1932) who made counts of fibres and cells in the vagus, the superior laryngeal nerve, and the nodose ganglion, in cats. For instance, in one cat, he found an equal number (2500) of unmyelinated fibres

in the rootlets and in the vagus above the ganglion, at which level there were some 5300 myelinated fibres. Distally, the unmyelinated fibres numbered about 9600, and the myelinated 5000 (3000 in the main trunk and 2000 in the superior laryngeal nerve). In another cat, the ganglion contained a little over 14,000 cells, while the myelinated fibres, proximally to it, only numbered a little over 4,000. It seems, therefore, that the great majority of the cells must be regarded as cells of origin of the unmyelinated fibres. Heinbecker and O'Leary (1933) could ascribe no recognisable afferent function to the unmyelinated fibres; they showed that unmyelinated motor fibres to the lungs and the intestine arise from cells in the ganglion. Since there is no evidence of any synapse in the ganglion, they concluded that there are motor cells with a peripheral and a central process.

It is difficult to make even a rough estimate of the number of pulmonary and cardio-vascular afferent cells in the nodose ganglion on the basis of the results of Jones and the Japanese workers, as many unmyelinated fibres in the pulmonary and cardiac branches must have been cut also. One may well conclude, however, that the cells which would give stretch and cardio-vascular responses are probably only a small proportion of all the cells present. A microelectrode is even more likely to miss the comparatively rare units if there is a functional

grouping of neurones as suggested by Molhant. To do a systematic examination of every part of the ganglion is very difficult. Some evidence in favour of Molhant's hypothesis was found in these experiments. It was noticed that, in the course of any one stab, stretch units were usually in a group. Evidence of localisation at different levels of the ganglion was not conclusive. It may not be possible with small electrodes which could probably record from both cells and fibres (when the latter are not in very tight bundles).

The description given of injury discharges agrees on the whole with previous observations made during experiments in which micro-electrodes were used (Renshaw et al, 1940, Therman et al. 1940, Brookhart et al. 1950.) Adrian (1930) made a study of injury potentials in peripheral nerves. He noted two main types of discharge. One was a continuous succession of impulses, at a rate exceeding 150/sec. The other was at a lower frequency and irregular. He thought that the permanent depolarisation at the site of injury stimulates the intact part of the nerve. If this potential is just above threshold value, nerve cells will fire off at about 150/sec - the refractory period is about 6 - 7 msec. Higher rates of discharge are produced by potentials greater than threshold, and irregular, slower discharges, due to fluctuations of the threshold, if they are only a little below the maximum threshold value.

It is probable that injury potentials in the nodose ganglion are caused by a similar mechanism, acting on the cells and the fibres. The decrease in the size of the spikes may be due to exhaustion, or to a form of Wedensky inhibition resulting from injury.

CONCLUSIONS

Using small micro-electrodes (2 - 4 μ), it was possible to record in the nodose ganglion the activity of most types of stretch fibres previously described (including the fast and slow adapting). Aortic depressor impulses were also recorded clearly and in addition, some probable venous and deflator discharges.

These results were only obtained after much trouble; the method does not seem to be as useful for the study of vagal afferent activity as had been hoped.

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REFERENCES.

1. Adrian, E.D. (1930). Proc. Roy. Soc. B.106,596

2. Adrian, E.D. (1933). J. Physiol. 79, 332.

3. Adrian, E.D. and Moruzzi G. (1939). J. Physiol. 97, 153.

4. Amann, A. and Schaefer, H. (1943). Pflug.Arch ges. Physiol. 246,757.

5. Baxter, I.G. and Pearce, J.W. (1950).J.Physiol. 112,26P.

6. Bishop, P.O. and Collin, R. (1950) J.Physiol. 112, 8P.

7. Brookhart, J.M., Moruzzi, G. and Snider, R.S. (1950. J. Neurophysiol. 13,465

8. Brooks, C.McC. and Eccles, J.C. (1947) J. Neurophysiol, 10, 247.

9. Cajal, S.R. (1909) Histologie du système nerveux de l'homme et des vertébrés. p.723, Paris, A. Maloine.

10. Chase, M.R. (1916). J. comp. Neurol, 26, 421.

11. Chase, M.R. and Ranson, S.W.(1914). J. comp. Neurol. 24, 31.

12. Creed, R.S. and Hertz, D.H.(1933) J. Physiol. 78, 85

13. Daley, R., Wade, J.D., Maraist, F. and Bing, R.J. (1951) Amer. J. Physiol. 164, 380.

14. Dickinson, C.J. (1950) J. Physiol. 111, 399.

15. Dunn, J.S. (1919). J. Physiol. 53, v.

16. Dunn, J.S. (1919). Quart. J. Med. 13, 129.

17. Gaskell, W.H. (1886). J. Physiol. 7, 1.

18. Gaskell, W.H. (1889). J. Physiol. 10,153.

19. Grundfest, H. (1939). Amer. J. Physiol. 127, 252.

20. Hammouda, M. and Wilson, W.H. (1932) J.Physiol, 74, 81.

21. Hammouda, M. and Wilson, W.H. (1935). J.Physiol. 85, 62.

22. Hammouda, M., Samaan, A. and Wilson, W.H. (1943). J. Physiol. 101, 446.

23. Head, H. (1889). J. Physiol. 10, 1.

24. Heinbecker, P. and O'Leary, J. (1933). Amer. J. Physiol. 106, 623.

25. Jarisch, A. and Zottermann, Y. (1948) Acta. Physiol. Scand. 16, 31.

26. Jones, R.L. (1932) Proc. Soc. Exper. Biol, and Med. 29, 1138.

27. Knowlton, G.C. and Larrabec, M.G.(1946) Amer. J. Physiol. 147, 100.

28. Langley, J.N. (1900) Shäfer's text-book of Physiology v.2, p.665, Edinburgh and London, Young G. Pentland)

29. Lorente de Nó. R. (1939) J. Neurophysiol. 2, 402.

30. Lorente de Nó, R. (1947) J. cell. and comp. Physiol. 29,207.

31. Molhant, M.(1913). Le Névraxe, 15, 525.

32. O'Leary, J.L. and Bishop, G.H. (1939) Amer. J. Physiol. 126,P594.

33. Parrach, H.O.(1942) J,Neurophysiol, 5, 423

34. Partridge, R. (1939) J. Physiol. 96, 233.

35. Pearce, J.W. (1951) (unpublished observations).

36. Ranson, S.W. (1912) J. comp. Neurol. 22, 159.

37. Ranson, S.W. and Mihalik, P.(1932) The Anat. Rec. 54, 355.

38. Renshaw, B., Forbes, A. and Morrison, B,R. (1940) J. Neurophysiol, 3, 74.

39. Therman, P.O., Forbes, A. and Galambos, R. (1940) J. Neurophysiol. 3, 191.

40. Torrance, R.W. and Whitteridge, C. (1947) J. Physiol. 107, 6 P.

41. Walsh, E.G. (1947). J. Physiol. 106, 466.

42. Walsh, E.G. and Whitteridge, D. (1944). J.Physiol 103, 37P.

43. Weale, R. and Lander, M.R.(1950).Nature, London. (in the press)

44. Weidmann, H., Berde, B. and Bucher, K. (1949). Helv. Physiol. Acta. 7, 476.

45. Whitteridge, D. (1948). J.Physiol. 107, 496.

46. Whitteridge, D. (1950). Physiol. Rev. 30, 475.

47. Wilson, W.H. and Samaan, A. (1947). Abstracts 7th Intern. Physiol, Congress, Oxford. p. 102

48. Worzniak, J.J. and Gesell, R. (1939). Amer. J. Physiol. 126, P658.