THE STUDY OF
RESPIRATORY AND CARDIOVASCULAR
REFLEX MECHANISMS INVOLVING THE LUNGS

A thesis submitted

for the degree of Doctor of Philosophy

by

A. S. PAINTAL

15th May, 1952.

ACKNOWLEDGEMENTS

I am deeply indebted to Professor D. Whitteridge under whom this investigation was carried out, for his valuable guidance, and generous hospitality in his department. My gratitude is also due to Dr. C. O. Hebb for much helpful criticism throughout the investigation, and to her and Dr. Swan for allowing me to do the joint experiment on the left lung perfusion with them; to Mr. W.T.S. Austin for helping me to build the apparatus and many valuable suggestions, to Mr. T. May for the many photographic reproductions of the records, and to Miss M. Forfar for the excellent operative assistance in the experiments.

Finally, I am most grateful to the Rockefeller Foundation, who made this work possible, for the grant of a Fellowship, and for defraying the expenses of this research.

CONTENTS

INTRODUCTION.

In the present study of Respiratory and cardio-vascular reflexes of the lungs attention has been particularly directed to the afferent mechanisms involved. This was considered necessary since there is little definite information about the nature of these fibres although much is now known about the possible reflexes in which they may participate. With this object a first attack on the problem was made as described in the first part of this thesis by the determination of the conduction velocities of the known vagal thoracic afferents. It is hoped that information so gained will be of value in the interpretation of results of previous investigators.

The second part of the thesis presents the results of investigation into the afferent fibre composition of the vagal rootlets as studied by the electrical recording of nerve impulses. This was undertaken because it was hoped that a functional differentiation of the vagal afferents might occur at this level as suggested by the work Beer & Kreidl (1895) and Cadman (1900), and thereby provide a means of selective stimulation. This was found not to be the case and further investigation in that field was therefore discontinued.

From the work of Dawes, Matt, and Widdicombe (1951) it appeared that certain amidines such as

phenyl diguanide are capable of producing a reflex respiratory inhibition which is blocked by cooling the vagi to 31°C. Because of the low blocking temperature it seems possible that this drug affords the means of selective stimulation of small afferents of the vagus and as will be shown in the 3rd section, this is certainly true. Further in order to test the hypothesis of Dawes et al that the pulmonary vascular afferents described by Whitteridge (1948) were probably concerned in the above reflex, the action of phenyl diguanide on these and other afferents has been studied. Evidence will be presented to indicate that none of the known vagal afferents including the pulmonary vascular ones are sensitized or stimulated by phenyl diguanide.

PART I

The conduction velocities of the thoracic visceral afferents in the vagus.

REVIEW OF THE LITERATURE CONCERNING THE RESPIRATORY AND CARDIOVASCULAR RECEPTORS

Histological Evidence. In the last sixty years a considerable amount of histological evidence has accumulated to provide the anatomical basis for the existence of afferents from the heart, lungs and the great vessels of the thorax.

As early as 1893, Berkley described the presence of nerve endings in the lungs, and in an extensive investigation Dogiel (1898, 1903) showed the presence of a variety of pulmonary receptors in various situations. Larsell (1921) found that sensory endings occured in the epithelium of the primary bronchi and at the points of division of the smaller ones. In addition he described a different type of ending in the walls of the pulmonary atria. Later (1922), he showed that a third type occurred in the smooth muscle bands which he called "smooth muscle spindles". These he believed to be innervated by large myelinated fibres. The occurence in mammals of sensory terminations between the alveoli described by Okamura (1930) adds yet another group of endings to those already described by Larsell. Further, Elftman (1943) found two types of nerve endings in the trachea, one within the smooth muscle, and the other in the subepithelial tissue - in addition to a variety of sensory endings

in a number of situations in the bronchial passages, atria and alveoli. In an earlier work, using the method of experimental degeneration, Larsell and Mason (1921) concluded that most of the pulmonary sensory endings were of vagal origin. They also found that there was a certain amount of crossing over of the nerve supply of the two lungs, thus confirming Mollgaard's (1912) findings, who, in addition, showed that the lungs probably received afferent fibres from the 2nd and 3rd thoracic ganglia.

It would thus appear that much histological evidence exists to account in part, at least, for the varying activity observed in pulmonay volume receptors during respiration, particularly in respect of differences in rates of adaption. In a recent work Widdicombe (1952) presented evidence to suggest that the more rapidly adapting fibres arise from endings in the trachea and larger bronchi, while the more slowly adapting ones come from the peripheral portions of the respiratory passages.

A number of investigators have described receptors in the pleura (Dogiel 1903, Larsell 1922, MacLaughlin 1933). Dogiel studying the costal pleura of mammals including man, found a number of sensory endings, some of them like typical Pacinian corpuscles. Larsell found evidence to suggest that the pulmonary pleura is at least in part innervated by afferent fibres from the dorsal root ganglia of

the upper thoracic spinal nerves. He observed that the endings in the dog are limited to the interlobar margins of the lungs and that these were supplied by fibres from the pulmonary periarterial plexus. In the cat, MacLaughlin demonstrated encapsulated endings in the visceral pleura but was not certain of their central innervation.

Evidence for the existence of receptors in the veins opening into the right and left atria, in the auricles and atria themselves, is now conclusive owing largely to the work of Nonidez (1937a). He emphasized the fact that the endings concerned were situated in the intra-pericardial portions of these structures. They were identical with other presso-receptors and were found in the subendothelial layer. They were supplied by the larger myelinated fibres and he concluded that those arising from the right side of the heart provided the anatomical basis for the Bainbridge reflex the existence of which has been questioned in recent years. The nervous endings of the left side he believed to serve the reflexes observed by Daly, Ludany, Todd and Verney (1937). He also noted in the wall of the atria another variety of ending which was supplied by smaller myelinated fibres.

Even before Nonidez, the work of several authors had indicated the presence of these receptors. Dogiel in 1898 found in cats and dogs a large number

of sensory nerve endings in the visceral pericardium immediately over the myocardium and which were most abundant in the region of the auricles. He also noted some receptors in the vena cavae. Nettleship, in addition to finding the greatest abundance of sensory endings in the region of the insertion of the great vessels, also found them to occur in large numbers around the auriculo-ventricular orifices and at the base of the interauricular septum.

Nonidez concluded that, in general, the fibres ending as receptors in the large veins are thicker than those ending as presso-receptors in the aorta. this point will be considered further in the discussion.

In 1935 Nonidez described in detail the presso-receptors in the arch of the aorta which are now recognised as the anatomical basis for the depressor reflex. He showed that in cats, rabbits and guinea pigs, terminations of the afferent fibres of the aortic nerves occur in the media and externa of the arch of the aorta, and in the right subclavian artery. Pressoreceptors were also found in the walls of the arteries supplying the carotid bodies. The afferents from the pressoreceptors run in the vagi of both sides.

In contrast to the above, the histological evidence for the existence of pulmonary vascular receptors (Whitteridge 1948) is fragmentary in comparison to a considerable amount of physiological

evidence produced in recent years. In three papers Takino (1932 a, 1932 b, 1932 c) described the afferent and efferent innervation of the pulmonary vascular bed, the distribution of which he found to vary from one animal species to another. Earlier, Dogiel (1898) had described certain endings which he thought were sensory. Larsell and Dow (1933) found in human beings a peri-arterial post-ganglionic plexus which formed a network around the pulmonary capillaries. In addition, they showed the presence of afferent endings in the adventitia, of not only the pulmonary artery, but in the secondary branches in the lungs as well. Elftmann (1943) made a deliberate search for afferent endings in the pulmonary arterioles, but could find only one definite ending in a small artery which consisted of a swelling at the termination of a thin myelinated fibre. However, she concluded that there were probably no afferent endings of importance in the pulmonary vessels, and explained the conflicting results of previous workers as being due to variations in the histological techniques used. It seems that the conclusions drawn regarding the functional identity of fibres innervating the blood vessels needs to be cautiously interpreted since there are evident difficulties in distinguishing between motor and certain types of sensory endings.

Several structures with typical glomus cells (De Castro 1928) have been described in the region of

the aorta and pulmonary artery in recent years. In 1931 Penitschka described a cell mass lying between the aortic arch and pulmonary trunk which he termed the 'paraganglion aorticum supracardiale', and in 1934, Palme described an additional mass lying close to the left coronary artery. In 1936, two more cell masses of both subelavian arteries were described by Nonidez, which he termed the "aortic glomi". Later (1937), in addition to the above, he described scattered epitheleoid bodies in the dog. The innervation of these masses of cells, presumably chemo-receptor in function, is through the vagus nerves of both sides, largely via the aortic nerves (Nonidez, l.c.)

Some uncertainty existed for sometime concerning the blood supply of the supracardial paraganglion which Nonidez in 1936 described as arising from the pulmonary artery. Later (1937), he changed his opinion after the work of Goormaghtigh and Pannier (1939) who showed that the pulmonary arterial branch to the paraganglion was obliterated in the adult cat and replaced by a branch from the coronary artery. This was confirmed by Hollingshead (1940) who had shown earlier (1939) that in kittens the aortic bodies were supplied with fibres arising from cells in the nodose ganglion.

In addition to the receptors described above, the behaviour patterns of which are well known,

certain others have been described, the existence of which is based largely on histological evidence. Takino and Watanbe (1937) described pressure receptors in the arterial ligament which persisted after the obliteration of the duct. This was confirmed by Nonidez (1941) in the dog, cat, rabbit and guinea pig. In the dog he observed that the pressure receptors were supplied by medium sized and small fibres, and that these were most numerous at the base of the ligament nearest the pulmonary artery, Karsner (1911), using the methyline blue technique found end bulbs at the nerve terminations in the pulmonary artery. Larsell (1922) found sensory receptors in the walls of the pulmonary artery near the hilum in mammals, and later in human pulmonary arteries as well. Further, Nonidez (1936) found a pressoreceptor type of nerve ending in the small arterial branch supplying the paraganglion supracardiale.

Histological studies of the vagus or its branches have been done by Foley & DuBois (1937a, 1937b); Heinbecker & O'Leary (1933); Jones, (1937); and recently by Dickinson (1951), and Daly & Evans (1952). Most of these investigators are agreed that the great majority of fibres in the vagus are unmyelinated and survive degeneration after section above the nodose ganglion.

Foley and DuBois observed that of the myelinated fibres 65% to 80% survived degeneration after section

of the vagal rootlets. Heinbecker and O'Leary (1933) found that normal motor reactions in the bronchial and duodenal muscles could still be obtained after section of the vagus central to the nodose ganglion. In conformity with this, Jones (1937) observed that more myelinated fibres existed distal to the nodose ganglion than in the proximal portion and concluded that some motor fibres originated from cells in the ganglion. Daly & Evans (1952), however, repeated the experiments of Heinbecker and O'Leary and were unable to confirm their conclusions.

A frequency distribution of the myelinated fibres of a cardiac branch of the vagus in the cat has recently been done by Dickinson whose work indicates that relatively few myelinated fibres degenerate after a supranodosal section. He finds too that the greatest number of fibres are about 2 - 3μ in diameter, a result which has been confirmed by Daly & Evans for the vagus of the cat and rabbit.

Electrophysiological Evidence. The recording of impulses from single fibre preparation was done by Adrian and Bronk in 1928 on the phrenic nerve. Subsequent examination of the cervical vagus by the same method has not only confirmed the presence of afferents previously believed to exist but has led to the discovery of certain new ones in this nerve trunk.

In 1933 Adrian described in considerable detail the well known slowly adapting pulmonary stretch

afferents which are responsible for at least part of the Hering-Breuer reflex. He also noted that some of these fibres had a super-imposed cardiac rhythm.

From an analysis of vagal pulmonary stretch fibres made on cats under positive pressure ventilation, usually with open chests, Knowlton and Larrabee (1946) concluded that apart from the slowly adapting stretch fibres there was another group of afferents with a much higher rate of adaptation and a high threshold to inflation. From somewhat insufficient evidence they inferred that these rapidly adapting fibres constituted a separate group of pulmonary stretch receptors and were responsible for exciting inspiration. Widdicombe (1952) observed identical impulse activity in fibres arising from the trachea and larger bronchi and found that they could be blocked at a temperature of about 10°C (Widdicombe 1952b).

It is interesting to note the observation of Weideman and Bucher (1949) that a large number of stretch afferents could be blocked by anaesthetising the pleura with procaine Hcl. directly but not by injecting the anaesthetic into the general blood stream.

Fibres firing on deflation of the lungs (Adrian, 1933) but believed not to fire during normal expiration may also be included in the respiratory group. Adrian concluded that suction was not so effective as external pressure applied to the chest in producing

a discharge from the receptors concerned. Fibres stimulated by deflation and believed to arise from endings near the diaphragmatic pleura have been observed by Whitteridge (1952). Hammouda and Stella (1935) found that deflation always gave an outburst of impulses from a point on the vagus central to a region that was cooled to a temperature at which the response to deflation still remained, but the inhibitory response to inflation had disappeared. Knowlton and Larabee (1941) however could not find any receptors firing only on deflation.

Impulses with a cardiac rhythm were recorded from the depressor nerve of the rabbit by Adrian (1926) and later (1933) similar impulses were recorded by him in the vagus of the cat. In 1931, Bronk and Kaltreider concluded that an important stimulus for the aortic nerve endings is a change in the pressure - the more rapid the change the greater the discharge. Whitteridge (1948) recorded aortic pressure and depressor fibre activity simultaneously and showed that the curve of frequency of discharge corresponded closely with the aortic pressure curve.

Amaan and Shaefer (1943) recorded afferent impulses from fibres presumably arising in the great veins and later Walsh and Whitteridge (1944) and Walsh (1947) recorded similar activity. Whitteridge (1948) who recorded venous pressure and fibre activity simultaneously showed that the frequency of discharge

in certain fibres corresponded closely to the affective venous pressure. Jarisch and Zotterman (1948) concluded that though the first presystolic auricular volley coincided with the rise in intra-auricular pressure it is not elicited by that event, while the 2nd volley, which starts before the T wave and finishes before the P wave of the E.C.G. is dependant on auricular pressure. On the other hand Dickinson (1950) showed a direct relation between auricular pressure and the frequency of discharge of impulses from these afferents.

Yet another type, the pulmonary vascular receptor, was described by Whitteridge in 1948. This was shown to be a distinct entity, and later from the close correlation of fibre activity and pulmonary arterial pressure, Pearce and Whitteridge, (1951) concluded that the afferents were probably situated on the arterial side of the pulmonary vascular bed. However conclusive histological evidence has not been forthcoming possibly because of the diffuse distribution of these afferents.

Although chemoreceptor impulses in the Carotid branch of the ninth cranial nerve have been recorded by several investigators there are relatively few records of vagal fibre activity which can be attributed to chemoreceptor stimulation. Landgren and Neil, (1951) studying chemoreceptor discharges in the aortic nerve found that they were considerably

increased following haemorrhage and could be abolished by substituting 100% oxygen for room air. On the other hand 4.5% oxygen in nitrogen greatly increased the discharge.

Several other afferents not so well recognised have been described from time to time. Adrian (1933) described the occurrence in the vagi of persistent discharges without cardiac or respiratory rhythm which he believed originated from the bifurcation of the trachea or lung roots.

In 1948 Whitteridge described a type of discharge occurring very early in systole at a time when the aortic valves were closed. These he believed probably arose from the right ventricular wall - the discharges occurring during the isometric phase of contraction. A similar type of discharge was recorded by Dickinson (1950) and by Pearce (1951).

Jarisch and Zotterman (1950) recording impulses from the cardiac branches of the vagus observed the occurrence of small sized impulses which could be produced by pinching the ventricules and which they believed to be the afferent mechanism for the cardiac depressor reflex. Their conclusion that these afferents belong to the delta group of small diameter afferents is based on the fact that their spikes were small relative to those of other afferents.

The compound action potential set up in the

vagus has been studied in conjunction with the reflex and direct effects produced by its stimulation in the work of Heinbecker (1930), Bishop and Heinbecker (1930) Bishop, Heinbecker and O'Leary (1934) and recently by Wyss and Rivkine (1950) and Middleton, Middleton and Grundfest (1950).

The conclusions of these investigations will be considered in the section of discussion.

The effects of applying a differential block such as cold or pressure to the vagus has been studied by Torrance and Whitteridge (1947); Partridge (1939); Hammouda and Wilson (1935,1943); Heinbecker and O'Leary (1933); Stefenson, Brookhart and Gessel (1937); Dawes, Mott and Widdicombe (1951); and Pearce 1951. The observations and conclusions of these investigations will be discussed later.

REFLEX FUNCTIONS OF THE AFFERENTS.

In contrast to a considerable amount of knowledge now existing concerning the part played by the pulmonary stretch, depressor and chemoreceptor afferents, relatively little information exists regarding any definite role played by the pulmonary vascular and venous afferents in various respiratory and cardiovascular reflexes.

On the basis that the pulmonary vascular receptors lie in the walls of the pulmonary arterioles as suggested by Pearce and Whitteridge (1951), their

function would presumably be to signal changes of pressure in the small vessels. One would therefore expect these receptors to be associated with reflexes arising from the pulmonary vascular bed. Apart from the local affects produced in the pulmonary vascular bed by such agents as carbon dioxide and oxygen lack as observed by Duke (1949), and Hebb and Nimmo-Smith (1949), a number of pulmonary vascular reflexes mediated through the vagus and acting on the cardio-vascular and respiratory systems have been described.

Schwiegk (1935) showed in chloralosed dogs that a fall in systematic blood pressure occurred following a rise in the static pulmonary arterial pressure. In his experiments he cannulated the left pulmonary artery and connected it to a reservoir containing a mixture of Tyrode solution and defibrinated blood; and tied off the left pulmonary veins. The pressure in the artery was varied between 10-20mm. of mercury. Schwiegk considered the response to a rise in pulmonary artery pressure to be a protective reflex protecting the pulmonary vascular bed from the effects of an undue rise in blood pressure. Schweitzer (1936) could not confirm Schwiegk's observation in the decerebrate or chloralosed cat. However it is possible as suggested by him that the destruction of the afferent nerves in the adventitia of the pulmonary artery was responsible for his negative findings. Schweitzer used a mixture of gum arabic

and Ringer-Locke solution instead of defibrinated blood. This difference may be a contributory cause for the difference in results.

Daly, Ludany, Todd and Verney (1937) using perfused preparations in dogs concluded that an obstruction to the outflow of blood from the left auricules was more effective than an increase in pulmonary arterial pressure in producing a reflex fall in systemic blood pressure. They believed that this was caused by the stretching of receptors on the venous side of the pulmonary vascular bed. Histological evidence of such receptors has been provided by Nonidez (1937).

Their view is not necessarily incompatible with Schwiegk's interpretation for it is possible that receptors on both sides of the pulmonary vascular bed could affect systemic reflexes in the same way.

Recently Donnet (1951) working on chloralosed dogs concluded that the reflex observed by Schwiegk did not operate normally but was probably effective at high pressures only.

The association between a rise of pulmonary arterial pressure and an increase in respiratory rate has been observed by Daly, Ludany, Todd and Verney in dogs and by Churchill and Cope (1929) in cats. Whitteridge (1950) in a review on rapid shallow breathing discussed the part played by pulmonary vascular receptors in the circulatory adjustments of the lungs and suggested the possibility that they

might be indirectly concerned in the production of tachypnoea by multiple pulmonary embolism.

The importance of the venous receptors has been rendered somewhat uncertain by some recent work.

After Bainbridge (1915) had described a reflex cardioacceleration occurring after the injection of saline into a cannulated iliac vein, it came to be assumed that the reflex that goes by his name originates from the right auricle or great veins. Confirmation of this conclusion was presented by Sassa and Miyazaki (1920) who observed a reflex cardio-acceleration on mechanical distension of the vena cavae and the right or left auricles. Subsequently Anrep and Segall, (1926) working on the innervated heart lung preparation observed a similar cardioacceleration. However, using a method similar to Sazza and Miyazaki, Tiitso (1937) observed that a cardioacceleration could be produced after bilateral vagotomy provided that the heart rate which is increased after vagotomy is kept low by stimulation of the peripheral end of the vagus. From this he concluded that the effect was a local one.

Ballin and Katz (1941) using both anaesthetised and unaenesthetised dogs could not produce any cardioacceleration by a mechanical distension of the superior vena cava or right atrium although they could consistently produce an increase in heart rate by saline infusion. They suggested that stimulation o

of receptors in the pulmonary vessels or other parts of the heart was responsible for the Bainbridge reflex, but no evidence is available so far to support this suggestion. However it is possible that the mechanical stimuli used by Ballin and Katz were not adequate for as shown by Dickinson (1950) the venous receptors show evidence of adaptation. It is worth noting that Megibow, Katz and Feinstein (1943) whose work confirms Ballin and Katz in that though they failed to produce cardioacceleration by distension of the superior vena cava or right atrium, they could always produce an increase in the rate and depth of respiration by the same stimulus.

In contrast to the meagreness of knowledge gained about chemoreceptor activity in the vagus by electo-physiological methods, studies by reflex methods have yielded more information and there is for example ample evidence to show that the aortic chemoreceptors produce reflex effects similar to those of the carotid body. Comroe (1939) showed that in the dog the rise of blood pressure produced by anoxia was largely due to stimulation of the aortic chemoreceptors, but in the cat the carotid bodies appeared to have an equal share in the vasomotor response. Neil, Redwood and Schweitzer (1949b) observed that chloralose injected into a decerebate cat converted the depressor response obtained on stimulation of the aortic nerve into a pressor one, an observation which had been seen earlier on the carotid sinus nerve. (1949a).

This was believed to be due to a selective depression of baroceptor activity leaving the chemoreceptors comparatively free to produce their characteristic effects of a rise in blood pressure and hyperpnoea.

McDowall (1924) observed that in animals suffering from the effects of severe haemorrhage, section of the vagi produced a fall in systemic blood pressure. To explain this he postulated the existence of receptors in the right auricle which were stimulated by a fall in venous pressure. No impulse activity from such receptors has been recorded in any vagal afferent so far. On the other hand the "McDowall effect" may, as suggested by the work of Coleridge, Kenny and Neil (1949), be due to the elimination of chemoreceptor impulses which are greatly increased during haemorrhage (Landgren and Neil, 1950).

NOTE ON THE FIGURES IN THIS THESIS

With the exception of the graphs, the figures in this thesis are reproductions of photographic records of impulse activity in nerve fibres. In all cases a record of an E.C.G. (lead I) accompanies that of fibre activity. In some, an intrapleural pressure record is incorporated. From above downwards therefore, in all cases the records are : E.C.G., fibre activity, and intrapleural pressure (where included). These three records read from left to right. In fig. 2 and Figs. 4 to 8, in addition to the above, there are the records of single sweeps consisting of two traces (uppermost), which are, respectively, time marks in milliseconds and conduction velocity determinations.

Fig.1. Photograph of the region of the right cervical vagus under liquid paraffin with electrodes in place. (1) recording electrode; (2) glass dissecting plate with vagus; (3)&(5) thermistors; (4) stimulating electrode; (6) trachea; ceph. cephalic end of dissected area; caud. caudal end of dissected area; V. vagus.

METHODS

All the experiments were done on adult cats anaesthetised with chloralose 80 mg/kg after induction first with ethyl chloride and then ether. By using ethyl chloride the amount of ether required was less and bronchial secretion was thereby reduced as well. The trachea was cannulated and the skin over the right side of the neck was freed from subcutaneous tissue over a length extending from the sternum to the mandible. The sternamastoid was cut between ligatures at the level of the nodose ganglion and the tonsilar lymph gland removed in order to expose the vagus. The nerve trunk was freed from the surrounding tissues including the sympathetic nerve at a point just below the nodose ganglion, and the dissection having been extended caudally for about 2 cm. it was placed on a smooth glass plate with a black background and was fixed to a stand from which it was fully insulated. The margin of the skin was then raised with hooks tied to dry string, and warmed paraffin (38° C) poured on the area of dissection. (fig. 1)

For the preparation of single units the nerve sheath was split with a sharp edge of a broken razor blade knife which led to bleeding from the nerve. With the aid of a binocular loupe (magnification = 3x) a thin nerve bundle was separated from the main trunk and severed at the cephalic end. This bundle was

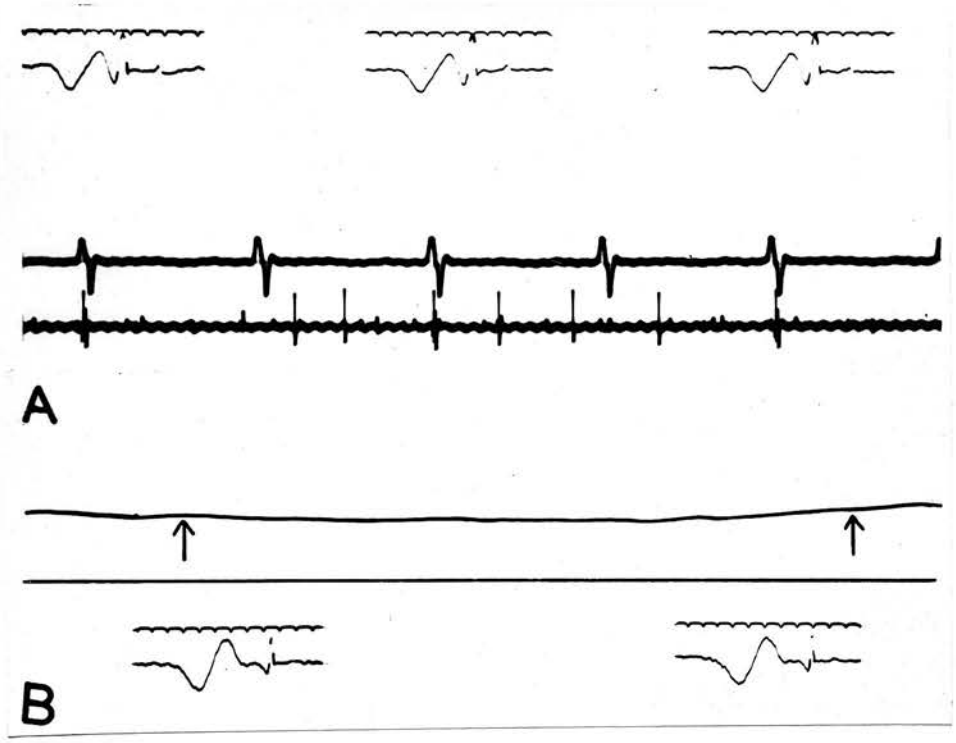

Fig. 2. The effect of diphasic muscle potential on the record of a single stretch fibre. In A, maximum stimulus was used, the sweeps (upper traces) showing the evoked spike and the muscle potential. In B, stimulus strength was subthreshold for the single unit; only, the muscle potential is present. Arrows indicate inspiration. See note on figures, page 21 .

then divided longitudinally into four or five smaller strands and spread out on the glass plate with fine sharpened needles. The recording electrodes were now inserted carefully under one nerve strand which was kept in position without attaching its free end to the distal electrode. The activity in it was recorded with a differential resistance capacity coupled amplifier connected to a double beam cathode ray tube and loud speaker. If there was more than one active unit in the strand it was divided further with needles - sometimes with the aid of a binocular microscope (x36).

To determine the conduction velocity of the single units a 5 mm. length of the whole vagal trunk was dissected out low down in the neck and placed on a pair of stimulating electrodes. At this stage the recurrent laryngeal nerve was cut where it lay beside the trachea in order to avoid the stimulation of the laryngeal nuscles which distorted the records by the picking up of diaphasic muscle responses (fig. 2). With this precaution, the use of the insulated Sherrington stimulating electrodes, which had been used in preliminary experiments, was no longer necessary. This was an advantage since their use occasionally interfered with the blood supply to the nerve. The vagal trunk was then stimulated and the action potential examined to see if it was compound which it invariably was in spite of the fact that

only one single active unit was being recorded. This was to be expected, however, as there were usually a number of inactive live fibres in the strand containing the active one. The next step and the most difficult of all therefore was to try to eliminate as many of these inactive live fibres as possible by further subdivision of the already fine nerve strand. This step was materially aided by a Zeiss binocular dissecting microscope. Subdivision of the nerve was accomplished by placing the sharp dissecting needles in the centre of the strand and separating the two bits apart with gentle pressure. After the final subdivision, and only then, the free end of the thin nerve strand was twisted around the distal electrode to ensure the recording of monophasic action potentials.

In the earlier experiments the animal was placed in a shielded chamber of the type used by Whitteridge (1948) moistened with steam to prevent drying of the nerve. Dissection of single units was done inside the box which had the disadvantages that it limited the movements of the operator, and suitable magnification either with a binocular loupe or microscope could not be conveniently used. In addition the steam which condensed on the glass plate through which the preparation was viewed impeded the dissection although the defect could be considerably minimised by applying a detergent to the glass plate. Finally, there was the danger that the nerve fibres

would be damaged if condensed drops of water vapour fell on them. The box, however, had the great advantage that the ambient temperature of the nerve could be kept constant. In view of the difficulties encountered further use of the box was discontinued.

The temperature of the nerve was kept between 36.5°C. and 38°C. by warmth applied to the area of dissection with a carbon lamp, and by maintaining the rectal temperature at about 38°C. The importance of the latter was considerable as the vagus between the recording and stimulating electrodes was not freed from the surrounding tissues and was thus approximately at body temperature. Initially a thermocouple was used to measure the temperature of the nerve at three points along its length. Later this was replaced by a thermistor bridge circuit (Grieve, 1951) connected to three thermistors type F 2311 - 300. The output from the bridge was coupled to the galvanometer of a three channel continuous recorder manufactured by Electroflo Meters Co. Ltd.

The conduction distance of the vagus from the cathode of the stimulating electrodes to the proximal portion of the recording electrodes was measured with a pair of dividers allowing for the tortuosity of the nerve. These measurements were frequently checked at the end of the experiment by measuring the length of the excised vagus and were found to be sufficiently accurate.

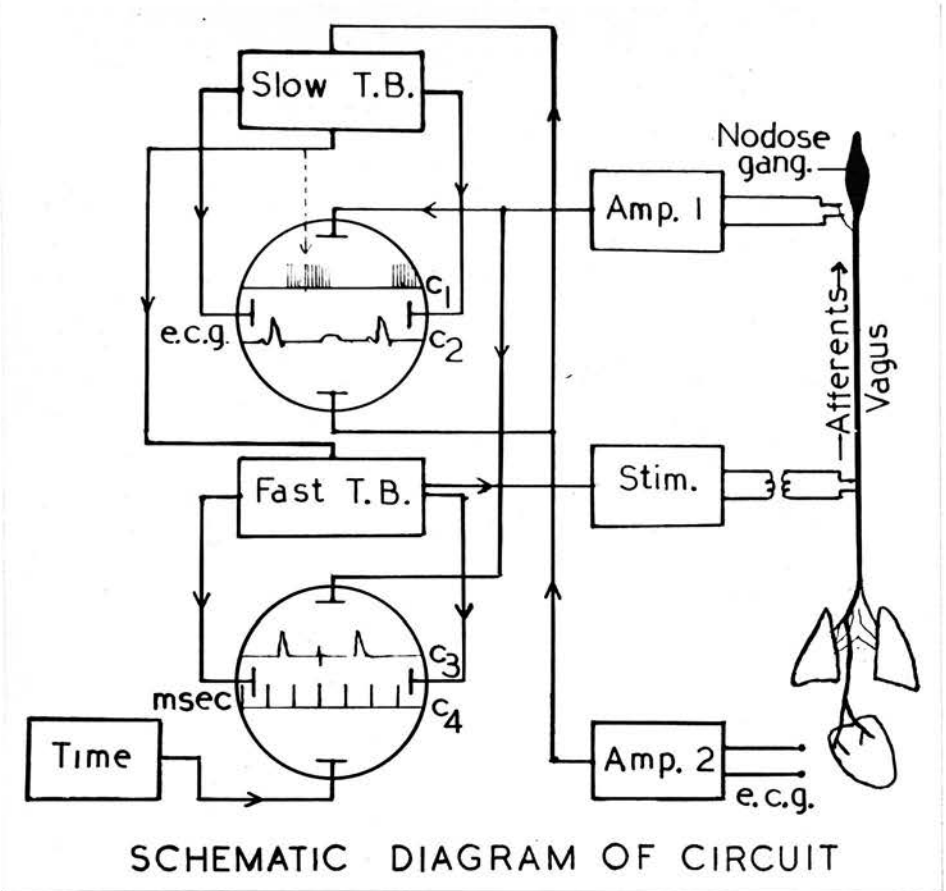

Fig.3. Note channels C_1 & C_3 are common.

To record the intrapleural pressure a wide bore intrapleural needle was inserted through the third or fourth intercostal space close to the sternum and connected to an air filled mirror membrane manometer of the Hamilton Wiggers type (1934).

Recording Circuit. Initially the circuit used had one time base which ran the sweep on two single beam 3" cathode ray tubes. With this arrangement a number of single units were recorded but only one of them, that of a pulmonary vascular fibre, has been considered in the present work. It soon became evident that an additional time base was necessary to provide a suitably fast sweep which would permit a detailed examination of the action potentials. A schematic diagram of the modified circuit is given in Fig. 3. In general the detailed circuits of the various parts have been based on those described by Dickinson (1950). Basically the circuit used consisted of two resistance capacity coupled differential amplifiers, the first one being used to amplify the action potentials of the nerve fibres and the second to amplify the E.C.G. Lead 1. Two time bases, a slow and a fast ran the sweep on two double beam cathode ray tubes respectively. A time marker recorded milliseconds on one beam of the second tube. To operate the circuit the E.C.G. after amplification was used to trigger the slow time base which ran a slow sweep with a duration of 0.2 to 0.5 seconds on

the first double beam cathode ray tube. The output from the Miller anode of the first time base was connected to a Schmitt trigger which then triggered the second fast time base. This ran the sweep at a much faster velocity on the second tube. By means of this any part of the slow sweep on the first tube could be examined in much greater detail on the second one thus enabling a simultaneous comparison of the spontaneous and evoked action potentials from nerve stimulation to be made.

A stimulator which was locked to the fast time base provided the stimuli which could be varied in duration and intensity. The usual duration of the stimuli was about 150 μsecs. and the intensity ranged from 10 to 30 volts.

An earth electrode of thick silver wire was embedded in the subcutaneous tissue in the neck placed near to the recording electrodes to reduce the size of the stimulus escape.

To take a photographic record (4:1 reduction) the spots on the first tube were stopped and the repetition frequency of the sweeps on the second tube reduced so that no overlapping of the sweeps occurred on the photographic record. With this procedure a record of the single units could be taken simultaneously with records registering the conduction velocity. The camera was run by a velodyne motor to ensure constancy of speeds which could be adjusted to

run the recording paper between 0.5 and 8 centimetres per second.

The frequency of discharge of impulses of some single units with a cardiac rhythm has been plotted. This was done by measuring the points of occurrence of impulses with respect to the Q wave of the E.C.G., recorded simultaneously, and plotting the reciprocal of the time interval between impulses against the midpoint of the intervals.

Sources of Errors:-

An estimate of the errors arising from the measurements of conduction distance was obtained by comparing the lengths of nerves measured in situ with those measured after excision. The differences between the two measurements was never greater than 3% - the determinations being made to the nearest 0.5 mm. In the determination of conduction velocity of nerves the errors due to variation in temperature are usually considerable, the Qio for frogs nerves being 1.7 (Gasser, 1931). Great care was therefore taken to maintain the temperature of the vagus as near the body temperature as possible. The average temperature of the vagus as measured at 3 points was kept fairly constant in different experiments at 37.5 $\pm$ 1°C. However there was reason to believe that the variation more often was less than 1°C as the greater part of the vagus was not separated from the surrounding tissues and was thus kept at

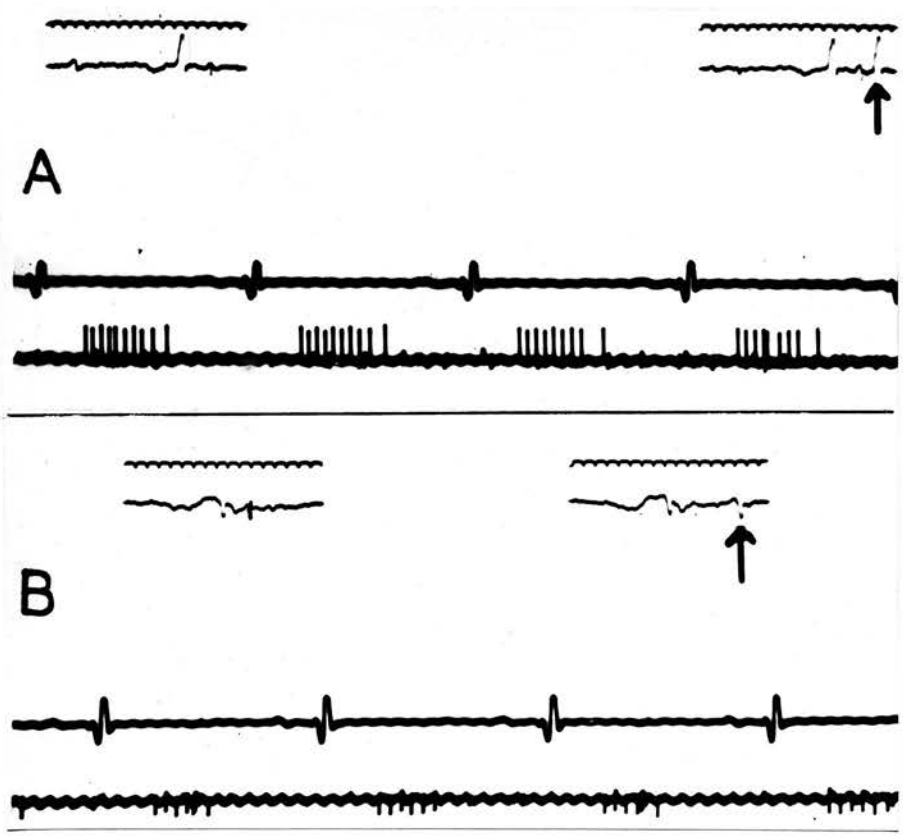

Fig.4. Conduction velocity in a depressor fibre determined with negative (A) and positive (B) spikes. In both cases the calculated conduction velocity was the same. Arrows indicate positions of spontaneous spikes. Conduction distance = 58mm. Temperature = 38° C.

body temperature.

The conduction time was measured on the photographs in which the reference points were the beginning of the stimulus escape and the beginning of the negative inflection of the monophasic spike. The distance between these points and corresponding measurements of the time trace were made with a travelling microscope. For each afferent fibre three pairs of such measurements were made in three different sweeps to allow for variations in the shock response time. In cases where the variation in conduction time for individual measurements exceeded 4% the final result was estimated by averaging five determinations in different sweeps. Hunt and Kuffler (1951) quoting Blair and Erlanger (1936) deducted 0.1 msec. from the stimulus response interval for 'setting up' time at the stimulating cathode in their measurements of the conduction velocity of mammalian nerves. The choice of this value of 0.1msec is apparently arbitrary and the allowance was therefore not made in the present determinations.

In some records in which the single unit preparation were very thin the action potential was triphasic with an initial small short-lived positive deflection. In such experiments the conduction time was measured up to the beginning of the negative inflexion of the spike. In other records the action potential consisted of an almost pure positive de-

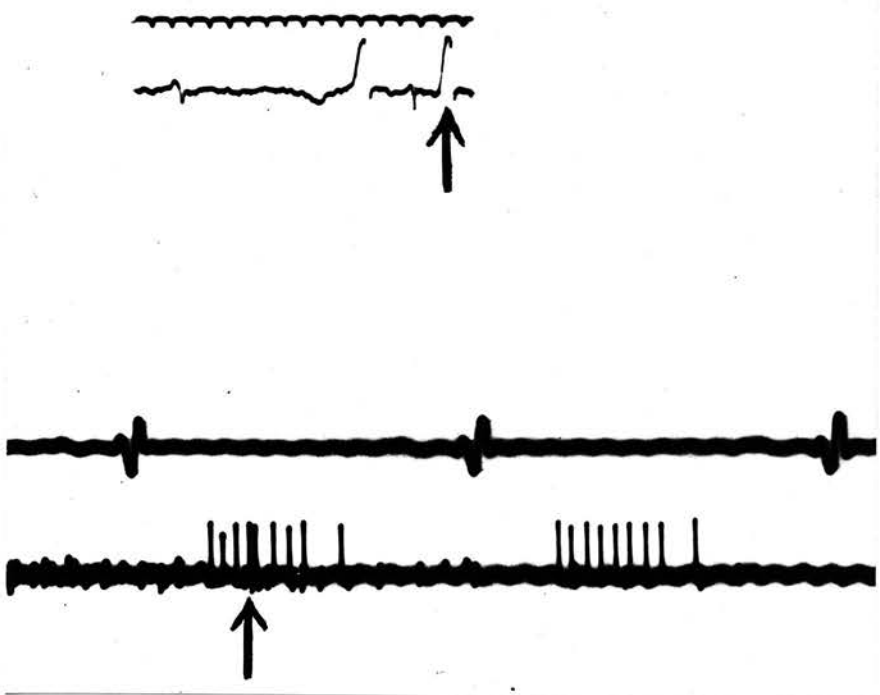

Fig.5. Similarity of spontaneous and evoked action potentials in a depressor fibre. In the upper trace the spontaneous action potential (at arrow) is followed by the stimulus artifact and then the evoked action potential. The two are seen to be identical and their position is indicated in the record of the single unit by the lower arrow.

flection and in these the conduction time was measured up to the beginning of the positive deflection. These changes in the action potential were believed to be due to injury in the nerve fibres just before they reached the recording electrodes since the positive deflection could be converted into a negative one by shifting the recording electrodes near the nerve trunk. Measurements of conduction velocity under the two conditions cited revealed no significant difference in several instances. (Fig. 4). However any error arising from this cause did not affect the conduction velocity determinations of the slowly conducting fibres while in those with a faster conduction velocity care was taken to reduce them to a minimum.

RESULTS.

In the determination of the conduction velocity of particular afferents it was necessary first of all to prove that the afferent fibre showing spontaneous activity in the form of a single functional unit was the same as the one whose conduction velocity was being determined by stimulation. In order to establish their identity the following criteria were used in the determination of the conduction velocity of each afferent fibre:-

1. The action potential produced by stimulation should be simple, and all or none in character.

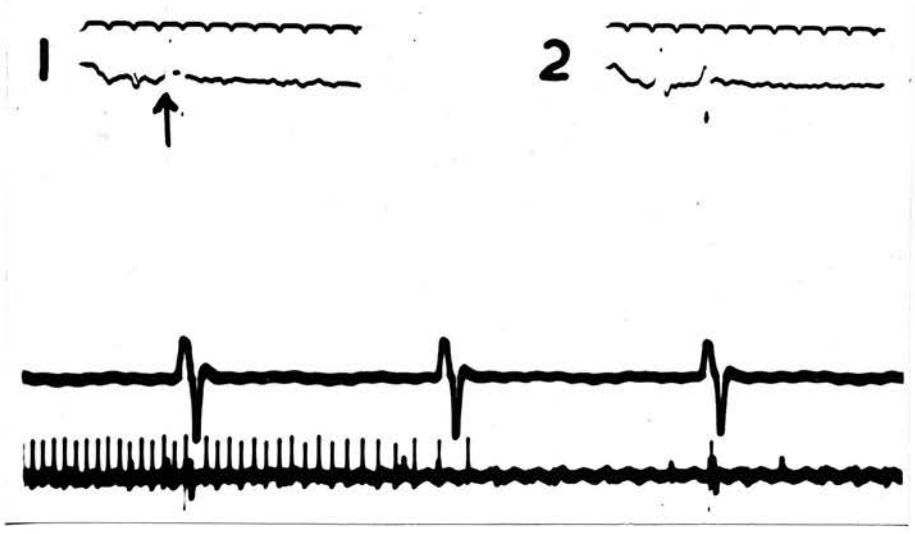

Fig.6. The effect of the absolute refractory period on the evoked impulse. In sweep 2, the stimulus escape is followed by the evoked spike. In sweep 1, a spontaneous impulse has appeared after the stimulus artifact (at arrow). The evoked spike is now absent owing to the fibre being in the absolute refractory state following the appearance of the spontaneous spike.

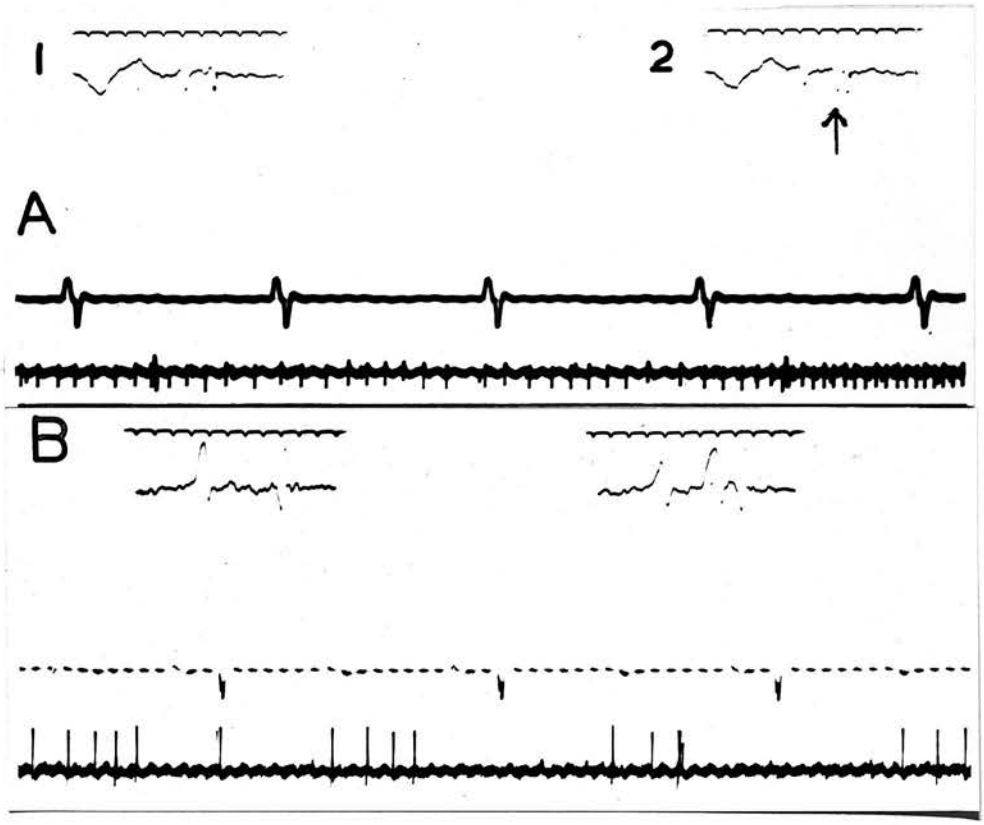

Fig.7. The effect of the relative refractory period on the evoked spike. A; In sweep 2 the occurrence of a spontaneous impulse (at arrow) has left the fibre relatively refractory which now conducts at a much slower conduction velocity as compared with sweep 1. In B, which is a record of a pulmonary vascular fibre the evoked spike in the 2nd sweep is reduced in size being in the relatively refractory period of the preceeding spontaneous impulse.

2. It should resemble the action potentials produced by spontaneous activity (viz. of the single unit) in amplitude and form.

3. It should disappear or be reduced in size and conduction velocity if it were so timed that it fell within the absolute or relative refractory period respectively, consequent upon the appearance of a spontaneous impulse.

Figures 4 - 7 show single action potentials produced by artificial stimulation which was ascertained to be all-or-none by varying the intensity of the stimulus and noting any change in the amplitude of the action potential. Further the duration of the spikes shown, about 0.6 msec., is approximately that shown by Gasser and Grundfest (1939) although it was found to vary in different fibres. It is possible, however, that two afferent fibres with exactly the same conduction velocity and threshold to stimulation could occur. Under the circumstances the action potential would be clearly summated and larger than the spontaneously occurring spikes. This could be tested by further subdivision of the strand although the result did not affect the determination of the conduction velocity of the fibre.

Figure 5 shows the occurrence of spontaneous and evoked action potentials in the same sweep. Measurements of their amplitude and duration show that they are identical. Further their rate of rise

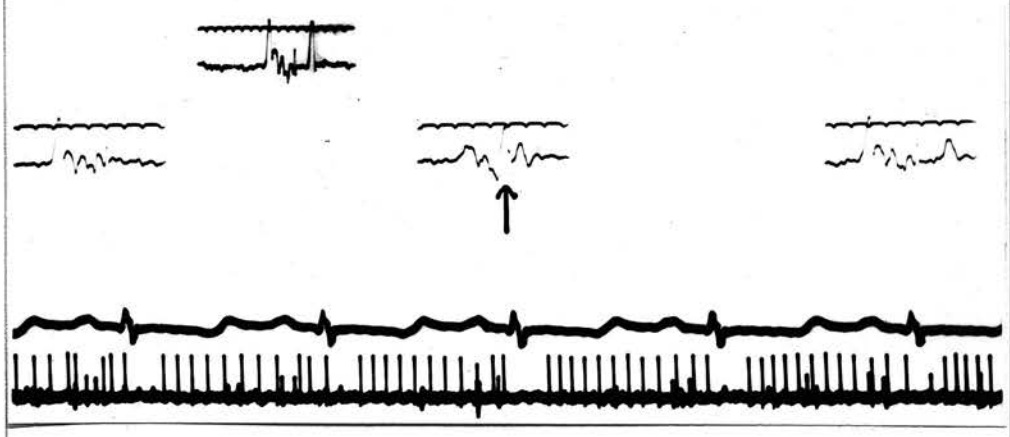

Fig.8. The determination of conduction velocity in a multifibre preparation. At arrow the appearance of a spontaneous impulse of the venous unit knocked out the particular component of the compound action potential. The top sweep at a slower velocity shows the similarity of the spontaneous spike with the portion of the compound action potential (large spike) knocked out in the middle sweep.

and fall is the same as could be confirmed by superimposing one on the other.

In most fibres, particularly those with a cardiac rhythm, a spontaneous impulse could be made to fall at a varying interval after the stimulus artefact by means of the circuit described earlier. This enabled one to put the fibre in an absolute refractory state for the duration of the spike (Adrian 1921, Gasser and Grundfest 1936) followed by a variable period of relative refractoriness lasting about 3 msec. If timed suitably this led to the complete disappearance of the evoked spike (Fig. 6), provided the fibre was totally refractory, or to a reduction in its amplitude and conduction velocity if it fell within the relatively refractory period (Fig.7). When such proof was obtainable it was not considered necessary to have a single live fibre preparation provided the compound action potential consisted of not more than two to three discreet and easily identifiable components. Figure 8 illustrates this clearly.

CONDUCTION VELOCITY OF PULMONARY STRETCH AFFERENTS

The conduction velocities of 42 pulmonary stretch afferents were determined. These afferents were identified by the increasing discharge of the impulses during a normal inspiration and by their response to a positive pressure inflation. Two types were distinguished according to their rate of

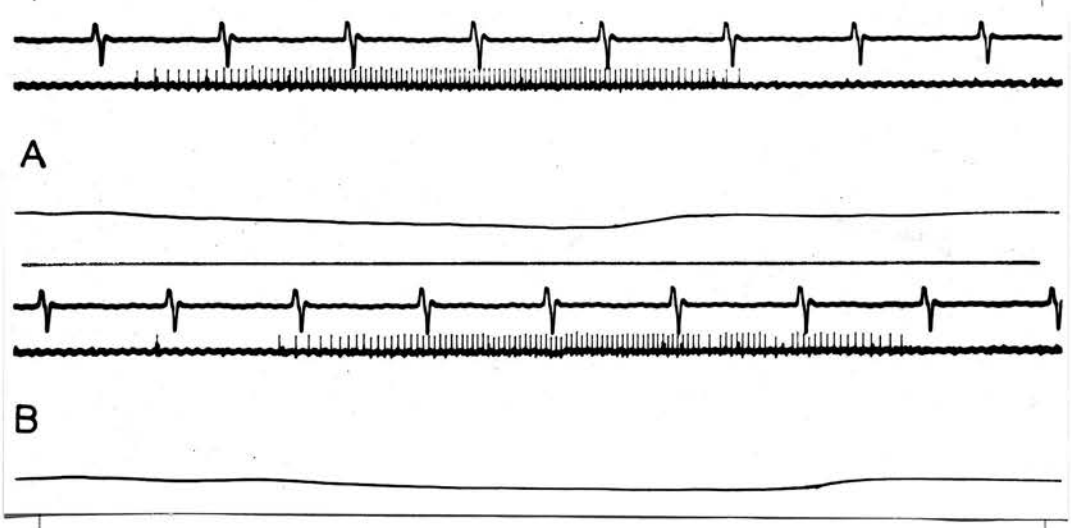

Fig.9. A slowly adapting stretch fibre during two normal respirations. A & B are continuous.

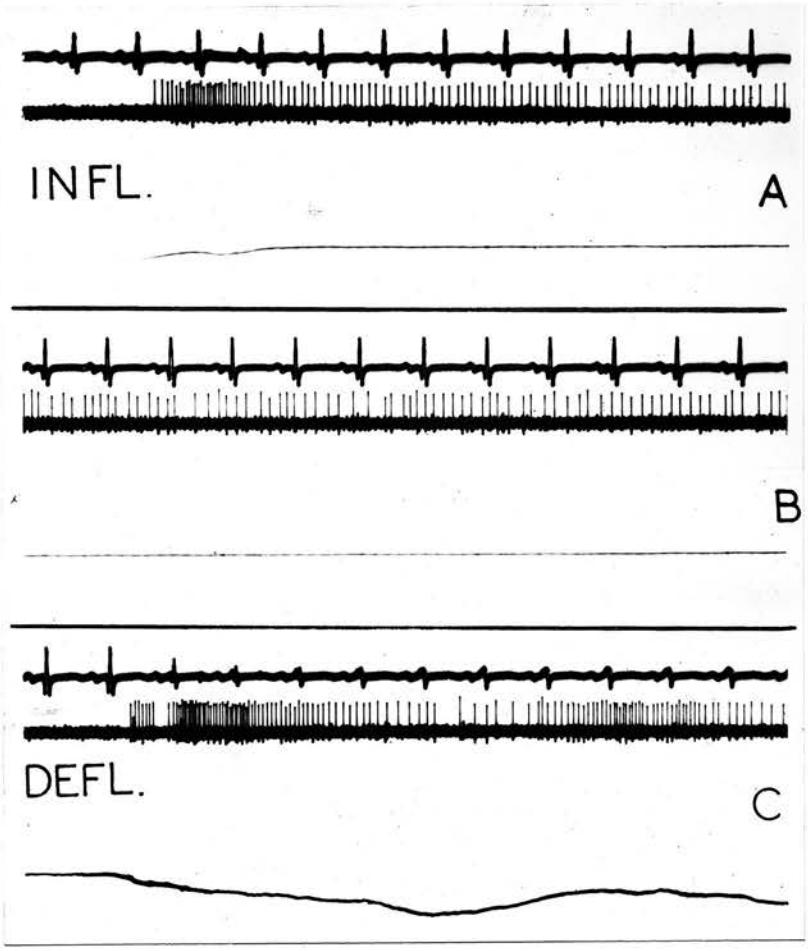

Fig.10. A, slowly adapting stretch fibre. A & B, which are continuous show response to an inflation. C, shows the slowly adapting response to suction of air from the trachea.

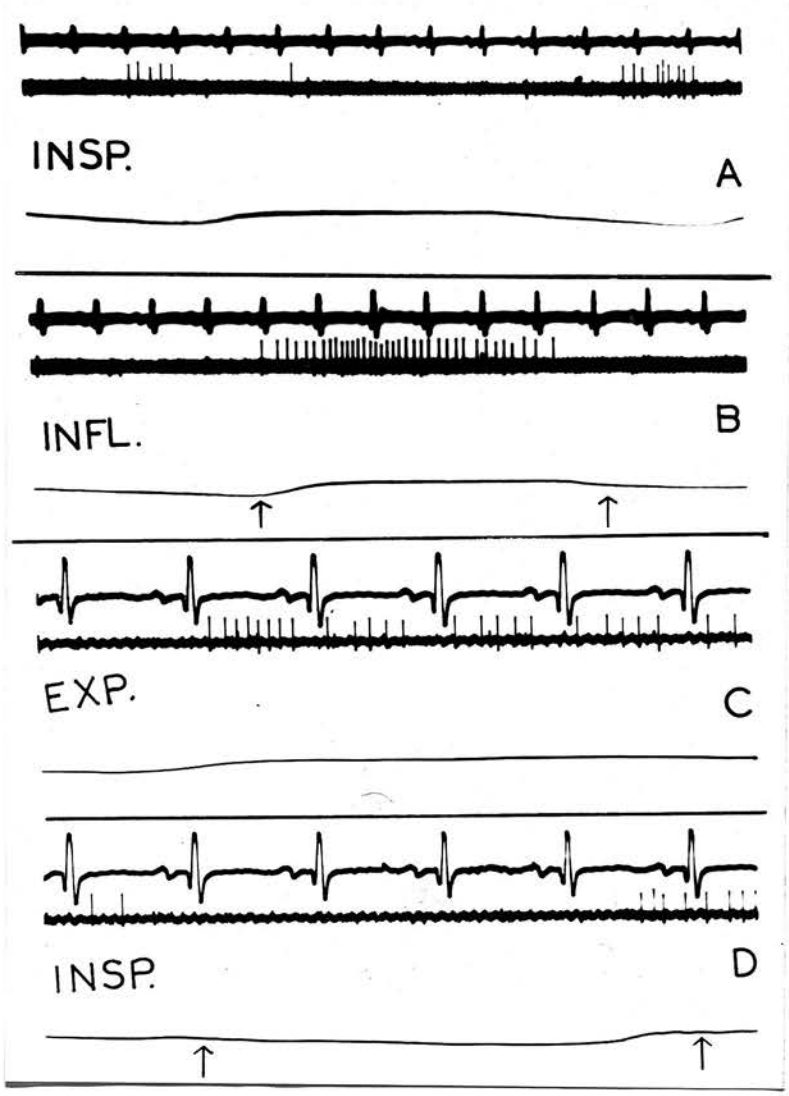

Fig.11. A slowly adapting stretch fibre. A, normal inspiration; B, inflation of the lungs; C & D, (continuous) with gentle pressure on the chest. Note that the fibre fires during expiration (C), and not during inspiration (D). Note the cardiac rhythm in C.

adaptation to a rapid and sustained inflation of the lungs by positive pressure.

The slowly adapting fibres (fig. 9) described in detail by Adrian (1933) formed the large majority (90.5%). It is very likely that the percentage is still higher since this figure takes no account of a large number which were encountered in the later part of this investigation and for which conduction velocities were not determined. As observed by Adrian many of these fibres fired on deflation as well as on inflation (fig.10). The external application of gentle pressure on the chest by the hand caused a few of these receptors to fire in the expiratory phase of the respiratory cycle with no activity driving inspiration (fig.11). Although this is explicable as being due to a partial collapse of the lung, the effect on the respiratory centre of such discharges during expiration if sufficient in number would need to be considered in the interpretation of reflex changes produced by a positive pressure deflation.

The rapidly adapting fibres of Knowlton and Larabee (1946) constituted the remaining 9.5%. This figure is lower than that given by Knowlton and Larabee for comparable experiments on animals with intact chests. Only units with an adaptation index of 100, namely those dropping out of action by the end of the first second of inflation, have been classified as rapidly adapting fibres. The effect

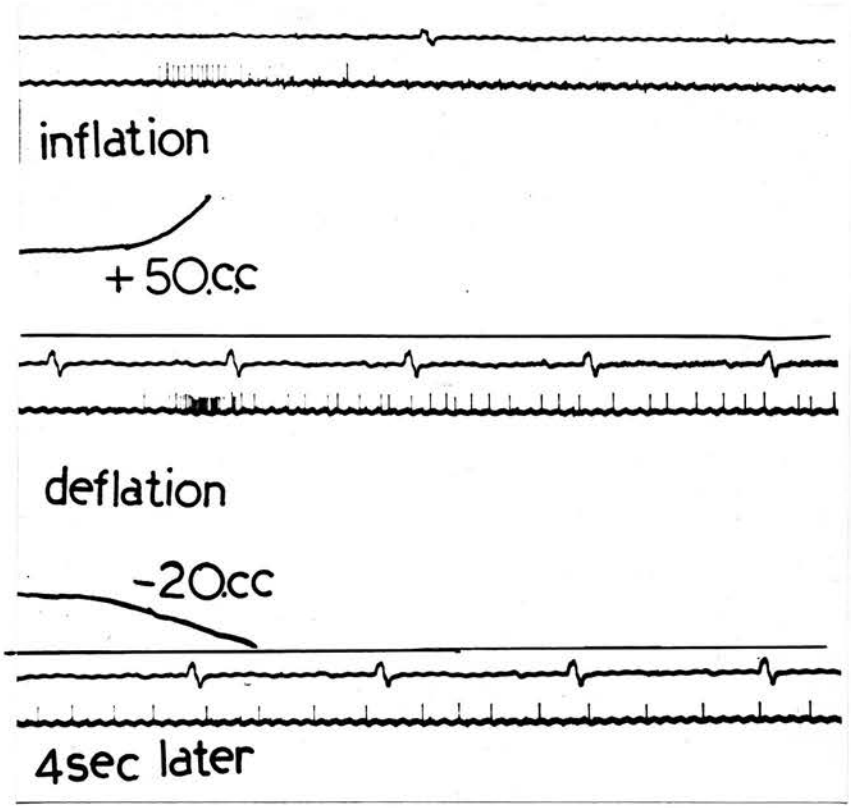

Fig. 12. Rapidly adapting stretch fibre. Upper record, response to a rapid and maintained inflation with 50 cc. air by a pump. Lower two, slowly adapting response to withdrawing 20 cc. air from the lungs.

of inflation on such fibres is shown in fig.12 which also shows the slowly adapting response of the same fibre to a negative deflation. The remaining fibres did not fire on a forced deflation at all. This observation differs from that of Knowlton and Larabee who found that about two-thirds of their rapidly adapting receptors could be stimulated by withdrawing 20 cc of air from the lungs. All the rapidly adapting fibres encountered here had a high threshold to inflation. The mean conduction velocity of these fibres was 26.3 metres/sec which is lower than the mean for all the stretch fibres. The difference is however not highly significant. Knowlton and Larabee found much larger differences. It is possible, however, that examination of a large number of rapidly adapting fibres might yield a significant difference.

A number of pulmonary stretch fibres with a superimposed cardiac rhythm first described by Adrian (1933) were observed (fig.11). These were chiefly of the slowly adapting type. Of the rapidly adapting fibres encountered, only one showed a cardiac rhythm. These fibres could be easily distinguished from the cardiovascular afferents with a cardiac rhythm by the loss of the cardiac rhythm on positive inflation of the lungs and its conversion into a steady continuous discharge. Further, their activity could be reduced or abolished by a negative

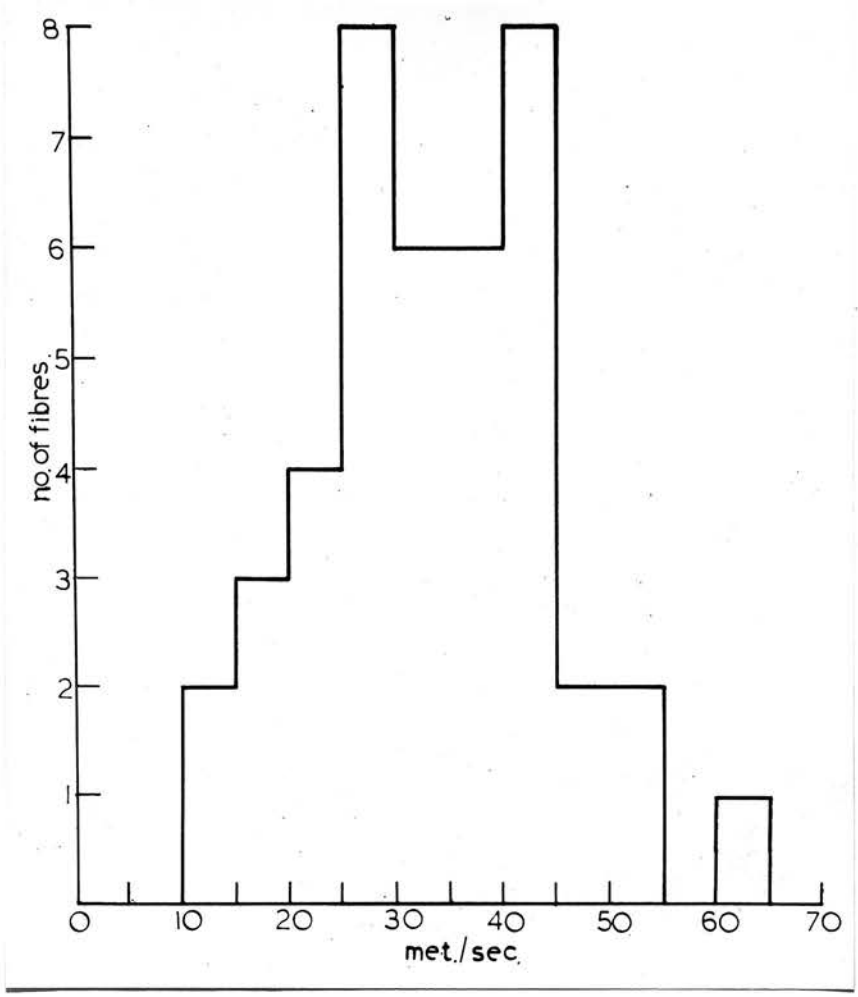

Fig.13. Frequency distribution of the conduction velocities of 42 pulmonary stretch afferents.

deflation. Presumably, as suggested by Adrian, these receptors are situated near the root of the lungs and are deformed by the pulsation of the heart and great vessels. It may be of interest to note that the mean conduction velocity of seven of these fibres is 27 metres/sec. The difference from the mean of all the stretch afferents is not necessarily significant however.

The frequency distribution of all the pulmonary stretch afferents is shown in fig.13 for which the pertinent data are as follows:-

Range - 13.9 - 60.5 mt/sec

Mean - 34 mt/sec

S. D. - 11 [illegible]

S. E. - 1.9 [illegible]

Although the range of conduction velocity is high it is likely that the lower limit is actually less than that presented. The sampling of fibres was subject to a slight bias leading to a selection of larger at the expense of smaller, that is slower conducting ones, since the chances of survival of the smaller fibres for experimental examination are poorer owing to the greater susceptibility to asphyxia.

CONDUCTION VELOCITY OF PULMONARY VASCULAR AFFERENTS

The pulmonary vascular afferents described by Whitteridge in 1948 have been identified by methods

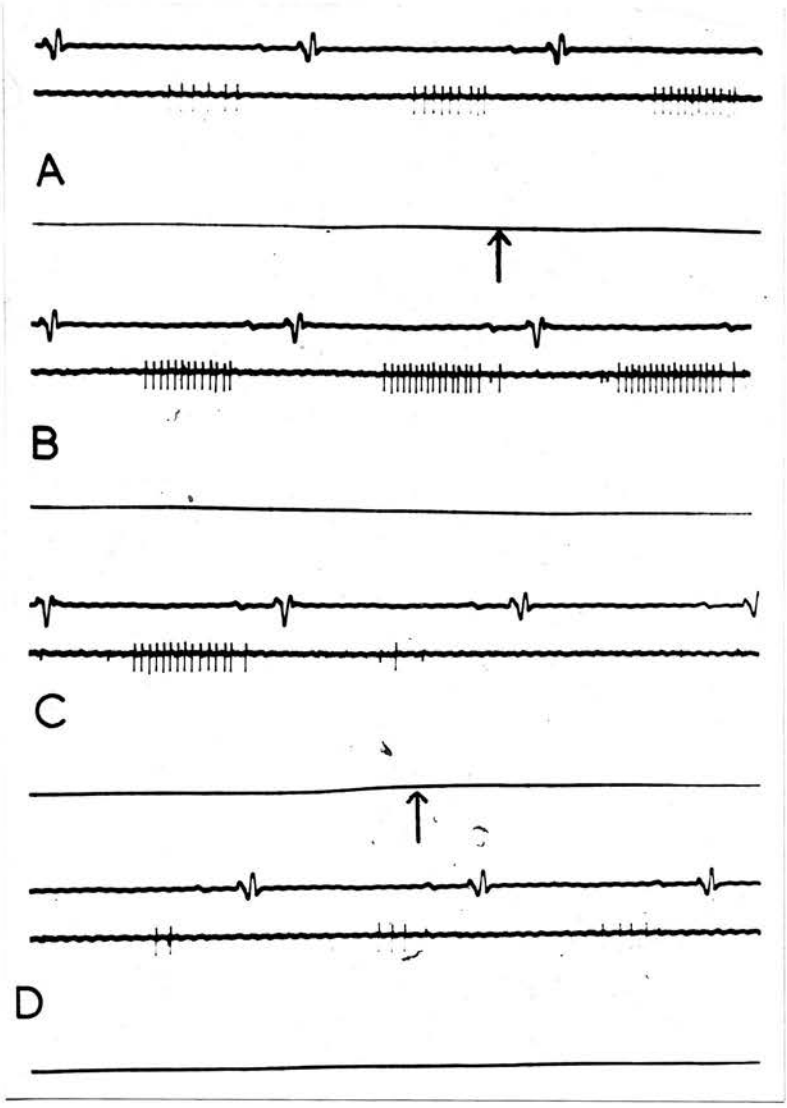

Fig.14. Pulmonary vascular fibre. Records are continuous. Note the effect of inspiration (between arrows) in increasing the activity of the fibre.

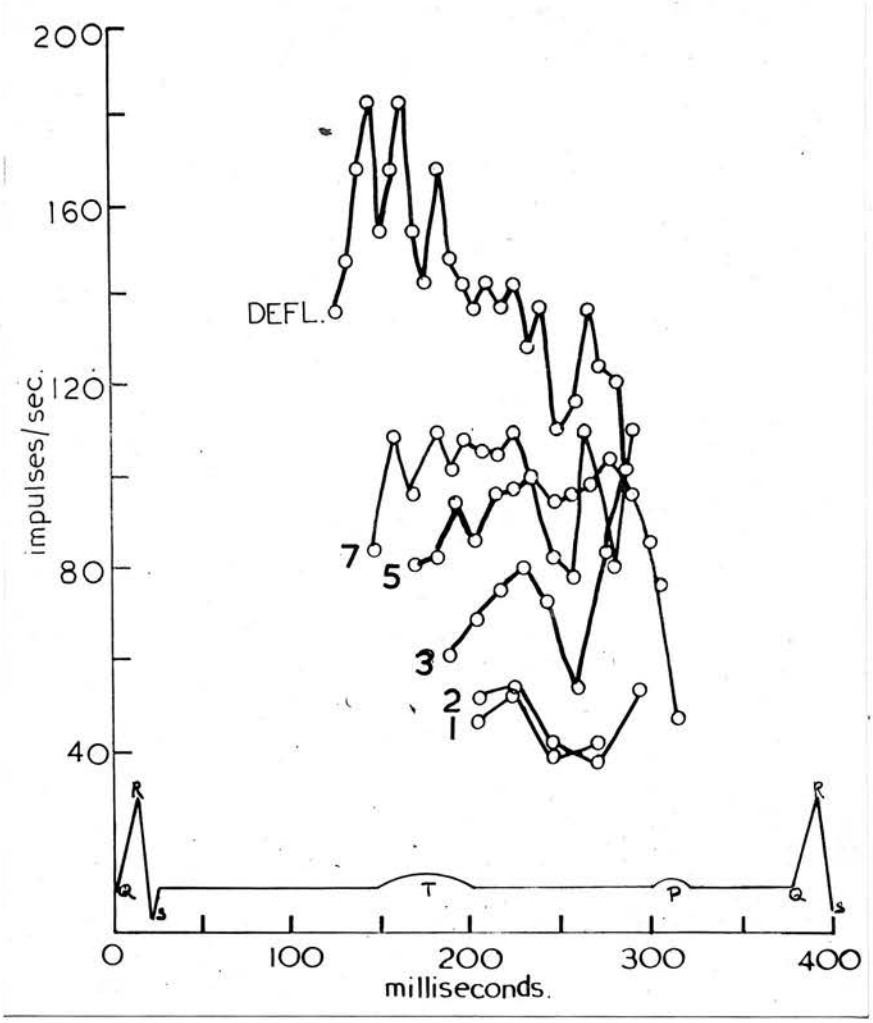

Fig.15. Plot of activity in a pulmonary vascular fibre in successive cardiac cycles during one inspiration 1-7, and during suction of air from the trachea (Defl.) Note the changing patterns. A representative E.C.G. is plotted below.

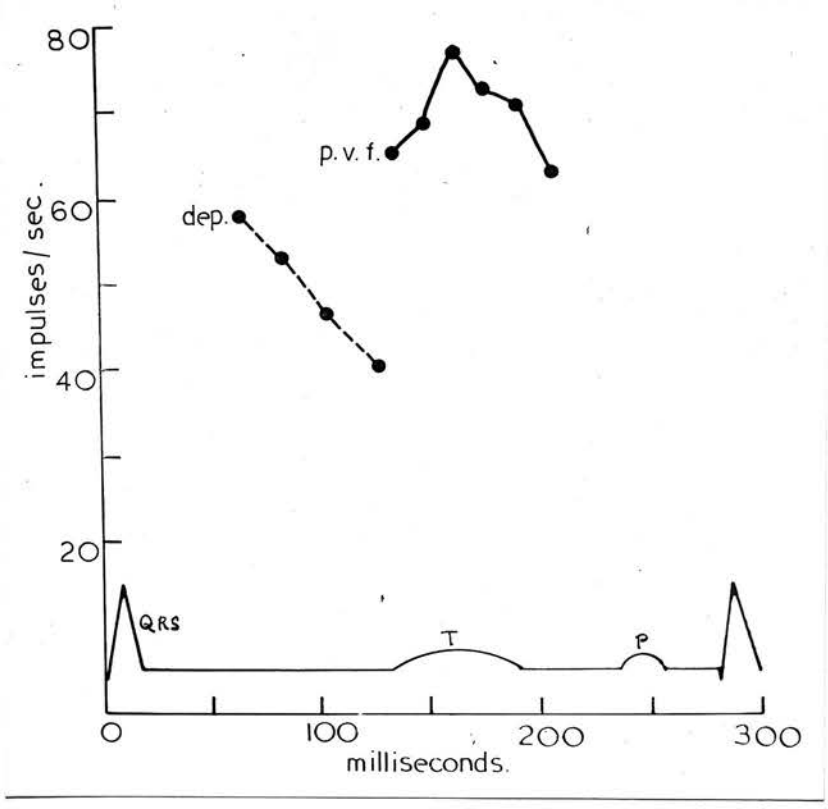

Fig.16. Plot of activity in a depressor and pulmonary vascular fibre recorded simultaneously.

used by him and later by Whitteridge and Pearce (1951). These fibres have a cardiac rhythm and their chief distinguishing feature is their late systolic discharge (figs. 14,17-19), the peak frequency of discharge occurring at a time when the aortic pressure pulse would probably be on the decline. The beginning of the discharge was found to range from 73 to 210 msec. after the Q wave of the E.C.G. In the present investigation some fibres were seen in which there was a short burst of impulses occurring early in systole during the isometric phase of contraction, followed by the usual late systolic discharge. This type of a discharge has been described by Whitteridge (1951) and by Pearce (1951) Such fibres have not been considered in the examination of conduction velocities; only those fibres conforming to the typical description have been considered.

The curves describing the frequency of discharge of these fibres using the Q wave of the E.C.G. as the reference point showed many variations (fig.20). Some of these were notched but most of them appeared to have a rounded peak. The occurrence of the peak frequency of discharge ranged from 135-276 msec. after the Q wave of the E.C.G. - the mean value being 184 msec. Although the mean has little significance in this case it does emphasize the difference between these fibres and the depressor fibres in which the mean value for the occurrence

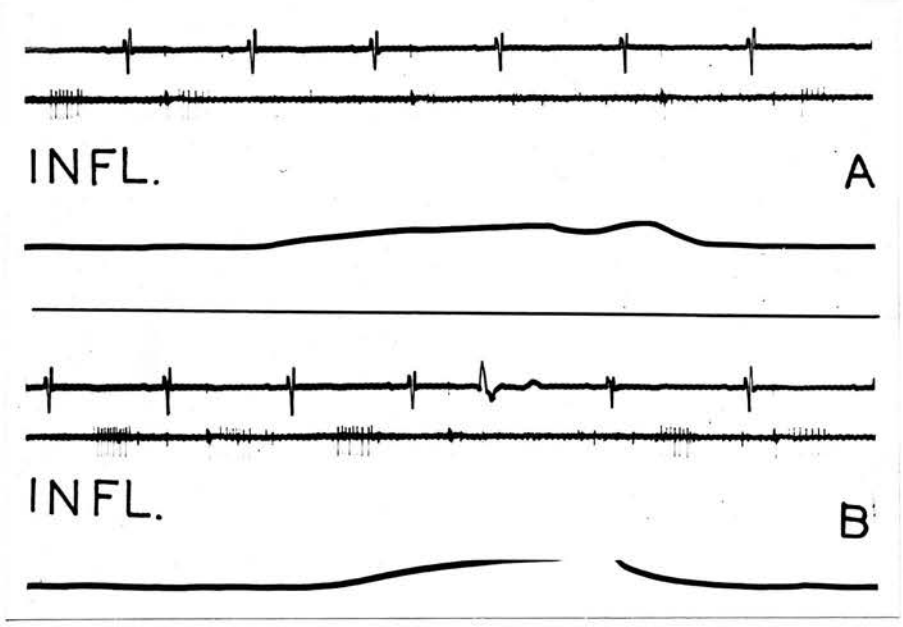

Fig.17. Effect of inflation of the lungs on the discharge of impulses in a pulmonary vascular fibre. Note the rapidity with which the activity returns after the inflation.

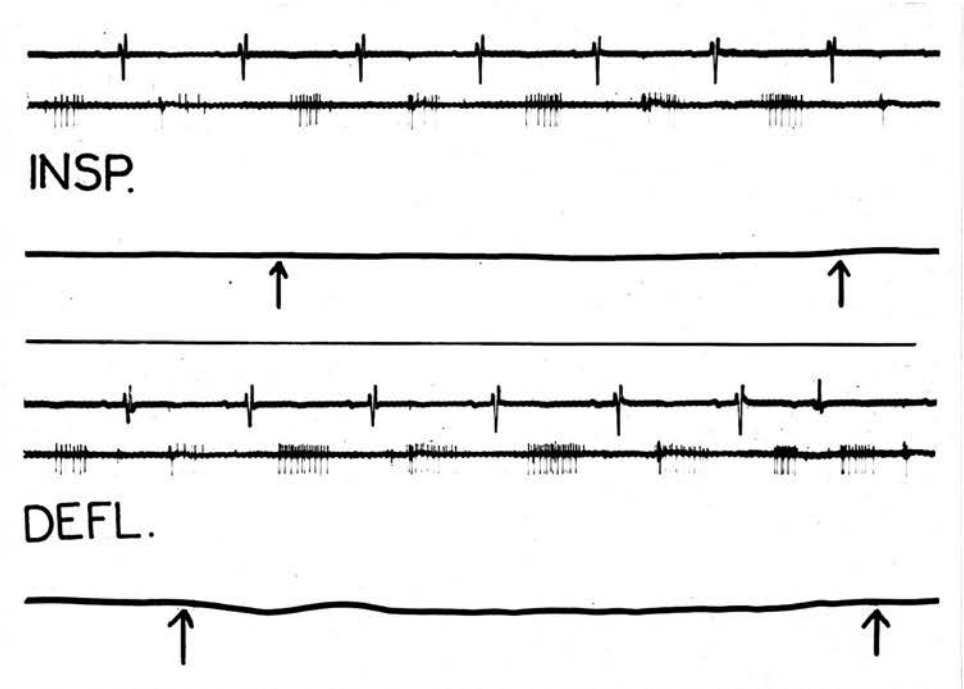

Fig.18. Pulmonary vascular fibre. Upper record shows the activity during inspiration (between arrows). Lower record response to sucking air from the trachea (between arrows).

of the peak frequency is 71 msec. In one experiment a depressor and pulmonary vascular fibre were recorded simultaneously and their activity has been plotted in fig.16.

In the pulmonary vascular fibres marked changes in the time of onset, number of impulses, and peak frequency of the volley occurred with various phases of the respiratory cycle (figs.15,16). As inspiration progresses the volley occurs earlier in systole and there is an increase in the number of impulses. The maximum change occurs at the height of inspiration while, as a rule, all activity ceases with the beginning of expiration. The number of impulses in each cardiac cycle during inspiration is plotted in fig 44. The increase may occasionally be 3 to 4 times the expiratory value. A similar increase is seen in the frequency of discharge, in which the peak value occurs at the height of inspiration. Fig.15 shows the variations in the frequency of discharge in successive stages of inspiration. It is clear that, apart from variations in activity in different fibres (fig.20), there is a considerable variation in the same fibre at different times.

The chief feature of these fibres which distinguishes them from other afferents is the rapid return of activity within two to three heart beats after the cessation of inflation (fig.17) which reduces or abolishes their discharge. This is a

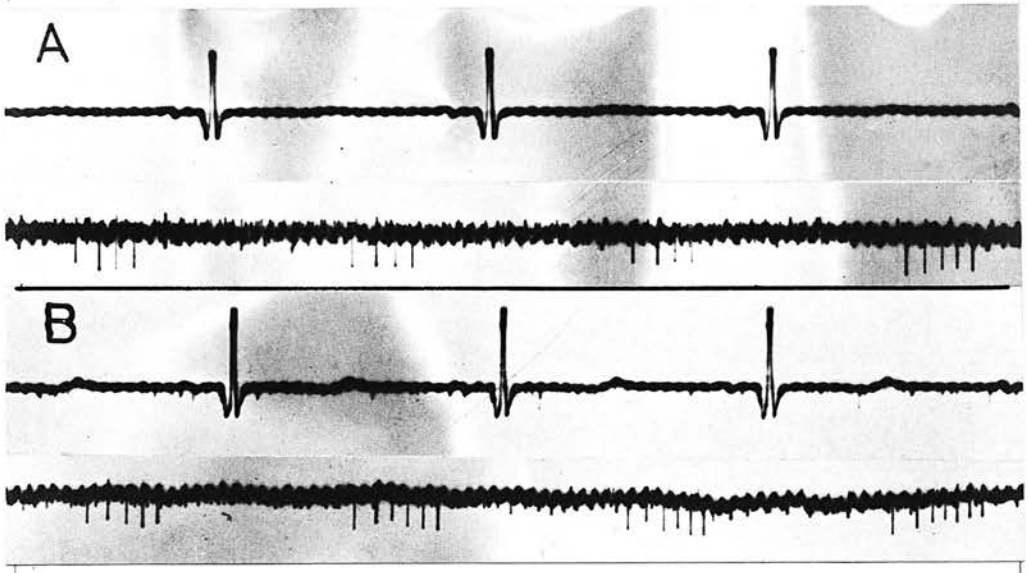

Fig.19. Pulmonary vascular fibre. A normal inspiration, (A); (B), Attempted inspiration after closure of the trachea.

TABLE 1.

PULMONARY VASCULAR AFFERENTS.

No. of fibre.	First impulse after ϛ wave in msecs.	Duration of discharge in msec.	Occurrence of peak frequency after ϛ wave in msecs.	Conduction velocity in m/secs.
1	209	101	276	11.4
2	134	140	227	14.8
3	76	138	145	12.1
4	111	97	199	17.0
5	97	139	169	11.2
6	73	149	135	23.2
7	150	51	161	8.4
8	129	87	166	9.8
9	169	94	188	7.9

Range of conduction velocity = 7.9 - 23.2m/sec.

Mean conduction velocity = 12.9 m/sec.

Standard deviation = 4.8

Standard error = 1.6

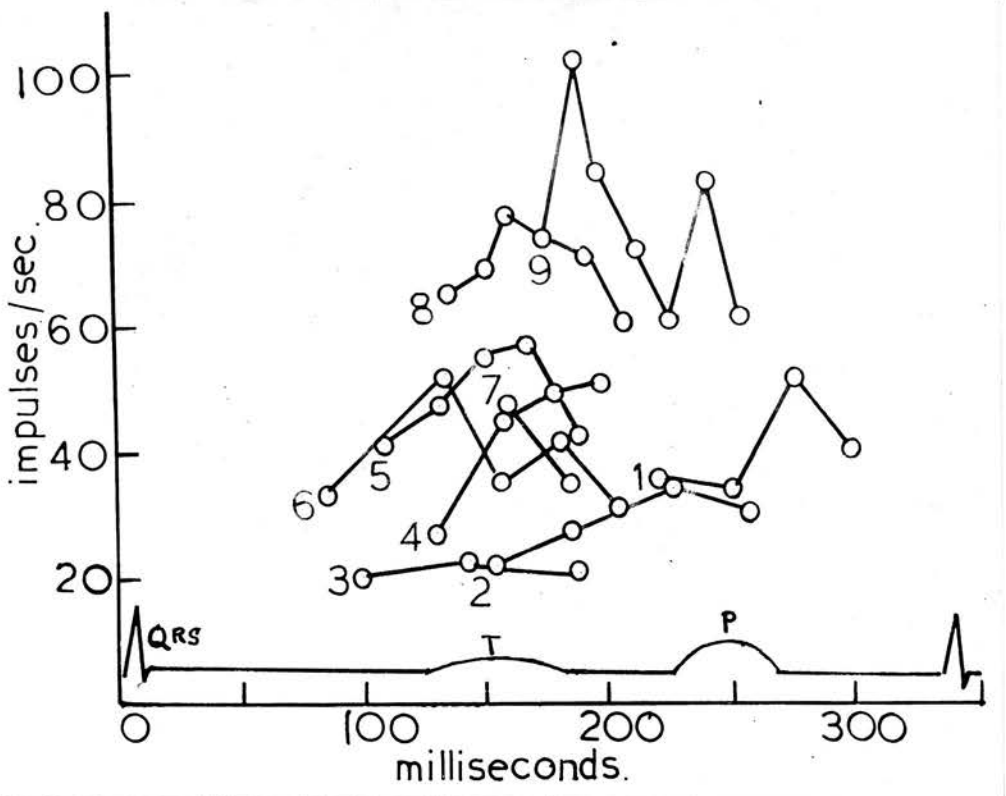

Fig.20. Plot of activity of all pulmonary vascular fibres studied showing variation in temporal configuration. A mean plot of the E.C.G. is made below.

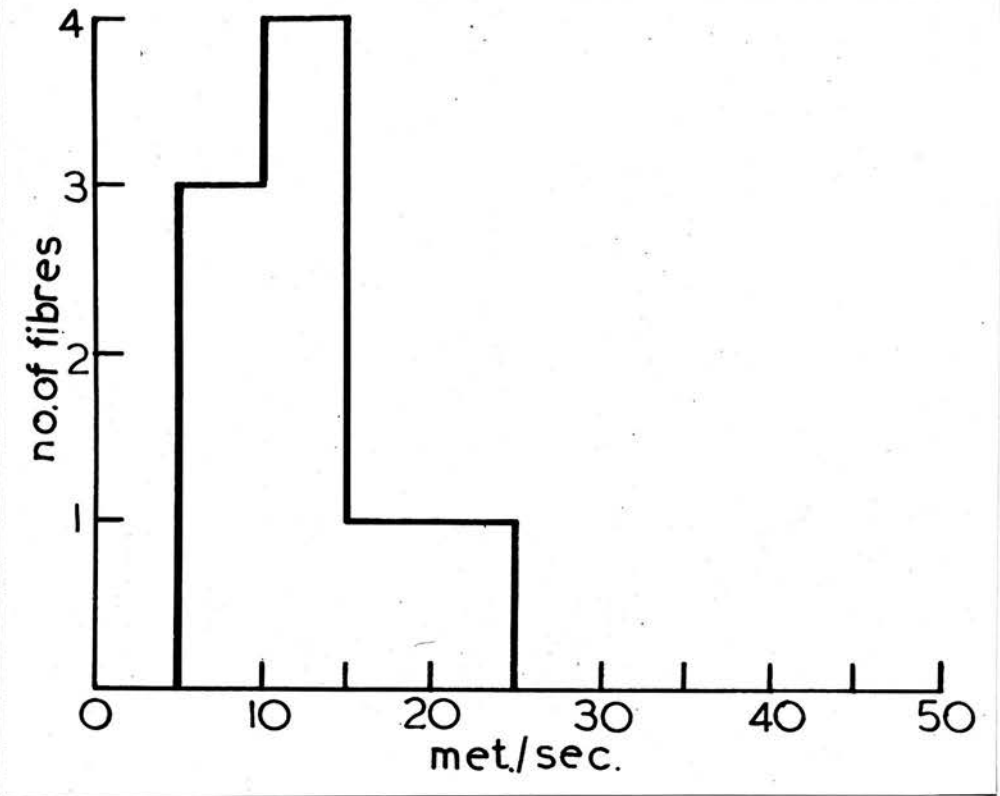

Fig.21. Frequency distribution of the conduction velocities of pulmonary vascular fibres.

consistent feature and has been observed in all the pulmonary vascular fibres encountered. The reasons for it have been discussed by Whitteridge (1948). This procedure successfully differentiated these fibres from stretch fibres with a cardiac rhythm in which the activity was increased on inflation with the frequent loss of the cardiac rhythm.

Suction of air from the trachea produced results similar to those observed by Whitteridge (1948), namely an increase in the number of impulses (fig.18) and the frequency of discharge (fig.15).

Another procedure which aided the differentiation of pulmonary vascular afferents from pulmonary stretch fibres was to close the trachea at the beginning of an inspiration. This invariably resulted in an increase of activity in the pulmonary vascular afferents (fig.19), while the pulmonary stretch afferents were unaffected by this procedure since no air entered the chest. The increased activity of the pulmonary vascular afferents was probably due to a reduction of intrapleural pressure consequent upon the animals attempts to breathe.

The conduction velocities of these fibres are presented in Table 1 along with other relevant data pertaining to each fibre; while fig.21 shows the frequency distribution of the conduction velocities, It can be seen that, although the mean conduction velocity is 12.9 m/sec., the majority of the fibres examined have a lower conduction velocity than this.

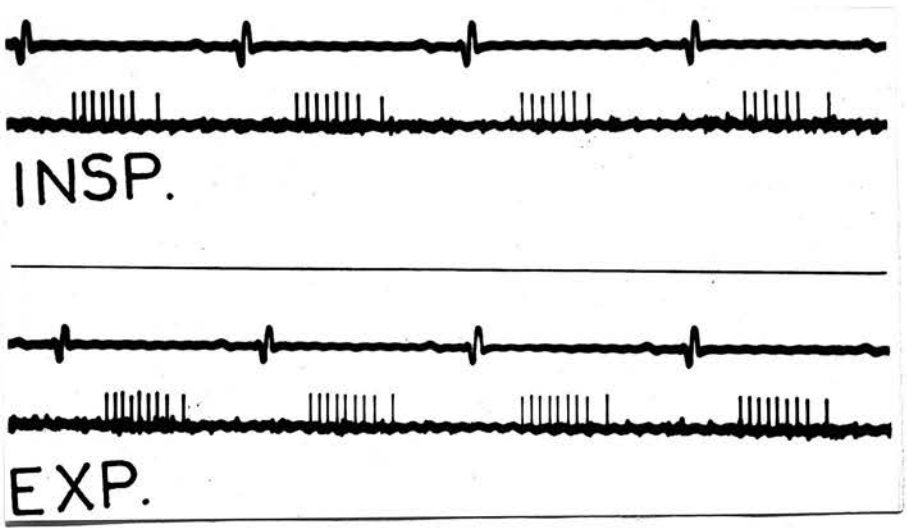

Fig.22. Record of a depressor fibre, illustrating the typical pattern of activity.

The range of values is narrower than that of the stretch fibres which is about 3 times greater.

CONDUCTION VELOCITY OF SYSTEMIC ARTERIAL PRESSORECEPTOR AFFERENTS

Nonidez (1935) has shown that, in addition to the pressure receptors found in the arch of the aorta, there are others in the walls of the subclavian artery, descending aorta, and the arteries to the aortic bodies. It is to be expected that the activity in fibres from all these receptors would be approximately synchronous since the systolic pressure wave should reach them at about the same time. Consequently all such fibres will appear to belong to a single group when classified according to the timing of their discharge. The aortic depressor fibres presumably formed the large majority.

These afferents are easily distinguished from others having a cardiac rhythm when their activity is heard on the loudspeaker. They are characterised by the early systolic volley (figs.22-23) which begins between 36 and 83 msec. after the Q wave of the E.C.G and reaches its peak frequency between 53 and 106 msec. (mean value 71 msec). This is very much earlier than the volley from the pulmonary vascular receptors and provides one means of distinguishing the two.

Another difference is that the activity of these fibres is much less influenced by respiratory move-

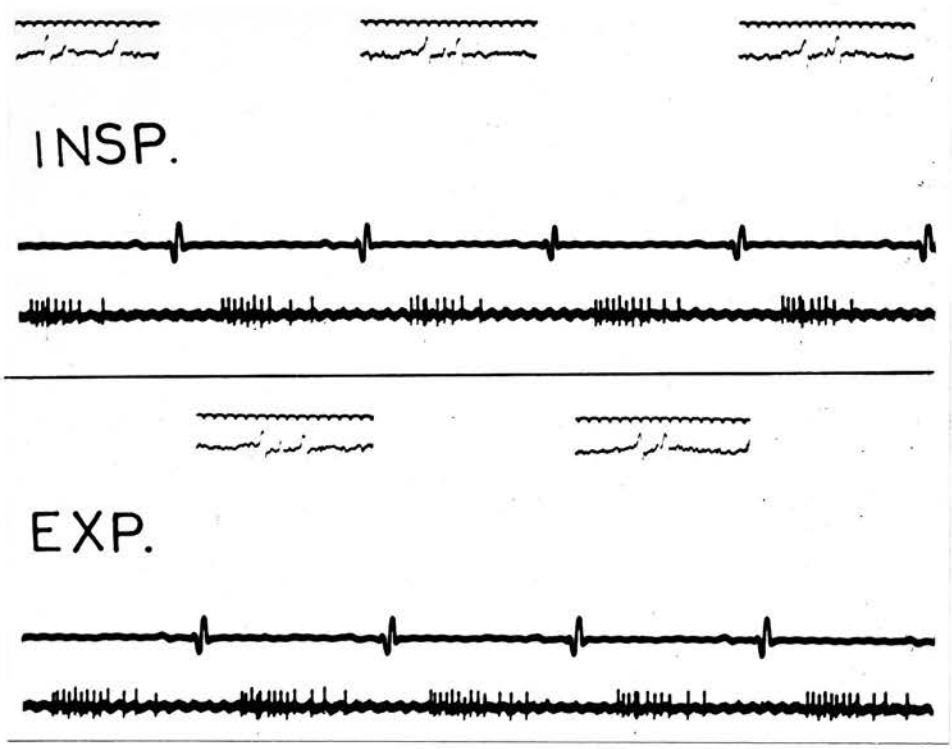

Fig.23. Depressor fibre showing increased activity during expiration. The occurrence of spontaneous impulses can be seen in all the sweeps. The frequency of discharge is plotted in figure 24.

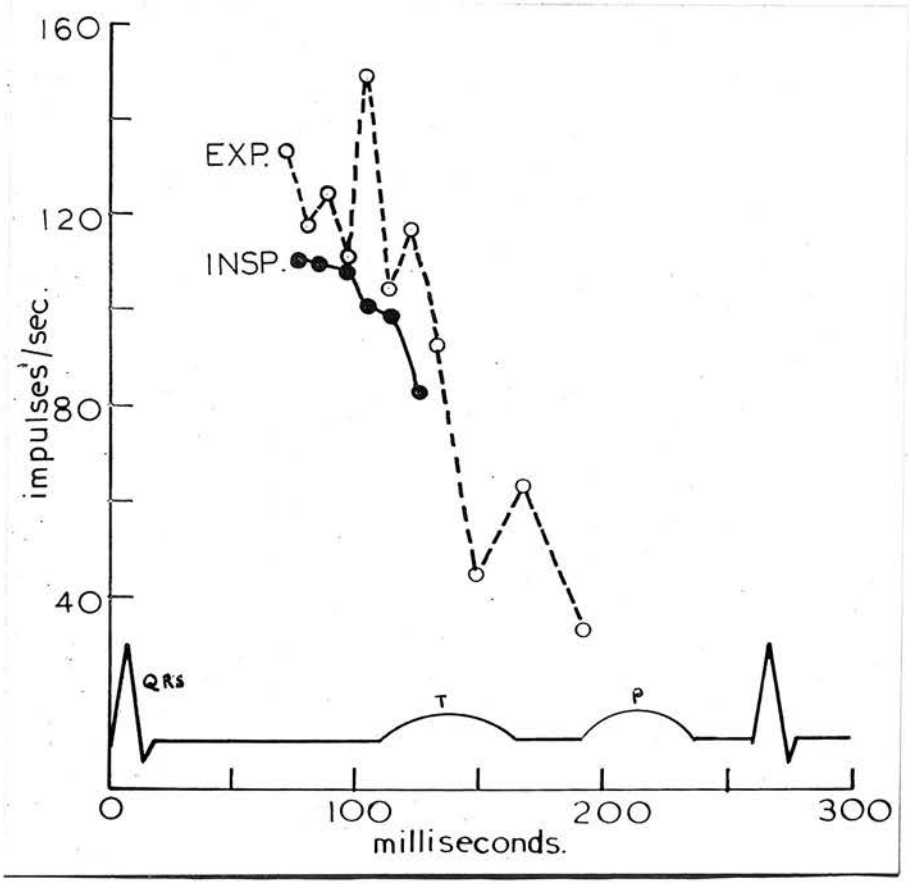

Fig.24. Plot of activity of a depressor fibre during inspiration and expiration.

ments (figs.22-23) than are the pulmonary vascular fibres. Further, the comparatively small variations in activity associated with respiration have a pattern which is different from that of the pulmonary vascular fibres. Maximum activity occurs during expiration (fig.24), usually at its beginning, whereas maximum activity in pulmonary vascular fibres occurs at the height of inspiration.

Inflation of the lungs with positive pressure reduces the activity of the systemic arterial receptors and this does not return to its original height for several heart beats after inflation has ceased. This is one of the most consistent features of these fibres for the purpose of distinguishing them from pulmonary vascular afferents in which maximum activity always returns within two to three heart beats.

Table 2 shows the conduction velocity of all the depressor fibres, the frequency distribution of which is shown in fig.26.

The range of conduction velocity of systemic arterial pressure of fibres is wide, being 21-46 m/sec. The entire range falls within the range of conduction velocity of the pulmonary stretch afferents. On the other hand, it is significant that there is hardly any overlap with the pulmonary vascular afferents.

The mean conduction velocity of 32 m/sec. is

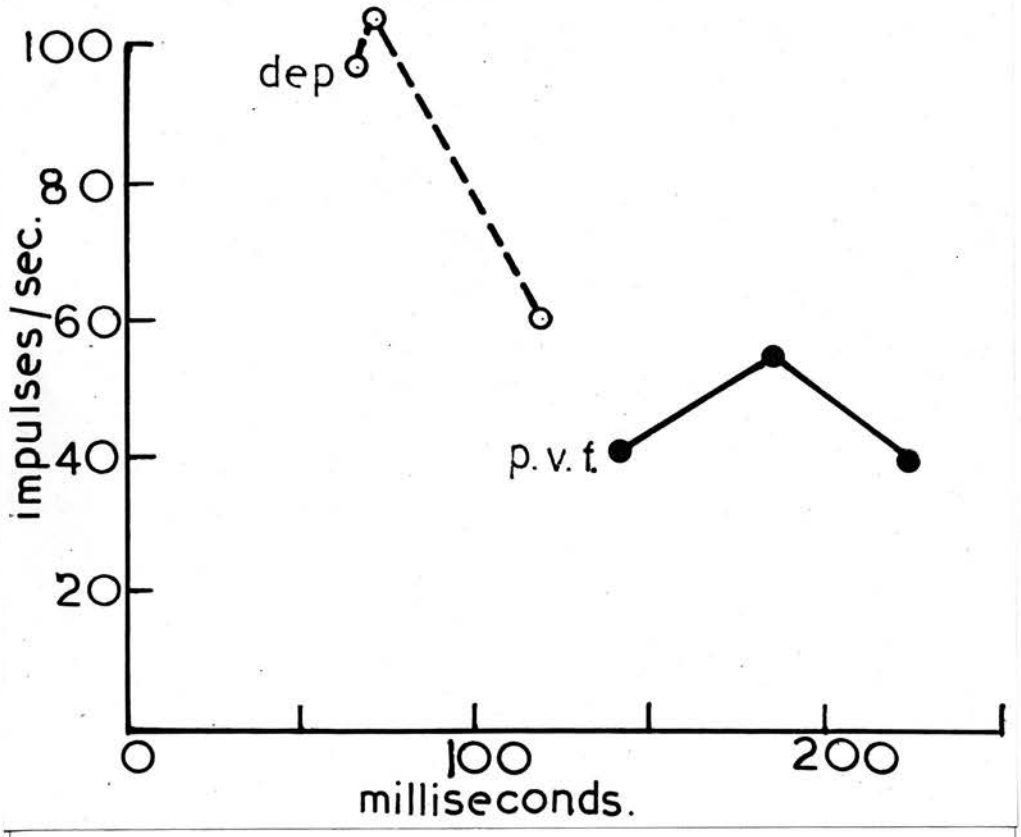

Fig.25. Plot of activity of all depressor and pulmonary vascular fibres. The points represent the mean values for the beginning, peak, and end of volley in each case.

TABLE 2.

SYSTEMIC ARTERIAL PRESSORECEPTOR AFFERENTS.

No. of fibre	Source of fibre D = Depressor V = Vagus.	First impulse after Q wave in msecs.	Occurrence of peak frequency after Q wave in msecs.	Conduction velocity in m/sec.
1	D	45	65	24.3
2	V	62	90	34.6
3	V	83	106	46.2
4	D	53	67	22.3
5	D	48	52	30.5
6	D	86	97	26.6
7	D	36	63	30.5
8	V	57	66	20.8
9	V	65	69	26.0
10	V	72	77	32.9
11	D	61	64	44.7
12	D	60	66	39.4

Range of conduction velocity = 20·8 - 46.2 m/sec.

Mean conduction velocity = 32 m/sec.

Standard deviation = 8.4

Standard error = 2.4

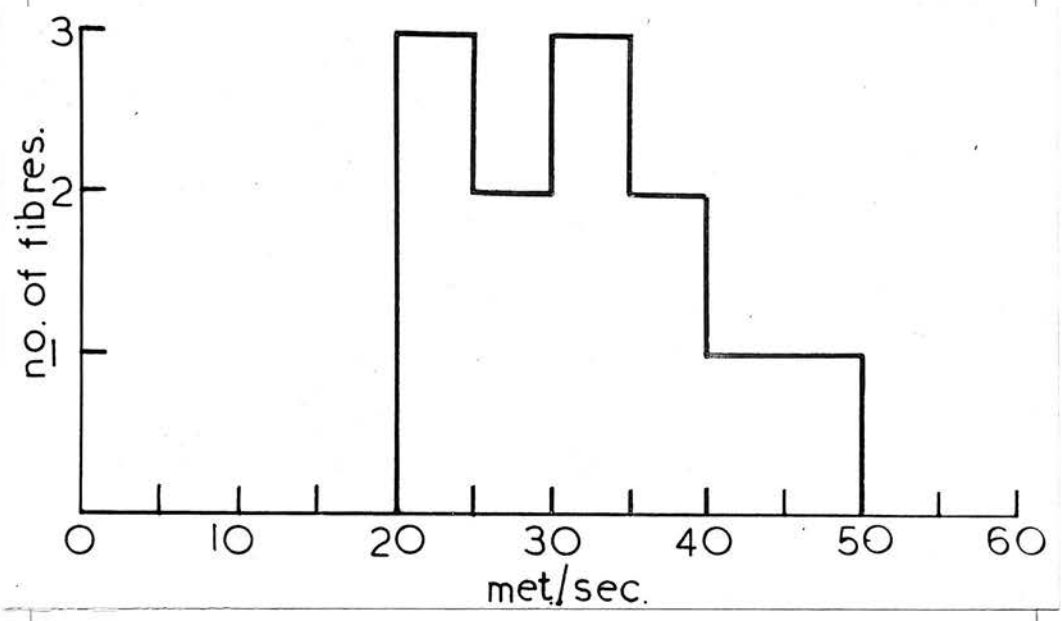

Fig.26. Frequency distribution of the conduction velocities of 12 systemic arterial pressoreceptors.

also near to the corresponding value for the pulmonary stretch afferents which is 34 m/sec. and both are much higher than the mean for pulmonary vascular afferents which is about 13 m/sec. From the data given in Tables 1 and 2 it can be calculated that the difference in means for the two groups of fibres is statistically highly significant.

There is no significant difference between the conduction velocities of the fibres which are found in the depressor and those which occur in the vagus (Table 2), the mean of the two being 31 and 33 m/sec. respectively. From the figures it appears that the assumption of Bishop, Heinbecker and O'Leary (1934) that fibres with depressor function belonging to the vagus are larger than those found in the depressor nerve itself, is probably incorrect.

When the activity in these systemic arterial pressure afferents is plotted as frequency of discharge against time, the constancy of behaviour of different fibres is well illustrated. The similar shapes of the histogram and the narrow range of time (54 msec.) within which the peak frequency occurs is remarkable. This is in contrast to the curves for the pulmonary vascular afferents which show a wide variation in shape and temporal cofiguration (fig.20).

In order to get a general picture of the activity in the depressor and pulmonary vascular afferents the mean valves of frequency of discharge occurring

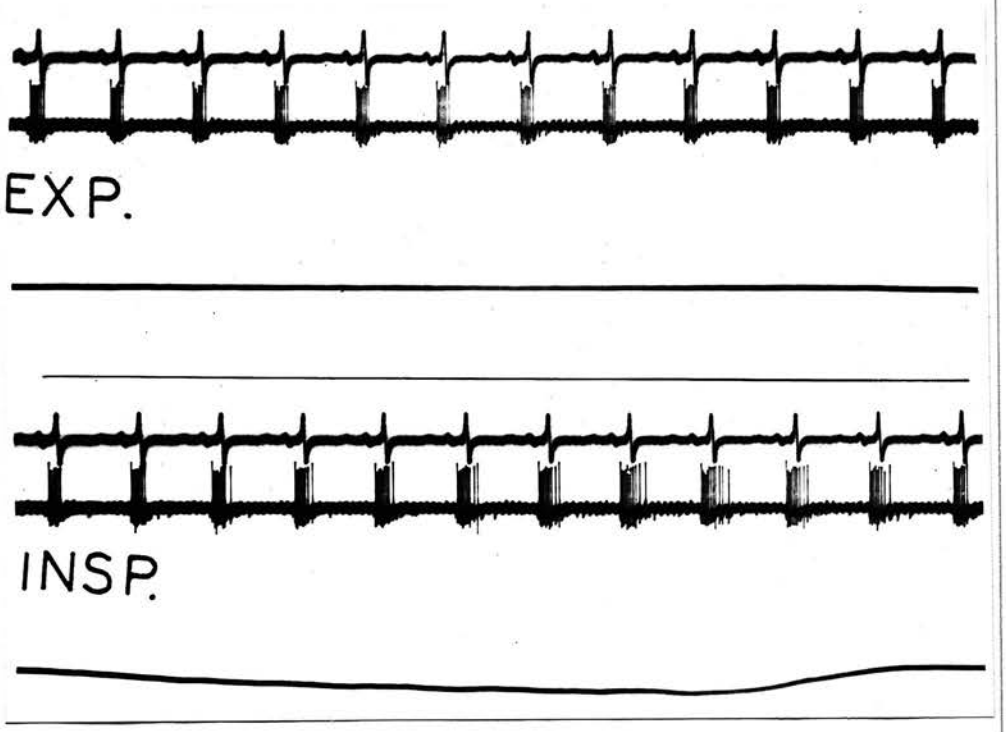

Fig.27. Venous fibre with a presystolic volley. There is increased activity during inspiration.

at the beginning, peak and end of the volley was plotted for all the depressor and pulmonary vascular fibres respectively (fig.25). This clearly shows that the time course of events for the two groups of fibres is quite different.

CONDUCTION VELOCITY.

VENOUS AFFERENTS.

It has been possible to determine the conduction velocities of only five venous afferents. This was partly because, in the absence of a venous pressure record, it was only possible to identify this number with certainty. Some other fibres examined may also have been venous or right arterial afferents with an atypical discharge.

The first criterion in the identification of venous afferents was that they should show a characteristic presystolic burst, with or without a following systolic or diastolic burst, in time with the 'C' or 'V' waves respectively of the venous pulse (fig.27,28).

Another characteristic was that the venous afferents showed an increase in activity during inspiration (fig.27,28) and during deflation, brought about by sucking air out of the lungs. One fibre (fig.8) showed an increase in activity during positive pressure inflation and was initially thought to be a pulmonary stretch fibre showing a cardiac rhythm. However, a plot of the frequency of discharge showed

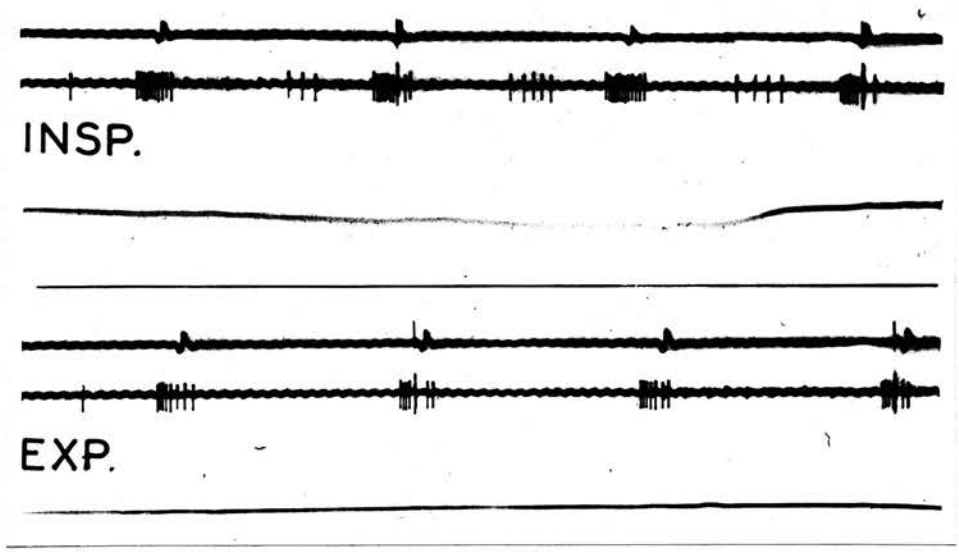

Fig.28. Venous fibre. The 'V' volley appears only on inspiration. The two records are continuous.

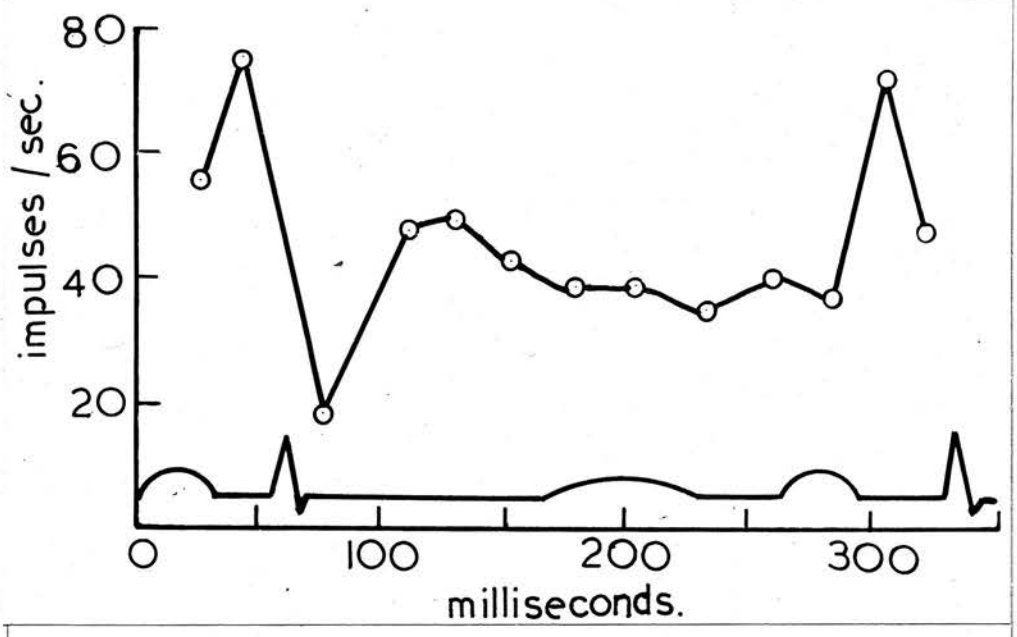

Fig.29. Plot of activity of the venous fibre shown in figure 8.

clearly the characteristic contour of the venous pulse (fig.29). All the other fibres showed a reduction in activity on positive pressure inflation of the lungs.

Two out of the five venous afferents showed a burst in time with the 'V' wave and one in time with the 'c' wave of the venous pulse. The 'v' discharge seen in fig.28 appeared only during inspiration or on deflation by suction.

The frequency of discharge was very high; for the fibre shown in Fig.27 the peak frequency reached was 405 impulses/sec.

The frequency distribution of the conduction velocities is plotted in fig.30 and the relevant data obtained is as follows:

Range = 12.5 - 27.3 m/sec.

Mean = 21.7 m/sec.

S.D. = 5.0

S.E. = 2.2

The range of conduction velocity overlaps that of the pulmonary vascular fibres although the means for the two groups differ sufficiently to be statistically significant.

The possibility was considered that a venous afferent fibre might show only a 'V' volley of impulses, thus making the differentiation from the pulmonary vascular fibres somewhat difficult. However, other investigators (Jarisch, & Zotterman 1948;

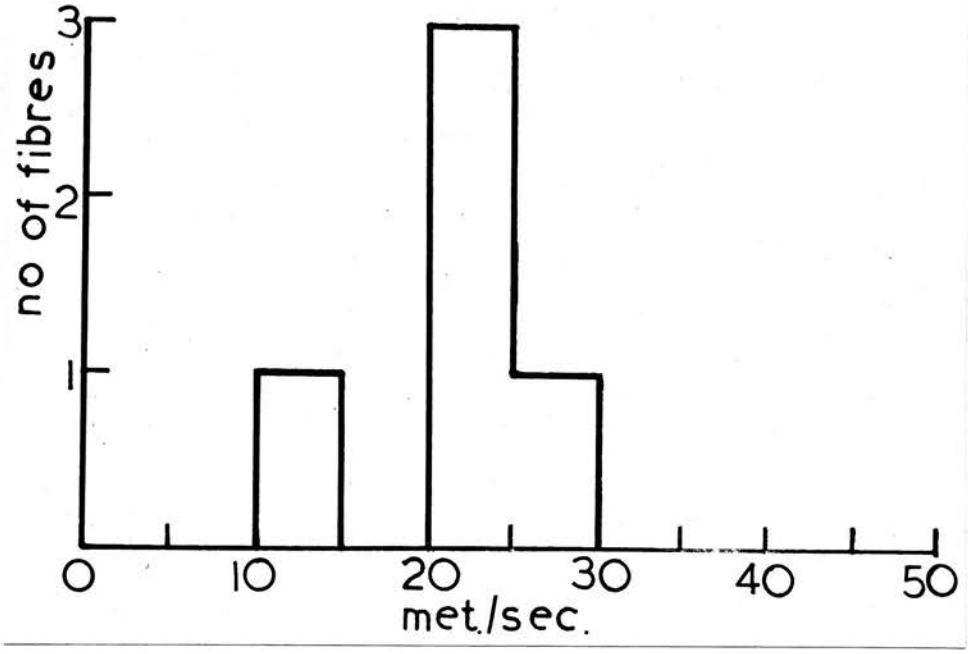

Fig.30. Frequency distribution of the conduction velocities of 5 venous afferent fibres.

Whitteridge 1948; Dickinson 1951;) who have recorded venous pressures and fibre activity simultaneously have not found any fibre with such a discharge.

CONDUCTION VELOCITY OF CHEMORECEPTOR AFFERENTS.

The main reason for the difficulty in the isolation of chemoreceptor single units is that they are normally inactive.

The observation of Landgren and Neil (1951) on the recording of chemoreceptor impulses in the aortic nerve first suggested the possibility of determining the conduction velocity of these afferents. Since the measurements were to be made on the right vagus and according to Neil, Redwood and Schweitzer (1948) a preponderance of the afferent fibres from the aortic body are contained in the right vagus and aortic nerves, the investigation seemed to offer a reasonable prospect of success. Nevertheless it has so far been possible to determine the conduction velocity of only three chemoreceptor afferents due to a reason already mentioned, and due to the sensitivity of these fibres to anoxia.

In the isolation of single units the fibres were dissected as already described. If on recording there was any evidence of the occurrence of occasional irregular impulses, then chemoreceptor activity was tested for by administering pure nitrogen for a brief period through a respiratory pump (Starling Ideal).

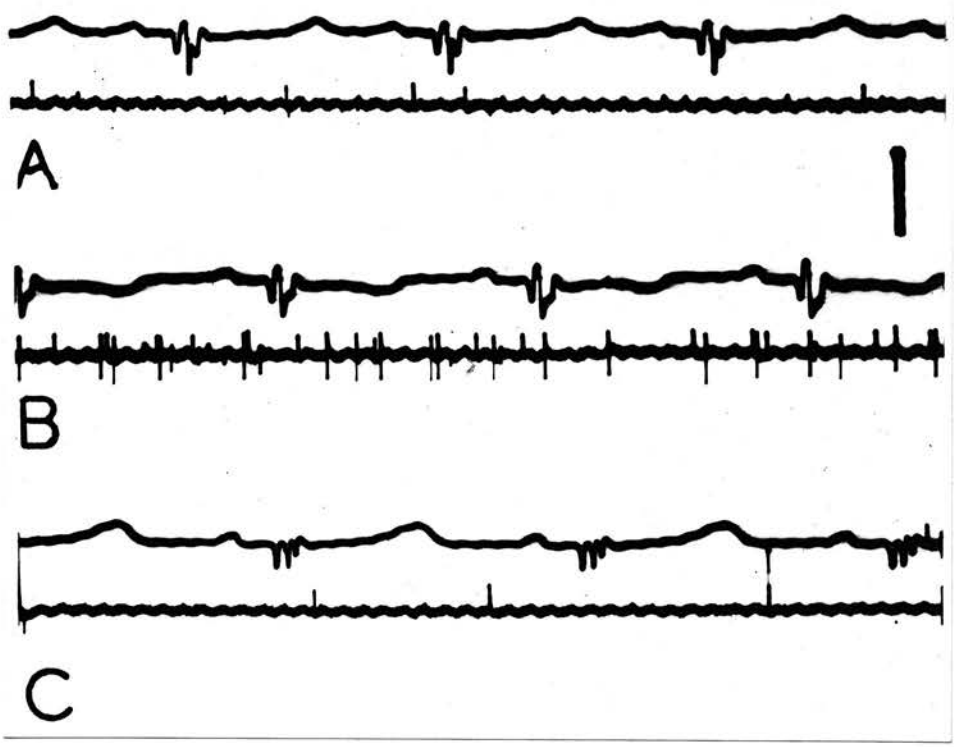

Fig.31. Chemoreceptor fibres. (A), before; (B), during; and (C), after, administration of nitrogen for 2 minutes by respiratory pump. The E.C.G. shows gross abnormalities.

If a continuous discharge appeared during N_2 inhalation and ceased a few minutes after it was discontinued, this was regarded as evidence that the nerve strand contained a chemoreceptor unit (fig.31). This test is based on the observation of Landgren and Neil (1951) that a continuous discharge is produced in the aortic chemoreceptor afferents on the administration of a high concentration of N_2 (96% with 4% O_2).

The administration of N_2 was found to cause changes in the E.C.G. which was very marked in one case (fig.31).

The conduction velocities of three chemoreceptor afferents identified in this way were as follows:-

Serial No.	Source	Conduction velocity
1	Depressor	11.7 m/sec
2	Vagus	10.4 "
3	Depressor	6.6 "

Mean = 9.6 m/sec.

S.D. = 2.2.

The range of conduction velocity appears to be rather narrow but this might be due to the small number of fibres osolated. It will be noted that the mean conduction velocity of the chemoreceptor afferents is not significantly different from the mean conduction velocity of the pulmonary vascular afferents.

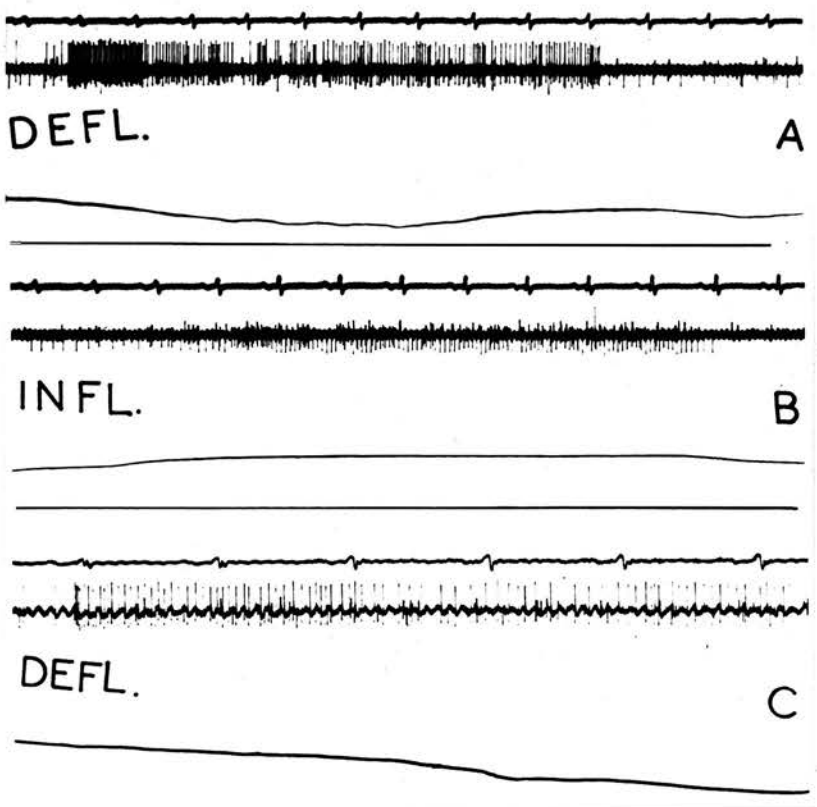

Fig.32. Record from a multifibre preparation showing a deflation fibre (large spike) and a pulmonary stretch fibre, (small spike). In A, activity is aroused in the deflation fibre on suction of air from the trachea. In B, an inflation produces a discharge only in the stretch fibre. In C, the response to deflation is taken with a faster camera speed.

CONDUCTION VELOCITY OF DEFLATION AFFERENTS.

Adrian (1933) reported the existence in the vagus (particularly the vagus of the rabbit) of fibres that responded by a burst of impulses during deflation. He found that none of these fired during normal respiration. Although a search for these was started only in the latter part of the present investigation, four fibres were found which were believed to be deflation afferents. It has been possible, however, to determine the conduction velocity of only two of these.

The behaviour of the four fibres has been tabulated as follows:-

Serial No.	Response to Inflation	Response to Deflation	Activity during Expiration	Conduction Velocity
1	-	+	+	3.2 m/sec.
2	-	+	-	-
3	-	Not recorded	+	-
4	-	+	-	2.8 m/sec.

Adrian observed that positive pressure applied to the animal's chest in an air tight box was more effective than suction of air from the trachea in producing a discharge from these fibres. Only suction of air from the trachea has been used in the present work to evoke a discharge.

Fig. 33 A shows the record from a multifibre preparation during a normal respiratory cycle. The

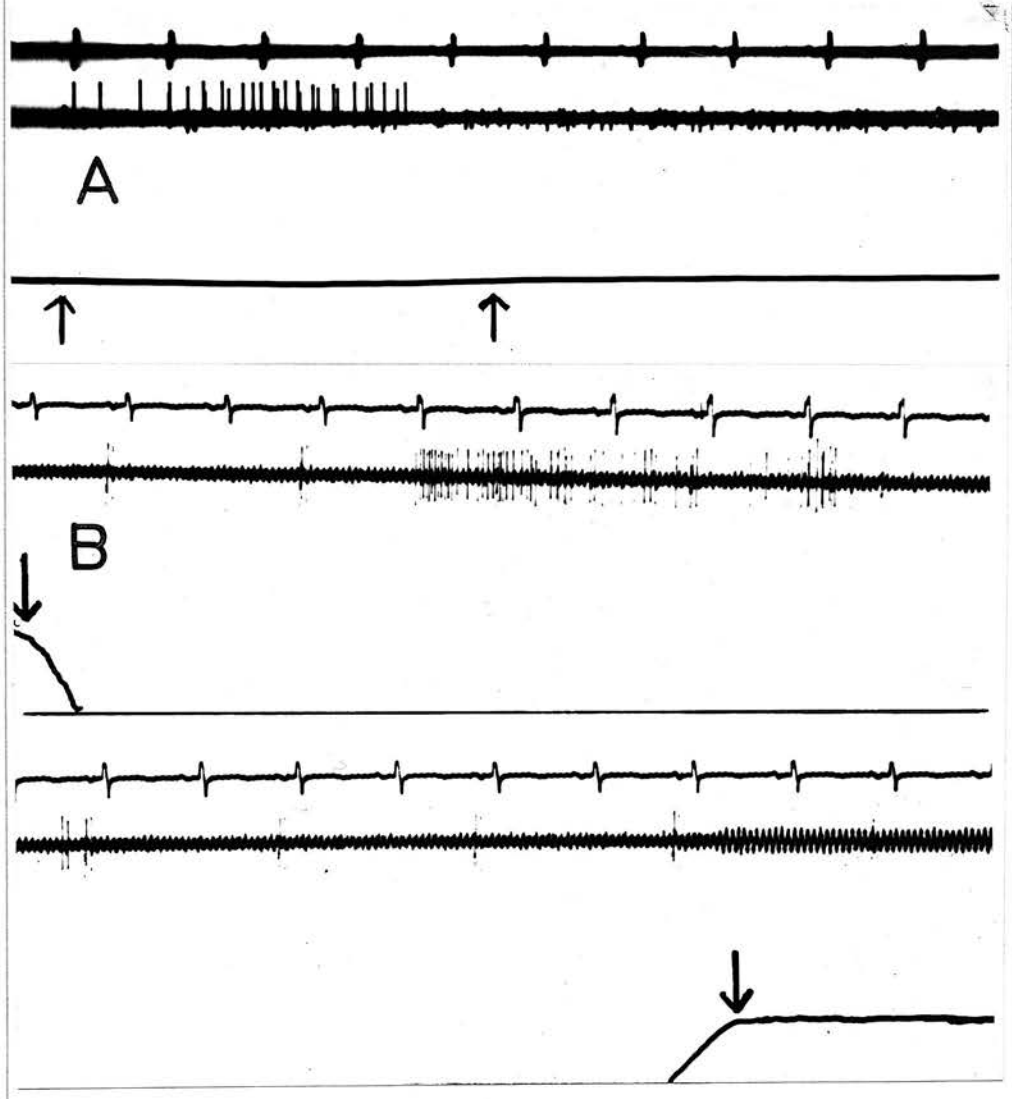

Fig.33. Deflation fibres: A, is a record from a multifibre preparation showing stretch and deflation fibres. During inspiration (between arrows) the stretch fibre is active. The deflation fibre discharges during normal expiration. B, Response of a rapidly adapting deflation fibre on forced deflation. These fibres were unaffected by an injection of 100 γ phenyl diguanide i.v.

pulmonary stretch fibre with the large spike becomes active during inspiration while the fibre with the small spikes identified as a deflation fibre shows a definite marked activity during expiration. It appears, therefore, that some fibres do come into activity during a normal expiration.

UNIDENTIFIED FIBRES WITH A CARDIAC RHYTHM

Three afferents have been found which could not be related to any of the known fibre types already described. It is probable that their location and function could have been determined by simultaneous recording of impulse activity and intravascular pressures, but this was not done in the present case.

Fibre 1.

This fibre with an early systolic burst (fig.34) was at first thought to be a ventricular fibre (Whitteridge 1948) which is presumably brought into activity during the isometric phase of contraction at a time when the tension on the ventricular wall is highest. The high frequency of discharge attained in the fibre accords with this view. However this was not substantiated by observations in the later part of the experiment when from the same fibre there appeared a few impulses before the Q wave of the E.C.G. at a time when the ventricles are relaxed. This occurred after the heart had been slowed by strong stimulation of the vagal trunk. It is conceivable that a powerful auricular contraction with increased

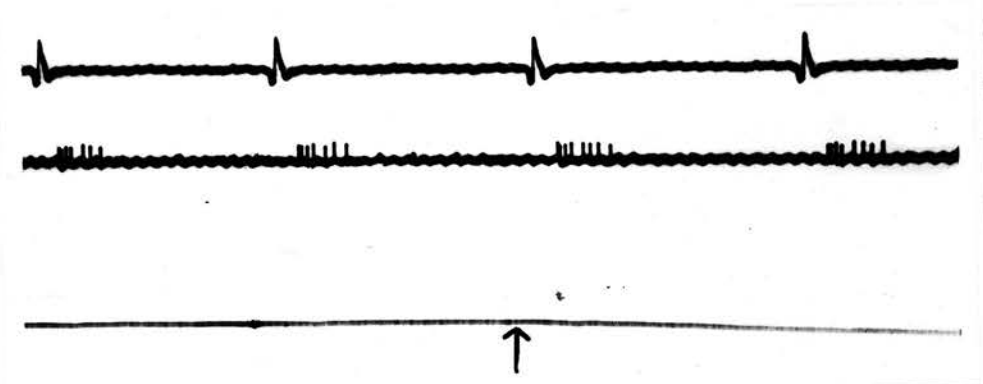

Fig.34. Ventricular fibre. The early systolic discharge corresponds closely to that shown by Whitteridge (1948.fig.8). Arrow indicates beginning of inspiration.

venous filling as the heart slowed might have succeeded in raising the tension on the ventricular wall enough to cause the receptor to fire a few impulses before the occurrence of the isometric contraction.

The activity of this fibre was reduced on inflation of the lungs and increased by suction of air from the trachea. These responses suggest that this fibre might be derived from the right atrium i.e. an atypical venous fibre firing off only during a C wave. The absence of the typical presystolic burst could be due to the occurrence of a contraction of that part of the atrial wall containing the receptor. This would have the effect of preventing any stretching of the receptor during the rise in pressure produced by the rest of the auricle. No venous afferent fibres have previously been recorded, however, which show only a burst of impulses occurring in time with the C wave of the venous pulse (Whitteridge 1948, Dickinson 1951). The conduction velocity of this fibre was 12.3 m/sec.

Fibre 2.

A record of this fibre is shown in Fig.35 and a plot of its frequency discharge in Fig.36. The fibre showed changes in activity during respiration - an increase occurring during inspiration. During inflation by positive pressure, activity was considerably increased. A similar increase occurred also

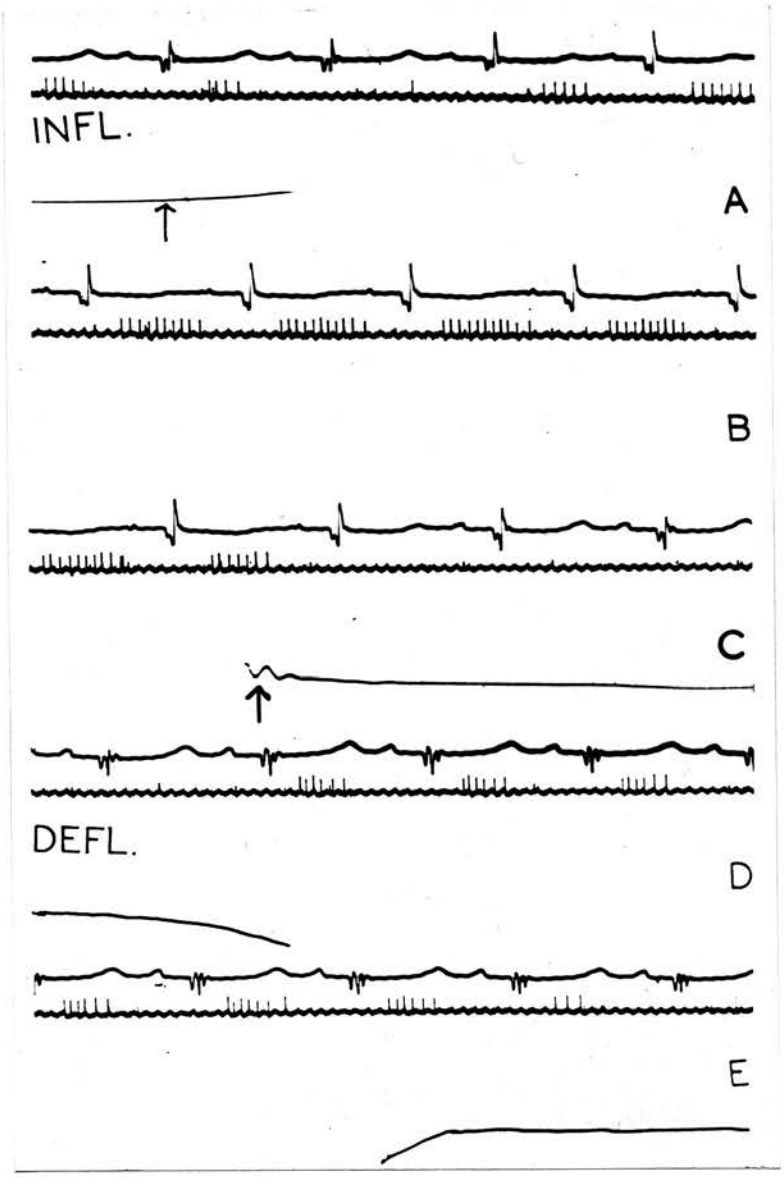

Fig.35. A pulmonary arterial fibre ? A, B, & C, are continuous records showing the response of the fibre to inflation (between arrows); D, & E, show the response to suction of air from the trachea.

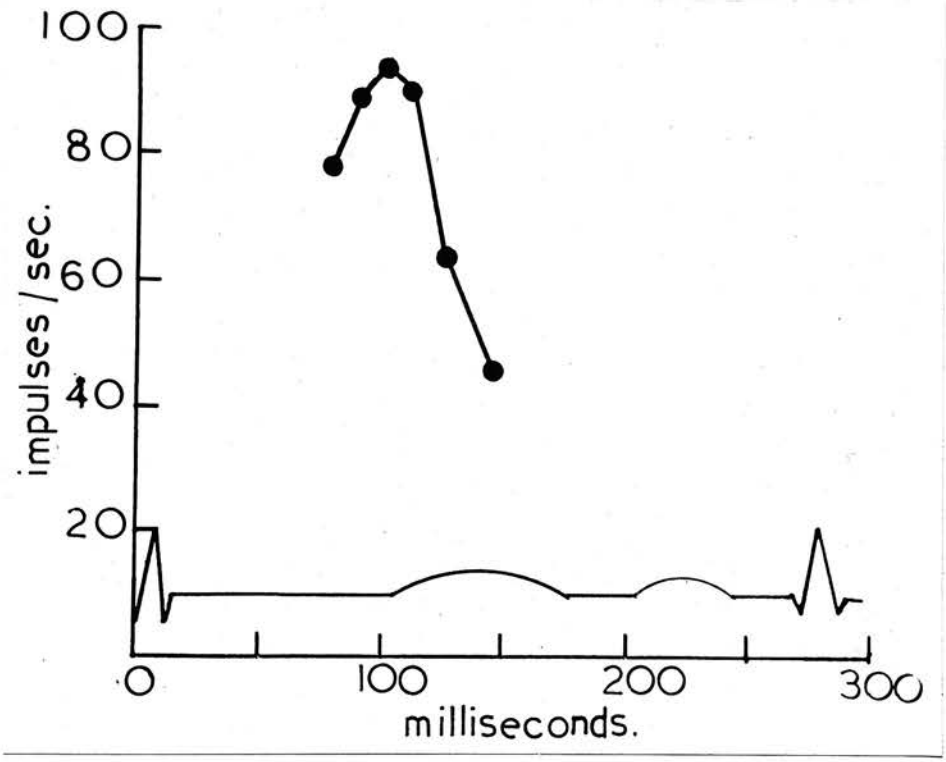

Fig. 36. Plot of activity of fibre shown in fig.35.

during suction of air from the trachea. After both proceedures, the return to the previous level of activity occupied about five or six heart beats.

The time of onset, peak frequency and duration of discharge (Fig.35) suggested that the fibre originated from the arterial side close to the heart. It was evidently not a depressor fibre from the aorta since these invariably show a reduction of activity during an inflation and for several heart beats thereafter.

The fibre could not have been a pulmonary stretch fibre with a cardiac rhythm as the impulses in these fibres lose their cardiac rhythm and become continuous during an inflation. For similar reasons it was unlikely to have been derived from the peripheral parts of the pulmonary vascular bed since no pulmonary vascular fibre previously recorded has shown an increase in activity during inflation of the lungs. Further, the peak frequency of discharge and its beginning occurred much earlier than they do in pulmonary vascular fibres. The only possibility that comes to mind is that the fibre originated from the pulmonary artery itself. Although no definite pressoreceptors have been shown to be present in the main trunk of the pulmonary artery they have been found at its bifurcation in the arterial branch to the supracardial paraganglion and the arterial ligament. (Takino and Watanbe 1937; Nonidez 1941). It could be conceivable that though

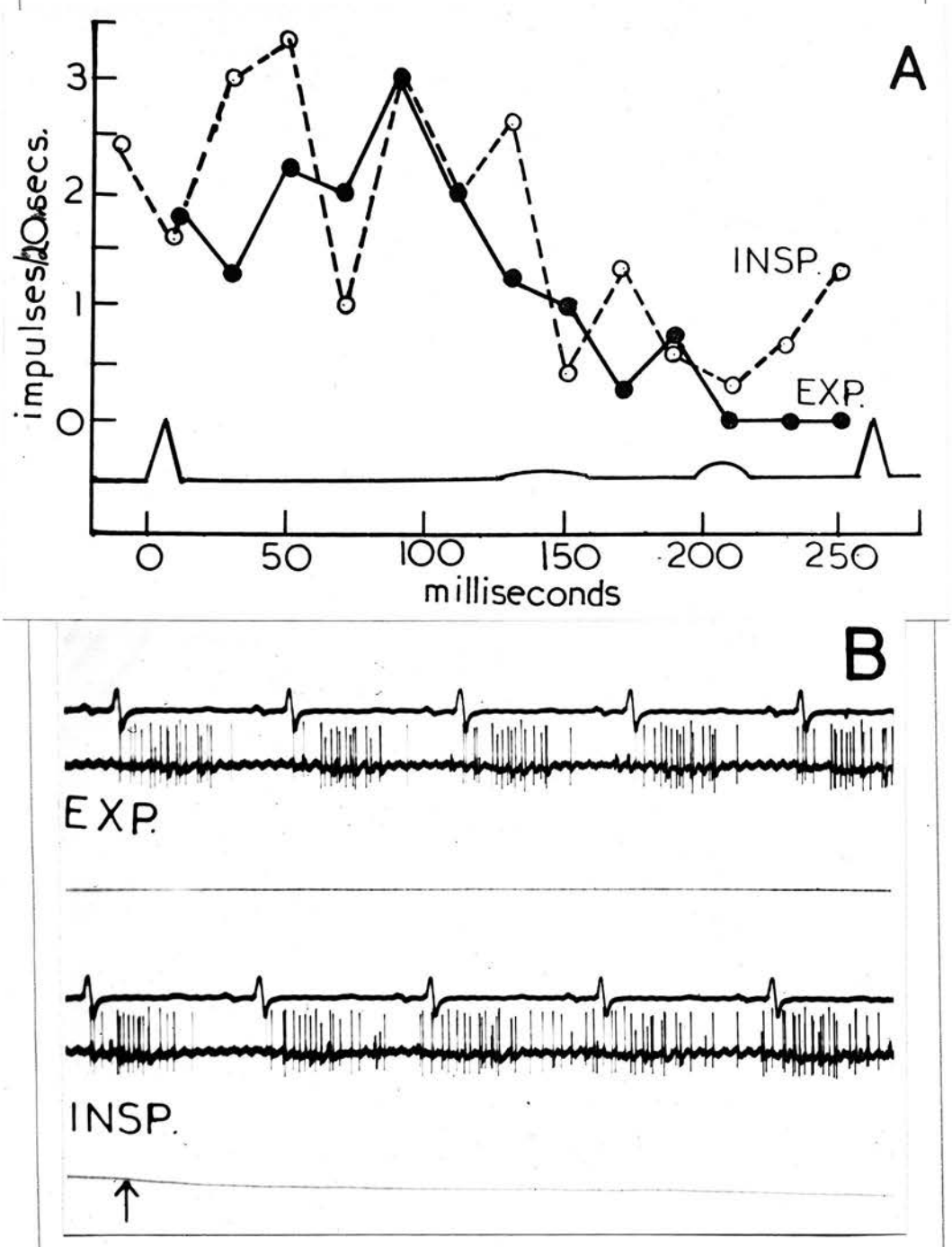

Fig. 37. Unknown fibre with a cardiac rhythm. In B, increased activity occurs during inspiration (at arrow) the discharge running into the presystolic period. In A, the activity was plotted by determining the mean number of impulses occurring in every 20 msec. of the record taking the Q wave of the E.C.G. as the reference point.

the effective pulmonary arterial pressure is usually reduced during inflation (Pearce and Whitteridge 1951) this might not always be the case for, although Sharpey-Shafer and Bain (1932) did not measure effective pressure, many of their records show very large increases in pulmonary arterial pressure in response to inflation.

The data for the fibre is as follows:-

Conduction Velocity	:	45.3 m/sec.
Beginning of discharge	:	102 m/sec. after Q wave
Peak frequency	:	102 m/sec. after Q wave
End of discharge	:	145 m/sec. after Q wave

Fibre 3

This multifibre preparation (fig.37) was isolated from a strand which yielded several fibres with a typical venous discharge. When examined on the face of the cathode ray tube the fibre to be described was thought to be a venous afferent, but on examination of the record no definite presystolic burst could be found.

Activity was greatly increased during inspiration as can be seen in the figure. Fig.37A is a plot of the average number of impulses occurring in every 20 milliseconds of the record in relation to the E.C.G. as determined during four heart beats each, during inspiration and expiration. It can be seen that the peak activity occurs between 50 and 90 milliseconds after the Q wave, and during expiration there is no activity in the presystolic period.

Because of these characteristics, and because the conduction velocity which was 24 m/sec. was near the mean value for the conduction velocity of the venous afferents, it was tempting to label the fibre as an atypical member of the group originating from the right atrium and showing a main burst of activity during the C wave of the venous pulse.

It is doubtful whether a simultaneous recording of the venous pressure would have been helpful in the identification of this fibre, for it can be presumed that it would probably have had a prominent "P" wave as can be judged from Fig.27 which is the record of a venous fibre obtained from the same animal a few minutes earlier.

It can be seen therefore that certain patterns of afferent activity exist which are quite different from already known patterns and which may either represent variations in the behaviour of established receptors or be characteristic of receptors hitherto unknown.

AFFERENTS STIMULATED BY PHENYL DIGUANIDE

These afferents are normally inactive and respond by a continuous discharge to an intravenous injection of 100 phenyl diguanide. They are discussed in detail in the third section of this thesis. The conduction velocities of two such fibres were 3.2 and 5.5 m/sec. respectively.

These fibres could be the ones responsible for

the inhibition of respiration produced by the drug, since this reflex effect is abolished by cooling the vagi to about 3° C (Dawes, Mott and Widdicombe (1951), a temperature which could be effective in blocking fibres with such low conduction velocities.

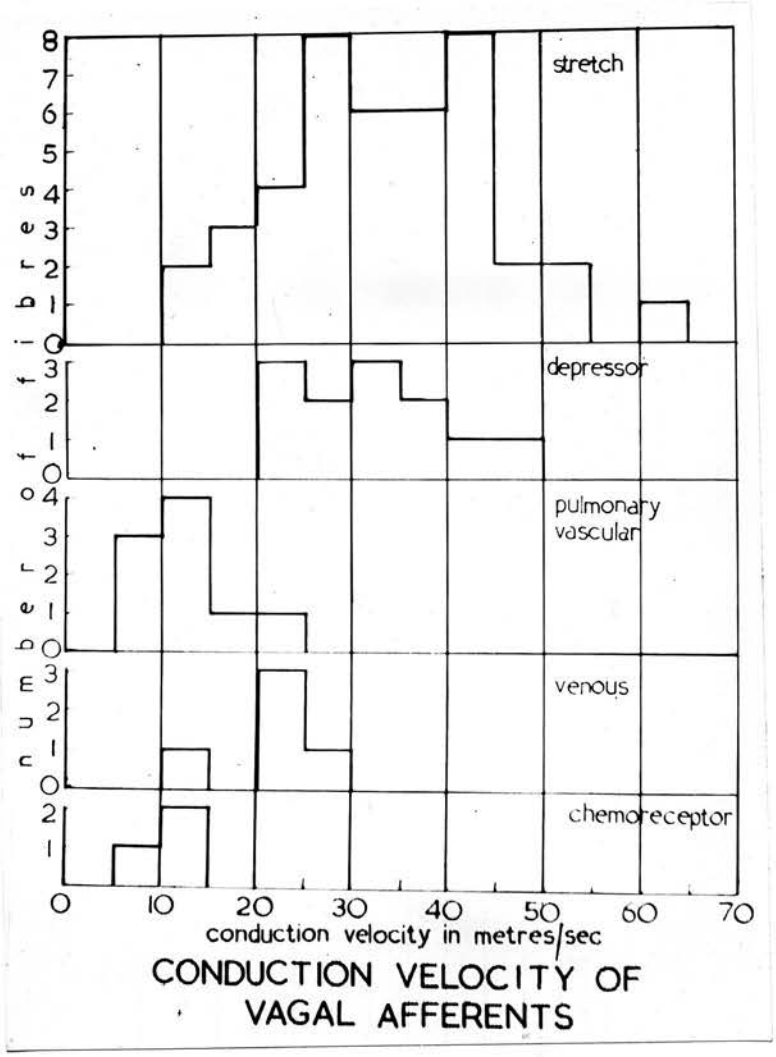

Fig. 38. Frequency distribution of the conduction velocities of various afferents in the vagus.

DISCUSSION

On the assumption that thermal blocking of conduction in nerve fibres varies according to their conduction velocity (Pearce 1951) the results presented here are in general agreement with those of Torrance and Whitteridge (1947). These authors showed that conduction in pulmonary stretch fibres is blocked between 12 and 18° C, in depressor and venous afferents between 8 and 12° C, and in pulmonary vascular fibres between 4 and 8° C. However the results of the present investigation indicate that some stretch fibres would survive cooling below 8 and 12° C. Further they do not support the conclusion which might be drawn from Torrance and Whitteridge's results that the conduction velocities of the various afferents are distinct from one another without any overlap whatsoever.

On examination of Fig.38 it is apparent that throughout the entire range of conduction velocities of the vagal afferents, only a narrow band from 50 - 65 m/sec. is occupied by one type of afferent; namely the pulmonary stretch fibres. Elsewhere, at least two types of afferents are found in every part of the range. The degree of overlap is thus seen to be considerable, the pulmonary stretch covering the entire range of conduction velocities for the depressor and venous afferents and a considerable part of the range of the pulmonary vascular afferents as well. It might be argued that this wide range is

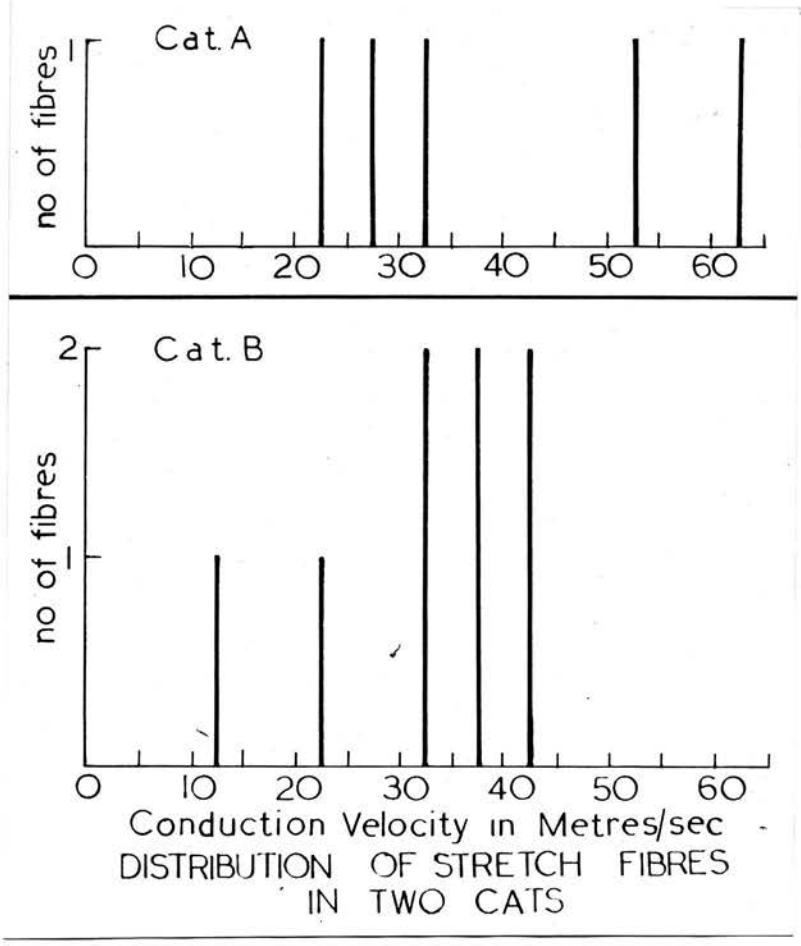

Fig. 39. Distribution of the conduction velocities of stretch fibres in two cats showing the wide range of conduction velocity existing in individual cats.

the result of variation found in a large number of cats, but on examination of Fig.39 it may be seen clearly that even in individual cats the stretch fibres have a very wide range of conduction velocities. The same is probably true of the other types of afferents.

From these facts it is unlikely that blocking conduction by pressure or cold could be selective for any one group of afferents excepting the stretch afferents with the highest conduction velocity. Consequently the reflex changes brought about by any cold block would be the result of action on more than one type of fibre. For instance, cooling to a temperature designed to block conduction in stretch fibres would probably block all the depressor, most of the venous, and some pulmonary vascular fibres as well. It would, therefore, seem important to consider this fact in the interpretation of reflex changes produced by applying a vagal block and stimulating the vagal trunk distal to it or stimulating the receptor themselves by various drugs. This question will be discussed in the third section of the thesis.

Heinbecker and O'Leary (1933) using pressure on the vagus as a means of differential block concluded that in the cat the stretch afferents had a range of fibre size of 4 - 10 μ in contrast to the depressor fibres with diameters of 3 - 6 μ with a significant

difference in their mean size. This is not borne out by the mean conduction velocities of these two groups which are 34 and 32 m/sec. for the pulmonary stretch and depressor fibres respectively. Their figures further indicate that depressor fibres with diameters smaller than pulmonary stretch fibres are to be found. Again this is at variance with the present results in which the stretch afferents were found to cover the whole range of conduction velocity of the depressor fibres. Finally no evidence for large "vasomotor" fibres (presumably pressor afferents) with diameters of 3 - 8μ have been found in the present investigation. It is not clear what type of fibre they had in mind but if there are any such fibres they are normally inactive and comparable in behaviour and reflex effects to the chemoreceptor afferents.

Bishop, Heinbecker and O'Leary (1934) assumed that there are in the depressor nerve, pressor fibres which are larger than the depressor fibres themselves. Although chemoreceptor afferents which produce a vasopressor reflex have been identified in the cats' depressor their conduction velocities are much lower than those of baroreceptor afferents. It is possible that the method of selective stimulation on which they based their conclusions excited the chemoreceptor afferents before the baroreceptor fibres, although the larger fibres usually have a lower

threshold to stimulation than have the smaller ones. Otherwise, it would be necessary to postulate the existence in the depressor of another type of afferent, hitherto unidentified and normally inactive. Further no confirmatory evidence for the existence in the vagus of depressor fibres larger than those found in the depressor nerve itself (15 μ against 5 μ are the figures given by Bishop, Heinbecker & O'Leary, 1934) could be obtained in the present investigation in which the mean conduction velocities of the arterial pressure afferents in the vagus and depressor were 33 and 31 m/sec. respectively.

The comparison made of the conduction velocities of the venous and depressor afferents with mean values of 21 and 32 m/sec. respectively does not support the observation of Nonidez (1941) that in general the fibres ending as receptors in the large veins are thicker than those which end as presso-receptors in the arch of the aorta.

In respect of their conduction velocities there does not appear to be a significant difference between the slowly adapting and rapidly adapting (Knowlton and Larabee 1946) pulmonary stretch afferents, A consideration of their high threshold to inflation, rate of adaptation, and their responses to forced deflation led Knowlton and Larabee to conclude that the rapidly adapting receptors excited inspiration and explained on that hypothesis the

inspiratory response to superinflation and forced deflation of the lungs. (Larabee & Knowlton, 1946). In the present investigation in contrast to the majority of slowly adapting stretch fibres, only one out of four rapidly adapting fibres encountered was found to fire on deflation. This observation is at variance with that of Knowlton and Larabee who found that the number of responses to deflation in rapidly adapting fibres was twice that of the slowly adapting group and that two thirds of the rapidly adapting ones could be stimulated by withdrawing 20 cc. of air from the lungs. The results obtained in the present experiments therefore do not tend to support Knowlton and Larabee's interpretation of the inspiratory responses obtained on deflation.

One possibility is that all pulmonary stretch fibres are functionally similar and that the very slowly adapting and very rapidly adapting afferents represent but extreme variations in activity of the same type of fibre. This could probably be shown if a random sampling of stretch afferents was made for the sole purpose of determining the rates of adaptation. Recently Widdicombe (1952) has shown that the rapidly adapting fibres end in receptors in the trachea and larger bronchi, and as suggested by him, it is possible that the rates of adaptation of stretch receptors are dependent on their anatomical situation in the respiratory tree - the rapidly

adapting ones being situated in the proximal, and the slowly adapting ones in the distal portions of the respiratory passages respectively. There is considerable histological evidence for the wide distribution of pulmonary receptors (Larsell, 1921, 1922; Elftman, 1943).

The observation of Adrian (1933) that deflation calls a new set of endings into play finds confirmation in the present investigation. These were observed by him chiefly in rabbits and were found to be rapidly adapting. They were believed to be part of the mechanism responsible for producing inspiration. However, he concluded that these receptors were not active during normal expiration although by applying external pressure to the rabbits chest in an airtight box, a discharge during expiration could be produced. Evidence has been presented in the present work to show that some do discharge impulses during expiration (fig.33A) and that both rapidly and slowly adapting types are found (fig.32 and 33B). The conduction velocity of one fibres (2.8 m/sec.) suggests that some of these afferents possess the characteristics required to account for some of the results of Hammouda and Wilson (1935 a & b) in experiments involving cooling of the vagi. It is however difficult to account for the acceleration of respiration on inflation of the lungs according to their views, on the basis of the deflation

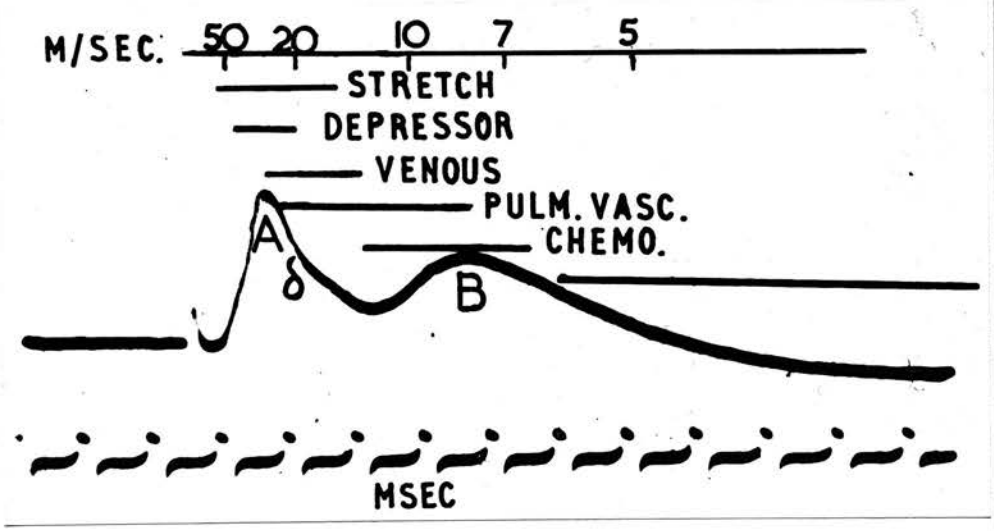

Fig. 40. Compound action potential of the vagus with the range of conduction velocities of various afferent fibres superimposed on it.

afferents described as none of these responded to an inflation. It is possible that the mechanisms concerned are complex for as shown by Steffenson, Brookhart, and Gesell (1937) the part played by extra-vagal structures cannot be ignored. The same argument would apply to the paradoxical effect of Head (1889).

An analysis of the compound action potential of the vagus on the basis of the conduction velocities of the afferent fibres and histological evidence provided by previous investigators would now seem possible. Various analyses have been done before among which may be mentioned the conclusions of Heinbecker (1930) and Middleton, Middleton, and Grundfest (1950).

Heinbecker concluded from a comparative study of the vagus and sympathetic of the cat and turtle that the A and B_1 elevations contained visceral afferents while the B_2 (with a conduction velocity of about 10 m/sec.) and C elevation were composed of autonomic motor fibres. The B_1 and B_2 elevations as pointed out by Erlanger (Erlanger & Gasser 1937) presumably refer to the A and B elevation respectively in his nomenclature. Recently Middleton, Middleton and Grundfest, showed that the fibres responsible for depressing and slowing the heart were of the B group. From an examination of the vagal compound action potential after a supranodosal section

they concluded "that one cannot assume that the B elevation is composed of afferent fibres because this assumption requires that there be a distribution in the vagus nerve of afferent A fibres of diameters below 3 μ far outweighing the distribution found in other sensory nerves which in these as well as in the vagus nerve produces the delta elevation". Their inference was that part of the δ elevation with a conduction velocity of 18 m/sec. and the B elevation with a maximum conduction velocity of 14.3 m/sec. were composed of sympathetic preganglionic fibres with an external influx.

An examination of fig. 40 shows that the conclusions of these two groups of authors are not entirely correct. That both the A and B elevation contain visceral afferents is borne out by the variety and large number of afferent fibres that have been isolated and found to have corresponding conduction velocities. On the other hand although it is highly probable that part of the B elevation is composed of preganglion afferents it would appear that the contribution by visceral afferent fibres is considerable. Not only is there a number of different types of afferent fibres in this group viz. pulmonary vascular, chemoreceptor, deflation and fibres with unknown function stimulated by phenyl diguanide, but as much as one fifth of all afferents encountered have a conduction velocity below 15 m/sec. which

according to the figures given by Middleton et al would all fall in the B elevation. It would therefore appear unnecessary to postulate an external influx from sympathetic preganglionics to account for the practically unchanged B elevation after a supranodosal section. Histological evidence of Foley and DuBois (1937 a and 1937 b), Dickinson (1951), Daly and Evans (1952) supports these conclusions. Dickinson did a frequency distribution analysis of the cardiac branch of the vagus before and after a supranodosal section and showed that the greatest number of fibres are in the 2 to 3 μ groups. A somewhat similar conclusion has been reached by Daly and Evans. It seems necessary therefore to adopt the alternative conclusion by Middleton et al "that there exist in the vagus nerve afferent A fibres of diameter below 3 μ - a distribution far outweighing that found in other sensory nerves". On these grounds as suggested by Whitteridge (1952) it would be more appropriate to designate the B elevation as 2.

Wyss and Rivkine (1950) observed that on stimulating the central end of the cervical vagus a strong inspiratory reaction with marked acceleration of breathing was associated with activity of distinctly slow fibres of the B_1 type (presumably delta). From fig.40 it is obvious that the delta elevation is a composite one, pulmonary stretch, depressor, venous and pulmonary vascular afferents being represented in it. The appearance of this

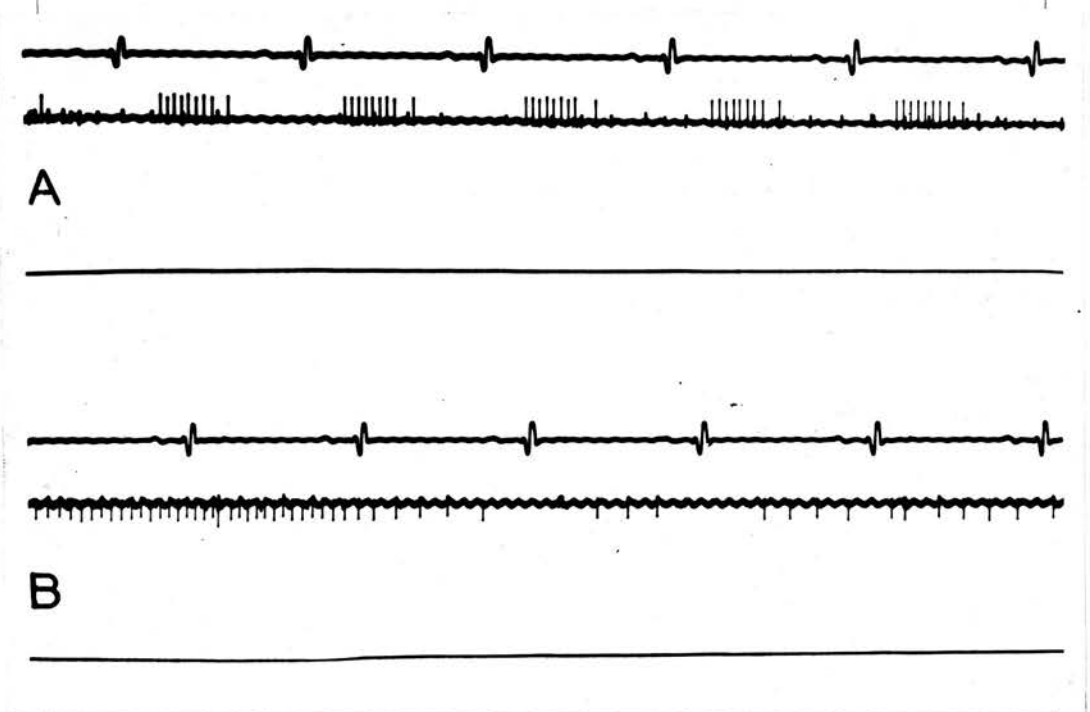

Fig. 41. Spike size and conduction velocity. In A, a depressor (large spike) and a stretch fibre with a cardiac rhythm (small spike) can be seen; B, record of the same stretch fibre after further subdivision. The conduction velocities of the depressor and stretch fibres were 26 and 39 m/sec. respectively.

elevation therefore implies that some or all of these afferents may have been stimulated - the resulting reflex changes being due to the composite effect of several functionally different types of afferents instead of to a single group. Caution is therefore necessary in the interpretation of results obtained by this approach.

It is generally recognised that the amplitude of the action potential varies as the size of the fibre, (Gasser and Grundfest 1939) and therefore, as its conduction velocity. Exceptions to this rule, however, have been observed on many occasions. An example is shown in an experiment (fig.41) in which it was found by later subdivision that the pulmonary stretch fibre with the smaller spike had a conduction velocity of 39 m/sec. and the depressor fibre with a large spike a conduction velocity of 26 m/sec. The spike size and conduction velocity relation need not therefore hold true when action potentials from fine strands are being recorded. This is mentioned, as conclusions about the size of fibres have often been drawn purely from measurements of the relative sizes of the spike. (Jarisch and Zotterman 1948).

PART II

Afferent fibres in the vagal rootlets.

AFFERENT FIBRES IN THE VAGAL ROOTLETS

INTRODUCTION

A great deal of work in the functional organisation of the vagal rootlets was done towards the close of the last century chiefly by German investigators. The value of this work is difficult to assess however since conflicting results have been obtained by different workers. This was due to the fact that the rootlets being so inaccessible do not lend themselves to a proper study of their reflex functions.

It is generally recognised that in the cat, dog and rabbit apart from individual variations the rootlets are arranged in three groups, upper, middle and lower, corresponding to the glossopharyngeal, vagus, and accessory nerves respectively. In the cat the middle one belonging to the vagus proper consists of 10 to 12 rootlets, (Cadman 1900; Foley and Dubois, 1934) and forms the largest group. The spinal root of the accessory is joined by a few bulbar rootlets. All of the three nerves leave the skull by the jugular foramen, the vagus and accessory in a common dural sheath, and the glassopharyngeal in a separate one. Due to this arrangement the glassopharyngeal rootlets are separated by a space from the vagal rootlets and the two nerves are easily distinguished from one another.

Chase and Ranson (1914) and Ranson & Mihalik

(1932) analysed the structure of the vagal and accessory rootlets according to their histological characteristics in studies made chiefly in the dog. They distinguished two types of rootlets depending on the size of their constituent nerve fibres and considered it probable that the afferent and efferent fibres of the vagus leave the medulla by separate roots. This arrangement was confirmed in the cat by Ranson, Foley, & Alpert (1933).

Using the method of experimental degeneration Foley and DuBois (1934) made a thorough study of the composition of the vagal rootlets in the cat after intracranial section of the rootlets in some cats, and nodose ganglionectomy in others. They concluded that the bulbar rootlets of the accessory are composed almost entirely of motor fascicles; and that of the vagal rootlets those consisting chiefly of motor fibres are the most caudal of the group, while the nodose bundles (vagal sensory) may pass into the brain stem in company with motor fibres, jugular fibres, or alone. This description emphasises the non-homogenous character of the vagal rootlets and the inconstancy of their arrangement. It is important to bear this in mind in the interpretation of the observations made by other investigators.

Experimental studies to determine the functional type of fibres present in the individual rootlets have on the whole yielded conflicting results, although there appears to be general agreement

regarding the course of the pulmonary stretch fibres. Kreidl (1895) concluded that among others the "Hering Breuer" fibres to the lungs run in the upper bundle of vagal rootlets. Later Beer and Kreidl (1895) found that respiratory standstill could be produced by stimulating the central cervical vagal stump when only the second highest rootlet of the upper group, presumably glossopharyngeal remained intact. Direct stimulation of this rootlet gave the same result. The studies of Kreidl (1897) on the monkey and of Cadman (1900) on the cat and dog confirmed these results.

There is less agreement about the composition of the other rootlets. Cadman (1900) believed that the efferent cardio-inhibitory fibres leave the bulb in the two lowest rootlets of the lower group, whereas Kreidl thought that they left the bulb by the upper two rootlets of the middle group. Grossman (1895) found that cardiac inhibition was only obtained when the lowest rootlet of the middle group and the highest one of the lower group were stimulated by unipolar stimulating electrodes. The value of such experiments is doubtful, however, since individual stimulation of the rootlets is scarcely possible owing to the fact that they are so close to one another some stimulus spread is almost unavoidable.

It is also interesting to note that whereas Grossman (1895) concluded that in the rabbit the inferior laryngeal nerve arises from the middle, and

the superior laryngeal from the upper group of rootlets Lemere (1932) found in dogs that the motor component of the laryngeal nerves is derived from the caudal bulbar accessory rootlets.

The function of the individual rootlets is therefore by no means settled and the lack of agreement between various workers emphasises the technical difficulties of the problem when approached in this way. In acute experiments the difficulty of recognising the individual rootlets is obvious. A second difficulty is to maintain the animal in a sufficiently good condition to test the various reflexes since the operation cannot be achieved without risk of serious haemorrhage.

On the other hand, the unanimity of evidence concerning the distribution of the inhibitory fibres of respiration suggests that the grouping of fibres into the separate bundles which form the rootlets may occur according to their type and function. If such a separation existed a knowledge of the course of the different kinds of fibres would be helpful in the study of various reflexes. It was with this consideration in mind that the present investigation was carried out, but as the following account will show the distribution of the afferent nerves studied does not support the idea that there is a functional separation of fibres at the level of the rootlets. Instead the results agree with the anatomical evidence already presented (Foley and DuBois 1934).

METHODS.

Experiments were done on 12 adult cats, although afferent fibre activity could be recorded from the vagal rootlets of only four of them. The experiments which failed were those in which owing to severe haemorrhage either the cats died before recording of fibre activity could be commenced, or the blood supply of the nerve was reduced to such an extent that the nerve fibres did not survive the manipulation necessary to obtain a record.

Dissection - In the first two experiments the ventral approach to the rootlets used by Beer and Kreidl (1895) in the rabbits was employed. The cats were anaesthetised with ethyl chloride followed by ether and then chloralose (intravenous injection of 80 mg/kg.) In the operation the trachea was cannulated and the larynx and pharynx, together with the muscles on the ventral surface of the basiocciput and obturator membrane, cut between ligatures. After making fine drill holes through the side of the basiocciput, and petrous temporal bone, the bone was cut away with cutting pliers and nibblers in order to expose the ventro-lateral curface of the medulla. The duramater was then incised and the cerebrospinal fluid allowed to flow out. With the resultant fall in intra-cranial pressure more bone could then be chipped away with reduced risk of damage to the medulla. When the field had been enlarged in this

way the rootlets could be just seen at the point of their entrance into the medulla. The spinal root of the accessory could be clearly identified but the other rootlets could not be distinguished from one another, since the space in which they were enclosed was a narrow conical recess and were often obscured by the considerable oozing of blood.

To record the action potentials a rootlet was detached from the medulla and placed on the recording electrodes. Subdivision of a rootlet was done by holding the free end between two pairs of forceps, then on pulling, two strands separated from one another. Bleeding that occurred from the cut sinuses vitiated the greater part of these experiments although it could be stopped temporarily by the application of gelatin sponge. Apart from certain small ones the two sinuses responsible for most of the bleeding were the sigmoid and inferior petrosal sinuses situated on either side of the jugular ganglion. Because of the problems created by bleeding this method of dissection was discontinued. The exposure in the subsequent experiments on ten cats was done by a postero-lateral approach. The best preparations were obtained when this was done rapidly by bone cutters and pliers. Bleeding occurred, but could be controlled by (1) scraping plasticine into the cut adges of bone and (2) use of a 'spinal' ligature to reduce haemorrhage by occluding the

vertebral arteries. The common carotids were temporarily occluded when the opening into the skull was made. These last proceedures were only used in one experiment, but this was the best of the series.

RESULTS.

In the first two experiments using the ventral approach pulmonary stretch activity was recorded in two rootlets of the middle group. The number of rootlets could not be identified for reasons already mentioned. However, in the subsequent experiments usually about 18 individual rootlets could be identified issuing from the medulla. These are arranged in three groups. The upper one belonging to the glossopharyngeal nerve consists of about 3 rootlets. Then follows caudally after a space the middle group of about 10 to 12 small rootlets which is derived from the vagus, and finally there is a lower group of about three rootlets that appears to join the spinal root of the accessory. As the bulbar rootlets of the accessory are known to join the vagus (Chase and Ranson, 1914; Foley and DuBois 1934) these have been considered as part of the vagus in the present investigation and the rootlets have therefore been enumerated from below upwards beginning from the first rootlet above the spinal root of the accessory which is always clearly visible.

Pulmonary stretch activity was recorded from the rootlets in four cats as follows:

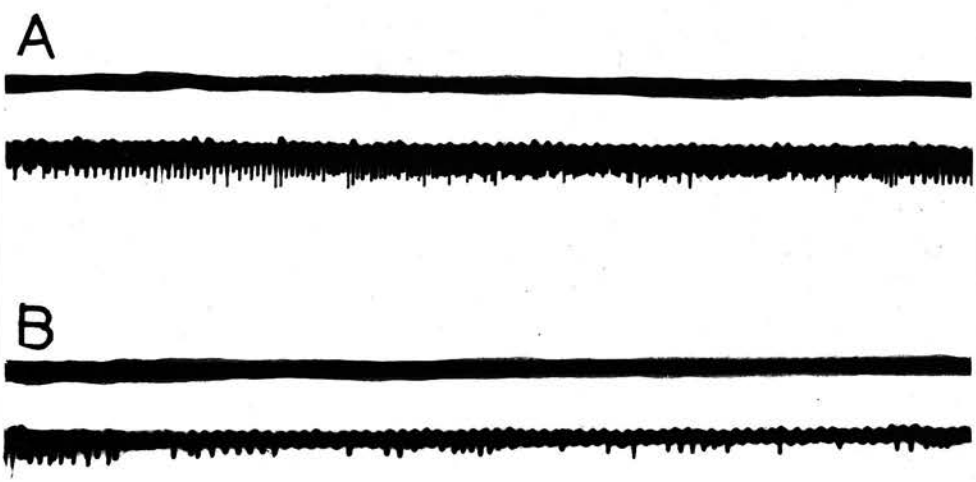

Fig. 42. Fibre activity in the fifth vagal rootlet. A, response of pulmonary stretch fibres to inflation; B, discharge with a cardiac rhythm in a fibre of the same rootlet recorded simultaneously. A and B are continuous. Upper trace in both records are the unused beam of the cathode ray tube.

Number of Cat	Rootlets in which activity was recorded
1	2 rootlets of middle group (vagal)
2	5th, 9th, 11th, 12th, 14th (vagal)
3	2nd (accessory)
4	4th and 5th (vagal)

No pulmonary stretch activity was recorded in the rootlets of the glossopharyngeal group.

In one instance, on leading from one rootlet a pulmonary stretch fibre and another fibre of cardiovascular origin were simultaneously recorded. The fibre showing a cardiac rhythm could not be identified because there was no E.C.G. record (fig.42). This is a further indication that as far as afferents are concerned the rootlets have a heterogenous composition.

DISCUSSION.

Although the analysis of the afferent fibre composition of the various rootlets is little more than begun the results indicate clearly that the separation of fibres into rootlets does not represent a functional separation since at least one type of afferent is found to be widely distributed through the vagal and accessory group. Although it is not denied that pulmonary stretch fibres may be present in rootlets of the upper group it is significant that no activity was recorded from them in the present investigation - a finding at variance with the results of previous investigators. It is not likely that this is due to a difference in the animal experimented

upon, for Cadman (1900) showed that the results obtained in the cat and dog were similar to those obtained in the rabbit by Beer and Kreidl (1895). It is difficult therefore to explain the results of Kreidl (1895), Beer and Kreidl (1895), and Cadman (1900), all of whom are agreed that it is the lower rootlets of the upper group which contain the fibres responsible for the inhibition of respiration. Although one can dismiss their results by direct stimulation of the vagal rootlets as being unreliable it seems clear that the results obtained by stimulation of the central end of the vagus after division of certain of the rootlets cannot be dismissed so readily. It is unlikely that any mistake would have been made about the identity of the rootlets since the glossopharyngeal nerve is easily distinguished from the vagus in it's intracranial course. The results imply that there are certain fibres in the cervical vagus that terminate in the glossopharyngeal rootlets. This point which might be settled by degenrative section was not investigated by Foley and DuBois (1934) who were only interested in the vagus and accessory groups. However, a considerable amount of intercommunication between the glossopharyngeal and vagus nerves is known to occur near the nodose ganglion. (Cunningham 1950). This might then provide an explanation for the observations of Beer and Kreidl and Cadman. It may be that afferent fibres

from the larynx and pharynx pass via the laryngeal and pharyngeal branches of the vagus to terminate in the glossopharyngeal rootlets, and it is these which were stimulated in their experiments.. This is only one possibility to account for the results for it is conceivable that other vagal fibres might terminate in the upper group of rootlets. However if there are any such fibres it is reasonable to conclude from the results obtained here that they are probably not the pulmonary stretch fibres as has been previously believed.

It is obvious that a more thorough investigation into the fibre composition of the vagal rootlets is necessary before any reliable conclusion about the central termination of the vagal afferents can be made as has been done by Oberholzer (1951).

PART III

The action of phenyl diguanide on Respiratory and cardiovascular afferents.

THE ACTION OF PHENYL DIGUANIDE ON RESPIRATORY AND CARDIOVASCULAR AFFERENTS

Introduction. Phenyl diguanide and other substances such as veratrine and its alkaloids, horse serum, mistletoe extract, ATP, other aromatic guanides, and isothiourea derivatives, when introduced into the blood stream are capable of producing certain characteristic respiratory and cardiovascular changes which are abolished by cutting the vagi. It is of interest that some of these presumably reflex effects are not abolished by cooling the vagi to low temperatures a result which suggests that small fibres are involved in their production.

The best known of these drugs is veratrine, an intravenous injection of which, as first shown by von Bezold and Hirt (1867) produces a reflex fall in blood pressure and heart rate. This was believed to be due to stimulation of cardiac receptors and several years later Jarisch and Richter (1939 a, 1939 b) obtained evidence from which they concluded that these were in the myocardium. Subsequently, Richter and Amaan (1940) pointed out that in addition to the heart receptors, others in the lungs were also implicated in the response to veratrine. The reflex nature of the response was demonstrated conclusively by Krayer, Wood and Montes (1943) who were able to produce a reflex vasodilation in the peripheral vascular bed on introducing a veratrine alkaloid into

the blood stream of a heart lung preparation which had intact nerve connections with the rest of the body.

Dawes, (1947) who tested the effect of veratridine by injecting it into the perfused coronary vessels showed that the most sensitive area for the production of the Bezold effect with this drug was the left ventricle. He also observed that a similar though small response could be produced by injection of the drug into the perfused left lung, thus confirming the observation of Richter and Amaan (1940). An interesting point about the reflex inhibition of respiration was brought out by Dawes, Mott and Widdicombe (1951) who presented evidence to show that veratridine sensitised the pulmonary stretch endings.

Brodie, (1900) observed that injection of serum into cats produced a bradycardia, fall in blood pressure and an inhibition of respiration which he attributed to a reflex arising in the lungs. Receptor areas for the action of the drug have also been shown to exist in the ventricles. (Dawes & Feldberg, 1949).

However, as a means of assisting the experimental study of the behaviour of certain cardiovascular afferents, the aromatic guanidines and isothiourea derivatives seem to surpass the other drugs mentioned. These drugs which have been investigated by Dawes & Mott (1950) and Dawes and Fastier (1950) are active in very small doses and do not show the phenomenon of

tachyphyllaris. The two groups of drugs both produce respiratory inhibition, bradycardia, and fall in systemic blood pressure, and differ from each other only in minor details. (Dawes, Mott & Widdicombe 1951). The actions cited have been attributed to three reflexes which are:-

1. A respiratory reflex the receptors for which are in the lungs.
2. A depressor reflex from the lungs.
3. A depressor reflex from the heart.

In the cat the afferent mechanism responsible for the inhibition of respiration was shown to be different from that due to veratridine in that the amidines did not excite or sensitise the pulmonary stretch fibres. Further the respiratory inhibition produced by veratridine could be blocked by cooling the vagus to about 10° C while the inhibition produced by the amidines was blocked only at 3° C. On the other hand no conclusive evidence was found to suggest that veratridine and the amidines acted on different receptors in the heart.

It was suggested (Dawes et al 1951) that the pulmonary vascular afferents might be responsible for the respiratory effect of the amidines. This as will be shown is probably not so.

The respiratory effects of the amidines observed in the rabbit differ from those seen in the dog. In the rabbit inhibition of respiration in the inspiratory position and an increase in the functional

residual air is observed, while an acceleration of respiration occurs in the dog. Reasons for this have been discussed. (Dawes et al, 1951,1952.).

A detailed investigation of the action of the amidines on various vagal afferents seems to be a necessary step if the nature of their actions is to be understood, but this has not been done although Dawes et al (1951) quote a few observations by Cabrera who found no sensitization of right venous or aortic depressor receptors. It was in view of this gap in knowledge that the present investigation was carried out.

METHODS.

The methods of dissection and recording were similar to those described in part I of this thesis.

To study the influence of phenyl diguanide on the vagal afferents single units were dissected and after identification, 100 γ phenyl diguanide dissolved in 1 cc.0.9% saline was injected intravenously through a cannula in the femoral vein. This was controlled by a separate injection of 1 cc. 0.9 % NaCl solution.

During a preliminary study of the effects of phenyl diguanide on the known vagal afferents it was found that injection of the drug aroused activity in a fibre which had previously been inactive and which had a conduction velocity of about 3 m/sec. In subsequent experiments in which the phenyl

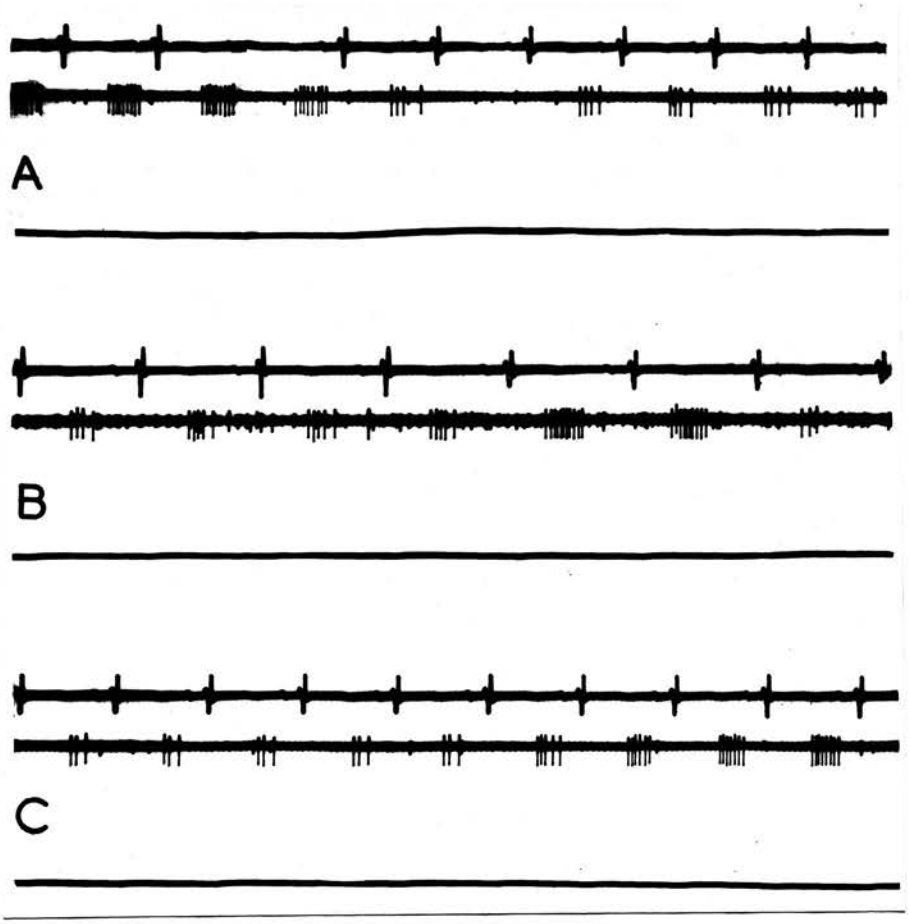

Fig. 43. Pulmonary Vascular fibre. A, before; B, 1 sec. after intravenous injection of 100 γ phenyl diguanide; C, 26 sec. later. Note the considerable small spike activity in B.

diguanide sensitive fibres were to be studied these were isolated in the following manner:-

After a strand had been dissected the vagal trunk was stimulated and the compound action potential examined for evidence of a slowly conducting component. When there was one, phenyl diguanide was injected and the response noted on the cathode ray tube. If an increase in base-line noise then occurred, the strand was subdivided and the proceedure repeated until a sufficiently fine strand was obtained to record a single unit.

RESULTS.

Phenyl diguanide when injected intravenously did not stimulate or sensitize the pulmonary stretch receptors. In some cases the activity of the fibre was reduced due to the inhibition of respiration produced by the drug. These observations are in agreement with those of Dawes, Mott and Widdicombe (1951). There was no effect on the rapidly adapting fibres either.

The results of the action of the drug on Depressor, Venous, and Pulmonary vascular afferents are not entirely conclusive owing to the absence of appropriate pressure records. However when considered in conjunction with the results of other investigators the conclusions arrived at here are reasonably justified.

No sensitization or stimulation of the depressor

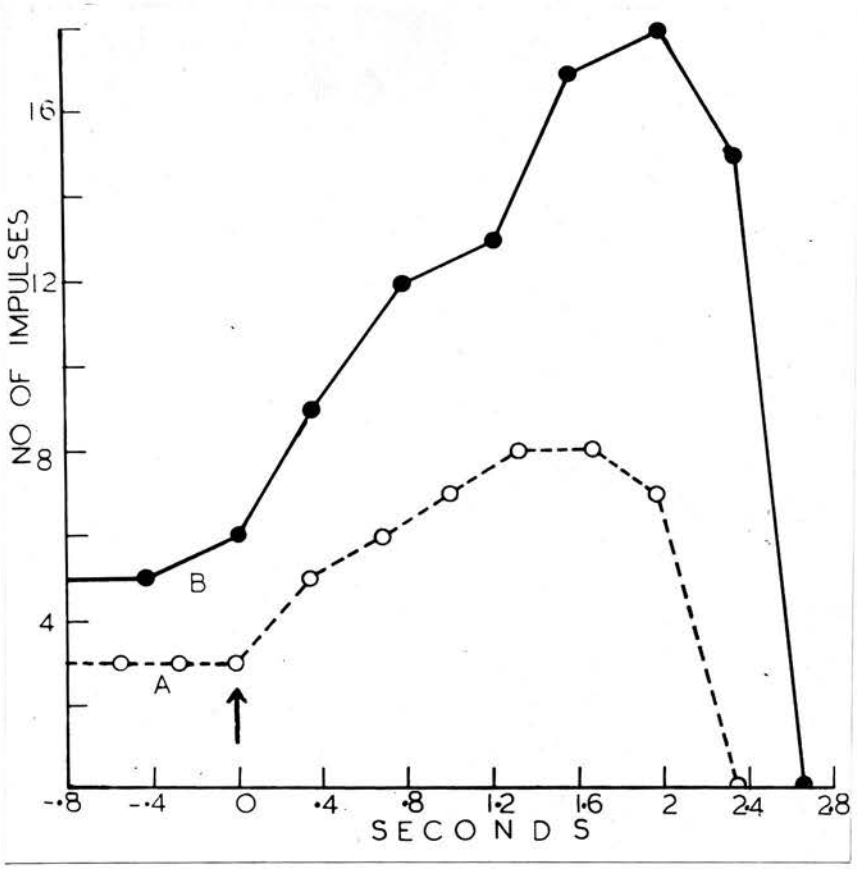

Fig. 44. Plot of activity in a pulmonary vascular fibre in successive cardiac cycles during inspiration. A, before; and B after inhalation of ammonia.

fibres was seen. In some cases there was a slight inconsistent initial increase in the discharge which was followed by a marked decrease. This was presumably due to the lowering of blood pressure that has been shown to occur. (Dawes et al, 1951). The discharge usually returned to its original level within a minute.

No significant increase in fibre activity was seen in any of the venous afferents tested. In a few instances a slight initial increase in the number of impulses was observed to be associated with the occurrence of bradycardia. It was possible that this was due to an increased filling of the right atrium consequent on the slowing of the heart. The slight initial increase was followed by a slight period of reduced activity with a gradual return to the original level. In one case while the activity of a venous fibre was being recorded there was seen to be a considerable increase in the background noise when 100 γ phenyl diguanide was injected. It appears that fibres firing on deflation are not stimulated or sensitized by phenyl diguanide. Both the fibres shown in fig.33 were unaffected by the drug.

The action on one pulmonary vascular fibre was investigated (fig.43). It is obvious that no excitation of the receptor occurred although the inhibition of respiration and bradycardia were marked.

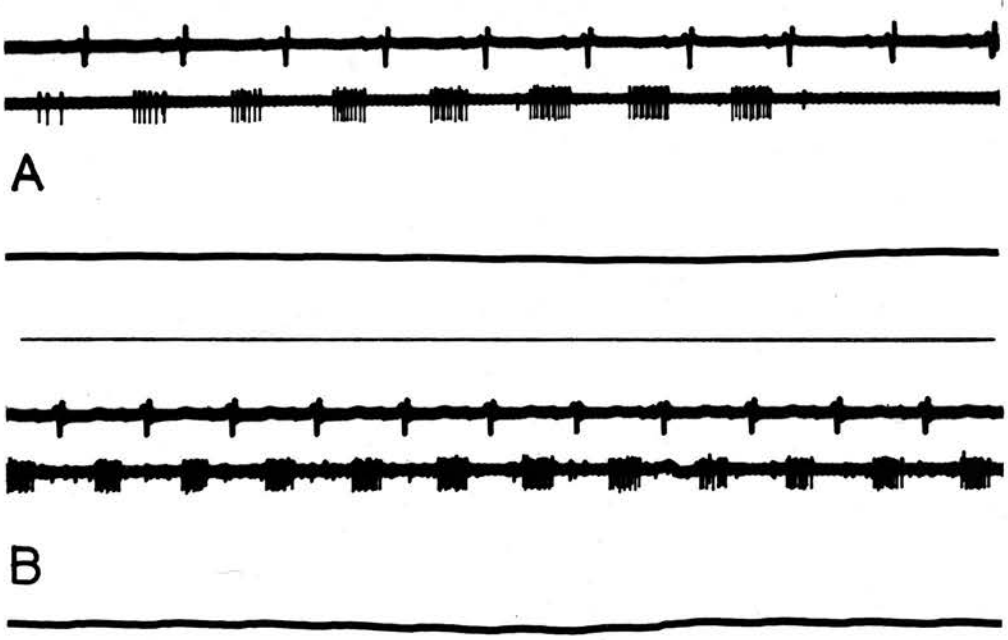

Fig. 45. Pulmonary vascular fibre. A, before; and B, after intravenous injection of 0.5 cc pituitrin.

The heart rate fell from an initial value of 188/min. to 130/min. There was however a slight increase in the number of impulses per cardiac cycle, increasing from 9 to 11. This was believed not to be due to a sensitization of the receptor but as shown by Pearce (1951) to a rise in pressure in the pulmonary vascular bed following upon an increased filling of the heart working at a slower rate.

The effect of ammonia on this fibre was also tested. The gas was administered through a side tube of the tracheal cannula. It is interesting to note that a more than twofold increase in activity resulted (fig.44), The exact cause of this could not be determined but it is presumed to have been due to a rise in the effective pulmonary arteriolar pressure caused by changes in intrathoracic pressure.

On the other hand it is not impossible that the pulmonary vascular receptors might have been actually sensitized for as shown by Banister, Fegler and Hebb (1949) ammonia may have an action on afferents in the lung in addition to a direct stimulating action on the bronchial smooth muscle.

When the effect of ammonia had passed off pituitrin 0.5 cc was injected intravenously. The result is shown in Fig.45. The respiratory, and heart rates increased considerably reaching a maximum gradually in about 30 seconds. From the large increase in fibre activity there was presumed to have

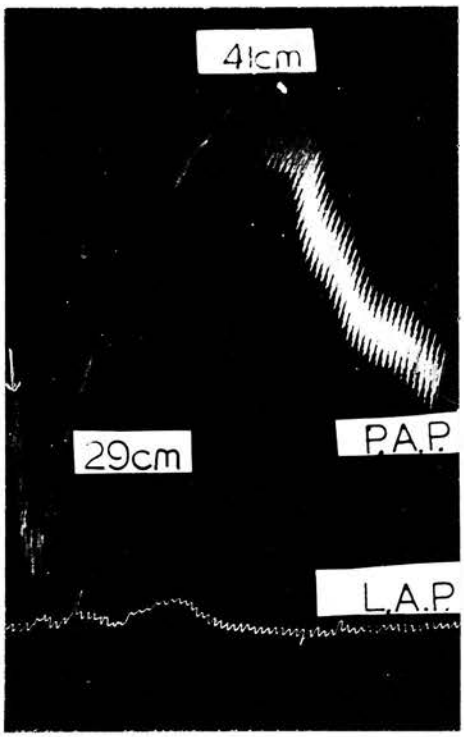

Fig. 46. Effect of 0.5 cc pituitrin on the pulmonary arterial pressure of a perfused left lung preparation. From above downwards, left pulmonary arterial pressure and left auricular pressure. Pituitrin injected at arrow.

been a rise in the pulmonary arterial pressure as this had been observed by Hebb (personal communication) in perfused lung preparations of the dog. It is noteworthy (fig.47) that although the number of impulses during inspiration are increased after pituitrin, the effect of the drug on the discharge during expiration is much more marked. Whereas, there may be no activity of the fibre during expiration, normally, the level of discharge after pituitrin is markedly raised.

In order to see whether an actual rise in the pulmonary arterial pressure occurred in the cat the effect of pituitrin was tried on a constant volume inflow left lung perfusion of the cat (joint experiment with Hebb and Swan). When the drug was injected intravenously into the general circulation or into the left lung perfusion a considerable increase in the pulmonary arterial pressure was observed as can be seen in fig.46. The effect of 100 γ phenyl diguanide tested on a similar preparation was not marked and consisted only of a slight initial rise followed by a small fall in pressure. This agrees with the observation of Dawes, Mott & Widdicombe (1951) and Pearce (1951) who could not detect any marked change in the pulmonary arterial pressure in response to phenyl diguanide.

The striking similarity of the curves in figs. 46, & 47, suggests that similar events are being

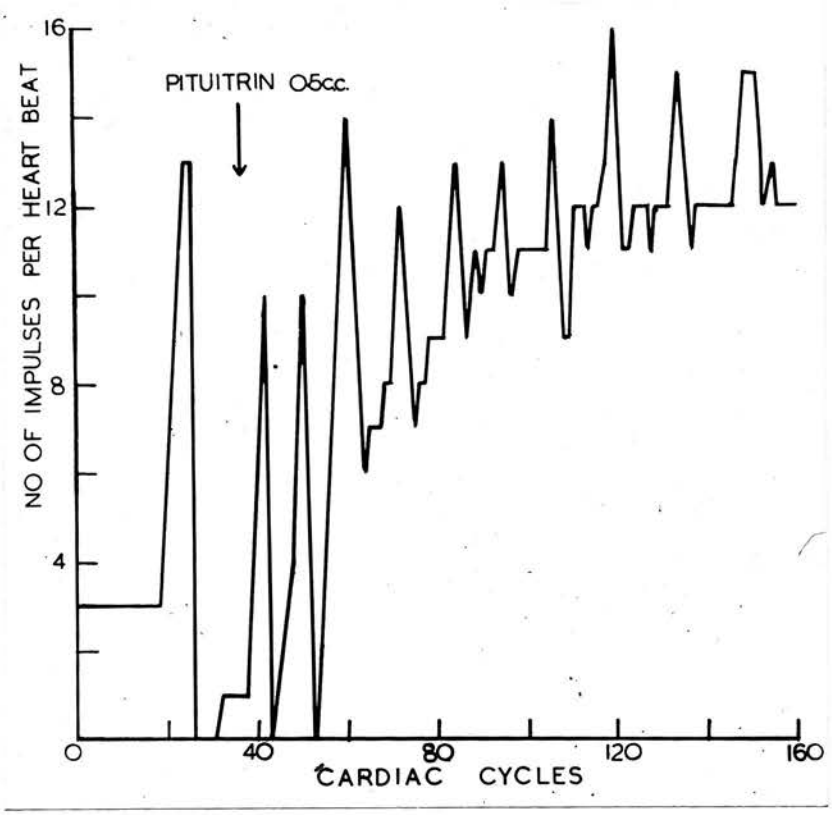

Fig. 47. Plot of activity in a pulmonary vascular fibre in successive cardiac cycles. At arrow, 0.5 cc pituitrin was injected. The fluctuations are due to the influence of respirations. Note the considerable increase in the expiratory level of the discharge.

recorded in the two cases to an injection of pituitrin. And since it has been shown that an increase in pulmonary arterial pressure occurs on injection of pituitrin it is reasonable to infer that the record of pulmonary vascular fibre activity does in fact reflect changes in the pulmonary arterial pressure, as shown by Pearce and Whitteridge (1951).

In contrast to its lack of effect on the afferents mentioned above phenyl diguanide was found on 4 occasions to excite fibres which had previously been quiescent. All were characterised by a sudden outburst of activity following almost immediately after intravenous injection of the drug. The time elapsing between injection into the femoral vein and the first appearance of the discharge varied from two to three seconds (fig.48). Activity was usually maximal in another 4-5 sec. and died away after about 10-25 sec. The response could be repeated at will and showed no diminution with subsequent injection. The conduction velocity of one of this group of fibres was found to be 3.2 m/sec., while another was 5.5m/sec In the remaining two a slow elevation of about 5 m/sec. in the compound action potential provided the only estimate of their velocity but there was no certainty that the phenyl diguanide sensitive fibre arose from the slow component.

The response of these fibres to phenyl diguanide

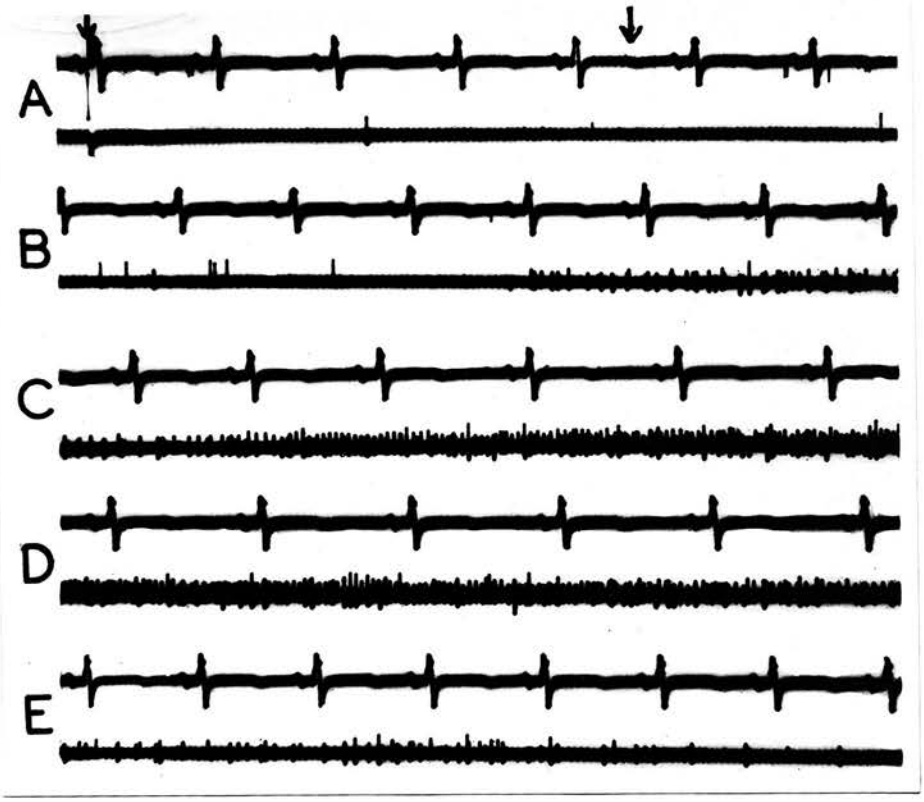

Fig. 48. Unknown fibre stimulated by phenyl diguanide. A, B, C, & D are continuous. E, 9 sec. later. 100 γ phenyl diguanide was injected between arrows in A.

appeared to be fairly specific. Many other stimuli were tested but with the exception of pituitrin none produced any activity in units responsive to phenyl diguanide. Among the stimuli tested and found to be ineffective were: (1) gradual or rapid inflations of the lungs (2) suction of air from the trachea (3) administration of 100% nitrogen and (4) administration of a mixture of 90% oxygen and 10% carbon dioxide. The gases were given by a respiratory pump for a period of 2 minutes. In one case the effect of trichlorethylene was also tested but it too, failed to arouse activity in the fibre.

A phenyl diguanide-sensitive fibre was present in the strand containing the pulmonary vascular fibre which has already been described (fig.43). It is interesting to note that the activity of this phenyl diguanide sensitive fibre occurred at a time when the pulmonary vascular fibre activity remained unaffected. On the other hand NH_3 failed to arouse activity in the phenyl diguanide-sensitive fibre although as already noted the discharge of the pulmonary vascular fibre was considerably increased. However 0.5 cc pituitrin stimulated the phenyl diguanide sensitive fibre as it did also the pulmonary vascular afferent. It is important to note that this was accompanied by an increase in heart and respiratory rates.

In discussing the effects of ammonia, Dawes (1951) suggested from a consideration of the struct-

of the amidines and their strongly basic nature that ammonia might resemble the latter in its actions. This is probably not the case, for as shown by Bannister, Fegler and Hebb (1949) ammonia causes an increase in respiration instead of the inhibition usually observed with phenyl diguanide. Further, the fact that a phenyl diguanide sensitive fibre was unaffected by ammonia would support this conclusion.

The possibility was suggested by Whitteridge (personal communication) that these phenyl diguanide sensitive endings might arise from the pleura, and to test this 500 γ of phenyl diguanide was injected into the pleural cavity of one cat. No alterations in the respiratory or heart rate were observed while an injection of 100 γ phenyl diguanide intravenously produced the usual marked bradycardia and inhibition of respiration.

The possibility that phenyl diguanide might stimulate all small fibres was also considered but the total absence of activity on injection of the drug in a number of strands showing the presence of a slow component (conduction velocity of 2-10 m/sec) in the compound action potential seemed to rule out this possibility. Thus, while the phenyl diguanide sensitive fibres belong to the slow conducting small-diameter group there are many others in the same group which are completely insensitive to this substance.

DISCUSSION

The results described do not support the suggestion of Dawes, Mott, and Widdicombe (1951) that the pulmonary vascular afferents are concerned in the inhibition of respiration produced by phenyl diguanide. These authors considering it inadvisable to postulate a new type of receptor, put forward their suggestion from a consideration of the fact that the blocking temperatures of the pulmonary vascular fibres (Torrance and Whitteridge 1947) are about the same as the temperatures at which the respiratory response to phenyl diguanide is blocked. The evidence does not justify the conclusion however, since several types of fibres with similar blocking temperature may exist. Moreover, a particular reflex effect produced by phenyl diguanide need not be due to the specific action of one type of fibre for Dawes et al (I.C.) have shown that at least three sets are involved in the production of the various reflex effects. Further the multiple nature of the action is illustrated by the varying responses observed by them in different animals.

On the other hand the phenyl diguanide sensitive fibre with conduction velocities of 2-6 m/sec. could be one of those responsible for the inhibition of respiration. Further its conduction velocity corresponds to the blocking temperature of this reflex which is about $3^{\circ}C$. However, the reflex

inhibition might well be the result-ant of the actions of more than one type of fibre for as has already been shown (see part I) a number of functionally different afferents may have similar conduction velocities. Among those that have been identified and found to have such low conduction velocities are the deflation and the chemoreceptor afferents. However, from the experimental evidence provided these would not appear to be in any way involved in the response to phenyl diguanide. Further, from what is known of the reflex function of these afferents, they would be expected to produce directly opposite effects to those observed after phenyl diguanide.

The phenyl diguanide-sensitive fibres which have been recorded are probably not concerned in the depressor reflexes produced by the drug from the heart and lungs, for as shown by Dawes, Mott and Widdicombe (1951) the fibres concerned in these responses are blocked at higher temperatures. These afferents so far remain unidentified.

The normal function of the phenyl diguanide-sensitive fibres however remains to be settled. They are certainly not any of the known thoracic afferents. It is possible they originate from the abdominal viscera although the number of myelinated fibres in the vagus caudal to the heart are very few. It has been suggested (Whitteridge, personal communication) that they may be pain fibres from the lungs.

This is possible for although pain is rarely observed during bronchiscopic manipulation it appears to be a prominent feature of bronchogenic carcinoma (Jackson 1942). However a satisfactory method of stimulating pain fibres specifically would be necessary if this hypothesis were to be tested adequately. Tryptamine and serotonin would appear to offer certain possibilities (Armstrong, Keel, & Markham, 1952).

The fact that phenyl diguanide and pituitarin both excite the same fibre but produce exactly opposite reflex effects namely, bradycardia, fall in blood pressure, and inhibition of respiration on the one hand and increase in the heart rate, rise in blood pressure, and rapid respiration, on the other, casts a certain amount of doubt on the validity of conclusions drawn from a study of reflex changes alone. At least it is clear that a study of effects of the drug on afferent fibre activity can only have a limited value in determining which fibres are responsible for particular reflex effects. In the case of phenyl diguanide as already pointed out there is reason to believe that a number of different fibres may be affected by the drug. On the other hand the method has revealed the presence of receptors which are normally inactive and therefore unobserved; and it is useful to the extent that it provides a way of studying the behaviour of these afferents.

SUMMARY.

Part I

1. A method of determining the conduction velocities in single respiratory and cardiovascular afferent units is described.

2. The conduction velocities of pulmonary stretch, depressor, pulmonary vascular, venous and chemoreceptor afferent fibres have been determined in the cat and it has been shown that:

   a) the range of conduction velocities of the thoracic vagal afferents extend from about 2 to 61 m/sec.

   b) there is a considerable overlap of the conduction velocities of the different afferents.

   c) a statistically significant difference exists between the mean conduction velocities of the Depressor & Pulmonary vascular fibres and also between the pulmonary stretch and pulmonary vascular fibres, but not between the depressor and pulmonary stretch fibres.

   d) there is a group of slowly conducting fibres with conduction velocities of 2 to 10 m/sec. consisting mostly of unidentified fibres in addition to deflation and chemoreceptor afferents and of fibres of unknown functions stimulated by an intravenous injection of 100 phenyl diguanide.

2. The compound action potential of the vagus has been analysed and it has been shown that a number of afferent fibres are represented in each of its components.
4. The value of studying reflex changes by stimulating the vagus or using differential nerve blocks has been discussed in the light of the present results.

Part II

1. The afferent fibre composition of the rootlets of the 9th, 10th and 11th cranial nerves has been studied in the cat by the electrical recording of nerve impulses.
2. Pulmonary stretch afferents were found in all the vagal and some bulbar accessory rootlets. None were found in the glossopharyngeal rootlets.
3. The rootlets have a heterogenous composition.
4. The observations of previous investigators are discussed.

Part III

1. The action of phenyl diguanide on pulmonary stretch, depressor, pulmonary vascular, venous and chemoreceptor afferents has been studied in the cat. The drug does not seem to stimulate or sensitize any of them.
2. Certain fibres of unknown functions stimulated by an intravenous injection of 100 γ phenyl

diguanide were found. These had a conduction velocity of 2-6 m/sec. and could be one of those responsible for the reflex inhibition of respiration produced by the drug.

3. The value of studying visceral reflexes by the use of drugs is discussed.

REFERENCES

ADRIAN, E.D. (1921). J.Physiol. 55, 193-225.

ADRIAN, E.D. (1926). J.Physiol. 61, 49-72.

ADRIAN, E.D., BRONK, D.W. (1928). J.Physiol. 66, 81-101.

ADRIAN, E.D. (1933). J.Physiol. 79, 332-358.

AMAAN, A. & SHAEFER, H. (1943). Pflüg.Arch.ges. Physiol. 246, 757-789.

ANREP, G.V. & SEGALL, H.N. (1926). J.Physiol. 61, 215-231.

ARMSTRONG, R.M.L., KEEL, C.A. & MARKHAM, J.H. (1952). J.Physiol. 117 in press.

BAINBRIDGE, F.A. (1915). J.Physiol. 50, 65-84.

BALLIN, I.R. & KATZ, L.N. (1942). Amer.J.Physiol. 135, 202-213.

BANNISTER, J., FEGLER, G. & HEBB, C. (1949). Quart.J. Exp. Physiol. 35, 233-250

BEER, T. & KREIDL, A. (1895). Pflüg.Arch.ges.Physiol. 62, 156-165.

BERKLEY, H.J. (1893). J.Comp.Neurol. 3, 107-111. Cited by Larsell, O. in J.comp. Neurol. 1922, 35, 97

BEZOLD, A.von & HIRT, L. (1867). Unters.Physiol.Lab. Würzburg. 1, 73- . Cited by Krayer, O. & Acheson, G.H. in Physiol. Rev. 26, 399.

BISHOP, G.H. & HEINBECKER, P. (1930). Amer.J.Physiol. 94, 170-200.

BISHOP, G.H., HEINBECKER, P. & O'LEARY, J. (1934). Amer.J.Physiol. 109, 409-421.

BLAIR, E.A. & ERLANGER, J. (1936). Amer.J.Physiol. 114, 309-316.

BRODIE, T.G. (1900). J.Physiol. 26, 48-71.

BRONK, D.W. & KALTREIDER, N.L. (1931). Amer.J.Physiol. 97, proc. 508.

CADMAN, A.W. (1900). J.Physiol. 26, 42-47.

CHASE, M.R. & RANSON, S.W. (1914).J.Comp.Neurol. 24, 31-60.

CHURCHILL, E.D. & COPE, O. (1929). J.exp.Med. 49, 531-537.

COLERIDGE, J.C.G., KENNEY, R.A. & NEIL, E. (1949). J.Physiol. 110, 27P.

COMROE, J.H.Jr.(1939).Amer.J.Physiol. 127. 176-191

CUNNINGHAM (1950) Text Book of Anatomy.

DALY, I.de B., LUDANY, G., TODD, A. & VERNEY, E.B. (1937). Quart.J.exp.Physiol. 27, 123-146.

DALY, M.de B., & EVANS, D.H.L. (1952).J.Physiol.in press

DAWES, G.S. (1947).J.Pharmacol.89,325-342.

DAWES, G.S. & FELDBERG, W.(1949).J.Physiol.108,362-369

DAWES, G.S. & FASTIER, F.N.(1950).Brit.J.Pharmacol. 5, 323-334.

DAWES, G.S. & MOTT, J.C.(1950)Brit.J.Pharmacol. 5, 65-76.

DAWES, G.S. (1951) Acta.Physiol.Scand.(1951).22,73-76.

DAWES, G.S., MOTT,J.C. &WIDDICOMBE, J.(1951). J.Physiol. 115,258-291., (1952) In press.

DeCASTRO, F. (1928). Trab.Lab.Invest.Biol.Univ.Mdr. 25, 331-380.

DICKINSON, C.J. (1950a) J.Physiol. 111, 399-407.

DICKINSON, C.J. (1950). Electrophysiological Technique London: Electronic Engineering.

DICKINSON, C.J. (1951). Unpublished observations.

DOGIEL, A.S. (1898).Arch.mikr.Anat. 52, 44-70.

DOGIEL, A.S. (1903).Arch.mikr.Anat.62. 244-250.

DONNET, V. (1951). Arch.Sci.Physiol. 5, 145-165.

DUKE, H. (1949).Quart.J.exp.Physiol. 35, 25-37.

ELFTMAN, A.G.(1943). Amer.J.Anat. 72, 1-27.

ERLANGER, J. & GASSER, H.S. (1937). Electrical signs of Nervous Activity.Philadelphia Univ. of Pennsylvania press.

FOLEY, J.O. & DuBOIS, F.S.(1934), J.Comp.Neurol. 60, 137-159.

FOLEY, J.O. & DuBOIS, F.S. (1937a)J.Comp.Neurol. 67, 49-67.

FOLEY, J.O. & DuBOIS, F.S.(1937b).J.Comp.Neurol. 67, 69-88.

GASSER, H.S. (1931). Amer.J.Physiol. 97,254-270.

GASSER, H.S. & GRUNDFEST, H. (1936).Amer.J.Physiol. 117, 113-133.

GASSER, H.S. & GRUNDFEST, H. (1939).Amer.J.Physiol. 127, 393-414

GOORMAGHTIGH, H. & PANNIER, R. (1939).Arch.Biol.Paris. 50, 455-533.

GRIEVE, J. (1951). Lancet, II, 431-432.

GROSSMANN (1895). Pflüg.Arch.ges.Physiol; 59, 1-8

HAMILTON, W.F., BREWER, G. & BROTMAN, I. (1934). Amer.J. Physiol. 107, 427-435.

HAMMOUDA, M., SAMAAN, A. & WILSON, W.H. (1943). J. Physiol.101, 446-459.

HAMMOUDA, M. & STELLA, G. (1935).J.Physiol.83.10P.

HAMMOUDA, M. & WILSON, W.H. (1935a).J.Physiol. 83, 292-312.

HAMMOUDA, M. & WILSON, W.H. (1935b).J.Physiol. 85, 62-72

HEAD, H. (1889).J.Physiol. 10, 1-70.

HEBB, C.O. & NIMMO-SMITH, R.H. (1949).Quart.J.exp. Physiol. 34, 159-163.

HEINBECKER, P.(1930).Amer.J.Physiol. 93, 284-306.

HEINBECKER, P. & O'LEARY, J.(1933a).Amer.J.Physiol. 106, 623-646.

HEINBECKER, P. & O'LEARY, J.(1933b).Proc.Soc.exp.Biol. N.Y. 30, 506.

HOLLINGSHEAD, W.H.(1939).J.Comp.Neurol. 71, 417-426.

HOLLINGSHEAD, W.H.(1940).Anat.Rec. 76, 283-287.

HUNT, C.H. & KUFFLER, S.W. (1951).J.Physiol.113,298-315.

JACKSON, C.L.(1942).Pain.Res.Publ.Ass.Nerv.Ment.Dis. 23. 271-273.

JARISCH, A. & RICHTER, H.(1939a).Arch.exp.Path. Pharmak. 193, 347-354

JARISCH, A. & RICHTER, H.(1939b).Arch exp.Path. Pharmak. 193, 355-371.

JARISCH, A. & ZOTTERMAN, Y.(1948). Acta.Physiol.Scand. 16, 31-51.

JONES, R.L.(1937). J.Comp.Neurol.67, 469-482.

KARSNER, H.J.(1911).J.exp.Med. 14, 322-325.

KNOWLTON, G.C. & LARRABEE, M.G.(1946).Amer.J.Physiol. 147, 100-114.

KRAYER, O., WOOD, E.H. & MONTES, G.(1943).J.Pharmacol. 79, 215-224.

KRAYER, O. & ACHESON,G.H.(1946).Physiol.Rev.26,383-446.

KREIDL, A. (1895).Pflüg.Arch.ges.Physiol.59, 9-16.

LANDGREN, S. & NEIL, E.(1951).Acta.Physiol.Scand. 23, 158-167.

LARRABEE, M.G. & KNOWLTON, G.C.(1946), Amer.J. Physiol. 147, 90-99.

LARSELL, O. (1921). J.Comp.Neurol. 33, 105-131.

LARSELL, O. (1922).J.Comp.Neurol. 35, 97-132.

LARSELL, O. & Dow, R.S. (1933).Amer.J.Anat.52,125-146

LARSELL, O. & MASON, M.L.(1921).J.Comp.Neurol. 33, 509-516

LEMERE, F.(1932).Amer.J.Anat.51, 417-437.

McDOWALL, R.J.S.(1924). J.Physiol. 59, 41-47.

McDOWALL, R.J.S.(1935). J.Physiol. 83, 37P.

McLAUGHLIN, A.I.G.(1933).J.Physiol. 80, 101 - 104.

MEGIBOW, R.S., KATZ, L.N. & FEINSTEIN, M. (1943) Arch. Int.Med. 71, 536-546.

MIDDLETON, S., MIDDLETON, H.H. & GRUNDFEST,H(1950). Amer.J.Physiol. 162, 545-552.

MOLLGAARD, H.(1912)Skand.Arch.Physiol. 26, 315-383.

NEIL, E.,REDWOOD, C.R.M. & SCHWEITZER, A. (1948). J.Physiol. 108, 8P

NEIL, E., REDWOOD, C.R.M. & SCHWEITZER, A. (1949a).J. Physiol. 109, 259-271.

NEIL, E., REDWOOD, C.R.M. & SCHWEITZER, A. (1949b). 109, 392-401.

NETTLESHIP, W.A. (1936). J.Comp.Neurol. 64,115-133.

NONIDEZ, J.F.(1935). Amer.J.Anat. 57, 259-301

NONIDEZ, J.F.(1936). J.Anat. 70, 215-224.

NONIDEZ, J.F.(1937a) Amer.J.Anat. 61,203-231.

NONIDEZ, J.F.(1937b).Anat.Rec. 69, 299-317.

NONIDEZ, J.F.(1941). Amer.J.Anat. 68, 151-189.

OBERHOLZER, R.J.H.(1951). J.Physiologie. 43,329-360.

OKAMURA, C,(1930). Z.Anat.Entw-Gesch. 92, 20-26.

PALME, F.(1934). Z.mikr-ant. Forsch. 36, 391-420.

PARTRIDGE, R.C. (1939). J.Physiol. 96, 233-239.

PEARCE, J.W. (1951). The Control of Pulmonary Blood Pressure and its relation to certain reflex respiratory phenomena. Unpublished D.Phil.thesis Oxford.

PEARCE, J.W. & WHITTERIDGE, D.(1951).Quart.J.exp. Physiol. 36, 177-188.

PENITSCHKA, W. (1931). Z.Mikr-ant.Forsch.24, 24-37.

RANSON, S.W.,FOLEY,JO. & ALPERT, C.D.(1933).Amer.J. Anat. 53, 289-316.

RANSON, S.W. & MIHALIK, P.(1932).Anat.Rec. 54,355-360.

RICHTER, H. & AMAAN, A.(1940).Arch.exp.Path.Pharmak. 196, 275-279.

SASSA, K. & MIYAZAKI,H.(1920).J.Physiol.54, 203-212.

SCHWEITZER, A.(1936). J.Physiol. 87,46-48P.

SCHWIEGK, H.(1935). Pflüg.Arch.ges.Physiol. 236,206-219.

SHARPEY-SCHAFER, E. & BAIN, W.A.(1932).Quart.J.exp. Physiol. 22, 101-147.

STEFFENSON, E.H., BROOKHART, J.M. & GESELL, R.(1937). Amer.J.Physiol.119, 517-526.

TAKINO, M.(1932a).Acta.Sch.Med.Univ.Kioto.15,303-307.

TAKINO, M.(1932b).Acta.Sch.Med.Univ.Kioto.15,308-320.

TAKINO, M.(1932c).Acta.Sch.Med.Univ.Kioto.15,321-354.

TAKINO, M. & WATANDE, S.(1937).Arch.Kreislauf. 2,18-27.

TUTSO, M.(1937).Pflug.Arch.ges.Physiol.238,738-748.

TORRANCE, R.W. & WHITTERIDGE, D.(1947).J.Physiol. 107, 6-7P.

WALSH, E.G.(1947).J.Physiol. 106, 466-470.

WALSH, E.G. & WHITTERIDGE, D. (1944).J.Physiol.113,37P.

WEIDMANN, H., BERDE, B. & BUCHER, K. (1949).Helv. Physiol.Acta. 7, 476-481.

WHITTERIDGE, D. (1948).J.Physiol.107, 496-512.

WHITTERIDGE, D. (1950).Physiol.Rev. 30, 475-486.

WHITTERIDGE, D. (1951). Unpublished observations.

WHITTERIDGE, D. (1952). Symposium on Visceral Circulation. Ciba conference.

WIDDICOMBE,J(1952a). J.Physiol. In press.

WIDDICOMBE, J.(1952b) unpublished observations.

WYSS, O.A.M. & RIVKINE, A.(1950). Helv.Physiol.Acta. 8, 87-108.