BUFFER NERVE REFLEXES IN BARBITURATE ANAESTHESIA.

THESIS

Submitted By

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INTRODUCTION.

Since the discovery of POURFOIS DU PETIT (1727), that section of the cervical sympathetic gave rise to dilatation of the vessels of the conjunctiva, great interest has attached to the nervous control of the blood vessels. The anatomical basis was provided by the early histologists, including WRISBERG (1784), who followed nerves in and around arteries, and by HENLE (1840) who described the muscular coat of these vessels. Close on a century ago nervous vasoconstriction had been established and soon thereafter the classical experiments of BERNARD (1858) demonstrated that the nervous influence might be dilator.

Subsequent work has amply confirmed the presence of constrictor and dilator nerves and has shown their importance in the control of the vascular bed and blood pressure. It has been found that these vasomotor nerves are governed by the central nervous system which can thus extend its co-ordinating influence to the vascular system to bring about alterations in the pressure and the distribution of the blood to meet the needs of the organism. The vasomotor activity of the higher nervous structures is not dependent solely on their immediate environment, but may be modified by a number of factors, psychic and nervous, external or internal. Many mechanisms, interoceptive and exteroceptive, can produce reflex changes in vasomotor activity and blood pressure, often to a considerable extent, but the majority of these come into play only in special circumstances and exert no constant influence/

influence under normal conditions. They are thus unsuited to the maintenance of a 'working level' of blood pressure. The essential nervous control of the blood vessels lies, not with these unspecific afferents but with special afferent nerves arising within the walls of the arteries. Thus the great tide of vasomotor activity, upon which the random waves are superimposed, is governed by impulses from within the vascular system itself, much as an engine modifies its own activity when fitted with a governor.

The nerves which possess this regulating activity, the so-called moderator nerves are bilateral structures arising from the great vessels some little distance from the heart. There are two pairs. The first, discovered by CYON and LUDWIG (1866) take origin from the arch of the aorta and are known as the depressor (or aortic) nerves. The second two arise, one on each side, from the carotid sinus region at the level of bifurcation of the common carotid artery. They were discovered by HERING (1924), and are named after that author or known as the sinus nerves. Both the aortic and the sinus nerves have similar functions, section of either causing a rise in blood pressure and stimulation a fall, and they may be regarded as exercising a steady depressor activity on the mechanisms responsible for maintenance of vascular tone and blood pressure. Notwithstanding the accumulating evidence pointing to the existence of afferent pathways primarily concerned with the regulation of the blood vessels/

vessels other than these four nerves, it is certain that they play a very minor rôle. Modern work has clearly established the constant inhibiting activity exercised by the sinus and aortic nerves on vascular tone and blood pressure, and it is beyond doubt that these nerves are the afferents of paramount importance.

It was in the light of this essentially depressor function that it was decided to investigate the responses to stimulation of the moderator nerves in animals under barbiturate anaesthesia. The problem had arisen out of an accidental finding in this department that electrical stimulation of the central ends of the vagus nerves in a cat overdosed with nembutal (sodium pentobarbital) caused, not a fall, but a rise in blood pressure. Now, although the expected response, and that usually obtained, is a fall in blood pressure due to activation of the contained aortic nerve fibres, pressor responses from vagal stimulation are not uncommon and have been reported by many authors. The pressor response in this animal might have been written off as something already observed and understood, or at least described. It was felt, however, that the special circumstances of its appearance, in an animal overdosed with a drug closely related to the modern barbiturate anaesthetics of medical practice, indicated the need for further research. Confirmatory experiments left no doubt as to the grossly altered nature of the vasomotor reflexes in deep nembutal anaesthesia.

This work presents the findings of a series of experiments carried/

carried out in the Physiological Laboratory of the University of Aberdeen by the author and devoted to the study of aortic and sinus nerve vasomotor reflexes, with special reference to the effect of nembutal. The matter upon which the paper is based was obtained from observations made upon more than ninety animals, the great majority cats.

PART 1.

ANATOMICAL AND FUNCTIONAL CONSIDERATIONS.

PREVIOUS WORK.

THE CENTRAL AND EFFERENT NERVOUS MECHANISMS INVOLVED IN VASOMOTOR ACTIVITY.

THE EFFERENT PATHWAYS.

The Peripheral Vasoconstrictor Mechanism.

After POURFOIS DU PETIT's description of the effect of cervical sympathetic section, many years were to pass before the nature of this phenomenon was to be fully understood. Certainly when a modified version of his experiment was performed by DUPUY and BRACHET in 1830, over a hundred years later, the fact that the increased temperature and redness of the eye of the horse following removal of the superior cervical ganglion was due to loss of nervous action on the blood vessels was not appreciated. The condition was ascribed to upset of the nutritive function of the 'grand sympathique'. To be sure, there was already in existence histological evidence showing the close relationship between nerves and arteries, for WRISBERG (1784), LUCAE (1810), RUDOLPHI (1821) and SCHLEMM (1828) had all indicated its presence. It must be pointed out, however, that the anatomical basis for arterial responsiveness, the contractile muscular coat of these vessels, was not demonstrated by HENLE until 1840. This worker recognised the significance of his discovery and interpreted VALENTIN's observation of arterial constriction from nervous stimulation as due to the action of the nerves on this muscular tissue. STILLING (1840) described such activity as

'vasomotor/

'vasomotor' and observed it as a result of stimulation of the spinal cord thus demonstrating the influence of the central nervous system over the arteries. AXMANN (1847) suggested that the sympathetic chain was essentially a vasomotor nerve.

Thus by 1851 there was a background, no doubt scanty but nevertheless sufficient, to point to the nervous control of the blood vessels. It was in this year, however, that certain experiments were performed which gave impetus to the study. WHARTON JONES demonstrated that the frog web capillaries dilated after sciatic nerve section and BERNARD showed that a similar condition occurred in the ear vessels of the rabbit following section of the cervical sympathetic. A year later BROWN-SEQUARD (1852) performed the complementary experiment of stimulation of that nerve and found that it caused constriction of the ear vessels. These experiments served to establish vasoconstriction and to associate the phenomenon with the sympathetic system. It remained now to follow up the work of STILLING and relate that system to the spinal cord.

PFLUGER (1855) showed that stimulation of the anterior roots of the spinal nerves going to constitute the sciatic caused vaso-constriction in the frog, and after a great deal of research GASKELL (1885) was in a position to state, "I am inclined boldly to assert that in mammals all the vasomotor nerves of the body of necessity leave the central nervous system in the outflowing stream of visceral fibres which occurs between the second thoracic/

thoracic and second lumbar nerves". He went on to show that the fine medullated fibres left the cord by the anterior roots, ran into the sympathetic chain and became 'changed' to non-medullated fibres in the ganglia of the chain, thereafter being distributed to their destinations directly or after communication with other ganglia.

DICKENSON and LANGLEY (1889), studying the effect of nicotine, showed that in moderate dose it paralysed the nerve cells of various sympathetic ganglia without paralysing the peripheral endings. Stimulation of the nerve before the ganglion under these circumstances was without result while stimulation distal to the ganglion was still effective. From this finding developed the knowledge of the synapse, and LANGLEY (1901), who coined the term preganglionic and postganglionic for the fibres involved, showed that there were always at least two neurones concerned in the conduction of an impulse from the central nervous system to smooth muscle or glandular tissue. GASKELL showed that the first of these, the preganglionic neurones, arose from cells lying in the lateral horn of the grey matter of the spinal cord. The researches of these two last workers resulted in the anatomical picture of the efferent vasoconstrictor path as at present understood.

The humoral aspect of sympathetic activity was indicated by LANGLEY (1901), who pointed out that adrenaline produced the same effects/

effects as stimulation of the sympathetic nerves. The conception that such nerves cause vasoconstriction by the liberation of adrenaline at their endings was advanced by ELLIOTT (1904) and supported by FINKLEMAN (1930), and for this reason DALE (1933) suggested that such fibres be termed 'adrenergic'. FELDBERG and GADDUM (1934) completed the chain from cord to artery by showing that transmission across the synapse was accompanied by the liberation of acetylcholine.

The vasoconstrictor efferent pathway may be summarised thus:- Preganglionic fibres arise from cell bodies located in the visceral efferent column (lateral horn of the grey matter) of the spinal cord and pass out between the first thoracic and the third or fourth lumbar segments (RANSON, 1943) as fine myelinated fibres in the anterior root of the corresponding segments. There they leave via the white rami to join the sympathetic trunk and synapse in the ganglia of the same segments or at some more distant ganglion. At the synapse these preganglionic fibres liberate acetylcholine to activate the postganglionic non-medullated fibres. These finally reach the vessels by way of the grey rami or cervical or lumbar chains and bring about constriction by the liberation of adrenaline or like substance.

The Peripheral Vasodilator Mechanism.

When the brothers WEBER demonstrated in 1845 that electrical stimulation of the cardiac vagus could stop the heart, they established/

established a new principle of nervous action. Some years later BARNARD (1858) demonstrated that the smooth muscle of the arteries also might have its activity inhibited by nervous action. He found that stimulation of the chorda tympani caused an increased flow of blood from the submaxillary gland. Subsequent work has confirmed the vasodilator action of this nerve and has showed the existence of similar fibres in the lingual, petrosal and pelvic visceral nerves (GASKELL, 1885) and all these have been regarded as special dilator nerves. Their existence did not then, and does not now, call for a modification of the conception of vasomotor activity as essentially involving constriction nerves.

The demonstration of a more generalised and significant dilator mechanism began with the work of DOGIEL (1872), who stimulated the sciatic nerve in curarized animals and found that vasodilation resulted. It has been shown since, that vasodilator fibres occur extensively in mixed nerves, although special technique to avoid interference from co-existent pressor fibres must be employed to reveal them. These dilators resemble the sympathetic in that they are widely distributed and have a large field of influence, but unlike the sympathetic they do not form macroscopic gross structures but run intermingled with the somatic nerves.

The manner of exit of these fibres from the spinal cord is of great interest. STRICKER (1876) obtained vasodilation from the stimulation of the peripheral end of the cut dorsal roots and suggested/

suggested these as the pathways. This was, however, contrary to the findings of Sir Charles Bell (1811, 1844) that the dorsal roots are sensory and the ventral roots motor, a conception known as BELL'S LAW. Interest was added when BAYLISS (1901) examined this system and confirmed that the dorsal roots did possess dilator fibres but that they were anatomically indistinguishable from the ordinary sensory afferent fibres, failing to degenerate when the roots are cut between the cord and the ganglion but degenerating when the dorsal root ganglia are removed. Inasmuch as it appeared to BAYLISS that these fibres were in fact carrying impulses in a direction opposite to the normal he called them 'antidromic' a term suggested to him by LANGLEY.

That the dorsal root dilators are merely sensory fibres acting antidromically has been doubted by several workers. MISLAWSKY and BISTRENIN (1905) claimed that section of the dorsal roots did not cause central degeneration of the dilator fibres, while MULLER (1924) suggested that the dilator fibres arose from cells in the dorsal horn of the spinal cord and were of parasympathetic nature. This view was taken up by KURE et al (1927) who, in a series of papers, stressed that the posterior root dilators constituted a spinal parasympathetic outflow, the parent cells of which lay in the grey matter of the cord at the base of the dorsal horn. They maintained that the fibres arising thus passed out by way of the dorsal roots to synapse in the ganglia of these structures with other fine medullated fibres which in

turn/

turn ran in the sensory nerves. In the cervical and lumbar regions they found that forty per cent. of all the nerve fibres of the posterior roots were of this parasympathetic type, while in the thoracic region such fibres constituted one third to one half of the fine medullated neurones. KURE et al (1932) concluded: 'the commonly held hypothesis that the vasodilator effects seen on stimulation of the dorsal roots of the spinal nerves in the thoracic region of the cord are produced by antidromic impulses passing down the sensory fibres is incorrect'. They point out that GAGEL (1932) found that in man examination of the central end of the cut degenerated dorsal root revealed the presence of small myelinated fibres only. BACH (1945) believes that the 'parasympathetic type' fibres arise in the ganglia of the dorsal roots. The precise nature of the dilator fibres is thus still an open question.

There is, however, closer agreement on the question of transmission of the dilator impulse. LEWIS and MARVIN (1924) indicated that histamine might be the substance revealed by the antidromic fibres, but DOI (1920) has shown that they are still effective despite previous capillary dilatation induced by histamine. WYBAUW (1936, 1937, 1938) has shown that the substance involved is acetylcholine. This has been confirmed by several workers among them BACH (1945), who has presented the evidence for the cholinergic nature of the dorsal root dilators.

There is a third dilator mechanism to be considered. In

1906/

1906 and 1913 DALE showed that after ergotoxine electrical stimulation of the splanchnic nerves might give rise to a vasodilation in the intestine, or stimulation of the abdominal sympathetic to vasodilation in the hind limb. This threw new light on the observations of DASTRE and MORAT (1880) indicating the presence of sympathetic dilators in the bucco-facial region. The question lay dormant for some years until taken up by BURN (1932). This worker made a study of the sympathetic vasodilators in various animals, and along with BULBRING (1935) showed that such nerves existed in the muscles of the cat and dog. In the cat these were fewer than in the dog and differed in that they were adrenergic whereas those of the dog were cholinergic. There were exceptions, however, for they point out that 'the fibres in the cat are in the main adrenergic though there are some which are cholinergic'. Extending the study to the skin and intestine (BULBRING and BURN, 1936), they were able to show that in the cat although sympathetic dilators were absent in the skin, fibres of this nature were present in the splanchnic nerves. These last they claim are not cholinergic. CLARK (1934) had also failed to find evidence of sympathetic vasodilators in the skin of cats or dogs.

In a later review, BURN (1938) attempted to ascribe a function to these fibres. He postulated a relation to the potentiating action of sympathetic chain stimulation on fatigued muscle brought about by stimulation of anterior roots, such as was found by ORBELI/

ORBELI (1923). While the function of the splanchnic dilators he thought was to innervate the peripheral branches so that while constriction of the larger arteries safeguards the general blood pressure, dilatation in the terminal arterioles or capillaries facilitates the blood flow to the intestines. He stressed the species difference, pointing out that the rabbit and monkey have no such fibres, and suggested that sympathetic vasodilator fibres acquire importance in animals of the chase which are capable of prolonged exertion.

Such then are the efferent pathways through which the central nervous system can influence the size of the blood vessels. Without doubt the sympathetic constrictor mechanism is the most embracing, and from the evidence would appear to be in the best position to influence the vascular bed as a whole. The dilator mechanisms, on the other hand, are not so comprehensive. The special dilator nerves, chorda tympani, nervi erigentes and the like have obviously a part to play in the local distribution of blood but can scarcely be looked upon as the counterpart of the sympathetic system having a wide influence over the general vascular system and blood pressure. So also the rôle of the sympathetic dilators, adrenergic or cholinergic, seems not to be that of causing generalised dilation of blood vessels and lowering of systemic blood pressure, but rather of modifying locally the vasoconstriction brought about by increased sympathetic constrictor activity. But against the conception of the/

the sympathetic system as the all important mechanism in generalised vasomotor reflexes, we must place the increasing amount of evidence indicating the comprehensive nature of the dorsal root dilator system.

Normal Vascular Tone.

The extent to which the nervous control of the blood vessels is responsible for their state of contraction or relaxation under normal conditions must be considered, for it gives an insight into the manner in which modifications in arterial state may be brought about. The problem is intimately concerned with the inherent muscular tonus of the vessels. If the nervous influence be disregarded, the volume of the vascular bed, or more simply, the size of the lumen of any vessel, is dependent upon the activity of its muscular coat. There is no doubt that blood vessels can exhibit a marked degree of tonus when completely isolated from all nervous influence. McWILLIAM (1902) examined this property in vessels isolated from the body, while GOLTZ and FREUSBERG (1874) showed that in the intact animal denervated vessels regained their tonus in a few days. Evidence has recently been led by BACH (1945) to show that the partially contracted state is the normal. Such tonus is not peculiar to the blood vessels; but is well known in other smooth muscle. For example, ROEPKE and HENDERSON (1934, 1935) have found this inherent tonus in the gut and bladder, while GULLBERG, OLMSTED and WAGMAN (1938) have shown 'idiotonus' in the pupillary muscles. Many years ago WHARTON JONES (1852) discovered that the veins of the bat's wing were 'endowed with rhythmical activity/

activity' and recently NICOL and WEBB (1946) have shown that the terminal arterioles and capillaries of the bat's wing evince vasomotion after denervation. The nature of this activity, and of vessel tonus is not understood, but it is generally believed to be a property of the smooth muscle cells themselves. It is of interest to note that the fine interstitial cells of Cajal, which are found intermingled with the smooth muscle tissue of the arteries and elsewhere, are considered by MEIJLING to be of essentially nervous nature. This author believes that they form a syncitial network closely embracing the smooth muscle to lie between the post-ganglionic fibres of the autonomic nervous system and the effector tissue. In a recent communication MEIJLING (1948) has postulated that this network is the intermediary in the transmission of post-ganglionic impulses, and suggested that humoral agents liberated by the nerves act upon this network which in turn excites the tissues concerned. It is possible that the powers of contraction of such tissues are dependent upon the integrity of this network. The intimate relationship of the plexus to the effector cells, coupled with the fact that they do not degenerate when post-ganglionic fibres are cut, nor when the tissue is injured, being in nature of a syncitium, has thus in fact not permitted the study of truly denervated organs. If the interstitial cells of Cajal are regarded as the 'nervous system' of a tissue upon which the central nervous system exerts its influence by intermediate neurones, it is conceivable that the idiotonus of the blood vessels might result

from inherent activity of these cells.

Be this as it may, normal vascular tone does not depend on inherent vascular tonus alone. The effects of denervation discussed above indicate a constant constrictor nervous influence and, indeed, this has been recorded electrically by many workers, recently by GERNANDT, LILJESTRAND and ZOTTERMAN (1946). Such sympathetic tonus is generally admitted.

The evidence for vasodilator tone is not so complete. BAYLISS (1893 - 1923), although believing in its existence, found great difficulty in demonstrating it. Recently BACH (1945) has stressed that the dorsal root dilators exert considerable tonus in the cat and has shown that after section of the dorsal roots the blood pressure rises. This finding is in agreement with the old theories of dual innervation and antagonism. If KURE's view that the dorsal root dilators are of parasympathetic nature holds, then in the control of the blood vessels there is further evidence for what HARE (1946) calls 'the dichotomous classification of the autonomic nervous system into sympathetics and parasympathetics which carry on a perpetual tug-of-war'. It is possible that the large measure of control over the blood vessels is sympathetic and constrictor, but dilator influence must certainly be borne in mind.

THE HIGHER CONTROL OF THE EFFERENT PATHWAYS.

The Medullary Vasomotor Centres.

The study of the higher mechanisms in the central nervous system/

THE MEDULLARY VASOMOTOR CENTRES.

1. THE POINTS ON THE FLOOR OF THE 4th VENTRICLE. (Ranson and Billingsley, 1916).

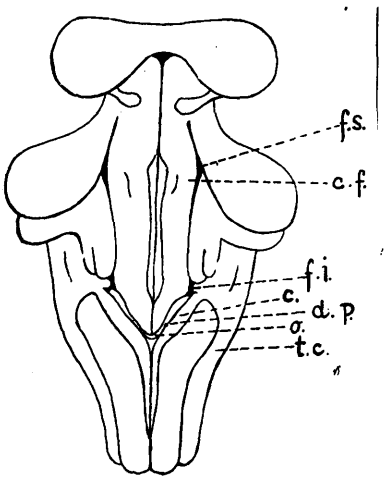

Fig. 1. Diagram of the floor of the fourth ventricle of the cat. *f.s.*, fovea superior; *c.f.*, colliculus facialis; *f.i.*, fovea inferior; *c.*, clava; *d.p.*, depressor point; *o.*, obex; *t.c.*, tuberculum cinereum.

2. THE LOCALIZATION OF THE CENTRES. (Alexander, 1946).

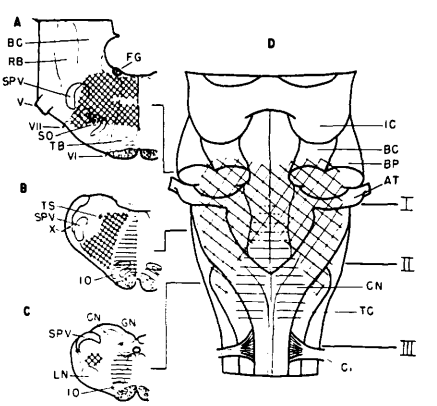

FIG. 1. Localization of pressor and depressor centers in the brain stem of the cat. Pressor regions indicated by cross hatching; depressor regions by horizontal ruling. A-C: cross sections through medulla at levels indicated by guide lines to D; D: semi-diagrammatic projection of pressor and depressor regions onto the dorsal surface of the brain stem viewed with the cerebellar peduncles cut across and the cerebellum removed.

Legend: AT: auditory tubercle; BC: brachium conjunctiva; BP: brachium pontis; C1: first cervical nerve; CN: cuneate nucleus; FG: facial genu; GN: gracile nucleus; IC: inferior colliculus; IO: inferior olivary nucleus; LN: lateral reticular nucleus; RB: restiform body; SO: superior olivary nucleus; SPV: spinal trigeminal tract; TB: trapezoid bodyi; TC: tuberculum cinereum; TS: tractus solitarius; V, VI, VII, X: corresponding cranial nerves; I, II, III: levels of transection discussed in text.

system which control the efferent pathways and regulate vasomotor activity began with work carried out in LUDWIG's laboratory at Leipzig. First DITTMAR (1870) found that central stimulation of the sciatic nerve gave a reflex rise in blood pressure after separation of the cord and medulla from the rest of the brain, and then OWSJANNIKOFF (1871) localised the part responsible for the mediation of vasomotor effects by the method of descending section of the brain. This worker, who carried out his research upon rabbits, showed that removal of the four to five millimeters of brain matter above the calamus led to the same results as cervical cord section, that is to say, a maximal fall in blood pressure and loss of pressor or depressor reflex excitability. DITTMAR (1873) histologically identified this 'vasomotor centre' in the cells of the superior olive.

The question was next taken up by RANSON and BILLINGSLEY (1916), who exposed the floor of the fourth ventricle in six cats and with unipolar electrical stimulation identified two points, a 'pressor point' and a 'depressor point' which gave a rise and a fall in blood pressure. As may be seen from their diagram which is reproduced below, the pressor point was found to be at the fovea inferior or ala cinerea and the depressor point in the area postrema just lateral to the obex. These authors at this time expressed the view that the depressor point might represent a true dilator centre, but they indicated that both regions might represent points on the afferent pathways, pressor or depressor.

SCOTT/

SCOTT and ROBERTS (1923) in attempting to confirm RANSON and BILLINGSLEY's work were unable to obtain the pressor response with any regularity. They were, however, able to obtain depressor responses from the depressor point, which they said also caused cardio inhibition. They believed it 'to represent a point where afferent vagal fibres belonging to the depressor arc occupy a superficial position'. With regard to the pressor point, they maintained that pressor activity could not be ascribed to any one point, and support their argument by pointing out that in the light of the progressive loss of tone shown to occur with descending section (OWSJANNIKOFF) this region should not be so confined. In a later paper, SCOTT (1925) showed that certain pressor reflexes persisted after destruction of the pressor point and certain depressor reflexes could be obtained after similar treatment of the depressor point, but that loss of these points resulted in loss of vagal pressor and depressor activity. This strengthened the view advanced previously by him and his co-worker and he stated at this time that 'the pressor points are merely points on the reflex pressor areas of the vagi'. The study of these points in the fourth ventricle was continued by CHEN, LIM, WANG and YI (1936), who showed that stimulation of the pressor point calls forth not only a rise in blood pressure but a 'sympathetic' response from a number of organs, and because of this considered it a 'myelencephalic sympathetic centre'. These workers showed that these effects persisted in the absence of the/

the hypothalamus (1937) and that they were abolished by cervical section at the second segment. Their pressor area was in agreement with RANSON and BILLINGSLEY's, but tended to be more diffuse. The depressor point was also studied in this light by LIM, WANG and YI (1938), who confirmed the original localisation and showed that in addition to the fall in blood pressure there were also visceral reactions opposite in sign to sympathetic, and concluded that this point was a 'sympatho-inhibitory centre'. These results were obtained in cats and dogs, and in the dog they found that section of the brain between the upper pressor point and lower depressor point did not abolish the depressor activity of the latter on stimulation and decided that the depressor centre was independent and not exerting its effect by inhibition of the pressor centre.

Thus far investigation had been confined to the surface of the brain. The development of the HORSLEY-CLARKE stereotaxic instrument, allowing of accurate localisation of electrodes in the brain substance, permitted the study of the deeper parts of the brain stem. WANG and RANSON (1939), employing this device, stimulated the brain stem of nembutalized cats with bipolar electrodes. They were able to confirm the points as originally described, but found, as SCOTT had stressed, that they were but points where the pressor and depressor regions reached the surface of the fourth ventricle. Indeed, the deeper lying pressor and depressor regions they found to be much more extensive/

extensive. The region giving rises of blood pressure they found was situated in the 'lateral reticular formation throughout the rhombencephalon', while the depressor area was 'at the level of the inferior olive and tended more to the midline'. They did not distinguish whether these areas giving rises or falls were afferent or efferent or association cells possibly forming a centre. MONNIER (1939), in a less extensive work, has also found pressor and depressor areas in similar situations. Recently ALEXANDER (1946), in a study of the functions of the cardio-vascular centres has confirmed WANG and RANSON's localisation of the pressor and depressor regions. He has constructed a composite diagram which is reproduced above. By brain stem section he has shown that the 'functional deficits resulting from transections at various levels are in close agreement with what would be anticipated from the exploratory experiments'. Following OWSJANNIKOFF, he has shown that sections removing increasing amounts of pressor area cause a progressive fall in blood pressure, that is section at 1 and 11, but further, he has shown that loss of the depressor area, by section at III, is followed by an increase in sympathetic activity as recorded in the inferior cardiac nerve. This, he indicates, is evidence of the tonic depressor activity exerted by this centre. In conclusion, he maintains that both the pressor and depressor centres are functionally significant and exert tonic pressor and depressor influence over the efferent pathways.

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The Spinal Vasomotor Centres.

It has long been known (GOLTZ, 1864) that after the loss of all brain substance above the cervical region there is a profound fall in blood pressure, and that after time for recovery there is a return of vascular tone to a considerable degree, which finally disappears and causes the blood pressure again to fall when the cord is destroyed. Much confirmatory evidence has accumulated, and the explanation generally offered is that there are 'subsidiary vasomotor centres in the cord capable of taking over vasoconstrictor activity in the absence of the medullary vasoconstrictor centres' (McDOWALL, 1938). This author stresses the minor part played by such centres, stating that 'the evidence that the spinal centres normally play an important part is not very strong', but goes on to show that where precautions are taken to avoid 'shock' these centres take over very rapidly. BARD (1930) points out that the evidence in favour of bulbar dominance over spinal vasoconstrictor mechanisms is based on comparison of the bulbo-spinal and spinal preparations, and suggests that the comparison is unfair as spinal shock is such a big factor in the spinal animal. ALEXANDER (1945), following up the old knowledge that the spinal centres were sensitive to the gaseous content of the blood (KAYA and STARLING, 1909), by recording the electrical activity of the sympathetic outflow in spinal preparations came to the conclusion that the oxygen tension in the spinal cord of the normal animal may contribute to the excitatory state of the spinal/

spinal cardiovascular centres. In a later paper, ALEXANDER (1946) expresses the opinion that these centres possess tonic activity which is modified by the medullary neurone pools. He maintains that the depressor centre of the medulla exercises a tonic depressor influence over these spinal neurones, and points out that the activity of the spinal vasoconstrictor centres increases when the depressor region is removed. This work then places greater importance on the normal function of the spinal centres and indicates that the sympathetic cells lying in the visceral efferent column of the grey matter of the cord do not act merely as relay cells on the vasoconstrictor pathway from the medulla, reflecting the activity of vasoconstrictor centre, but are subject to inhibiting influence from the depressor centre, a view also held by LIM, WANG, and YI (1938), and subscribed to by GERNANDT and ZOTTERMAN (1946). It is of interest to note here, that DALE and EVANS (1922) observed that in the spinal animal a higher blood pressure was obtained when the cervical cord was cut at the third segment rather than the first.

In the light of these researches it appears that the cells in the lateral horn may normally exert spontaneous constrictor tonus and that increased sympathetic activity and vasoconstriction may arise, not only as is commonly agreed, from increase in the excitor impulses descending from the pressor centre but also from a diminution of the inhibitor impulses from the depressor centre, while decreased sympathetic activity may result from a similar reciprocal/

reciprocal mechanism of opposite nature.

The spinal centres to which reference has been made above are formed by the aggregations of preganglionic sympathetic cells and are, of course, 'pressor' centres. Whether or not the dilator cells held by KURE (1932) to exist in the posterior horn constitute spinal dilator centres in an analogous fashion is unknown.

The pathways through which the higher medullary centres influence the efferent mechanisms have been investigated by several workers. CHEN, LIM, WANG and YI (1937) found that the tract from the pressor centre, the 'myelencephalo-spinal sympathetic tract', passed 'unilaterally down the spinal cord in the ventrolateral column'. This tract terminates round the preganglionic cell bodies and is excitor. The fibres descending from the depressor centre to inhibit these cells are said by LIM, WANG and YI (1938) to travel in the dorsolateral columns of the cord. The descending tract to the posterior root dilator cells has not been experimentally localised, but ROSENBLUETH and CANNON (1934) showed that they could activate this mechanism by stimulating the depressor point on the floor of the fourth ventricle, while BACH (1946) states that descending paths from the depressor centre in the medulla travel down the cord and out by the dorsal roots to activate the 'parasympathetic type' fibres in the dorsal root ganglia.

The Hypothalamus.

It/

It is well established that the hypothalamus is intimately concerned with the autonomic nervous system. BARCROFT (1934) has concluded that the controlling mechanisms for autonomic function are centred on the hypothalamus or near about, and BEATTIE (1938) has stated that 'the physiology of the hypothalamus is the physiology of the internal environment' and has pointed out that the evidence clearly indicates the presence of two distinct mechanisms in the hypothalamic region, one producing a co-ordinated response of numerous sympathetic reflexes and the other a similar response of the parasympathetic reflexes. Direct electrical stimulation of the hypothalamus and surrounding regions has been carried out by several workers, among them KABAT, MAGOUN and RANSON (1935), WANG and RANSON (1939) and HARE and GEOHEGAN (1941), and they have shown that pressor or depressor responses may result. It is generally agreed that the hypothalamus can invoke the activity of pressor or depressor mechanisms, but the part played by that region in reflexes involving the afferent vasomotor nerves is unknown. Certainly vasomotor reflexes do not appear to be greatly altered by decerebration, for many workers employing intact and decerebrate animals for the study of such reflexes have not seen fit to indicate any difference in the two preparations. Furthermore, BRONK, PITTS and LARRABEE (1940) have shown that the activity of the inferior cardiac nerve, which is dependent on the afferent discharge/

discharge from moderator nerves, is in no way dependent upon the hypothalamus. Nevertheless, in a later paper BRONK, PITTS and LARRABEE (1941) point out that stimulation of the hypothalamic pressor regions can block the depressor activity of the moderator nerves, while strong buffer nerve stimulation can suppress the sympathetic activity brought about by hypothalamic stimulation. These authors, in their analysis of hypothalamic cardiovascular control, suggest that one function of this system is to permit deviation from the normal - of a degree directly related to the intensity of hypothalamic activity. On this basis, although vasomotor reflexes are not thought to be mediated through, and appear perfectly well in the absence of the hypothalamus, this part of the brain would appear, nevertheless, to be capable of modifying the extent of the reflexes in one direction or the other.

THE SPECIAL FUNCTIONS OF THE BUFFER NERVES.

Before going on to detail the part played by the vasomotor mechanism in response to afferent discharge of the sinus and aortic nerves, the special function of these nerves will be considered.

THE AORTIC NERVES.

These were discovered in the rabbit by CYON and LUDWIG (1866) who called them the 'depressor' nerves, as stimulation of their central ends caused reflex cardiac inhibition and vascular dilatation. They supposed the nerves to come from the heart.

BERNARD/

BERNARD (1868) studied the anatomy of the nerves in cats, and this work was extended by ANUFRIEW (1928) who gives a detailed account of their paths and distribution. LIDDELL and SHERRINGTON (1929) stimulated them in the cat, and found that they produced the same effects in that animal as in the rabbit. WOOLDRIDGE (1883) showed that nerves giving similar depressor responses occurred in the dog and that they arose from the aorta. On this account he termed them the 'aortic nerves'. Since then, functionally similar nerves from the aortic region have been described in other mammals, and their truly afferent nature shown by the fact that stimulation of the central end of any of them results in the depressor effects described while stimulation of the peripheral end is without effect.

TELLO (1924) has shown from the study of embryos that the fourth branchial arch receives innervation from the superior laryngeal nerve, and that these fibres become the aortic nerve terminating in the wall of the aorta in a fine basket work of nervous filaments. This confirms and enlarges upon the research on the subject carried out by ROEVER (1869), who was the first to show that the 'depressor' fibres came not from the heart but from the aorta. Subsequent work has shown that the distribution involves both the heart and the aortic arch (ANUFRIEW, 1928 and HEYMANS, 1933). A detailed study from the histological viewpoint by HAMMOND (1941) shows that the depressor fibres arise from pressure sensitive endings in the aortic arch region and terminate/

terminate in cells situated in the ganglion nodosum of the vagus. Whether indeed the actual baroreceptors are the tortuous terminal filaments of these fibres or are the yet more peripheral structures (cells of Cajal) demonstrated by MEIJLING (1938) is an open question.

The path the depressor fibres take through the neck is usually in association with the vagus. In the rabbit the nerve is quite discrete and easily made out, lying close to the tenth cranial nerve. In other animals it travels cephalad more closely bound up with the vagus, although by careful dissection it is possible at times to separate out a bundle of depressor fibres which may be called the depressor nerve, as LIDDELL and SHERRINGTON (1929) have done. Nevertheless, even in the rabbit it has been shown (by O'LEARY, BISHOP and HEINBECKER (1934)) that the depressor afferents may be divided between the vagus and the depressor nerve, and they point out that where a depressor nerve gives a powerful depressor response on stimulation the vagus tends to be pressor and where the depressor nerve response is feeble the vagus tends to be depressor. In the cat a similar state would appear to exist, for LIDDELL and SHERRINGTON (1929) have indicated that the response of the central end of the vagus to stimulation, after separation of the depressor nerve, may be a rise or a fall in blood pressure. They suggest that the study of depressor function can be readily undertaken by stimulating the central end of the vagus which contains the depressor fibres. WRIGHT (1928) states 'in my experience/

experience a separate depressor nerve in the cat is uncommon'. Thus, while the aortic nerve is undoubtedly a functional entity, its anatomical definition is by no means simple, and this can be said to hold especially for the cat.

The function of the depressor nerves was early the subject of speculation. In 1876 LATSCHENBERGER and DEANHA had put forward a theory of regulation of blood pressure according to which a rise of blood pressure in an artery caused a discharge of afferent depressor impulses due to the stimulation of depressor endings, while a fall in blood pressure resulted in a decrease in depressor impulses and a rise in general arterial blood pressure. This, of course, implied that the normal blood pressure is sufficient to stimulate the depressor mechanism. Their theory has come to have weighty support, not only from the anatomical basis which is firmly established, but also from numerous studies of aortic nerve function. Mention has already been made of the results of stimulation, and in accord with the view that the nerves exert a tonic depressor function, it is found that their section results in a rise in blood pressure. Early attempts to show that cutting the aortic nerves caused a rise in blood pressure (BAYLISS, 1923) were ill rewarded, for at the time the existence of the other buffer mechanism provided by the sinus nerves was not established, and the restraining influence of these prevented any marked hypertension. Where the sinus nerves have first been inactivated, it is an easy matter to demonstrate the tonic depressor activity of the aortic nerves

by/

by such an expedient. Further proof has come from studies of electrical activity. From these it is well established that the aortic nerves transmit afferent impulses under normal conditions and that the magnitude of the afferent discharge is related to the aortic blood pressure. The electrical activity was first shown by KOSTER and TSCHERMAK (1902), and ADRIAN (1926), using valve amplification, demonstrated that showers of action potentials ascend the nerve in rhythm with the heartbeats and during the periods corresponding to cardiac systole. Further emphasis was placed on the importance of the nerves by BRONK and KALTREIDER (1931), who found action currents in the rabbit depressor when the arterial blood pressure was below 60 mm. of mercury, and by RIJLANT (1932) who observed in unanaesthetised rabbits that, although electrical activity was greatest during the systolic and dicrotic phases, it was continuous under normal conditions and even when the intraortic pressure had fallen considerably. KARASEK (1933) confirmed this last work and showed that the changes in nervous activity followed closely the variations in the arterial blood pressure. The basis for the continuous depressor activity has been provided by BARANY (1943), who has found that the aortic baroreceptors are non-adapting and thus suited to their homeostatic function. GERNANDT (1946) has shown that afferent discharges similar to those found by RIJLANT (1932) occur also in the aortic nerve of the cat.

Were the aortic nerves then to be regarded in the light of these findings, CYON and LUDWIG's appellation 'depressor' would appear/

appear to be quite satisfactory, for it appears that their function is to exert a tonic moderating influence upon the blood pressure. But there is another aspect which must be considered.

For many years it had been known to histologists that 'chromaffin' tissue exists around the heart and great vessels of the neck region. Thus WEISEL (1906) had found it near the human heart, while TRINCI (1907) discovered similar tissue around the base of the heart in mammals and reptiles. A number of morphologists, notably NONIDEZ (1935) and BOYD (1937), have extended the study and located cell masses of the same nature in many situations more or less closely associated with the heart and large arteries. The function of this 'paraganglionic' tissue was in the dark until the researches of HEYMANS and HEYMANS (1924 - 1927) shed a new light on the function of the aortic nerves. They found that in the dog respiration could be stimulated by impulses arising in the aortic region and passing centrally by way of the vagi, and that three agencies aroused such activity, fall in pH, rise in CO_2 tension or fall in O_2 ~~and~~ tension of the aortic blood. Not only was respiration affected, but the 'chemoreflex' was accompanied by a rise in blood pressure due, they concluded, to a stimulation of the vasoconstrictor centre. Since then, these respiratory and 'pressor' reflexes from the aorta have been amply confirmed. The proof that the chemosensitivity was associated with the paraganglionic tissue was not forthcoming until COMROE (1939)/

(1939) demonstrated that in dogs the chemosensory zone of the aorta was confined to a small area from which a minute artery ran to the 'aortic body'. This was the name given by NONIDEZ (1937) to that mass of paraganglionic tissue which lies just below the aortic arch. COMROE (1939) was able to show that a nerve ran from this structure to join the vagodepressor trunk. In the cat, he found that the chemoreceptors lay beneath the aorta near the coronary orifices and received their blood supply from the coronary arteries. Recently, GERNANDT (1946), studying the action potentials in the aortic nerve of cats, has confirmed this localisation, and demonstrated that the chemosensory fibres run in this nerve.

It is thus apparent that the chemosensory mechanism has a dual rôle, stimulating both the respiratory and vasoconstrictor mechanisms, and on this point there is general agreement. Are the barosensory reflexes to be regarded in the same light? HEYMANS (1933) and his co-workers maintain that they are, and have led evidence to show that a rise in blood pressure, which calls into play the barosensory fibres, is accompanied by inhibition of respiration, while a fall has the opposite result. They attribute these effects to a play on the respiratory centre by these fibres. This is the commonly accepted interpretation. Recent work has tended to belittle the influence of sinus baro-sensory mechanisms on respiratory activity, and this will be discussed/

discussed later along with other sinus nerve functions. No evidence of this point is available with regard to the aortic nerves. If the dual rôle of the baroreceptors be accepted, then the functions of the two sets of aortic afferents are seen to be antagonistic. For activation of the chemosensory fibres, which have central connections similar to those of the barosensory fibres, gives rise to effects which are just the opposite. Where increased barosensory activity leads to inhibition of respiration and lowering of blood pressure, chemosensory activity causes increased respiration and a rise in blood pressure. It is apparent that 'depressor' is a far from satisfactory term for a nerve which is thus composed.

THE SINUS NERVES.

Prior to 1924, many workers had produced evidence for there being a specially sensitive zone in the neck concerned with blood pressure reflexes. It was known, for instance, that clamping of the common carotid arteries caused a rise of blood pressure, and that pressure over a certain point (which corresponds with the point of bifurcation of the carotid) led to a slowing in heart rate, but these effects were ascribed to cerebral anaemia in the first instance or to pressure on the vagus in the second. PAGANO (1900), however, had shown that the application of chemical agents to the region of the bifurcation of the common carotid arteries caused cardiac slowing, and suggested that the effects of carotid occlusion were reflex, and SICILIANO (1900) had demonstrated/

demonstrated that occlusion of the internal carotid artery (calculated to produce cerebral anaemia) did not result in the rise of blood pressure that common carotid clipping gave. Despite these findings, the reflex nature of these phenomena was not generally accepted, and the theory of cerebral anaemia held sway. Even when SOLLMAN (1908) discovered that traction on the cephalic end of the carotid artery caused a fall in blood pressure, the significance of the findings was missed. SOLLMAN and BROWN (1912) concluded 'the afferent path is through the carotid plexus and the impulse is transmitted to this structure through the intimate connection with the internal carotid artery'. In their paper they make no reference to earlier work and it appears that they did not associate the reflex with any special innervation of the blood vessels, but rather to the mechanical excitation of surrounding nerves 'composing the plexus'.

It was not until 1924 that HERING demonstrated conclusively that the various reflex effects from occlusion, traction and pressure and the like were initiated in the region of the bifurcation of the common carotid artery and carried centrally by a special nerve, the sinus nerve, (which he named thus because of its origin from the dilated portion of the internal carotid artery known as the carotid sinus). He was able to show that section of the sinus nerves had the same effect on blood pressure as carotid occlusion, and that the fall in blood pressure and cardiac inhibition obtained by traction could be reproduced by faradic stimulation/

stimulation of their central ends. Because of these facts, HERING (1927) maintained that like the aortic nerves the sinus nerves were true depressors. He believed that they exercised a solely tonic inhibitory influence on the circulation and that the afferent discharge was dependent on the adequate distension of the sinus by the blood pressure. This was supported by MOISSEJEFF (1926), using what has been termed the 'cul-de-sac' preparation. This consists of an arrangement whereby the sinus region is subjected to changes of pressure through the injection and withdrawal of fluid by a cannula tied into the cephalic end of the cut common carotid, escape of the fluid being prevented by having all the branches of the common carotid ligated. This worker showed that a rise in perfusion pressure caused a reflex lowering of systemic blood pressure while a fall had the opposite effect. This finding was confirmed by HEYMANS (1929) using the cross perfusion technique evolved by him, and by HEYMANS and BOUCKAERT (1929) employing mechanical perfusion with a DALE-SCHUSTER pump. Shortly thereafter, the electrical activity of the sinus nerves was studied, as had been done in the case of the aortic nerves. BRONK (1931) was able to record the action potentials set up by a single pressure sensitive end organ in the sinus nerve of the rabbit and found that as the endosinusal pressure was raised, action potentials made their appearance and increased in frequency, the relation between blood pressure and frequency being linear. In the whole nerve he found that below

forty/

forty millimeters of mercury no impulses could be detected, but as the pressure was raised, the receptors of lowest threshold began to discharge, further rises being accompanied by the addition of more active end organs and an increase in the frequency of discharge of the individual end organs already taking part. Studies of the electrical activity were taken up by BRONK and STELLA (1932), HEYMANS and RIJLANT (1933), EULER and LILJESTRAND (1936) and others since, and it is well established that the sinus nerves exhibit cardioinhibitory and vasodepressor activity from low pressures, and that this activity increases with rise in endosinusal blood pressure. Like their aortic counterparts, the sinus nerves may then be regarded in this light as 'depressor' nerves, but again, as with the aortic nerves, they have been found to be compound. BOUCKAERT, DAUTREBANDE and HEYMANS (1930) showed that, like the aortic region, the sinus region was sensitive to CO_2, H ion and anoxaemia, and that these stimuli caused the same respiratory chemoresponse. In a later paper HEYMANS, BOUCKAERT, EULER and DAUTREBANDE (1932) further indicated the similarity in function between the two pairs of nerves by finding that the same excitants again not only stimulated respiration, but caused a rise in blood pressure by the carotid chemoreflex. Unlike the aortic chemoreceptors, however, those of the carotid region also have connection with the cardioinhibitory centre, which they excite. The dual nature of the sinus nerve reflex was made manifest by HEYMANS and BOUCKAERT (1933) who destroyed/

destroyed the fibres from the sinus proper after which the response to pressure changes was abolished, although the effects due to chemoreflexes remained.

The site of origin of the chemoreflexes was suggested by the morphological finding that some of the fibres going to constitute the sinus nerve ended in a mass of chromaffin tissue known as the carotid body (DE CASTRO 1926, BOYD 1937). Further work by SCHMIDT (1932) and GOLLWITZER-MEIER (1934), who abolished chemoreceptor activity in dogs by occluding the occipital artery at its origin, narrowed the issue. The proof that the chemosensitivity lay in the carotid body fell to COMROE and SCHMIDT (1938), who were able to show that by occluding the artery to that structure, a small branch of the occipital, they could suspend chemoreflexes, and could cause them to reappear by disoccluding that vessel.

Evidence of the tonic chemoreceptor activity in the sinus nerves was early forthcoming. In their electrical records BRONK and STELLA (1932) observed a continuous discharge which they suggested might be chemical, a finding confirmed by HEYMANS and RIJLANT (1933), who indicated that their records showed two types of action potentials, large 'pressor' spikes and smaller 'chemical' spikes. These were also observed by ZOTTERMAN (1935), who recorded the chemoreceptor impulses after cutting the stretch receptor fibres and found them to be only ten to twenty per cent. of the size of the largest baroreceptor impulses.

It/

It has been mentioned above that HEYMANS (1933) believed that the baroreceptors also had this dual function and acted in an inhibitory fashion on the respiratory centre. This view was based on the original demonstration by MOISSEJEFF (1926) that raising the cul-de-sac pressure led to inhibition of the respiratory movements, and on the work carried out by HEYMANS himself and his co-workers. It is shared by COMROE (1940), but has recently been subjected to criticism by BJURSTEDT and EULER (1942), who found that sinus baroreceptor stimulation in the dog did not effect respiration with the vagodepressors intact, and gave but feeble inhibition after cutting these nerves, and by EULER and LILJESTRAND (1943), who have indicated that in the occlusion test in the cat, which gives rise to increased respiratory activity and is often instanced as evidence of baro-sensory influence on respiration, a considerable part is played by the chemoreceptors. GERNANDT (1946) lends further support to the views of these workers. But if the dual rôle of the baro-sensory fibres be accepted then the sinus nerves are composed, as are the aortic, of two sets of afferent fibres having directly opposite action on the respiratory and vasomotor centres. The sinus nerves, however, possess the additional chemoreceptor influence over the cardioinhibitory centre.

THE AORTIC AND CAROTID MECHANISMS CONSIDERED TOGETHER.

Further discussion of the aortic and sinus nerves is best carried out by considering them together, for they have much in

common.

The Receptor Mechanisms.

Both the carotid and the aortic bodies have a dual origin from ectoderm and mesoderm, from the glossopharyngeal and vagus nerves and from the neighbouring blood vessels, the third and fourth branchial arches (BOYD 1937), while the baroreceptor endings of the aorta are identical with those of the carotid sinus (NONIDEZ, 1935).

Reference has been made to the view of MEIJLING (1938) that the terminal representation of the barosensitive mechanism may lie more peripheral to the endings of the fibres of the aortic and sinus nerves. This author maintains that the nervous network formed by the Cajal cells is the true receptor mechanism, and that the barosensory fibres act merely as relays on the afferent pathway. In both aortic and carotid sinus regions these interstitial cells are especially well developed, this the author has been able to verify in specimens kindly demonstrated by Dr. Meijling. The matter is yet controversial.

Much more interest has attached to the physiology of the chemoreceptor mechanisms. Both aortic and carotid bodies are essentially masses of specialised cells, the so-called glomus cells, held together by a minimum of connective tissue and being extremely highly vascularised. The glomus cells of each organ are identical (HAMMOND, 1941) and are closely enveloped by the terminations of the chemosensory afferents which again are identical/

identical in each site and are typically afferent endings (NONIDEZ, 1935). Thus far there is agreement. Difference of opinion exists, however, as to the excitation mechanism and its relation to the structure of the carotid and aortic bodies. It is generally considered that the changes in the blood affect the glomus cells in such a manner as to cause a stimulus to be transmitted to the afferent nerve endings surrounding them in a manner analogous to the taste bud mechanism. Again MEIJLING (1938) places a different interpretation on the matter. He maintains that the glomus cells are syncytially connected autonomic ganglion cells (modified Cajal cells) and that the connection between the receptor cells and the chemosensory fibres may thus be interpreted as nervous. This view receives support from HAMMOND (1941) who considers the glomus cells as 'essentially neuro-epithelial sensory cells', and from GOORMATIGH and PANNIER (1939) who hold that the connection is in the nature of a synapse. From the functional aspect, the conception of a synapse has been supported by the work of EULER, LILJESTRAND and ZOTTERMAN (1939), who have led evidence to the effect that the cause of excitation of the chemosensory fibres is a change in the reaction of the glomus cells (after the reaction theory of WINTERSTEIN), and go on to show that ammonia abolishes the response to O_2 lack, CO_2 excess and KCN, but fails to abolish the response to lobeline. (HEYMANS and his co-workers (1933) first showed that both cyanides and lobeline excite the chemoreflexes and since then they/

they have been used by many workers to elicit chemoresponses). They stress the fact that lobeline, and other agents which can be used to elicit chemoreflexes, such as nicotine, K ions and acetylcholine, all act on post-ganglionic fibres and that the persistence of the lobeline effect when the receptors are knocked out by alkali indicates the existence of a synapse on the chemo-pathway. HOLLINSHEAD and SAWYER (1945) seek to co-ordinate the functional and morphological aspects. They point out that there are but three significant elements in the carotid body, blood vessels, nerve fibres and chemoreceptor cells and that the synapse must be between the nerve endings and the chemoreceptor cell. This is in accord with MEIJLING's (1938) view. They state that the normal mechanism is two-phase, firstly a change of pH of the chemoreceptor cell, and secondly the liberation of a cholinergic substance to excite the afferent fibres. The first phase may be brought about by a fall in the pH of the blood or a rise in the CO_2 content, or by O_2 lack (which acts indirectly, raising cellular acidity by depressing intracellular respiration), or indeed by chemicals which inhibit oxidative processes, such as cyanides or sulphides. The second phase follows on and arises from the first, or may be stimulated by drugs such as nicotine, lobeline or acetylcholine.

Tonic Functions of the Nerves.

The tonic depressor barosensory activity of the sinus and aortic nerves has been discussed in some detail above and shown to/

to exert a powerful influence over the cardiovascular system. The less significant rôle played by the baroreceptors in respiratory reflexes has been indicated and the matter need not be discussed further except to note that EULER and LILJESTRAND (1943), although agreeing that the barosensitive mechanism could affect respiration, stated that 'no evidence has been adduced that respiration is in any way reflexly influenced by the baroreceptors of the sinus or aortic regions under normal conditions'.

Tonic chemoreceptor activity was held by HEYMANS (1933) and his school to be of great importance in the control of respiration, although SCHMIDT and COMROE (1939) regard it as accessory. The question is inevitably concerned with the sensitivity of the chemoreceptor cells and EULER, LILJESTRAND and ZOTTERMAN (1939) have shown that chemosensory fibres signal the presence of CO_2 and lack of O_2 even at the normal arterial pressure of these gases. They did not, however, evaluate the central effect of this afferent discharge. Numerous studies of the effects of denervation have not served to settle the issue and, like the effect of tonic chemosensory impulses on the vasomotor and cardioinhibitory centres, the question must be left open.

Compound Effects.

Since the two pathways, chemosensory and barosensory, can influence both respiratory and vasomotor centres in opposite directions, there are certain circumstances where the afferent discharge may be so compounded that either exciting or inhibiting impulses/

impulses predominate, or indeed cancel one another out. This has been shown by GESELL, LAPIDES and LEVINE (1940) who found that cold blocking of the sinus nerves decreased, increased or had no effect on respiration. The existence of similar mixed effects on the vasomotor mechanism is not unlikely, for the chemo-sensory fibres can exert considerable pressor activity, and it might well be that where there is both low blood pressure and asphyctic blood the activity of the nerve, sinus or aortic might be pressor. COMROE (1939) suggested that the fall in blood pressure observed by McDOWALL (1924) after section of the vagi might have such an explanation. The electrical records showing both barosensory and chemosensory fibres active under near physiological conditions would suggest that there must be present normally a damping of one effect by the other. This assumes, however, that the barosensory and chemosensory impulses recorded under these conditions each possess dual function, whereas it has not been shown that they have. There is no proof that the same afferent barosensory fibre plays on both centres or the same chemosensory fibre on both.

The Significance of the Sinus and Aortic Nerves.

There is general agreement that the four nerves possess similar functions and that they are afferent pathways of great importance in the regulation of blood pressure, heart rate and respiration.

It is without doubt that they exert a tonic influence over the/

the vasoconstrictor and cardioinhibitory mechanisms, restraining the former and increasing the discharge from the latter, and in this light they have been regarded as the principle bodily defences against hypertension and cardiac stress. Looked at from a different viewpoint, it is apparent that through exerting this tonic depressor activity they ensure that a margin of cardiovascular activity remains which can be released should the blood pressure fall. In this sense they can also be considered as defences against hypotension. That there may be other afferent pathways modifying blood pressure and heart rate does not detract from the main issue that an animal deprived of the moderator nerves, aptly described as 'debuffered', is without such defences. The unbridled cardiovascular discharge following upon section of these nerves and resulting in hypertension, rapid heart rate and cardiac irregularity bears this witness.

Just as opinions are divided on the sensitivity of the chemoreceptors, so too is there disagreement over the part played by the chemoreflex in normal respiration. It has been pointed out that the sensitivity of the respiratory centre to changes in carbon dioxide tension is such that under normal circumstances there is no call for a reflex stimulus. Be this as it may, there are certain conditions where central sensitivity and spontaneous activity are greatly diminished or even lost, and respiration becomes dependent largely or entirely upon afferent chemosensory drive. Such a state may be brought about by/

by a variety of depressants among them carbon dioxide excess, oxygen lack and drugs. Speaking of man, DRIPPS and COMROE (1944) have said that 'whatever resistance the intact individual has against anoxia is due to a flow of afferent impulses from the chemoreceptors'. As the ultimum moriens of the respiratory regulating system, these structures play a most important rôle in the maintenance of respiration during the central depression produced by narcotic drugs. Thus it has been frequently found that respiration fails when deeply anaesthetised animals are subjected to loss of chemosensory afferents. Similarly in man, physiological denervation during anaesthesia, brought about by the administration of pure oxygen, may lead to complete respiratory arrest. DRIPPS and DUMKE (1943) have shown how the balance shifts from central to chemoreceptor control as narcosis deepens and how the reflex control assumes a special significance where the anaesthetic employed is particularly liable to have a pronounced central respiratory depressant effect relative to the depth of anaesthesia, such a drug in fact as the much-used barbiturate compounds.

THE PARTICIPATION OF THE CENTRAL AND PERIPHERAL VASOMOTOR MECHANISMS IN SINUS AND AORTIC REFLEXES.

CYON and LUDWIG held that stimulation of the depressor nerve caused a fall in blood pressure by inhibiting the vaso-constrictor centre. OSTROVMOV (1876) believed, however, that the effect was due to stimulation of the dilator mechanisms. It/

THE VASOMOTOR REFLEXES.

(Bayliss, 1908).

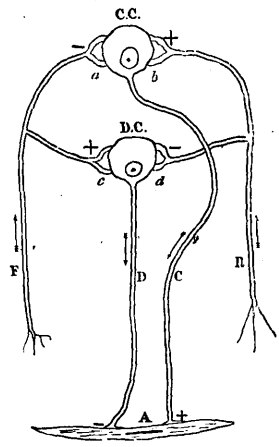


Figure 40.

Diagram of Vaso-motor Reflexes.

A. Arteriole muscle.
D. Vaso-dilator fibre, inhibiting natural tonus of *A.*
C. Vaso-constrictor fibre, causing contraction of *A.*
D. C. and *C. C.* — Dilator and constrictor centres, resp.
F. Afferent depressor fibre, with two collaterals, one (—) inhibiting constrictor centre, the other (+) exciting the dilator centre.
R. Pressor fibre of ordinary sensory nerve, exciting *C. C.*, inhibiting *D. C.*
a, b, c, d. — Synapses of the above fibres with the efferent neurones. Intermediate neurones omitted for simplicity. (Bayliss, 1908, Fig. 27.)

It remained for BAYLISS (1893) to show that both might be involved. After demonstrating the participation of both dilators and constrictors in vasomotor reflexes, he concluded 'the simplest explanation is the hypothesis that the vasomotor centre consists of a constrictor and a dilator part, the depressor nerve acting in an inhibitory manner on the former and in an exciting manner on the latter, while pressor nerves act in an opposite way on both'. BAYLISS' diagram is shown opposite. The fact that a reflex rise or fall in blood pressure is usually accompanied by an increase or decrease in the tonic activity of the vasoconstrictor centre is generally accepted. The participation of an active dilator mechanism in generalised vasomotor reflexes is not so well established. BAYLISS (1893-1923), the chief protagonist of the dilator mechanism repeatedly showed activation of the 'antidromic' dilators in depressor reflexes but found great difficulty in demonstrating the inhibition of dilator tonus in pressor reflexes, although he believed firmly in its existence. Evidence is gradually accumulating to support his view. FREEMAN and ROSENBLUETH (1931) were able to obtain a rise in blood pressure from sciatic or median nerve stimulation and a fall from depressor nerve or vagal stimulation in completely sympathectomised cats, and concluded that there was a definite vasodilator tone which could be reflexly diminished or augmented to give these pressor and depressor effects. Again in sympathectomised cats, ROSENBLUETH and CANNON (1934) showed that stimulation of the depressor/

depressor point gave a fall in blood pressure. BACH (1945) has presented further evidence for the reflex activation of the dorsal root dilators. He claims that in the cat they are an essential pathway for the reflex vasodilation brought about by depressor nerve stimulation.

A similar involvement of extrasympathetic mechanisms in sinus nerve 'depressor' reflexes has been indicated by BACQ, BROUHA and HEYMANS (1932, 1934), who found that in the sympathectomised cat occlusion of the common carotids gave a marked rise in blood pressure (40-50 mm. Hg.), while stimulation of the sinus nerve gave a fall of the same order. They could not obtain these results in the dog. Recent studies by GERNANDT, LILJESTRAND and ZOTTERMAN (1946), in which the electrical activity of the sympathetic (splanchnic nerve) in sinus and aortic baro-reflexes was studied, while in no way excluding extra sympathetic participation, confirm the established view that increased baro-sensory activity leads to decreased sympathetic activity and vice versa.

These last authors have also shown that the splanchnic sympathetic path is brought into play by both aortic and carotid chemoreflexes. Whether or not in the cat there is concomitant participation by the dilator mechanisms, as shown to occur in the baroreflexes, is not known. In the dog BERNTHAL, MOTLEY, SCHWIND and WEEKS (1945) conclude that the 'thoracico-lumbar autonomic fibres constitute the sole efferent pathway for all vascular reflexes/

reflexes originating at the carotid body'. The species difference observed in the baroreflexes disallows of analogy and the question of alternative pathway in the cat must be left open. BERNTHAL et al (op. cit.) have said that 'the whole question of the participation or non-participation of vasomotor fibres other than the thoracico-lumbar autonomics in generalised vascular reflexes is confused by unexplained differences in the experimental results of numerous investigators'. This is the view to which one must necessarily subscribe after a study of the evidence to date, but what emerges is that in the cat the sinus and aortic baroreflexes have been shown to involve mechanisms other than the sympathetic, while the possibility of an alternative pathway in the chemoreflexes cannot be excluded.

THE EFFECTS OF STIMULATION OF THE AORTIC, VAGUS AND SINUS NERVES.

DEPRESSOR RESPONSES.

Depressor Nerve.

CYON and LUDWIG (1866) were the first to show that electrical stimulation of the central end of the cut depressor nerve of the rabbit brought about cardioinhibition and a fall in blood pressure. The latter was shown by them to persist when the vagi were cut, demonstrating that it was due to reflex vasodilation. BAYLISS (1923) supported these findings and, observing no effects other than those mentioned, maintained that the nerve contained no fibres other than those producing reflex inhibition of the heart/

heart and vasodilation. LIDDELL and SHERRINGTON (1929) obtained similar responses from the depressor nerve of the cat.

Vagus Nerve.

Where the depressor fibres run mixed with the fibres of the vagus, stimulation of the central end of this nerve has been shown to produce the same effect as stimulation of the depressor nerve. Thus SHERRINGTON (1929) maintains that in the cat stimulation of the vagus gives much the same results as aortic nerve stimulation and refers the reader to the study of depressor vasomotor reflexes carried out by WRIGHT (1928), who employed the aortic nerve in the rabbit but the central end of the vagus in the cat. McDOWALL (1938) states, 'it is interesting to note that even where a separate (aortic) nerve is present, stimulation of the central end of the vagus gives a similar depressor response'.

Sinus Nerve.

The discoverer, HERING (1924), showed that faradization of the sinus nerve gave rise to marked cardiac slowing of a greater order than that caused by aortic nerve stimulation and that there was in addition reflex vasodilation which persisted after section of the vagi. He obtained these responses in the dog, cat, rabbit and monkey.

These three nerves have been stimulated repeatedly by many workers for a variety of purposes, and it has been generally accepted that the characteristic response to electrical stimulation of any one is a slowing of the heart and vasodilation.

Indeed/

Indeed, with the exception of the sinus nerves, which are not readily accessible, they are employed regularly in the classroom to demonstrate these reflex effects. Nevertheless, responses contrary to those expected have been reported and are as follows.

PRESSOR RESPONSES.

Depressor Nerve.

Potential pressor activity of this nerve was demonstrated by TSCHIRWINSKY (1896), who obtained signs of pain in unanaesthetized rabbits and a rise in blood pressure after curare. Also by FRANK (1880), who observed increased respiratory activity. Both these instances were evidence of activity other than vasomotor, and led LANGLEY (1912) to state 'the depressor nerve is potentially capable of causing a rise in blood pressure by means of higher centres of the brain'. Pressor responses without evidence of involvement of such centres were obtained by O'LEARY, HEINBECKER and BISHOP (1934), who made a careful study of the effects of electrical stimulation of the rabbit aortic nerve. They obtained pressor responses differing from those seen by the earlier workers in that they were produced by feeble currents and were unaccompanied by agitation. They concluded that not all depressor nerve fibres have a depressor effect, some fibres being present which are pressor, and emphasised that the fibre constitution varied from animal to animal and similarly also the amount of pressor component present. In the cat no such analysis has been made, but it is reasonable to assume that in this animal,

where/

where the nerve is not a separate discrete structure as in the rabbit, but runs intermingled with vagal fibres from which it must be teased, there will be an even greater liability to variation and 'contamination' by pressor vagal afferents.

Vagus Nerve.

v. BEZOLD (1803) found in curarised rabbits that vagal stimulation caused a rise of blood pressure, but when the cerebral hemispheres were removed a fall only was obtained. LANGLEY (1912) showed that the effect in rabbits varied greatly 'indicating a struggle with varying pre-potence of pressor and depressor action'. This author obtained the pressor response with weak currents just above threshold and without agitation, and further, also observed it in the decerebrate animal, showing that the higher centres were not essential. The fact that the vagus contains pressor fibres is not disputed and there are certain conditions in which they manifest themselves. WRIGHT (1928) has stated that in the cat 'both vagi may be depressor (perhaps the left more so than the right), or they may give rise to a mixture of pressor and depressor effects. Occasionally, they may produce pressor effects only'. This author believed the variation in response occurred since 'the proportion of depressor to pressor fibres in the vagus of the cat seems to differ in different animals'. This is certainly the case in the rabbit, where O'LEARY, HEINBECKER and BISHOP (1934) were able to demonstrate that the response varied with the pressor and depressor fibre content. They found the depressor/

depressor fibres variably distributed between the vagus and aortic nerves, and that, where the depressor function of the aortic nerve was poor, the vagus tended to be depressor and vice versa. CHANG, CHIA, HSU and LIM (1937) observed that a pressor response might be obtained from vagal stimulation due to the release of the pressor principle of the pituitary gland.

The nature of the pressor afferents has not been defined by any of these workers, and whether they are unspecific visceral fibres from the lungs or abdomen as shown by NEWMAN (1914), or primarily cardiovascular, such as the accelerator fibres from the heart, or those said by McDOWALL (1924) to be responsible for vagopressor reflexes, remains obscure.

Sinus Nerve.

Mechanical stimulation of the sinocarotid region may sometimes give rise to pressor effects. This was found by DANIELOPOLU (1928), and later DANIELOPOLU, MARCU, PROCA and ASLAN (1932), in a paper purporting to show the amphotropic nature of the sinus nerve reflexes in a variety of animals, demonstrated that either inhibitor or excitor vasomotor reflexes might predominate. In the cat they found that pinching the sinus region initiated reflexes which were pressor, depressor, diphasic or 'dissocié', that is, showing a fall in blood pressure accompanied by a rapid heart rate or a rise with slow rate. In the same animal it was sometimes pressor and at others depressor, varying from one trial to another. Although HEYMANS (1933) has criticised the interpretation put upon these findings by their author, stressing that mechanical/

mechanical or electrical stimulation of the sinus nerve does not necessarily reproduce the physiological functions of the nerve, nevertheless the demonstration of pressor activity stands. TOURNADE and MALMEJAC (1930) sometimes obtained a rise in blood pressure on faradic stimulation of the sinus nerve in dogs, and although they were unable to interpret the finding they defined the conditions in which it was obtained. It was most often seen at the end of prolonged experiments in chloralosed dogs with the vagi cut and sinuses denervated, and was usually accompanied by signs of agitation. The response persisted, however, in curarized animals, but was abolished by the administration of more chloralose or chloroform, when once again a depressor response could be obtained. HEYMANS (1933), who also observed such rises in blood pressure, stresses that the reaction is due to the use of excessively powerful stimuli which bring into play pain reflexes, which, by their secondary action in raising the blood pressure, mask the true reflexes.

This last author classified the pressor responses occurring in the aortic and sinus nerves under similar conditions as 'douloureux' and of no physiological significance.

Pressor Responses Associated with Drugs.

Up until the time this research was undertaken no anaesthetic had been shown to cause the normal depressor response from stimulation of any of the buffer nerves to be replaced by a pressor response. Such a 'reversal' had been observed to occur, however, with/

with strychnine and ergotamine.

Strychnine had been shown by REY and ADUCCO (1888) to paralyse the depressor in the rabbit and the vagus in the cat and dog. SHERRINGTON found that it converted the fall from depressor stimulation to a rise, and suggested to BAYLISS (1908) that further study was called for. This last author took up the work and found that the phenomenon could be regularly observed both in the rabbit depressor and cat vagus when large doses of the drug were administered, and considered that the effect was due to a change of action on the part of the afferent depressor fibres rendering their effect excitor. LANGLEY (1912) contested this view maintaining that no such reversal of inhibitory impulses to excitatory occurred, but that the change occurring on stimulation of these nerves may be most simply explained by the fact that pressor as well as depressor fibres occur in the nerves, and that strychnine exerts a 'different action on the centres connected with the pressor and depressor fibres'. SCOTT (1925) has confirmed this view.

Ergotamine was found by ROTHLIN (1923) to abolish the depressor reflex in rabbits. WRIGHT (1930) observed that, when stimulation of the central end of the vagus in cats gave a mixed effect, ergotamine might convert it into a pure rise 'by an action on the afferent side of the vasomotor centre'.

CHAPTER I.

PART 11.

AUTHOR'S WORK.

C H A P T E R I.

ELECTRICAL STIMULATION OF THE VAGUS NERVE.

The original observation upon the cat overdosed with nembutal, out of which this research arose, had been that a rise of blood pressure occurred when either vagus was electrically stimulated. Whether or not this effect could be ascribed to the anaesthetic was conjectural. Accordingly, further experiments were carried out to obtain evidence on this point.

Methods:

Cats were employed. They were anaesthetized with nembutal (Pentobarbital, ABBOTT) given intraperitoneally in aqueous solution (40 mg/cc.), the amount being adjusted so that 40 mg/Kg. bodyweight were administered. No difficulty was experienced, although the animal was not under the effect of any volatile anaesthetic when the injection was made. This amount it was found, produced in most animals a depth of anaesthesia sufficient to permit of operative interference within fifteen minutes. In others, additional doses were administered (20 mg. or so per injection) until anaesthesia was satisfactory.

When anaesthetized, the animal was transferred to an operating table, placed upon its back on the warmed copper top, which was covered with a layer of towelling, and its position maintained by loops attached to each leg and secured over cleats. A second towel was used as a blanket to conserve the animal's

temperature/

temperature.

Readings of temperature were taken from a rectal thermometer placed in situ, and any tendency for this to alter during the experiment was countered by appropriate adjustment of the heating arrangements of the copper and disposition of towels.

The dissection was begun by making a midline incision in the neck and retracting the skin to expose the muscles overlying the trachea. These were parted and the trachea exposed, looped and cannulated with a glass cannula of as large bore as could readily be accommodated. Adequate dead space was ensured by the addition of suitable lengths of rubber tubing. Spontaneous respiration was allowed.

Next, the carotid sheath on one side was exposed and the vago-sympathetic trunk separated out from the common carotid artery and internal jugular vein. By careful dissection the sympathetic was stripped from the vagus, and the vagus tied and cut low in the neck in such a manner that a length was obtained adequate for stimulation. The structures were kept moistened with warm Ringer-Locke solution throughout dissection and during the experiment. The nerve thus prepared was then buried by closing the muscles over it and attention directed to the opposite side, a precisely similar technique being followed there.

Blood pressure was recorded from a cannula inserted, in most instances, into the left carotid artery. In several animals, however/

however, the femoral artery, right or left, was employed. Sodium citrate (3.8% or 2% in 0.6% saline) or heparin was employed as anticoagulant. Tracings were obtained by a mercury kymograph.

Artificial ventilation was provided, when necessary, by a Starling pump attached to the tracheal cannula.

The nerves were stimulated by bipolar platinum electrodes. The stimulator used was that designed by WHITFIELD (1946), which provides brief condenser shocks discharged through neon tubes. The amplitude and frequency scales of the instrument were checked and recalibrated with the aid of an oscillator and cathode ray oscilloscope before it was employed in this series of investigations.

The experiments were confined to electrical stimulation of the vagi in the lightly (normally) anaesthetized animal and repetition of the stimulus after additional doses of nembutal.

Results:

It was found that where sufficient nembutal had been given, electrical stimulation of the central end of either vagus failed to elicit the expected depressor response, but caused instead a marked rise of blood pressure.

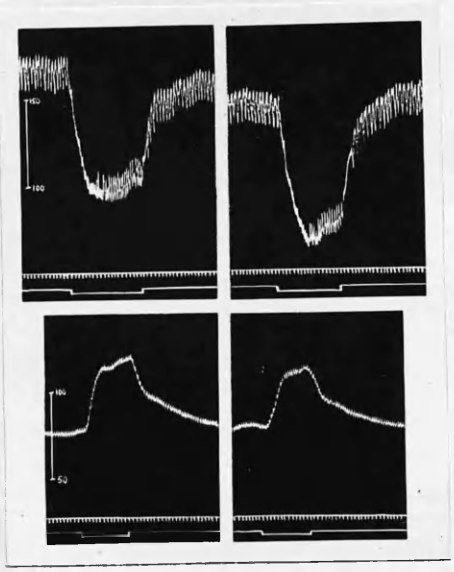
One typical experiment will be described in some detail. The animal, a cat weighing 2.75 Kg. was injected, intraperitoneally, with nembutal (40 mg/Kg.) at 10 a.m. Dissection was begun at 10.14 a.m. and was carried out as described. Both vagi were made ready for stimulation. Blood pressure was recorded

from/

ELECTRICAL STIMULATION OF THE VAGUS NERVE.

THE EFFECT OF NEMBUTAL.

A B



C D

Fig. 1. Cat. Double vagotomy. Spontaneous respiration. Arterial B.P. (left carotid artery).

Time 5 sec. Scale mm. Hg.

Upper 2 Tracings obtained 2 hr. after induction. (50 mg/Kg. nembutal).

A. Stimulation of right vagus nerve 20 V. 30/sec. (Whitfield).

B. Stimulation of left vagus nerve 20 V. 30/sec. (Whitfield).

Between upper and lower 2 Tracings nembutal injected to a total of 108 mg/Kg. and artificial respiration begun.

C. Stimulation of right vagus nerve 20 V. 30/sec. (Whitfield).

D. Stimulation of left vagus nerve 20 V. 30/sec. (Whitfield).

from the left femoral artery. Dissection was completed and recording begun by 11.5 a.m.

11.06. Anaesthesia light. 10 mg/Kg. nembutal injected intraperitoneally.

12.09. Stimulation of the right vagus with a stimulus of 20 volts (peak-base) and 30 shocks per second (referred to as 20 V. 30/sec.) gave a marked fall in blood pressure (Fig. 1A.).

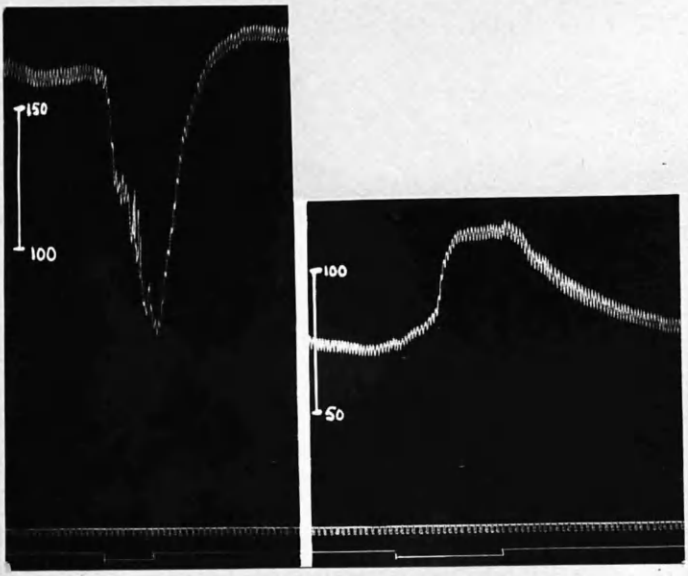
12.14. Stimulation of the left vagus gave a similar response. In each instance stimulation was across the nerve, the electrodes being placed so that one lay above the nerve and the other below, the vagus being threaded between in such a manner that the anodal electrode was the more distal (i.e. was nearest the cut end of the nerve).

The stimulus was applied to each nerve on several more occasions, and each time the electrodes were reapplied so that the nerves were stimulated in a variety of positions. Similar responses were obtained in each case. This was done since it was felt unwise to keep the nerve on the electrodes over the duration of the experiment (up to nine hours) and it was thought desirable to establish the fact that with the stimuli and electrodes used, rearrangement of the electrodes could not in itself be responsible for any gross change observed.

12.50/

ELECTRICAL STIMULATION OF THE VAGUS NERVE.

THE EFFECT OF NEMBUTAL.



A B

Fig. 2. Cat. Double vagotomy. Artificial respiration. Thorax intubated. Arterial B.P. (left femoral artery). Time 5 sec.

A. Nembutal 45 mg/Kg. Stimulation of right vagus 30 V. 30/sec. (Whitfield).

B. Nembutal total 95 mg/Kg. Three hours after A. Stimulation of right vagus 30 V. 30/sec.

12.50. Having shown that the stimulus used (20 V. 30/sec.) when applied to either vagus caused a marked fall in blood pressure, the animal was now given nembutal (40 mg/cc.) intraperitoneally in several doses.

12.55. 1 cc. nembutal (I.P.).

1.10. 1 cc. nembutal (I.P.).

1.25. 1 cc. nembutal (I.P.).

The response to vagal stimulation was elicited between each dose.

1.35. Animal respiring feebly. Artificial respiration begun. Thorax intubated.

1.40. 1 cc. nembutal (I.P.).

2.0. Stimulation of the vagi (20 V. 30/sec.) now caused, not a fall, but a rise in blood pressure. (Fig. 1, C & D).

Interpretation:

The change in response cannot be ascribed to the effects of opening the thorax, or to altered vagal activity when the animal is under artificial respiration, as can readily be seen from Fig. 2. In this animal, the thorax was intubated through the diaphragm and artificial respiration instituted before any stimulation was undertaken; both responses were readily obtained.

These two examples cited show the typical vagal responses found in several preliminary experiments, firstly, the normal expected depressor effect obtained in light nembutal anaesthesia, and secondly, the pressor effect produced by application of the same stimulus after the addition of further amounts of nembutal.

Such/

Such a 'reversal' of the normal responses could be regularly obtained in several animals provided that the nembutal was appropriately given. No attempt is made here to define the dosage of nembutal required, the method of administration, or the nature of the vagal response under various dosages. These will be detailed in a later chapter. Suffice it to say that the original chance finding could be repeated at will.

C H A P T E R II.

ELECTRICAL STIMULATION OF THE SINUS NERVE.

It was early realised that the further study of the phenomenon would most satisfactorily be carried out on a nerve of more purely 'moderator' nature than the vagus which, as is well known, has a mixed content of pressor and depressor afferents. In the cat, where the aortic nerve is not a discrete structure, the sinus nerve was the obvious choice.

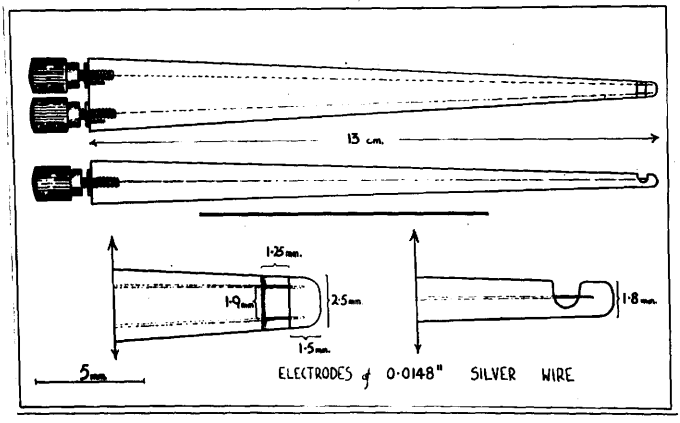
Accordingly, experiments were undertaken to determine the effect of electrical stimulation of that nerve in deep nembutal anaesthesia.

IDENTIFICATION OF THE SINUS NERVE.

The anatomy of the sinus region was carefully studied post mortem in several animals employed in the early experiments on the vagi. Little difficulty was found in identification of the sinus region, as this is marked by the crossing of the common carotid artery by the hypoglossal nerve. By dissecting carefully with the aid of a large lens, the sinus nerve could be identified coursing dorso-cephalad from the deep surface of the sinus region through the fat and connective tissue which invests the area, to reach the glossopharyngeal nerve as it lay on the tympanic bulla.

It was obvious from these preliminary explorations that, by nature of its small size and close relationship to the base of/

THE SINUS NERVE ELECTRODES.



Legend. Electrodes are of silver wire encased in 'Perspex'.
The lower drawings are three times actual size.

of the skull, the nerve would be inaccessible to electrical stimulation by electrodes of conventional design. The problem was solved by adopting two expedients. Firstly, an especially small pair of electrodes was designed (shown opposite) such as would penetrate the little space available, and secondly, a technique was evolved which gave an adequate exposure of the nerve while, at the same time, minimising trauma to it and the important structures in the vicinity.

In these dissections in the dead animal, identification and exposure of the sinus nerve was by no means simple, despite the fact that there was no consideration other than the dissection. In early experiments on the live animal it proved, in addition, to be hazardous and time-consuming, as indeed was to be expected, for the region abounds in blood vessels. Notwithstanding the difficulties, a technique was soon evolved which permitted of rapid identification and exposure of the nerve. The method was employed in over forty of the experiments necessitating sinus nerve exposure and was found to be very satisfactory. It is given here in detail.

METHOD OF EXPOSING THE SINUS NERVE.

(1) The anaesthetized cat is placed on its back with all limbs extended and secured. An ellipse of skin is removed from the neck, from the manubrium to within an inch of the symphysis of the mandible. The trachea is exposed by separating the right sterno-thyroideus muscle from the left and is cannulated.

(2)/

(2) The carotid sheath lying on the prevertebral muscles is exposed by blunt dissection, the line of cleavage being between m. sternothyroideus mediad and m. sterno-mastoideus laterad. (At this point the vagus is prepared before pursuing the sinus nerve exposure. The vago-sympathetic trunk is separated from the carotid sheath and the cervical sympathetic stripped off the lower 2 cm. or so of vagus and removed. The vagus is ligatured and cut low in the neck).

(3) The cleavage plane is continued cephalad, division of likely bleeders being made between cotton ligatures, and the carotid sheath followed headwards. The communicating branch from the anterior facial vein of the other side is cut between ligatures and the carotid sheath exposed as it lies between the large lymph gland and m. thyroideus. M. stylohyoideus is divided to reveal the lingual branch of the external carotid artery. The lingual artery and the accompanying hypoglossal nerve are now laid bare by separating m. mylohyoideus from m. digastricus which, in turn, is retracted laterad exposing the external carotid artery as far as its external maxillary branch.

(4) The external carotid artery is now divided between stout cotton ligatures, and the cardiac end lifted gently by the uncut ligature and retracted ventrad. By blunt dissection the tympanic bulla is approached. The glosso-pharyngeal nerve is identified, carefully separated out from the adherent connective tissue binding it to the bone, and is looped, tied and cut as far distally as possible. The central end of the nerve is gently raised/

raised by the ligature and the small twigs on the cranial aspect are cut, and the nerve cleared along its length to within a few millimetres of its exit from the skull. Now, attention is focussed on the caudal aspect of the nerve (that is to say, the length presenting to the sinus region and to which the sinus nerve runs). Soft blunt dissection of the fatty tissues lying between the raised glossopharyngeal nerve and the sinus region soon reveals a slender twig running from the sinus region to join the glossopharyngeal nerve. The nerve is minute but can be identified readily, being almost constant in position and characteristically joining the glossopharyngeal nerve in such a fashion that the division between the two is apparent for one or two millimetres. About 5 - 8 millimetres of the nerve are cleared. If exposure is unsatisfactory, the approach is opened up by cutting between ligatures the muscular and glandular branches of the common carotid which sometimes obscure the infero-lateral approach.

Methods:

Cats were used. They were anaesthetized with nembutal as before. Both vagi were cut, both sinus nerves exposed, and the trachea canmulated as detailed. Blood pressure records were taken from the left carotid artery. Citrate was used as anti-coagulant. All animals were artificially ventilated from the beginning of the experiment, care being taken to avoid over or under-ventilation. One or two animals had the pleural space opened from below by tubes inserted through the upper abdomen and/

and diaphragm. This method was soon abandoned as it proved inadequate to prevent secondary changes in blood pressure arising as a result of the vigorous respiratory activity caused by sinus nerve stimulation. Accordingly, bilateral open pneumothorax was routinely carried out, a portion of a rib on each side being removed. This proved adequate in preventing thoracic movements from influencing pulmonary ventilation or blood pressure. The technique adopted is here detailed.

METHOD OF OPENING THE CHEST.

When the chest is to be opened, this is carried out immediately before the blood pressure cannula is inserted and recording commenced. At this stage artificial ventilation is begun by attaching the Starling pump to the tracheal cannula.

The procedure is as follows:

(1) An ellipse of skin is taken from the postero-lateral aspect of the chest of sufficient size to provide adequate exposure of three of the lower ribs.

(2) The next step consists of ligaturing the rib, a section of which is to be removed - and also the rib above and below, (if this is not done trouble from bleeding is encountered). Ligatures are placed round the rib at the lower end of the cleared area, that is, just posterior to the most lateral point of the rib. Before each ligature is passed round the rib, (by an aneurysm needle) the way is cleared by piercing the chest with

a/

a closed haemostat. Once the pleural space has been punctured it is held open, the Starling pump is stopped, and the lungs allowed to collapse safely out of reach of the instrument or ligature. The stout cotton thread is rapidly passed round the rib and firmly tied. Artificial ventilation is then reinstituted. The whole process takes but a few seconds. This manoeuvre is adopted as it is easy to injure or catch the lungs if such precautions are not taken.

Each of the three ribs is ligatured in similar fashion, artificial ventilation being stopped each time.

(3) The intercostal vessels having been thus secured, the centre rib is freed for about $\frac{3}{4}$" on each side ventral to the ligature. A large haemostat is then applied to each end of this cleared portion of rib. Again, as each is applied the lungs are collapsed by stopping the pump. The portion of bone between the haemostats is then removed with bone nibbling forceps. Each free end of the rib is now firmly crushed between the holding haemostat to compress the spongy bone and prevent bleeding. A stout ligature is then passed round each rib end and the surrounding muscle before the haemostat is removed. In the case of the ventral stump this is a very necessary precaution for no attempt has so far been made to prevent haemorrhage from the anastomosing vessels tracking toward the field from the ventral aspect/

aspect of the chest. On the dorsal stump it serves as extra protection.

The operation results in a hole in the chest wall about 1.5 cm. in diameter. This is kept covered with loose web gauze moistened with warm Ringer-Locke solution which prevents drying of the thoracic contents and excessive heat loss, while interfering little with the efficiency of the opening. The warmth of the gauze is maintained by suitably sited electric bulbs.

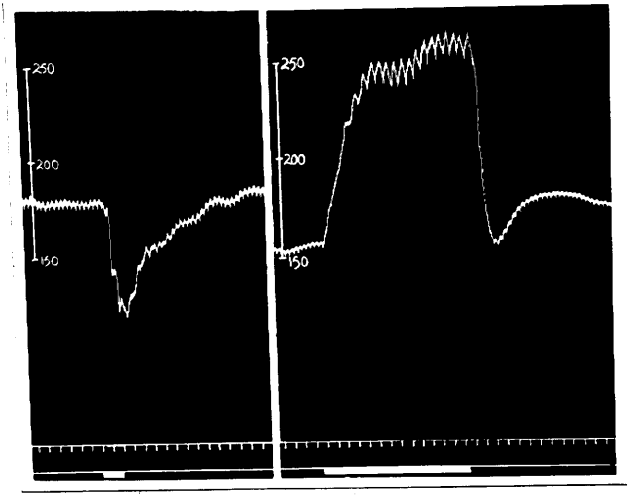
The whole procedure is repeated on the other side of the chest.

The temperature of these animals must be carefully watched for considerable loss of heat may occur from the open pneumothorax. During experiments the room is kept well heated, usually at 70°F. or more. An especially careful watch is kept over the animal's rectal temperature, which is recorded throughout by indwelling thermometer.

When the animal had been thus prepared, the experiment was begun. The sinus nerve (usually the right but sometimes the left) was carefully laid across the chlorided electrodes, which were inserted up to the sinus region by a caudo-lateral approach. The nerve was left intact, for it was found that as it lay in its natural position it was ideally placed to receive the electrodes. Moreover, experience showed that such a preparation lasted much longer than if the nerve were cut. Control stimulations carried out with the sinus nerve tied and cut gave results/

ELECTRICAL STIMULATION OF THE SINUS NERVE.

THE EFFECT OF NEMBUTAL.



A B

Fig. 3. Cat. Anaesthetic nembutal. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec.

A. Nembutal 40 mg/Kg. (total).
Stimulation of right sinus nerve 30 V. 30/sec. (Whitfield).

Between A and B 15 mg/Kg. nembutal were given intraperitoneally and 40 mins. have elapsed.

B. Nembutal 65 mg/Kg. (total).
Stimulation of right sinus nerve 30 V. 30/sec. (Whitfield).

results in every way comparable to those with the nerve intact.

The experiments were conducted along similar lines to those already carried out in assessing the effects of nembutal on the vagal responses. Condenser discharges from the Whitfield stimulator were applied to the sinus nerve in the normally (lightly) anaesthetized cat, and a stimulus was selected which gave pronounced depressor effects on the blood pressure on repeated tests. Thereafter, the animal was given additional doses of nembutal intraperitoneally and the same stimulus repeated.

Results:

In each of several animals, it was found that where electrical stimulation of the sinus nerve in the normally nembutalized cat produced a fall in blood pressure, repetition of the same stimulus in deep nembutal anaesthesia had the opposite effect, causing a pronounced pressor response (Fig. 3).

Interpretation:

The experiments clearly indicated that the action of nembutal was not confined to the vagus nerve, but that it produced a similar 'reversal' in the more essentially vasomotor sinus nerve.

C H A P T E R III.

THE EFFECT OF NEMBUTAL MORE CLOSELY EXAMINED.

The last two sections have been devoted to a description of technique, and to illustration of the alteration of the 'normal' sinus and vagus responses brought about by nembutal. Little detail was given as it was intended only to introduce the problem. Now the findings may be related in greater detail and presented together, for it is apparent that the phenomenon concerns both nerves.

It has been shown that administration of nembutal in excess brings about a 'reversal' of the normal expected depressor responses both in the vagus and sinus nerves, and tracings have been selected which illustrate the fall in blood pressure obtained in light nembutal anaesthesia and the rise observed when the anaesthesia is deepened. The two responses, however, do not occur the one immediately after the other. Between them there is a period when vagus or sinus nerve stimulation yields neither the one effect nor the other.

It will no doubt have been observed that to bring about the abnormal response, nembutal was given in several small doses over an interval of time. This was done for two reasons, firstly, to assess the total amount of nembutal required to produce the reversed response and, secondly, to permit of examination of the stages between the normal depressor and the fully established pressor effect. It transpired that this method of piecemeal

administration/

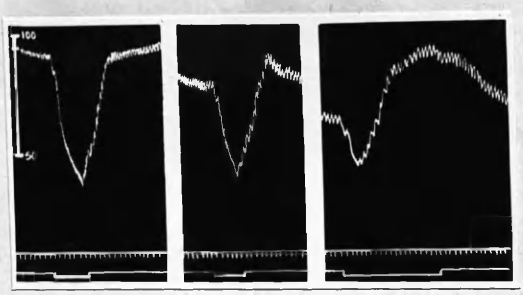
administration of the anaesthetic was unsatisfactory from the point of view of obtaining powerful pressor responses. It was useful, however, in that it clearly revealed the various stages in the production of the pressor response.

Where the nembutal was given intraperitoneally in 20 mg. doses at intervals of more than 20 min. there was an increase in the depth of anaesthesia and a tendency for the blood pressure gradually to fall. In most animals, repeated stimulation of the vagus as the total injected nembutal mounted, revealed a tendency for the percentage fall in blood pressure to decrease. In some, this diminished fall during stimulation was followed by a distinct after-rise when the stimulus was withdrawn. In others, the depression of blood pressure was ill sustained and tended to return to the normal level during stimulation, sometimes falling short of it and at others rising above it. As more nembutal was given the depressor component of these responses diminished while the pressor component increased, so that there resulted a biphasic response, the initial phase always being depressor. Finally, the depressor component was lost and the first deviation observed was a rise.

Assay of the amount of nembutal required to produce the pressor response proved impracticable by this method of successive small doses, for there was much variation among different animals and indeed the 'reversed' response was not always obtained. In three cats submitted to such methods, results were obtained which were, although qualitatively similar, certainly/

TRANSITION RESPONSES.

EFFECT OF REPEATED SMALL DOSES OF NEMBUTAL GIVEN INTRAPERITONEALLY.

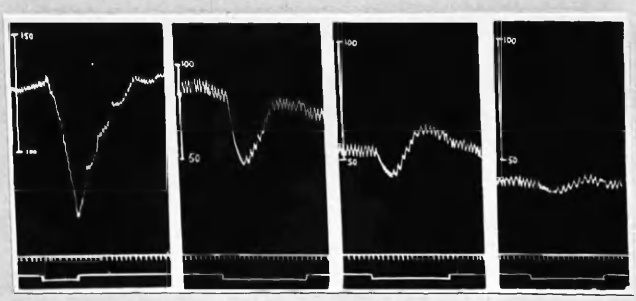


A B C

Fig. 4. Cat. Anaesthetic nembutal. Double vagotomy. Artificial respiration. Thorax intubated. Arterial B.P. (left femoral artery). Time 5 sec. At each signal the left vagus is stimulated 15 V. 30/sec. (Whitfield). Between stimuli nembutal given intraperitoneally in small doses.

A. Nembutal 50 mg/Kg. (total) 2 hr. after induction
B. " 75 mg/Kg. " 4 hr. " "
C. " 98 mg/Kg. " 6 hr. " "

EFFECT OF INTRAVENOUS INFUSION OF NEMBUTAL.



A B C D

Fig. 5. Cat. Chloroform. Nembutal I.V. 35 mg/Kg. Double vagotomy. Artificial respiration. Thorax intubated. Arterial B.P. (left carotid artery). Time 5 sec. At each signal the right vagus is stimulated 20 V. 30/sec. (Whitfield).

A. Nembutal 35 mg/Kg. (total) 1 hr. 10 min. after induction. Between A and B constant intravenous infusion of nembutal begun (40 mg/hour).
B. Nembutal 51 mg/Kg. 2 hr. 20 min. after induction
C. Nembutal 73 mg/Kg. 3 hr. 50 min. " "
D. Nembutal 92 mg/Kg. 5 hr. 10 min. " "

certainly very different quantitatively. Thus, in one female cat (2.8 Kg.) anaesthetized with nembutal (50 mg/Kg.), intraperitoneal injection of additional doses of nembutal (30 mg.) at ½-hourly intervals begun 2½ hours after induction, resulted in the appearance of a biphasic response in 4 hours, and a fully developed pressor response without depressor component in 5 hours. In another female cat (2.6 Kg.), however, again anaesthetized with 50 mg/Kg., in which the same course was followed, a biphasic response did not appear until 6 hours after induction, while even then the response was feeble and more anaesthetic resulted in the death of the animal. The results of the third experiment are shown opposite (Fig. 4).

It was thought that the variation in the amount of nembutal required, might be due to different rates of absorption of the anaesthetic, possibly attributable to injection of some of the drug into the gut, although such an accident was not discovered post mortem. To obviate the possibility of such a complication, nembutal was given intravenously to two animals. These were anaesthetized with chloroform and the nembutal (20 mg/cc.) slowly administered by the saphenous vein. The chloroform was withdrawn as the injection proceeded. 35 mg/Kg. of nembutal were given in just over three minutes. Thereafter dissection was carried out and the vagi prepared for stimulation. At this stage, constant intravenous infusion of nembutal (20 mg/cc.) was begun, the pump delivering 40 mg/hour, and the effects of vagal stimulation observed at intervals as the injection proceeded/

proceeded. In the first animal (Fig. 5) the depressor response had become poorly sustained when the total nembutal had reached 51 mg/Kg. and biphasic at 73 mg/Kg. Further nembutal did not suffice to produce the pressor effect and the animal died. In the second cat the same procedure was followed. This animal died when the total nembutal administered reached nearly 105 mg/Kg. without showing any indication of a pressor response, but merely a gradual fall in blood pressure.

It was apparent that although these experiments had indicated the nature of the intermediate responses to vagal stimulation, the quantitative aspect could not be readily determined by such methods unless considerable time was spent modifying the technique, rates of dosage and the like. But on consideration of the evidence provided it became clear that the appearance of the pressor response was a function of time. In the five animals cited, slow administration of the anaesthetic failed to produce a pressor response, or resulted in a feeble one, yet the amount of anaesthetic given was of the same order as that found adequate to produce vagal pressor responses in earlier experiments in which the anaesthetic was given more rapidly. In several succeeding experiments therefore, after the preliminary studies of the 'normal' responses, and when it was required to produce a 'reversed' response, this was done by giving 30 mg. or so at shorter intervals of about 15 min. This procedure certainly yielded pressor responses more readily and also confirmed that the intermediate responses were as described, but/

THE SINUS NERVE PRESSOR RESPONSE APPEARING AT A LIGHTER PLANE OF NEMBUTAL ANAESTHESIA THAN ITS VAGAL COUNTERPART.

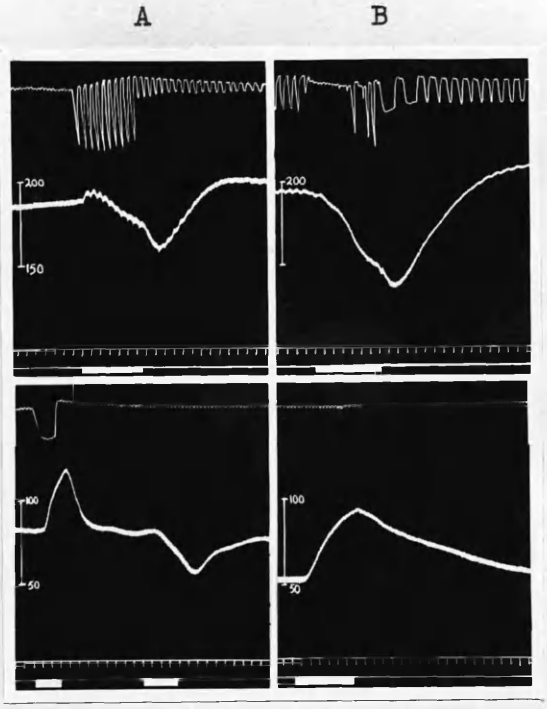


Fig. 6. Cat. Anaesthetic Nembutal. Double vagotomy. Left sinus nerve exposed and cut. Artificial respiration. Bilateral open pneumothorax. Records of chest movements (stethograph). Arterial B.P. (left carotid artery). Time 5 sec. Stimuli throughout 40 V. 30/sec. (Whitfield).
Upper 2 Tracings nembutal 50 mg/Kg. 1 hr. 35 min. after induction.
A. Stimulation of right sinus nerve.
B. " " " vagus "
Between B and C 15 mg/Kg. nembutal injected intraperitoneally.
C. Stimulation of right sinus nerve and right vagus 2 hr. 50 min. after induction.
Between C and D 15 mg/Kg. nembutal injected intraperitoneally.
D. Stimulate right vagus 3 hr. 30 min. after induction.

but in turn, was abandoned in favour of a still more massive scheme of dosage. By this last method, the pressor response was produced by giving intraperitoneally, in a single dose, half the amount of nembutal found to be required in the particular animal to produce an adequate depth of anaesthesia. Such an amount usually caused a profound fall in blood pressure which reached a minimum at or about 50 mm. Hg. within a few minutes. At this stage, which lasts for varying periods of time, both weak and powerful stimuli are without any effect whatsoever. Such 'areflexia' is, however, transient. Gradually the blood pressure rises, and as it does, the application of stimuli previously depressor causes faint pressor responses which become more and more pronounced, reaching a maximum in about 45 min., and lasting for an hour or more without decrement.

It must be emphasised that there is considerable variation in all these factors, fall in blood pressure, magnitude of response and times of the phases described. Further, in some animals, administration of the half-anaesthetic dose gives a short 'areflexic' period, after which a biphasic response is obtained. In these cases, injection of small doses of nembutal (20 - 30 mg.) rapidly leads to the establishment of a pure pressor response. Where the pressor response is tending to diminish, it can be restored in a similar manner.

So far, no mention has been made of the effects of sinus nerve stimulation, but much that has been said of the transition from the depressor to the pressor response in the vagus has been found/

THE EFFECT OF SPLANCHNIC NERVE STIMULATION DURING THE AREFLEXIC PERIOD FOLLOWING UPON A LARGE DOSE OF NEMBUTAL.

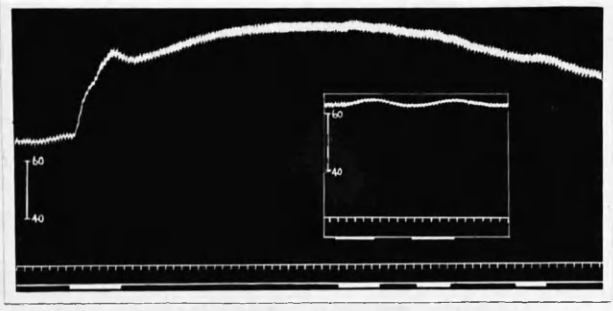


Fig. 7. Cat. Anaesthetic nembutal. Double vagotomy. Artificial respiration. Arterial B.P. (left carotid artery). Time 5 sec.

Inset: Stimulation of right sinus nerve and right vagus 20 V. 30/sec. (Whitfield) 10 min. after injection of half anaesthetic dose of nembutal (25 mg/Kg.).

Large tracing. The effect of stimulation of the peripheral end of the right splanchnic nerve 3 min. later with the same strength of stimulus. Superimposed on the rise of B.P. are seen the effects of stimulation of the right sinus nerve (X 2) and the right vagus. Stimulus in each case 20 V. 30/sec.

found to hold for the sinus nerve. Like that of the vagus, the sinus nerve depressor response diminishes, becomes biphasic, the phases being depressor and pressor in order of appearance, and finally pressor. The main difference lies in the fact that it often needs but very small doses of nembutal (sometimes less than 10 mg/Kg.) rapidly to convert a marked fall on stimulation into a powerful rise. Thus it is that a sinus nerve 'reversed response' appears much sooner than its vagal counterpart (Fig. 6). As a result, biphasic responses are not so readily observed. Nevertheless, they do occur, and make their appearance, as in the vagus, when anaesthesia is not sufficiently deep. An example is illustrated in Chapter IV.

As with the vagus reflexes, large amounts of nembutal, such as the excess half anaesthetic dose, result in an areflexic stage where sinus nerve stimulation is quite without effect.

It was of interest to study the response of the peripheral vasoconstrictor mechanism during the reflex 'paralysis' and, accordingly, in one animal the splanchnic nerve was exposed and cut at this stage and the peripheral end stimulated. The effect of this manoeuvre can be seen in Fig 7. Inset are the responses to vagus and sinus nerve stimulation 3 min. prior to the splanchnic nerve stimulation, and on the blood pressure trace resulting therefrom are seen the responses from both vagi and the right sinus nerve. They are still feebly pressor although the blood pressure lies between 175 and 150 mm. Hg. and one splanchnic nerve is cut.

When/

A TYPICAL VAGUS NERVE RESPONSE IN DEEP NEMBUTAL ANAESTHESIA.

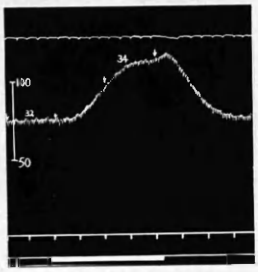


Fig. 8.

Cat. Anaesthetic nembutal. Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Respiratory movements (stethograph). Time 5 sec. Stimulation of right vagus 40 V. 30/sec. (Whitfield). Record taken 4 hr. 30 min. after induction (45 mg/Kg) and 50 min. after 'reversing' dose of 25 mg/Kg. Small numbers refer to heart rates in the ten second periods indicated by the arrows. Small movements on the respiratory tracing are caused by the pump. There is no spontaneous respiration.

A TYPICAL SINUS NERVE RESPONSE IN DEEP NEMBUTAL ANAESTHESIA.

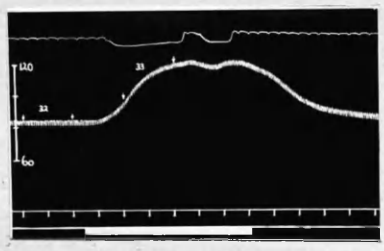
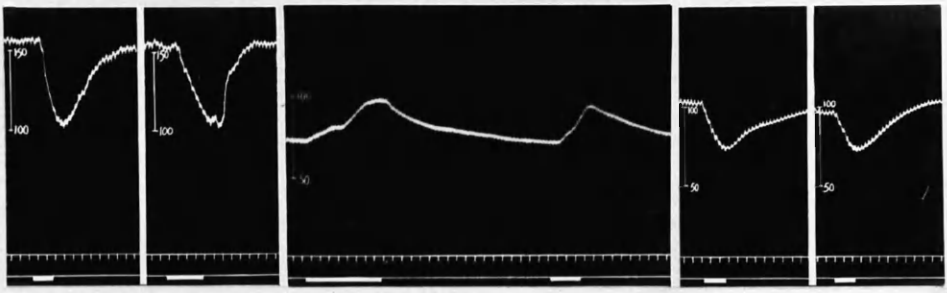


Fig. 9.

Cat. Anaesthetic nembutal. Preparation and records as in animal above (Fig. 8). Stimulation of right sinus nerve 40 V. 30/sec. (Whitfield). Record taken 3 hr. 20 min. after induction (40 mg/Kg.) and 1 hr. after 'reversing' dose of 25 mg/Kg. Two respiratory movements are superimposed on waves due to pump.

When well developed, the reversed responses show several characteristic features. Thus, pressor effects obtained from any one of the buffer nerves present a remarkably uniform appearance, the height and configuration of successive blood pressure tracings, in response to the same stimulus, being strikingly similar. Each nerve may, however, tend to give a response differing slightly in extent or form from those obtained from the others, although the difference between members of a pair such as the sinus nerves is usually small. In general, large pressor responses from the vagi usually occur in company with marked sinus nerve reversals and vice versa. It must be remembered, however, that sinus nerve reversed responses occur at a much earlier stage. What is being discussed at present is the nature of things in the animal showing depressor responses from none of the buffer nerves, the kind of preparation one can produce at will by injecting a 'half anaesthetic dose' of nembutal (or little more), and thereafter waiting until the blood pressure rises to about 75 mm. Hg. At such a stage the blood pressure and heart rate, even in animals with all buffer nerves cut, are characterized by rock-like steadiness. It is then that the inflexible, uniform pressor responses make their appearance. Two have been chosen for illustration as the typical pressor responses from the vagus (Fig. 8) and sinus nerve (Fig. 9) in the over-nembutalized cat. It must be stressed that they are examples typical of many scores of 'reversals' observed and are not selected as being especially marked. Much more

SPONTANEOUS REVERSION.



A B C D E F

Fig. 10. Cat. Anaesthetic nembutal. Double vagotomy. Left sinus nerve cut. Artificial respiration. Thorax intubated. Arterial B.P. (left carotid artery). First 2 Tracings obtained after 50 mg/Kg. nembutal (total).

A. Stimulation of right vagus nerve 40 V. 30/sec.

B. " " " sinus nerve 40 V. " "

Next 2 Tracings obtained 1 hr. 20 min. later. Nembutal 80 mg/Kg. (total).

C. Repeat vagus nerve stimulation.

D. " sinus " "

Last 2 Tracings obtained 3 hr. after 'reversed' responses.

E. Repeat vagus nerve stimulation.

F. " sinus " "

powerful responses do occur, and some are illustrated elsewhere. In some animals the rise is unaccompanied by increase in heart rate, in others (Figs. 8, 9), there is a very small increase despite the vagotomy, but it is apparent that the pressor effects are independent of this and must be ascribed to vasomotor activity.

So much, then, for the developing and established 'reversed' responses. It was found that if animals showing pressor responses due to nembutal overdosage were left for some hours to recover these effects disappeared and were replaced by depressor responses of much the same kind as were found at the outset of the experiment during light anaesthesia (Fig. 10). The return to the expected response is very slow and goes through the same phases as does the developing reversed response, undergoing diminished and biphasic stages before becoming purely depressor. The biphasic stages are similar to those already described in that the first component is always depressor. As the depth of anaesthesia lessens, this initial phase becomes more and more marked and the rise becomes less, so that finally no trace of it remains. The administration of more nembutal at this stage has been found to cause the reappearance of the reversed response.

There was no doubt then that both in the case of the sinus nerve and the vagus, the normal depressor responses to electrical stimulation could be converted to a rise of blood pressure by increase in the dosage of nembutal. The few animals in which the pressor responses failed to appear, when reviewed/

reviewed in the light of later experience, were found to be those in which the nembutal was administered in inadequate manner. A great number of experiments (detailed in the chapters to follow) were carried out to analyse the pressor response, and showed that the reversed responses could be obtained regularly, in every animal. That the 'reversal' was due to the anaesthetic and to no other change in the preparation could not be doubted, for the various responses could all be seen to be closely dependent on the depth of anaesthesia, or concentration of the nembutal at any one time. The normal depressor responses in light anaesthesia, either at the commencement of the experiment or several hours after overdosage with nembutal when recovery was taking place, the biphasic responses at slightly deeper planes, the 'reversed' responses after considerable overdosage, and indeed the areflexic periods following upon massive injection, all bear witness to this.

C H A P T E R IV.

PICROTOXIN.

Having established that nembutal altered the effects of stimulation of the buffer nerves, it was of interest to determine the effect of a barbiturate antidote given to the animal showing 'reversed' responses. Picrotoxin, which is used extensively in the clinical treatment of barbiturate poisoning, was the obvious choice.

Methods:

Cats were employed; they were anaesthetized with nembutal. The vagi were cut and in some, one sinus nerve. Artificial ventilation was instituted and the blood pressure recorded from carotid or femoral artery. The vagus and sinus nerves were then stimulated, and control stimuli selected which produced powerful depressor responses. Thereafter, reversal of the response to electrical stimulation was caused by injecting a further dose or doses of nembutal. When marked pressor responses had been obtained, picrotoxin (B.P.C. Abbott: 0.3%) was injected intravenously, and stimulation of the vagus or sinus nerve with the control stimulus repeated after the lapse of some minutes, usually less than five. This procedure of injection followed by stimulation was repeated where necessary at intervals of a few minutes.

Results:

(1) The Effect of Picrotoxin on the 'Reversed' Response/

THE EFFECT OF PICROTOXIN ON THE 'REVERSED' RESPONSE.

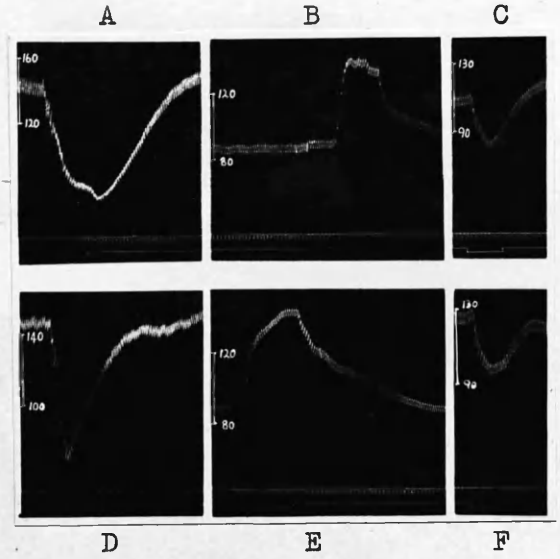


Fig. 11. Cat. Anaesthetic nembutal. Double vagotomy. Left sinus nerve cut. Artificial respiration. Thorax intubated. Arterial B.P. (left carotid artery). Time 5 sec.

Upper 3 tracings: stimulation of the right sinus nerve 40 V. 30/sec. (Whitfield).

A. 2 hr. after induction. 43 mg/Kg. Nembutal.

B. 4 hr. 35 min. after induction. Nembutal 83 mg/Kg. (total).

C. 4 hr. 43 min. after induction. Picrotoxin 1.1 mg/Kg. I.V. 7 min. earlier.

Lower 3 tracings: stimulation of right vagus nerve 20 V. 30/sec. (Whitfield).

D. 1 hr. 50 min. after induction.

E. 4 hr. 28 min. after induction. Nembutal 83 mg/Kg. (total).

F. 4 hr. 47 min. after induction. Picrotoxin 1.1 mg/Kg. I.V. 11 min. earlier.

Response.

The procedure outlined has been carried out in nine animals. In eight of these, results were obtained which were qualitatively similar. In each, picrotoxin caused a rapid conversion of the pressor to a depressor effect. The control depressor responses obtained from the sinus nerve and the vagus, the reversed responses obtained after nembutal overdosage, and finally, the conversion of these to depressor responses by the administration of picrotoxin are all illustrated in Fig. 11. This animal, the second of the series, has been chosen for illustration, not merely to demonstrate the return to the expected response which follows injection of this drug, for indeed the falls in blood pressure obtained in this case are not so striking as were obtained in some subsequent experiments, but also to show that even where very marked depressor responses are obtained from the sinus nerve and vagus, under certain conditions the administration of nembutal may cause the appearance of equally striking rises in blood pressure.

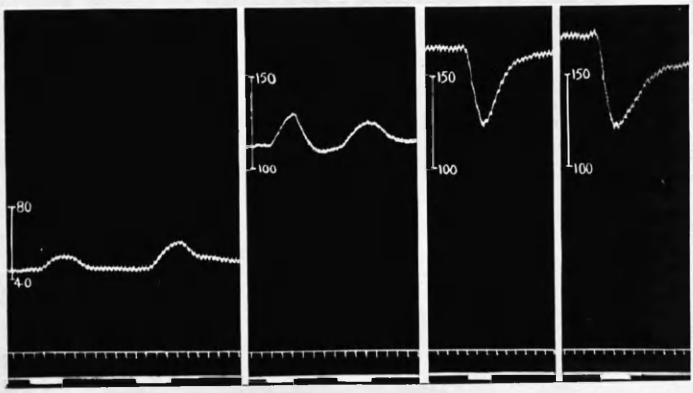
(2) The Effect of Picrotoxin on the Blood Pressure.

Although picrotoxin had the same ultimate effect in each animal, the establishment of the depressor response was accompanied by different effects on the level of the systemic blood pressure. These are of special interest and will be related in some detail.

In one cat, that illustrated in Fig. 11, a single injection (1 cc.) of picrotoxin served to bring about a marked rise in blood/

THE EFFECT OF PICROTOXIN ON THE ARTERIAL

B.P. AND THE REVERSED RESPONSES.



A B C D

Fig. 12. Cat. Anaesthetic nembutal. Double vagotomy. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec.

A. Stimulation of right sinus nerve and right vagus (read left to right). 40 V. 30/sec. (Whitfield). 3 hr. 50 min. after induction (4.5 mg/Kg.). 40 min. after 'reversing' dose nembutal (25 mg/Kg.).

B. Stimuli repeated 5 min. later, 3 min. after Picrotoxin 0.9 mg/Kg. I.V.

C and D stimuli repeated 9 min. after 'B' and 6 min. after further 0.3 mg/Kg. Picrotoxin.

blood pressure and clearly defined depressor responses. In three cats, 1 cc. (3 mg.) of picrotoxin, injected into one or other femoral vein, caused a rise of blood pressure, although the pressor responses persisted when the reflexes were tested a few minutes later. Subsequent administration of several (1 - 3) smaller doses (0.3 cc.) further raised the blood pressure and caused the appearance of the depressor response. Each of these animals was very profoundly anaesthetized at the moment of injection (Fig. 12).

In two cats, at a somewhat lighter plane, administration of picrotoxin (1 cc.) led to comparatively small rises in blood pressure but to the reappearance of depressor responses at this level (Fig. 13).

In two other cats there was a fall of about 20 mm. Hg. after intravenous injection of picrotoxin (1 cc.), but the blood pressure slowly rose to pass the 'pressor' level, and electrical stimulation of the buffer nerves yielded 'depressor' responses. These two last animals were perhaps at a somewhat lighter plane of anaesthesia than the others.

(3) Transition Responses.

In several of the eight successful experiments, it was observed that where the vagus or sinus 'reversed' response was in process of being converted to a fall, transition responses made their appearance which were of the same nature as those observed during the development of the 'reversal' or during spontaneous reversion (Chap. III). They persisted for very brief/

THE EFFECT OF PICROTOXIN ON THE ARTERIAL

B.P. AND THE REVERSED RESPONSES.

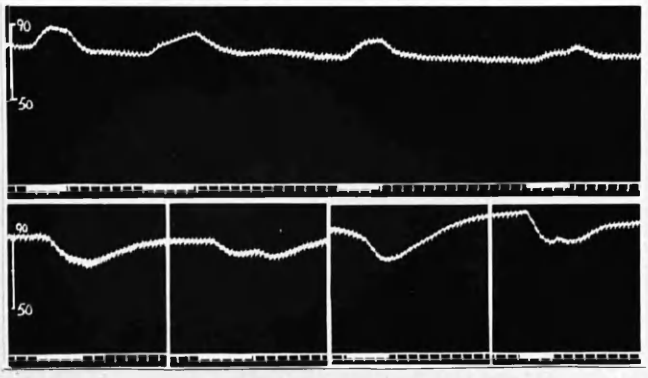


Fig. 13. Cat. Anaesthetic nembutal. Double vagotomy. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec.

Upper tracing: (left to right). Stimulation of right vagus, right sinus nerve, left vagus and left sinus nerve. 40 V. 30/sec. (Whitfield).

3 hr. 35 min. after induction 50 mg/Kg.

1 hr. after 'reversing' dose nembutal 25 mg/Kg.

Lower tracing: the same stimulations repeated 7 to 14 min. after the upper, and 6 min. after 1.2 mg/Kg. Picrotoxin I.V.

brief periods, however, for the transition brought about by picrotoxin was usually very rapid, being complete within a few minutes. One such occurrence, favoured by cautious administration of the drug, is shown in Fig. 14 (D). This figure illustrates phenomena also described in the last chapter, namely, a partial spontaneous reversion occurring in the sinus nerve response (B), and its conversion to a pure pressor response by the addition of nembutal (C).

(4) Picrotoxin - Nembutal Antagonism in the Intact Animal.

It has been found that just as picrotoxin reinstates a depressor response after its reversal by nembutal, so too does this latter drug re-establish a pressor effect abolished by the former. The rise due to nembutal and the fall due to picrotoxin are, in effect, both reversed by administration of the other drug. This has been found to hold good in all of three experiments in which it has been attempted, both for the sinus nerve and for the vagus. In these animals the responses could be altered at will, see-saw fashion (Fig. 14, A, C, E, F, G).

(5) Other Effects Accompanying Picrotoxin Administration.

The effects of picrotoxin were not, of course, confined to restoration of the expected depressor activity to the buffer nerves, or to alteration of the level of systemic blood pressure, although such actions were those of particular interest and were those most closely studied. In most animals, other indications of/

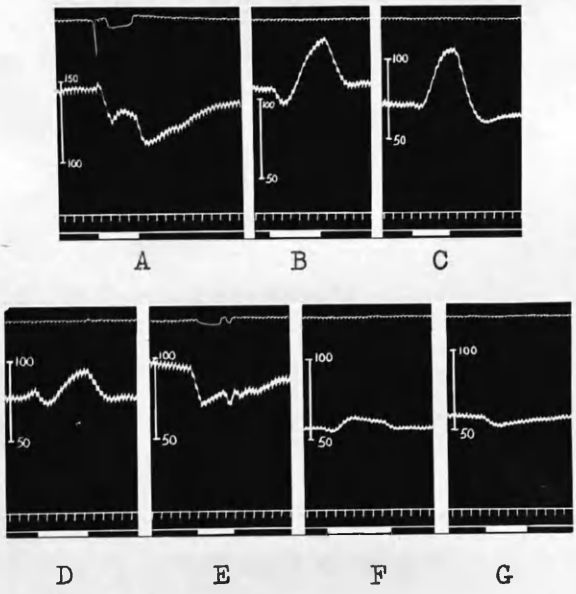


Fig. 14. Cat. Anaesthetic nembutal. Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Respiratory movements (stethograph). Arterial blood pressure (left carotid artery). Time 5 sec.

9.40 a.m.	Induction nembutal (40 mg/Kg.).
11.02 a.m.	Stimulate right sinus nerve 40 V. 30/sec. (A).
12.10 p.m.	'Reversing' dose nembutal given (20 mg/Kg.).
1.50 p.m.	Repeat sinus nerve stimulation.(B).
3.15 p.m.	Nembutal 10 mg/Kg. injected intraperitoneally.
3.33 p.m.	Repeat sinus nerve stimulation (C).
3.57 p.m.	0.3 cc. Picrotoxin intravenously (0.3 mg/Kg.).
4.01 p.m.	0.3 cc. " intravenously (0.3 mg/Kg.).
4.06 p.m.	Repeat sinus nerve stimulation (D).
4.11 p.m.	0.3 cc. Picrotoxin intravenously.
4.26 p.m.	Repeat sinus nerve stimulation (E).
4.40 p.m.	Nembutal 10 mg/Kg. intravenously.
5.02 p.m.	Repeat sinus nerve stimulation (F).
5.05 p.m.	1.0 cc. Picrotoxin 1.V. (1 mg/Kg.).
5.09 p.m.	0.5 cc. " 1.V. (0.5 mg/Kg.).
5.10 p.m.	Repeat sinus nerve stimulation (G).

of the analeptic powers were manifest, and it is of interest to relate these well known effects to the reappearance of the depressor reflexes.

At a time when the drug had been given in sufficient amount to cause the return of the depressor response, there was usually present some other indication of its action, such as an increase in respiratory irritability as shown by sinus nerve stimulation (Fig. 14, E) or by the return of spontaneous respiration, or a return of the corneal or pad withdrawal reflexes, or even twitching of the vibrissae. But in some cats in which the dose of picrotoxin was insufficient to cause any of these effects, and the animal was seemingly yet deeply anaesthetized, the amount of the drug given sufficed to restore the depressor effect.

(6) Failure to Regain the Depressor Response.

The one experiment in which a depressor response was not observed can be discounted as the technique of administration of the picrotoxin was at fault. In this animal, 3 mg. of picrotoxin caused a rise in blood pressure from 75 mm. to 95 mm., but without conversion of the pressor response. Another 3 mg. given after 4 min. led to a further rise to 130 mm., and to such marked twitching that the animal had to be destroyed with chloroform without testing the reflex. Administration of the drug had been incautious. In later experiments smaller injections, usually 1 mg., were given, and a longer interval was allowed between injections.

(7) The Dosage of Picrotoxin.

Since/

Since these experiments were not devoted to the study of the effects of picrotoxin alone, and other aspects of the nambutal 'reversal' were also being investigated, there was considerable variation in the technique. Thus, at the time of administration of the drug, the animals were at varying depths of anaesthesia, and at different intervals both from the induction and the 'reversing' doses of nembutal (which varied in themselves). In consequence they provided only a rough indication of the amount of picrotoxin required. A quantitative assay was outwith the scope of a series intended primarily to determine the qualitative effects. The amounts of picrotoxin injected in the eight animals (expressed as mg/Kg. body weight), and after which depressor responses appeared were as follows: 0.9, 1.1, 1.2, 1.2, 1.2, 1.3, 1.5, 1.8.

The interpretation of these findings will be discussed later in conjunction with evidence provided by other experiments.

C H A P T E R V.

DECEREBRATION EXPERIMENTS.

In the light of evidence of participation of nervous structures above the medulla in vasomotor reflexes, it was decided to devote several animals to the study of the effect of nembutal overdosage upon the vagus and sinus nerve reflexes of the decerebrate preparation. In this way, it was hoped further to localise the site of action of nembutal.

It is customary to decerebrate animals under a volatile anaesthetic, and dosage must be very heavy to obviate the gross disturbances and failures which otherwise readily occur. Such a method, however, was felt to be unsuited to the purpose in view, for in addition to the complication of an additional anaesthetic, there had also to be reckoned with two other factors, firstly, that the animals were to be vagotomized, and section of the vagi increases the liability to failure (WRIGHT, 1928), and secondly, that dissection of a long and extensive nature had to be carried out. The uncertainty of obtaining a preparation in sufficiently good condition to endure the fluctuations of blood pressure consequent on buffer nerve stimulation, or to survive over the period of time taken for the 'reversed' response to develop, caused such a technique to be unfavourably viewed. It was realised that another line of approach might well be adopted. Mention has been made already/

already of the stability of the profoundly nembutalized animal (Chap. III), and it was decided to utilise this state for the performance of the decerebrations. By this means it could be discovered whether or not the 'pressor' responses were dependent or independent of the brain matter above the level of decerebration.

Methods:

Six cats were employed in the study. Anaesthesia was obtained by injecting nembutal intraperitoneally (40 + mg/Kg.). A tracheal cannula was inserted. Both vagi and both sinus nerves were exposed and the vagi cut. Bilateral open pneumothorax (rib resection) was performed and artificial respiration begun. Records of the arterial blood pressure were taken from the left carotid artery (citrate was used). The right carotid artery was tied.

When dissection had been completed, control responses to electrical stimulation of the vagus and sinus nerves were obtained. Stimuli were chosen which gave distinct depressor responses. Thereafter a 'reversing' dose of nembutal was given, and if necessary, supplemented with further doses to produce an especially profound anaesthesia, so that buffer nerve stimulation showed the pressor response. This was allowed to develop until it was well marked, but as far as possible care was taken to prevent there being too great an interval between the 'reversing' dose and the decerebration, for such would allow the anaesthesia to lighten and increase the risk of the operation. When control 'reversed'/

'reversed' responses had been obtained, the animal was rapidly decerebrated. This step was carried out by turning the animal spine up to expose the back of the head which was then firmly held. A mid line incision was made from the orbits to below the lambdoidal ridge. The skin was retracted laterally. The medial part of the floor of the left temporal fossa was stripped of m. temporalis and the bone cleared by separating this muscle laterally. A $\frac{3}{4}$" trephine opening was made through the left parietal eminence and extended with bone-nibblers to the level of the bony tentorium caudally. The dura was cut with fine scissors and retracted. A blunt metal decerebrator was introduced below the occipital pole of the left cerebral hemisphere, care being taken to avoid stripping the dura off the skull, and the brain stem was sectioned with a single sweep of the instrument. This allowed all brain matter above the level of the section to be carefully scooped out. Again the dura was disturbed as little as possible. Special attention was paid to the hypophysis, this was carefully removed with forceps. Bone wax was now applied to the exposed cut bone to prevent bleeding and several layers of gauze moistened with Ringer-Locke placed over the opening in the skull. Finally the skin was closed over the swabs with clips. The animal was now placed on its back so that the dorsum of the skull rested on a layer of cotton wool warmed by the copper table top. There was remarkably little bleeding, and the vertebral arteries were manually occluded at the longest for 3 min., and in two instances not at all. (Both carotids were

of course tied early in the experiment). The reasons for the lack of haemorrhage were not hard to find. Firstly, decerebration was always carried out at a low level of blood pressure due to the nembutal overdose, and secondly, tracings of the blood pressure taken throughout the operation showed that this level never rose by more than 40 mm. Hg., and usually by about 20 mm. Hg., despite the irritant nature of the trauma involved, and the effect of occlusion of the vertebrals. This too must be ascribed to the nembutal, for it has been observed in several other decerebrations carried out under different anaesthetics that the rise in blood pressure, from trauma and asphyxia, accompanying the operation, was of a much greater order. Apart from the first experiment where the blood pressure fell from 70 to 50 mm. Hg., the level after decerebration was found to have risen some millimetres in every animal.

Within a few minutes of completion of the operation, stimulation of the buffer nerves was repeated.

An especially careful watch was kept on the rectal temperature once decerebration had been performed.

Results:

(1) The First Experiments. The Method Justified.

The first animal showed that the 'reversed' responses, sinus and vagus, persisted after decerebration was carried out at a level between the upper and lower corpora quadrigemina. As has been mentioned, the blood pressure was low, due to haemorrhage from insufficiency of bone wax, but there was no doubt/

doubt that in this animal removal of all brain matter above the inferior corpora had not caused the disappearance of the pressor responses. That being so, it was obvious that the effect of picrotoxin should be investigated. Accordingly, 1.0 cc. of this drug (3 mg.) were given intravenously. There was no appreciable effect on blood pressure four minutes later and responses to stimulation were doubtful. A further injection (1.0 cc.) caused the appearance of twitching but stimulation of the vagi and the right sinus nerve now caused a definite although feeble fall in blood pressure. Shortly thereafter the animal was killed. The skull and its contents were then placed in formalin and after hardening the brain was removed and the level of section studied.

This experiment confirmed the belief that such an approach was practicable and would yield satisfactory results.

(2) The Second Experiment. The Effects of Nembutal and Picrotoxin Persist.

A second animal was subjected to the same procedure (Fig. 15). The details of this experiment are as follows:-

Time from induction.

0 hrs. Induction. 40 mg/Kg. nembutal I.P.

0 hr. 15 min. Began dissection.

1 hr. 35 min. Dissection completed: (Trachea cannulated. R. and L. vagi and R. sinus nerve prepared for stimulation. L. sinus nerve cut. R. carotid artery tied. L. carotid artery cannulated/

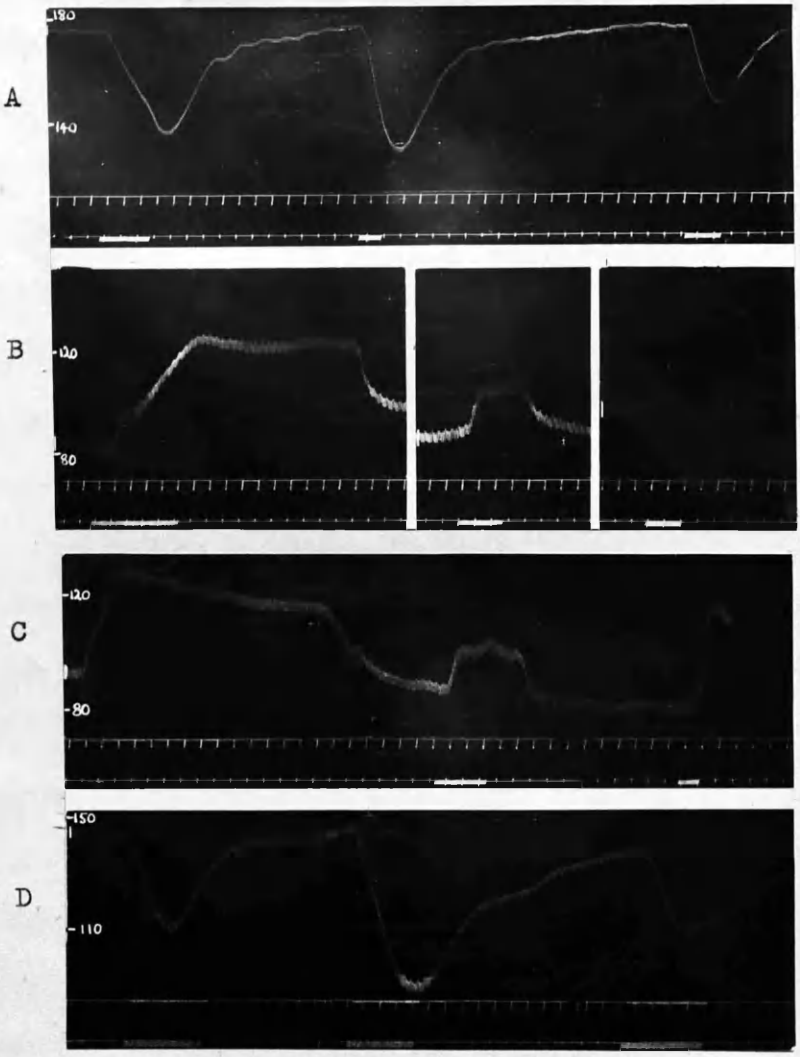


Fig. 15. (See text for full description).
Each series A, B, C and D shows the effects of stimulation of the right vagus, left vagus and right sinus nerve (left to right). Stimuli 40 V. 30/sec. (Whitfield).

A. 2 hr. after induction. Nembutal 52 mg/Kg. (total).

B. 3 hr. 15 min. after induction. Nembutal 78 mg/Kg. (total).

Between B and C animal decerebrated.

C. 3 hr. 31 min. after induction. Nembutal 78 mg/Kg. (total).

Between C and D picrotoxin 1.8 mg/Kg.

D. 4 hr. 4 min. after induction.

cannulated to record B.P. Bilateral open pneumothorax (artificial respiration).

1 hr. 40 min. Anaesthesia too light. Further 12 mg/Kg. nembutal given.

2 hr. Control stimulation (40 V. 30/sec. Whitfield). R. and L. vagi and R. sinus nerve (Series A, Fig. 15) several tests.

2 hr. 15 min. 'Reversing' dose nembutal I.P. 25 mg/Kg. (to total 78 mg/Kg.).

3 hr. 15 min. 'Reversed' responses obtained when control stimuli repeated (Series B, Fig. 15). (The responses are somewhat unusual, the effect lasting long after stimulation has ceased. This is especially the case with the right vagus and right sinus nerves. Because of this, the responses were tested several times. The same kind of tracings were obtained. In some subsequent experiments somewhat similar responses have been observed).

3 hr. 21 min. Decerebration. B.P. rose 30 mm. during operation which took just under four minutes to complete, from incision to waxing of the bone after removal of brain matter. The vertebral arteries were occluded for 2 min.

3 hr. 27 min. Animal on back.

3/

3 hr. 31 min. Repeated control series stimuli (Series C, Fig. 15). B.P. now 10 mm. above pre-decerebration level, but the operation has had but little effect on the configuration of the responses.

3 hr. 46 min. 1 cc. picrotoxin intravenously. (0.9 mg./Kg.).

3 hr. 50 min. B.P. now 110 mm. Hg. pressor persists.

3 hr. 53 min. 1 cc. picrotoxin I.V. (total 1.8 mg/Kg.).

4 hr. 4 min. Repeated control series stimuli (Series D, Fig. 15).

Examination of the brain stem after hardening revealed section just below the inferior corpora posteriorly and involving the superior edge of the pons arteriorly.

(3) Further Localization by Descending Section.

Four subsequent experiments were carried out to further localise the effects of the two drugs.

The technique employed was similar to that just detailed but more extensive. After decerebration at or about the inferior corpora quadrigemina, and after the 'reversed' responses had been obtained from buffer nerve stimulation as in experiment 2, the brain stem was sliced from above down. In order to do this, the opening in the skull was extended across the midline with bone nibbling forceps to the parietal of the other side. Then the caudal aspect of this enlarged opening was further nibbled away, removing the posterior part of the parietals, including the bony tentorium, the interparietal, and encroaching more or less/

less on the lambdoidal ridge. Bone wax was again liberally applied. In this way the cerebellum was revealed, and adequate exposure was obtained allowing of further interference with the brain matter under direct vision. Sections were made with a single sweep of the blunt decerebrator. As each was removed, it was transfixed with a needle in such a manner that the several sections were built up on it to reproduce the whole, thus giving additional guiding information on the level of section reached. After each sectioning the responses were again tested. The cerebellum was removed in all four animals to permit exposure for the lower sections to be made.

Findings.

Confirmation of previous experiments.

The initial 'high' decerebrations, usually about the inferior colliculus, were performed without difficulty and the preparations obtained showed no evidence of mal effect. In each, the level of blood pressure showed no deterioration from that found prior to the operation, and indeed was found to have risen. Stimulation of the buffer nerves in three animals gave pressor responses similar to those obtained before decerebration.

In a fourth, the effects of electrical stimulation of both vagi and both sinus nerves, pressor before decerebration, were found to be feebly depressor after decerebration. After a few minutes they were more strongly depressor. This was possibly ~~to be~~ due to the fact that decerebration was carried out when the effects of the nembutal were wearing off, and perhaps the

operative/

DECEREBRATION EXPERIMENTS.
DIAGRAM OF CAT BRAIN STEM TO SHOW LEVELS OF SECTIONS.

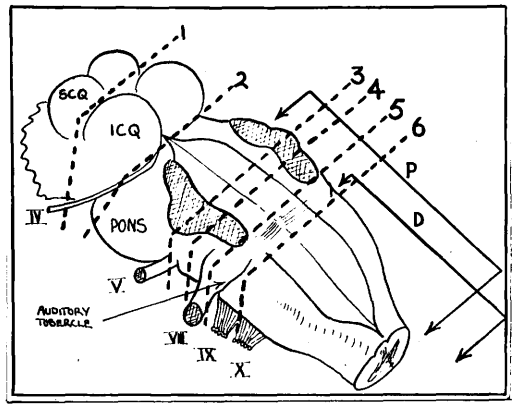


Fig. 16. Dotted lines indicate lowest levels of sections reached in each of the 6 experiments. Shown also is the approximate extent of the pressor (P) and depressor (D) centres according to Alexander. (Presentation after Lumsden).

RESULTS.

Expt.	Pressor Responses after high decerebration	Pressor Responses after section shown	Nerves Stimulated	Depressor Responses after Picrotoxin
1	Obtained	Obtained	Both vagi. Right Sinus N.	Obtained
2	Do.	Do.	Do.	Do.
3	Do.	Do.	Both vagi. Both Sinus N's.	Do.
4	Do.	Do.	Both vagi.	Do.
5	Do.	Do.	Both vagi. Right Sinus N.	Do.
6	Do.	Absent (Nerves injured)	Both vagi. Both Sinus N's.	-

operative interference hastened the 'spontaneous reversion' which was taking place. Be this as it may, by injecting nembutal (10 mg/Kg. + 5 mg/Kg.) intraperitoneally the depressor responses obtained in this decerebrate proportion were converted to pressor.

All four animals then confirmed experiments 1 and 2.

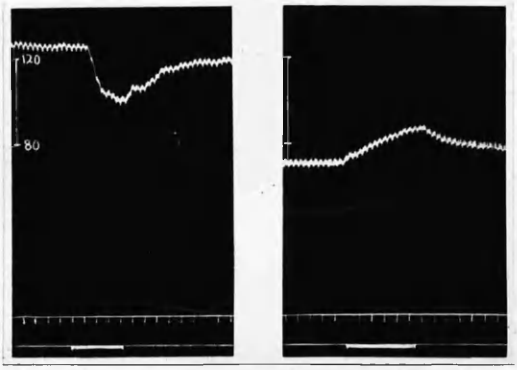
(b) The more accurate localization.

In the four animals further section of the brain stem after the 'reversed' responses had been obtained, showed that these pressor effects persisted almost unchanged as increasing amounts of the brain were removed.

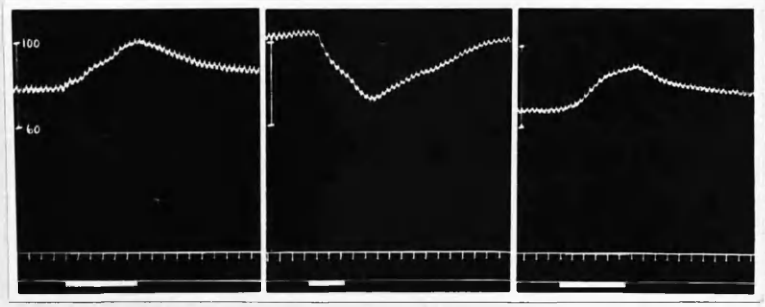
In one instance, the 'reversed' responses were present when the entire pons and brain matter above the level of the eighth nerve were removed (Fig. 16, Section 5). In this animal, as in the others in which serial section stopped short of this point (Fig. 16, Sections 3, 4), it was found that picrotoxin caused much the same effect as in the intact animal, that is, a rise in blood pressure and a return of the depressor effects of buffer nerve stimulation.

In the last animal the pressor responses disappeared when lower section was attempted. It was found in this case that section had involved the point of origin of the ninth and tenth nerves (Fig. 16, Section 6).

The table accompanying Fig. 16 summarises the findings. The extent of the medullary vasomotor centres is indicated in the figure, and it can be seen that no section encroached on the depressor/



A B



C D E

Fig. 17. Cat. Anaesthetic nembutal. Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B. P. (left carotid artery). Time 5 sec.

Each tracing shows the effect of stimulation of the right vagus (40 V. 30/sec. Whitfield).

A. C.N.S. intact. Nembutal 60 mg/Kg.

B. C.N.S. intact. Nembutal 130 mg/Kg.

Animal decerebrated between B and C.

C. Brain stem sectioned below pons (Section 4, Fig. 16).

Between C and D Picrotoxin 6.9 mg/Kg. I.V.

D. After Picrotoxin.

Between D and E nembutal 20 mg/Kg. I.V.

E. After Nembutal.

depressor region (nor could do so without severing the connections of the buffer nerves with the brain). Sections 3, 4 and 5 remove some of the pressor area, yet as can be seen (Fig. 17) the pressor response is little affected. This might be explained by the small extent of the pressor area removed, or by ALEXANDER's (1946) finding that the upper limits of the pressor area are more poorly endowed with pressor points than the lower and more significant areas.

(4) Picrotoxin-Nembutal Antagonism in the Decerebrate Preparation.

The same antagonistic effects of these drugs, as have been described already as occurring in the intact animal, were also found in the decerebrate preparation. Thus, in each of two cats, the administration of nembutal re-established the pressor effect abolished by picrotoxin (Fig. 17).

(5) The Dosage of Picrotoxin in the Decerebrate Preparation.

In the five animals to which picrotoxin was given, it was found that the following amounts (in mg/Kg. body weight) were required to convert the pressor effects of buffer nerve stimulation to depressor: 2.1, 1.8, 2.5, 6.9 and 3.8.

These values are certainly higher than those found necessary in the intact animal, but this difference between the two series is probably explained by the difference in depth of narcosis, the decerebrate preparations being especially heavily overdosed. This was certainly the case in animals four and five, particularly/

particularly the former. In this instance, nembutal had been administered to a total of 130 mg/Kg. Nevertheless it is remarkable that here the very massive dose of 6.9 mg/Kg. picrotoxin (5.75 cc.) was required.

Interpretation:

The experiments clearly indicate that nervous structures above the known medullary vasomotor centres are not required for the 'reversal' of the vasomotor effect of buffer nerve stimulation brought about by nembutal overdosage. Further, the similarity of the 'reversed' responses obtained in the absence of such structures, with those found in their presence suggests that the nervous mechanisms above the medulla play no part whatsoever in their production.

The experiments have also shown that the site of action of both nembutal and picrotoxin must lie at or below the level of the medullary vasomotor centres.

C H A P T E R VI.

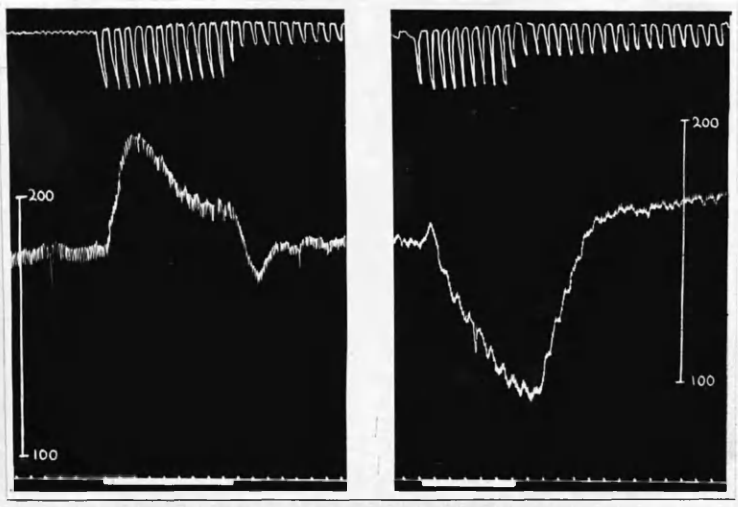
PRESSOR RESPONSES FROM THE SINUS NERVE IN THE NORMALLY ANAESTHETIZED ANIMAL.

It has been pointed out earlier (p. 48) that the normal expected response to electrical stimulation of the sinus nerve is vasodilation and a fall in blood pressure, and that a rise in blood pressure has been observed only rarely, and then under abnormal conditions where the depth of anaesthesia has been insufficient or the strength of stimulus used excessive. These 'reflexes douloureux' have been observed frequently in this series of experiments, and familiarity with such responses and the conditions under which they manifest themselves has been acquired. It transpired, however, that pressor responses of a quite different nature were readily demonstrable.

From the very first experiment it was apparent that, in normal nembutal anaesthesia, a rise of blood pressure was much more readily obtained than a fall when either of the sinus nerves was stimulated with the Whitfield device. Indeed, so regularly did the pressor responses appear (in every animal) that doubt was cast on the identity of the structure stimulated. Consequently, steps were taken to verify its identity (these are detailed later - Chap XI), and it was proved to be the sinus nerve as described by HERING.

It will have been observed that where experiments involving electrical stimulation of the sinus nerve have been carried out, mention/

THE EFFECT OF STIMULUS STRENGTH ON THE SINUS NERVE REFLEXES IN THE NORMALLY NEMBUTALIZED CAT.



A B

Fig. 18. Cat. Anaesthetic nembutal 40 mg/Kg. Double vagotomy. Artificial respiration. Bilateral open pneumothorax. Arterial blood pressure (left carotid artery). Time 5 sec.

A. Stimulation of right sinus nerve 8 V. 30/sec. (Whitfield).

B. " " " sinus nerve 40 V. 30/sec. (Whitfield).

mention has been made of the fact that stimuli were 'selected' which gave depressor responses. Now it has just been stated that electrical stimulation of the sinus nerve caused a rise in blood pressure in every animal. This also is true, and the rises of blood pressure were often very considerable. The two statements become reconcilable when the character of the stimulus employed is examined, for after one or two experiments it became apparent that this was of the greatest importance. It was found that weak stimulation was responsible for the pressor responses, while depressor responses of the nature expected from the work of others were only obtained with much stronger stimuli, often of the maximum strength of which the Whitfield stimulator was capable (40 volts). It was for this reason that such stimuli were adopted to obtain normal responses before the effect of excess nembutal was assessed.

What, in fact, had been found, was that during light nembutal anaesthesia vasomotor responses of quite opposite sign could be regularly elicited from the sinus nerve by appropriate electrical stimulation.

Now the fact that such a phenomen had not previously been described, coupled with the finding that nembutal in excess converted the expected depressor response to a rise, hinted at this pressor effect observed in light nembutal anaesthesia being associated in some way with that particular anaesthetic. Nevertheless, it was considered possible that the two effects, the rise and the fall, might be independent of the anaesthetic and be functions of the stimulus strength.

CHAPTER VII.

THE EFFECTS OF SINUS NERVE STIMULATION IN THE NON-ANAESTHETIZED (DECEREBRATE) PREPARATION.

Whether or not the pressor effects brought about by sinus nerve stimulation were independent of the anaesthetic might be shown by experiment on the non-anaesthetized animal. To this end several such preparations were studied.

Methods:

Three cats were employed. They were anesthetized with volatile anaesthetic (1 part chloroform to 3 parts ether). A tracheal cannula was inserted and light anaesthesia was continued by a chloroform-ether swab lightly applied to its opening. The vago-sympathetic trunk was exposed and cut, and the carotid arteries clipped. These steps were done as rapidly as possible and were completed in a few minutes. The animal was now turned back up and anaesthesia was deepened while exposure of the skull was taking place. This and the actual decerebration were carried out as described for the deeply nembutalized animal, except that the process was completed much more quickly. Anaesthetic was withdrawn immediately after section of the brain stem, and the brain matter above the section (at about the superior corpora) scooped out. During this, and for about a minute thereafter the vertebral arteries were manually occluded. The animal was now laid on its side, head raised, and the wound covered with swabs moistened with warm Ringer-Locke. Bleeding

was greater than with nembutal, but plugging with cotton wool was avoided. The animal was left undisturbed for one hour permitting the anaesthetic to be washed out. The head was then lowered by stages, a minute or so between each, until the animal was again lying on its back. The carotid arteries, which had been clipped throughout, were now tied off. The right sinus nerve was dissected out and made ready for stimulation. The left carotid artery was cannulated to record the blood pressure. Ventilation was recorded by a modified version of GADDUM's apparatus (described in Chap. XVIII).

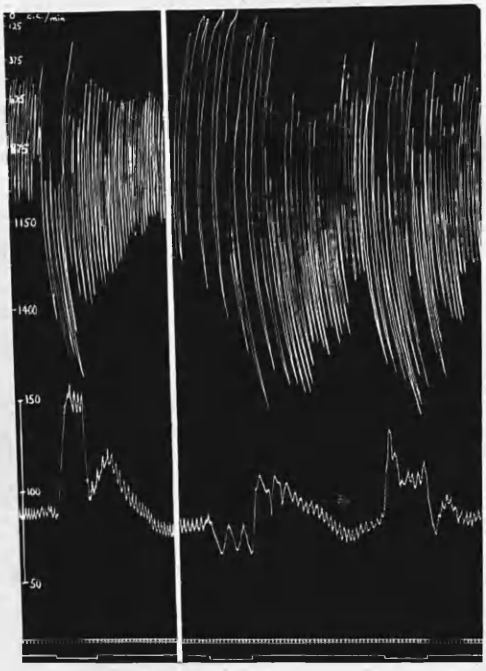
When these stages had been completed, the silver-silver chloride electrodes were fixed in position and the effects of sinus nerve stimulation observed.

Results:

In two animals, stimulation of the right sinus nerve with many stimuli between 2V. 30/sec. and 20V. 30/sec. (Whitfield stimulator) caused marked rises in blood pressure. No depressor responses were obtained. At the lower voltages these pressor responses were unaccompanied by any signs of agitation or spread. At higher voltages there was marked agitation, twitching, convulsive movements and gasping respiration, so much so that the stronger stimuli found effective in producing depressor responses in the nembutalized cat could not be employed.

In the third animal, however, although powerful pressor responses were readily obtained with weak stimulation (4V. 30/sec.), it was found that a stimulus of 40V. 30/sec. could be employed/

THE EFFECT OF STIMULUS STRENGTH ON THE SINUS NERVE REFLEXES IN THE NON-ANAESTHETIZED (DECEREBRATE) CAT.



A B C

Fig. 19. Cat. Decerebrate. Double vagotomy.
Spontaneous respiration (recorded modified Gaddum apparatus). Arterial blood pressure (left carotid artery). Right carotid artery tied.

A. Stimulation of right sinus nerve 4 V. 30/sec.

B. " " " " " 40 V. 30/sec.

C. " " " " " 4 V. 30/sec.

employed without such disturbances and that this stimulus provoked only a fall (Fig. 19). The pressor and depressor responses could be repeated. Furthermore, although the animal was not being artificially ventilated, these effects could not be ascribed to medullary or spinal vasomotor activity consequent on the altered quality of the blood resulting from concomitant respiratory changes. Rather was the opposite the case, for the rise in blood pressure was accompanied by increased ventilation and the fall by decreased ventilation.

It is of interest that this animal afforded the only example of inhibition of respiration brought about by electrical stimulation of the sinus nerve in the large series of animals studied.

Interpretation:

These experiments sufficed to indicate that the pressor responses obtained with the less powerful stimulations of the sinus nerve were not dependent on the presence of nembutal, and could readily be obtained in the absence of anaesthetic.

CHAPTER VIII.

ANALYSIS OF SINUS NERVE RESPONSES.

It was apparent that the demonstration of a regular pressor response to electrical stimulation of the sinus nerve, in addition to the previously described depressor activity, was of the greatest significance in the interpretation of the reversed responses observed after nembutal overdosage. For this reason it was essential that the responses, especially the pressor, be thoroughly analysed before the problem of reversal of the normal response be further pursued.

Besides this consideration peculiar to the main theme of investigation, it was hoped that such an analysis would contribute materially to the knowledge of the physiology of this highly specialised sensory nerve.

1. EXPERIMENTS CONDUCTED UNDER LIGHT NEMBUTAL ANAESTHESIA.

Investigations carried out in over a score of cats, prior to this immediate study, had shown that weak stimulation of the sinus nerves caused a rise in blood pressure, while powerful stimulation was required to elicit the 'normal' depressor effect. These experiments had been primarily concerned with events appertaining to the normal depressor response, and apart from observing the pressor responses and avoiding them by increasing the strength of stimulation, no detailed analysis correlating stimulus with effect was undertaken. In the experiments that follow/

follow, a close study was made of the effects of stimuli of different strengths, during which three stimulators of different characteristics were employed.

(A). Analysis with the WHITFIELD Stimulator.

Methods:

(a) Preparation of the Animal.

Cats were used. They were anaesthetized with nembutal (40 mg/Kg. intraperitoneally), and this was supplemented, where necessary, by further doses (a few mg/Kg.) until anaesthesia was sufficiently deep to permit of operation. The trachea was cannulated, both vagi were cut low in the neck, both sinus nerves were carefully dissected out and one or the other was cut. Artificial ventilation was begun and the chest was opened on each side. The left carotid artery was cannulated, citrate being employed as anticoagulant. Records of the blood pressure were taken by mercury manometer, and of respiration by a stethograph attached round the lower ribs and held in position with a stout ligature. Tracings as usual were made on a smoked paper.

The dissection was extensive, but could be performed in most instances in under an hour, indeed in some animals recording was begun in less than one hour from the injection of the anaesthetic. It was found that this rapid dissection usually resulted in a preparation still sufficiently anaesthetized to permit of stimulation at the commencement of recording. In some animals, however, additional nembutal was given, again a few/

THE WHITFIELD STIMULATOR.

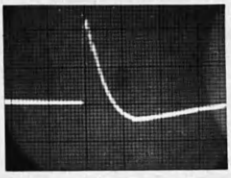


Fig. 20. A single condenser discharge from the Whitfield stimulator. Photograph of a horizontal sweep. (Cathode ray oscilloscope).

1 large square = 0.4 millisec.

few mg/Kg. at a time, until a satisfactory plane was reached. Pulmonary ventilation was adjusted to that which just permitted spontaneous respiratory movements at the beginning of the experiment, thereafter the Starling pump was fixed and the amount kept constant throughout the experiment.

(b) The Stimulator.

The instrument designed by WHITFIELD (1946) was employed. This provided brief condenser discharges (Fig. 20) up to 40V., and at various frequencies. Both the amplitude and frequency scales had been specially calibrated for the present series of investigations, but it was felt that it would be unwise to rely on dial readings alone, since it was possible that in this essentially 'class purposes' instrument, characteristics might alter to a degree sufficient to upset quantitative estimations. In consequence, the electrical technique was so altered that every stimulus was checked for frequency and amplitude before and during its application to the nerve. This was done by monitoring the electrode circuit with a cathode ray oscilloscope.

Further, it had been found that by employing the 'make and break' switch on the stimulator, the initial discharge from the condenser was often very much greater than that prevailing during the major part of any stimulus. This characteristic was eliminated by having the stimulator discharge continuously at the desired strength and frequency, and having an external switch allowing stimuli of suitable duration to be 'tapped off' to excite the nerve.

(c)/

(c) Conduct of the Experiment.

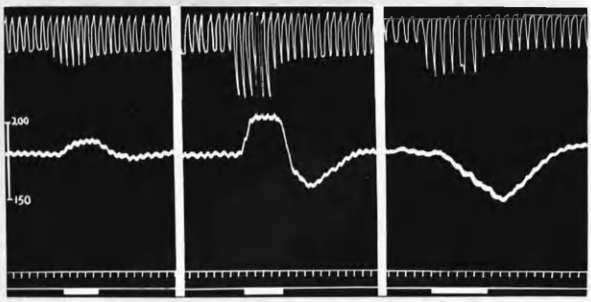
Five cats were studied. When dissection had been completed, the sinus nerve electrodes were chlorided, placed under the intact sinus nerve (right or left), and firmly clamped in position. The nerve was not moved on the electrodes during any series of tests, but when there was considerable delay between successive runs, the electrodes were removed and the nerve bathed with warm Ringer-Locke. During tests, care was taken to remove moisture which might 'short' the electrodes. This was done by mopping out the channel with tiny wisps of cotton.

The effects of stimuli of various strengths were studied. In each animal stimulation was begun with feeble discharges (about 1 V.) and increased until a B. P. response was elicited. Thereafter the effect of increasing the voltage by small steps was tried until the maximum output was reached (40 V.).

Results:

The right sinus nerve was stimulated in three cats and the left in two. It was found that in each animal, and in both nerves the results were qualitatively similar. In each, the first response observed was a rise in blood pressure. This increased in magnitude as the stimulus strength was increased until a stage was reached when it began to diminish. Decrement of the pressor component was accompanied in every case by increment of the depressor, the increased depressor activity manifesting itself either during the stimulus (Fig. 22 B), and giving rise to a biphasic response, or following upon the stimulus/

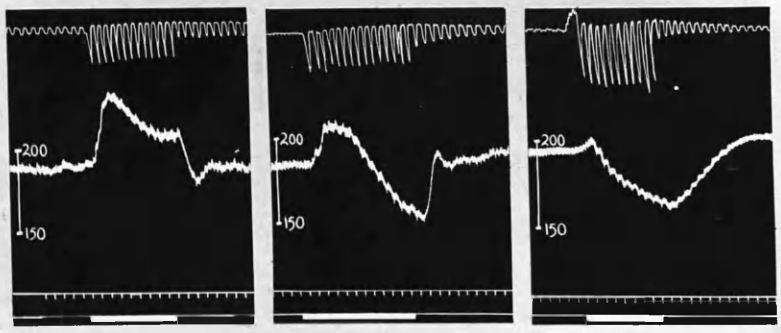
ANALYSIS OF SINUS NERVE RESPONSES
WITH THE WHITFIELD STIMULATOR.
THE EFFECT OF INCREASED VOLTAGE.



A B C

Fig. 21. Cat. Anaesthetic nembutal (40 mg/Kg.). Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec. Respiratory movements stethograph.

A. Stimulation of right sinus nerve 2 V. 30/sec.
B. " " " " " 20 V. 30/sec.
C. " " " " " 40 V. 30/sec.



A B C

Fig. 22. Cat. Anaesthetic nembutal (45 mg/Kg.). Double vagotomy. Right sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec. Respiratory movements stethograph.

A. Stimulation of left sinus nerve 5 V. 30/sec.
B. " " " " " 20 V. 30/sec.
C. " " " " " 40 V. 30/sec.

stimulus, and resulting in after fall (Fig. 21 B). As the voltage was increased there was a further diminution of the initial rise, and ultimately an almost pure depressor response was obtained.

To exclude the possibility that these different responses were caused by some change in the preparation, such as alteration in the degree of anaesthesia, or some local change in the nerve, responses elicited in one series were immediately repeated in the reverse order and at random. This technique was adopted throughout the present series of experiments, and in all those entered upon later in which the effects of different stimuli were to be compared.

The illustrations chosen (Figs. 21 and 22) show the ultimate development of well marked depressor responses in two of the animals. In another, however, only a feeble fall (10 mm. Hg.) was obtained with the most powerful stimuli (40 V. 30/sec.), although this animal had again shown the change from rise to fall on increasing the voltage.

These experiments, carried out under carefully controlled conditions, served to confirm the results observed in earlier investigations. There was no question but that increasing the stimulus strength caused a transition from the pressor to the depressor response. There was, however, no evidence to indicate that the process was complete when a strength of 40 V. was employed, rather was there much against it. In several earlier experiments, the 'normal' depressor response could not be achieved/

achieved despite the use of such a stimulus, and although such was not the case in the present series, one of the three animals showed but a feeble response, while even in the other two, the slow decline in blood pressure (C Fig. 21) and the persistence of a small initial rise (C Fig. 22) suggested that the transition was incomplete. It was decided to continue the investigation with a device capable of stimulation of a higher intensity.

(B). Analysis with the Thyratron Stimulator.

In this next series, the study was continued using a more powerful stimulator.

Methods:

(a) Preparation of the Animal.

This was as described for the series just discussed, ('A' above).

(b) The Stimulator.

The only stimulator available at the time which would be capable of providing a more powerful stimulus than that of the WHITFIELD circuit, was a Thyratron valve device. This allowed of stimulation up to 100 V., with the usual condenser discharge of exponential wave form similar to those shown in Fig. 23. The output characteristics were dependent partly on the use of a battery of condensers of different values, and this arrangement proved to be of distinct advantage, for unlike the WHITFIELD circuit, it provided a means other than alteration of amplitude (peak to base voltage), whereby the total current delivered/

THYRATRON STIMULATOR.

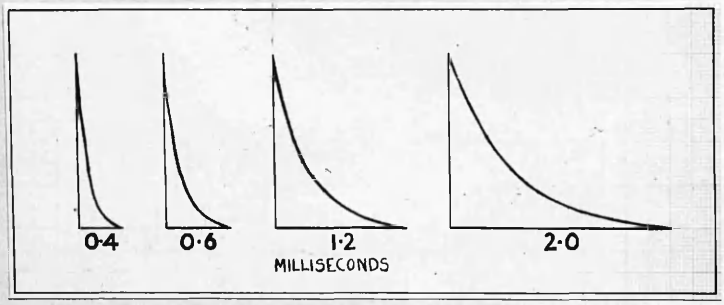


Fig. 23. Pulses of different duration.

Grid Bias (Volts)	Potentiometer	Condenser	Frequency cyc/sec.	Duration m secs.
- 1½	4	1	100	3.4
- 3	5	1	10	3.2
- 3	8	2	30	2
- 3	8	4	100	0.6
- 4.5	7	2	10	2.2
- 4.5	9	3	30	1.2
- 4.5	8.5	5	100	0.4
- 6.0	6.5	3	10	1.2
- 6.0	7	4	30	0.6
- 7.5	5	4	10	0.6
- 7.5	9	5	30	0.4

Fig. 24. Table showing the various alterations made to the Stimulator circuit in order to obtain pulses of different duration, but of the same voltage and frequency. For each of three frequencies (10, 30 and 100/sec.) several pulses were available. Those obtained at a frequency of 30/sec. are illustrated in Fig. 23.

delivered in any one pulse could be changed. In fact, pulses of the same voltage occurring at the same frequency could be made to differ widely in their excitant properties by varying their duration. For example, at a frequency of 30/sec. and at any voltage between certain wide limits, pulses of 0.4, 0.6, 1.2 and 2 msec. duration could readily be obtained (Fig. 23). The change from one value to another was accomplished by making three simultaneous alterations to the stimulator circuit, involving condenser capacitance, charging resistance and grid bias of the thyratron valve. A table was constructed (Fig. 24) from which the appropriate alterations in the circuit could rapidly be determined and brought into effect. Fine adjustment was then carried out with the impulse visualised on the oscilloscope, and the pulse duration modified as necessary.

Amplitude and frequency were also checked before and during each stimulus with the aid of the oscilloscope and oscillator.

By 'modelling' each and every stimulus in this way, no call was made on dial readings.

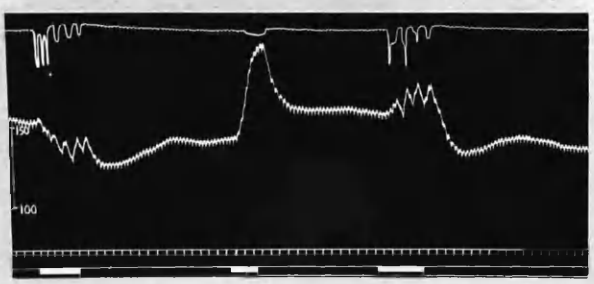
(c) Conduct of the Experiment.

Experiments were carried out with this Thyratron stimulator on four cats. The technique was precisely similar to that adopted in the analysis carried out with the WHITFIELD stimulator, except that the effects of various pulse durations as well as voltage was investigated, and that the upper limit of stimulus strength was not determined by the output of the stimulator, but only by the necessity for avoiding damage to the/

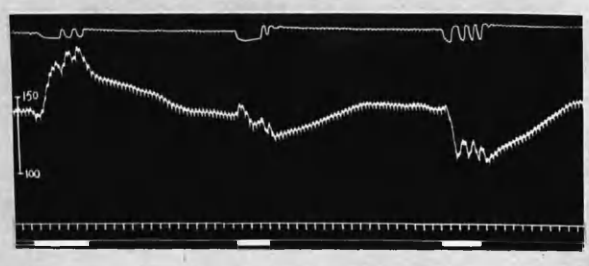
ANALYSIS OF SINUS NERVE RESPONSES

WITH THE THYRATRON STIMULATOR.

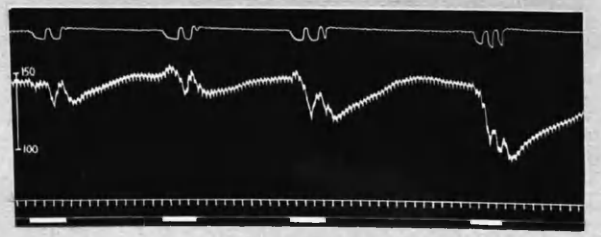
THE EFFECT OF INCREASED VOLTAGE AND PULSE DURATION.



Pulse 0.6 m.sec. 30 V. 6 V. 15 V.



Pulse 1.2 m.sec. 6 V. 15 V. 30 V.



Pulse 3.4 m.sec. 3 V. 6 V. 15 V. 30 V.

Fig. 25. Cat. Anaesthetic nembutal. 45 mg/Kg. Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec. Respiratory movements stethograph. Stimulation of right sinus nerve throughout with constant frequency (30/sec.). Voltages and pulse durations as shown.

the nerve.

Frequently during the course of each of the four experiments, the WHITFIELD device was substituted for the Thyratron stimulator. In this way a measure of continuity with the previous work was achieved and the effects of the two stimulators could be compared.

Results:

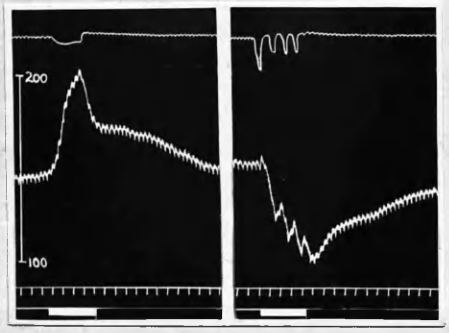
The right sinus nerve was stimulated in three animals and the left in one. In each experiment the effects of increasing the voltage were essentially the same as in the animals studied with the WHITFIELD stimulator, the pressor response appearing at low values, and going through similar stages as the voltage was increased, waxing, waning and finally yielding to a depressor effect.

As was to be expected, increasing the pulse duration increased the effective strength of any stimulus and augmented the depressor component of the response.

A series of tracings (Fig. 25) from one of the experiments servesto illustrate these points. It shows how the response is affected by increase of voltage or pulse duration. It will be seen that with pulses of 0.6 msec., stimulation with 6 V. gave a rise of over 50 mm., 15 V. gave a rise only during stimulation but an after fall, while 30 V. gave a pure fall only, which was, however, slow in developing and comparatively feeble. When the same three voltages were again employed with doubled pulse duration, 6 V. gave a rise of less than 50 mm. which was slower in/

ANALYSIS OF SINUS NERVE RESPONSES
WITH THE THYRATRON STIMULATOR.

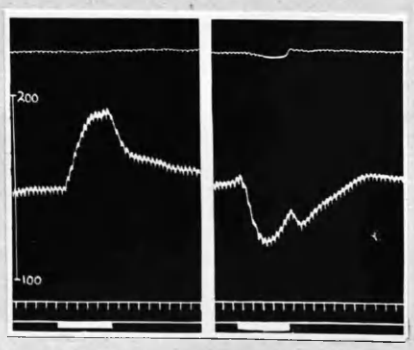
REVERSAL OF THE RESPONSE BROUGHT ABOUT
BY ALTERATION OF VOLTAGE.



A B

Fig. 26. Legend as Fig. 25.
Stimulation of right sinus nerve
A. 3 V.
B. 45 V.
Pulse duration and frequency constant
(1.2 m sec. 30/sec.).

REVERSAL OF THE RESPONSE BROUGHT ABOUT
BY ALTERATION OF PULSE DURATION.



A B

Fig. 27. Legend as Fig. 25.
Stimulation of right sinus nerve.
A. Pulse 0.6 m sec.
B. Pulse 3.4 m sec.
Voltage and frequency constant (3 V. 100/sec.).

in developing, while 15 V. now gave a fall with only a small initial rise, and 30 V. gave a depressor response which developed more rapidly and reached a lower level. When the pulse duration was further lengthened to 3.4 msec. all these voltages caused depressor responses, while even 3 V. sufficed to lower the blood pressure some 15 mm.

The markedly differing excitant properties of the various pulses meant that a reversal of the blood pressure response could be brought about by altering the pulse duration of the stimulus alone. Thus by appropriate selection two stimuli could be employed each of the same voltage, and occurring with the same frequency, the one of short pulse duration producing a rise in blood pressure and the other, of longer duration, a fall. Figs. 26 and 27 summarize the findings. They show the reversal from pressor to depressor, firstly by increasing the voltage alone, and secondly by increasing the pulse duration alone.

When the two stimulators were compared, it was found that if the voltage and frequency of each were the same, the briefest pulse from the Thyratron (0.4 msec.) had effects comparable to the WHITFIELD stimulus.

If the transition from the pressor to the depressor response were taken as far as the WHITFIELD stimulator would permit with its maximal stimulus (40 V.), further increase in the depressor component could always be brought about by employing more powerful stimuli from the Thyratron device. This could be produced/

produced either by employing a pulse of approximately the same duration (0.4 msec.) and taking advantage of the higher voltage range to increase the amplitude, or by employing longer pulses and using appropriate voltages. For reasons which will be given in the discussion later in this chapter, the latter was the method usually employed, and by employing a pulse of several times the duration of the WHITFIELD stimulus, depressor responses could be obtained with stimuli of relatively feeble amplitude.

These experiments confirmed the belief that the maximal stimulation from the WHITFIELD device was insufficiently strong regularly to elicit the most powerful depressor response which the nerve was capable of producing, and was, therefore, unsuited to the study of sinus nerve reflexes. It was found, indeed, that a maximal depressor response was not achieved until stimuli considerably in excess of the WHITFIELD 40 V. 30/sec. were employed

(C). Analysis with the Square Wave Stimulator.

Although the methods adopted during the experiments with the Thyratron stimulator were both time and labour-consuming, each stimulus being 'tailor made' before being applied, and as many as 240 stimuli being required in one experiment, they provided, in conjunction with earlier work, sufficient evidence to permit of certain conclusions which are discussed later in this chapter.

At this time, however, a stimulator was acquired which did away/

away with the necessity for such tedious manoeuvring. Partly to provide a link between experiments yet to be performed with this device and those already carried out with other stimulators, and partly to confirm the latter, experiments were carried out in which this third stimulator was used in conditions similar to those prevailing in the WHITFIELD and Thyratron series.

Methods:

(a) Preparation of the Animal.

This was as in the two earlier series (A and B).

(b) The Stimulator.

The B.N.I. stimulator designed by WALTERS and RITCHIE was used. It provided a means whereby pulses could be obtained of square (more properly rectangular) form, of duration from 0.02 msec. to 10 msec., of voltage 0 - 100, and of various frequencies. When employed it was substituted in the electrical lay-out already described, in place of the Thyratron device, the oscilloscope being retained so that in every experiment each stimulus was monitored as before.

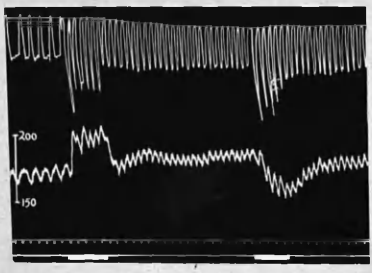
(c) Conduct of the Experiment.

Experiments were carried out on two cats. The effects of weak and strong stimulation of the sinus nerve were studied, and special attention was paid to the effects of pulse duration. The responses to square wave stimulation were compared with those to the Thyratron and WHITFIELD stimulators by substituting one device for the other at intervals throughout the experiments.

Results/

ANALYSIS OF SINUS NERVE RESPONSES
WITH THE SQUARE WAVE STIMULATOR.

REVERSAL OF THE RESPONSE BROUGHT ABOUT BY
ALTERATION OF THE PULSE DURATION.



A B

Fig. 28. Cat. Anaesthetic nembutal 45 mg/Kg.
Double vagotomy. Left sinus nerve cut.
Artificial respiration. Bilateral open
pneumothorax. Arterial B.P. (left carotid
artery). Respiratory movements stethograph.
Time 5 sec.
Stimulation of right sinus nerve 3 V. 70/sec.

A. Pulse duration 0.02 m sec.

B. Pulse duration 10 m sec.

Results:

The right sinus nerve was stimulated in one animal and the left in the other.

The findings regarding the nature of the sinus nerve response were in close agreement with those already obtained. Thus pressor responses were elicited with low intensity stimulation and depressor effects with strong stimulation, the usual transition stages being encountered.

Further emphasis was placed on the need for adequately long pulses in eliciting the depressor response, for, by employing a pulse of long duration (10 msec.) a fall in blood pressure could readily be obtained with stimuli of low voltage (= 3 V.), while much higher voltages 30 - 60 had to be used to obtain a depressor response when pulses of the shortest duration (0.02 msec.) were employed. Indeed, the difficulty in obtaining the expected response with such brief shocks was in distinct contrast to the ease with which it could be elicited with pulses of longer duration.

The reversal of blood pressure response was especially readily obtained using this stimulator with its wide range of pulse durations (Fig, 28).

Besides confirming previous results, these experiments yielded further information on the intensities of stimulus at which the pressor response appeared, decreased, and was replaced by a fall, and some degree of familiarity was acquired regarding the voltages needed to elicit these responses with various/

various stimuli (pulses). Indeed much of the value of these experiments can be said to lie in the information of this nature obtained, and with the comparison with the other stimulators afforded, for in this way the response of the sinus nerve elicited by square wave stimulation could be assessed against the background of work carried out by the other stimulators, and the future studies more closely linked with those already undertaken.

Interpretation of the Sinus Nerve Responses.

The Effects Attributed to Differential Response of the Nerve.

It is felt that the results obtained and related so far might well be discussed at this point, for although the experimental analysis of sinus nerve vasomotor reflexes has been told only in part, the purpose of the experiments yet to be described will be better understood if the principles underlying them are outlined at this stage.

It has been shown that in cats normally anaesthetized with nembutal (i.e. not profoundly), two distinct responses are usually elicited by electrical stimulation of either sinus nerve, a rise in blood pressure, and a fall, the pressor effect being achieved with relatively feeble stimulation and the depressor effect with powerful stimulation. The pressor response has been shown in the non-anaesthetized decerebrate preparation and/

and cannot therefore be due solely to any effect of the anaesthetic.

The few pressor effects afforded by the literature have been obtained in abnormal conditions where anaesthesia has been insufficiently deep and where excessively powerful stimuli have been used. The pressor responses obtained in this investigation have occurred in animals adequately anaesthetized and showing no signs of pain. Furthermore, they tended to diminish and become depressor as the strength of stimulus was increased, which is certainly not the case with the 'reflexes douloureux'. Nor could the effect be attributed to spread to surrounding tissues, for not only did close inspection fail to reveal any such happening, but stimuli of many times the strength could be employed still without evidence of spread, and indeed, brought about diminution of the pressor response. Moreover, feeble stimuli which elicited marked rises of blood pressure when applied to the sinus nerve, failed to give any such response when applied to any of the immediately surrounding tissues. The regularity with which the response was obtained in a large number of animals bore further witness against the likelihood of such a chance happening. Some other explanation had to be forthcoming, and obviously had to account for the blood pressure effects of opposite sign on the basis of differential response by the sinus nerve to varying strengths of stimulus.

The Differential Response Ascribed to Pressor and Depressor Fibre Groups.

It has long been known that mammalian nerves are composed of fibres of different irritabilities (LAPIQUE & LEGENDRE, 1913), and since GASSER & ERLANGER (1922) adopted the cathode ray oscilloscope for the study of nerve function, this and other properties of the fibres have been carefully studied and related to their morphology. The nerve fibres have been found to be divided into three groups, each characterized by its action potential and called A, B, and C (ERLANGER and GASSER 1937), the A group being further divided into α, β, γ and δ subgroups. Although there is a certain amount of overlap, these groups demonstrate a gradation of certain properties. Thus, through the A and B to the C groups, fibre diameter, conduction rate, action potential and excitability all diminish, from the large fast conducting A fibres of large action potential and low threshold, to the small slow conducting C fibres of small action potential and high threshold. Both the conduction velocity and the action potential have been shown to be in linear relation to the diameter of the fibre (GASSER and GRUNDFEST 1939). The relation of excitability to fibre diameter is more complex, but again, the smaller the fibre the less the excitability (BLAIR and ERLANGER 1933). The significance of this may be judged from the statement made by ERLANGER and GASSER (1930) to the effect that "the differences in irritability of the three types of fibres are such that by increasing the strength of stimulus it is possible to elicit in succession, with intervening gaps, the A elevation of the action/

action potential, the B elevation and the C elevation". In the same paper these authors point out that after all A fibres have been activated the strength of stimulus (voltage) must be increased seven times to obtain the first appearance of the C elevation, and increased one hundred times before all C fibres are stimulated. The same effect may be shown among the subgroups of the A component. Thus, LEKSELL (1945) states, 'at a stimulus strength necessary for including the whole α complex there is never more than a small fraction of the γ fibres engaged - a successive increase of stimulus strength will therefore engage all the fibres of the α group before a significant part of the γ fibres has become molilised'.

If the responses obtained from sinus nerve stimulation in the present investigation be interpreted in the light of this fundamental work, they might well be ascribed to two groups of fibres of different thresholds having directly opposite effects on the level of the blood pressure. There is ample precedent for such an interpretation in the literature appertaining to vasomotor afferents. Strong electrical stimulation of the cut end of a sensory nerve has long been known to cause a reflex rise in blood pressure, and after LATSCHENBERGER and DEANHA (1876) and others had found that weak stimulation had the opposite effect, HUNT (1895) interpreted the effects on a fibre basis. He maintained that the pressor and depressor reflexes were served by two different types of afferent fibres, the pressor having a larger threshold, and this, the classical

view, has been commonly accepted. It has, however, had its critics, among whom have been RANSON and BILLINGSLEY (1916) and RANSON (1921), who favoured the hypothesis that there was but a single type of afferent fibre, each fibre making such connections in the central nervous system that the impulses which it carried, could, under appropriate conditions each call forth one or other vasomotor response. This view will be considered later in greater detail as it affects the results of this present investigation (Chap. XVI). It will suffice here to point out that RANSON's hypothesis was partly attributable to the fact that he found it difficult to believe that the excitability of individual neurones varied to the enormous degree necessary for the explanation of the vasomotor effects on a different fibre basis. This objection has been met with. The thresholds of the least irritable fibres have been shown to be two hundred or more times as great as for the largest and most easily excitable. More recent work does not favour RANSON's interpretation. For instance, by a technique involving simultaneous electrical stimulation of an afferent nerve, recording of the action potentials produced, and determination of the resulting changes in blood pressure, O'LEARY, HEINBECKER and BISHOP (1934) showed that weak stimulation excited large fibres and caused a fall in blood pressure, while stronger stimulation excited in addition small fibres and resulted in a fall in blood pressure. While this certainly demonstrates that the different vasomotor responses appear synchronously with the activation/

activation of different fibres, it might of course be argued that the small fibres were in no way responsible for the change in vasomotor activity, which might be attributed to the effects of the stronger stimulus on the large fibres. Such, however, has been disproved by CLARK, HUGHES and GASSER (1935), and by GORDON (1943), who have shown, by selectively knocking out one or other set of fibres, that the pressor response in the mammalian sensory nerve is mediated by the small fibres, and the depressor by the large. Their results confirm the time honoured view of HUNT, showing that in the 'unspecific afferent' stimulation, the reversal from depressor to pressor is brought about by the successive mobilization of afferent groups of low and high excitability threshold. It would be reasonable to assume that where a nerve is composed of large pressor fibres and small depressor fibres, stimulation with increasing strengths of current would elicit first a rise and then a fall; in other words a reversal of a different sort, but one more in keeping with the results of the present investigation. Such a reversal has been shown sometimes to occur in the cat vagus by WRIGHT (1928), and also in the rabbit depressor by O'LEARY, HEINBECKER and BISHOP (1934) who have found, again by recording the action potentials as the nerve is stimulated, that the pressor response is due to the activation of large pressor fibres which are occasionally present, but that the effect of these is rapidly overcome as the stimulus strength is increased to include the smaller, specific, depressor fibres.

Against/

Against such a background, the dual response from the sinus nerve was considered to be due to stimulation of two groups of fibres, the first of low threshold causing a rise in blood pressure, and the second of considerably higher threshold causing a fall in blood pressure. That is to say, pressor and depressor fibres, the former being the larger.

The Findings Related to Sinus Nerve Function.

It was apparent that one possible source of pressor activity to be reckoned with in the sinus nerve was the chemosensory fibre content. The existence and function of these fibres were well known at the time when HEYMANS (1933) wrote off all sinus nerve pressor responses to electrical stimulation as unphysiological, and not indicative of the true function of the nerve. Nevertheless, it seemed a priori that the explanation of the clear cut pressor responses obtained in this series demanded a functional basis, and when it was borne in mind that the rise in blood pressure was accompanied by a very marked increase in respiratory activity, it seemed reasonable to adopt as a working hypothesis the explanation that the pressor responses obtained were due to excitation of chemosensory fibres. On further relating the effects of electrical stimulation to the known function of the nerve, it followed that the depressor effect must be attributed to excitation of the specific depressor neurone content, the barosensory fibres.

Now at once there appeared an obvious discrepancy between this tentative conclusion, and the current concepts of the fibre/

fibre content of the sinus nerve, for although no attempt has been made specifically to correlate chemosensory and barosensory activity with the morphology of sinus nerve fibres, it has been assumed from the earliest electrophysiological studies of the nerve, that the barosensory fibres are large while the chemosensory are small. Thus the first study by BRONK (1931) had shown the presence of large action potentials deriving from large fibres activated by pressure sensitive endings, while later work by BRONK and STELLA (1932) demonstrated smaller impulses, which again were to be attributed to small fibres, and which they suggested were due to discharge by chemosensitive endings. Their findings were confirmed by HEYMANS and RIJLANT (1933), BOGUE and STELLA (1934 and 1935) and by ZOTTERMAN (1935). The last named author states that the 'chemospikes' are but 10 - 20% of the amplitude of the largest pressure spikes. Now if such potential differences be translated into terms of axon diameters (whether by the newer formula $P \propto D$ (GASSER and GRUNDFEST 1939) or by the older $P \propto D^2$ (GASSER and ERLANGER 1927), it is apparent that the chemosensory fibres should be considerably smaller than the barosensory, and should be less irritable. This is at marked variance with the hypothesis adopted. The discrepancy was emphasised when an attempt was made to correlate the strength of stimulus required to elicit the depressor response, with the expected strength on the basis of the action potential studies, assuming the large fibres to be responsible for the depressor effect. It was at once

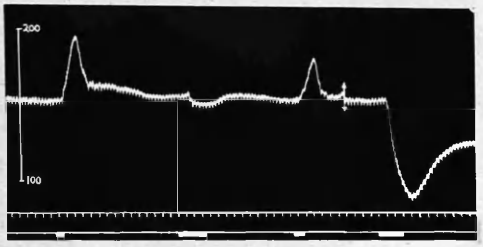
apparent/

apparent that the stimulus strengths (voltage at a given pulse duration) required to mobilize the depressor fibres were considerably in excess of those estimated to activate the large fibres, as calculated from the data of BLAIR and ERLANGER (1933). Without relying on absolute values, the same conclusion may be drawn from the fact that the intensity required to elicit the depressor response was found to be thirty times or more that which just caused the appearance of the pressor response, whereas, belonging to the A group these large fibres would be expected to be completely mobilized at a stimulus strength approximating much more closely to the minimal or threshold stimulus (ERLANGER and GASSER 1930). This finding called for a reconsideration of the position. Firstly, it suggested that the depressor response observed in this series, and taken as being the typical response of the sinus nerve, was being obtained by activating fibres much smaller than those responsible for the large action potentials on the electroneurograms, and secondly, if this were so there was no need as yet to abandon the hypothesis that the pressor response was due to excitation of chemosensory fibres. But what then of the large fibres shown to convey impulses set up by pressure sensitive endings? Before carrying out any further experiments to detect any effect attributable to these fibres, a search was made of the records already obtained. Tracings were selected which showed the effect of stimulation of the sinus nerve with currents insufficiently strong to evoke the pressor response. In nine animals/

ANALYSIS OF SINUS NERVE RESPONSES.

THE FEEBLE DEPRESSOR RESPONSE OBSERVED

WITH WEAK STIMULATION.



A B C D

Fig. 29. Cat. Anaesthetic nembutal 40 mg/Kg. Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec. Stimulation of right sinus nerve at each signal (Whitfield).

A. 14 V. 30/sec.

B. 1.5 V. 30/sec.

C. 4 V. 30/sec.

D. 40 V. 30/sec.

Tracing cut at arrow.

animals, that is in about a quarter of the relevant experiments, it was found that such stimulation had been carried out. In three of these nine experiments, it was found that the first response observed as the stimulus strength was increased from zero, was a faint fall of blood pressure (Fig. 29) but this rapidly yielded to a pressor response as the stimulus strength increased, and became depressor again when more powerful stimulation was employed. It was decided that the analysis be continued, special attention being given to the effect of weak stimulation.

11. EXPERIMENTS CONDUCTED UNDER URETHANE ANAESTHESIA.

In continuing the analysis of sinus nerve responses a certain modification of the technique was adopted. It is well known that conclusions based on experiments carried out under any one anaesthetic may be biased, if not frankly erroneous. Reference to the monographs of BAYLISS (1923) and McDOWALL (1937) yields many such instances in the general vasomotor field, while HEYMANS (1933) has indicated in some detail the variation in results observed in sinus nerve and aortic nerve reflexes under different anaesthetics. This apart, the profoundly altered nature of the buffer reflexes during deep nembutal anaesthesia, was sufficient to hint at the possibility of some 'colouring' of the results obtained with normal dosage, although experiments on the unanaesthetized preparation precluded the pressor effects being entirely due to that drug.

It was decided to continue the study with urethane, for the major point of difference between the results of this investigation and those obtained by others was the demonstration of a powerful pressor response and evidence has been led to the effect that urethane favours depressor reflexes (FLOREY and MARVIN 1928). The persistence of the response under this anaesthetic would therefore be especially significant.

Apart from aiding the study of the sinus nerve responses, it was hoped to obtain information on the nature of the sinus and vagus responses after urethane overdosage. It was of interest to determine whether this anaesthetic would have the same effect as nembutal and convert the depressor responses into pressor. If it did, then apart from any other significance, it might well be argued, as with nembutal, that the responses obtained in light anaesthesia were already biased. It was decided, therefore, to study the effects of urethane overdosage at the outset so that the responses in light anaesthesia could be better evaluated.

(A). Buffer Nerve Responses in Urethane Overdosage.

Methods:

(a) Preparation of the Animal.

Cats were employed. They were anaesthetized by injecting urethane (25% in water) intraperitoneally (1 gm/Kg. body weight). As with those anaesthetized with nembutal, no other anaesthetic was employed during this operation which, although involving large amounts of fluid (4 cc/Kg.), caused no distress to/

to the animal. Induction was rapid. In a few instances too much so, for respirations tended to cease and the animals had to be artificially ventilated for a few minutes. Recovery to a normal anaesthesia of satisfactory depth was rapid, however, and the complication was of little consequence.

Dissection was along the usual lines. The trachea was cannulated, both vagi were cut in the neck, and the cervical sympathetic stripped off each. Both sinus nerves were exposed and one or other was cut. Artificial ventilation was instituted and the chest opened on each side. Records of blood pressure and respiratory movements were taken by mercury manometer and stethograph.

(b) Stimulation.

The sinus nerve was stimulated with the silver-silver chloride electrodes already described, and the vagus with platinum electrodes of conventional design. The B.N.I. stimulator was employed.

(c) Conduct of the Experiment.

Five experiments were carried out as follows: First the effect of stimulation of the buffer nerves was studied under light anaesthesia (usually the induction dose, but in two animals supplemented with 0.25 gm/Kg. to give sufficient depth). Thereafter further amounts of urethane were given intravenously and the nerves again stimulated. This process was continued until the animal died from overdosage.

Results/

Results:

Four animals yielded the following results:

(1) In each, stimulation of the right or left vagus during light anaesthesia elicited the normal expected depressor response with moderate and strong stimulation (no attempt was made to obtain vagal pressor responses).

(2) Stimulation of the sinus nerve (in three animals the right, in the fourth the left) elicited either a pressor or a depressor response during light anaesthesia. The pressor responses were of considerable magnitude (25 - 50 or more mm.Hg.), and were obtained with a wide range of stimulus strengths, increasing in magnitude as the strength of stimulus was increased, and then falling away and becoming replaced by depressor effects when powerful stimulation was employed.

(3) The effects of repeated intravenous injections of urethane showed striking differences from administration of equivalent amounts of nembutal. To procure overdosage, the drug was given in doses equivalent to one quarter the amount required to anaesthetize the animal, and although the blood pressure fell during each slow injection it rapidly recovered, in some instances attaining a level higher than that prevailing before injection. (This was notably the case with injections made after a series of stimulations, during which there had occurred a progressive decline in the blood pressure, as is apt to occur especially after repeated sinus nerve stimulation). When the animal had been considerably overdosed, however, the blood/

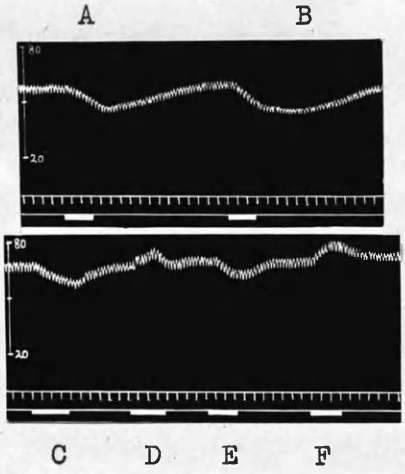


Fig. 30. Cat. Anaesthetic urethane 1 gm/Kg. (intraperitoneally). Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec.
Tracings obtained 1 hr. 50 min. after induction and after overdosage with urethane (total 2 gm/Kg.). Square wave stimulator.

Upper tracing: A. Right vagus 20 V. 70/sec. pulse 1 m/sec.
B. Left " 20 V. 70/sec. pulse 1 m/sec.

Lower tracing: C. Right sinus nerve 20 V. 70/sec. pulse 0.02 m/sec.
D. " sinus nerve 20 V. 70/sec. pulse 1 m/sec.
E. Repeat C.
F. Repeat D.

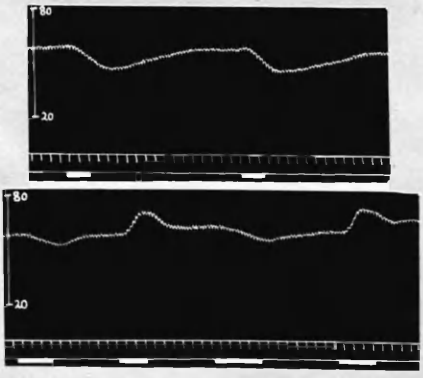


Fig. 31. The same experiment: Stimuli repeated 2 hr. 10 min. after induction and after additional urethane to total 2.75 gm/Kg. (Addition of 0.25 gm/Kg. caused the B.P. to fall away rapidly and the animal died.)

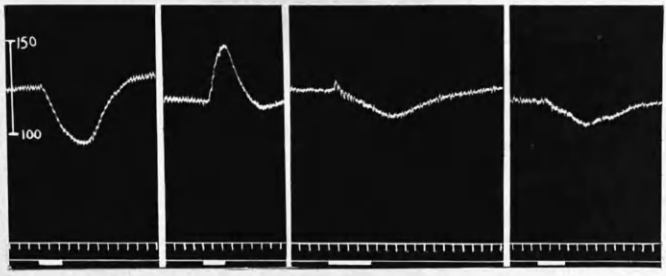
blood pressure always fell away after injection of more urethane. In two instances this decline was progressive, in the others it developed slowly and then rapidly worsened. Transient areflexia, which would certainly have occurred with injection of equivalent doses of nembutal, was not found. Only when the blood pressure had fallen to a very low level, after about three times the anaesthetic dose, was this unresponsiveness observed. Usually, stimulation of the buffer nerves within a minute or two of injection elicited responses showing remarkably little evidence of impairment.

(4) In each of these experiments on overdosage, urethane was given in amount sufficient to cause the death of the animal, and observations were made to the end. The total anaesthetic given to the several animals was 2.5 gm/Kg., 2.75 gm/Kg., 3 gm/Kg. and 3.25 gm/Kg. These amounts were reached within three hours of induction.

(5) Overdosage with urethane did not cause reversal of the responses. In each animal the three responses elicited (the vagus depressor, the sinus depressor and the sinus pressor) all persisted as the depth of anaesthesia increased. Each tended to show a diminution in terms of percentage change in blood pressure, but this was resisted in some animals until the blood pressure had fallen very considerably.

Fig. 30 shows the persistence of the vagus and sinus responses after twice the anaesthetic dose of urethane. (The responses have all become markedly diminished, but are qualitatively/

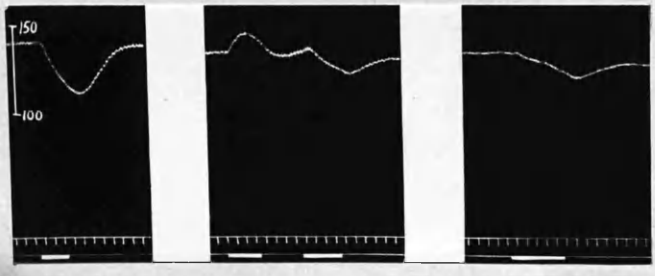
ANALYSIS OF VAGUS AND SINUS NERVE RESPONSES UNDER URETHANE.



A B C D

Fig. 32. Cat. Anaesthetic urethane 1.25 gm/Kg. (intraperitoneally). Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec. Tracings obtained 1 hr. 15 min. after induction. Square wave stimulator.

A. Right vagus nerve 20 V. 70/sec. pulse 1.0 m/sec.
B. Right sinus nerve 20 V. 70/sec. pulse 0.02 m/sec.
C. Right sinus nerve 50 V. 70/sec. pulse 0.02 m/sec.
D. Right sinus nerve 3 V. 70/sec. pulse 10 m/sec.



A B C D

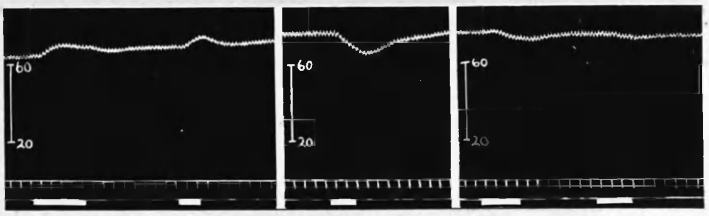
Fig. 33. The same experiment. Stimuli repeated 2 hr. after induction and after additional urethane to total 2.25 gm/Kg.

qualitatively similar to those obtained with identical stimuli earlier in the experiment during lighter anaesthesia). While from Fig. 31 it may be seen that addition of a further $\frac{3}{4}$ anaesthetic dose of urethane (three injections of 0.25 gm/Kg. given in less than 20 min.) has had little effect on the responses, yet this amount of anaesthetic was almost the maximum which the animal would tolerate (further 0.25 gm/Kg. causing death).

A fifth experiment, the third of the present series, produced results of a somewhat different nature in that there did occur a 'reversal' of the response to sinus nerve stimulation, although not to vagal stimulation. In this animal, stimulation of the vagus in light urethane anaesthesia gave the normal depressor response, and stimulation of the sinus nerve gave either a rise or a fall depending on the strength of stimulus (Fig. 32). It can be seen, however, that the sinus nerve depressor effect was somewhat feeble despite the strong stimulus. Buffer nerve stimulation after additional urethane (to a total of 2.25 gm/Kg.) produced very similar changes (Fig. 33). Thus far there is qualitative agreement with the other experiments. It was found in this animal, however, that for a short period following upon a further intravenous injection of urethane, stimulation of the sinus nerve with a stimulus previously 'depressor' produced a definite rise in blood pressure (Fig. 34 A, B). The effect of vagal stimulation at this stage was still depressor, and indeed, when the sinus nerve/

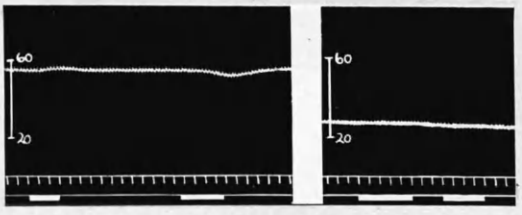
ANALYSIS OF VAGUS AND SINUS NERVE RESPONSES

UNDER URETHANE.



A B C D E

Fig. 34. The same animal as in Figs. 32 and 33. The experiment continued. More urethane injected to total 3 gm/Kg. 2 hr. 30 min. after induction.
A obtained 1 min. and B 2 min. 10 sec. after last dose urethane.
A. Stimulation of right sinus nerve 3 V. 70/sec. pulse 10 m/sec.
B. " " " sinus nerve 50 V. 70/sec. pulse 0.02 m/sec.
C. Two min. later, stimulation of right vagus 20 V. 70/sec. pulse 1 m/sec.
D. and E. Two min after C.
D. Stimulation of right sinus nerve 3 V. 70/sec. pulse 10 m/sec.
E. " " " sinus nerve 50 V. 70/sec. pulse 0.02 m/sec.



A B C D

Fig. 35. The same experiment as above continued. More urethane injected.
A and B. obtained 2 hr. 45 min. after induction, and 4 min after injection urethane to total 3.25 gm/Kg.
A. Stimulation of right sinus nerve 3 V. 70/sec. pulse 10 m/sec.
B. " " " vagus nerve 20 V. 70/sec. pulse 1 m/sec.
C and D obtained 2 hr. 58 min. after induction and after 0.25 gm/Kg. urethane to total 3.5 gm/Kg.
C. Stimulation of right sinus nerve 3 V. 70/sec. pulse 10 m/sec.
D. " " " vagus nerve 20 V. 70/sec. pulse 1 m/sec.

nerve stimulation was repeated four minutes later a feeble fall in blood pressure was obtained (Fig. 34 D, E). Another dose of urethane led to the reappearance of a faint rise in blood pressure while the vagus response was still a fall (Fig. 35 A, B.). Finally no response at all could be elicited (Fig. 35 C, D).

Interpretation:

Out of five experiments designed specifically to bring to light any abnormality of the buffer nerve reflexes in profound urethane anaesthesia, only one animal showed any tendency whatsoever to a 'reversed' response. This single instance was observed in the sinus nerve only, was of little magnitude, was transient and moreover appeared only after administration of three times the anaesthetic dose. It is worthy of note that the sinus nerve in this animal gave but a feeble depressor effect in light urethane anaesthesia even with very powerful stimuli. In every animal, including that just discussed, over 2 gm/Kg. urethane could be given with no qualitative change in any of the buffer nerve reflexes, and in four of these considerably more had as little effect.

It was apparent that urethane, unlike nembutal, interferes little with the vasomotor responses elicited from stimulation of the buffer nerves. This finding justified the adoption of the drug for the further study of such responses.

The fact that successive doses of urethane can be given without favouring either the pressor or depressor responses elicited/

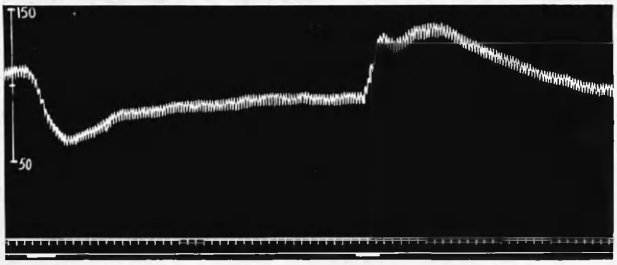
elicited from the sinus nerve, renders it unlikely that the one or the other occurring in light urethane anaesthesia is due to any effect of this drug. This implies that such effects are independent of the anaesthetic, a finding which agrees with the conclusions arrived at from the experiments in the non-anaesthetized decerebrate preparation, and which supports the view that the pressor effects obtained under light nembutal anaesthesia are not dependent on that drug despite any effect it may have in overdosage.

(B). Analysis of Sinus Nerve Responses in Light Urethane Anaesthesia with Special Reference to the Effects of Feeble Stimulation.

The preceding series of experiments, although primarily concerned with the effects of urethane overdosage, had yielded much useful data on the effects of electrical stimulation of the sinus nerve with different intensities during light urethane anaesthesia. The immediate purpose of these experiments, however, demanded that additional doses of urethane be given as quickly as possible after induction so that a high concentration of the drug was reached with the shortest delay. This was necessary lest through deterioration there was a diminution of the absolute quantity of the drug which the preparation could tolerate. In consequence the lengthy procedures associated with estimation of the effects of different stimulus strengths, such as repeated application of one stimulus or the repetition of a series of stimuli in the reverse/

ANALYSIS OF SINUS NERVE RESPONSES UNDER URETHANE.

THE EFFECT OF VOLTAGE.



A B

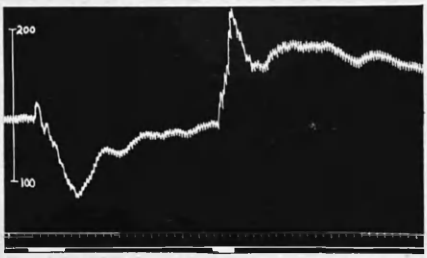
Fig. 36. Cat. Anaesthetic urethane (1.25 gm/Kg.). Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec.

A. Stimulation of right sinus nerve 20 V.

B. " " " " " 2 V.

(pulse duration (1 msec.) and frequency (70/sec.) constant.)

THE EFFECT OF PULSE DURATION.



A B

Fig. 37. Cat. Prepared and anaesthetized as above (Fig. 36).

A. Stimulation of right sinus nerve. Pulse 10 msec.

B. " " " " " Pulse 1 msec.

(Voltage(1 V.) and frequency (70/sec.) constant).

reverse order, could not be carried out. Neither could any detailed study of the effects of feeble stimulation be carried out.

It was the purpose of the present series to perform such investigations.

Methods:

(a) Preparation of the Animal.

Cats were anaesthetized and prepared as in the immediately preceding urethane series. Sufficient depth of anaesthesia was maintained by intravenous injection of small amounts of urethane as necessary.

(b) Stimulation.

The B.N.I. stimulator and the sinus nerve electrodes were employed. The latter were chlorided before use.

(c) Conduct of the Experiment.

Six animals were studied. Experiments were confined to stimulation of the sinus nerve with a wide range of stimulus strengths, from subminimal to supramaximal values.

Results:

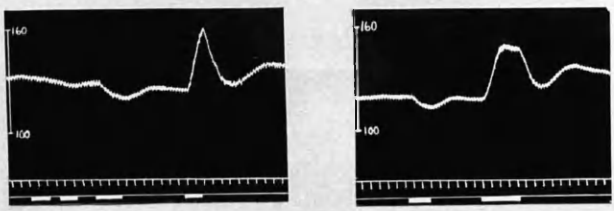
(1) In each animal powerful pressor responses were obtained over a wide range of stimulus strengths.

(2) In every instance these pressor responses diminished and became replaced by depressor effects when more powerful stimulation was employed.

(3) Reversal of the vasomotor effects could be easily affected by selecting stimuli of appropriate intensity. Thus by/

ANALYSIS OF SINUS NERVE RESPONSES UNDER URETHANE.

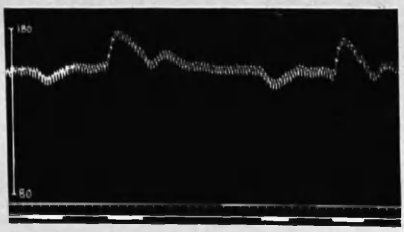
THE INITIAL DEPRESSOR RESPONSE OBTAINED WITH WEAK STIMULATION.



A B C D E F

Fig. 38. Cat. Anaesthetic urethane (1 gm/Kg.). Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec.

A. Stimulation of right sinus nerve 0.5 V.
B. " " " " " 1 V.
C. " " " " " 2 V.
D. " " " " " 5 V.
(Pulse duration (0.02 msec.) and frequency (70/sec.) constant.)
E. Stimulation of right sinus nerve 0.5 V.
F. " " " " " 1 V.
(Pulse duration (1 msec.) and frequency (70/sec.) constant).



A B C D

Fig. 39. Cat (another animal prepared as Fig. 38). Total urethane 1.5 gm/Kg.
A and C. Stimulation of right sinus nerve 3 V.
B and D. " " " " " 10 V.
(Pulse duration (0.02) and frequency (70/sec.) constant).

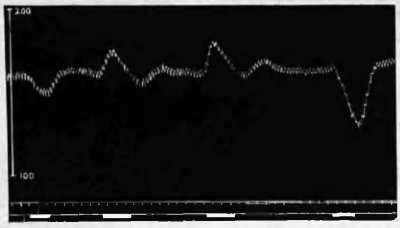
by adopting a suitable pulse duration a rise could be converted to a fall by increasing the voltage alone, while by selecting an appropriate voltage the same effects could be achieved by suitable alteration in the pulse duration. To illustrate that the process is reversible and may be carried out in either direction tracings have been selected in which a fall in blood pressure has been converted to a rise by a diminution of stimulus strength (Figs. 36 and 37).

(4) By carefully testing the effects of feeble stimulation in each animal, it was found, in three out of six, that a depressor response could be elicited with stimuli weaker than those required to bring about the first appearance of the pressor response. The stimulus range over which this response appeared was remarkably narrow, any slight increase tending immediately to evoke a pressor effect while decrease caused its disappearance and indeed was without detectable effect on the blood pressure. Once found, however, the effect could be repeatedly obtained (Figs. 38 and 39). Despite the fact that the preparation had the three other buffer nerves cut, the response was feeble and was quite different from that obtained by powerful stimulation not only as regards magnitude, but also in that the fall was often transient and tended to diminish markedly while the stimulus was yet being applied.

In these animals then, as had been found in certain of the nembutal experiments, the sinus nerve demonstrated three distinct responses to electrical stimulation, firstly a feeble fall/

ANALYSIS OF SINUS NERVE RESPONSES UNDER URETHANE.

THE EFFECT OF INCREASING VOLTAGES.



A B C D

Fig. 40. Cat. Anaesthetic urethane (total 1.5 gm/Kg.). Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec.

A. Stimulation of right sinus nerve 3 V.

B. " " " " " 8 V.

C. " " " " " 10 V.

D. " " " " " 30 V.

(Pulse duration (0.02 msec.) and frequency (70/sec.) constant).

fall appearing with liminal stimulation, secondly a rise obtained with stimulation of somewhat greater strength and persisting over a considerable intensity range, and thirdly a marked depressor response obtained by powerful stimulation (Fig. 40).

In the other animals it was not found possible to elicit this initial depressor effect, the first response observed, as the stimulus strength was increased from subliminal values, being pressor.

A DISCUSSION AND INTERPRETATION OF SINUS NERVE RESPONSES TO ELECTRICAL STIMULATION.

Evidence has been presented to show that electrical stimulation of either sinus nerve, in unanaesthetized (decerebrate), nembutalized or urethanized cats, gives rise to effects on the blood pressure which are dependent on the intensity of stimulus employed. In animals prepared by any of these methods it has been found that besides the normal expected depressor response, which is obtained only with stimuli of considerable intensity, there can also be elicited, in every case, marked pressor responses when weaker stimuli are employed. Further, it has been found that in certain instances very feeble stimulation causes a slight fall in blood pressure, and in animals showing this effect three responses are then observed, the initial feeble depressor effect with liminal stimulation, the terminal characteristic depressor/

depressor effect only obtained with very powerful stimulation, and an intermediate pressor response obtained with stimuli which lie between those responsible for the two depressor effects. Thus, in most animals, as the sinus nerve was stimulated with increasing intensities from values below threshold, the first observed response was a rise in blood pressure which yielded and became depressor when very powerful stimuli were applied. In the others, the first observed response was a fall in blood pressure, feeble and transient, and rapidly being converted to a pressor effect as the stimulus intensity increased, thereafter, however, the course of events in these animals was similar to that in the majority. In experiments following either pattern, once the depressor effect with strong stimulation had been obtained, further increase in the stimulus strength caused the fall to be of greater extent until a maximum was reached. After this, increasing the stimulus strength caused neither increase or decrease in the magnitude of the depressor effect.

If these results be interpreted as due to the effects of pressor or depressor fibres of differing excitabilities, and considerable evidence has already been led in justification of such a procedure, they permit of analysis of the fibre constitution of the sinus nerves.

Firstly, consider the initial depressor response. This must certainly be due to large fibres of low threshold. The experiments/

experiments, however, neither prove nor disprove the presence of such depressor fibres in every sinus nerve, for they have been demonstrated in but six animals from rather less than twenty in which appropriate low intensity stimulation was carried out. At first sight this would appear to suggest that they were present only irregularly, and the variation in results might be attributed to a variation in the fibre content of the nerve such as O'LEARY, HEINBECKER and BISHOP (1934) have shown to occur in the rabbit aortic nerve. On closer examination and analysis of the experimental evidence, however, these large depressor fibres assume a greater significance, for when the technical difficulties involved in their demonstration are considered it is surprising that they can be shown at all. To dwell for the moment on the question of excitability, both the fibres responsible for the initial depressor effect and the pressor fibres of neighbouring threshold are highly excitable. They are mobilized by feeble stimuli (one or two volts. of brief exponential condenser discharge of 0.3 - 0.4 msec. or of rectangular pulse of 0.02 msec.), and are therefore A fibres. Now it is well known that over a large range of fibre sizes (which includes the A group), the threshold of excitability rises but little, before rapidly increasing for the small fibres (BLAIR and ERLANGER 1934). For example, ERLANGER and GASSER (1930) found that the least excitable of the A fibres are activated with stimuli which are little over twice the strength needed to mobilize the most highly excitable fibres

of the same group. Now applying this to the problem in hand, it is apparent that the excitability thresholds of these depressor and pressor fibres mobilized with the weaker stimuli, approximate so closely one to the other as to render improbable any regular demonstration of their opposite effects by selective stimulation. From another point of view, when the two fibre groups are mobilized in succession with increasing voltages, they bring about a change of the vasomotor response, from a depressor to a pressor effect, in much the same way as electrical stimulation of many mixed nerves yields first a fall and then a rise in blood pressure as the strength of stimulus is increased. This latter phenomenon, the familiar 'reversal' obtained in such nerves as the popliteal or tibial and many others, is caused, however, not by excitation of fibres within the same group, but by successive mobilization of depressor fibres of the A group and pressor fibres of the C group (GORDON 1943). Now ERLANGER and GASSER (1930) have shown that a stimulus which is adequate to activate all the A fibres must be increased seven times to mobilize the most highly excitable of the C fibres, and over one hundred times to include all the C fibres. That being so, nerves of this type would appear to be ideally constituted to permit of the demonstration of afferents of directly opposite effect by the method of electrical stimulation with increasing intensities, for the two groups of afferents responsible for the effect of opposite sign are of widely differing thresholds. Not so the sinus nerve, however, for the similar reversal has

not/

not the same mechanism. No C fibres are involved. It has already been pointed out that the current intensities employed to elicit both effects are such as would be expected to activate only large fibres of low threshold, and the argument may be further supported by consideration of the following facts. In attempting to demonstrate the presence of the large depressor fibres in the sinus nerve, advantage had been taken of the fact that the relative irritabilities of fibres of different size are functions of the time constant of the stimulus, and that the greater irritability of the coarse fibres is most prominent with stimulating currents of short duration. Care was taken to employ brief shocks, either the short (0.3 msec.) pulses from the WHITFIELD stimulator or the shortest (0.02 msec.) from the B.N.I. device. The very fact that such brief stimuli were employed precluded the reversal being due to the mobilization of C fibres. To excite these with such short stimuli much higher voltages would have to be employed, ten times or more the value of those found effective in bringing about a transition from the depressor to the pressor response. This may be calculated from the data given by BLAIR and ERLANGER (1934). Further, from the standpoint of differential stimulation of vasomotor afferents, GORDON (1943) has stressed the difficulty of exciting C fibres with brief shocks. Thus with a stimulus of short time constant (0.1 msec. condenser discharge), this author found it <u>impossible</u> to produce any pressor reflexes from sensory nerves, such as the medial popliteal/

popliteal of the cat, even with 60 - 80 volts. The sinus nerve reversal, depressor to pressor, is not then to be considered in the same light as the similar effect occurring in a great number of other nerves of less specific vasomotor function, but can be seen to depend on successive mobilization of fibres of depressor and pressor function, which lie within the same A group. It would, therefore, be unjustifiable to expect it to be demonstrable with anything like the regularity with which its counterpart may be elicited in these other nerves. Where the two blood pressure responses of opposite sign are dependent upon selective stimulation of afferents of such closely approximated thresholds of excitability, it is probable that the large depressor fibres will make themselves manifest only under favourable circumstances, possibly where the nerve is so disposed on the electrodes as to favour their excitation. When it is further borne in mind that the index of selective stimulation of the fibres is not the sensitive method of recording the action potentials set up in the nerve, but is the nature of the blood pressure effects brought about in the animal itself, it is remarkable that the depressor fibres of low threshold have been observed in even as many as six out of twenty experiments.

From such arguments it follows that the absence of an initial depressor response to feeble stimulation does not necessarily imply the non-existence of large depressor fibres. The absence of the effect might justifiably be attributed to

a/

a failure, although an understandable one, of the technique employed in demonstrating them. It is therefore possible that large depressor fibres are a normal content of the sinus nerves. On the other hand it is possible that they are not regularly present.

Extending the analysis to the consideration of the pressor and depressor responses obtained with higher intensity stimulation, it is at once apparent that the experimental results permit of much more definite conclusions. It can be said at the outset that all the nerves stimulated showed evidence of pressor fibres of a wide range of excitability and of depressor fibres of higher threshold. This statement holds, despite the fact that in some animals a depressor response was not obtained at all, for in these, depressor fibres were making themselves manifest as a diminution of the pressor effect as the stimulus was increased,and further, the strength of stimulus used was later shown to be insufficient to mobilize enough small fibres to bring about the maximum fall of blood pressure. What is of especial interest is the very high intensities required to cause the normal depressor effect. At once they point to the depressor fibres being very small, certainly smaller than the pressor fibres. There are several indications that some at least of the depressor fibres may belong to the C group. For example, the maximum stimulus of which the WHITFIELD device was capable (40 V. 0.3 msec.), was early abandoned as being insufficiently strong regularly to bring about the expected depressor/

depressor response. This stimulus sometimes mobilized the depressor fibres only in sufficient number to bring about a decrement in an established pressor effect. Usually, however, it brought about a fall in blood pressure, but in these instances the effect was never shown to be maximal, and in each of the experiments where the effect was compared with that brought about by stronger stimulation from another stimulator, an increased depressor component testified to the fibres it left inactivated. Similarly with the B.N.I. stimulator it was found that although a depressor effect could be achieved with pulses of 0.02 msec. when 40 - 50 volts. were used, it was usually feeble and could always be augmented by increasing the time constant. These absolute values in themselves speak in support of the depressor fibres belonging to the C group (BLAIR and ERLANGER 1934). The same conclusion may be reached in a different way, however, without reference to absolute values. For instance, an effect on the blood pressure, rise or fall, is always observed in response to stimulation of the sinus nerve with a few volts, of either of the two stimuli mentioned, and often with less than one volt. Yet in some instances this must certainly be increased over one hundred times before depressor fibres are mobilized in any significant amount. Again, the difficulty with which the small fibre response is obtained with the short pulses, and the comparative ease of its demonstration with pulses of 10 msec. duration, point to a similarity between this series of experiments and those carried out/

out by GORDON (1943), to which reference has already been made, and where the small fibres of the 'unspecific' nerves were considered to belong to the C group.

It is certain that the fibres responsible for the classical depressor response are very small, and considerable evidence has been adduced in favour of their belonging, at least in part, to the C group. This latter conclusion, however, cannot be considered as proved in the absence of such other evidence as might be derived from differential fibre blocking, or simultaneous recording of action potentials, both of which are outwith the scope of the present work.

With regard to the pressor fibres. The great majority of these certainly belong to the A group. Their irritability, however, extends over a very large range, and the masking of their effect as depressor fibres are mobilized precludes any estimate of the values required to stimulate the least excitable. They may extend down to the smallest A fibres or even into the C group.

Finally, quantitative differences in the total pressor or depressor potentialities in the nerves would suggest considerable variation in the pressor and depressor fibre content.

Such then are the conclusions arrived at in the analysis of the fibre content of the sinus nerve. It remains to relate these to the known functions of the nerve.

First, consider the known pressor fibre content, the chemosensory fibres. Pressor responses from electrical stimulation of/

of the sinus nerve have not previously been ascribed to activation of these fibres, but where stimulation is carried out over a wide range of intensities, from liminal to supra-maximal values, it is obvious that these fibres must be excited at some stage. In the present study, no evidence of pressor activity has been found, except in the range of fibres with thresholds intermediate between the initial and terminal depressor fibres. Such evidence has been deliberately sought in view of the fact that in allocating the chemosensory fibres to a group of higher excitability than the powerful depressor fibres, a conclusion directly at variance with the well known action potential studies of BRONK (1932) and others has been arrived at. Increase of the intensity of stimulation in the depressor range, even with the most powerful stimuli of 10 msec. and to the destruction of the nerve, failed to demonstrate any decrement in the depressor response or other evidence of pressor fibre activity. The significant pressor content had thus been mobilized before all the depressor fibres had been activated.

The chemosensory fibres must therefore be assigned to the intermediate pressor group. Whether or not they form the whole group is a different matter, for the range of thresholds has been shown to be large, and it is possible that pressor fibres other than chemosensory fibres are involved.

As to depressor activity, the sinus nerve has a known complement of depressor fibres, the barosensory fibres, and the present series of experiments have shown that two quite distinct depressor/

depressor afferent groups exist. The first group of low threshold is in agreement with the action potential studies, but produces only a feeble fall in blood pressure and indeed may not be regularly present. The second group disagrees with the established teachings, but is responsible for producing the classical depressor response and must be assumed to include the barosensory fibres. It might be held that the small fibres causing the depressor response are not barosensory, but the argument here is the same as with the chemosensory group, simply that the depressor barosensory content of the nerve has to be accounted for somewhere over the comprehensive range of electrical stimulation, and this has failed to show powerful depressor activity other than in the small fibre group. As for the large depressor fibres, it may well be that they are those responsible for the large spikes of the sinus nerve neurograms shown to be set up in barosensory fibres. The feeble effect they have been found to produce on the blood pressure does not preclude this, for the arguments that such large fibres are responsible for the reflex fall in blood pressure following stimulation of the baro-receptors are indirect, the conclusions being derived from two isolated facts. Firstly, that a rise in intrasinusal pressure causes such spikes to appear, and secondly, that the same change is accompanied by a reflex fall in systemic blood pressure. Such an argument would be sound only if the large fibres alone could be shown to be active during the reflex. This is not the case, however. Indeed since BRONK's (1932) study there has been known/

known to be a continuous background of small fibre activity. This has been ascribed to the chemosensory fibres on the basis that excitation of the chemoreceptors produces just such activity as is seen between the showers of large potentials. There is no evidence, however, that the chemoreceptors account for all such small fibre activity, or that during a barosensory reflex there is not indeed an increase in the number of small fibres active. These might well exist behind a masking barrage of large pressure sensitive fibres. There is then no proof that the large spikes are responsible for all, or even part, of the reflex vasodilation in the sinus reflex. The present series of experiments certainly would deny this, and attribute the effect to barosensory fibres of much smaller size, the very smallness of the fibres being perhaps a reason for their not being easily seen on electroneurograms. It is possible then that the large fibres picked out by feeble stimuli are these barosensory fibres producing the large action potentials. If so, they are of little importance in the production of the reflex fall of blood pressure in the vagotomized animals, although they may well be subserving some other function. On the other hand, these large depressor fibres may be afferents other than 'barosensory'.

Such conclusions are opposed to the classical conceptions of the fibre constitution of the sinus nerve, but do not stand alone in this respect for EULER, LILJESTRAND and ZOTTERMAN (1939), 1941) have also led evidence which necessitates a reconsideration of the older views. Working from the

complementary/

complementary standpoint, the recording of action potentials, they have found that in addition to the large spikes, there are also smaller spikes in the sinus nerve electroneurogram which respond to variations in endosinusal pressure, and are moreover, insensitive to chemical stimuli. Further, selective abolition of these small impulses with nicotine or lobeline in high concentration abolishes the depressor reflex although it leaves the large spikes active. For these reasons, and also because the large pressure impulses are few in number compared with the small ones, they considered 'the latter to be of greater importance in the sinus pressure reflexes'. They do not draw any distinction in size between the chemosensory and small barosensory fibres. The present work suggests that the barosensory are the smaller. The results are reconcilable, however, for owing to the rapid increase in excitability thresholds amongst the very small fibres an exceedingly small decrease in diameter may well reflect itself as a marked rise of threshold and thus permit of easier determination of relative size by estimation of thresholds than by difference in action potentials characteristics.

To summarize, the experiments indicate that the sinus nerve of the cat is regularly composed of two groups of vasomotor afferents, the first of very small depressor fibres probably of the C group, and the second of somewhat larger pressor fibres. It is considered that the barosensory fibres responsible for the characteristic depressor vasomotor effects belong to the first

group/

group, while the second group of larger fibres includes those having their origin in chemoreceptors. A third and less easily demonstrable component, which may or may not be regularly present, is made up of yet larger fibres of feeble depressor function.

CHAPTER IX.

THE EFFECT OF NEMBUTAL OVERDOSAGE ON THE SINUS NERVE REFLEX CONSIDERED IN THE LIGHT OF THE FIBRE CONSTITUTION OF THE NERVE.

It was thought that the pressor response to powerful sinus nerve stimulation in the cat overdosed with nembutal (the 'reversed' response) might be due, not so much to a reversed effect of the depressor component, but rather to the pressor content. Such a hypothesis rests on two premises, firstly that the nerves contain pressor fibres capable of producing the rises of blood pressure observed, and secondly that these fibres are activated with the stimulus strengths used to elicit both the 'normal' depressor and abnormal pressor or 'reversed' responses. Both these conditions prevail, for it has been shown that the sinus nerve regularly contains pressor afferents of considerable power and that these are certainly mobilized at a stimulus strength necessary to bring about the classical depressor response. The same stimulus, it will be remembered, was also used to elicit the 'reversed' response after excess nembutal, and must therefore be expected to stimulate the same fibres.

The relation of the 'reversed' response to the pressor fibre content of the nerves was early apparent. If it be assumed that the vasomotor response is the algebraic sum of the effects of the different afferents (BAYLISS 1893), then it may be said to/

to depend upon the pressor and depressor fibre content of the nerve if the stimulus be sufficient to excite all the fibres, and upon the relative activation of depressor and pressor components if it is not. In a sinus nerve yielding feeble depressor responses, the depressor fibres are obviously being mobilized in less effective proportion to the pressor fibres than where the nerve is causing a powerful depressor response. Now, if the phenomenon of 'reversal' were brought about by the former it would be poorest in such nerves, whereas the converse is the case. Again, the sinus nerves giving the greatest pressor responses in light anaesthesia, when the normal depressor responses could also be elicited, tended to give the more powerful pressor effects when overdosed with nembutal.

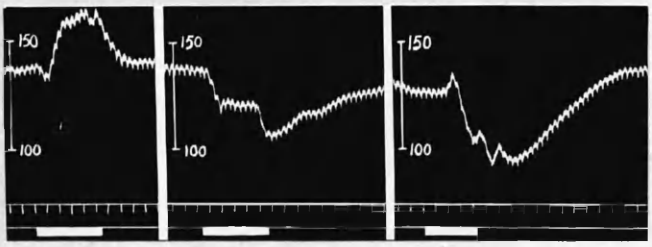
In the normally anaesthetized animal the pressor potentialities of the sinus nerve are always striking, indeed it is sometimes difficult to obtain a marked depressor response. Such is not the case in the vagus which usually manifests very much greater depressor activity over a wide range of stimulus strengths. But the sinus nerve 'reversal' always appears before its vagal counterpart, which further supports the hypothesis.

Assuming the 'reversal' to involve the two afferent groups, it was considered that the phenomenon might be the result of a shift in the threshold intensities of stimulus required to convert a rise into a fall. In other words an action of the excess nembutal tending to augment the efficacy of the pressor component or diminish that of the depressor component. There

ANALYSIS OF THE REVERSED RESPONSE.

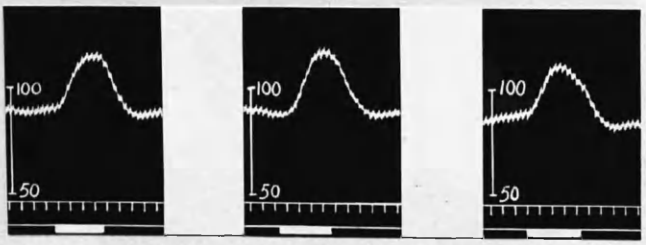
THE EFFECT OF DIFFERENT STIMULUS STRENGTHS.

DURING NORMAL NEMBUTAL ANAESTHESIA.



3V. 15V. 45V.

DURING PROFOUND NEMBUTAL ANAESTHESIA.



3V. 15V. 45V.

Fig. 40a. Cat. Anaesthetic nembutal. Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec.

Stimulation of the right sinus nerve throughout with constant frequency (30/sec.) and pulse duration (1.2 msec.). Thyratron Stimulator.

Upper series. Nembutal 45 mg/Kg.
Lower series. Nembutal 70 mg/Kg.

was no opportunity to pursue this in the early experiments, for the nerve was stimulated with the maximum strength of stimulus of which the WHITFIELD device was capable, but in later experiments where a greater amount of the depressor component could be mobilized with more powerful stimuli, it was found that there was indeed a shift in threshold. In some instances where a submaximal stimulus had caused a depressor response in light anaesthesia and then a pressor effect after excess nembutal, a depressor response could be regained by increasing the stimulus strength. As the nembutal was increased, however, this manoeuvre failed, so that a depressor effect could not be obtained no matter how strong the stimulus (in several animals currents were applied of sufficient strength to destroy the nerve). Here again the significant feature is that stimuli greater than the control strengths for normal depressor and 'reversed' affects served to decrease the pressor effect. If the 'reversed' response were due to a true reversal of the effect of normally depressor fibres then it would be augmented.

These findings suggested that the reversal was brought about by an alteration in the relative efficacy of the pressor and depressor components, in such a way that the former became prepotent. Now nembutal is essentially a depressant drug and after massive overdosage abolishes not only the depressor but also the pressor effects of sinus nerve stimulation (Chap. III). It cannot readily be conceived therefore that at a somewhat lighter phase of anaesthesia the drug causes a 'reversal' of the effects of/

of powerful stimulation by increasing the absolute potency of the pressor fibres. It would be much more reasonable to view their enhanced effect being relative and due to more markedly diminished effectiveness of the depressor fibres.

If the 'reversal' be regarded thus, as a selective abolition of the efficacy of the depressor reflex, the response should not be greatly altered if the depressor fibres are not stimulated at all, and indeed this has been found to be the case (Fig. 40a). In this animal, during light nembutal anaesthesia, stimulation with 3 V. caused a marked pressor response indicating if not stimulation of the pressor fibres alone, at least a prepotence of these over any depressor fibres that had been activated. When the stimulus had been increased to 15 V. the pressor fibres had been overcome by the activation of a sufficiency of small depressor fibres, while it will be seen that 45 V. further increased the fall, presumably by mobilizing yet more depressor fibres. This last stimulus gave the maximal effect. After a 'reversing' dose of nembutal, however, it can be seen that each stimulus causes much the same effect. The simplest explanation is that these pressor responses are dependent on the pressor fibre content alone, for stimuli shown to mobilize the depressor content neither increase nor decrease the response. In this animal at the time of obtaining these reversed responses the depressor mechanism must have been completely inactivated.

It is concluded that the reversed response to stimulation of/

of the sinus nerve after nembutal overdosage is brought about by a selective depressant effect of that drug on the effectiveness of the small fibre depressor reflex leaving the larger fibre pressor reflex comparatively vigorous.

CHAPTER X.

PERFUSION OF THE ISOLATED CAROTID SINUS.

It has been argued that the normal depressor effect of electrical stimulation of the sinus nerve is brought about by stimulation of both pressor and depressor afferents, the effect of the latter predominating (Chap. VIII), and that the reversal of the effect observed after nembutal overdosage is caused by a selective depression of the potency of the latter (Chap. IX). Reasons have also been given for considering that the pressor afferents stimulated are, or at least include those subserving chemosensory function, and that the depressor afferents are the barosensory fibres involved in the normal sinus depressor reflex (Chap. VIII).

It is apparent, therefore, that the degree of activity of barosensory and chemosensory reflexes during profound nembutal anaesthesia has a most important bearing on these views.

Experiments were carried out to estimate these functions by activating the two fibre groups selectively through their peculiar receptor mechanisms.

Methods:

Cats were used. They were anaesthetized with nembutal (40 mg/Kg. intraperitoneally). One animal required another 10 mg/Kg. before dissection could be carried out. When anaesthesia had been established, the trachea was cannulated and the left vagus cut. The left sinus nerve was then exposed and/

and carefully dissected out to as near its origin as possible, whereupon it was tied with fine cotton and cut distal to the ligature. Care was taken to obtain a good length of nerve, and a clear approach was ensured in each instance by removing the large neighbouring lymph gland. Having cut the buffer nerves on the left side and prepared the way for stimulation of the left sinus nerve, attention was turned to the right side. First the vagus was cut and tied. Then the right common carotid artery was separated off from all the neighbouring tissues from below up, all the small branches being tied between ligatures. This process was continued to within about 5 mm. of the bifurcation of the vessel. Next the hypoglossal nerve was cut and stripped off the lingual artery and the sinus region. The lingual artery was then tied and cut, and the glossopharyngeal nerve was sought, not by the usual lateral approach, but by gently retracting the lingual artery away from the midline and approaching from the medial side. When found, it was tied well distally and the sinus nerve identified and dissected out. The other branches of the external carotid artery were then tied and about one centimetre of this vessel exposed by removing the right digastric muscle. The blood flow through the external carotid itself was left intact. Now the sinus region was carefully dissected free from the closely adherent superior cervical ganglion of the sympathetic chain and the ganglion nodosum of the vagus. The fatty and nervous tissues binding these structures together were carefully removed, until there remained/

remained only the sinus nerve and the occipital and internal carotid arteries. These two vessels were double looped as far dorsally to the sinus region as possible, but left untied. Within a few minutes this prepared sinus could be isolated and perfused, but meanwhile it remained nourished in continuity with the blood stream.

The left femoral artery was now cannulated. Heparin was employed as anticoagulant. A stethograph was fixed in position and recording begun.

The dissection required about two and a half hours to complete.

The sinus nerve electrodes were now clamped in position on the left side, and the cut left sinus nerve laid across them. Control depressor responses were obtained from this nerve. The animal was then given additional nembutal intraperitoneally ($\frac{1}{2}$ anaesthetic dose in two animals, rather more in the other) until the control stimulus caused a rise in blood pressure. Now the prepared right sinus region was isolated and perfused. First the internal carotid artery and the occipital artery were tied off and divided. Next the common carotid was clipped low down and the external carotid cannulated as far from the sinus as possible and divided. Finally the perfusing cannula was inserted into the lower end of the preparation through the common carotid artery, which was then cut caudal to the cannula. Both cannulae were firmly clamped to maintain the isolated arterial tube in proper relationship to the animal, for it was connected thereto only/

only by the minute sinus nerve. At once perfusion was begun with oxygenated Ringer-Locke saline, the temperature being kept at 38°C. (a thermometer was incorporated in the perfusion cannula). The perfusion fluid was composed of water 100, NaCl 0.9, KCl 0.042, $CaCl_2$ 0.024, glucose 0.2 and $NaHCO_3$. The amount of bicarbonate added was much less than usual, being about 1 cc. 0.3% $NaHCO_3$ to the litre of fluid, the exact amount being controlled by titration with bromothymol blue to give a pH of 7.4. The fluid was supplied by a DALE-SCHUSTER pump (the method originally used by HEYMANS and BOUCKAERT 1929 in their experiments on the dog). A fairly well marked but not over vigorous pulsation of the perfused fluid was obtained by damping the output with a variable air buffer (STRAUSS (1940) maintains that stimulation of the baroreceptors is more effective if pulsatile pressure is employed, although BARANY (1943) denies this). The rate of flow could be readily determined as drops issuing from the return cannula to the oxygenating reservoir, and was kept close to 10 cc. per minute.

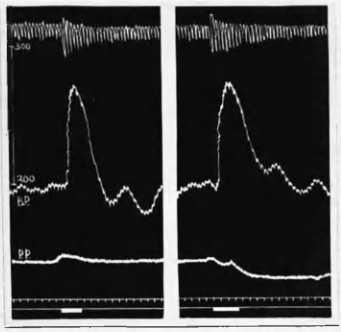
The conversion from the normal blood circulation to this artificial saline perfusion took but a few minutes. This 'delayed perfusion' technique provided a preparation in which the reflexes from a freshly prepared sinus could be tested at a time when control stimulation of the other sinus nerve showed a reversal of the normal effect.

Barosensory reflexes were tested by raising or lowering the perfusion pressure. To do this, the pump rate (about

PERFUSION OF THE ISOLATED CAROTID SINUS.

RESPONSES OBTAINED IN LIGHT NEMBUTAL ANAESTHESIA.

1. STIMULATION OF THE CHEMORECEPTORS.



A B

Fig. 41. Cat. Anaesthetic nembutal (40 mg/Kg.). Double vagotomy. Left sinus nerve cut. Spontaneous respiration recorded by stethograph. Arterial B.P. (left femoral artery) and sinus perfusion pressure (P.P.) in mm. Hg. Time 5 sec.

A. Injection of acetylcholine (25 γ) into perfused sinus.

B. " " lobeline HCl. (20 γ) " " "

11. STIMULATION OF THE BARORECEPTORS.

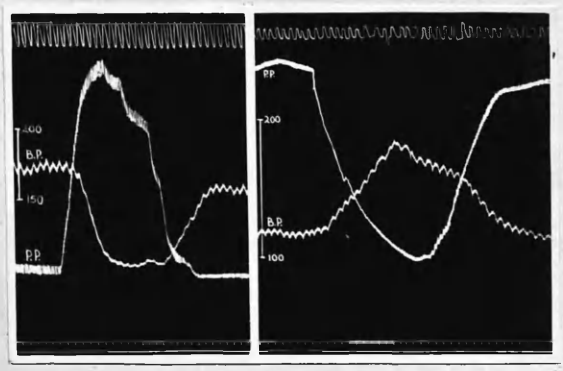


Fig. 42. Tracings from two animals (preparation and records as Fig. 41) showing the typical responses brought about by changes in the perfusion pressure.

160/min.) was not interfered with, but the stroke volume and the resistance on the return tube were simultaneously altered to bring about the desired change in pressure while keeping the flow through the sinus constant.

Chemosensory reflexes were tested by injecting acetylcholine into the perfused sinus. This drug was first shown to excite the chemosensory fibres by HEYMANS, BOUCKAERT and HANDOVSKY (1935), and this has been amply confirmed since (HEYMANS 1935, HEYMANS, BOUCKAERT, FARBER and HSU 1936, COMROE and SCHMIDT 1938, EULER 1938, WINDER 1938, SCHWEITZER and WRIGHT 1938), both in dogs and cats, while EULER, LILJESTRAND and ZOTTERMAN (1941) have shown that intrasinusal injection of acetylcholine in the cat causes the appearance of a shower of 'chemical' impulses. (The mechanism of its action has been discussed in Part 1). Acetylcholine was favoured, not only because it was found to be just as effective as the more commonly used lobeline (see Fig. 41), but because its use was unattended by the dangers of deterioration or even paralysis of the receptor mechanisms which may accompany the use of that drug (EULER, LILJESTRAND and ZOTTERMAN 1941). To make a test, the acetylcholine (100 µg/cc. in normal saline) was injected through a ureteric catheter threaded into the perfusion cannula, so that the drug was introduced directly into the lower end of the isolated artery and carried through it by the perfusing saline. The fluid issuing from the sinus after such a test was shunted off momentarily so that no contamination of the reservoir should take/

take place.

Several tests of barosensory and chemosensory function were made in each animal and at the same time control responses to electrical stimulation of the left sinus nerve obtained. In one animal electrical stimulation of the vagus was also carried out. Picrotoxin was then given intravenously and further tests made.

It will be observed that perfusion was carried out only when the animal was profoundly anaesthetized, and the barosensory and chemosensory reflexes tested during that state alone, or after picrotoxin. For this reason controls were not obtained during light anaesthesia. Such indeed would have necessitated the establishment of perfusion an hour or so earlier, before the excess nembutal was given, and would have entailed the crucial tests in the overdosed animal being carried out in a sinus preparation subjected to grossly artificial conditions over this prolonged period of time. The inadvisability of such a procedure had been clearly indicated in four preliminary experiments, in which a progressive loss of sensitivity of the receptor mechanisms had rendered tests after additional nembutal valueless. These four experiments, however, besides indicating the course to be taken in the later experiments, provided a valuable background which clearly indicated the adequacy of the sinus preparation and the methods adopted to provoke the two reflexes. For in these animals, where the sinus was prepared as for the three later experiments, powerful/

PERFUSION OF THE ISOLATED CAROTID SINUS.

RESPONSES OBTAINED IN DEEP NEMBUTAL ANAESTHESIA.

First Animal.

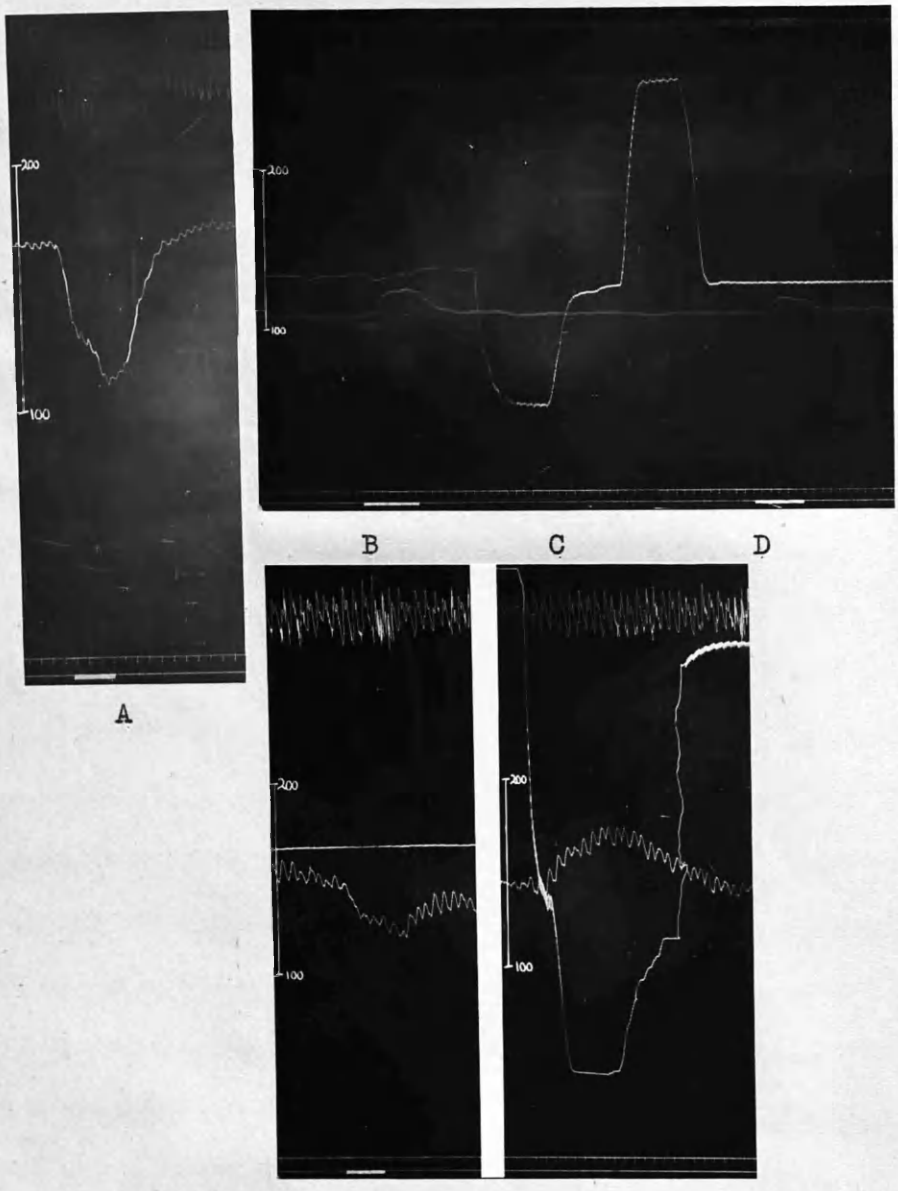


Fig. 43. Cat. For preparation, see text. Records as Fig. 41.

A. Control stimulation of the left sinus nerve (10 V.70/sec. 1.0 msec.).

B. Acetylcholine (100 γ) injected into perfused right sinus.

C. Perfusion pressure lowered and raised.

D. Stimulation of left sinus nerve (10 V. 70/sec. 1.0 msec.).

E. After picrotoxin D repeated.

F. After picrotoxin C repeated.

powerful chemosensory responses could be elicited by chemical excitants such as lobeline and acetylcholine (Fig. 41) and marked barosensory responses by varying the endosinusal pressure (Fig. 42). The demonstration of brisk barosensory reflexes is of much value as a control, especially as in every case there was but little decrease in efficacy after thirty minutes' perfusion, and as in each of two experiments in which observations were made over a longer period the responses were still well marked, if somewhat diminished, after an hour's perfusion.

Results:

Three cats were studied by the 'delayed perfusion' technique. In the first animal (Fig. 43) the following results were obtained:

(1) Control stimulation of the left sinus nerve during light nembutal anaesthesia (50 mg/Kg.) produced powerful depressor effects (Fig. 43 A).

(2) One hour later, after nembutal had been given to a total of 80 mg/Kg. and the blood pressure had fallen to below 100 mm. Hg., the control stimulus now evoked feeble (5 mm.) pressor responses. The right sinus was now perfused and it was found that injection of 100 g. acetylcholine caused a very feeble rise in pressure (4 mm.) while gross changes in the perfusion pressure (of more than 200 mm. Hg.) had no effect on the systemic blood pressure.

(3) Ten minutes later systemic blood pressure had risen a few/

PERFUSION OF THE ISOLATED CAROTID SINUS.

RESPONSES OBTAINED IN DEEP NEMBUTAL ANAESTHESIA.

Second Animal.

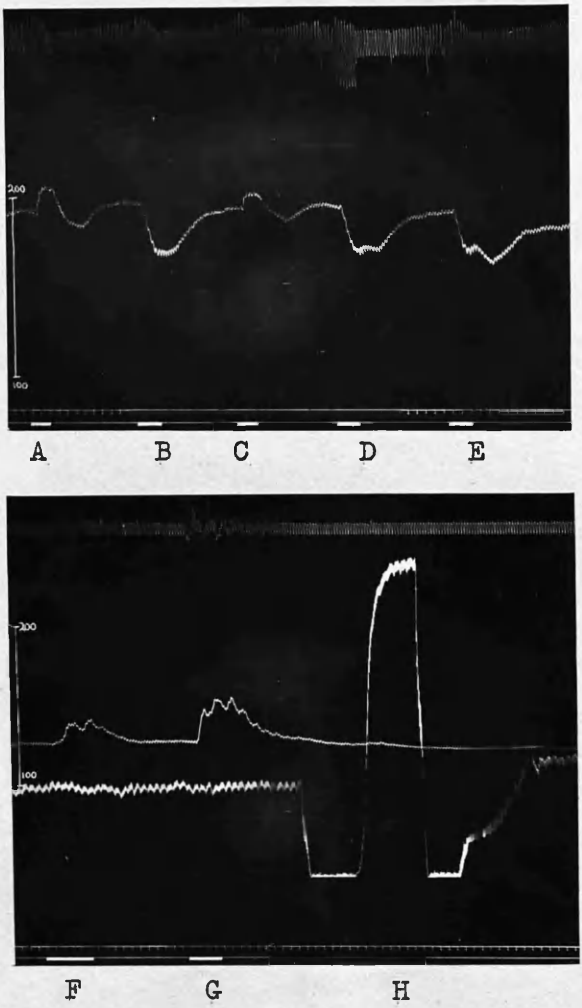


Fig. 44. Cat. Anaesthetic nembutal. Double vagotomy. Left sinus nerve cut. Right sinus perfused. Respiratory movements (stethograph). Arterial B.P. (left femoral artery) and sinus perfusion pressure in mm. Hg. Time 5 sec.
Upper tracing: nembutal 40 mg/Kg. Spontaneous respiration. Stimulation of left sinus nerve.
A and C. 10V. 70/sec. 0.02 msec.
B and D. 50V. 70/sec. 0.02 msec.
E. 10V. 70/sec. 1.0 msec.
Lower tracing: nembutal 60 mg/Kg. Artificial Respiration.
F. Stimulation of left sinus nerve 10 V. 70/sec. 1.0 msec.
G. Injection of acetylcholine (100γ) into perfused right sinus.
H. Perfusion pressure lowered and raised.

few mm. and the pressor effects of both electrical stimulation and of acetylcholine had increased. Alteration of the perfusion pressure was again without any visible effect whatsoever.

(4) Three subsequent series of tests repeated at intervals of ten minutes gave similar results; a progressive slow rise in blood pressure and increase in the pressor effects of electrical stimulation and acetylcholine being observed. The last of these tests is illustrated (Fig. 43 B, C and D).

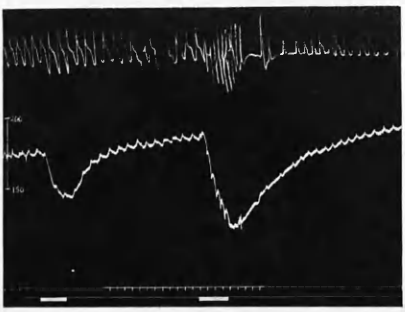
(5) At this point, 1 hr. after sinus perfusion had begun, 1 cc. of picrotoxin was given intravenously (0.9 mg/Kg. body weight). Sinus nerve stimulation was still pressor 5 min. later. Another 1 cc. was given. After a few minutes the response was depressor (Fig. 43 E), and there was now a response to alteration of the endosinusal pressure (Fig. 43 F). As the anaesthesia was now becoming light the animal was killed.

The second animal gave essentially the same results (Fig. 44). Control stimulations during light anaesthesia gave the usual responses, a pressor effect with weak stimulation and a powerful fall with strong stimulation. After a further half anaesthetic dose of nembutal the characteristic depressor response was 'reversed', endosinusal injection of acetylcholine causing a rise in blood pressure (in the last test a rise of 46 mm. Hg., nearly a 50% increase, from injection of 100 μg.) and gross changes in the perfusion pressure were without effect. Three series of tests gave similar results. Again, after picrotoxin (1.3 mg/Kg.) there was a reappearance, in some measure, of the sinus pressure reflex:/

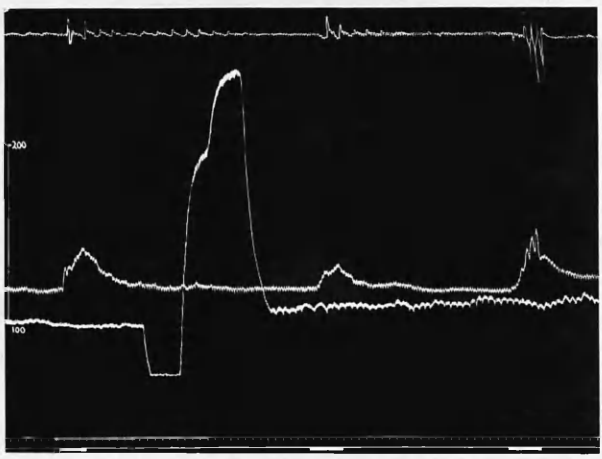
PERFUSION OF THE ISOLATED CAROTID SINUS.

RESPONSES OBTAINED IN DEEP NEMBUTAL ANAESTHESIA.

Third Animal.



A B



C D E F

Fig. 45. Cat. Anaesthetic nembutal. Double vagotomy. Left sinus nerve cut. Right sinus perfused. Respiratory movements (stethograph). Arterial B.P. (left femoral artery) and sinus perfusion pressure in mm.Hg. Time 5 sec.
Upper tracing: Nembutal 40 mg/Kg. Spontaneous respiration.
A. Stimulation of left sinus nerve 10 V. 70/sec. 1.0 msec.
B. " " right vagus " " "
Lower tracing: Nembutal 60 mg/Kg. Artificial respiration.
C. Injection of acetylcholine (100 γ) into perfused right sinus.
D. Perfusion pressure lowered and raised.
E. Stimulation of left sinus nerve 10 V. 70/sec. 1.0 msec.
F. " " right vagus " " "

reflex: a fall of 20 mm. in the systemic blood pressure being brought about by a rise in the endosinusal perfusion pressure from 40 to 280 mm. Hg.

In the last animal, control depressor responses for both the left sinus nerve of the right vagus were obtained in light anaesthesia (Fig. 45 A and B). After addition of nembutal to a total of 60 mg/Kg. it was found that both the sinus nerve and the vagus response was 'reversed', and that as in the other two animals, change in the perfusion pressure was without effect while intrasinusal acetylcholine gave a pressor response. Three trials gave similar results (Fig. 45 C, D, E and F). After picrotoxin (1.2 mg/Kg.) endosinusal pressure changes produced visible effects on the blood pressure, a fall of 12 mm. occurring in response to a rise in the perfusion pressure from 30 to 230 mm. Hg.

Interpretation:

In all three animals overdosed with nembutal, and showing 'reversed' responses to electrical stimulation of the left sinus nerve (and in one instance also the right vagus), it has been found that injection of acetylcholine into the isolated and perfused right sinus causes a well defined pressor response, while alteration of the perfusion pressure is without any effect on the systemic blood pressure whatsoever.

Acetylcholine can only have its effect over the sinus nerve, there being no other connection with the animal, and evidence has been led to the effect that it activates the chemosensory fibres/

fibres (see 'methods'). Certainly a typical chemoreceptor response has been obtained in the control animals (Fig. 41), and the response in deep nembutal anaesthesia is qualitatively the same, there being increased respiratory activity and a rise in systemic blood pressure. Two other possibilities remain, firstly that the effects are due to stimulation of baroreceptors or secondly that a hypothetical third group of fibres, neither chemosensory or barosensory, is involved. With regard to the first, it has been shown that barosensory reflexes cannot be elicited by chemical stimuli (BOUCKAERT, DAUTREBANDE and HEYMANS 1931 and COMROE and SCHMIDT 1938) and EULER, LILJESTRAND and ZOTTERMAN (1941) have found that synaptotropic substances are without effect on the impulse frequency in either the large or the small barosensory fibres. It has also been shown that at this depth of anaesthesia excitation of the barosensory fibres by increasing the perfusion pressure is without any effect whatsoever. As to the effects being due to a third group of fibres, it can only be said that no afferents in the sinus nerve, other than those from the chemoreceptors, have been demonstrated to possess such functions, nor has acetylcholine been shown to excite any afferent nerve fibres whatsoever besides those of chemosensory function (ZOTTERMAN 1944). It must be concluded that the chemosensory fibres and the central and peripheral mechanisms involved in the chemosensory reflex are active, at least in some measure, during deep nembutal anaesthesia.

With regard to the effects of gross alteration of the perfusion/

perfusion pressure, analysis fails to account for the absence of response on the basis of faulty technique, either through an inadequate stimulus or through damage to the barosensory mechanism. The barosensory reflexes are easy to demonstrate, much more so than the chemosensory. If damage were done to the preparation it would be more likely to involve the latter. Further, the method adopted is reliable and well established being almost as old as sinus perfusion itself (HEYMANS and BOUCKAERT 1929), and indeed in the control animals it has proved entirely satisfactory, allowing of the demonstration of barosensory reflexes for upwards of an hour. When it is remembered that the dissection was precisely the same, and that the tests were first made within a few minutes of isolation and interruption of a normal circulation through the sinus region in the deeply anaesthetized animals just as in the controls, the possibility of a technical basis for the failure becomes more remote. The final proof is provided by the positive results of the same stimulus repeated after the injection of picrotoxin into the animal. In every case there was demonstrable depressor activity, certainly not of the magnitude of the controls, but all the more significant considering the deterioration in the pressure reflex which was found to occur in these preliminary experiments after equivalent periods of time. The appearance of depressor activity in the sinus connected to the animal only by a minute nerve indicates that the effect of picrotoxin is not on the depressor receptor mechanisms. It further implies that/

that these structures have been activated during the earlier tests when the same stimulus was applied. The absense of response cannot therefore be ascribed to inadequacy of the stimulus. Nor can the depressor effect after picrotoxin be ascribed to fibres other than from baroreceptors. Chemoreceptors are unaffected by pressure (COMROE and SCHMIDT 1938), and indeed their activation by acetylcholine or lobeline after the injection of picrotoxin has been found to cause a pressor effect. Moreover, no depressor fibres activated by increased endosinusal pressure other than from baroreceptors are known to exist in the sinus nerve. It must be concluded that the barosensory reflexes from the carotid sinus are absent in the animal overdosed with nembutal, and further, that the effect of picrotoxin is to restore the function of these reflexes.

Certain other findings are of importance. In each animal repetition of the same dose of acetylcholine in successive tests has caused increasingly marked pressor responses and more powerful effects on respiration. This has corresponded with a recovery of systemic blood pressure and tendency to spontaneous respiration, so that the maximum response has always been obtained in the last test, that is at the longest interval after injection of excess nembutal. No such phenomenon was observed in the control animals, the converse being the case. Further, the pressor effects obtained in the overdosed cats have been considerably smaller than those obtained in the controls. The relationship between the depth of anaesthesia and magnitude of the/

the response is striking, especially in the first animal which was grossly overdosed, and in which the initial chemosensory reflex was very feeble although becoming stronger as anaesthesia lightened. The chemosensory activity is then not unaffected by the nembutal, but is also diminished, although not to the same extent as the barosensory reflexes. It will be recalled that in the second animal a chemosensory response of considerable magnitude (46 mm. Hg.) was unattended by any evidence of barosensory activity whatsoever. It is possible that had the anaesthesia been allowed to lighten still further the contrast would have been even more striking. This of course was undesirable for it would have jeopardized the subsequent course of the experiment. The difference in the action of nembutal on the two mechanisms is then quantitative, and markedly so.

In every experiment, and in each test, it was found that electrical stimulation of the contralateral sinus nerve had effects qualitatively the same as the chemosensory reflexes initiated in the other sinus. When the latter caused a rise in blood pressure, so did the former, and where respiratory activity was brought about by one, it resulted also from the other. There was, however, an obvious and constant difference in the quantitative results. In each instance the effects brought about by acetylcholine were greater than those brought about by electrical stimulation. Now it has been earlier argued (Chap VIII) that a stimulus of the strength employed to excite the sinus nerve in these experiments would be expected

to/

to mobilize all the chemoreceptor fibres. A priori, the difference might be ascribed to a greater pressor content in the right sinus nerve, but during deep nembutal anaesthesia both nerves tend to give similar responses to electrical stimulation. Ideally the comparison of the effects of electrical stimulation and acetylcholine should be made on the same side. Such a procedure was not regularly adopted, for it endangered the preparation. In one instance, however, (the third animal) it was found that when the right sinus nerve was electrically stimulated it gave a similar response to the left, and a lesser effect than acetylcholine. It would appear that the former is less efficacious than the latter, but this does not imply that electrical stimulation of the intensity employed does not mobilize all chemoreceptor fibres, the difference may lie in the frequency of discharge induced by the two methods. The more significant value of the differences discovered, lies in the fact that it has been found that the reversed effect brought about by electrical stimulation of the sinus nerve in the cat overdosed with nembutal, can readily be accounted for by chemosensory fibre activity alone. Likewise the powerful pressor effects which can be elicited from the sinus nerve by appropriate electrical stimulation in light nembutal or urethane anaesthesia or in the decerebrate preparation have never exceeded the enormous responses obtained in the control animals (Fig. 41) and certainly do not require explanation on a basis of hypothetical pressor fibres other than chemosensory.

The/

The results clearly indicate that the sinus nerve 'reversal' is not attributable to a true reversal of the baro-sensory reflex which is totally absent in nembutal overdosage, but that the effect may readily be accounted for by activation of the yet functioning chemosensory mechanism. If in the place of 'barosensory reflex' one substitutes 'small fibre depressor reflex', and in place of 'chemosensory reflex', 'larger fibre pressor reflex' the interpretation becomes in effect precisely similar to that arrived at in Chap. IX, and it is therefore considered that besides throwing light on the mechanism of the action of nembutal, these perfusion experiments have tested and supported the assumptions which allowed of this earlier interpretation, that is to say, the analysis of the sinus nerve fibre content achieved in Chap. VIII.

During the present investigation NEIL, REDWOOD and SCHWEITZER (1948) reported that electrical stimulation of the sinus nerve in the chloralosed cat yielded pressor responses. It is possible that chloralose may act in a similar fashion to nembutal. These authors, however, employed stimulus intensities which have been found in the present series to be quite inadequate to obtain the classical depressor response, and it is therefore not clear to what extent the effects are due to that anaesthetic.

PART 111.

AUTHOR'S WORK (continued).

CHAPTER XI.

CERTAIN CAROTID SINUS REFLEXES AND THE EFFECT OF NEMBUTAL.

In this chapter an account will be given of a variety of experiments which were carried out for two main reasons, firstly, to prove that the structure taken as being the sinus nerve, and which yielded such unexpected responses to electrical stimulation, was indeed that nerve and no other, and secondly, to determine the state of the carotid sinus reflexes in relation to the effects of electrical stimulation of the sinus nerve, both in the normally and in the profoundly nembutalized cat.

(1) EXPERIMENTS TO CONFIRM THE IDENTITY OF THE SINUS NERVE.

In an earlier chapter (Chap. II) reference has been made to such experiments, and they will be indicated briefly here.

Methods:

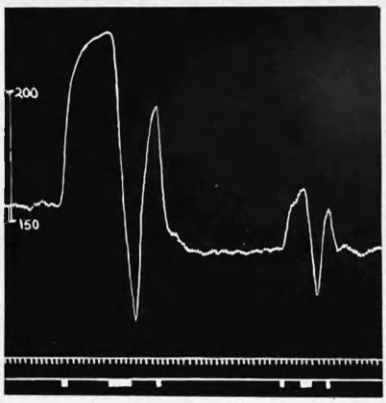
Cats were employed. After the effects of 'sinus' nerve stimulation had been studied, the nerve was 'proved' by methods similar to those first adopted by HERING (1927). Firstly the common carotid artery was clipped. This caused the usual reflex rise in blood pressure. Secondly, the effect of occlusion was repeated after section or cooling of the structure in question. And thirdly, in the cooled nerve, the test was repeated after warming.

Results:

In/

THE OCCLUSION AND TRACTION TESTS IN

LIGHT NEMBUTAL ANAESTHESIA.



A B

Fig. 46. Cat. Anaesthetic nembutal (40 mg/Kg.). Double vagotomy. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left femoral artery). Time 5 sec.

Two tests are shown. In each the signals, from left to right, denote application of occlusion, traction and release of occlusion.

A. The tests applied to both common carotid arteries.

B. " " " " the right common carotid artery alone.

In five animals it was found that either cutting or cooling abolished the reflex rise of blood pressure consequent on occlusion, while in the three animals in which the nerve had been cooled, the reflex was regained by warming the nerve.

Interpretation:

These experiments proved that the structure stimulated was, in fact, the sinus nerve. The perfusion experiments put the matter beyond all doubt.

(2) EXPERIMENTS TO DETERMINE THE STATE OF THE CAROTID SINUS REFLEXES IN NEMBUTAL ANAESTHESIA.

(a) Carotid Sinus Reflexes in Light Nembutal Anaesthesia.

Methods:

The carotid sinus reflexes were studied in more than twenty lightly anaesthetized cats in which the effects of sinus nerve stimulation were being investigated. The reflexes were tested by occlusion or traction, the former by clipping the common carotid artery low in the neck with a bulldog clip, and the latter by looping the occluded vessel and pulling manually in a caudal direction.

Results:

In every animal, results similar to those of Fig. 46 A were obtained, that is to say the characteristic rise of systemic blood pressure on occlusion and fall on traction.

Quantitative differences were observed, however, the response as usual being greatest when both sides were tested, or/

or when the opposite sinus nerve was cut, or had its buffering power otherwise lessened, and least when such conditions did not hold (Fig. 46 B).

Variation in the magnitude of the responses was observed from causes other than technical, and appeared to be related to the depth of anaesthesia or dosage of nembutal, increase in depth or dosage showing a distinct tendency to diminish the response.

Interpretation:

Electrical stimulation of the sinus nerve, carried out at the same time as the above-mentioned tests, showed all the effects already described (Chap. VIII) i.e. powerful pressor responses in each animal becoming depressor in most instances with increase in stimulus intensity, but in a few animals remaining pressor, although diminished, despite the maximum stimulus from the WHITFIELD device.

Now in every animal the effects of occlusion and traction demonstrated an active depressor barosensory pathway, even in those where maximal stimulation with the WHITFIELD device yielded only a pressor response. It was apparent that electrical stimulation was failing to reproduce the depressor function of the nerve, and that, unless such stimulation of the barosensory fibres were credited with having an effect quite the opposite from activation of the fibres through their receptor mechanisms, the pressor effects were not to be attributed to any altered effect of the barosensory fibres caused by the anaesthetic. The evidence/

evidence indicated that true pressor fibres were involved, and that the pressor response in light nembutal anaesthesia was not due to the anaesthetic, but was a function of the stimulus. Arguments are advanced elsewhere that the pressor fibres include those from the chemoreceptors (Chaps. VIII and X).

The value of these experiments did not rest solely, however, in the indication of the mechanism of the pressor response. The normal nature of the sinus reflexes in the lightly nembutalized animal allowed the analysis of the sinus nerve responses to be made under this drug. This was of distinct advantage, in that the effects of nembutal overdosage could also be studied in the same animals at a later stage in the experiments. The adoption of this course was later justified by the urethane series.

Finally, the experiments provided valuable controls for the similar tests carried out after excess nembutal which may now be detailed.

(b) Carotid Sinus Reflexes in Deep Nembutal Anaesthesia.

Methods:

The carotid sinus reflexes elicited by occlusion and traction were studied in more than a dozen cats after excess nembutal. In most of these, controls had been obtained in light nembutal anaesthesia.

Results:

Whereas the tests had yielded straightforward classical responses/

THE OCCLUSION AND TRACTION TESTS IN

DEEP NEMBUTAL ANAESTHESIA.

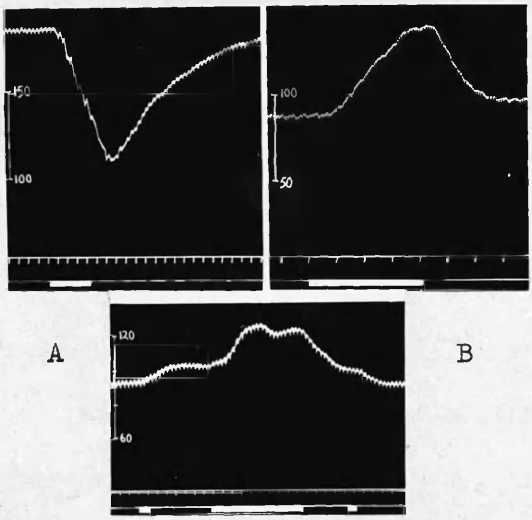


Fig. 47. Cat. Anaesthetic nembutal. Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec.

A. Light anaesthesia. Total nembutal 45 mg/Kg. Control stimulation of right sinus nerve.

B. Deep anaesthesia. Total nembutal 70 mg/Kg. Control stimulation of right sinus nerve repeated.

C. A few minutes after B. The occlusion and traction tests applied to the right common carotid artery as in Fig. 46.

responses in light nembutal anaesthesia, they produced very different effects after excess of the drug.

The occlusion test became less effective as successive doses of nembutal were administered, in some animals a few mg/Kg. causing marked diminution of the normal pressor response. This effect was especially well marked a few minutes after intraperitoneal injection. When nembutal had been given in sufficient amount to cause a 'reversal' of the effects of electrical stimulation of the sinus nerve, occlusion in most instances was quite without effect on the blood pressure. In four animals, however, feeble but distinct pressor effects were observed.

The effect of nembutal on the traction test was much more remarkable. In every case a distinct and usually powerful rise in blood pressure resulted (Fig. 47).

Interpretation:

The depressant effect of nembutal on the barosensory mechanisms activated by occlusion would appear to be similar to that of other barbiturates tested by VERCAUTERAN (1932) and NOWAK (1934).

The reversal of the effects of traction, at a time when the response to electrical stimulation of the sinus nerve was also reversed, suggested at first sight that the latter response was due to a true reversal of barosensory fibres, since these are the afferents commonly accepted as being activated by traction. Now both tests are generally regarded as testing baroreceptor/

baroreceptor reflexes, occlusion decreasing the number of stretch receptors active and traction having precisely the opposite effect. In four animals, however, both occlusion and traction caused pressor effects (Fig. 47). The former test, then, implied that baroreceptors activated by the blood pressure in the sinus were having a normal depressor effect, while the baroreceptors activated by traction were having a pressor effect. This appeared to be improbable. The need for some other explanation was further stressed by the finding that section of the sinus nerve did not cause a rise in blood pressure in the profoundly nembutalized animals in which occlusion had this effect (such as that illustrated in Fig. 47). Nerve section in these instances, as in the others where occlusion was ineffectual, resulted in no change in the systemic blood pressure whatsoever, and therefore inferred that the pressor effect of occlusion was active and not due to decrease in tonic inhibiting baro-sensory activity.

It was decided that further investigation into the mechanism of the two tests was necessary before their effects in profound nembutal anaesthesia could be analysed.

(c) The Occlusion and Traction Tests More Closely Examined.

The Occlusion Test:

Occlusion of the common carotid artery has long been known to cause a rise of systemic blood pressure. The early interpretations of the phenomen have already been discussed

(Part 1), and need no further mention. Suffice it to say that the experiments of HERING (1927) proved that the pressor effect was reflex, and due to a loss of tonic depressor activity passing over the sinus nerve, and that occlusion is commonly regarded as abolishing the buffering power of the sinus nerve (McDOWALL 1935). Certain findings in the present series, however, suggest that the effect on the barosensory mechanism is more complex and these are discussed below. Apart altogether from the pressure reflexes, which are those most obviously concerned in the test, there would appear to be a concomitant effect on the chemosensory mechanisms. Thus, EULER and LILJESTRAND (1943) maintain that the increased respiratory activity consequent on common carotid occlusion is largely due to stimulation of chemoreceptors caused by the altered blood supply to the carotid body.

It was of interest to determine the change in sinus nerve activity following on occlusion.

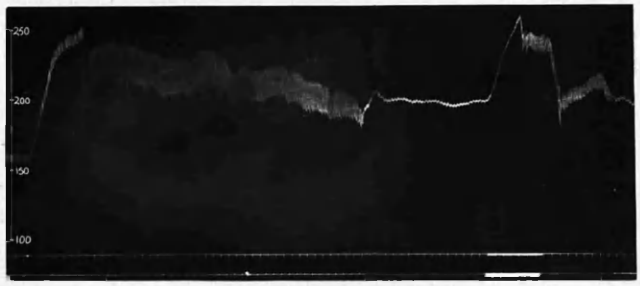
Methods:

Animals were not specially set aside for the study, but experiments were made on vagotomized cats lightly anaesthetized with nembutal, in which the effects of buffer nerve stimulation were being investigated. The effect of occlusion of the common carotid artery on the tonic activity of the sinus nerve was estimated by sectioning or cooling the nerve after the vessel had been clipped or tied. The nerve was cut with sharp scissors or cooled with a device specially constructed for the purpose.

This/

THE OCCLUSION TEST.

1. BUFFER ACTIVITY PERSISTING AFTER OCCLUSION.



A B

Fig. 48. Cat. Anaesthetic nembutal (45 mg/Kg.). Double vagotomy. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec. The left carotid artery has been tied and sectioned well below the sinus region. The left and right sinus nerves are intact.

A. Occlusion of right common carotid artery.

B. The left sinus nerve cooled.

2. THE COLLATERAL CIRCULATION IN THE OCCLUDED SINUS.

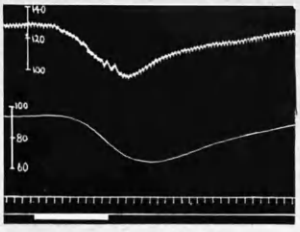


Fig. 49. The same animal as in Fig. 48.

Upper record: Systemic B.P. (cardiac end left common carotid artery).

Lower record: Endosinusal B.P.(cephalic " " ").

The right sinus nerve was stimulated at the signal.

This apparatus consisted of a small copper channel, into which the sinus nerve could be fitted, brazed to the loop of a narrow gauge copper tube doubled acutely on itself and through which a stream of iced brine could be pumped from a pressure bottle. In all the experiments buffering by the other sinus was minimized by occluding the vessel on that side (the nerve was not cut as it was to be stimulated later).

Results:

Section (2 cats) or cooling (3 cats) of the sinus nerve serving the occluded carotid sinus caused a marked rise in systemic blood pressure in each instance. One experiment is illustrated in Fig. 48. In this animal the left common carotid artery was tied, and with the right patent the blood pressure ran about 160 mm. Hg. When the right common carotid was also occluded there was the usual marked pressor response, in this case accompanied by marked cardiac irregularity. After a few minutes the systemic pressure had fallen considerably and normal cardiac rhythm had been re-established. At this stage, however, cooling the left sinus nerve caused a further pressor effect and reappearance of cardiac irregularity, both of which were abolished by warming the sinus nerve (with a stream of Ringer-Locke at 38°C.).

Interpretation:

The results indicated that occlusion of the common carotid artery did not abolish the tonic depressor function of the sinus nerve, and suggested that the endosinusal blood pressure/

pressure under these circumstances was of sufficient magnitude to maintain a significant degree of barosensory activity. Certainly the sinus region has a well-marked collateral circulation by way of the occipital and internal carotid arteries which is readily demonstrable on dissection. The influence of this collateral circulation on the chemoreceptor effects of occlusion have already been indicated by EULER and LILJESTRAND (1943). In one animal the pressure within the 'occluded' sinus was measured. It was found to be of a magnitude sufficient to account for considerable barosensory activity, and moreover, was found closely to follow coincident changes in systemic blood pressure (Fig. 49).

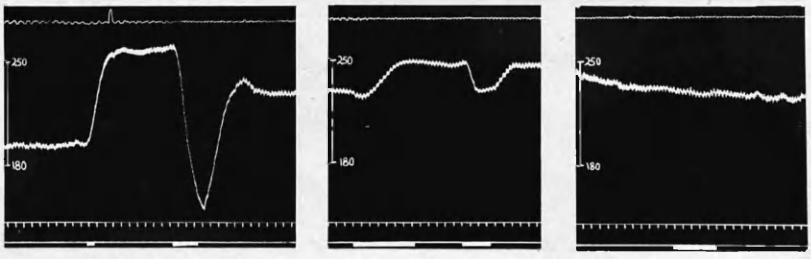
It was apparent that common carotid occlusion, besides setting up chemosensory reflexes as suggested by EULER and others (see page 37), might leave a considerable degree of barosensory activity and buffering power. These findings made it desirable to denervate the occluded sinus where such effects were to be avoided, as in many of the present series, and furthermore, had to be borne in mind in interpreting any results brought about by the test.

The Traction Test:

SOLLMAN and BROWN (1912), who discovered the depressor effect of traction on the common carotid artery, attributed the response to mechanical excitation of the nervous plexus in the sinus region. This view was abandoned when HERING's (1927) studies revealed the pressure sensitive functions of the sinus nerve, and it is commonly believed that the effect is brought about/

THE TRACTION TEST.

THE DEPRESSOR EFFECT PERSISTING WHEN THE SINUS NERVE IS FROZEN.



A B C D E

Fig. 50. Cat. Anaesthetic nembutal (40 mg/Kg.). Double vagotomy. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Respiratory movements stethograph. Time 5 sec.

A. Right common carotid artery tied.

B. Traction on this vessel.

C. The right sinus nerve frozen.

D. Traction repeated.

E. Traction repeated after section of the sinus nerve.

about by excitation of the stretch receptors in the walls of the carotid sinus. During the present series traction was carried out in animals in most of which the sinus region had been dissected to expose the nerve, either for stimulation or section, and it was early seen that the test caused considerable distortion of the sinus region and some tension on the sinus nerve itself. So much so, that sinus nerve responses were not studied in nerves which had been thus treated. This finding, moreover, suggested that these mechanical changes might play some part in the traction reflex and the matter was put to experimental test.

Methods:

Lightly nembutalized cats with both vagi cut were employed. One carotid artery was occluded throughout the tests. The other was tied off well below the sinus region and the ligatured vessel tugged firmly in a caudal direction. The sinus nerve on this side was then frozen solid and traction repeated. Finally, the sinus nerve was cut and the test again performed.

Results: Three animals were studied. Similar results were obtained in each instance. Traction before freezing gave the expected powerful depressor response. During the time when the sinus nerve was frozen solid, traction gave a distinct but less pronounced fall. After section of the nerve no effect whatsoever was obtained on repeating the test (Fig. 50). The buffering action of the occluded sinus is again noticeable

(Fig/

(Fig. 50 C).

Interpretation:

Any effect of traction brought about when the sinus nerve was frozen could not be attributed to activation of the receptor mechanisms, or indeed to any effect on that part of the sinus nerve peripheral to the cold block. The abolition of the effect by sinus nerve section indicated that it was dependent on the anatomical integrity of the nerve. It is concluded that traction exerts its effect, not only by exciting the receptor mechanisms, but by mechanical stimulation of the sinus nerve itself.

(d) The Carotid Sinus Reflexes in Deep Nembutal Anaesthesia. An Analysis.

Occlusion:

The gradual diminution in the efficacy of the test witnesses the depressant action of nembutal on the barosensory mechanisms. The pronounced but transient effect which follows a short time after intraperitoneal injection would appear to be analogous to the 'areflexia' seen after heavier dosage. The complete absence of the reflex after nembutal overdosage sufficient to cause 'reversal' of the effects of sinus nerve stimulation is in accord with the effect of the drug on the barosensory mechanism as demonstrated in the perfusion experiments. On the other hand, the persistence of a pressor effect on occlusion is not. Reasons have been given (3 above) for considering this effect as indicative of excitatory activity/

activity set up in the sinus nerve. If this is so, then one possible explanation is in the test giving rise to chemoreceptor activity (4 above). This view would receive support from the results of perfusion, and is of considerable importance, for in the perfusion experiments the chemosensory reflexes were elicited with a synaptotropic agent (acetylcholine) which, of course, by acting on a point on the afferent pathway does not test the integrity of the true receptor mechanism. If the pressor effect of occlusion be accepted as caused by chemosensory activity, it implies that the chemoreceptor cells are responsive to their normal excitant, that is change in the chemical composition of the blood. The fact that the pressor effect was not observed in eight animals out of twelve in which the test was made does not indicate abolition of the chemosensory mechanisms effectiveness. EULER and LILJESTRAND (1943) found they could abolish the chemoexcitant effects of occlusion by administering oxygen, and it is possible that in these animals the combination of well oxygenated blood (the animals were artificially ventilated) and adequate collateral circulation prevented any significant local asphyxia and consequently no effective stimulus.

Traction:

The 'reversed' effect of traction must be interpreted in the light of the findings laid out in section C. Two facts are of obvious importance, first the mechanical effect on the sinus nerve itself, and second the distortion of the sinus region/

THE TRACTION TEST IN DEEP NEMBUTAL ANAESTHESIA.

EXPERIMENTAL ANALYSIS.

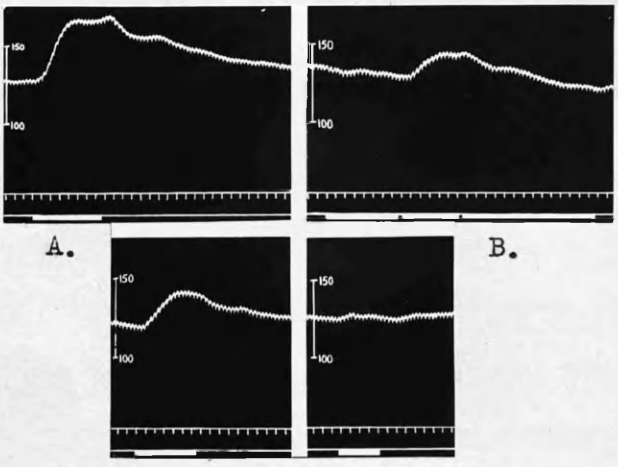


Fig. 51. Cat. Anaesthetic nembutal. (Total 65 mg/Kg.). Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec. The right common carotid artery is occluded throughout.

A. Traction on the right common carotid artery.
B. The test repeated when the right sinus nerve is frozen.
Between B and C the right sinus nerve has been cut.
C. Mechanical stimulation of the central end of the cut right sinus nerve.
D. Traction repeated after sinus nerve section.

region. The first point lends itself to investigation in overdosage just as in light anaesthesia, and similar experiments involving nerve freezing and traction were carried out in two animals. In both, similar results were obtained, the pressor response persisting despite the sinus nerve being frozen solid, being absent when the test was repeated after nerve section, and being caused by mechanical stimulation of the sinus nerve (pinching and traction) (Fig. 51). Part of the effect of traction, therefore, as in the lightly anaesthetized animal, can be attributed to its mechanical stimulation of the sinus nerve itself. It is of interest that the maximum pressor effect in both animals was obtained when carotid traction was carried out without excluding the receptor mechanisms. A possible explanation lies in the second effect of traction, the gross distortion of the sinus region. This, without doubt, must markedly diminish, if not abolish entirely, the collateral circulation, and might well lead to excitation of the chemoreceptors.

It is apparent that the pressor effect of traction does not necessarily imply a 'reversed' action of baroreceptors, but can well be explained on a basis of persisting pressor fibre activity in the sinus nerve itself. Certainly the perfusion experiments preclude the effect being due to the former, and support the view that it is due rather to an unmasking of chemosensory activity by the selective depressant action of the nembutal on the barosensory mechanisms.

C H A P T E R XII.

VAGUS AND SINUS NERVE REFLEXES AT LOW BLOOD PRESSURE.

Pressor activity in the buffer nerves associated with low systemic or intrasinusal blood pressure, has been observed by several workers, among them McDOWALL (1925), HEYMANS, BOUCKAERT and REGNIERS (1933) and WRIGHT (1932). In many of the early experiments on the 'buffer' nerve reflexes in deep nembutal anaesthesia, the 'reversed' response was observed only at the low pressures associated with gross overdosage. Steps were therefore taken to ascertain the effect of buffer nerve stimulation in the normally anaesthetized animal when the blood pressure was low.

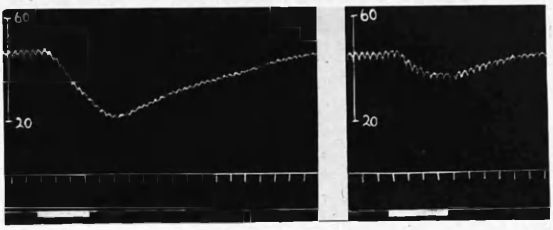
Methods:

Animals were not specially set aside for the study, but use was made of nembutalized cats which had been employed in other investigations. In two of these, the blood pressure had fallen markedly of its own accord as a result of lengthy experimental procedures. In another two, the pressure was lowered by repeated bleedings. Two more animals completed the group, these were under urethane anaesthesia, the low pressure being consequent upon the combined effects of operative interference on the skull and bleeding.

Results:/

THE EFFECTS OF ELECTRICAL STIMULATION OF THE VAGUS AND SINUS NERVES AT LOW BLOOD PRESSURE.

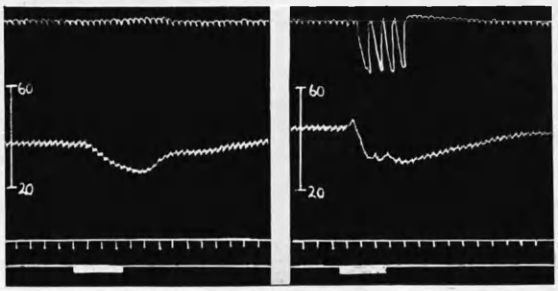
1. AFTER PROLONGED EXPERIMENT.



A B

Fig. 52. Cat. Anaesthetic nembutal. Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec.
A. Stimulation of right vagus nerve 40 V. 30/sec. (Whitfield).
B. " " " sinus nerve 40 V. 30/sec. (Whitfield).
Tracings obtained 8 hr. 10 min. after induction. Repeated small doses of nembutal had been administered to maintain anaesthesia.

2. AFTER REPEATED BLEEDINGS.



A B

Fig. 53. Cat. Anaesthetic nembutal (40 mg/Kg.). Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Respiratory movements stethograph. Time 5 sec.

A. Stimulation of right vagus nerve 40 V. 30/sec. (Whitfield).
B. " " " sinus nerve 40 V. 30/sec. (Whitfield).

Tracings obtained 2 hr. 35 min. after induction, and after repeated bleedings to lower B.P.

Results:

In all these animals, buffer nerve stimulation yielded marked depressor responses (Figs. 52 and 53).

Interpretation:

The experiments indicated that the 'reversal' seen in nembutal overdosage was not due to low blood pressure. This view found ample support from the later experiments in which the reversed response was achieved when the blood pressure was much higher, either as a result of lighter nembutal dosage or from the action of picrotoxin.

CHAPTER XIII.

THE AORTIC NERVES.

In the analysis of the effects of nembutal and picrotoxin on the response to sinus nerve stimulation, it has been shown that the phenomenon of 'reversal' is concerned with the chemosensory and barosensory afferents of that nerve (Chap. X). The vagus certainly contains similar pressor and depressor afferents from the baroreceptors and chemoreceptors of the aortic arch region, and indeed LIDDELL and SHERRINGTON (1929) maintain that electrical stimulation of the cat vagus yields results much the same as from the depressor nerve itself which is the chosen pathway for these two groups of afferents, but, nevertheless, the vagus does contain known pressor afferents from other sources, and this very fact detracts from the value of any comparison between that nerve and the sinus nerve. More significant would be a comparison of the effects of the two drugs on the sinus and aortic nerves, each of which is composed essentially of barosensory and chemosensory fibres. This was done in a few animals.

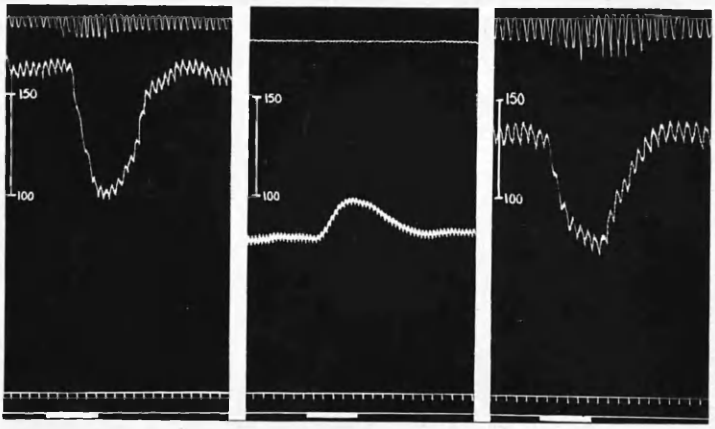
Methods:

(a) Preparation of the Animal.

The aortic nerve reflexes were studied in three cats. In each, the trachea was cannulated, the left sinus nerve divided and both vagi cut low in the neck. The right sinus nerve and the/

ELECTRICAL STIMULATION OF THE AORTIC NERVE.

THE EFFECTS OF NEMBUTAL AND PICROTOXIN.



A B C

Fig. 54. Cat. Anaesthetic nembutal. Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left femoral artery). Respiratory movements stethograph. Time 5 sec.

A. Nembutal 45 mg/Kg. Control stimulation of left aortic nerve (10 V. 70/sec. 1.0 msec.).

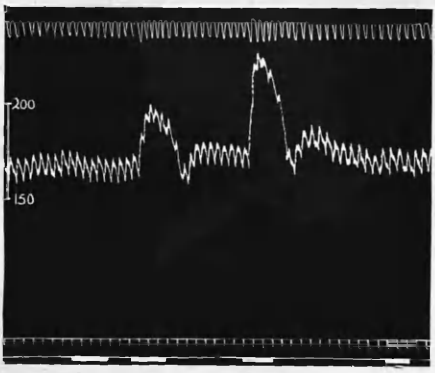
B. Nembutal 70 mg/Kg. Control stimulus repeated.

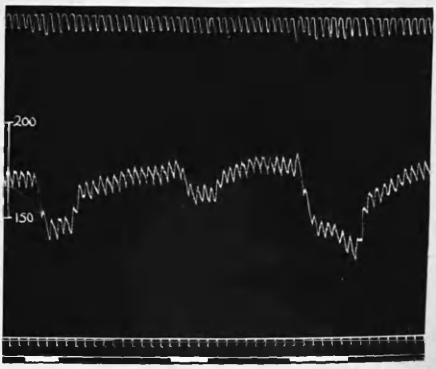
C. Twenty minutes after B. Three injections of picrotoxin have been given (2.4 mg/Kg.). Control stimulus repeated.

ELECTRICAL STIMULATION OF THE SINUS AND AORTIC NERVES.

THE RESPONSES CONTRASTED.

A.





B.

2V. 5V. 20V. 3V. 5V. 0.5V. 20V.

Fig. 55. The same animal as in Fig. 54 (during light anaesthesia).

Series A. Stimulation of the right sinus nerve.
Series B. " " " left aortic "
Stimulation throughout with 0.02 msec, pulses at 70/sec. voltages as shown. (B.N.I. stimulator).

the left aortic nerve were prepared for stimulation. (The aortic nerve was more easily defined on the left side in each of these animals, and was identified high up as it left the superior laryngeal nerve to join the vagus. It was separated from the latter for a few millimetres).

(b) Stimulation.

The sinus nerve electrodes and the B.N.I. stimulator were employed for both nerves.

(c) Conduct of the Experiment.

Both nerves were stimulated in normal nembutal anaesthesia, again after excess of the drug and finally, in two of the animals, after picrotoxin.

Results:

(1) In each of the three animals it was found that the powerful depressor responses to aortic nerve stimulation could be converted to pressor effects by injecting excess nembutal (in two animals half the anaesthetic dose, in the third, three-quarters).

(2) Picrotoxin was given to two of the animals when 'reversed' responses were established. In both, it caused a prompt reappearance of the depressor effect. (1.1 mg/Kg. body weight in one animal, 2.4 mg/Kg. in the other, which was that most heavily overdosed).

These effects are illustrated in Fig. 54. The aortic nerve has thus shown the same phenomena as have been repeatedly found in the vagus, and would appear to be affected by nembutal and picrotoxin/

picrotoxin just as is that nerve, or indeed the sinus nerve.

It must be pointed out, however, that although there is close agreement between the sinus and aortic nerves as regards the changes brought about by the two drugs, there are striking differences in the effects of electrical stimulation during light nembutal anaesthesia. In all three animals, pressor responses were easily elicited from the sinus nerve with the less powerful stimuli (Fig. 55 A), but in none was this the case with the aortic nerve, in which the first response to liminal stimulation was invariably a fall in blood pressure, which increased in extent as the intensity of the stimulus was raised (Fig. 55 B).

Interpretation:

The demonstration that the aortic nerve component of the vagus is affected by nembutal and picrotoxin in a similar fashion to the whole nerve, opens up the way for an interpretation of the effects of these drugs by analogy with their known effects on the sinus mechanism. Comparisons are certainly justified on a functional basis, for the recent work of GERNANDT (1946) has clearly indicated the similarity between the two nerves, as regards both barosensory and chemosensory content. It may be then, that the effects of nembutal and picrotoxin on the aortic nerve reflexes are brought about by a selective abolition and restoration of the barosensory mechanism relative to the chemosensory. The matter has not been put to experimental proof, and no arguments other than analogy may be offered.

Whatever/

Whatever the functional similarity between the sinus and aortic nerves, and GERNANDT (1946) has shown that both large and small barosensory fibres exist in the latter as they do in the former, it is remarkable that the two should yield such widely differing results on stimulation in the normally anaesthetized animal. It is possible that there are large depressor fibres in the aortic nerve, of such power that they effectively mask the chemosensory pressor component, which moreover appears to be relatively weak in the aortic nerve as compared with the sinus nerve (GERNANDT, 1946).

CHAPTER XIV.

RESPIRATORY REFLEXES AND THE EFFECT OF NEMBUTAL.

Although the present investigation was primarily concerned with the effect of nembutal on the vasomotor responses to buffer nerve stimulation, one or two experiments sufficed to show that the drug also affects the character of the respiratory reflexes. Close attention was thereafter paid to this aspect of the problem.

Methods:

Except in a few of the early experiments, respiratory movements both in light and deep nembutal anaesthesia were recorded by chest stethograph throughout any investigation involving stimulation of the sinus or vagus nerves. In this way observations were made in thirty-four cats.

Results:

(a) Vagus Nerve.

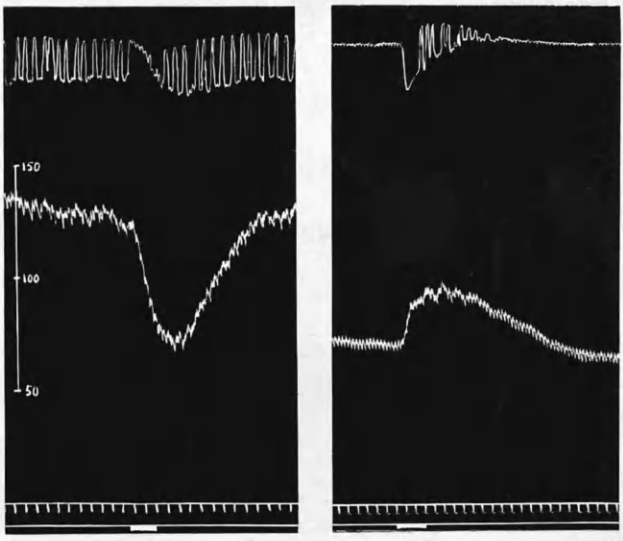
In light (normal) nembutal anaesthesia the strong stimulus employed to obtain the control depressor effect usually inhibited breathing. The duration of inhibition seemed to depend on the degree of spontaneous respiratory activity present (all animals were being artificially ventilated). Where breathing was brisk, the effect tended to be confined more or less to the duration of stimulation (Fig. 56 A), while a much more prolonged effect was the rule where breathing was sluggish (Fig. 57 A).

When/

RESPIRATORY REFLEXES

AND THE EFFECT OF NEMBUTAL.

ELECTRICAL STIMULATION OF THE VAGUS NERVE.

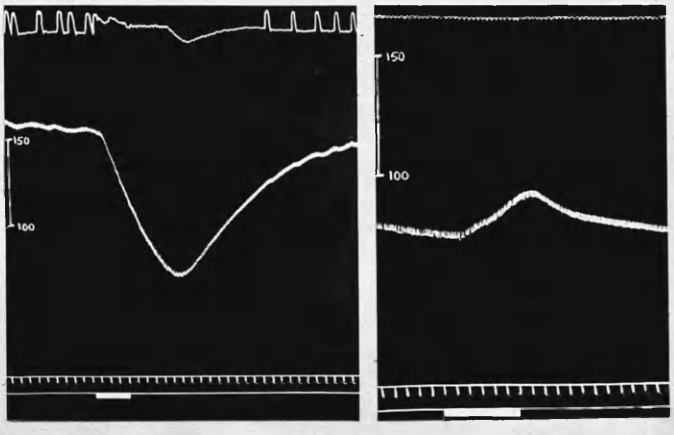


A B

Fig. 56. Cat. Anaesthetic nembutal. Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Respiratory movements stethograph. Time 5 sec.

A. Stimulation of right vagus (20 V. 70/sec. 1 msec.). Nembutal 40 mg/Kg.

B. " " " " (20 V. 70/sec. 1 msec.). Nembutal 65 mg/Kg.



A B

Fig. 57. Another animal. Preparation and records as Fig. 56.

A. Stimulation of right vagus (20 V. 70/sec. 1 msec.). Nembutal 45 mg/Kg.

B. " " " " (20 V. 70/sec. 1 msec.). Nembutal 75 mg/Kg.

When nembutal had been added in excess to 'reverse' the vasomotor response, spontaneous respiratory activity was absent, presumably due to the well known depressant effect on the medullary centres which were of course deprived of the defensive central and reflex excitatory stimuli - CO_2 excess and O_2 lack - by virtue of the artificial ventilation. In thirteen animals, however, control stimulation of the vagus caused a burst of respiratory activity in addition to the pressor effect (Fig. 56 B). In the others it did not, and the pressor effect was unaccompanied by any respiratory activity. This was notably the case where overdosage was pronounced or where breathing had been sluggish in light anaesthesia (Fig. 57 B).

(b) Sinus Nerve.

In normal anaesthesia, a wide range of stimulus strengths caused only respiratory stimulation. In no lightly nembutalized animal was there observed any other effect. It is of interest to note that in most animals very powerful respiratory effects could be obtained with stimulus intensities insufficient to evoke the depressor response, and that further increase in intensity usually, although not always, served to augment the effects. Where spontaneous respiratory movement was absent, from somewhat excessive pulmonary ventilation aided in some instances by slightly greater depth of anaesthesia than usual, it could be re-established readily by stimulation of the sinus nerve at longer or shorter intervals. In such instances, the marked excitant effects during each stimulus became less on

cessation/

RESPIRATORY REFLEXES

AND THE EFFECTS OF NEMBUTAL.

THE EFFECTS OF ELECTRICAL STIMULATION OF THE

SINUS AND VAGUS NERVES COMPARED.

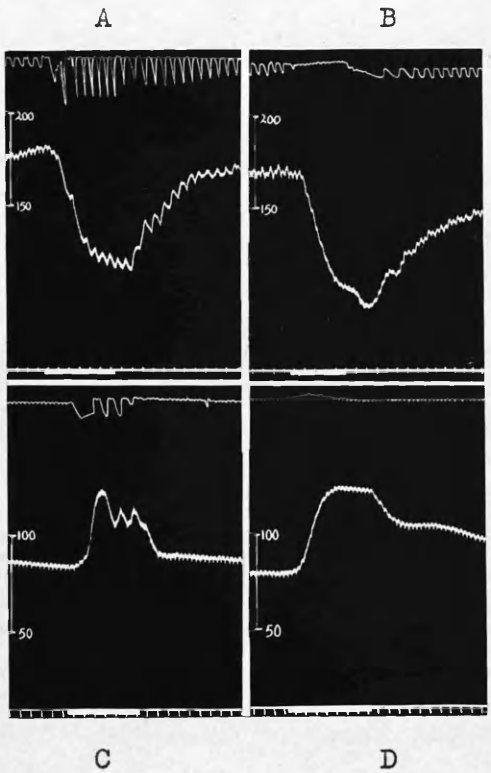


Fig. 58. Cat. Anaesthetic nembutal (45 mg/Kg.). Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Respiratory movements stethograph. Time 5 sec.
Upper two tracings during light anaesthesia.
A. Stimulation of the right sinus nerve. (30 V. 30/sec. 1.2 msec.).
B. " " " " vagus " (30 V. 30/sec. 1.2 msec.)
Lower two tracings during deep anaesthesia (70 mg/Kg.).
C. Stimulation of the right sinus nerve (30 V. 30/sec. 1.2 msec.)
D. " " " " vagus " (30 V. 30/sec. 1.2 msec.).

(Thyratron stimulator employed).

cessation of stimulation, but persisted, although gradually declining, until the next stimulus was applied. Again it must be emphasized that this pronounced stimulatory effect on breathing was readily obtainable with the moderate voltages yielding pressor responses.

When reversal of the vasomotor response had been achieved by nembutal overdosage, the control stimulus in twenty-one of the animals showed a persistence of the respiratory stimulant effect, although to a diminished extent. The effect was much more readily observed than in the vagus (Fig. 58). Again it tended to be absent in those animals in which overdosage with nembutal was excessive, or where spontaneous respiratory activity in light anaesthesia had been feeble.

Interpretation:

(a) Sinus Nerve.

During light anaesthesia, the powerful, regular, sustained respiratory efforts resulting from electrical stimulation, and the prolonged stimulant effect on the quiescent respiratory centre, strongly suggest chemosensory excitation. The fact that such effects are sometimes maximal and usually very powerful with stimuli insufficiently strong to mobilize the depressor fibres in significant number, lends further support to the view that chemosensory afferents are included in the 'pressor' group of fibres activated with these less powerful stimuli. More powerful 'depressor' stimulation of the nerve may be regarded as causing its augmented respiratory effects

by/

by mobilizing more chemosensory fibres within the excitability range of the depressor afferents. Such an overlap has been suggested elsewhere (Chap. VIII, P. 106).

It is of interest to note, in the light of doubt cast by the Scandinavian workers (vide Part 1, page 37) on the respiratory depressant action of barosensory fibres, that increase in stimulus intensity to include the small depressor fibres in no instance diminished the increased breathing consequent on stimulation.

In the overdosed animal, the bursts of respiratory activity accompanying electrical stimulation are similar to those observed in the perfusion experiments in response to chemoreceptor stimulation (Chap. X, Figs. 43, 44, 45), and may be interpreted as due to excitation of the yet functioning chemosensory mechanism.

(b) Vagus Nerve.

During lighter nembutal anaesthesia the inhibition of spontaneous respiratory activity caused by strong stimulation of the vagus presents no unusual feature. It does merit discussion, however, for it raises two considerations essential to any analysis of the effects observed during profound anaesthesia. Firstly, it must be borne in mind that the inhibition derives from a complex afferent discharge, for as has been pointed out elsewhere (Chap. XIII), powerful vagus stimulation as employed in the present series must activate aortic chemosensory fibres, so that the resulting inhibition must be regarded/

regarded as the consequence of the algebraic sum of the excitor activity of these, and the inhibitor activity of other vagus afferents, the latter predominating. PARTRIDGE (1939) has indeed shown this to be the case. Secondly, due regard must be given to the essentially depressant nature of the action of nembutal on the respiratory mechanism, an action which is well known and which the present series of experiments has emphasized, the drug having been found consistently to diminish spontaneous respiratory activity and to render it increasingly susceptible to the inhibitory effect of vagus stimulation.

During profound anaesthesia, the absence of any respiratory response to vagus stimulation, as in the animals illustrated by Fig. 57, requires little comment, being as it were the expected response bearing in mind the inhibitory effect of the procedure in light anaesthesia and its augmentation by nembutal. What does demand careful analysis, however, is the phenomenon observed in the group of animals illustrated by Fig. 56 in which vagus stimulation causes a burst of respiratory activity. In these instances, there exists the situation in which the 'normal' inhibition has become replaced by 'abnormal' excitation. In other words, a 'reversal' of the respiratory response analogous to the 'reversal' of the vasomotor response simultaneously observed. It is clear that any explanation of the former is not only of interest in its own right, but has a considerable bearing on the mechanism of the latter, which is the main point of investigation. In analysing the respiratory phenomenon

advantage is taken of the fact that it does not involve the complexity of efferents that does the vasomotor. Thus the active respiratory movements in response to vagus stimulation in the overdosed animal must be attributed to rhythmical activity of the appropriate motor cells in the grey matter of the cord. This, in turn, implies an intermittent excitatory discharge from the medullary neurone pool forming the respiratory centre. Such behaviour of the centre is precisely opposed to that observed in lighter anaesthesia, and it is thus apparent that the 'reversal' must be caused by an action of nembutal on the centre itself or on the afferent pathway. This much seems clear, and is in itself a conclusion of considerable importance. A more accurate localisation of the site of action of the drug cannot be made with any certainty, nevertheless, consideration of the factors involved leads to certain tentative conclusions. The problem is best viewed from two angles, central and afferent, in each of which consideration must be given to the complexity of the afferent discharge set up in the vagus. From the former viewpoint, the respiratory 'reversal' may be regarded as an altered response on the part of the centre itself to afferent bombardment of similar pattern to that obtaining in light anaesthesia, and from the latter, as due to a normal response of the centre to an altered afferent discharge or pattern.

If the nembutal be conceived as acting on the centre, then a 'reversed' response might be accounted for either by the drug rendering the neurone pool insensitive to the inhibitor element

of the compound afferent discharge, while interfering little with the excitor component, that is to say rendering the centre more susceptible to excitation and less susceptible to inhibition, or by it actually causing the afferent discharge of normal inhibitory quality or pattern, to have excitor effect, in fact an action similar to that postulated for strychnine by SHERRINGTON (1905) and BAYLISS (1908). Both these hypotheses, however, attribute to nembutal an excitant property contrary to its well known depressant actions, and indeed at variance with its action in lighter anaesthesia, which has been found in the present series essentially to favour inhibition.

A simpler explanation is forthcoming if the nembutal be considered as acting on the afferent side of the centre, converting the vagus discharge from inhibitory to excitatory. In this case, unless the action of the drug is to increase the absolute potency of the excitor component of the discharge, or to alter the character of the normally inhibitor component so that it becomes excitor, and here again such mechanisms are out of keeping with its known pharmacological actions, then it only remains to explain the excitant nature of the afferent discharge as being due to selective suppression of its inhibitory component. No matter the site, whether on the afferent neurone itself or some synapse on its pathway, such a mechanism ascribes to nembutal a more acceptable rôle. Moreover, a patent excitor pathway, a sine qua non of this last hypothesis, would appear to exist in the form of aortic chemo-

sensory fibres. Although the integrity of these vagal afferents has not been proved experimentally, it would seem probable in view of the persistence of activity in the analogous fibres of the sinus nerve under similar conditions (Chap. X). Finally, the mechanism postulated, which has been arrived at by argument, cannot be discounted on the score of improbability, for it is that which has been found to obtain in somewhat different circumstances. For instance PARTRIDGE (1939) has shown that a similar 'reversal' of the respiratory effects of vagus stimulation can be accomplished by cooling the nerve to a temperature which inactivates the inhibitor fibres but interferes little with the function of the excitor fibres from the aortic body.

CHAPTER XV.

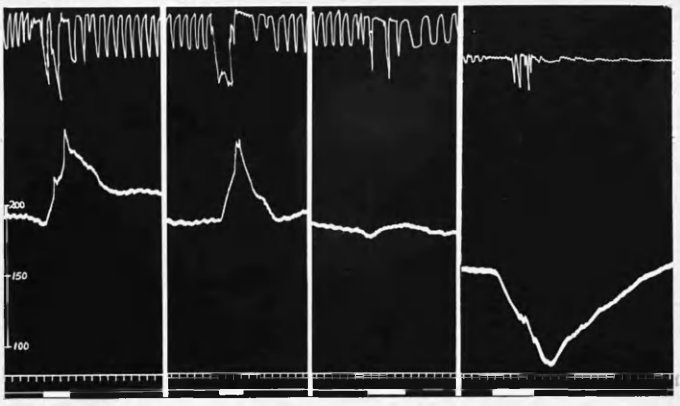
RÉFLEXES DOULOUREUX.

It has long been known that stimulation of the vagus, aortic or sinus nerves, under certain conditions, usually where anaesthesia is light or the stimulus excessive, may cause signs of pain associated with hypertension (LANGLEY, 1912, TOURNADE and MALMEJAC, 1930, and others). These 'réflexes douloureux' have been said to be of no physiological significance, and to rank among the abnormal, accidental or even pathological reflexes such as may be set up in angina pectoris (HEYMANS, BOUCKAERT and REGNIERS, 1933).

In the present series, reflexes of this type have been observed in several experiments. In each instance, but especially in the vagal reflexes, the depth of anaesthesia has been insufficient. Although presenting the same pattern in all the buffer nerves, the phenomenon was much more frequently observed on sinus nerve stimulation, so that the effect of powerful vagus stimulation in the same animal was often quite normal. Usually with feeble stimulation the hypertension was mild, associated with moderately increased breathing and without sign of agitation, in other words, an effect little different from that already described for adequately anaesthetized animals. But as the stimulus strength was increased, the hypertension became more pronounced, violent gasping respiratory movements occurred and these effects were accompanied by tremors, agitation/

REFLEXES DOULOUREUX.

ELECTRICAL STIMULATION OF THE VAGUS NERVE IN INADEQUATE ANAESTHESIA.



A B C D

Fig. 59. Cat. Anaesthetic nembutal. Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Respiratory movements stethograph.
Time 5 sec.
Tracings A, B and C all during shallow anaesthesia. (2 hr. after induction with 40 mg/Kg.).

A. Stimulation of right vagus 40 V. 30/sec. (Whitfield).

B. " " " " 20 V. 30/sec. "

C. " " " " 2 V. 30/sec. "

Between C and D 10 mg/Kg. nembutal injected intraperitoneally.

D. Stimulation of right vagus 40 V. 30/sec. (Whitfield).

agitation and sometimes convulsions. All this occurred when the stimulus was calculated to be yet insufficiently strong to mobilize a significant number of depressor fibres. Raising the stimulus intensity further, to the values required to elicit the normal depressor effect, worsened the picture, so that in fact no depressor response at all could be obtained. When additional amounts of nembutal or urethane were injected, these 'painful reflexes' disappeared. The sinus nerve then yielded pressor or depressor effects according to the intensity of the stimulus, or where vagal responses had been affected the normal depressor effect was restored (Fig. 59).

These results emphasize the need for a fair depth of anaesthesia in the study of the sinus nerve depressor reflex which involves fibres of high threshold and therefore demands powerful stimulation. Moreover, they make it quite clear that the pressor effects obtained from sinus nerve stimulation in adequate anaesthesia are not 'réflexes douloureux'.

Finally, it is of interest to note that these 'abnormal' reflexes can be obtained from the sinus nerve with stimulus strengths which excite chemosensory fibres, for recent work indicates that these possess functions relevant to the matter under discussion. For instance, SCHMIDT and COMROE (1940) have found, in lightly narcotized or unanaesthetized decerebrate dogs and cats, that stimulation of the carotid chemoreceptors (by anoxia, cyanide, lobeline, etc.) may cause very marked disturbances in the central nervous system including gross hypertension/

hypertension and respiratory activity, generalized muscular activity, micturition, defaecation and convulsions, which they state points to a far-reaching and powerful distribution of effects from chemoreceptors. SCHMIDT (1941) who reports convulsions from aortic body stimulation, postulates a physiological rôle for the powerful C.N.S. effects, suggesting that chemosensory excitation of this nature might assist in maintaining the activity of the system in the face of depression by anoxia.

The similarity between these two phenomena, the one caused by electrical stimulation and the other by chemosensory mechanisms, suggests a common basis. Certainly excitation of the chemoreceptors must be regarded as capable of eliciting the signs of pain after which the abnormal reflexes have been named, and in this respect ADRIAN's (1932) dictum - 'there is no single afferent system either for pain reactions or for pain itself' - is brought to mind.

Might not the explanation of 'réflexes douloureux' lie in electrical stimulation of the chemosensory afferents, a known content of the sinus nerve? And might not electrical stimulation in these lightly anaesthetized animals have a functional basis as suggested by SCHMIDT (1941), and in a sense reproduce one aspect of the physiology of the chemosensory afferents, an aspect which is readily masked by 'adequate' anaesthesia?

CHAPTER XVI.

THE EFFECT OF FREQUENCY OF STIMULATION OF THE SINUS NERVE.

In an earlier chapter (Chap. VIII), in discussing the interpretation to be placed on the phenomena associated with increased intensity of electrical stimulation of the sinus nerve, it has been pointed out that the great mass of evidence demands that these be explained by the stronger stimulus activating afferents of higher threshold. From time to time, however, it has been suggested that the effects of the increase are due not so much to the more powerful stimulus calling into play an additional group of afferents, but to its altering the effectiveness of the discharge set up in those already involved at the lower intensity. This it has been held to do by increasing the frequency of their transmitted impulses, the greater central summation in some manner sufficing to alter the response. This view, favoured by RANSON (1921) after a study of the vasomotor reversal effected either by increase in the intensity or rate of application of a given stimulus to sensory nerves, has found little support from subsequent work on similar structures. Nevertheless, it has gained interest more recently through the finding that in direct electrical stimulation of the hypothalamus (HARE and GEOHEGAN 1939; PITTS, LARRABEE and BRONK 1941) or medulla (BERRY, McKINLEY and HODES 1942), the frequency of stimulation determines the sign/

sign of the vasomotor response.

GORDON (1943), however, has re-examined the mechanism of the vasomotor responses to various strengths of stimulus in the light of RANSON's theory, and has been unable to find any evidence in its support. He concluded, as did BISHOP, HEINBECKER and O'LEARY (1934), that the reversal observed on increasing the intensity was dependent upon mobilization of fibres of higher threshold.

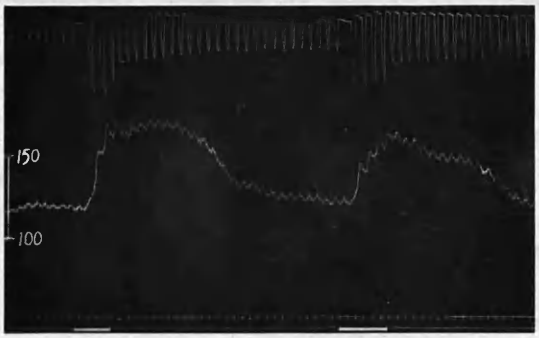
Although a similar study involving exhaustive investigation of the effects of frequency on the sinus nerve response would be of interest, the weight of argument against the repetitive theory would appear to obviate the necessity for excluding such a mechanism in explanation of the intensity reversal observed in the present series, which is essentially similar to those discussed by the opponents of the theory. In any event, time did not allow of such study being undertaken. This being so, the interpretation of the phenomenon on the basis of successive mobilization of afferents of increasing thresholds, such as has been offered in Chap. VIII, stands or falls by the classical conceptions set out in that chapter and by the findings of GORDON and others. Nevertheless, certain observations have been made regarding the effect of frequency upon the sinus nerve response which support the interpretation. These may now be detailed.

Methods:

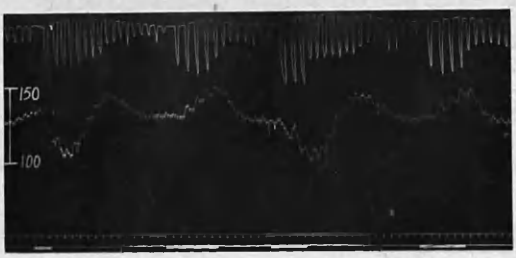
Throughout the investigation, both in cats anaesthetized with/

ELECTRICAL STIMULATION OF THE SINUS NERVE.

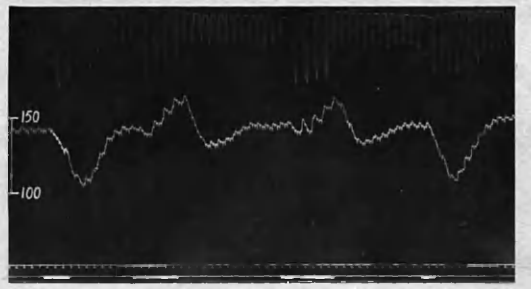
THE EFFECT OF FREQUENCY OF STIMULATION.



7/70 7/300



30/100 30/10 30/100 30/10



30/70 25/70 30/15 30/70

Fig. 60. Cat. Anaesthetic nembutal (45 mg/Kg.). Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left femoral artery). Respiratory movements stethograph. Time 5 sec.

The right sinus nerve was stimulated in each series with 0.02 msec. pulses (B.N.I. stimulator).

The voltage and frequency of each stimulus is indicated thus:- 7/70 = 7 V. 70/sec.

with nembutal and with urethane, opportunities arose which permitted observation of the responses to different rates of stimulation: in addition, one cat was devoted entirely to such a study. It was anaesthetized with nembutal and prepared in the usual way (see legend Fig. 60), the right sinus nerve being placed on the silver-silver chloride electrodes and stimulated with rectangular pulses of short duration (0.02 msec.) calculated to favour differential stimulation. The B.N.I. stimulator employed was modified to give a selection of eleven frequencies between 1.5/sec. and 350/sec.

Results:

In this last experiment it was found that:-

(1) With a stimulus intensity of seven volts., marked pressor effects were obtained with each of the frequencies over the whole range tested (between 1.5/sec. and 300/sec.). Two of the responses may be seen in the uppermost photograph in Fig. 60.

(2) When the stimulus intensity was increased, however, to a value (30 V.) that was just sufficient to produce an unequivocal fall in blood pressure at a frequency of 70/sec., it was found that by increasing the rate of stimulation to 100/sec. the depressor effect was enhanced, but that by lowering the frequency to 10/sec. the effect was reversed and a rise of blood pressure resulted. This is illustrated in the middle photograph in Fig. 60.

It seemed that the effectiveness of frequency change was dependent on the intensity of the stimulus, and this hypothesis was/

was next subjected to test.

(3) The intensity threshold was first determined, at which, at a frequency of 70/sec., a 'pressor' stimulus became 'depressor'. It was found to lie between 25 and 30 V. Thus 25 V. 70/sec. elicited a rise in blood pressure and 30 V. 70/sec. a fall. Thereafter each of the two intensities was subjected to a series of variations in frequency, each series, the 25 V. and the 30 V., being intermingled with, and running parallel to, the other. By this means, the effects of widely varying frequencies on these neighbouring voltages could be readily compared, and a possible misinterpretation due to change in intensity threshold obviated. It was found that 25 V. caused only a rise in blood pressure whether the frequency was low (15/sec.), or high (350/sec.), or indeed of any intermediate value. On the other hand 30 V. regularly had pressor effects at frequencies below 30/sec., but depressor effects when the rate was 70/sec. or more. Between these two frequencies the vasomotor response at 30 V. was indeterminate. These results could be obtained repeatedly and in mixed order. Part of the series is shown in Fig. 60 lower photograph.

This last experiment did no more than to assemble and subject to examination, phenomena which had already revealed themselves in other animals. For instance, it had been found on several occasions that altering the frequency of stimulation when the stimulus was supraminimal (pressor) left unaltered the sign of the vasomotor response, but that around the intermediate intensities/

intensities, the sign could be changed and reversals brought about by altering the frequency alone, a depressor response being favoured by the higher frequencies and vice versa.

Interpretation:

Any argument which attributes the effects of increased intensity of stimulation of a nerve to an altered effectiveness of the same fibres as activated at lower intensities, must necessarily, in the light of the fundamental concepts of neurone behaviour, hold the effect as due to the more powerful stimulus setting up an increased frequency of afferent discharge in these fibres. Where the frequency of the stimulus is the same at low and high intensity, this can result only from the latter setting up repetitive discharge in the nerve fibres. In accord therefore, with RANSON and BILLINGSLEY's (1916) view, it would be expected that increase in stimulus intensity or frequency, each by contributing to the number of afferent impulses in a temporal sense, would have the same effect. For the sinus nerve this implies that either manoeuvre might be expected to effect a reversal from the pressor to the depressor response. Under certain conditions, however, it has been found that gross alterations in stimulus frequency fail to do so, whereas a relatively minor increase in intensity is successful. The disparity in effectiveness between the two manoeuvres may be illustrated by an example from the experiment cited. Here, with a 25 V. stimulus, a five-fold increase in frequency (from 70 to 350/sec.) had little effect upon the response, whereas/

whereas an increase of intensity of but a fifth (from 25 to 30 V.) led to a reversal. In this same experiment a two hundred-fold increase in the rate of application of a 7 V. stimulus (from 1.5 to 300/sec.) was effected without altering the quality of the response. It is extremely difficult to reconcile such findings with the repetitive theory, for to do so, it must be assumed that the intensity increases bring about an even greater rate of afferent discharge in the nerve than do these large increases of stimulus frequency. The improbability of such a hypothesis is apparent when consideration is given to the fact that each stimulus lasts but 0.02 msec., that is to say about one twentieth of the duration of the absolute refractory period (even assuming the fibres to belong to the group of shortest refractory period). In brief, it would appear that the sinus nerve reversal, effected by an increase of intensity, is no more to be attributed to a 'frequency' effect than are the similar phenomena examined by GORDON (1943) and others, and that it is most readily explained in the alternative and classical manner as being caused by successive mobilization of pressor and depressor afferents.

Now, in the present series it has been found that under certain circumstances an increase in frequency, far from being without effect, alters markedly the excitant properties of the stimulus, causing the vasomotor response to be increased where it is already depressor, or on the other hand to be diminished, or even converted to a fall, where it is pressor. The clue to the/

the efficacy of the manoeuvre in each of these instances lies with the strength of stimulation. Above a certain minimal intensity, relatively small increases in frequency acquire a depressor effectiveness absent below this value (vide the 25 and 30 V. series referred to above). Consideration of this fact renders compatible with the conclusion arrived at in the preceding paragraph, findings which at first sight appear to contradict that view, for if the stronger stimulus be regarded as activating a significant number of small depressor afferents - whether the resultant afferent discharge causes a rise, a fall or an equivocal response - it is only necessary to offer in explanation a phenomenon long recognized by workers in this field which is in effect, that 'the small fibres respond better to high frequency' (GRUBER 1917), or as BISHOP, HEINBECKER and O'LEARY (1934) have said, that 'the optimum frequency for the small fibres is greater than the large'. Upon such a basis, the depressor effectiveness of increased rate of sinus nerve stimulation may be ascribed to a disproportionally increased effectiveness of the small depressor fibres. Presumably this is due to a greater central summation of their impulses over those carried by the large pressor fibres. The greater power of central summation, seemingly possessed by the small fibres over their larger fellows, is a property not peculiar to vasomotor afferents, both ADRIAN (1932) and ZOTTERMAN (1939) have discussed its existence and significance in other sensory systems.

C H A P T E R XVII.

THE MECHANISM OF THE REVERSED VASOMOTOR RESPONSE.

Analysis of the 'reversed' response to sinus nerve stimulation brought about by nembutal, either by methods depending on the relative excitabilities of the two major afferent groups (Chap. IX), or by methods involving selective stimulation of their peculiar receptor mechanisms (Chap. X), has indicated that the phenomenon is probably due to persistence of the chemosensory reflex at depths of anaesthesia at which the barosensory reflex is abolished. No indication has been given of the manner in which the drug may bring about this effect. Consideration is given to that aspect of the problem in the present chapter.

In showing that the reversed response may readily be accounted for by the normal pressor component of the nerve, and that it cannot be attributed to any action of the barosensory depressor fibres whatsoever, the problem is certainly simplified and resolves itself to a determination of the site of the nembutal block of the depressor mechanism. Despite this, the complexity of the nervous pathways involved in vasodilation precludes any one site of action being claimed without experimental proof. Especially is this so when it is considered that, besides their more obvious actions on the neurones and synapses of the central nervous system, barbiturates, including nembutal, have been shown to have an effect on various

peripheral structures, the autonomic nervous system in particular.

The precise location of the site of action by experiment proved beyond the scope of the present work. Several experimental approaches were made, however, and a certain amount of information gained.

(1) THE AFFERENT DEPRESSOR MECHANISM.

The perfusion experiments clearly indicate that the significant depressant effect of nembutal is not on the baro-receptors. Moreover, the effect of the drug on the response to electrical stimulation demonstrates that its action is central to the point of excitation. The first explanation to be considered therefore, is an effect on the depressor fibres as they course in the nerve. Such an action, however, does not seem likely, for under the conditions of the experiment the nerve is separated from the investing tissues and repeatedly washed with saline, which must considerably reduce its exposure to circulating nembutal. Moreover, the influence of circulating drugs on the exposed nerve fibres has been further diminished by perfusing the carotid sinus during light nembutal anaesthesia (control animals Chap. X), and in these animals also the effect of electrical stimulation of the sinus nerve has been 'reversed' and restored by systemic injection of nembutal and picrotoxin respectively. Nevertheless, a blocking action of nembutal on the depressor fibres as they course within the skull or medullary substance may well occur.

(2)/

(2) THE DEPRESSOR MECHANISMS OF THE CENTRAL NERVOUS SYSTEM.

A depressant action on the medullary mechanisms concerned in the barosensory reflex is the next explanation which must be entertained. Several experiments were carried out to investigate this possibility.

(a) Application of Nembutal to the Floor of the Fourth Ventricle.

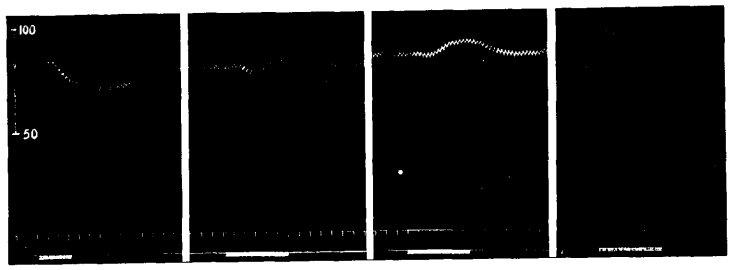
Methods:

Vagotomized cats under urethane anaesthesia were employed. In most of the animals the effects of buffer nerve stimulation were being studied as part of another series. All were artificially ventilated with bilateral open pneumothorax. When the effect of nembutal was to be studied, additional urethane was injected intravenously (0.25 gm/Kg. body weight per dose) in two or more doses until profound anaesthesia had been achieved. The animal was then turned nose down, and the back of the skull around and below the external occipital tubercle exposed. The skull was trephined through the tubercle and much of the bone forming the dorsal aspect of the cerebellar fossa (occipital and interparietal bones) removed with nibbling forceps. The cut bone was then waxed. When bleeding had thus been controlled the cerebellum was removed by sectioning the peduncles. Fresh bleeding was stemmed by inserting cotton wool pledgets into the antero-lateral angles of the fossa. In this way the entire floor of the fourth ventricle was exposed along with the medulla to 3 mm. or more below the obex. The opening

in/

APPLICATION OF NEMBUTAL TO THE FLOOR OF THE FOURTH VENTRICLE.

First Animal.

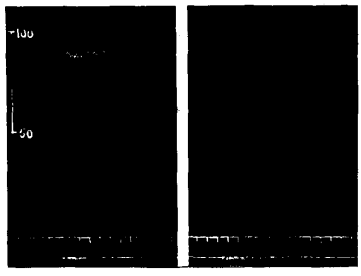


A B C D

Fig. 61. Cat. Anaesthetic urethane (1.75 g/Kg.). Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec.
The cerebellum has been removed and the floor of the fourth ventricle exposed.

A. Control stimulation of the right vagus nerve (20 V. 70/sec. 1 msec.).
B. " " repeated after applying 2 nembutal pledgets.
C. " " repeated after applying 3 nembutal pledgets.
D. " " repeated some minutes after C.

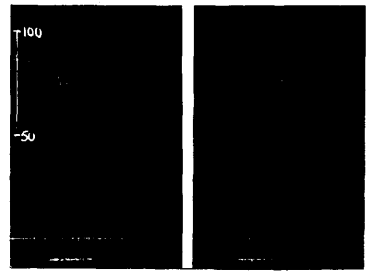
Second Animal.



A B

Fig. 62.

Third Animal.



A B

Fig. 63.

Cats. Preparation and records as in Fig. 61. Urethane 1.5 and 1.75 g/Kg. respectively.
A. Control stimulation of the right vagus nerve.
B. " " repeated after applying 3 (Fig. 62) and 2 (Fig. 63) nembutal pledgets.

in the skull was covered with gauze moistened with warm saline. The animal was now turned on its side so that both the right vagus and the exposed brain were accessible. The head was elevated and fixed in this position. The right vagus was laid across the electrodes and left untouched throughout the experiment. It was now stimulated and control depressor responses obtained. A flat cotton wool pledget of sufficient size to cover the floor of the fourth ventricle and dorsum of the medulla around the obex was saturated with 0.25 cc. of warmed nembutal solution (40 mg. nembutal per 1 cc. normal saline) and placed in position. Thereafter the vagus reflexes were tested at intervals and the pledget replaced with a fresh one as necessary.

Results:

Five successful experiments were made. In each animal the depressor effect of vagal stimulation was abolished by successive applications of nembutal.

In three animals a 'reversal' of the effect of control vagal stimulation was observed at some time after the application of nembutal. In the first of these the control depressor response became biphasic (several tests) after the second application (Fig. 61 B). After a third pledget six successive pressor responses were obtained (Fig. 61 C). These were somewhat feeble but quite definite. Ten minutes later without there being any interference whatsoever, only depressor responses could be obtained (Fig. 61 D). The two other animals provided/

provided between them only four unequivocal reversals. These were observed during short periods between the normal depressor effect and a complete absence of response, in the one case before a third pledget had abolished all effect (Fig. 62 B), and in the other as the effect of a second pledget was wearing off (Fig. 63 B). In neither of these two animals could the effect be repeated. Subsequent application of nembutal served only to abolish the response completely.

In the two remaining cats, no pressor effects were observed whatsoever despite resort being had to weaker and stronger concentrations of the drug.

Interpretation:

The experiments are somewhat similar to those carried out by SCOTT (1925). That author sought to elucidate the site of action of strychnine, which also caused a 'reversal' of the normal depressor effect of vagus stimulation in the cat, by applying that drug in high concentration in a tiny pledget to the 'depressor point', which he regarded as a point on the afferent vagal depressor path. Having shown that strychnine so applied caused vagal stimulation to yield a pressor response, he concluded that this then was the site of action of the drug, and that the strychnine 'reversal' was due to the selective paralysis of the depressor vagal afferents leaving the pressor afferents active. But any manoeuvre which is calculated to destroy the function of depressor afferents would be expected to have just such a result, and indeed the author himself (SCOTT/

(SCOTT 1925) has shown that by cauterizing the depressor point, a similar 'reversal' can be brought about. He makes no mention of any experiment in which the pressor afferents were exposed selectively to the same concentrations of strychnine.

In the present series this objection was met, as far as possible, by applying the drug over the dorsal projection of the entire pressor and depressor areas of ALEXANDER (1946), which includes both the pressor and depressor points. Nevertheless it may still be argued that the depressor mechanism, even under these conditions, is that exposed to the greatest concentration of the drug, and that the persistence of the pressor response is indicative of a selective exposure rather than selective effect. This apart, in the absence of evidence to the effect that nembutal cannot cause a reversal by action at another site, it must be concluded that the experiments merely indicate one possible explanation of the phenomenon. That is to say, an action on the vasomotor region of the medulla.

(b) Application of Picrotoxin to the Floor of the Fourth Ventricle.

This, the complementary experiment to those just described, would certainly have provided more significant evidence were it shown that by such a manoeuvre a normal response could be re-established in the overdosed animal, providing of course that the amount applied was insufficient to allow of other interpretation.

In two profoundly nembutalized cats the brain stem was exposed/

exposed as above and picrotoxin in various dilutions, 0.03 - 0.3%, applied directly to the vasomotor areas. The weaker concentrations were without effect. The stronger solutions abolished all response entirely. No useful information was therefore gained, although the method might be worthy of further trial.

(c) Stimulation of the Vasomotor Areas in the Profoundly Nembutalized Cat.

ALEXANDER (1946) and others have claimed that reflex vasodilation comes about by the 'depressor' afferents exciting the medullary dilator centre, which then actively inhibits the tonus of the spinal vasomotor (constrictor) centres. Others such as BACH (1945) postulate an excitatory discharge from the medulla activating the spinal dilator cells. It is possible then that the nembutal dilator block is spinal. An experimental approach was designed to test this hypothesis.

Methods:

Both authors mentioned consider the respective cell groups to be appropriately affected by discharge from a functional depressor centre in the medulla. The method adopted therefore, was to stimulate the depressor area in the cat showing the 'reversed' response. The brain stem was exposed as above (series 'a'). Stimulation of the vasomotor regions in the substance of the medulla (ALEXANDER 1946) was effected by unipolar excitation using pulses (5 V. 100/sec. 0.02 msec.) from the B.N.I. device. A fine silver wire electrode shielded by/

ELECTRICAL STIMULATION OF THE MEDULLA.

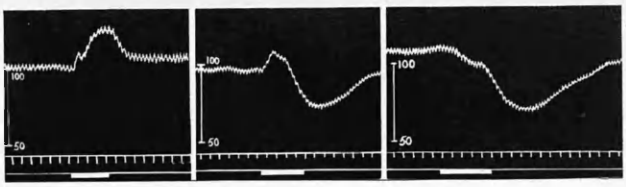


Fig. 64. Cat. Preparation and records as in Fig. 61. The responses to unipolar stimulation of three regions in the substance of the medulla with stimuli of 5 V. 100/sec. 0.02 msec. (B.N.I. stimulator).

THE RESPONSE TO ACETYLCHOLINE IN DEEP NEMBUTAL ANAESTHESIA.

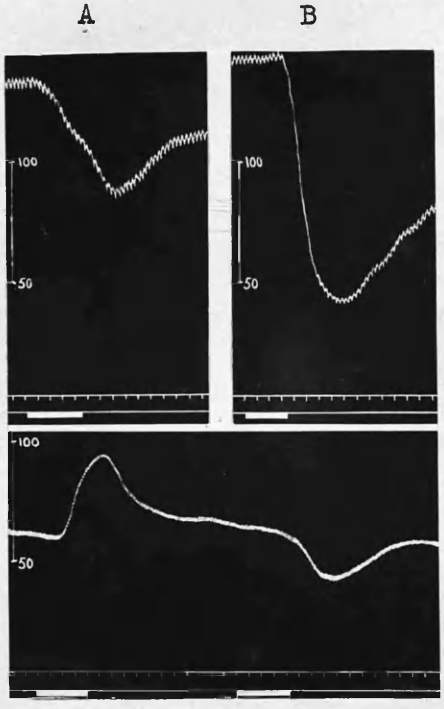


Fig. 65. Cat. Anaesthetic nembutal. Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec. Upper two tracings during light anaesthesia. Nembutal 40 mg/Kg.

A. Control stimulation of right vagus (5 V. 70/sec. 1 msec.).
B. " injection acetylcholine (10 μg.) into right femoral vein.

Lower tracing during deep anaesthesia. Nembutal 70 mg/Kg.

C. Stimulation of right vagus repeated.
D. Injection of acetylcholine (10 μg.) repeated.

by drawn capillary glass was used.

Results:

In three control experiments on urethanized decerebellate preparations exploration of the medullary tissue yielded pressor and depressor effects (Fig. 64). The former were readily obtainable while the latter were more elusive, indeed this difficulty rendered any negative result in the overdosed animal obviously valueless. One nembutalized decerebellate preparation only was investigated. Pressor responses only could be obtained. In the interest of more promising investigations the matter was not further pursued.

(3) THE PERIPHERAL DEPRESSOR MECHANISMS.

If, as is generally held (GERNANDT, LILJESTRAND and ZOTTERMAN 1946), both chemosensory pressor reflexes and barosensory depressor reflexes are mediated by variations in sympathetic tonus, it is clear that both cannot have simultaneous efferent representation in this common pathway. When they are activated at the same time, the sympathetic efferent outflow does not carry both dilator and constrictor influences but rather a constrictor tonus which represents the end result of the algebraic summation of the two effects of some earlier stage in the reflex arc. Powerful sinus nerve stimulation, therefore, although exciting both chemosensory and barosensory afferents, causes a depressor effect in the normally anaesthetized animal only by a diminution of sympathetic constrictor tonus. Even if the nembutal in excess were assumed

to/

to render the vessels insensitive to this lessening of tonus, it is quite clear that such an action would fail to account for the reversed response, for there exists under such conditions no contemporaneous pressor activity which can assert itself.

On the other hand, if there is no common pathway, but the barosensory depressor influences are relayed by depressor centres and dilator nerves and the chemosensory pressor effects by separate pressor centres and constrictor nerves, excitation of both mechanisms simultaneously can then be regarded as effecting a vasomotor response which is the resultant of the two opposing influences. Abolition of the prepotent depressor component by excess nembutal could readily effect a 'reversal' under these conditions. Two points considered together lend interest to this hypothesis. In the first place, the dilator fibres are held to be cholinergic, and indeed BACH (1945) maintains that such fibres are entirely responsible for the depressor reflex. In the second place, there is a wealth of evidence to the effect that barbiturates block peripheral cholinergic structures.

In 1925 De WAELE found that somnifene affected the vagal inhibitory mechanism of the heart. LIEB and MULINOS (1929) followed with the demonstration that amytal also depressed or paralysed the cardiac vagus, and GARRY (1930) found the effect even with normal anaesthesic doses. Since then, nembutal (KOPPANYI, LINEGAR and DILLE 1936), evipan (EMMELIN 1941) and

a/

a host of other barbiturates have been found to behave similarly (GRUBER and GRUBER 1941). Nor are the effects confined to the heart, for a diminished or abolished effectiveness of the cholinergic nerves has been found in a large number of organs including the submaxillary gland (STAVRAKY 1931), somatic neuromyal junctions (HUSTON, MARTIN and DILLE 1947) and the pupil, gut, uterus, bronchi and sweat glands (EMMELIN 1941). The last mentioned author has shown that 'the atropine-like action of evipan on the stomach is even stronger than that of atropine itself' and concludes with the statement that, 'there is nothing to contradict the assumption that the barbiturate acts on the specific receptor system which transfers the impulse in autonomic nerves to the effector cells'. To explain the 'reversal', it need only be postulated that the action of nembutal is to cause failure of the 'specific receptor system' of the blood vessels (? plexus of Cajal) to respond to cholinergic influence. The fact that picrotoxin abolishes the reversed response cannot be held as evidence against the nembutal block being peripheral, for GRUBER, GRUBER and COLOSI (1938) have shown that the former drug antagonizes the depressant affect of the latter on the cardiac vagus.

Faced with such evidence, and the lack of information on a possible effect of nembutal on the cholinergic dilator mechanism, the matter was put to experiment.

Methods:

In brief, the method adopted consisted of assessing the effectiveness/

effectiveness of acetylcholine injected into the intact cat manifesting the reversed response to buffer nerve stimulation. Four cats were studied. They were lightly anaesthetized with nembutal (40 mg/Kg. I.P.) and control depressor responses to buffer nerve stimulation and acetylcholine injection obtained. After nembutal overdosage these manoeuvres were repeated. To minimize cardiac effects direct intra-aortic injection by indwelling ureteric catheter was considered, but proved unnecessary since virtually no cardioinhibition occurred in the overdosed animal when the drug was administered by the femoral vein, and this route was therefore used throughout. Acetylcholine was freshly prepared for each experiment and made up in normal saline. For each test 1 cc. was employed, the amount of active principle being varied where necessary.

Results:

Eighteen injections were made in all. The table below contains the data of importance in each experiment, dosage of nembutal, vasomotor response to powerful electrical stimulation of the buffer nerves, dosage of acetylcholine and the fall of blood pressure produced by that drug (expressed as a percentage of the blood pressure prevailing before the test, e.g. B. P. before injection 150 mm. Hg. - acetylcholine injected - B. P. fell to 51 mm. Hg. Fall in B. P. = 66%).

THE RESPONSE TO ACETYLCHOLINE IN DEEP NEMBUTAL ANAESTHESIA.

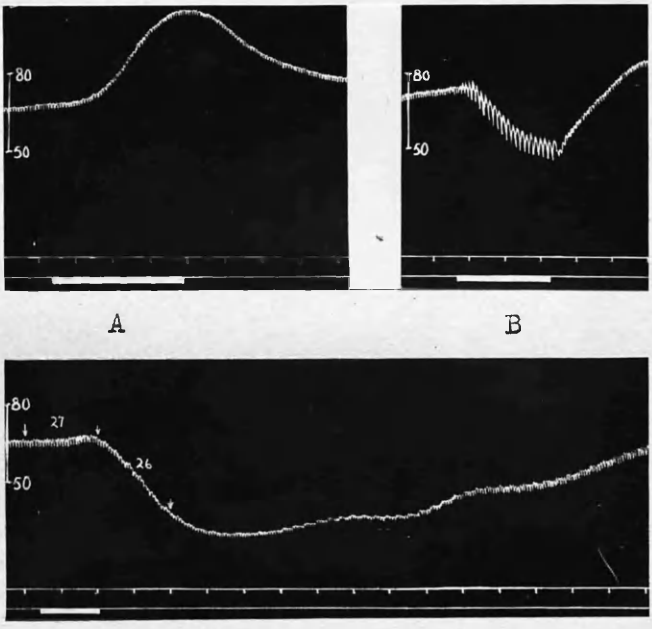


Fig. 66. Cat. Anaesthetic nembutal. Double vagotomy. Left sinus nerve cut. Artificial respiration. Bilateral open pneumothorax. Arterial B.P. (left carotid artery). Time 5 sec.
Tracings all obtained in deep anaesthesia. Nembutal 65 mg/Kg.

A. Stimulation of central end of right vagus (10 V. 70/sec. 1 msec.).
B. " " cardiac " of right vagus (10 V. 70/sec. 1 msec.).
C. Injection of acetylcholine (100 μg.) into right femoral vein.

Cat Number	DURING LIGHT ANAESTHESIA				DURING PROFOUND ANAESTHESIA			
	Nembutal mg/Kg.	Sinus n. and Vagus Stim.	Acetyl-choline Hg.	B.P. fall %	Nembutal mg/Kg.	Sinus n. and Vagus Stim.	Acetyl choline Hg.	B.P. fall %
86	40	Both depressor	10	54	65	Both pressor	10	41
87 (Fig.65)	40	Do.	10	69	70	Do.	10	32
89 (Fig.66)	45	Do.	10 10 50 50 100	49 57 62 67 75	65	Do.	10 10 50 50 100	37 31 48 40 55
90	40	Do.	10 50	41 79	68	Do.	10 50	33 64

The blood pressures during normal anaesthesia in the four animals ranged from 140 to 170 mm. Hg. and during profound anaesthesia from 55 to 75 mm. Hg. Although cardiac slowing was quite a prominent feature following acetylcholine injection in two of the animals (86 and 87) it was less marked in the others. During nembutal overdosage no significant degree of bradycardia whatsoever occurred in any of the animals (Fig. 66 C.), although in such instances powerful stimulation of the peripheral (cardiac/

(cardiac) end of the cut vagus (three animals) produced a fair degree of slowing (Fig. 66 B).

Interpretation:

Be the absence of cardioinhibition as it may, the fact that acetylcholine causes a substantial fall in blood pressure in the presence of excess nembutal and the 'reversed' vasomotor effect, which cannot be ascribed to cardiac slowing, clearly indicates that the vascular response to acetylcholine is dilator, and would imply that the block on the depressor pathway is not due to the abolition of the response of the vessels to acetylcholine.

CONCLUSION.

No satisfactory experimental demonstration of the site of action of nembutal for the reversal of the vasomotor response has been made. It is tempting to adopt the explanation that the effect is due to a 'depressor' block on the afferent side of the 'vasomotor centre'. This view has the merit of simplicity, is in accord with the commonly accepted belief in a tonic vasoconstrictor centre and efferent sympathetic common to dilation and constriction, and allows for the well established presence of pressor and depressor afferent fibres. Furthermore it is the explanation which would appear to hold for the 'respiratory' reversal (Chap. XIV). There the matter must rest.

C H A P T E R XVIII.

EXPERIMENTS ON THE RABBIT.

This chapter is concerned with experiments which were carried out early in the investigation in an attempt to elucidate the mechanism of the 'reversing' effect of nembutal on the cat vagus reflexes. It had been recognized from the beginning that the 'reversal' might depend on vagal afferents other than those constituting the aortic nerve proper, and to gain information on this point it was desirable to study the response of the aortic nerve free from other afferents. At a later period this was done in the cat, and the results have already been given (Chap. XIII). As a first approach, however, recourse was had to the rabbit where the aortic nerve is a separate and well defined structure. The results are worth recording for they differ from those obtained in the cat.

Methods:

The rabbits were anaesthetized with nembutal given intraperitoneally in 40 mg/cc. solution. In two animals the drug was injected, as was the practice with cats, without induction by volatile anaesthetic. This method was abandoned as the gut was readily punctured and absorption thereby hindered. The difficulty was overcome by inducing with light ether and tying a glass cannula into the peritoneum through which all nembutal was subsequently injected. This was done in another two animals.

When/

THE MODIFIED GADDUM APPARATUS EMPLOYED

TO RECORD BREATHING.

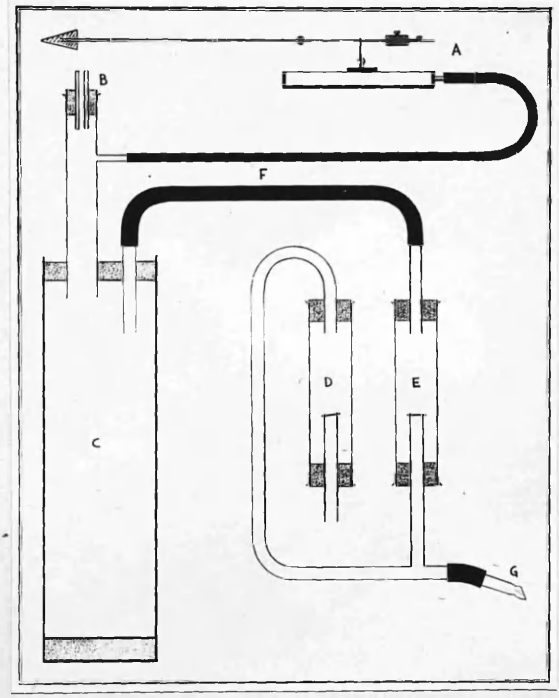
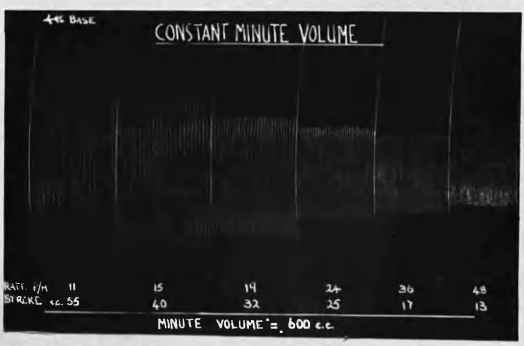


Fig. 67. Illustration of apparatus.
A. Large flat tambour. B. Resistance.
C. Reservoir (500 cc). D and E. Inspiratory and expiratory valves. F. Rubber tubing.
G. Tracheal cannula.



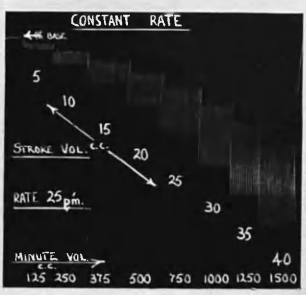


Fig. 68. Calibration tracings obtained with the apparatus.

When the rabbit was anaesthetized, the trachea was cannulated, both vagi and aortic nerves exposed and divided low in the neck and the femoral artery cannulated to record blood pressure. Respirations were then recorded by attaching to the tracheal cannula a modified GADDUM flowmeter. This device recorded individual breaths and rate of breathing and is illustrated in Figs. 67 and 68. (It was also used in experiments on the decerebrate cat and has been referred to in Chap. VII).

The experiment was conducted along the usual lines. The central ends of the cut aortic nerves and vagi were stimulated in light anaesthesia and control responses obtained. Thereafter fairly large amounts of nembutal (20 - 40 mg/Kg. per injection) were given in several doses and control stimuli repeated. This process was continued until the animal was killed. The WHITFIELD stimulator was employed. The conventional platinum electrodes were used for vagal stimulation, and the sinus nerve electrodes for the aortic nerve.

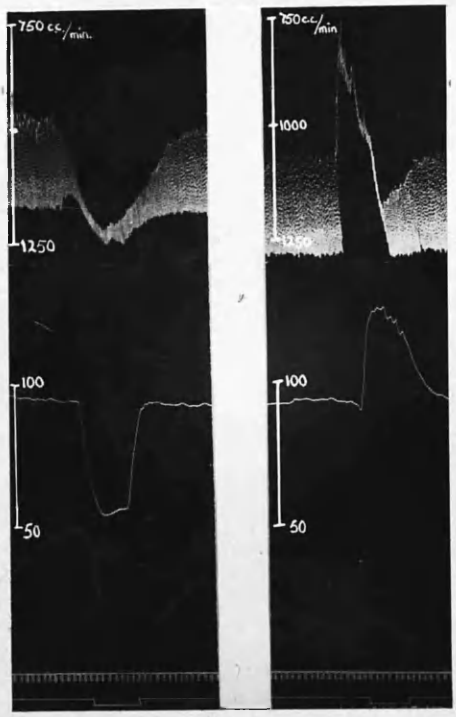
Results:

(a) In the Lightly Anaesthetized Animal.

Stimulation of the vagus in each animal yielded pressor effects alone, with intensities from 2 V. - 40 V. and frequency 20 - 40/sec. The respiratory response varied. With weak stimulation the effects were indeterminate, but with stronger stimulation inhibition was the rule (Fig. 69). Stimulation of the aortic nerve in each animal caused brisk depressor effects only over the range of stimuli last mentioned. Powerful stimulation/

ELECTRICAL STIMULATION OF THE AORTIC AND VAGUS NERVES

IN THE RABBIT.

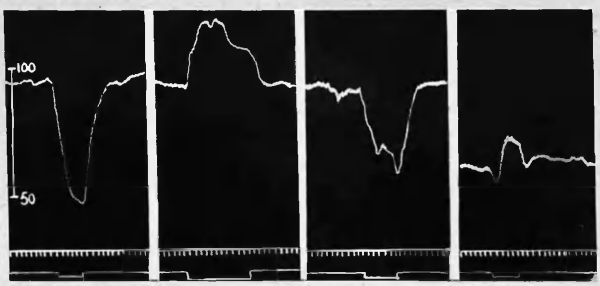


A B

Fig. 69. Rabbit. Anaesthetic nembutal (60 mg/Kg. intraperitoneally). Double vagotomy. Both aortic nerves cut. Spontaneous respiration recorded by modified GADDUM apparatus. Arterial B.P. (left femoral artery). Time 5 sec.

A. Stimulation of right aortic nerve (20 V. 30/sec. Whitfield).

B. " " " vagus " " " ").



A B C D

Fig. 70. Rabbit. Prepared as Fig. 69.
A, B and C during light anaesthesia.
A. Stimulation of right aortic nerve (20 V. 30/sec. Whitfield).
B. " " " vagus " " " "
C. " " " aortic and vagus nerves (20 V. 30/sec. Whitfield).
Between C and D nembutal added to total 128 mg/Kg.
D. Stimulation of right aortic and vagus nerves (20 V. 30/sec. Whitfield).

stimulation in three of the animals resulted in markedly increased respiratory activity (Fig. 69).

(b) In the Heavily Overdosed Animal.

After injection of excess nembutal, the marked depressant effect of nembutal on the blood pressure and spontaneous ventilation was soon manifest. Areflexic periods such as those which follow massive dosage in the cat were not observed, rather was there a progressive decline in the effectiveness of stimulation of the aortic and vagus nerves. Additional doses of nembutal further depressed the blood pressure, respiration, and the response to nerve stimulation. Eventually, when considerable amounts of nembutal (> 110mg/Kg.) had been given, there was a sudden failure of breathing and despite artificial ventilation the heart failed and the animal died. In no instance was a 'reversed' response observed, although terminally there was almost complete abolition of the vagal and aortic reflexes. The former appeared to be the more resistant and the vagal pressor effect could yet be achieved when the aortic depressor reflex was absent. As a result of this, stimulation of both nerves simultaneously (by laying both across the same electrodes) resulted in a pressor effect although a similar manoeuvre in normal anaesthesia yielded a fall in blood pressure (Fig. 70).

Interpretation:

Although repeat experiments are necessary before it can be concluded finally that nembutal does not bring about a reversal of the depressor effect of aortic nerve stimulation in the/

the rabbit, it is apparent that the phenomenon - if it does exist - is much less readily obtained in that animal than in the cat. Indeed the picture produced by nembutal excess in the two species differs widely not only in respect of the vasomotor response, but with regard to the areflexic period, stability of blood pressure and onset of collapse.

The demonstration that nembutal can bring about a reversal of the vasomotor response to compound afferent stimulation by differential depression of one component, is in agreement with the mechanism which has been advanced in explanation of the sinus nerve reversal.

The aortic nerve reversal in the cat having been attributed to persistent activity of the aortic chemosensory reflexes, it is of interest to note in connection with the failure of nembutal to effect reversal in the homologous structure of the rabbit that GERNANDT (1946) has been unable to detect any chemosensory fibres in the aortic nerve of the latter.

PART IV.

SUMMARY.

Following upon the observation that electrical stimulation of the central end of the vagus nerve caused a rise of blood pressure in a cat overdosed with nembutal, experiments have been carried out to analyse the phenomenon, which is referred to as the 'reversed' response.

Part 1. The anatomical and functional considerations are first presented in some detail and their complexity indicated.

The author's work is then set out in two sections. In the first are described the experiments forming the main theme of the investigation which culminate in a broad interpretation of the 'reversed' response. In the second are discussed various other aspects of the buffer nerve reflexes which have been studied, including a more detailed consideration of the mechanism of the 'reversed' response.

Part 11. The original observation is confirmed by experiment, and 'reversed' responses to vagal stimulation achieved by administration of excess nembutal. Chap. 1.

It is found that the drug has a similar effect on the normal depressor response to stimulation of the more purely 'moderator' sinus nerve. The effect of nembutal on these two buffer nerve reflexes is then discussed in greater detail, and the transition from the normal to the abnormal response is shown to involve intermediate stages which are dependent on the Chap. II.

Chap. III.

method/

method of dosage. Single massive doses cause periods of complete absence of any response whatsoever, despite the fact that during this areflexic stage the afferent sympathetic mechanism is active. This mode of administration most readily effects a 'reversal', and the total nembutal required (including the anaesthetizing dose) is then about 60 mg/Kg. body weight, although less may be required in the case of the sinus nerve. When similar, or even greater amounts are given slowly - either intravenously or by repeated small intraperitoneal injections - biphasic effects appear and 'reversed' responses are less readily gained. The abnormal buffer nerve responses revert spontaneously to normal after some time, but can be readily regained by additional nembutal.

Having firmly established the effect of nembutal, the barbiturate antidote picrotoxin is then studied. It is found rapidly to restore the depressor response in the overdosed animal, the transition responses being similar to those encountered in spontaneous reversion. The antagonism is reciprocal, for the 'reversed' response can be regained by subsequent administration of nembutal, and again abolished by picrotoxin. The antidotal effect of picrotoxin on the buffer nerve reflexes may occur without any other evidence of its analeptic actions. The mechanism of action of the two drugs is further pursued by studies involving serial section of the brain stem in the decerebrate preparation, and they are shown to be effective even when all brain matter

Chap. IV.

Chap. V.

above/

above the eighth nerve is removed. It is concluded that the locus of action of both nembutal and picrotoxin responsible for the vasomotor phenomena under discussion, lies at or below the known medullary vasomotor centres. With this established, attention is focussed on the afferent sinus nerve mechanisms,
Chap. VI. for it is found that in addition to the classical depressor response, stimulation of that nerve readily elicits a pressor effect in the cat normally (lightly) anaesthetized with nembutal. No such effect has been previously described, and this fact, coupled with the knowledge that nembutal in excess reverses the normal response, suggests that it might be due to the anaesthetic. Nevertheless, since this pressor response is obtained regularly with stimulus intensities less than those effective in lowering the blood pressure, it is considered as possibly a function of the stimulus. Experiments on the unanaesthetized decerebrate preparation confirm the
Chap. VII. latter view, pressor effects being very prominent with less powerful stimuli. It is essential, therefore, that an analysis of the sinus nerve responses be made before the results observed in nembutal overdosage can be
Chap. VIII. interpreted. This is done in a series of animals lightly anaesthetized with nembutal, using three stimulators of different output characteristics, and the earlier findings amply confirmed. Powerful stimulation is required to elicit the 'normal' reflex fall in blood pressure, while weaker stimulation has the opposite effect. When, however, an attempt

is made to relate these two responses to the known functional content of the nerve an obvious discrepancy arises, for the barosensory depressor fibres are generally considered to be larger than the chemosensory pressor afferents and therefore more excitable. In the light of this, the analysis is continued using urethane, which is first shown to have no significant distorting effect on the buffer nerve reflexes. Search over a wide range of stimulus intensities, however, fails to reveal any significant pressor or depressor activity other than in the ranges already determined under nembutal, although additional feeble depressor activity is manifest in some animals when very weak currents are used. The results are interpreted as indicating that the sinus nerve of the cat regularly contains a pressor afferent group, to which the chemosensory fibres must be assigned, and a depressor afferent group composed of very small fibres (probably of the 'C' group) which must include the barosensory fibres responsible for the characteristic depressor reflex. The third element detected - the large depressor fibres - may or may not be regularly present in the nerve. When this analysis of the fibre constitution of the sinus nerve is adopted as a basis for the interpretation of the 'reversed' response

Chap. IX.

observed in nembutal overdosage, it is concluded that the phenomenon is not due to any 'reversed' effectiveness of the depressor mechanism, but rather to a selective depression of that reflex leaving the larger fibre pressor reflex comparatively vigorous. Now the former is earlier/

earlier identified with the barosensory reflex and the latter with the chemosensory. It is therefore decided to determine experimentally whether the sinus nerve reversal can, in fact, be interpreted in terms of these two mechanisms. Using the isolated perfused carotid sinus, and activating the two reflexes through their receptors, it is shown that it can, for the chemosensory reflex has been found to retain a considerable measure of pressor activity at depths of nembutal anaesthesia which abolish the barosensory reflex entirely. Moreover, the pressor effectiveness of the chemosensory mechanism under such conditions is sufficient in itself to account for the entire 'reversed' response. The perfusion experiments lend considerable support to the interpretation of the sinus nerve fibre constitution arrived at earlier in the investigation.

Part III. Chap. XI. The first series of experiments discussed in the second section of the author's work, is that concerned with 'proving' the identity of the sinus nerve, and with the responses to the familiar occlusion and traction tests. Both tests are normal during light nembutal anaesthesia, and this finding substantiates the conclusion that the pressor effect of sinus nerve stimulation under the same circumstances is a function of the stimulus, not the drug. During profound nembutal anaesthesia, however, occlusion causes little or no reflex rise in blood pressure and the response to traction is reversed, being strongly pressor. Both tests are shown to be more complex than is generally believed. The former, besides decreasing/

decreasing barosensory activity, excites chemoreceptors and allows of considerable buffering (depressor) power by virtue of the collateral circulation, and the latter not merely involves stretch receptors, but excites the fibres in the sinus nerve itself by distortion, and probably stimulates chemoreceptors by further depleting the blood supply. The pressor effects observed in profound nembutal anaesthesia on occlusion or traction are therefore considered to be due to activation of the yet patent chemosensory reflex. In view of reported pressor activity of the sinus and vagus nerves at low blood pressures, consideration is given to this as a possible explanation of the 'reversed' responses. Experimental evidence does not support such a mechanism, however.

Chap. XII.

Electrical stimulation shows the aortic (depressor) component of the vagus to be influenced by nembutal and picrotoxin just as is the whole nerve. It is considered therefore, that an explanation of the vagal 'reversed' response in terms of its barosensory and chemosensory content may hold for that nerve as for the sinus nerve.

Chap. XIII.

Although the present investigation is primarily concerned with the effect of nembutal on the vasomotor response to vagus or sinus nerve stimulation, a study of the concomitant effects on respiratory activity provides results of considerable significance. They are in close agreement with the view that the 'reversed' vasomotor effects are due to persistent chemosensory activity, for both the vagus and sinus nerve/

Chap. XIV.

nerve 'reversed' responses are in many instances accompanied by increased respiratory activity. Since the effect of vagal stimulation during light anaesthesia is to diminish breathing, the respiratory response may also be considered as 'reversed'. This is of especial interest, for it would appear that the effect must be ascribed to a selective depression of the respiratory inhibitory fibres on the afferent side of the medullary respiratory centre, an interpretation which is pertinent to the vasomotor phenomena.

Chap. XV. During the course of the investigation 'réflexes douloureux' have been elicited from time to time. A hypothesis is advanced which attributes the effects to chemosensory excitation, rather than to some ill defined pain pathway.

Chap. XVI. A study is made of the effect of frequency of stimulation on the sinus nerve response. Although a transition from a rise in blood pressure to a fall is effected under certain conditions by increasing the rate, the evidence fails to support RANSON's theory, but favours the assumption that the different intensities of stimulation cause their various effects by exciting afferent groups of higher or lower threshold.

Chap. XVII. Detailed consideration is given to the mechanism of the 'reversed' vasomotor responses. The accumulated evidence is discussed, along with the results of experiments on the afferent, central and efferent mechanisms thought/

thought to be involved. Although experiment has resolved the problem to a determination of the site of the nembutal block on the barosensory mechanism, it is not found possible to demonstrate the site with any certainty. A tentative conclusion that it lies on the afferent side of the medullary vasomotor centre is largely inspired by the interpretation of the vagal respiratory reversal.

Finally, attempts to reverse the aortic nerve responses in the rabbit, by administration of large doses of nembutal, meet with no success, and a possible explanation of the failure is considered.

Chap. XVIII.

ACKNOWLEDGMENTS.

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