M.Sc. EXPERIMENTAL SURGERY

An experimental study of a method of producing chronic remote vagal stimulation by radiofrequency with particular interest in the effects on gastric secretion.

by

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PREFACE

This thesis is the result of a year spent in the Experimental Surgical Laboratories at McGill University, as a member of the McGill Diploma Course in Surgery. The subject of gastro-intestinal physiology is a stimulating one, and although no conclusive results have been obtained, the experience of carrying out a research project and the realization of the problems impeding further knowledge of this subject, have been invaluable.

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CHAPTER I

INTRODUCTION

The electric excitability of nerves has been known since 1780-83 when Galvani[48,29] and his coworkers first applied electric sparks to exposed nerves and muscles. Since then many famous investigators have devised new or improved means of conducting a known current to a definite nerve for a prescribed period of time. The problem has always been to control the action of the nerve over a flexible period of time and to attempt to evaluate the changes caused by its action. The most common type of nerve stimulation has been the utilization of flexible wire leads to conduct the electrical impulse to the nerve. This has been satisfactory in acute experiments and has contributed greatly to our knowledge of nerve physiology. However, in true chronic experiments, technical difficulties have always been encountered, due to movement of the unanaethetized animal and the presence of a sinus along the wire leads. Thus infection of the wound and breaking of the leads has interfered with the course of prolonged studies. Methods used in an attempt to overcome these problems will be discussed in a later chapter and the difficulties will be clearly demonstrated.

Of all the methods employed, that of Lafferty and Farrell[84] seemed the most effective. By means of radiofrequency and a small receiver implanted on the thoracic vagus of a dog, they attempted to produce chronic nerve stimulation. This idea has been modified by us and an effort has been made to perfect this technique of remote nerve stimulation.

Chronic remote nerve stimulation may be defined as a technique whereby a nerve in an intact animal is stimulated for periods of weeks or months by an electrical current, the ultimate source of which is removed from and not connected to the animal. By this method the effect of stimulation of a specific nerve in unanaethetized, intact animals can be assayed, thus eliminating disturbing emotional factors due to restraint. Chronic remote nerve stimulation permits close imitation of normal and certain abnormal physiological states.

The influence of the vagus on gastric secretion has been stressed by many workers, with special reference to the work of Pavlov[3] and his school. This experimental work has more recently been emphasized by the revival of the operation of vagotomy by Dragstedt[32] and has led to increased work on the factors affecting the cephalic phase of gastric secretion. It was thought that if a method of chronic stimulation of the vagus nerve could be perfected, it would then be comparatively easy to produce peptic ulcers in experimental animals by prescribed periods of stimulation. With the tremendous volume of work performed on the experimental production of peptic ulcer - 557 methods being listed by Ivy, Grossman and Bachrach[(75)] - no worker has yet produced a true peptic ulcer by stimulation of the vagus nerve.

The influence of the psyche on the appearance and recurrence of peptic ulcer is widely recognized and its influence is probably principally concerned with the cephalic phase of digestion. It is generally agreed that episodes of psychic stress play an important part in the appearance of an ulcer. Furthermore recurrent stress is closely associated with the return of symptoms in the convalescent patient. In a study which includes significant case reports, Wolf[(134)] summarizes the importance of the psyche in these patients as follows : "The causes and mechanism of peptic ulcer are still unexplained, but a large body of experimental evidence supports the view that this disorder, in many instances at least, occurs as a sequel to disturbed gastric function in reaction to significant stress in the life situation. In subjects with peptic ulcer, gastric hyperfunction may be accentuated, with the production of hyperacidity, hypermotility and pain, by a vigorous discussion of significant personal problems. Such gastric hyperfunction is apparently mediated through vagus innervation, and is associated with two serious physiological hazards: (a) a lowering of the pain threshold in the stomach and (b) increased fragility of the membranes."

These emotional influences on the formation of peptic ulcer are of first importance and explain much about its recurrence and about the frequent failure of medical therapy. They may be, in part at least, the cause of the "vagotonia which is the underlying cause of the ulcer diathesis" of which Beattie[11] speaks and the control of these factors may lie in the anticholinergic field of pharmacology that Grossman[54] mentions. The value of producing physiological situations in dogs over long periods, comparable to the daily stress of people in the modern world, was recognized and it was thought that the stimulation of the vagus nerve would produce this picture. If successful in producing a peptic ulcer, further investigation could be carried out in the field of ulcer therapy.

Thus it was decided to attempt to perfect a method of chronic vagal stimulation and to assess the changes in gastric secretion and gastric morphology.

CHAPTER II

THE HISTORY OF ELECTRICAL STIMULATION

A. Evolution of Electrical Stimulation

This subject has been very concisely summarized by Chaffee and Light(23) and much of the following history is attributable to their research.

In his studies of function, the physiologist has been greatly dependent on the basic sciences, not only in the attempt to identify the separate processes, but also in the interpretation of their actions. Particularly has this been true of investigations of the neuromuscular system where electrophysics has paved the way to many important discoveries. It may even be said that the history of neurophysiology has been decided in large part by the development of electric-recording instruments on the one hand, and by the increasingly effective use of electric currents for stimulating on the other. As Adrian(1) has written: "It would be hard to think of any other method which has done so much to show us how the body works, for it gives us a means of throwing a nerve or muscle into activity at will by an agency which does no damage and can be precisely controlled."

The early developments of these two sciences went forward hand in hand, since many of the discoveries in electricity were due to the tell-tale sensations and spasms caused by the passage of a current; in the absence of precise instruments for measuring electrical currents, the unique susceptibility of the neuromuscular system to electrical excitation made it an indispensable detector. The Leyden phenomenon occurred in 1746 when a group of scientists unexpectedly encountered the "capacity" of an electrified glass of water, because of the dramatic shock which accompanied its discharge. This has been vividly described by J. Priestley[111] : "Mr. Muschenbroeck, who tried the experiment with a very thin glass bowl, says in a letter to Mr. Reaumur, which he wrote soon after the experiment, that he felt himself struck in his arms, shoulders and breast, so that he lost his breath, and was two days before he recovered from the effects of the blow and the terror. He adds that he would not take a second shock for the kingdom of France." Thus the Leyden jar introduced to the world of science the possibilities of electrical stimulation.

The physicians of that time were quick to apply this spark phenomenon to a multiplicity of ailments, apparently believing that if odoriferous medicines were confined in glass vessels, and the vessels were excited electrically, the medicinal virtues would transpire through the glass to be absorbed by the patients in whose hands the containers were placed[112]. The fallacy of these ill-judged methods was soon to be exposed by the Abbe Nollet[113] but the procedure of electrification meanwhile had been transferred directly to the patient, and numerous cures of paralysis were reported. Among the most optimistic of the electro-therapists was John Wesley the divine, who, although possessing neither medical nor scientific degrees, yet organized dispensaries for the treatment of disease and used "this unparalleled remedy" as one of his principal therapeutic agents. His book[133] published in 1759 is replete with miracles which tested the patience of men of stricter scientific discipline. The remedy fell into better hands when Jean Paul Marat adopted it in his lucrative medical practice, but he too wrote with an enthusiasm which exceeded the evident results[97]. As can be easily imagined, a large series of charlatans, including the notorious Graham[126] milked the public unmercifully with the mysterious electricity as a further disguise of their deceptions.

Probably the first honest approach to the therapeutic value of electricity, was made by the ubiquitous Benjamin Franklin. He treated a series of paralysed patients in Pennsylvania in the early 1850's by application of a series of condenser discharges of fairly high capacity. His battery of jars yielded sparks great enough to produce local hyperthermia, petechiae and paraesthesias. Occasionally the treatments resulted in some increase of voluntary motion and he wrote to a friend: "These appearances gave great spirits to the patients and made them hope a perfect cure; but I do not remember, that I ever saw any amendment after the fifth day; which the patients perceiving, and finding the shocks pretty severe, they became discouraged, went home, and a short time relapsed; so that in palsies, I never knew any advantage from electricity that was permenent and how far the temporary advantage might arise from the exercise of the patient's journey, and coming daily to my house, or from the spirits, given by the hope of success, enabling them to exert more strength in moving their limbs, I shall not pretend to say(44)".

As has been previously stated, Galvani(48) discovered the electrical excitability of nerves. This opened up new methods in the study of neurology and also provided a detection of electric currents far better than tactile sensation, and thus paved the way for Galvani's second great contribution - the discovery of chemically generated electric current in 1789(49). The quantity of electricity necessary to evoke a convulsion in the freshly killed frog's leg is exceedingly minute, and Volta(130) found it to be 50 to 60 times less then that which could be detected by the most sensitive electrometer of the day, so that for 30 years the nerve-muscle preparation - called by Volta, "the animal electrometer" - occupied a unique position in the physicists laboratory.

Instruments of greater precision were obviously needed and the epochal work of Oersted(105) in 1820 in linking the two forces of galvanism and magnetism provided a basis for the detection of a current by its magnetic effect. This instrument was called a "galvanometer" and the appellation is proper not only for its descriptive force, but because the device marked the first advance over Galvani's other "meter", the animal electrometer.

At this time, Magendie(91), while utilizing the galvanic current to supplement his studies of the spinal roots, demonstrated that stimulation of the anterior roots causes muscular contraction and that of posterior roots sensation. This of course was of fundamental importance and gave rise to a type of experiment which has been steadily repeated: first, to reduce the activity of an organ through nerve section and second, to increase its activity (or certain phases thereof) through nerve stimulation.

It was already known that muscular contraction occurs at the beginning and at the end of current flow while the muscle remains quiescent during the period of steady flow. This characteristic made possible a series of repeated stimuli from a source of interrupted current, and in 1845 Dubois-Reymond(34) began to apply the methods for generating alternating currents recently uncovered by Faraday to the uses of physiology. His device, an induction coil with a magnetically driven breaker in the primary circuit, produced a series of closely spaced stimuli as the current went through its cycles. The frequency of the current pulses was varied in a rough way through adjustments of the spring tension, and the amount of current could be controlled by changes in the degree of magnetic coupling. This mechanism, simple and effective, is still to be found in every physiological laboratory today. It has, however, three disadvantages:

1. It operates at unknown frequencies except when regulated by tuning forks.
2. The precise current developed is unknown.
3. The wave form used for stimulation is irregular and varies in shape from coil to coil and with varying conditions of use.

The latter characteristic has come into much prominence of recent years, largely through the demonstrations by Lapicque[85] and Keith Lucas[90] of the quantity called chronaxie, but even the careful studies of such men as Erlanger and Garrey[36] have not succeeded in stabilizing the output characteristics of the Dubois-Reymond (or Harvard) coils. Thus a standardized method that could be used in widely separated laboratories was still unknown.

Electrical stimulation of peripheral nerves has revealed the functions of many tissues, through the exaggerated activity of the parts to which they lead. However, nerves are merely conduction paths, and the controlling action is known to lie deeper, in the spinal cord or brain. For many years, attempts had been made to elicit responses from the application of currents directly to the surface of the brain, but these resulted in failure until Fritsch and Hitzig[46] (1870) announced that electrical excitation of certain small areas of the cerebral cortex would give rise to muscular movements in the opposite side of the body. Much interest attaches to this finding, as it has led to the modern views of localization of cerebral function in the hands of Ferrier[42], Beevor and Horsley[12], Sherrington[122] and the host of modern investigators who have added definition to the picture of cerebral activity by the use of this method. The studies have not been confined to animals alone, however, for as early as 1909 Cushing[28] pointed to the results of electrical stimulation of the post-central gyrus during the course of surgical removal of intracranial tumors.

As soon as one considers the use of stimulating currents in physiological investigations, he must choose between the employment of two closely spaced electrodes (bipolar) and the method of grounding the body to a large indifferent plate, leaving only one wire as an active electrode (monopolar). The relative efficiency of the two systems has been the cause of much controversy, although so far as the brain is concerned, it appears that properly constructed electrodes are as satisfactory in pairs as in a grounded circuit[123]. In peripheral nerve studies, however, an important distinction exists, for it must not be forgotten that Galvani's original experiments were made with monopolar stimulation, and that Volta's successful argument against "animal electricity" hinged on the application of both electrodes to the nerve, thus showing that a nervous impulse and not a conducted current traversed the remaining nerve trunk to set the muscles into contraction.

Until very recently, physiologists have been content to observe the effects produced by direct electrical excitation during the short period of a crucial experiment in the decorticate animal, or in one under general anaesthesia. With the growing recognition of the immense duties of the central nervous system in the regulation of autonomic functions, it has become apparent that no method of study can succeed in revealing these central controlling mechanisms, that does not permit the student to reproduce artificially a controlling force which is similar to the function under investigation. This concept is best examined by a comparative consideration of the motor apparatus, for in this system the activities are directive, precise and capable of attaining full accomplishments in a few minutes. They respond to individual electric stimuli, even under anaesthesia, and the shocks may be given with fair rapidity since the cycle of activity can be completed many times in the course of a second. Central autonomic centers, on the other hand, are invested with the regulation of the ceaseless mechanism of internal life, in which changes are of degree and not of the fundamental act, and ebb and flow succeed each other at a comparatively leisurely pace. The very existence of higher autonomic centers has only been suggested, largely as the result of accident or disease, and they have proved extraordinarily refractory to experimental demonstration. It is quite possible that the difficulty rests with the investigative method employed, for not only does it lack the continuing character of automatism, but it is usually applied together with a general anaesthesia, the depressing action of which is exerted on the very region under consideration.

Several efforts have been made to stimulate more nearly the functional character of these processes, as well as to reach the other neurological systems during their normal phase of activity. In 1915 Keeton and Becht[81], during an experimental study of the pituitary body in dogs, implanted some iron filings directly into the gland. The dogs were allowed to recover, and were then brought within the range of an electromagnet, in the hope that local stimulation might occur at the site of the implanted iron. The experiment failed to demonstrate any resulting change, and this was attributed to the weak magnetic field available although in any case there would have resulted only a movement of the filings (mechanical stimulation) and not an induced electric current. In Zurich, Hess(66) has developed a technique for bringing conducting wires out through the skin to form a direct electric circuit, and this in his hands has been quite successful. In this continent, Mussen(100), Bradford Cannon(19) and many others have adopted similar means. These workers have succeeded in prolonging the duration of the experiment from a few hours at the time of the operation, to a period of several days after recovery. The drawback to the method lies in the wires and clamps used, which project through the skin, and are therefore susceptible to accident and facilitate infection, so that it is seldom that the experiment can be continued longer than two or three weeks. Furthermore, the arrangement required the constant watch and restraint of the animal during the time that wires are connected to the source of current, so that the subject does not enjoy real freedom, nor can the experiment be adjusted to the changing phases of physiology.

However, even with these difficulties excellent work has been done. Cressman and Blalock[27] in 1939 described attempts at chronic stimulation of splanchnic nerves in order to elicit chronic vasoconstriction in the splanchnic area with an accompanying rise in systolic blood pressure. Unipolar electrodes were applied to the splanchnic nerves between the diaphragm and the upper poles of the adrenal glands. Lead wires were brought out through the skin and stimulation potentials, consisting of 60 cycle alternating current at 0.1 to 0.5 volts, were raised until the animal was annoyed by the stimulus but still did not feel pain. The current was applied alternately for three minutes and interrupted for 30 seconds in an attempt to avoid nerve fatigue. The procedure was tried in three dogs who lived for 17, 11 and 4 days respectively. Only one dog showed a continued rise in blood pressure post-operatively while under stimulation and this effect was noted for four days only.

Kottke, Kubicek and Visscher[83] in 1938 reported successful production of hypertension in dogs by chronic stimulation of renal artery and accompanying sympathetic nerves for a period of 27 days. Transmission was by means of wire leads and the current used was a sine wave having a repetition rate of 10 per second.

B. Improved Methods of Chronic Nerve Stimulation:

All the above experiments share common faults and indicate that in order to obtain the desired results, the experimental animal must be completely freed from restraint and encouraged to live as normal an existence as possible. The complications of the sterile surgical field and the time limit set by the operative procedure, must be avoided. Anaesthesia, which suppresses many functions, should not be used during the investigation of the nervous system, yet the animal must not be led into awareness of the experiment through pain or restraint. Under the best conditions the animal would be allowed to lead a normal life throughout the duration of the experiment, without interference of eating and drinking, sleeping, or exercising. The actual excitation should be confined to the small area of the nervous system under consideration and be applied without fear of current spreading. Since the degree of stimulation depends in part upon the current density in the excited tissue, and also on the manner in which the current varies with time[25], these factors must be under ready control of the operator. Because, too, the character of the response is often dependent on the number of times per minute or per second that the stimulus is repeated, provision must be made for accurate and rapid adjustments of the rate of stimulation. Various ingenious methods have been devised in an effort to overcome these problems.

Chaffee and Light[23] in 1934 developed a system of remote stimulation for the study of excitable regions in the nervous system during the normal life of an animal. A field of electromagnetic force was set up by a periodically charged condenser of high capacity, discharging through a primary coil 36 inches in diameter. The subject was confined in a cubical cage suspended in the centre of the primary coil. In order to ensure allignment of primary coil and implanted secondary coil, it was necessary to have three primary coils about the cage in planes perpendicular to one another. The three coils had to be energized in sequence which required the use of an elaborate vacuum tube switching device to handle the heavy current. Much distortion of wave form occurred before application to the nerve and the equipment was bulky and expensive. However they did carry on their experiments while the animal - usually a monkey (adult Macacus rhesus) - was living comfortably in its cage and they showed the possibilities of such a method.

In 1936 Fender[40,41] developed a method of prolonged stimulation of the nervous system. Like that of Chaffee and Light it was based on the principle of an induction coil but differed in that the primary coils were energized by a 60 cycle alternating current. Six primary coils were arranged on the faces and at the ends of a shell which was incorporated into a cage - thus setting up electromagnetic fields in three primary planes. The paired primary coils were energized in sequence by a mechanical switching device. The secondary coils were small and consisted of 6000 turns of fine copper wire, the unit being coated on the outside with molded rubber. Leads consisted of multiple braided strands of fine silver wire. Stimulation was described as adequate but the high voltages required in the primary coils necessitated close confinement of the animal in order that it would be within the dense portion of the field.

C. <u>The Role of Radiofrequency in Nerve Stimulation</u>:

Improved means of nerve stimulation were still being sought and investigation naturally was directed toward the use of radio frequency. This idea was first carried out by Feser(43) who reported the successful transmission of radio waves to an exposed nerve-muscle preparation, without the use of any receiving "pick-up" mechanism other than the nerve itself, which he believes acts as a dipole antenna. His experiments have not been substantiated but are interesting if only for historical purposes.

In 1937 Newman, Fender and Saunders(101) described the use of radiofrequency potentials for energization of the primary coils. High frequencies allowed them to take advantage of the increased induction incident to the increased rate of change of flux within the field and the resulting ability to use smaller currents in the primary coils. A frequency of 430 kilocycles was used. Induced current in the receiving coils was rectified by small copper oxide rectifiers. The resonating frequency of the receiver coil was adjusted to that of the primary coil by means of a suitable condenser placed across it. The current delivered to the electrode depended for its characteristics, both as to frequency and wave form, on the modulation of the source of the high frequency current energizing the primary coil. The form of the resonating receiver coils permitted construction of a unit consisting of three coils placed in three primary planes in such a manner that they shared a common centre point. Each coil's output was rectified by four copper oxide rectifiers, then led to a common terminal. The entire unit had a diameter of $1\frac{1}{2}$". A vacuum tube oscillator modulated at 60 cycles delivered 150 watts at 430 kilocycles to a single coil of six turns of No. 14 insulated copper wire placed in the walls of a room five feet square. An output of 4.0 milliamperes was obtained from a receiving unit in the center of this room through a resistance of 2000 ohms. Trial stimulation of the cerebral cortex, nerves to skeletal muscles and sympathetic chains was carried out. It will be noted that modulation was fixed at 60 cycles and that the article contained no description of receiver casings.

Greig and Ritchie[53] designed a simple apparatus for remote nerve stimulation in the unanaethetized animal. They postulated that the electrical stimulation of nerves normally inaccessible in the conscious animal may be effected by the use of induced electric currents excited from an external source. They describe the apparatus in detail in their article. The size of the complete pick-up unit consisting of pick-up coil, miniature rectifier and reservoir condenser, all imbedded in wax, depends to a considerable extent on the voltage required, but for normal purposes, where the requisite voltage does not exceed 0.5 volts, a space some 3 cm. square by 0.5 cm. deep is adequate. The output voltage is very considerably increased if the pick-up unit is operated at or near resonance, but this condition is necessarily much more sensitive to slight maladjustments.

In 1949 Lafferty and Farrell[84] reported a technique for chronic remote nerve stimulation by means of radiofrequency. The receiver consisted of a flat pick-up coil tuned to resonate at one megacycle by means of a suitable condenser placed across it.

The radiofrequency output was rectified by a germanium crystal and then applied to the nerve which was to be stimulated. A by-pass condenser was connected across the output terminals of the receiver to prevent r-f voltage from being applied to the nerve and to increase the receiver sensitivity. An exciting coil eight feet in diameter was used to establish an r-f field of electromagnetic force, the potentials being developed by a vacuum tube oscillator. Modulation of the r-f potentials was accomplished by means of a square wave generator which developed a series of impulses whose repetition rate, duration and potential could be accurately adjusted over a wide range by means of calibrated control knobs.

Close attention was given to the construction of leads and receiver casings. A flexible copper lead was made by overwinding a cotton thread 0.015" in diameter with two copper ribbons 0.0005" thick and 0.02" wide. The lead was insulated by a polyethylene tube having an inside diameter of 0.023" and a wall thickness of 0.014". The electrode which wrapped around the nerve was made from 0.002" thick silver foil, having a length of $\frac{1}{4}$" and a width of 3/8". This was attached to the flexible lead by inserting the lead and a small wedge of silver foil into a small metal tube, then crushing the tube. The electrode was covered by a $\frac{1}{2}$" length of polyethylene tube having an inside diameter of 0.118" and a thin flexible wall. This tube was cut lengthwise on one side to allow insertion of the nerve. The side of the tube opposite the longetudinal cut was pierced in the center to permit passage of the flexible lead, leaving the electrode lying within the tube. The electrode casing was then fused to the polyethylene cover of the lead. After joining the proximal end of the flexible lead to the receiver terminus, the lead casing was fused to the polyethylene shell of the receiver. The receiver was mounted in two polyethylene shells having a hemispherical outline, one and one-half inches in diameter. Sealing of the receiver was accomplished by fusing the two shells at the equator where they join.

The receiver consisted of three coils - each of which lay in one of three primary planes, mounted mutually at right angles. The spherical receiver is essentially three separate receivers with their output terminals connected in parallel. By mounting the coils mutually at right angles the receiver will respond to a r-f magnetic field in any direction. With this receiver the subject may move around fully within a single primary coil 6-10 feet in diameter and receive nearly uniform stimulation at all times. Dr. Farrell no longer uses this type of receiver as he demonstrated to us in his laboratory in Albany on 1st February 1952. At the present time he is using a flat polyethylene disc with a single pick-up coil surrounded by three primary coils - each made as before - mounted mutually at right angles. He believes that this permits the implanting of a smaller receiver in the dog while the three primary coils still allow nearly uniform stimulation at all times.

The coil consists of $17\frac{1}{2}$ turns of No. 26 enamelled copper wire wound on a form having a diameter of $\frac{3}{4}$" and a width of 3/16". The coil is painted with collodion before removal from its form. Next a 2500 mmfd resonating condenser, germanium crystal and 3000 mmfd by-pass condenser are mounted inside the coil. The electrode and ground leads are then attached to the circuit. The circuit is cast into a plastic disc of polyethylene. The ground plate is a tantalum or silver disc $\frac{3}{4}$" in diameter and 0.02" thick with edges rounded and smooth.

An analysis of the receiver circuit shows that there are optimum values of the circuit elements for maximum sensitivity. The voltage output of the coil under optimal conditions is proportional to the 3:2 power of the coil diameter and to the 1:2 power of the radiofrequency. This would indicate that, within the limits of the experiment, optimal conditions may be obtained with a pick-up coil as large in diameter as possible and of a high radiofrequency. The crystal rectifier should have as low a resistance as possible. The number of turns on the coil should be adjusted so that the product of its inductive reactance and Q is equal to the equivalent load resistance presented by the nerve and crystal rectifier. Sufficient capacitance should be added across the coil to produce resonance.

There are many advantages to the use of radiofrequency for transmitting the signal to the receiver and then applying the rectified output of the receiver to the nerve. Relatively low power equipment can be used. The stimulating voltage applied to the nerve can be made to have practically any wave form by proper modulation of the r-f transmitter. The use of rectangular pulses has various advantages:

1. Electrode polarization may be made negligible.
2. Pulse width is kept very short (a few milliseconds).
3. Repetition rate is kept well below what appears to be the fatigue level of the neuromuscular junction.
4. Detailed analyses are possible in determining optimal conditions for efficient and effective stimulation of specific nerves.

This technique worked successfully on the radial nerves of dogs for 8½ months. Stimulation of the splanchnics and the vagi in the lower thorax are also being carried out but no results have been reported.

Particular emphasis has been placed on the work of Lafferty and Farrell and the technique has been explained in moderate detail since it is this paper which initiated our present project. The basic ideas have been followed while considerable modification of details has been done, as will be explained in a later chapter.

CHAPTER III

PRINCIPLES OF NERVE PHYSIOLOGY

In order to understand the problems encountered in this type of research, a basic knowledge of neurophysiology is required[47]. The withdrawal of a hand or foot from an unpleasant stimulus is a reflex, involving a sequence of reactions beginning with excitation by the external agent of sensory end-organs. The excitation is conducted to the central nervous system by impulses in afferent neurons and transmitted therein to other nerve cells lying wholly within the nervous system. These are known as internuncial neurons, or for brevity, interneurons. From interneurons, the excitation is transmitted to efferent neurons, or motoneurons, by which the excitation is conducted away from the central nervous system to the muscles, causing the contraction which withdraws the hand or foot. Thus the operation of the reflex sequence depends upon three fundamental properties:

a. excitation.

b. conduction.

c. contraction.

Other, and more complex, events in the nervous system are mediated in a like manner.

A. Electrical Phenomena at Rest and in Action:

The prime function of neurons is the conduction of impulses. The nerve impulse is always associated with an electrical change of characteristic time course - the action potential. Conversely an action potential is never encountered in resting neurons. There are other changes - chemical and thermal - that occur in active neurons, but these appear to play a supporting role in contrast to that of the electrical change. Erlanger and Gasser in 1922 found it possible to measure the electrical events in excitable tissues with the greatest accuracy by means of the cathode ray oscillograph. In principle, the use of the cathode ray oscillograph in nerve physiology is quite simple. A beam of electrons is focused on a fluorescent screen to yield a luminous spot that can be photographed. The beam can be deflected by a system of charged parallel plates, the electrons being repulsed by a negatively charged plate and attracted by a positively charged plate. By varying the charges on plates set parallel to one another, the 'spot' can be made to 'sweep' in a horizontal plane across the fluorescent screen face of the cathode ray tube. The electrical changes occurring in a nerve are feeble and must be amplified by means of radio tubes. To accomplish this, the electrical charges are led off to an amplifier by a pair of electrodes suitably placed on the nerves. As the spot sweeps across the screen in a horizontal plane, the amplified nerve signals are impressed upon another pair of parallel plates so placed as to deflect the beam in a vertical plane. The resulting movement of the spot, deflected by the two sets of plates, traces out the nerve activity as a function of time. Thus in most recordings of action potentials, the vertical components of the records indicate the potential difference between two points on the nerve and the horizontal components, the course of time.

A distinction must be made between the demarkation potential and the spike potential. Hermann(63) established the fact that the difference of potential found in excised muscle or nerve is due to the injury at the cut ends. The potential difference between the intact longitudinal surface and injured end is known as the demarkation (or injury) potential, the resulting flow of current as the demarkation (or injury) current. It is generally agreed that the seat of the electromotive force is at the uninjured surface, the injured region serving only to provide an electrical connection from the outside to the inside of the nerve fiber. This is the concept embodied in the membrane theory of Bernstein. At about this time DuBois-Reymond had noted that the flow of current diminished when the muscle or nerve was stimulated into activity. This fact indicated that the potential difference between intact longitudinal surface and cut end was diminished - that is to say, the active surface becomes negative with respect to the resting surface. The effect was accordingly spoken of as the "negative variation". However, Bernstein, in 1871, proved that this negative variation takes the form of a wave and that the wave is propagated along a nerve at a finite velocity. This wave was originally called "current of action" or "action potential", but since it has been found that the action potential contains several elements - the wave is now designated the "spike potential".

Once it was proved that the spike potential is not, like the demarkation potential, a steady state but rather a wave propagated along a nerve or muscle at finite velocity, the concept of local self-propagating electrical change gained general acceptance. Only a small region of a long nerve fiber is active at any one time as a single nerve impulse passes along the fiber. The region is about six centimeters in the case of the largest mammalian nerve fibres down to a few millimeters for the smallest. The length of nerve occupied by the monophasic spike potential is sometimes called the wave length of the impulse. Maintained activity of a nerve fiber take the form of a series or train of impulses, the effect being more like the succession of machine gun bullets than the steady stream of a water pistol. Monophasic and diphasic spike potentials can be recorded - the diphasic differing in that here both electrodes are placed on living tissue. The fundamental processes involved are identical, only the disposition of electrodes being changed.

B. Distribution of Electric Current in Volume Conductors[89].

Up to this point nerve and muscle have been considered as linear conductors surrounded by an insulating medium. For many reasons the isolation of tissues in air or other suitable insulating media is a most useful procedure but frequently, as in electrocardiography, this cannot be done. Within the living organism the natural environment of excitable tissues - nerves, muscles, heart, brain and spinal cord - is an extensive watery medium containing dissociated electrolytes. Such a medium conducts electricity and is known as a volume conductor. Consequently, to appreciate the electrical activity of tissues in situ, as attempted in this project, it is necessary to gain at least an elementary understanding of the distribution of electrical currents in volume conductors. The principles of volume conduction may be presented by a study of the external field of an impulse travelling in a straight length of nerve situated in a volume conductor.

It is helpful before considering the external field of a nerve impulse to examine the relatively more simple situation existing when leads from the two poles of a battery are placed in an electrolytic medium. Current will flow through the medium from one pole to the other in the manner indicated by the solid lines in Figure 1 on page 35. If the conducting medium is sufficiently extensive, only a

FIGURE I

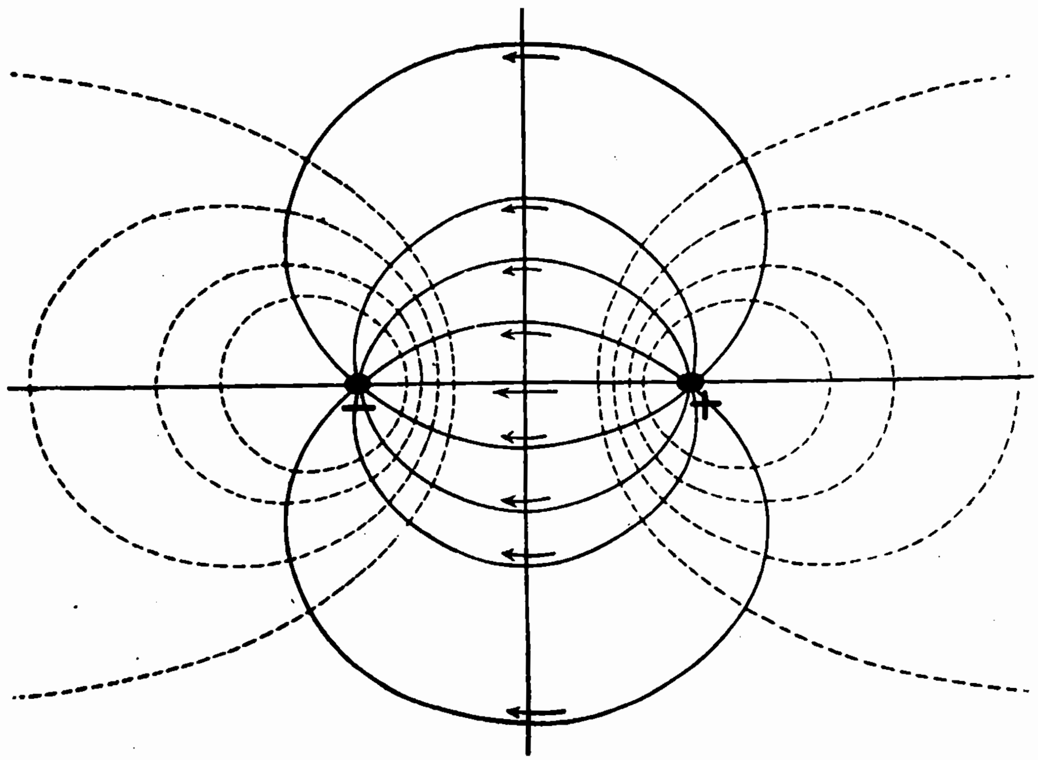


Fig. 6.—Diagram of the potential field and current flow about a dipole set in a conducting medium. Hatched lines = isopotential contours. Solid lines = current flow, the direction being indicated by arrows.

Potential Field and Current Flow about a dipole. Reproduced from Fulton, J.F.: a textbook of Physiology. Sixteenth edition. 1949. Figure 6, Page 13.

negligible current will flow through a point distant from the two poles. By employing a suitable electrometer, isopotential curves may be plotted for the conducting medium. They are indicated by the hatched lines as shown in Figure 1 on page 35. The lines of current flow and the isopotential lines intersect one another at right angles. An exploring electrode pitted against another electrode placed at a distance from the two poles will record a positive potential when located near the source of current flow and a negative potential when located near the sink of current flow. The magnitude of the potential difference depends upon the proximity of the exploring electrode to either source or sink of current flow.

In principle the situation with active nerves in a volume conductor is similar to that just presented. The external field is determined by the spatial arrangement of the sources and sinks of current. During the course of a monophasic spike potential, current first flows out through the membrane, then in through the membrane, and finally out through the membrane; that is to say, there are two reversals of membrane current during the monophasic spike potential. There is therefore a sequence of source - sink - source.

As the impulse is propagated along the nerve fibers, certain potential sequences may be recorded at points along the nerve. It is important fully to realize one essential difference between the recording of potential changes in nerve when the nerve is surrounded by an insulating medium and when immersed in a conducting medium. In the former instance an oscillograph will record nothing until the travelling spike potential reaches the point of electrode contact. In a conducting medium, however, a fluctuating field of current flow exists throughout the medium during the whole time that an impulse exists in any part of the nerve in the conducting medium. Now consider a nerve lying in a conducting medium and conducting an impulse from one end to the other. The passage of the impulse is recorded by four electrodes each pitted against an electrode located at some distance from the nerve. Electrode 1 is placed at the point where the nerve enters the conducting medium. Electrodes 2 and 3 are placed at two points along the length of the submerged nerve. Electrode 4 is placed at the other end of the nerve. Each of the electrodes will detect the existence of currents as long as the impulse occupies any part of the nerve within the conducting medium. The form of the recorded potential will be different at each electrode position. Electrode 1, being at the point of origin of the impulse, will record a diphasic wave, first negative, then positive. At electrodes 2 and 3, the recorded potential will be triphasic - positive, negative, positive - indicating that the nerve at electrodes 2 and 3 acts in succession as a source, a sink and a source. The relative size and duration of the three phases of the triphasic potential recorded along the nerve will vary according to electrode position. At electrode 2, the first positive phase will be brief, for that region will rapidly become a sink as the impulse travels. At electrode 3, the first phase will be relatively long, for it will take a longer time before the impulse reaches that point. At electrode 4 the recorded potential is diphasic, positive while that point acts as a source and negative when the impulse reaches electrode 4. Since the end of the nerve is at electrode 4, the impulse can go no further. Therefore a sink exists at electrode 4 until the spike process is over. To sum up: as an impulse approaches a point, that point acts as a source; as the impulse reaches that point, it acts as a sink; as the impulse recedes from that point, it again acts as a source.

C. Electrical Excitation:

A brief electrical (cathodal) shock is the method of choice for stimulating excitable tissues, one very important advantage being that it can be graded accurately in strength. A shock may be subliminal, in which case the conducted spike potential response is not elicited, but once the strength is liminal (that is, of threshold strength) further increase does not increase the response. The actual threshold strength for a given fiber will vary up or down, depending upon the condition or "excitability" of the membrane. Hence the exact strength of a shock necessary to reach threshold forms a measure of the excitability of the membrane. The preceding statement applies only when a single unit (nerve fiber) is considered. A nerve contains hundreds or thousands of such units having different thresholds. When a whole nerve trunk is stimulated, further grades of stimulation are recognized. There is first the subliminal stimulus which does not reach threshold for any of the constituent fibers. Next there is the liminal or threshold stimulus for the nerve, at which strength the most excitable fiber in the nerve discharges an impulse. As the strength is progressively increased (submaximal stimuli) more and more fibers are brought to threshold and discharge impulses until all the fibers have responded. The stimulus is then maximal. The stimulus, thereafter called supramaximal, may be still further increased without evoking a greater response.

A brief subliminal electrical shock applied to a nerve results in a change in excitability that extends in space beyond the region of electrode (cathode) contact and that lasts in time beyond the duration of the applied shock. The course of this excitability change can be plotted by applying a second subliminal shock at several time intervals after the first, and finding by trial the exact strength required of the second shock just to reach threshold. One stimulus is usually called the "conditioning" shock; it has an effect on the stimulated tissue and that effect may be gauged as to temporal, spatial and intensity characteristic by the action of a suitably chosen second stimulus, the "test" shock.

The local disturbance of excitability instituted by such a conditioning shock, or LOCAL EXCITATORY STATE as it is frequently called (Lucas), subsides along an exponential course at a rate characteristic of the tissue and comparable to that of the electrotonic potential. By stimulating with two pairs of electrodes, one for conditioning, the other for testing, and by separating the cathodes of the two pairs of electrodes, it is found that the local excitatory state extends a short distance along the nerve with an exponential decrement. The excitability change evoked by a brief shock corresponds, in space and time, to the electrotonic potential similarly produced. As conditioning shocks of progressively increasing size are applied to a nerve, both electrotonic potential and local excitatory state increase, until the critical threshold strength is reached, at which time a propagated impulse is initiated.

Changes in excitability during the passage of a constant current are studied by the same technique of testing outlined above. In place of the conditioning shock, a constant current of subthreshold strength is substituted, and a brief shock is again employed for test stimulation. Erlanger and Blair[35] demonstrated the changes in excitability occurring at or near the cathode of an electrode pair when a subthreshold constant current of finite duration is passed through a nerve. Excitability increases for a short period of time and then tends to fall off again while the current is still flowing. After the current ceases to flow, excitability falls below the resting level before returning finally to normal. Considerable interest attaches to the fact that excitability initially increases only to decrease again while the current continues to flow unchanged. This secondary fall in excitability is known as ACCOMMODATION, by which is meant the nerve reacts to the flow of current in such a way as to oppose, by an active process, the tendency of the current to excite. The decrease in excitability below the resting level after current ceases to flow is called POSTCATHODAL DEPRESSION.

Consideration should now be accorded to the course of events when constant currents of various strengths are imposed upon a nerve. Let R, in Figure 2 on page 43, represent the resting excitability and T the threshold. Constant currents of increasing strength are caused to flow through the nerve. By

FIGURE 2

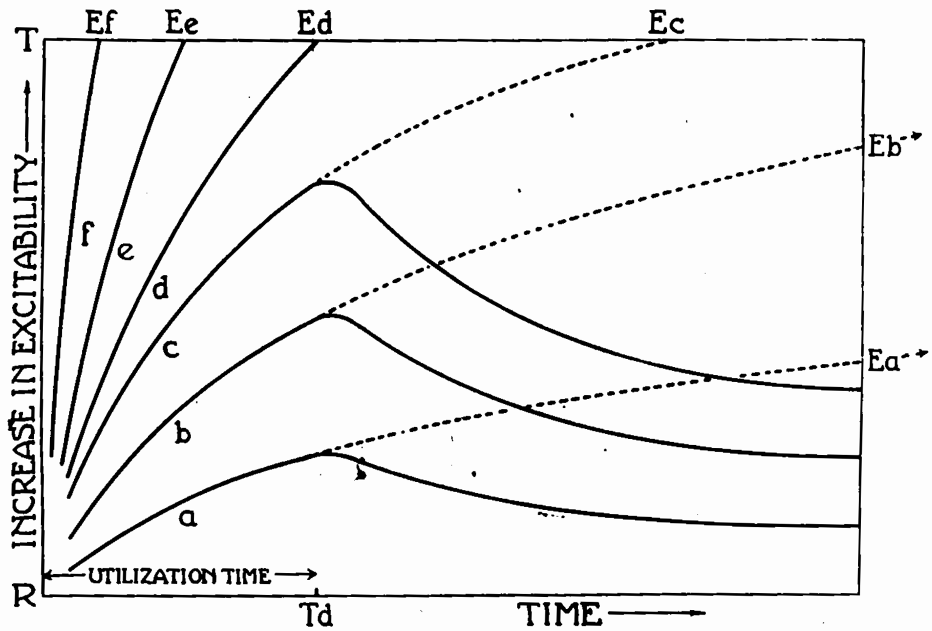


Fig. 13.—Effect measured by the same technique outlined in Figure 10 of constant currents of various intensities on the excitability of a nerve fiber. R = resting excitability. T = threshold. Curves a–f result from the application of successively greater currents. Curves a, b, c are subliminal and show the course of excitation and accommodation. Such currents never reach threshold because accommodation intervenes. In the absence of accommodation the curves would continue as extrapolated by dotted lines to Ea, Eb and Ec when excitation would occur. The current strength giving curve d is just sufficient to excite (it is *rheobasic*) and it does so after a time delay known as *utilization time* (Td). Curves e and f show that excitation occurs earlier with stronger currents.

Excitability Curves of Nerve.
Reproduced from Fulton, J.F.:
A textbook of Physiology.
Sixteenth edition, 1949.
Figure 15, Page 23.

means of test shocks, the excitability can be plotted. The curves a, b, c are obtained by allowing successively greater but still subliminal currents to flow. Curve d is obtained by the use of a current that is just sufficient to excite by itself. The curves e and f are obtained by the use of currents of supraliminal strength, f being stronger than e. Inspection of this family of curves illustrates a number of facts concerning excitation. If a response is not evoked within a certain time, there will be no response regardless of the duration of current flow. For instance, if the excitability continued to increase in a manner represented by the hatched lines of curves a, b, and c, threshold would be eventually reached at E a, E b and E c respectively, but accommodation supervenes before threshold is reached and excitability decreases. When a current of strength d flows through the nerve, as shown by curve d, excitability just reaches threshold and a response occurs at E d. The current which is just able to excite when flowing for a long time is called RHEOBASIC. The time during which the rheobasic current must flow in order to excite (T d in diagram 2 on page 43) is known as the UTILIZATION TIME.

When the current is further strengthened (e and f), excitation occurs earlier, at Ee and Ef respectively; i.e., the stronger the current, the shorter the time during which it need flow in order to excite. This relationship forms the basis of the STRENGTH-DURATION CURVE.

Since the work of Hoorweg (1892) and Weiss (1901) it has been known that, within certain limits, the longer the duration of current flow the weaker may be the current required to excite. As a consequence of the fact that threshold excitation by the constant current itself forms the end-point of measurement, the excitability changes evoked by subliminal currents find no expression in the resulting curves relating strength and duration. There is a finite strength of current below which excitation will not occur, however long the current may flow. This critical strength of current is called by Lapicque the RHEOBASE. The minimum time during which a rheobasic current must flow in order to excite is again the utilization time. Lapicque and others have found it useful, for the purpose of comparing tissues, to stimulate with a current twice the rheobasic strength and to find the minimum duration of flow required to excite. This duration of time has been called EXCITATION TIME (Lucas) or CHRONAXIE (Lapicque). Some tissues are slow in action, others fast; in fact, a graded series of excitabilities may be found with smooth muscles at one extreme and the largest nerve fibers at the other. The values of chronaxie or excitation time for the several tissues give a measure of the relative scales required for coincidence of the curves, and so form a basis for comparing in a general way the excitability of one tissue with another.

All of the remarks so far made have been concerned with changes in excitability at the cathode. These are of particular importance, for it is the excitability change at the cathode that has the direction leading to excitation and response of the tissue; it is current flowing through the membrane in an outward direction that leads to stimulation. At the anode, current flows inwardly through the membrane, and similar electrotonic and excitability changes to those at the cathode occur, but in the opposite direction. Accordingly, one frequently encounters the terms catelectrotonus and analectrotonus, or more simply, CATHODAL and ANODAL POLARIZATION.

Accompanying these opposed electrical states are the excitability changes which may be called cathodal enhancement and anodal depression respectively.

D. Concept of the Nerve Impulse.

Having now considered electrical events in nerve and the physiological effect of current flow in terms of excitability change, it is possible to form some idea of the mechanism of propagation. As we have seen, an applied current builds up a potential difference across the membrane in proportion to its strength. At the anodal region the added charge of the membrane has the same sign as the charge that exists in the resting state. At the cathodal region it has the opposite sign to the resting charge. The membrane is thus discharged at the cathode; that is, the membrane electromotive force is reduced. When the membrane is discharged to a critical level (i.e. threshold) at the cathode, the membrane at that point continues, of itself, to lose its remaining electromotive force. The applied current plays no further role once the critical discharge level is reached. The nature of the sudden change by which the membrane collapses without further external intervention is not known. The following points must be considered:

a. Local Circuit Theory - The active or depolarized region of the nerve fiber may for some purposes be likened to a cathode applied to the nerve. Just as current flows out through the extrapolar region of the membrane to a cathode, current flows out through the region of the membrane adjacent to the depolarized zone to enter again at the depolarized zone. The flow of current out through the membrane progressively depolarizes the region adjacent to the active zone and, since the flow of current in the local circuit is several times the critical strength required to bring the resting membrane to threshold, the membrane in that region in turn "collapses". In terms of the analogy, the collapse of the membrane and movement of the depolarized zone to a new position are equivalent to moving the cathode along the nerve. A new fresh segment of the nerve is thus subjected to the flow of current. The wave front is pushed a little further along the nerve and the whole process is repeated. The impulse, then, is propagated by a process of electrotonic extension into the resting membrane.

Although the nature of the change that occurs at threshold is not yet understood, attempts have been made to describe the change in terms of core-conductor theory by supposing an alteration in one or more of the equivalent electrical components of the membrane. A local increase in the capacity of the membrane has been suggested by Schmitt. As a result of this, the charge on neighbouring condensers would be drained. Recovery would take place as the local capacity returns to its original size and is re-charged by the source of electromotive force. Cole and Curtis(26) regard a drop in membrane resistance as the major factor, since, in their studies on the isolated single giant nerve fiber of the squid, they found an increase in transverse conductance during activity. Rushton(117) assumes that the battery disappears in the active region without any other change. The resulting loss of electromotive force at the active region would start the characteristic current flow. One should emphasize that these formal representations are nothing but ways of thinking about the actual event in living nerve, and are as artificial as the model upon which they are founded.

It should be noted that the local circuit theory is an integral part of all schemata of conduction. It is the part that rests on the most secure experimental foundation. Hodgin's[67] work on nerve blockage is an outstanding recent piece of evidence. He has found that a potential can be recorded beyond a block in nerve as a travelling spike potential reaches the upper margin of the block, although no impulses are transmitted through the block. The EXTRINSIC POTENTIAL, as this electrical charge beyond a complete block is called, is easily distinguished from a spike potential by the fact that it decrements exponentially along the stretch of nerve beyond the block, whereas a spike potential is conducted with uniform amplitude. Other support is derived from a number of experiments on different types of excitable cells in which a conducting bridge placed across a killed region permits the spike potential to pass what would otherwise be a complete block. The prototype of these experiments was performed by Osterhout and Hill[106] on the elongated excitable plant cell, Nitella. With the strict time relations that obtain, it is inconceivable that any influence other than an electric current could flow through the salt bridge to excite the region beyond the block.

Remaining details of the concept of impulse propagation may now be summarized. It will be noted that the point of division - in either space or time - between the electrotonic extension of the impulse and the impulse itself falls at the first reversal point of the membrane current. The foot of the spike potential, that part in front of the first reversal point of membrane current, therefore, is an electrotonic potential. The depolarized zone between the two reversal points draws current from in front of the first reversal point and from behind the second reversal point. The nerve impulse may be regarded as coextensive with that part of the depolarized zone that accepts current from the region in front of the first reversal point. The remainder of the current flow, from the region behind the second reversal point, is part of the recovery process.

b. Energy Relations - All of the currently accepted views represent the membrane as a leaky condenser system that is only maintained in the charged state by the constant performance of metabolic work, as evidenced by heat production, oxygen consumption and carbon dioxide production of a nerve at rest. The immediate energy for propagation of the impulse is supplied by the charged membrane in front of the impulse. Behind the impulse the membrane charge must be reconstituted. Presumably the energy source for recharging the membrane is the same as that for maintaining the charge in the resting state. The conduction of impulses involves an increase in heat production and respiration.

c. Refractory Period - The region of nerve, occupied by an impulse cannot be stimulated by the most drastic means. It is said to be refractory. In a manner of speaking, the membrane of the region not being polarized cannot be depolarized. There is not yet general agreement as to the duration of the refractory period relative to that of the spike potential, nor is it possible to say at this time whether some fixed relation holds between them. Theoretically, it would seem that as a minimum the stretch of nerve between the two reversal points would be refractory, but how much more would also be refractory is an open question. Before normal excitability returns, the nerve passes through a period of relative refractoriness, during which time it cannot be excited by stimuli of greater than normal, resting, threshold strength. The transition from absolutely refractory state to normal resting state is smoothly progressive.

E. Compound Spike Potential of Nerve and Types of Nerve Fibers:

Our modern knowledge concerning the properties of the individual fibers that together constitute a nerve trunk began with the discovery by Erlanger and Gasser(37) of the compound nature of the spike potential. The fibers of a nerve conduct impulses at various velocities; hence if a nerve be stimulated by a single shock, all the impulses will start out together, but, as they travel along the nerve, the fast impulses will outstrip the slow.

The required experimental procedure for studying the spike potential as it progresses along a nerve is to place recording electrodes at one end of the nerve and then to locate several pairs of stimulating electrodes on the nerve at known distances from the recording electrodes. The nerve is then stimulated by single shocks through each pair of stimulating electrodes in turn, with the result that successive recordings show the spike potential after different distances of conduction. When this is done and the records are arranged in order of increasing conduction distance, it is found that the spike potential of the whole nerve forms a simple elevation at or near its point of origin, but that it increases in duration and breaks up progressively until, at the far end of the nerve, a series of more or less distinct elevations is formed. Several features of the nerve response are immediately discernible. In the first place, the change in form of the spike potential as it travels along the nerve is the result of the conduction of two elevations at different linear velocities. Secondly, not only do distinct components separate from one another as a result of conduction, but, in addition, each component tends to broaden and become lower progressively with increasing conduction distance. Finally, measurement of the area enclosed by the recorded elevations and the base line (the so-called area of the spike potential) would show that it remains constant despite the changes in configuration with conduction distance. Therefore, each elevation is formed by the sum of a number of smaller elevations travelling at a range of velocities, the unit response, which does not change in amplitude nor in duration by virtue of conduction, being that of a single nerve fiber. The elevations in the compound spike potential of a nerve appear in succession as the strength of stimulus is progressively increased.

Systematic study[50] of a number of different nerves has revealed the fact that there are three major groups of nerve fibers as judged by significant discontinuities in properties. These groups are known by the alphabetical designations "A", "B" and "C". The A group includes all of the myelinated axons of the somatic nerves, these varying in diameter from 20 microns to 1 micron and in conduction rate from 120 meters per second to approximately 6 meters per second. The A fibers may show four daughter elevations in the spike potential, referred to as alpha, beta, gamma and delta elevations respectively. The B fibers are myelinated fibers of 3 microns diameter or less, found in the autonomic nerves but not in somatic nerves and with conduction velocity between 15 and 3 meters per second. The C fibers are unmyelinated and conduct impulses at velocities between 2 and 0.6 meters per second.

Hursh[70] carried out a study of conduction velocity and diameter of nerve fibers, summarizing the following formulae for the relationship between the size of nerve fibers and the velocity of conduction of impulse:

1. $V = kD$ (Gasser and Erlanger 1927[51])
2. $V = kD^2$ (Blair and Erlanger 1933[14])
3. $V = kD^{0.5}$ (Pumphry and Young 1938[114])

The basis of these methods depends on the accepted assumption that in any given nerve, the largest fiber conducts at the highest velocity. Procedure is to select nerves with different maximal velocities, measure the velocities and then prepare histological sections of the nerve in which measurement of the diameter of the largest fibers can be made. In order to measure the velocity, condenser discharges controlled by a thyratron were used for stimulation. Oscillographic records of the action potential were made at five or more distances of conduction, and then the shock-response times were measured on enlarged projections of the records. The series of times, plotted against the distances, yield a straight line graph, the slope of which gives the velocity freed from these sources of error. The first deviation of the action potential from the baseline in the records made for velocity measurements could be referred to the single largest fiber in the nerve. When small fibers were being studied, it was not possible in nerves of the sizes employed to record single spikes with certainty, because of interference by the noise level.

The fiber diameters were measured in the following way. When the nerves were removed, they were tied to glass rods to prevent a change in length. The excised nerves had previously been stimulated while mounted on silver-chloride electrodes in a moist chamber at 37.5° centigrade, using Krebs-Ringer solution as a moistener and in an atmosphere of 5% CO_2 in oxygen. The nerves were then fixed in 1% osmic acid to measure large fibers and in 10% formalin followed by staining according to the Kultschitzski[94] method for smaller nerves. The following sources of error were noted:

1. Tapering or breaking of the nerve fibers.
2. Random variation of the fiber diameters.
3. Differential distortion of large and small fibers.

F. Experiments Concerning Spike Potentials of the Dog's Vagus

In spite of a thorough review of the literature, no previous precise work on the spike potentials of the dog's vagus could be found. It was realized that this knowledge was of fundamental importance in any effort to stimulate successfully the vagus in an intact experimental animal. Thus a series of six experiments were carried out in the Department of Physiology and an effort was made to measure the spike potentials of the isolated vagus nerve of the dog - the cervical, upper thoracic and lower thoracic segments being studied. This work is discussed in the chapter on the experimental work done and is to be found on page 184 to page 193 inclusive.

CHAPTER IV

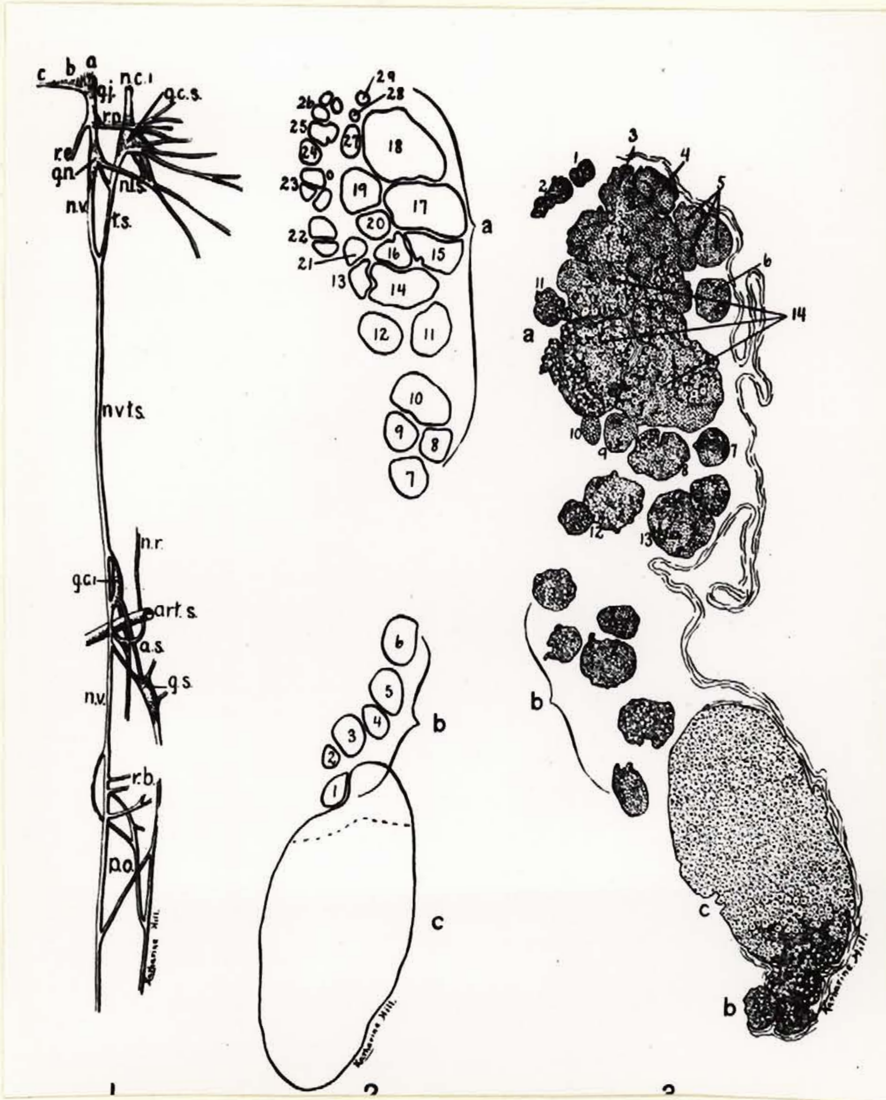
ANATOMY OF THE VAGUS NERVE IN THE DOG

This question has been considered at length and an effort has been made, by microscopic sections as well as varied types of stimulation, to ascertain if there are marked differences at various levels in the vagus and whether the types of nerve fibers change as the nerve descends.

A. Gross Anatomy

The vagus nerve in the dog[24] arises by means of a large number of small rootlets from the dorso-lateral sulcus of the medulla oblongata, in series with the roots of the glossopharyngeal and accessory nerves. In Figure 3 on page 60, (a) the rootlets of the vagus proper are seen running into the jugular ganglion and joining the vagus at the side of the ganglion is seen the spinal root of the accessory nerve (c). Scattered fine rootlets (b) which have come from the medulla are seen joining the spinal root. These two nerves - vagus and accessory - are fused into a single trunk at the level of the jugular ganglion. Below the jugular ganglion the pharyngeal

FIGURE 3



Structure of the Vagus Nerve

Reproduced from Chase, M.R. and Ranson, S.W.: The structure of the roots, trunk, and branches of the vagus nerve. J. Comp. Neurol., 24: 31, 1914. Page 37. Legend on Page 61.

LEGEND OF FIGURE 3

a.	vagus rootlets.
b.	bulbar rootlets of the accessory nerve.
c.	spinal root of the accessory nerve.
g.j.	ganglion jugulare.
r.p.	ramus pharyngeus.
r.e.	ramus externus n. accessorii.
g.n.	ganglion nodosum.
n.e.s.	nervus laryngeus superior.
n.v.	nervus vagus.
n.c.i.	nervus caroticus internus.
g.c.s.	ganglion cervicale superius.
t.s.	truncus sympathecus.
n.v.t.s.	nervus vagus and truncus sympathecus.
g.c.i.	ganglion cervicale inferior.
art.s.	arteria subilavia.
n.r.	nervus recurrens.
a.s.	ansa subclavia.
g.s.	ganglion stellatum.
r.b.	rami bronchiales.
p.o.	plexus oesophageus.

branch of the vagus and the external branch of the accessory are given off from the common trunk. The superior laryngeal nerve leaves the vagus at the level of the nodose ganglion. Somewhat below this level, the sympathetic trunk joins the vagus and runs downward in the same connective tissue sheath with it. Communicating branches from the sympathetic join the pharyngeal and superior laryngeal branches of the vagus. Just above the subclavian artery there is developed in close connection with the vagus, the inferior cervical ganglion of the sympathetic. From this, the ansa subclavia runs to the stellate ganglion. From the right vagus, immediately below the inferior cervical sympathetic ganglion, there is given off the recurrent nerve. This receives a branch from the inferior cervical sympathetic ganglion and from it a large branch runs to the posterior cardiac plexus. Kiss[82] regards the unmyelinated fibers - which predominate in the thoracic vagus - as sympathetic fibers which join the vagus trunk, mainly in the region of the superior cervical sympathetic ganglion. He also denies the existence of unmyelinated fibers in the vagus rootlets, disagreeing with the work of Ranson and Chase[24] and others. In the thorax the vagus gives off branches to the root of the lung and is then continued into the oesophageal plexus. Here the right vagus, joined by a branch from the left, runs on the right side and posterior aspect of the oesophagus to reach the posterior aspect of the stomach. It gives off many fine branches to the stomach. Figure 4 on page 64 shows the gross anatomy of the stomach with its nerve supply.

B. <u>Microscopic Anatomy</u>

A summary of histological studies of the vagus by osmic acid and pyridine silver techniques was compiled by Chase and Ranson[24]. They concluded:

1. The various rootlets of the vagus and accessory nerves differ markedly in structure. The spinal root of the accessory is composed almost entirely of large medullated fibers with a very few small ones. The bulbar rootlets of the accessory are composed of large and small medullated fibers with the small fibers predominating. These rootlets contain few if any non-medullated fibers.

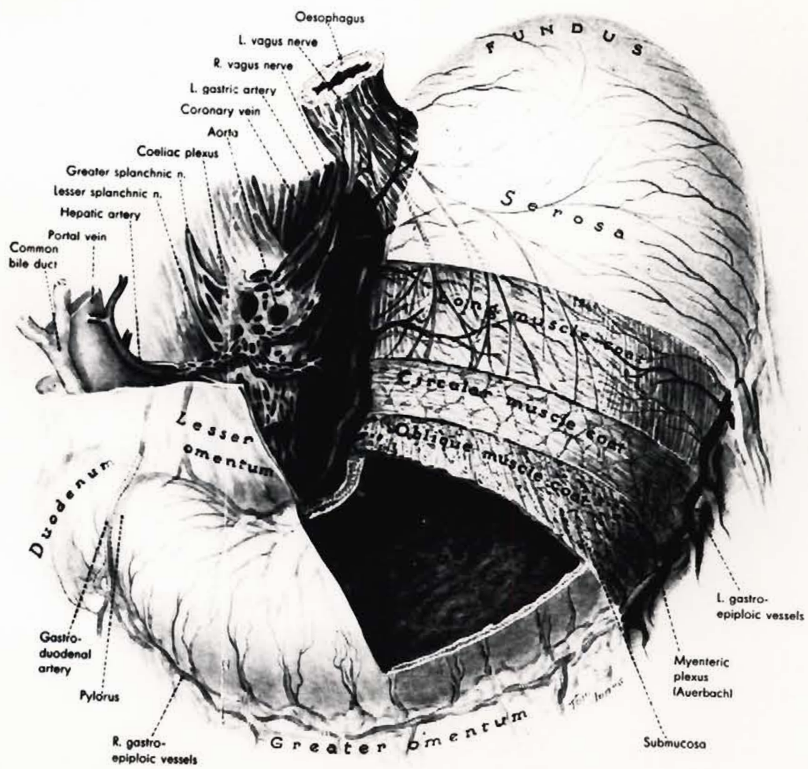
The rootlets of the vagus are of two kinds. Those of type 1, probably efferent in function, are composed of many fine and fewer coarse medullated

FIGURE 4

The Stomach

The illustration shows a stomach distended for greater detail, with the several layers composing its wall exposed. The blood supply is shown as is also the main vascular bed in the mucosa.

The parasympathetic innervation is represented by the right and left vagus nerves which terminate mainly in the myenteric plexus of Auerbach. The sympathetic innervation is derived from the celiac plexus and reaches the stomach as fibers coursing along the gastric arteries.



The Stomach

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fibers. The medullated fibers are evenly distributed through these rootlets and there are few if any non-medullated fibers. The vagus rootlets of type 2, probably afferent in function, contain large and medium-sized medullated fibers and fewer small ones. The medullated fibers are widely separated by enormous numbers of fine, non-medullated axons.

2. At the level of the upper part of the jugular ganglion the vagus and accessory nerves are fused into a common trunk in which it is possible to distinguish three areas derived respectively from the spinal root of the accessory, the bulbar roots of the accessory and the roots of the vagus. Each area presents the same histological characteristic as the corresponding roots, except that the fibers from the two types of vagus rootlets are now intimately mingled.

3. Below the level of the jugular ganglion, the spinal part of the accessory, which has maintained its independence throughout the common vagus-accessory trunk, now leaves it in the external branch of the accessory nerve.

4. The bulbar fibers of the accessory become intimately mingled with the vagus fibers at or above

the level of the nodose ganglion. The so-called internal branch of the accessory does not exist as a separate nerve but is only a fascicle of the common vagus-accessory trunk.

5. While the vagus and sympathetic nerves are intimately associated in the neck, it is clear that no considerable part of the non-medullated fibers of the vagus are of sympathetic origin.

6. The pharyngeal branch is composed for the most part of large medullated fibers but also contains a considerable number of medium and small medullated fibers. It contains few, if any, non-medullated axons.

7. The superior laryngeal branch contains large, medium and small medullated fibers with the medium and small ones predominating. It contains non-medullated fibers in considerable numbers but these are much less numerous then in the vagus trunk.

8. The recurrent nerve contains an area of large medullated fibers destined for the larynx and an area of medium and small medullated fibers which are given off in its oesophageal, trachial and cardiac branches.

9. The pharyngeal, superior laryngeal and

recurrent nerves take out from the vagus nerve almost all of its large medullated fibers, so that the vast majority of the medullated fibers in the thoracic vagus are either small or medium in size. These are widely separated by non-medullated fibers.

10. The non-medullated fibers are present in much greater proportion in the thoracic portion of the vagus than in the upper part of the nerve. This is to be accounted for by the fact that a large number of medullated fibers have been taken away by the cervical branches while practically all of the non-medullated fibers are carried down into the thoracic vagus. This increase in the proportion of non-medullated fibers in the lower portion of the nerve also is probably due in part to pre-ganglionic fibers losing their myelin sheath in their downward course.

11. Most of the medullated fibers in the thoracic vagus leave it through the bronchial and oesophageal branches so that the vagus as it passes through the diaphragm may properly be called a non-medullated nerve. It is composed entirely of non-medullated axons and contains only a few scattered medullated fibers.

This change in type of vagus fibers was substantiated by R.L. Jones[80]. Comparison of the vagus trunk proximal and distal to the nodose ganglion showed a great difference in the ratio of myelinated to unmyelinated fibers. Proximal to the ganglion the greater part of the cross-sectional area of the trunk is occupied by myelinated fibers, while distal to it the greater part of the cross-sectional area is occupied by unmyelinated fibers. Serial sections through the nodose ganglion show that this change is effected by a gradual increase downwards through the ganglion in the number of unmyelinated fibers. Another factor in the change is the deviation from the vagus trunk of the fibers which make up the superior laryngeal nerve. They showed in cats that in the left vagus above the nodose ganglion there were 5,377 myelinated fibers and 2,500 unmyelinated fibers, while below the nodose ganglion the left vagus has 2,848 myelinated fibers and 9,024 unmyelinated fibers. They also counted the number of cells in the nodose ganglion and found they closely approximated the number of fibers counted in the vagus trunk distal to the ganglion. They conclude that the greater number of the cells of the nodose ganglion therefore must be regarded as the cells of origin of the unmyelinated fibers. This was also proven by section of the vagus above the ganglion and after a degeneration period of fifteen days, the vagus nerve distal to the ganglion was studied. These showed only partial degeneration of the unmyelinated fibers, indicating the major portion of these fibers arise from the ganglion. The degenerated fibers in these preparations presumably represent preganglionic components of the vagus which terminate in ganglia located further distally. These results confirm the physiological evidence of the existence of efferent cells in the nodose ganglion reported by Morgan and Goland(99).

C. Functional Properties of the Vagus Fiber Groups

An interesting study of the functions served by the fiber groups in the mammalian vagus nerve has been done by Heinbecker and O'Leary(61). They have correlated the potential form and nerve fiber structure as revealed in osmicated cross-sections of nerve in turtles and cats, and showed the first potential complex to be associated with activity in the large myelinated fibers, the second with activity in the small thinly myelinated fibers and the third with activity in the non-myelinated fibers. The first of these fiber groups was shown to have physiological properties characteristic of somatic nerve fibers, the other two of autonomic efferent fibers(60).

Their results establish the afferent nature of the large myelinated fibers responsible for the first potential complex other than those motor to the larynx and similar skeletal muscle structures and confirm the efferent nature of the small thinly-myelinated and non-myelinated fibers responsible respectively for the second and third potential complexes. Of these efferent fibers, some have their cells of origin within the central nervous system; others within the nodose ganglion. No synapse has been demonstrated in the nodose ganglion.

The essential feature of the physiological investigation has been the utilization of the established correlation between potential form as recorded by the cathode ray oscillograph from a nerve trunk after conduction and the nerve fiber content. By the coincidental recording of the potential, and of the effect on the organism resultant from electrical stimulation of a nerve trunk still functionally attached, it is possible to correlate such body functions with fiber group activity. The electroneurogram, like the electrocardiogram, thus could become an index of function. Owing to the considerable differences in threshold of the various fiber groups, it is possible to study the functional effect of each by increasing the strength of stimulation.

The differential study of the fiber groups is further facilitated by utilizing the results of studies by Erlanger and Gasser[52] demonstrating that pressure blocks the large fibers in excised nerves before small ones. By applying pressure to the vagus nerve still functionally attached, it was possible for them not only to separate out functional sub-divisions within an extensive fiber group but also to study the function of the non-myelinated fibers separately from the myelinated ones.

To summarize briefly: of the afferent myelinated fibers of a cat vagus nerve, some 10 to 4 microns or less are concerned with respiratory reflexes, some 8 to 3 microns in vasomotor reflexes and the depressor fibers ranging from 6 to 3 microns, which is also the range for the few pain fibers in the nerve. The afferent fibers in the recurrent laryngeal nerve range in size from 8 to 24 microns. No non-myelinated afferents are found in the vagus nerve. The conclusion is that all afferent fibers of the vagus are of the myelinated somatic type.

Concerning the Efferent Fiber Groups, they were unable to trace any evidence that the fibers of the cervical vagus trunk are sympathetic in origin, thus refuting the Kiss Theory which has been discussed above.

In normal animals it was demonstrated that constriction of the bronchi and intestinal motor excitatory or inhibatory effects are produced by impulses conveyed by the non-myelinated fibers of the vagus. One must conclude that there are fibers in the vagus nerve whose cells of origin are in the nodose ganglion which are efferent in type - which again agrees with the work of Jones and others quoted above. Other fibers, including certain of those to the heart, are of similar character but their cells of origin are in the central nervous system.

By testing action potentials twenty days after section above the nodose ganglion, it was shown that the majority of fibers of each group of the vagus nerve trunk below the nodose ganglion arise from cells in the vagus nerve. By section of the vagus above and below the nodose ganglion it was seen that:

1. Most of the fibers reaching the viscera of the abdominal cavity are non-myelinated.
2. Most of these have their cells of origin in the nodose ganglion.
3. Those fibers, if any, which pass directly from the medulla with cells of origin central to the jugular ganglion are too few or too scattered to be detected by degeneration technique. In view of the functional results reported above, it may be concluded that the vagus below the diaphragm is almost purely a motor nerve.
4. The vagus nerve at the level of the nodose ganglion shows two anatomical divisions, one containing the motor fibers from the larynx and autonomic fibers to the heart, the other motor and sensory fibers to the lungs and abdominal viscus.

5. The mammalian vagus nerve contains four functional fiber groups - somatic motor and somatic sensory fibers, autonomic myelinated and non-myelinated fibers.
6. The potential recorded from the cervical vagus after conduction shows three major potential maxima - the first derived from the somatic fibers, the second and third from the autonomic fibers.

More specific work has been done on the afferent fibers in the vagus nerve from the abdomen by Harper, McSwiney and Suffolk(57). They reported that examination of the literature revealed the fact that while a number of investigators have obtained evidence of afferent fibers in the vagus trunks of the abdomen, few of these have set out to definitely investigate the course and pathway of these fibers. The study of afferent fibers in the vagus trunks of the abdomen in all instances has depended upon the elicitation of some reflex response in the animal. Many reflexes have been used. Rogers(116), working on decerebrate dogs, found that stimulation of the central end of one abdominal vagus trunk or of the vagus in the neck, produced spasmodic contraction of the entire stomach, if the other vagus was left intact. Harper, McSwiney and Suffolk[57], using dilatation of the pupil in animals anaethetized with chloralose as an index of afferent motor activity, obtained evidence that afferent fibers exist in the abdominal vagus nerve of the cat. Two groups of visceral afferent fibers have been identified in the abdominal vagus nerve. Fibers of the first group pass from the ventral and from the dorsal vagus trunk directly to the medulla by both cervical vagus nerves. Fibers of the second group leave the thoracic vagal trunks, accompany the intercostal arteries from the aorta, join the intercostal nerves close to their exit from the intervertebral foramina and enter the spinal cord by the dorsal roots from the second to the eighth thoracic roots inclusive. The medullary fibers have no synapses in the nodose ganglion and the spinal fibers have no synapses in the dorsal root ganglion, comparable to those in the sympathetic ganglia in the efferent sympathetic pathway. The impulses which pass to the central nervous system by the medullary and by the spinal fibers ascend as high as the midbrain at least.

Irving, McSwiney and Suffolk[72] did more precise work on the stomach and intestine. They used dilatation of the pupil of a cat anaethetized with chloralose, as above, and reflex changes of blood pressure in the spinal and decerebrate cat, and in the cat anaethetized with chloralose, as indices of visceral afferent impulses. These methods do not indicate the character of the sensation. They conclude:

1. The gastric and duodenal mesenteries are innervated by the vagi and splanchnic nerves while the jejunal mesentery appears to be supplied by the splanchnic nerves. The mesenteries are sensitive to traction.
2. The body of the stomach, pyloric antrum and pyloric sphincter are sensitive to distension and are innervated by afferent fibers in the vagus and sympathetic nerves. The lowest threshold pressure was obtained from the pyloric sphincter.
3. The stomach and duodenum are supplied by afferent fibers from the right and left

vagus nerves and from the right and left splanchnic nerves.

4. The jejunum and ileum are much less sensitive to distension than the stomach and duodenum. The right and left splanchnic nerves appear to be the main afferent pathway from these regions.

D. Cross-Sections of the Vagus Nerve of a Dog

Because of the difficulty in obtaining information on the type and distribution of nerve fibers in the dog, it was decided to have sections made by Dr. Jerzy Olszewski of the Montreal Neurological Institute. Sections were made of the normal cervical and thoracic vagus at the areas where the receivers are normally implanted. These sections were removed as atraumatically as possible from a dog anaethetized by intravenous nembutal and were tied by each end to a glass rod before being placed in the required fixative. Considerable difficulty was experienced by him in obtaining satisfactory cross-sections of the nerve as the bundles seemed irregular and usually appeared obliquely in the cross-sections.

The first series of sections were made from the thoracic portion of the left vagus and stained by Bodian's Method for axis cylinders[92]. These sections show that at this level the vagus nerve is composed exclusively of very small fibers, the diameter of which lies in the range of 1 - 2 microns.

The second series of sections were made from the thoracic portion of the left vagus at the same level as the first, but were stained with osmic acid to show the myelin sheaths after fixation in Flemming's solution[93]. This nerve is composed of very fine fibers as above and are practically unmyelinated. Only a very few large fibers - about twenty in the whole nerve - are slightly myelinated and have larger diameters.

Teased sections were also made of the thoracic vagus and stained with osmic acid to show the myelin sheaths.

The third series of sections were made from the cervical vagus and were stained with osmic acid to show the myelin sheaths. The nerve shows a definite division into two bundles. One smaller bundle is composed of medium-sized fibers which are very uniform in diameter. In this bundle, there is also a small group of fibers of larger caliber, which are separated into a very small single nerve in some sections. The second larger bundle shows great variety of fiber diameter - including thick, heavily myelinated fibers, medium sized fibers with thinner coats of myelin and some very fine fibers which are practically unmyelinated. Teased sections were also made of the cervical vagus and stained with osmic acid to show the myelin sheaths.

The fourth series of sections, stained by Bodian's method, were made from the cervical vagus at the site of implantation of a receiver which had been in position for 54 days and had been stimulated for 121 hours, the last 96 hours being continuous stimulation. An agar-saline bridge type of electrode had been used with a variety of types of stimulation.

The nerve shows a marked perineural fibrosis but no signs of degeneration of nerve fibers. Otherwise the nerve appears normal and was still responding to stimulation when excised.

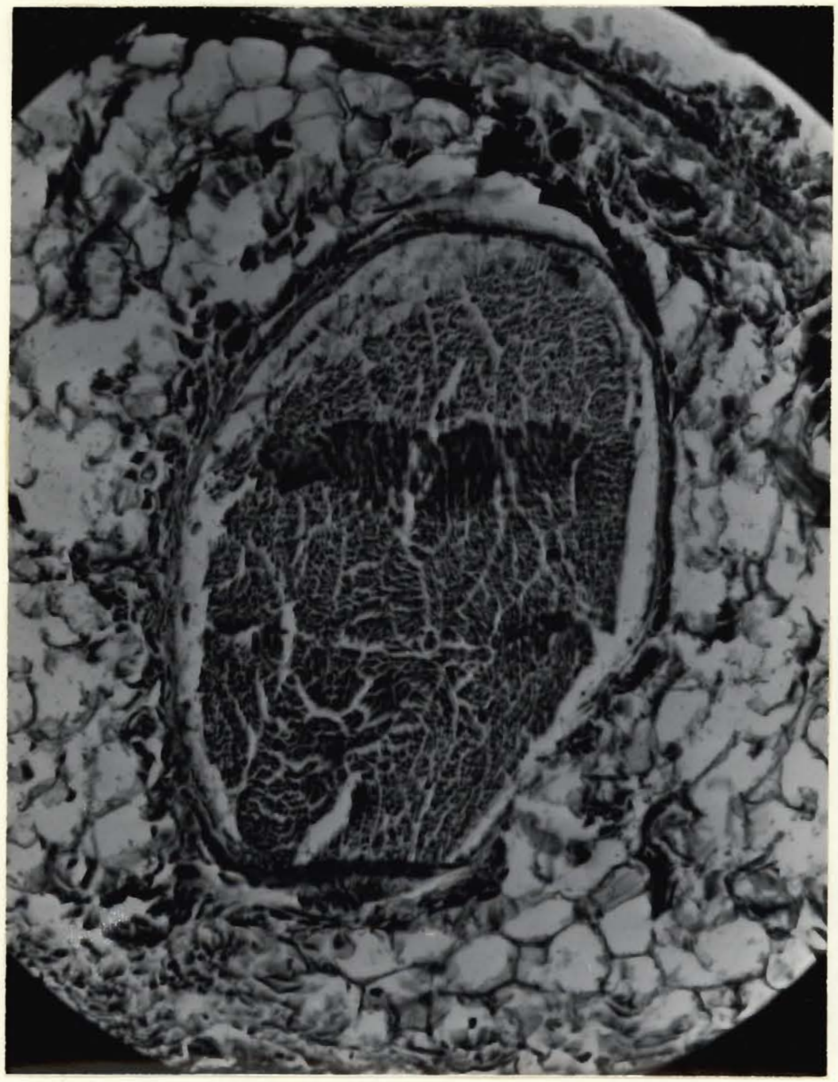
Thus it can be seen that the vagus nerve has an unusual distribution of fibers which doubtlessly is related to the physiological properties listed in this chapter. On the following twenty-one pages, photographs of these slides at magnifications of fifty, two hundred and five hundred are shown.

FIGURE 5

Thoracic Vagus (50 x)

Stained by Bodian's method for axis cylinders. The main nerve trunk with surrounding connective tissue and blood vessels is shown under low power.

FIGURE 6



Thoracic Vagus (200 x)

Stained by Bodian's method for axis cylinders. The main nerve trunk is shown under a higher magnification, and the whorled appearance in cross-section can be seen. These are all very small fibers, diameters ranging up to 1 to 2 microns.

FIGURE 7

Thoracic Vagus (500 x)

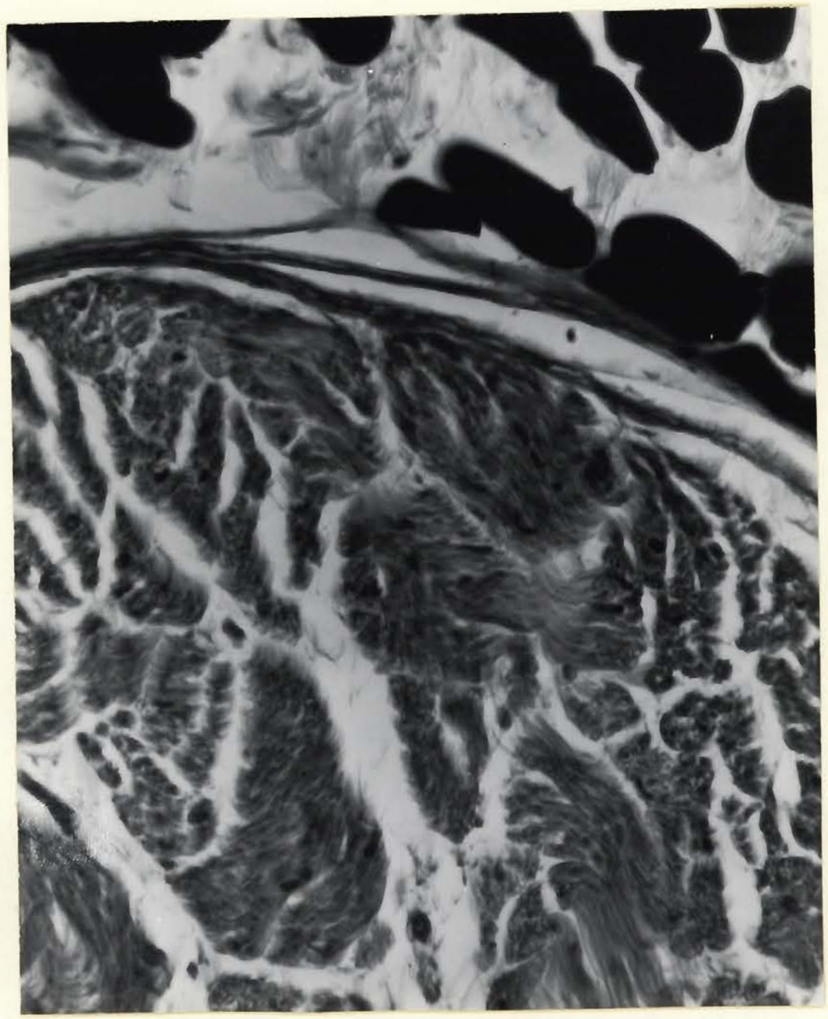
Stained by Bodian's method for axis cylinders. A section of the main nerve trunk is shown under very high power, the small diameter of the fibers being easily seen. Although this is a true cross-section, it is impossible to avoid the appearance of oblique, irregular fibers in this nerve.

FIGURE 8

Thoracic Vagus (50)

Stained with osmic acid to show myelin sheaths. Under low power, the very fine fibers of the nerve trunk can be seen with some surrounding connective tissue.

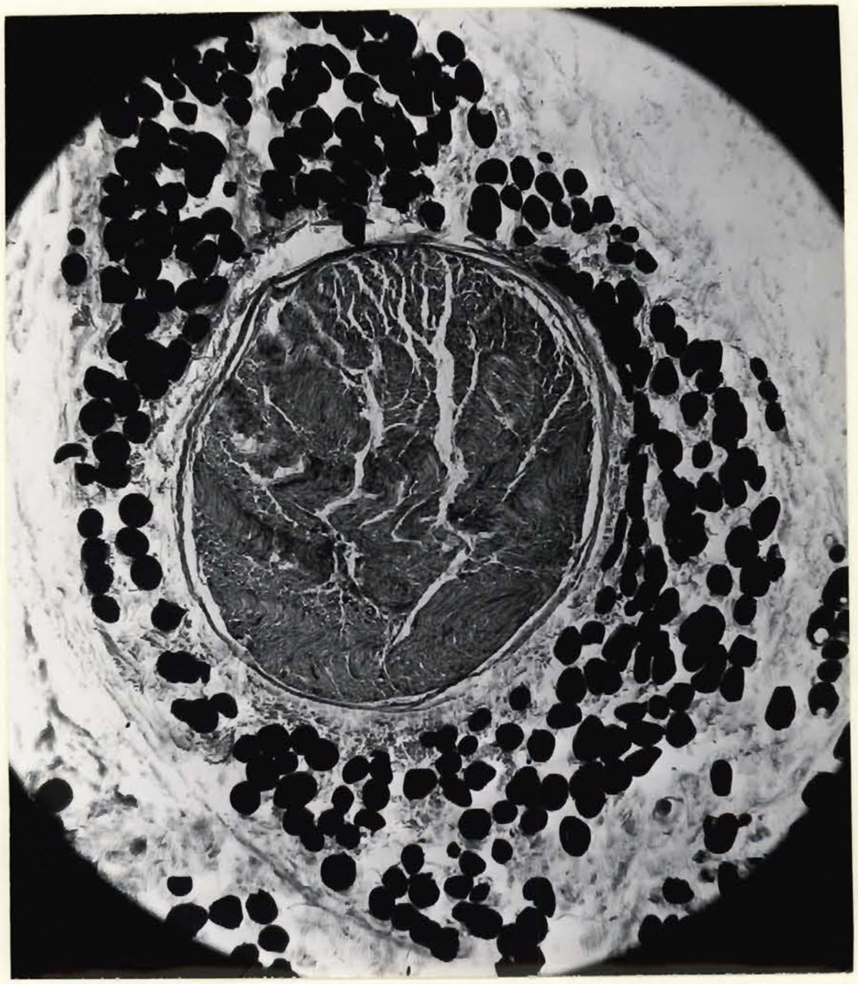
FIGURE 9



Thoracic Vagus (200 x)

Stained with osmic acid to show myelin sheaths. Under higher magnification, the whorled, very fine fibers can be more clearly seen. These very fine fibers are practically unmyelinated. Only a few larger fibers - about twenty in the whole nerve - are slightly myelinated and have larger fibers.

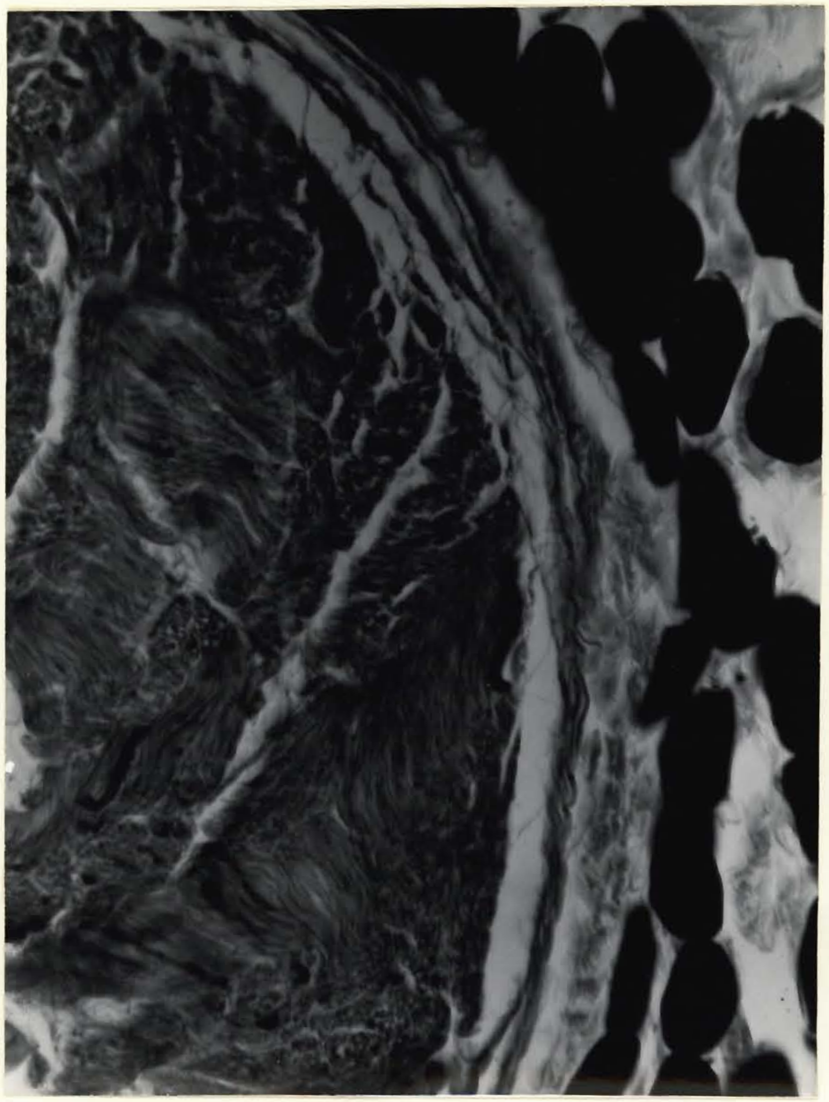
FIGURE 10



Thoracic Vagus (50 x)

Stained with osmic acid to show myelin sheaths. Under low power, this nerve shows more clearly than the previous similar cross-section, the appearance of the thin, irregular nerve fibers.

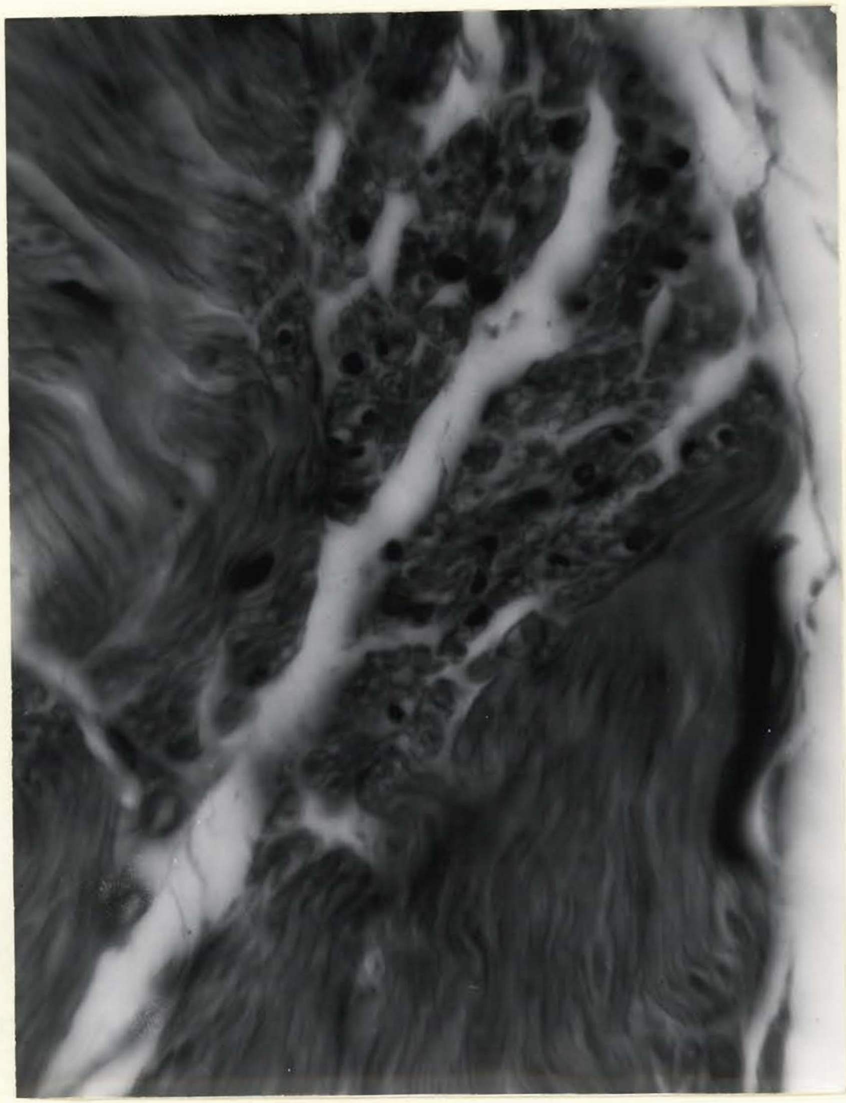
FIGURE 11



Thoracic Vagus (200 x)

Stained with osmic acid to show myelin sheaths. Under higher magnification, the few larger myelinated fibers can be clearly seen against the irregular background of thin, non-myelinated fibers.

FIGURE 12



Thoracic Vagus (500 x)

Stained with osmic acid to show myelin sheaths. A section of the thoracic vagus is seen under very high power. Both oblique and transverse views of nerve fibers are seen, with the few myelin sheaths showing clearly in black.

FIGURE 13

Thoracic Vagus (50 x)

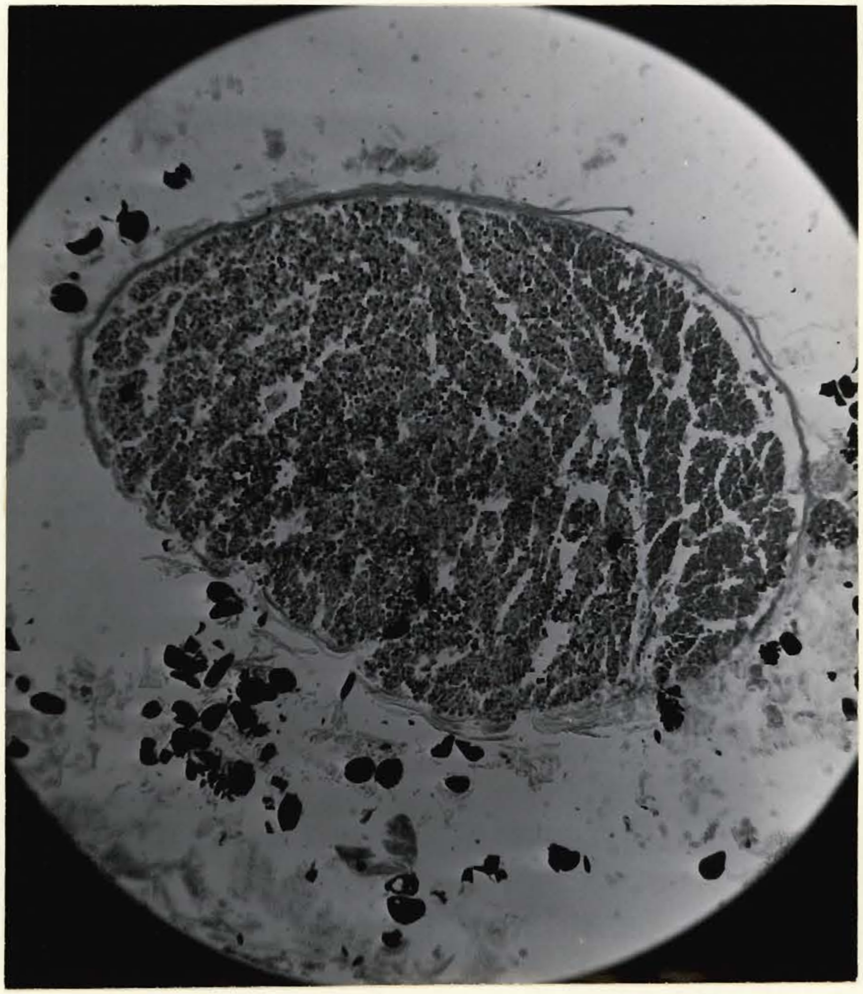
Stained with osmic acid for myelin sheaths. This shows a teased preparation of the nerve fibers showing the fine fibers in longitudinal section.

FIGURE 14

Thoracic Vagus (500 x)

Stained with osmic acid for myelin sheaths. This shows a section of the teased nerve under high magnification - the groups of nerve fibers being separated, with a few single nerve fibers visible.

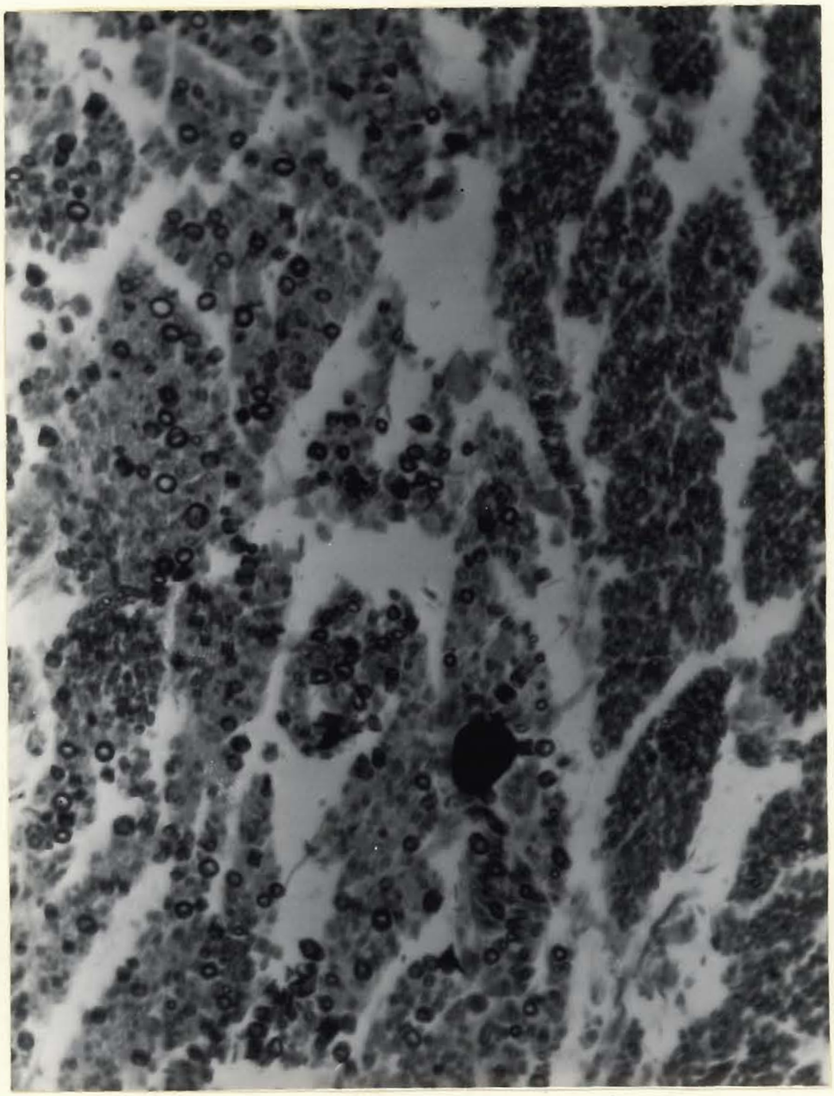
FIGURE 15



Cervical Vagus (50 x)

Stained with osmic acid for myelin sheaths. Under low power, this slide shows the division of the cervical vagus into two bundles, the smaller bundle being on the right of the picture.

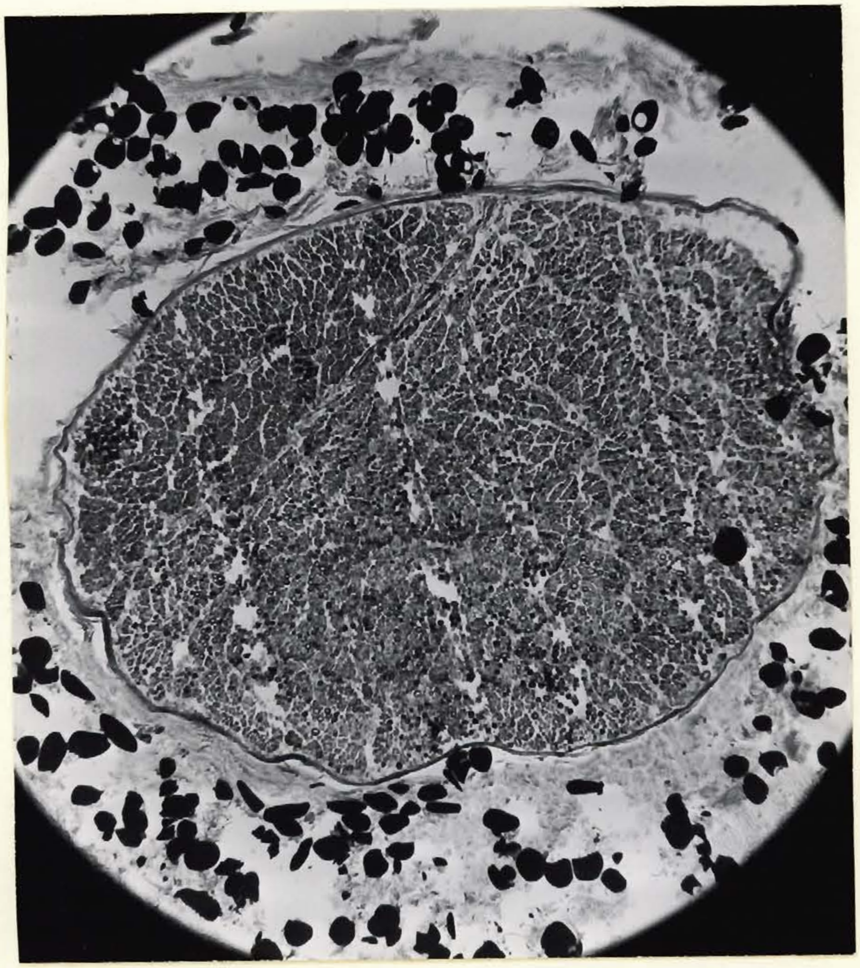
FIGURE 16



Cervical Vagus (200 x)

Stained with osmic acid for myelin sheaths. Under this magnification, the two bundles are distinct. The smaller bundle is on the right of the picture and shows uniform, medium-sized fibers. The larger bundle is on the left and consists of fibers of varying diameters up to 20 microns, their size being emphasized by the appearance of the black myelin sheaths.

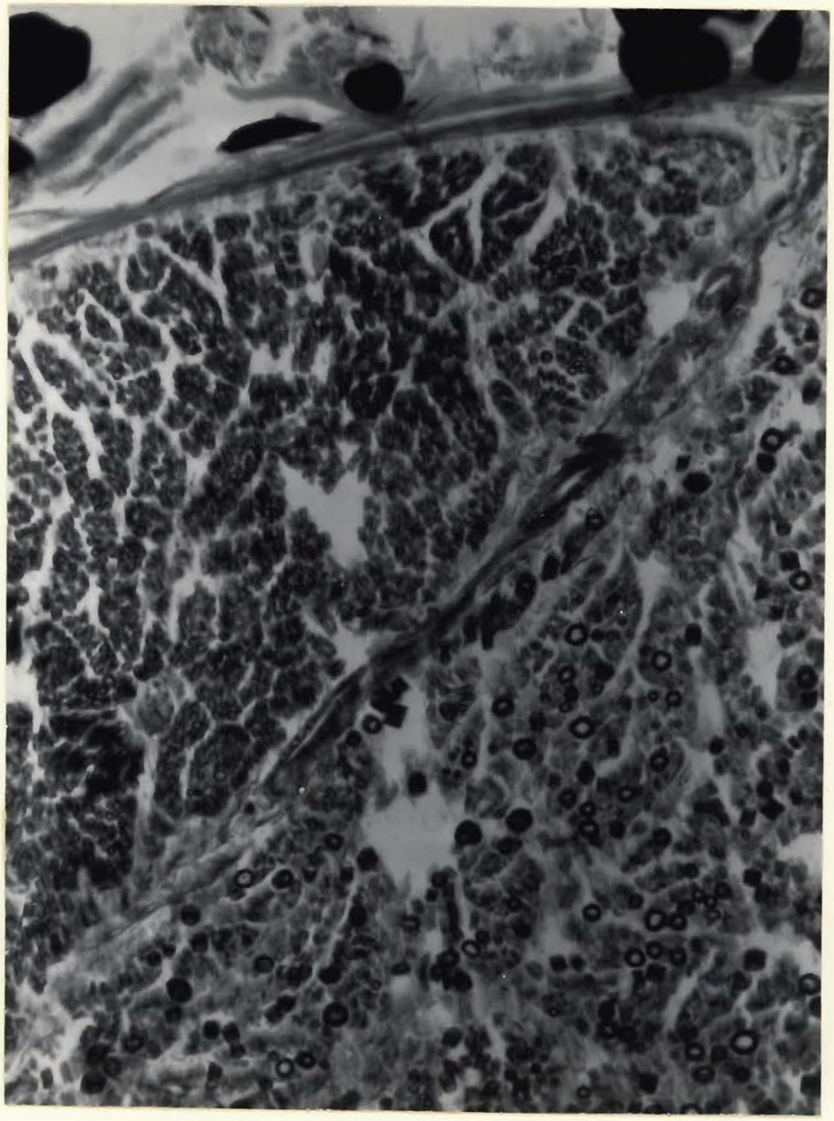
FIGURE 17



Cervical Vagus (50 x)

Stained with osmic acid for myelin sheaths. This is another section of the cervical vagus and once again the division into two bundles is distinct, the smaller bundle occupying the upper left portion of the nerve trunk in the picture.

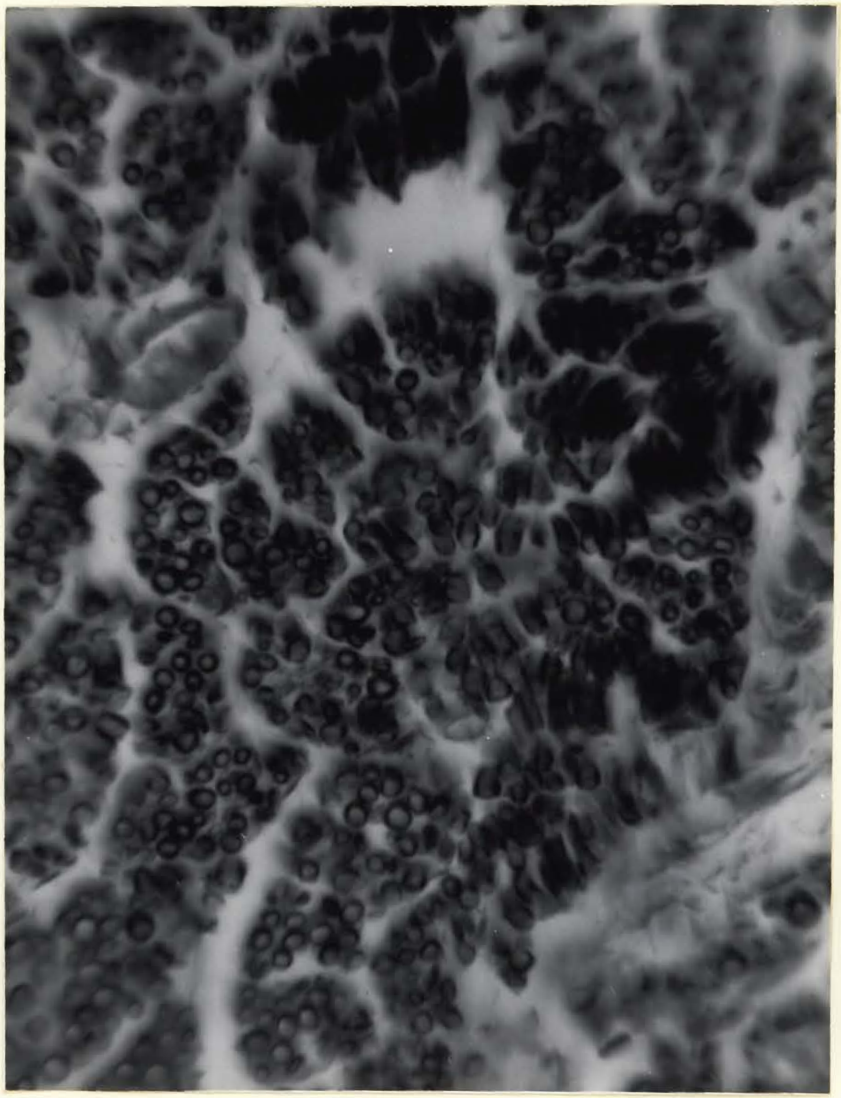
FIGURE 18



Cervical Vagus (200 x)

Stained with osmic acid for myelin sheaths. This is a higher magnification of the picture on the preceding page, showing the upper part of the junction between the two bundles, with the smaller bundle still occupying the upper left portion of the picture. The uniformity of the smaller bundle and the variation in size of fibers of the larger bundle are clearly seen.

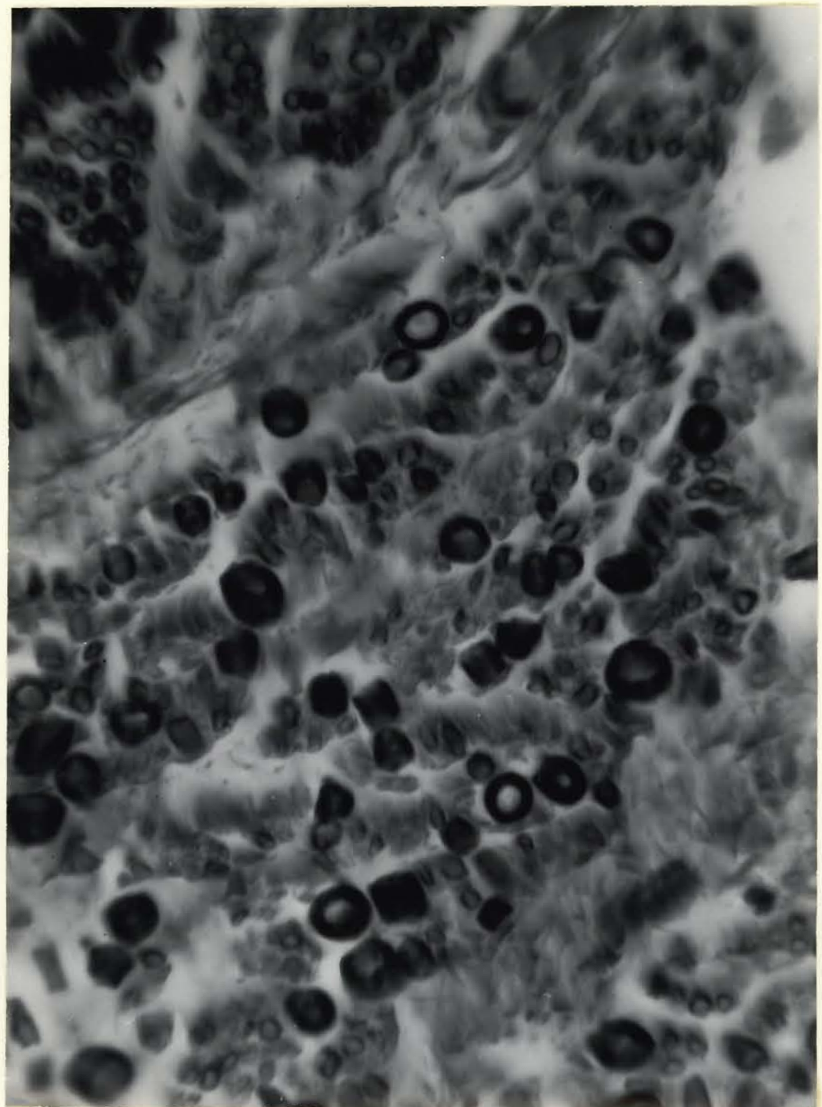
FIGURE 19



Cervical Vagus (500 x)

Stained with osmic acid for myelin sheaths. This shows a section of the smaller bundle of the nerve trunk under high power. The uniform diameter of the fibers, around 2 to 4 microns, is seen and the higher proportion of myelinated fibers as compared to the picture of the thoracic vagus under a similar power on page 87 can be appreciated.

FIGURE 20



Cervical Vagus (500 x)

Stained with osmic acid for myelin sheaths. This shows a section of the larger bundle under high power, with part of the smaller bundle and the division between these being seen in the upper left portion of the picture. Here the great variety of fiber diameters and myelination can be seen. There are all types; thick, up to 20 microns in diameter, and heavily myelinated; medium-sized with thinner coats of myelin; very fine fibers, around 2 to 4 microns in diameter, which are practically unmyelinated.

FIGURE 21

Cervical Vagus (50 x)

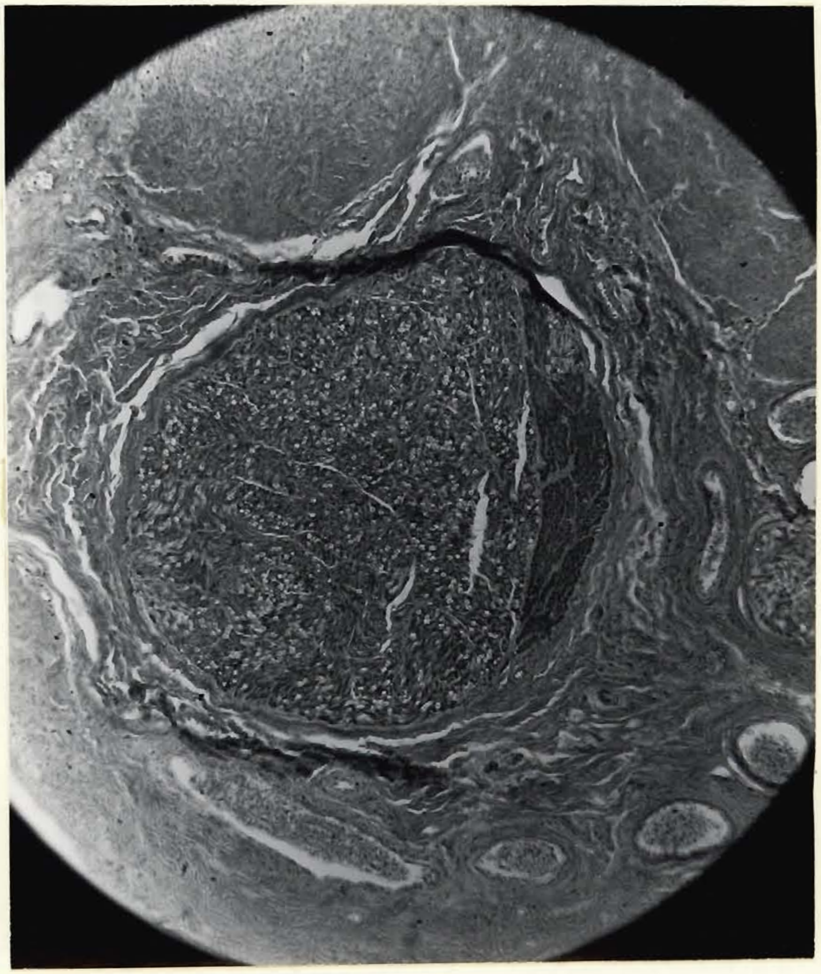
Stained with osmic acid for myelin sheaths. This shows a teased preparation of the cervical vagus, with groups of nerve fibers and some individual fibers being seen.

FIGURE 22

Cervical Vagus (500 x)

Stained with osmic acid for myelin sheaths. This shows the teased preparation of the cervical vagus under high power. Various nerve fibers can be seen but most of the nerve fiber groups are blended together.

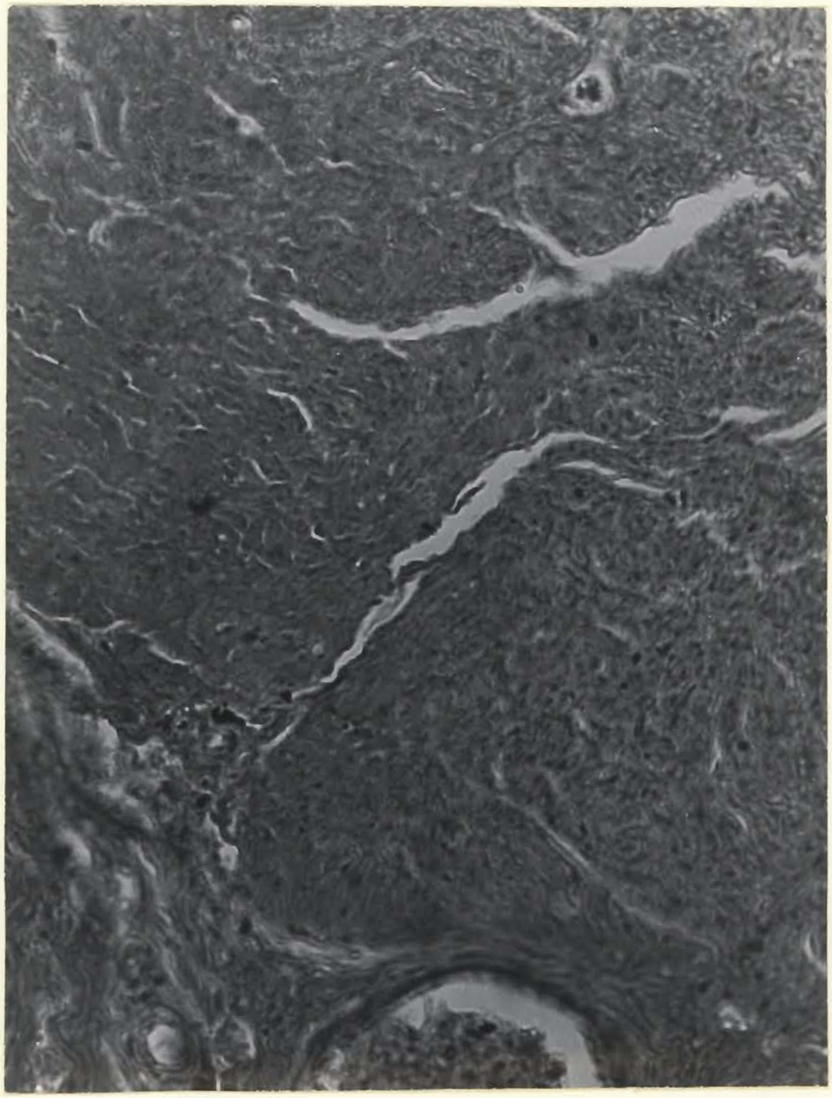
FIGURE 23



Cervical Vagus (50 x)

Stained with Bodian's stain for axis cylinders. This is a section of the cervical vagus which had been stimulated for 121 hours and which had been in contact with the agar-saline bridge type of electrode for 53 days. There is a moderate fibrosis in the area around the trunk but the two bundles appear essentially normal.

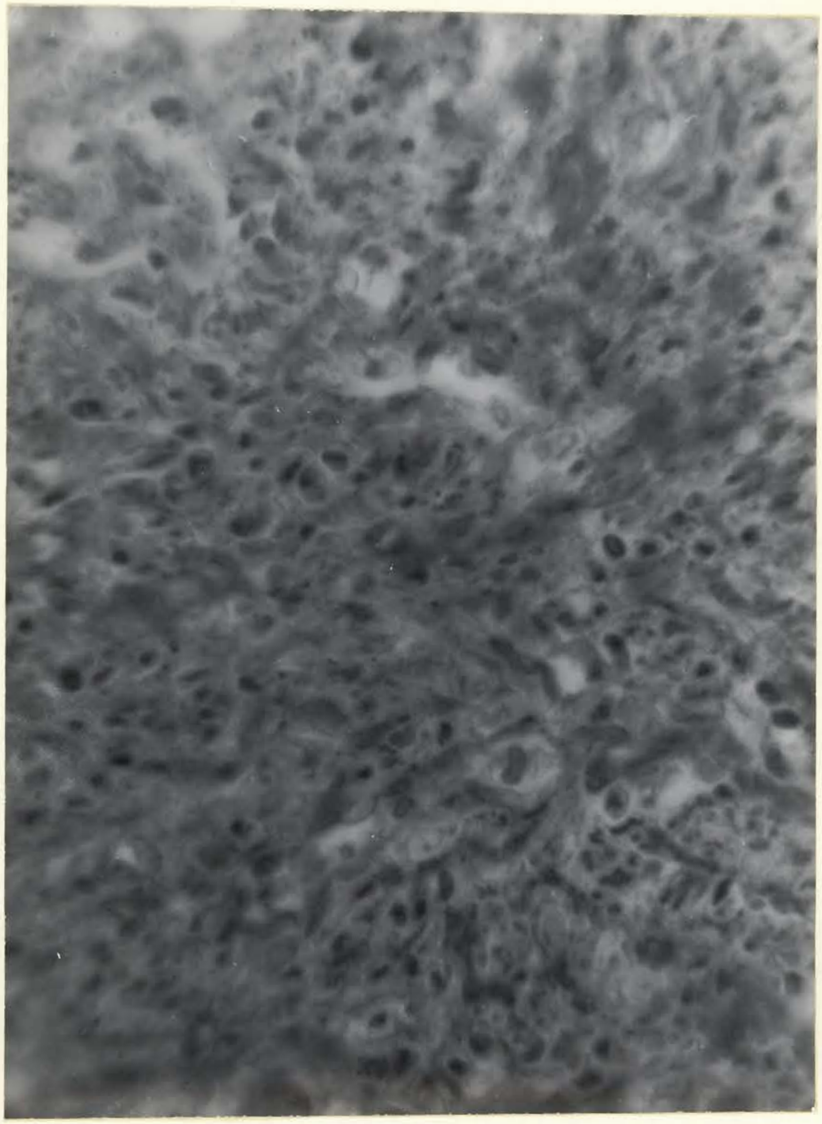
FIGURE 24



Cervical Vagus (200 x)

Stained with Bodian's stain for axis cylinders. Under higher power, this section of stimulated cervical vagus comprises mainly the lower portion of the smaller bundle which is on the right of the picture on the preceding page. These fibers appear quite uniform in size and show no areas of degeneration. A few of the more variable size of fibers from the larger bundle are seen in the lower left portion of this picture.

FIGURE 25



Cervical Vagus (500 x)

Stained with Bodian's stain for axis cylinders. This represents a section of the larger bundle of the cervical vagus after 121 hours of stimulation. The variation in fiber diameter is still clearly seen and no signs of degeneration are present.

CHAPTER V

PHYSIOLOGY OF GASTRIC SECRETION

A. Physiologic Anatomy of the Gastric Glands:

In man[74], monkey, dog, cat, rabbit, and certain other mammals, the stomach is divided into two major areas, each characterized by the type of gastric gland it contains. The proximal two-thirds of the stomach is occupied by the proper gastric glands whose function, in general, is to produce the active digestive elements of the gastric juices - pepsin and hydrochloric acid - while the distal third is provided with the pyloric glands, whose function is, in the main, the production of mucus.

There are, in addition, two minor zones which represent areas of transition between the major epitheleal divisions. The first, or upper of these minor zones is the cardiac zone, a narrow rim of mucus glands surrounding the oesophageal orifice and separating oesophageal from fundic mucus membrane. The second is the intermediate zone, a narrow strip of mixed glands lying between the proper gastric glands and the pyloric glands.

The various divisions of the gastric glands just outlined are all composed of the following four basic cell types in varying combinations and proportions:

a. surface epithelial cells.

b. neck chief cells, which are histologically identical with pyloric and cardiac gland cells.

c. parietal cells.

d. body chief cells.

The surface epithelial cells occur in all parts of the stomach; they line the entire surface of the stomach and the foveolae gastrica, or gastric pits, where they become continuous with the epithelium of the tubular glands. These cells contain mucinogen granules and contribute mucus - the visible mucus - to the gastric secretion.

The neck chief cells also occur in all parts of the stomach; in the fundic glands they are concentrated in the neck of the glands, whereas in the pyloric and cardiac parts they constitute the only cell type in the glands. The secretory product of the neck chief cells is a mucin whose character differs from that of the cells of the surface epithelium in being more soluble and less viscous.

Parietal cells and body chief cells occur only in the fundic part; it has been established that they secrete the hydrochloric acid and pepsin, respectively, of the gastric juice. The parietal cells lie not against the lumen but between the chief cells and the basement membrane. Though separated from the gland lumen by the chief cells, the parietal cells communicate with the lumen by means of delicate canaliculi, which pass between the chief cells. The canaliculus is the terminal conduit of an extremely fine meshwork of channels within the parietal cell.

B. Composition of Secretion of Gastric Glands:

Hydrochloric acid is the characteristic and identifying product of the parietal cell and other substances secreted by this cell must vary in concentration in the gastric juice directly with the acidity. Substances not secreted by the parietal cell must vary in concentration in gastric juice inversely with the acidity. Substances whose concentration does not vary must be secreted in the same concentration by all the cells contributing to the gastric juice.

The chief enzyme of the gastric juice is the protrolytic principle, pepsin, which is secreted by the body chief cells of the fundic glands. An inactive precursor of pepsin called pepsinogen has been isolated in the form of a crystalline protein[103]. Pepsinogen probably represents the immediate secretory product of the chief cells. Unlike pepsin, it is relatively resistant to alkali and heat, losing little of its activity by short exposure to pH values as high as 11 or to temperatures as high as 70°C, which cause immediate and irreversible inactivation of pepsin. If pepsinogen solutions are made more acid than pH6, the pepsinogen is transformed into pepsin by an autocatalytic reaction, i.e., the product of the reaction, pepsin, catalyzes the further formation of pepsin from pepsinogen. Thus in the acid gastric juice the enzyme is always present in the active form. Pepsin has also been isolated in the form of a crystalline protein[103] and has been shown to possess no nonprotein prosthetic groups.

Recent studies[64] indicate that crystalline pepsin is a solid solution of several forms of pepsin possessing different proteolytic potencies.

The distinguishing characteristics of peptic digestion are that it:

a. occurs only in a distinctly acid medium.

b. will not attack all peptide linkages indiscriminately but only those in which the amino groups are attached to aromatic amino acids.

Complete peptic digestion results in a predominance of proteoses and peptones and relatively few amino acids and polypeptides. The optimum pH for the proteolytic activity of pepsin varies with the substrate protein but in general is about pH2.

Mucus is secreted by the surface epithelial cells, the neck chief cells of the fundic glands, the pyloric gland cells and the cardiac gland cells. This appears in two forms - visible mucus and dissolved mucin[132] - the former appearing as shreds or strands and may be separated from the gastric juice by filtration or centrifugation, while the dissolved mucin can be precipitated by the addition of two volumes of acetone or alcohol to the gastric juice. Visible mucus is believed to be secreted by the surface epithelial cells and dissolved mucin by the neck chief cells.

C. <u>Mechanism of Secretion of Hydrochloric Acid</u>:

The secretion of hydrochloric acid may be divided into the following periods and phases[120]:

<u>Period I</u>. The period of interdigestive secretion.

<u>Period II</u> The period of digestive secretion.

<u>Phase I</u> The cephalic phase

A. Unconditional reflexes

B. Conditional reflexes

<u>Phase 2</u> The gastric phase

A. Mechanical distension

B. Secretagogues

<u>Phase 3</u> The intestinal phase

A. Secretagogues

<u>The Interdigestive Period of Secretion</u> -

There has been discussion for many years whether the stomach secretes hydrochloric acid in the absence of stimuli which are responsible for acid secretion in response to a meal. When the fasting stomach is continuously drained by means of a stomach tube, the secretions recovered in man and in the dog almost always contain free hydrochloric acid. Bloomfield[15] has applied the term basal anacidity to the condition in which there is an absence of free HCL in the fasting secretions of a person whose gastric glands are capable of responding with acid formation to histamine stimulation. According to Bloomfield, basal anacidity in man is of rare occurrence. It has been shown that interdigestive secretion is present in variable amounts throughout a fast of forty days[68]. Experimentally it has been shown that all gastric pouches - with or without their original extrinsic nerve and vascular supply - perform interdigestive secretion of acid. Thus it may be stated categorically that the normal human and canine stomachs periodically secrete HCL during the interdigestive period. This must be recognized to avoid erroneous interpretation of gastric response to stimulation.

Pavlov[109] believed that the gastric glands do not secrete acid during the interdigestive period and that, if acid is present, it is due to the thought or odor of food. Essentially the same viewpoint is taken by Babkin[4] who, however, believes that other factors such as swallowing of saliva or regurgetation of duodenal juices, mechanical stimulation of the mucosa of the stomach, or food masses in the intestine also might contribute to interdigestive secretion; however, none of these factors can explain the occurrence of interdigestive secretion in the subcutaneously transplanted pouch, unless they operate to produce a hormone. According to Babkin[5] vagal denervation of the gastric glands induces a continuous "paralytic" secretion of acid gastric juice and he considers this interdigestive secretion to be due to an abnormal mechanism of unknown nature. The Pavlov-Babkin school believes that after vagal denervation the stomach secretes HCL continuously while other workers[74] believe that healthy dogs with vagally denervated gastric pouches secrete hydrochloric acid intermittently during the interdigestive period and not continuously.

The cause of the intermittent secretion of hydrochloric acid during the interdigestive period is not known. It is at least in part independent of vagal impulses and therefore of conditional reflexes. There is a tendency for the phasic increases in interdigestive secretion to be synchronous with the periods of hunger motility of the empty stomach and the same humoral factor may control both of these phenomena(62).

The Period of Digestive Secretion

This is arbitrarily divided into:

Phase I - The Cephalic Phase: This denotes that the stimuli are acting in the region of the head. The stimuli are the sight, smell, taste, and thought of food. They act through conditional and unconditional reflexes.

A. The Conditional Reflex (psychic or appetite) secretion of gastric juice is exemplified by the occurrence of secretion in response to the sight, smell and thought of food. It occurs only in the presence of a desire for food, or an appetite, and is dependent on the cerebral cortex for its development and operation.

B. The Unconditional Reflex secretion of gastric juice is demonstrated by "sham feeding" a decorticate dog with an oesophageal fistula[136]. Such a dog does not secrete in response to the sight and smell of food, but does so secrete, after a brief fast, to sham feeding or to the taste and swallowing of food. Thus, the unconditional reflex secretion occurs in the fasting state on the adequate stimulation of the taste nerves, and it is independent of the cerebral cortex.

There is no good evidence that the excitation of nerves in the stomach and intestine unconditionally stimulates secretion through a long reflex to the medulla. But the stimulation of such nerves evidently facilitates the unconditional reflex mechanism.

The amount of juice stimulated during the cephalic phase in man varies from 50 to 150 cc's per twenty minutes and is extremely variable. The gastric juice of the cephalic phase is rich in all the elements secreted by the gastric glands. It has a high acidity, a high pepsin content, and a high concentration of dissolved and visible mucus.

The vagi contain the sole efferent excitatory nerves for the cephalic phase of gastric secretion. Sections of the vagi or administration of atropine will abolish this phase. Electric stimulation of the vagi will, under appropriate experimental conditions, excite the formation of a copious amount of gastric juice similar in composition to that produced during the cephalic phase. Under vagal stimulation the zymogen granules of the chief cells and the mucinogen granules of the mucoid cells are depleted. Thus the above summary is the basis for any experimental study of vagal stimulation.

The Gastric and Intestinal Phases will be discussed briefly for completeness of this picture. The gastric phase includes two types of stimuli acting in the stomach to excite the parietal cell secretion:

a. mechanical distension

b. secretagogues - including two types:

   1. those naturally present in food, e.g. lean meat, liver, whey of milk and yeast.

   2. those which arise from the digestion of food, such as products of protein digestion and soups.

The Intestinal Phase is stimulated by the secretagogues listed above which stimulate the stomach.

D. <u>Humoral Mechanism in Gastric Secretion</u>:

When a meal not containing too much fat is fed to a dog with an autotransplanted pouch, hydrochloric acid is secreted by the pouch[73]. The nature of the humoral agent is still not definitely established but Edkins[38] and others showed that the subcutaneous injection of acid extracts of pyloric mucosa would stimulate gastric secretion. He called the active principle gastrin and it was found to be present in extracts of fundic mucosa and many other tissues. Since those tissue extracts which stimulated gastric secretion also decreased blood pressure, it was suspected that the active constituent of the extracts was histamine, or a histamine-like substance. This was substantiated by Sacks, Ivy, Burgess and Vandolah[118] who concluded that Edkin's gastrin is histamine but do not conclude that histamine is necessarily the gastric hormone.

E. Inhibition of Hydrochloric Acid Secretion.

1. Nervous inhibition - The sympathetic nerves are believed to convey the impulses responsible for the inhibition of HCL secretion in the presence of emotions such as fear and anger[20]. The sympathetic nerves are known to mediate the inhibition of gastric motility under these circumstances. There are also inhibitory secretory fibers in the vagi, since, following vagotomy, it is only after these nerves degenerate that the excitatory secretory nerve fibers in the vagi can be demonstrated readily by electric stimulation.

2. Enterogastrone - This is a substance prepared from upper intestinal mucosa, which upon intravenous injection reproduces some of the action of fat in the intestine. According to Schaefer's terminology, this is a chalone formed by the action of neutral fat or sugars on the mucosal cells of the upper small intestine from where it enters the blood and is conveyed to the stomach, where it exerts an inhibitory effect on muscular and parietal cell activity.

3. Acid inhibition - This expression is used to indicate that the presence of acid in the stomach or intestine inhibits gastric secretion. Acid inhibition occurs only when a certain threshold of acidity - 0.36% - has been reached and it operates only against certain stimuli. The cephalic phase of gastric secretion is refractory to the inhibitory action of acid in the stomach or duodenum[30].

F. Control of the Secretion of Pepsin.

Pepsin is secreted continuously. Unlike hydrochloric acid, it is constantly present in gastric juice although it cannot, of course, act in the absence of acid. Thus pepsin is constantly being released into the stomach even in the absence of specific stimuli for its secretion. During the interdigestive period, when only small amounts of pepsin are being secreted, pepsin is stored in the body chief cells, as evidenced by the accumulation of zymogen granules in these cells.

The major stimuli for pepsin secretion are vagal nervous impulses. Sham feeding, electric stimulation of the vagus, and insulin - which stimulates gastric secretion by hypoglycemic excitation of the vagal center - produces a gastric juice very rich in pepsin. Similarly parasympathomimetic drugs such as pilocarpine and acetylcholine are strong excitants of pepsin secretion. There is some evidence that acid and secretagogues acting in the intestine simulate pepsin secretion[45] and certain impure extracts of intestinal mucosa (secretin preparations) contain a pepsin - stimulating principle[8].

The concentration of pepsin in the gastric contents is highest in the first hour of digestion and declines as the volume of gastric juice increases; in the later stages of digestion, when the volume of gastric juice is diminishing, the peptic activity tends to increase. Pepsin is a preformed secretory product and cannot be elaborated as rapidly as it is secreted during the early phases of active secretion. Therefore, all stimulants of pepsin secretion produce an initial spurt, during which stored pepsin is rapidly extruded, followed by a levelling off to a rate of secretion characteristic of the stimulant which is acting.

Depletion of pepsin cannot be accomplished even by prolonged and intense stimulation(76), the rate of anabolism being probably dependent on the rate of extrusion. No instance has been recorded in which pepsin is absent from gastric juice which contains hydrochloric acid.

Although it has been demonstrated that most or all of the inhibition of motility and HCL secretion caused by fat can be accounted for by the action of the hormonal agent, enterogastrone, in the case of inhibition of pepsin secretion by fat in the intestine the vagus nerves are involved(55). The effect is due to true inhibitory fibers in the vagus and not merely to decrease in tonic excitatory impulses.

G. Control of the Secretion of Mucus.

There is no good evidence showing that the extrinsic nerves play a significant role in the control of mucus secretion. The major stimuli for mucus secretion are the local action of chemical and mechanical irritants, acting either directly upon the cells or through local reflex arcs. Vagal stimulation produces an increase in mucus production but this is probably secondary to mechanical stimulation in the form of rubbing of the mucosal folds together as a result of the increased motility accompanying vagal stimulation. Pilocarpine does not cause an increase in mucus secretion by a pouch of the pyloric part of the stomach when mechanical stimulation secondary to motility is prevented[76]. Mucus is secreted continuously. Increased visible mucus production occurs in response to mechanical stimuli such as a rough diet, to chemical stimuli such as hydrochloric acid, alcohol and spices, but most markedly during the secretory period following sham feeding, especially toward the end[131].

Another view of this subject has been presented by Jennings and Florey[115]. They carried out vagal stimulation in decapitate cats and produced evidence that the vagus controls the secretion of the cardiac and pyloric mucus glands and the mucus neck cells of the fundal glands. They saw no effect of vagal nerve stimulation on the surface epithelial cells of the stomach. They also showed that innervated pyloric pouches cease to secrete on starvation but secrete at once following the ingestion of food. As stated, these experiments were performed on decapitate cats and were necessarily very traumatic, needing blood transfusions to sustain the animals.

H. Role of the Sympathetic Nervous System in Gastric Secretion

Experimental evidence concerning this controversial matter has been quite incomplete. Baxter[9] performed long-continued rhythmic stimulation of the freshly-sectioned splanchnic nerves in dogs and cats and produced a steady secretion of alkaline mucus possessing a low digestive power. The same effect was obtained whether the stimulation was produced by currents of high or of low frequency. Repeated injections of small doses of adrenaline had an effect similar to that of splanchnic stimulation. He also showed that twenty-four to seventy-two hours after aseptic section of the splanchnic nerves below the diaphragm, there was a spontaneous secretion of alkaline mucus in the stomach. This "paralytic mucus secretion" was increased in volume by electrical stimulation of the partly-degenerated splanchnic nerves as well as by repeated injections of adrenaline. Baxter showed that when massive doses of adrenaline were injected, the spontaneous mucus secretion was inhibited for one or two hours, but thereafter it increased in volume, became slightly acid and very rich in pepsin. Babkin[7] believed that a probable explanation of these facts might be that adrenaline increases the permeability of the peptic and parietal cells to a parasympathetic chemical transmitter which is liberated in very small amounts by the post-ganglionic vagal fibers. Under normal conditions it is not present in sufficiently strong concentration to stimulate those cells in the gastric glands which secrete acid and pepsin. The striking effect of adrenaline and in a lesser degree of stimulation of the sympathetic nerves, on increasing the permeability of the secretory cells was demonstrated in the submaxillary gland by Hebb and Stavraky[59].

It was also shown that atropine did not abolish the adrenalo-sympathetic mucus secretion, that cocaine increased the response of the mucosa and ergotamine inhibited it. Baxter also separated the stomach into three pouches - fundic, body and pyloric - and found that the pylorus was the chief source of the sympathetic mucus. The body of the stomach was less prolific in the secretion of mucus and the fundus was much less so. Weak stimulation of the vagi produced a mucus secretion from the stomach of the dog and of the cat. Atropine inhibited this secretion, whereas ergotamine had no effect on it, which indicated that the secretion was due to stimulation of parasympathetic fibers, and not of sympathetic fibers present in the vagi.

Baxter(10) also carried out experiments in cats with oesophagotomy, gastric fistula and aseptic bilateral severance of both major splanchnic nerves. His investigation established the fact that the sympathetic nervous system does not play an essential part in the first, or nervous, phase of gastric secretion. It would seem that this phase is mediated chiefly through the parasympathetic nervous system as is generally agreed. In his work, he concluded that the sympathetic nervous system has some relation to the secretion of gastric mucus. Another important conclusion was that the trophic effect on the peptic glands is exerted by the vagus and not by the sympathetic nerves, disagreeing with the work of Bickel[13] but agreeing with most other workers in this field.

Volborth and Kudryavzeff[129], in Pavlov's Laboratory in 1926, investigated the action of the splanchnic nerve as a secretory nerve to the gastric glands. They reported a minimal increase in the secretion of the fundus glands of the stomach. They obtained this by rhythmical stimulation of the left splanchnic nerve which had been severed five days before to permit the vasoconstrictor fibers to degenerate. The stimulation was done in unanaethetized dogs, equipped with a Heidenhain pouch[98] to permit collection of secretion and also to ensure vagotomy.

In 1950 Smithwick and Kneisel[124] reported that following splanchnicectomy in man, the free hydrochloric acid level of the gastric content was not increased, but that following insulin hypoglycemia, a somewhat greater output of hydrochloric acid was obtained than before the operation. Shafer and Kittle[121] reported that total stomach pouches, prepared in animals in which bilateral transthoracic preganglionic sympathectomy with excision of the sympathetic chain and splanchnic nerves from the region of the sixth thoracic to the second lumbar segments had been carried out before construction of the pouch, secreted more gastric juice per hour than similar animals whose sympathetic nerves were intact.

The most recent work has been done by Oberhelman, Woodward, Smith and Dragstedt[104] at the University of Chicago. With the development in their laboratory of a technique for making quantitative twenty-four hour collections of gastric juices in experimental animals in an optimum nutritional state and without anaesthesia, observations were made on the secretion of gastric juice in vagus innervated total stomach pouches both before and after division of the sympathetic nerve supply to the stomach.

They prepared vagal innervated pouches in eleven dogs, of which seven showed a good secretory response to insulin hypoglycemia, indicating functional vagus innervation[69]. All dogs were then subjected to a bilateral transthoracic sympathectomy, removing both sympathetic chains from the level of the sixth or seventh thoracic vertebra to, and including, the splanchnic nerves. The data obtained in this investigation demonstrate that when gastric secretion is measured quantitatively in vagus-innervated, total pouch dogs, sympathectomy produces a definite and significant increase in the 24-hour output of hydrochloric acid and an increase in the quantitative secretory response to insulin hypoglycemia and to a standard dose of histamine. This increase in gastric secretion following sympathectomy was not observed at all, or was very meager in amount, in animals with absent or relatively deficient vagus innervation to the stomach. These findings suggest that the sympathetic nerves to the stomach contain inhibitory fibers to gastric secretion, which are in tonic activity, and which, presumably, act on the vagus secretory mechanism rather than independently on the glands of the mucosa. Following section of the sympathetic nerves, the vagus fibers are left to act unopposed in stimulating gastric secretion.

I. <u>Anaesthesia and Gastric Secretion</u>:

The study of gastric secretion has been hindered in acute experiments by the undoubted effects of anaesthesia on the normal flow of gastric juices. The chloralose-urethane combination has been widely used by Babkin and his school while chloralose anaesthesia has been popular with Heslop(65) and others. Because of the popularity of this type of anaesthesia, Schachter(119) investigated its effect on gastric secretion. He concluded that both chloralose and chloralose-urethane anaesthesia cause a marked secretion of very acid gastric juice in dogs previously equipped with gastric fistulae and not subjected to any trauma at the time of anaesthesia. Urethane, pentothol and to a much lesser degree nembutal anaesthesia also resulted in the secretion of varying amounts of gastric juice of high acidity under similar conditions.

The chloralose-urethane phenomenon was especially investigated. This secretion was regularly found to approach a pH of 0.9 and to possess minimal amounts of pepsin and visible mucus, thus resembling the secretion evoked by histamine. It was completely abolished by the traumatic procedure of preparing the gastric fistula at the time of the experiment, and was at times completely prevented by slight mechanical manipulation of the vagi in the neck. In those instances where the phenomenon was not abolished by exposure of the cervical vagi it was immediately arrested following acute vagal section. The secretion returned to full intensity within a few days after vagal section only to almost completely disappear again as time for nerve degeneration elapsed. The secretion was not associated with a reduced blood sugar level but was regularly abolished by atropine. It was markedly reduced or unobtainable in chronically vagotomized dogs.

The consistent minimal amounts of mucus and pepsin plus the high acidity of the juice obtained with chloralose - urethane raised the question whether or not histamine liberation might be an effective link in the mechanism of the secretion. Babkin(6) suggested the possibility that histamine may play a physiological part in the nervous phase of gastric secretion. However, the fact that insulin, amino acids and ethyl 3:3 dimethylallyl barbituric acid(2), which are believed to act through the vagi, all result in a juice containing considerable mucus and pepsin tended to contradict this theory. The fact, however, that chloralose-urethane secretion markedly resembles that of histamine yet is largely dependent on integrity of the vagi indirectly supports the possibility of the mediation of histamine in this phase of secretion.

Browne and Vineberg(18) showed the influence of the CO_2 content of the blood on gastric secretion. It had been noted that artificial respiration greatly diminished or almost entirely arrested the activity of the gastric glands and made the production of a flow of acid gastric juice under vagal stimulation almost impossible. Experimentally they showed that vigorous aritificial respiration applied for eighty minutes lowered the CO_2 content of the arterial plasma to 25.4 volume per cent from a normal of 34.8 to 43.3 volumes per cent and raised its pH to 7.70. During the same period the volume of the gastric secretion decreased and its acidity was reduced. When CO_2 was pumped in, and normal values restored, the rate of hyperventilation being kept constant, the gastric response became normal. They thus believe that for the elicitation of gastric secretion via the vagus nerves, a CO_2 content of 30 volumes per cent of the arterial plasma is essential. This factor must be realized in any type of experimental procedure.

CHAPTER VI

PRODUCTION OF GASTRIC SECRETION BY VAGAL STIMULATION

A. Historical Review.

The function of the vagus nerve with respect to gastric secretion has puzzled investigators for many years and all are not yet agreed upon its true role. As early as 1814 Brodie[17] observed that, when dogs were killed by an intravenous injection of arsenic, there was a copious secretion of mucus and watery fluid in the stomach. He found, however, that if the vagi were severed previous to the administration of the lethal dose of arsenic, the stomach was always empty at autopsy. Work was done for many years in this field but in 1890 Pavlov and Schumov-Simanovski[110] discovered that the vagus nerve is a secretory nerve to the gastric glands. Pavlov advanced the hypothesis that stimuli received by receptor surfaces e.g. eyes, ears, nose and mouth, were transmitted to the gastric glands by the vagus nerves. He showed this by preparing an oesophagotomized dog with a gastric fistula and with the right vagus cut below the recurrent laryngeal nerve. This dog was then sham-fed and the gastric juices thus obtained were analysed. The left vagus nerve was then severed. Sham feeding in the doubly vagotomized animal now initiated not a single drop of secretion. In the second type of experiment, a dog was prepared in exactly the same manner except that the left vagus was cut and allowed to remain in the wound beneath the skin. After two or three days the peripheral end of this nerve was stimulated by an induction current and a few drops of highly and juice were obtained.

These results were confirmed by Oushakov[107] in 1896 who used an acute type of experiment. Gastric juice was obtained after stimulation of the peripheral cut ends of the vagi in the neck by an electrical induction current. The animals were first immobilized by section of the spinal cord below the medulla and the secretion collected through a gastric fistula. The acidity of the gastric juices thus obtained was on the average much lower than in normal and oesophagotomized dogs, but the digestive power was high. Approximately one-half of the total volume of the secretion was composed of mucus, this accounting for the relatively low acid values. Oushakov concluded that, in addition to the fibers responsible for the stimulation of acid and pepsin, there were special mucus - stimulating fibers in the vagus. There was a long latent period - from one to one and one half hours - between the beginning of stimulation and the appearance of secretion.

Pavlov(108) drew attention to the difference between the latent period of the gastric and of the salivary glands following nervous stimulation. He subscribed to the explanation offered by Oushakov that in the vagus there are secretory inhibitory as well as secretory fibers. He suggested that both groups of fibers are stimulated simultaneously but that at the onset of stimulation, the inhibitory fibers are more easily excited than the secretory fibers. After a time they become relatively less sensitive, thus permitting the action of the secretory fibers.

Stahnke(125) has been misquoted frequently in reports of vagal stimulation. He placed specially constructed electrodes through a rubber tube into the oesophagus, so the electrodes rested at the level of the cardia. He then stimulated for twenty minutes twice daily for ninety-six days and produced hypermotility, hypersecretion, pylorospasm, chronic gastritis and ultimate erosion of the gastric mucosa in a few of his dogs. This technique appears simple but it is impossible to know when the vagi are being stimulated and how far the electrical stimulus spreads and involves other nervous centers. The work has never been confirmed.

The above findings are generally accepted but Farrell[39] in 1928 failed to obtain gastric secretion after vagal stimulation but did obtain the same results as Pavlov after cutting the vagi in an oesophagotomized dog with a gastric fistula.

B. <u>Effects of Weak and Strong Stimulation of the Vagus</u>

Vineberg[128] showed the effects of weak and strong stimulation of the vagi. He isolated the stomach in dogs, anaethetized with chloralose and urethane, by ligation of the oesophagus and the pyloro-duodenal junction and insertion of a metal fistula into the stomach. The cervical vagi were then isolated, severed as high as possible and the animal was placed in a stand so gastric secretions could be collected easily. The vagus nerves were placed on platinum electrodes and stimulated alternately and rhythmically - fifteen interruptions per minute by Meltzer's metronome - for a period of ten minutes each. The induction coil employed was of the A.H. Baird (Edinburgh) type, and was calibrated to give the following currents for the corresponding centimeter readings on the scale: 11 cm., 0.20 milliamperes; 10 cm., 0.50 m.a.; 9 cm., 1 m.a.; 8 cm., 1.80 m.a.; 7 cm., 2.90 m.a., etc. At the beginning the electrodes were always applied at the central end of the severed vagi and during the course of the experiment were gradually moved along toward the peripheral ends of the nerves. It was established by him that weak stimulation of the vagi - coil distance not more than 9 centimeters corresponding to a current of 0.50 milliampere or less - produces a secretion of alkaline or very slightly acid mucus, and that a strong stimulation - coil 9 to 7 centimeters or 1 milliampere or more - produces a continuous and copious flow of gastric juice, possessing high acidity and strong digestive power. According to his results, weak stimulation produced a scanty secretion of alkaline mucus, having a low concentration of total chloride and low digestive power. On strong stimulation of the vagi the secretion became definitely acid, the total chloride rose and the peptic power increased enormously. This has since been proven by other workers and has been confirmed in the cat by S.G. Baxter[9].

In Vineberg's experiments in which gastric secretion was elicited by vagal stimulation, the latent period was much shorter than in Oushakov's. When strong stimulation was preceded by weak stimulation, the latent period in twelve experiments averaged twenty-one minutes - a maximum of thirty-five and a minimum of seven minutes. When only strong stimulation was applied from the beginning of the experiment, the average latent period in sixteen experiments was twenty-seven minutes - maximum of forty-two minutes and a minimum of fifteen minutes. This indicates that weak stimulation of the vagus facilitates the effect of strong stimulation, although the additional impulses initiated by the increased strength of the stimulus presumably exert their main effect on a new group of secretory cells which previously were more or less quiescent. No explanation of this phenomenon can be given or of the extraordinary long latent period in reflex gastric secretion, but Babkin believes they support the theory of the chemical transmission of nerve impulses to the gastric glands.

Heslop[65] in 1938 stimulated the vagi in the chest of the cat, using a thyratron stimulator and a rate of fifty per second, and found that the latent period for secretion was from three to five minutes. It is difficult to decide whether the shortness of the latent period was due to the use of the thyratron instead of the induction coil or to the fact that in Heslop's experiments the gastric glands were usually in a state of slight to moderate activity and the stimulation of the vagi only produced an increase in the volume and the acidity of the juice.

Manning, Hall and Banting[96] stimulated the vagus nerve over a period of time and observed the effects on the myocardium and gastro-intestinal tract. Hall, Ettinger and Banting[56] had shown that myocardial and coronary artery damage resulted from long continued, daily administration of acetyl-choline to unanaethetized dogs. Since the effects of vagus nerve stimulation are due to the action of liberated acetylcholine[88], they decided that long-continued stimulation of the vagus nerve, through liberated acetylcholine should likewise produce damage to the heart and possibly the gastro-intestinal tract. In order to stimulate the animals continuously, without anaesthesia and with perfect freedom, a new type slip-ring-contact described by Manning and Hall[95] was used to stimulate the vagus. Changes in heart-rate and respiration were taken as the index of effective vagus stimulation. They carried out vagal stimulation for seventy to ninety hours and in two instances, for one hundred and twenty hours. Diarrhea and vomiting were frequently observed, except in one animal which received atropine. The vomitus at first contained much bile-stained mucus and later coffee-ground material. These effects were seen on the second day. The heart changes will not be discussed here. At autopsy congestion was noted in the gastric and intestinal mucosa. It is of interest to note that in a series of dogs given a continuous injection of dilute eserine through the external jugular vein, in addition to the vagal stimulation, all symptoms were much increased and at autopsy, the upper gastro-intestinal tract appeared congested and haemorrhagic and in one animal definite ulcerated areas were seen in the duodenum and pylorus. It is thus logical to conclude that the occurrence of gastro-intestinal disturbances appears to be related to an exaggerated vagal tone.

An interesting method of producing prolonged vagal stimulation with the hope of causing peptic ulcer was that of Jefferson, Phillips and Necheles(77). In their work, an anastomosis was made between the central end of the left phrenic nerve and the distal stump of the left vagus nerve. It was thought that the constant discharge of respiratory impulses from the phrenic into the vagus and then into the stomach would lead to constant irritation similar to that which is thought to produce peptic ulcer in man. In the first dog that underwent this operation, acute dilatation of the stomach occurred ten months after operation, and the animal died from throttling of the venous return to the heart. Upon immediate autopsy, about ten ulcers were found surrounding the cardia, in a tremendously dilated stomach, which had pushed aside all other organs in the abdominal cavity. Such a reaction was not obtained subsequently in a series of dogs subjected to the same anastomatic operation. However it was found that within twenty months a functioning anastomosis had formed between the left phrenic and vagus nerves, with nerve fibers going from the phrenic into the vagus. In acute experiments, stimulation of the phrenic nerve above the anastomosis was followed by distinct contractions of the stomach.

C. Composition of Gastric Secretion Elicited by Vagal Stimulation

The mucoid secretion produced by weak stimulation of the vagi is scanty in amount and does not exceed six to eight cubic centimeters per hour. Under strong stimulation a huge volume of regular acid gastric juice can be produced in the dog during a long experiment, as much as 100 cc. per hour in a 25 kilogram dog. Vineberg(127) noted that to maintain a uniformly high level of secretion, care must be taken to move the electrodes gradually along the nerves. As soon as the excitability of the nerves at the point of contact with the electrodes begins to decrease, the volume, the acidities and the peptic power of the secretion diminish, though the percentage of mucus often rises. The secretion produced by weak stimulation consisted of a gelatinous mucus. It was alkaline or faintly acid to litmus, very rarely possessed any free acidity and had a very low total acidity. In the large volume of clear, acid gastric juice obtained under strong vagal stimulation, the percentage of mucus was always less than in the juice obtained with weak stimulation, while the absolute amount was rather slightly less or practically the same. However, "vagus" gastric juice invariably contains a certain amount of mucus.

The free and total acidities of the gastric juice during the copious secretion produced by strong stimulation corresponded to the normal figures for these values, reaching for example, 0.47 per cent hydrochloric acid for free acidity, and 0.56 per cent hydrochloric acid for total acidity.

The total chloride was low in the mucus secretion, if the secretion was not contaminated with acid, the lowest chloride figures ranging from 332 to 336 milligrams per cent. While the stimulation of the nerves was being increased from weak to strong, the concentration of total chloride gradually rose, reaching normal values - 560 to 580 milligrams per cent - when the secretion became established.

The peptic power was low in the mucus secretion, averaging 45 Mett's Units. The peptic power of the acid gastric juice produced by strong stimulation of the vagi was extremely high, ranging from 400 to 1200 Mett's Units or more. Such values were never observed by Babkin and his group in canine gastric juice under normal conditions of secretion even in sham-feeding experiments. This shows that the vagus has a special relation to the peptic cells. The absolute figures for the peptic power varied in different experiments, a fact which might be attributed in some measure to differences in the amount of pepsinogen already stored in the peptic cells before the stimulation of the vagi. Although care was taken to ensure that animals were always in the same state of nourishment before an experiment, some secretion of gastric juice due purely to conditioned stimulation could not be prevented. The stores of pepsinogen in the peptic cells are limited and it is not possible to cause the formation of new pepsinogen by means of artificial stimulation of the vagi: When long-continued rhythmic stimulation with a strong induction current was applied to the vagi in the neck of a dog, the curve of the peptic power gradually mounted and then gradually declined. This happened in spite of the fact that the electrodes were moved to a new position on the nerves. There was no reduction in the volume of the secretion nor in its acidity during the second part of the experiment. This phenomenon may be satisfactorily explained by supposing that the stores of pepsinogen in the peptic cells had been exhausted under the impulses received by these cells from the strongly stimulated vagi, whereas the activity of the parietal cells was not at all impaired, for the latter cells do not secrete preformed material.

Thus, in summary of the effects of rhythmic stimulation of the vagi with a strong induction current, a gastric secretion is produced which is

1. copious in volume.
2. of high free and total acidity.
3. of high total chloride content.
4. fairly rich in mucus.
5. of exceptionally high peptic power.

It is evident that stimulation of the vagi causes the gastric mucus membrane to secrete a composite fluid which is the product of the activity of different groups of glandular cells.

D. The Histological Changes Produced in the Gastric Glands by Prolonged Vagal Stimulation

This subject has been neglected by many investigators but the following authors have mentioned changes produced. Noll and Sokoloff(102), using dogs with oesophagotomy and a gastric fistula, investigated the histological changes occurring in the mucus membrane of the stomach after sham feeding. They found that during the period of secretion there was a moderate enlargement of the parietal cells and a diminution of the chief cells.

Di Cristina(31) fed an animal and one hour later stimulated the vagus nerve for a period of ten minutes, He then found that the granules in the chief cells had diminished in size and that occasionally there was vacuole formation. The parietal cells were observed to be filled with granules.

Bowie and Vineberg(16) carried on a series of experiments in which the peripheral ends of the cut cervical vagi were stimulated alternately with an interrupted strong induction current for a period of nine hours. The usual curve of response to vagal stimulation was observed and has been described above. Histological examination showed nearly all of the peptic cells of the control to be well filled with pepsinogen granules but after nine hours of stimulation the great majority of the peptic cells were quite free of granules. Some of the cells in the deeper portion of the gland contained a few granules located along the luminal border. From the position of these remaining granules it was assumed that they had become involved in the process which caused extrusion of them from the cell. They also conclusively proved that the vagal influence was responsible for the extrusion of the pepsinogen granules from the cells, showing an injection of atropine before vagal stimulation abolished the above histological changes. The results may be summarized as follows:

1. The peptic cells contained fewer granules than before stimulation.
2. The peptic cells became somewhat shortened, this resulting in a widening of the lumen of the gland.
3. The cytoplasm in the stimulated peptic cells presented a denser arrangement than that observed in the resting cells.

4. The nuclei in the stimulated peptic cells shifted towards the center of the cell. This was in contrast to the more peripheral and basal position occupied in the resting cell.

E. <u>The Effect of Vagotomy on Gastric Secretion</u>

The clinical importance of the influence of the vagus on gastric secretion is emphasized by the work done by Dragstedt in which he has popularized the operation of vagotomy as a treatment for peptic ulcer. He bases this operation on the experimental changes produced by vagotomy in dogs.

Lim, Ivy and McCarthy(86) described the use of a pouch of the entire stomach in studying the physiology of gastric secretion in the dog. This preparation is sometimes called a Fremont pouch, since Fremont described this procedure in 1895. Dragstedt and Ellis(33) described a total gastric pouch with the vagus nerves intact in which much greater quantities of highly acid gastric juice are secreted and which will kill the animal in three to ten days from hypochloremia and alkalosis unless daily intravenous infusions of Ringer's solution are given.

These animals develop large penetrating peptic ulcers in the gastric pouch and frequently die of perforation or haemorrhage.

Dragstedt and others[135] studied the effects of vagotomy in these total pouch dogs. They concluded:

1. The totally isolated stomach with intact blood and nerve supply secretes large amounts of gastric juice even in the absence of food-taking.
2. On the ingestion of food, there occurs an immediate augmentation of secretion followed by a period of inhibition lasting three to seven hours and then a period of profuse secretion.
3. Section of the vagus nerves above the diaphragm reduces the secretion of gastric juice in the isolated stomach by an average of 56 per cent, and the output of hydrochloric acid by 77 per cent. Nervous factors are thus more important than other mechanisms in determining gastric secretion in these animals.
4. Chronic progressive peptic ulcers occur

frequently in these isolated stomachs and cause death by haemorrhage or perforation. They rarely develop in such preparations that have been denervated, and following vagotomy, they tend to heal.

5. Partial vagotomy has little or no effect on gastric secretion.

6. After complete vagotomy, the secretory response to a standard dose of histamine is markedly reduced.

Thus it will be seen that the role of the vagus in producing gastric secretion has very valuable clinical significance.

F. The Double Innervation of the Gastric Glands

As stated earlier in this chapter, Pavlov[108] believed the digestive glands to be supplied with special secretory-inhibitory nerves. He thought that he had proved their existence in the case of the gastric glands and of the pancreas. It was supposed that these nerves diminished the secretion of the glands by direct action on the secretory cells and not through vasoconstriction. However, in the light of new facts, the evidence on which

Pavlov based his theory cannot be considered entirely convincing.

According to Pavlov, the main proof that the gastric glands were supplied with secretory-inhibitory nerves was the extremely long latent period preceding the gastric secretion elicited by the faradization of the vagus nerves. Pavlov thought that the secretory -inhibitory fibers were more easily excited than the secretory nerves and therefore dominated the latter in the initial period of faradization of the vagi. It is now known that this long latent period was due to the removal of CO_2 from the blood by the forced artificial respiration which was applied to the animal. As stated before by Browne and Vineberg[18], a certain CO_2 content is necessary in the blood to produce a gastric secretion when stimulated by an induction current.

Babkin[7] in his studies of the double innervation of the gastric glands, concluded that each cell in a group receives only one nerve fiber from the division of the autonomic nervous system which is in control of this set of epithelial cells. In spite of the anatomical distinction between the two nerves to the digestive glands, functionally they were dependent to a certain degree on one another. While it was hardly possible to demonstrate a true secretory antagonism between the sympathetic and parasympathetic nerves of the digestive glands, their synergistic effect was clearly evident. This put the digestive glands in a class by themselves, distinguishing them from other internal organs, where the two divisions of the autonomic nervous system usually act antagonistically.

In the gastric glands the relationship between the sympathetic and parasympathetic innervation is far more complicated than in the salivary glands and is not as well understood. The muscular sheath of the stomach of course possesses a double, antagonistic innervation - motor and inhibitory, parasympathetic and sympathetic. However, as discussed in Chapter V, Section H of this thesis, there is no clear proof that the sympathetic nerves supply the gastric glands with secretory fibers which might stimulate the production of acid gastric juice.

CHAPTER VII

A METHOD OF CHRONIC REMOTE NERVE STIMULATION BY RADIOFREQUENCY

A. Purpose

The purpose of devising a method of chronic remote nerve stimulation is to better simulate normal - or more rigidly controlled abnormal - physiological states in an intact experimental animal. As stated earlier, the chief disadvantages of the common method of using flexible wire leads, have been the breaking of the leads and the entry of infection around the leads. It was thus deduced that a method obviating the need for such leads and still allowing the animal moderate freedom of movement in a cage, would be of great value in studying various results of nerve stimulation over long periods of time. This type of project has been attempted before and the basic part of our method is similar to that of Lafferty and Farrell[84]. The construction of such equipment presented many problems which will be discussed with reference to each component of the apparatus.

B. Basic Problems

1. Type of Receiver to be placed in the dog. This receiver must be:

a. small enough to fit without impairment of the animal's normal physiological functions.

b. constructed so movement of the dog will not decrease the constant receiving capacity of the receiver.

c. resistant to the deleterious action of body fluids.

d. non-reactive to body tissues.

e. equipped with suitable electrodes to stimulate the nerve.

f. capable of receiving sufficient current to stimulate the nerve.

II. Construction of a suitable transmitter and primary coil. This must:

a. transmit sufficient power to adequately stimulate the nerves to be studied.

b. be calibrated on a scale consistent with optimal nerve response - that is, of optimal frequency, voltage and duration.

c. transmit a current with minimal polarization of the nerve.

d. utilize a primary coil around a cage large enough to permit relatively normal activity of the dog.

e. have a screen to shield against radiation of the radiofrequency and consequent interference with normal radio reception in the area.

III. Construction of a suitable type of electrode and foot in which to hold the nerve. These must ensure:

a. minimal trauma to the nerve.

b. minimal chemical damage to the nerve.

c. firm but not excessive retention of the nerve.

d. easy technical application to the nerve to minimize nerve trauma.

e. construction of the foot of dielectric material.

IV. Knowledge of the Neurophysiological properties of the vagus nerve. These include:

a. types and diameters of nerve fibers.

b. amplitude of spike potentials of these fibers.

c. chronaxie and rheobase of the vagus.

d. reaction of the nerve to chronic stimulation.

e. type of current best suited to the nerve.

f. amount of polarity caused between the electrodes by the current used.

V. Knowledge of investigative procedures in gastric physiology. These include:

a. type of fistula or pouch to be used to collect gastric secretion.

b. methods to eliminate extraneous factors.

c. innervation of the stomach.

d. analysis of gastric secretions.

e. measure of gastric motility.

f. training of experimental dogs.

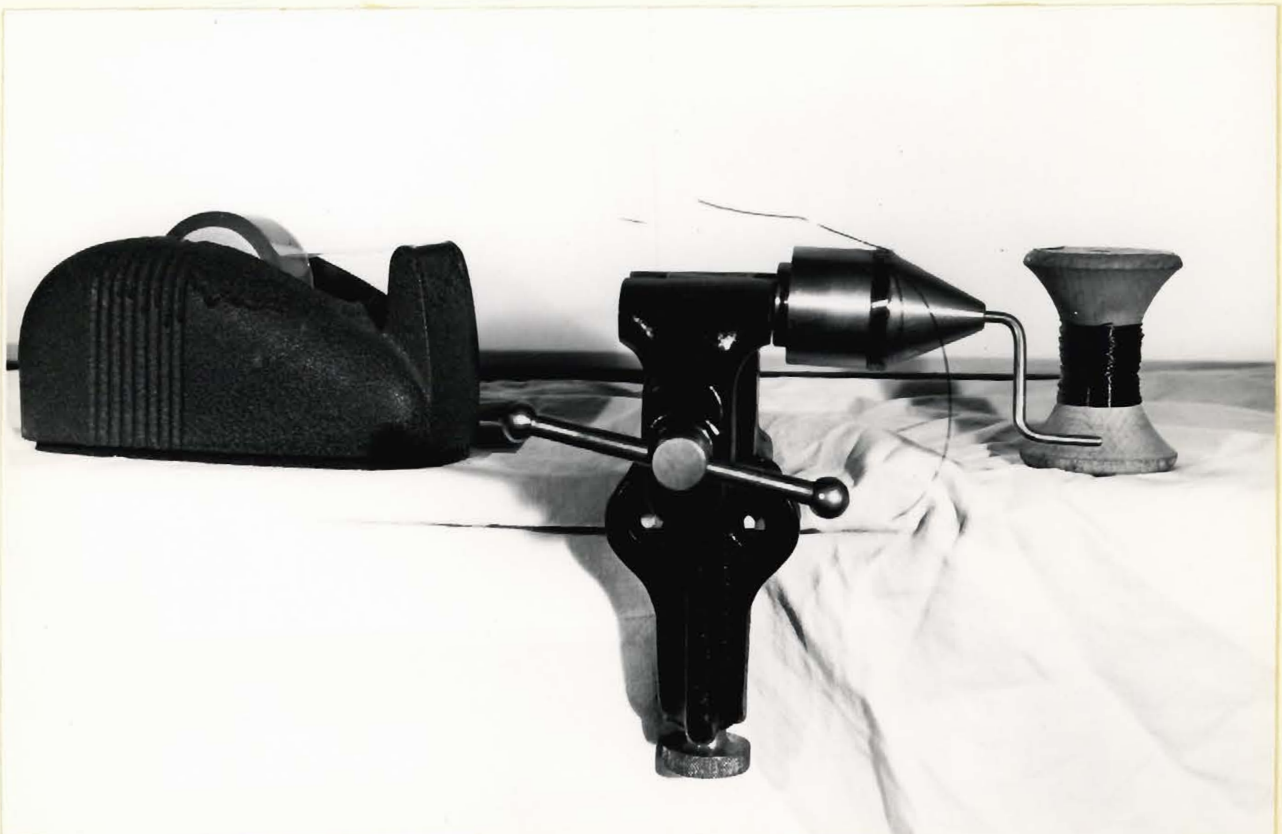
C. Construction of the Apparatus

I. Construction of a receiver and casing:

a. The Receiver:

The receiver is similar to that of Lafferty and Farrell(84) with several modifications. Three coils are turned on the designed brass spool, as shown in Figure 26 on page 152, having a diameter of 1.25 inches and a width of 1/8" - each coil con-

FIGURE 26



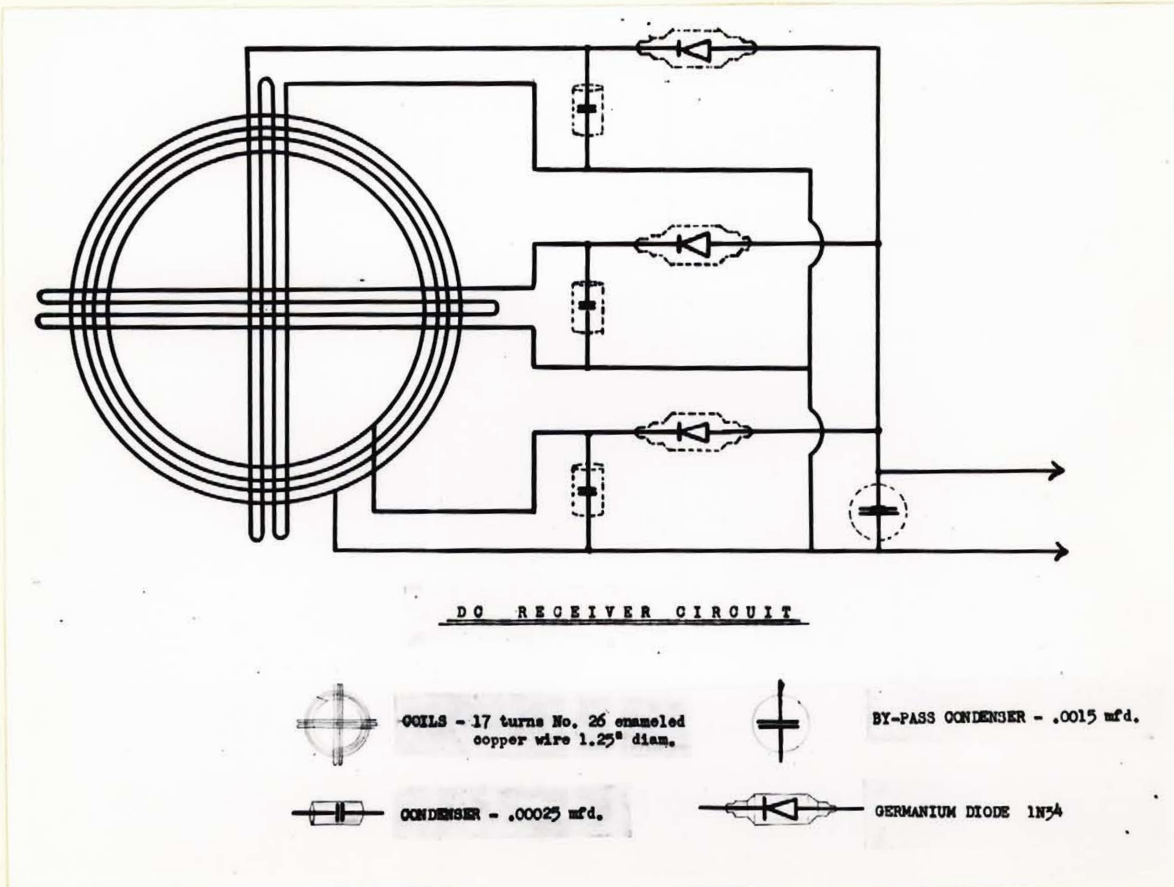
Brass Spool

The brass spool is shown held in the vice with a completed coil in position. The coil has three layers of scotch tape and is painted with collodion.

sisting of 17 turns of number 26 enamelled copper wire, arranged in two layers, the inner layer having nine turns. The two layers are separated by scotch tape and painted with collodion. A 250 micromicrofarad resonating condenser was then placed across each coil and a germanium rectifier was joined to one side of each resonating circuit. The three units were then arranged to lie at right angles and a 1500 micromicrofarad bypass condenser was placed across the terminals. Thus the receiver consisted essentially of three interlocked tuned circuits, one in each plane, with crystal diodes as detectors, and a bypass condenser as a radiofrequency filter. This direct current receiver circuit is represented in Figure 27 on page 154. The receiver has a capacity of 25 to 30 volts and an average of 15 to 20 volts when 1000 ohms resistance is placed across the leads. This is the estimated resistance of the vagus nerve. Also, due to arrangement of the coils at right angles, movement of the animal in the cage does not alter their efficiency.

This arrangement proved satisfactory for short periods of square wave stimulation but because of polarization effects leading to damage to the nerve,

FIGURE 27



Direct Current Receiver Circuit

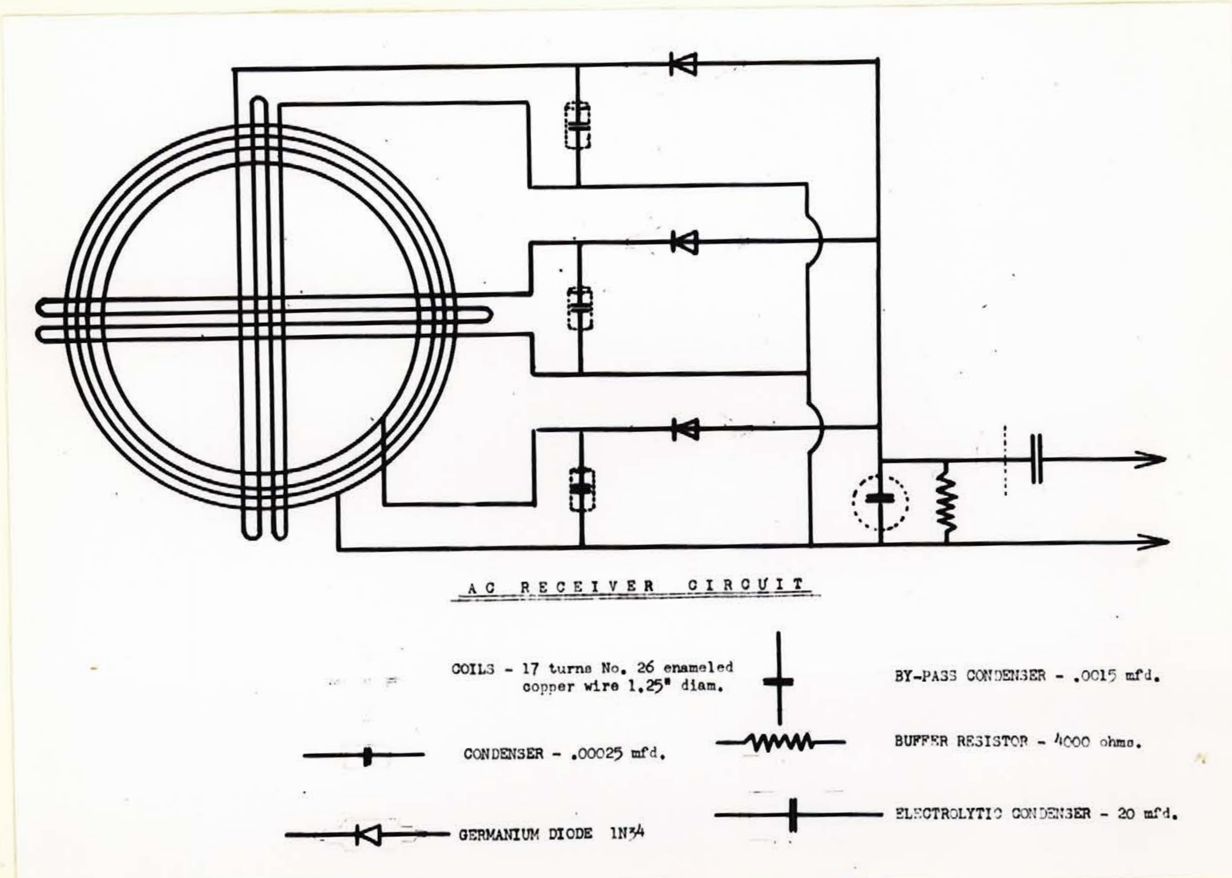
there was introduced a large capacity (20 microfarads) condenser in series with the electrodes to prevent flow of direct current through the nerve. A shunting resistor (4000 ohms) was introduced to allow the condenser to discharge after each pulse. This alternating current receiver circuit is represented in Figure 28 on page 156. The resistor resulted in some power loss but was essential to the circuit.

Figure 29 on page 157 represents the wave form before and after the introduction of the 20 mfd. condenser. It may be seen that the current in one (positive) direction is balanced before the next pulse by an equal current flow in the opposite (negative) direction. The darkened areas in the diagram represent the quantity of current flowing. By this method, polarization was effectively reduced to a minimum.

b. <u>The Receiver Casing</u>:

The construction of a casing for the receiver which would meet the stipulated requirements presented many problems. The substance of choice for building the casing was determined to be Alkathene[71], the Imperial Chemical Industries Limited brand of polythene. These plastics are

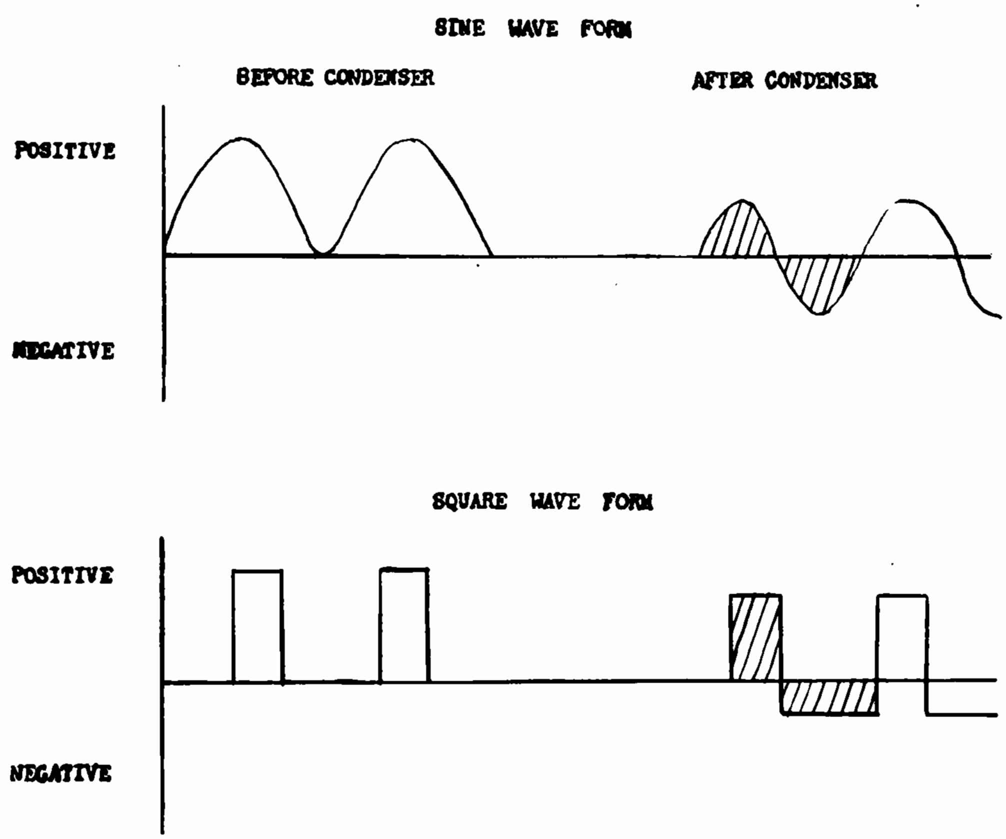
FIGURE 28



Alternating Current Receiver Circuit

This is the circuit now exclusively used in the construction of the receivers.

FIGURE 29



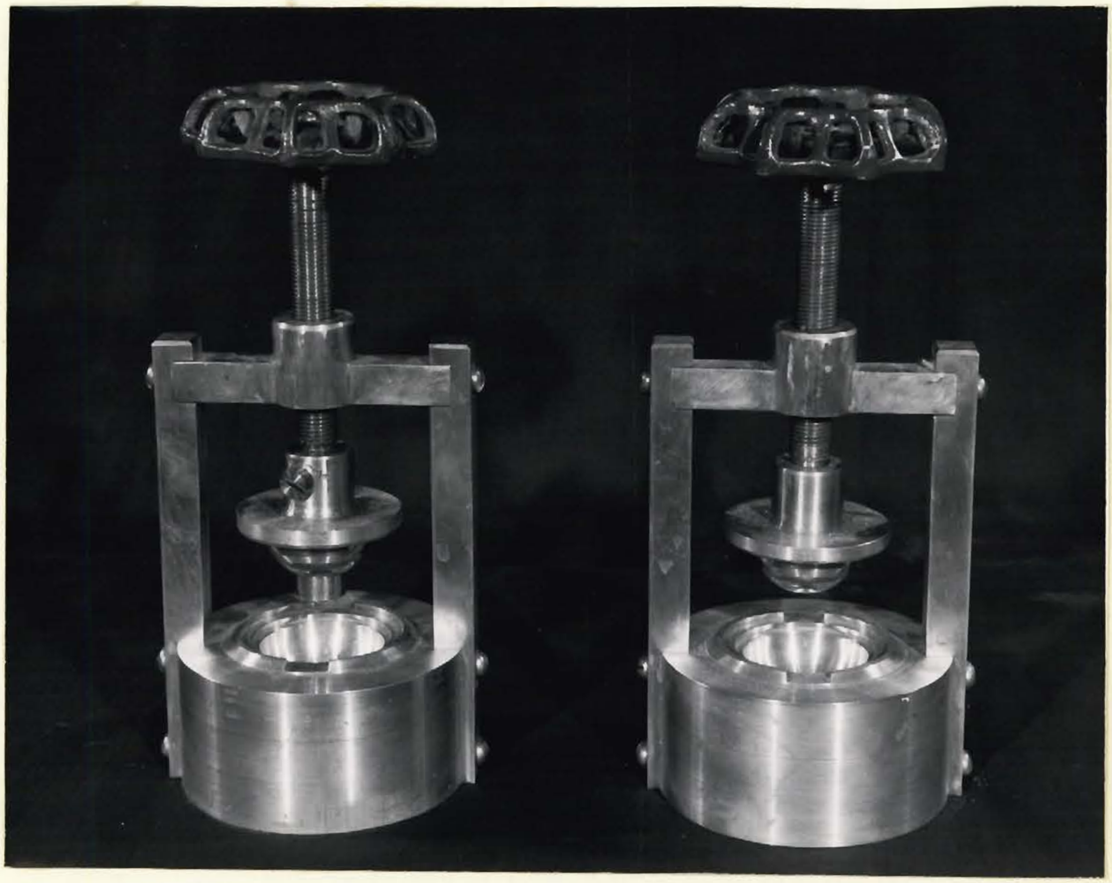
Wave Forms

This shows sine and square wave forms before and after the introduction of the 20 microfarad condenser. The darkened areas in the diagram represent the quantity of current flowing.

valuable electrical insulants, particularly at radio-frequencies, and have many applications in industry where chemical inertness of a high order is required. The Alkathene was obtained in crystalline form and melts at 110°C, needing considerable pressure at this temperature to be moulded. In order to overcome this and to facilitate the construction of the casings, two brass extrusion moulds were made as shown in Figure 30 on page 159. Each mould is essentially half a sphere but one of these moulds has a superimposed boss 10/16" wide and 5/16" deep to form a site for the attachment of the electrical leads on the moulded casing. These two halves of the casing are shown in Figure 31 on page 160.

Various methods were used to conduct the current from the receiver through the casing but in each attempt, seepage occurred at this site, destroying the efficiency of the receiver. This was overcome by using metal screws 5/16" long and 1/16" in diameter, screwed through small drilled holes 3/16" apart in the base of the boss. The rounded heads of the screws are on the inside, separated from the alkathene by a rubber washer and are tinned to allow soldering to the receiver leads. The outside tip

FIGURE 30



Brass Extrusion Moulds

The mould on the left is tapered to make the half-sphere with the hollow boss. The one on the right makes a normal half-sphere.

FIGURE 31

The Receiver Casing

This shows the two half-spheres, as they appear on removal from the moulds before the receiver and leads are attached.

of each screw is also tinned and held in place by a nut separated from the casing by a rubber washing.

The electrode foot can then be attached in one of two ways - by a fixed or flexible lead. The flexible lead has the following advantages:

a. decreased traction and trauma on the nerve.

b. application of the electrode casing at a site on the nerve where there is not room for the receiver casing.

c. permits application of the receiver subcutaneously if required.

These advantages were recognized but due to original inability to prevent seepage at the joints of the lead, the fixed lead was first employed.

1. The Fixed Lead:

Two insulated wires were soldered to the outer tips of the screws and were then encased in a polyethylene tube 10/16" long with an inside diameter of 6/16" and a wall diameter of 1/16". This was then welded to the boss with polyethylene and a polyethylene foot was welded to the outer end of the tube. This type of lead is shown in Figures 32 and 33 - anterior and lateral views respectively - on pages 162 and 163.

FIGURE 32

Anterior View of Fixed Lead

This shows the early type of fixed lead receiver, a polyethylene tube joining the casing to the electrode foot.

FIGURE 33

Lateral View of Fixed Lead

In this lateral view of the receiver, the polyethylene reinforcing the foot and junction with the casing can be seen. The two perforating electrodes are visible.

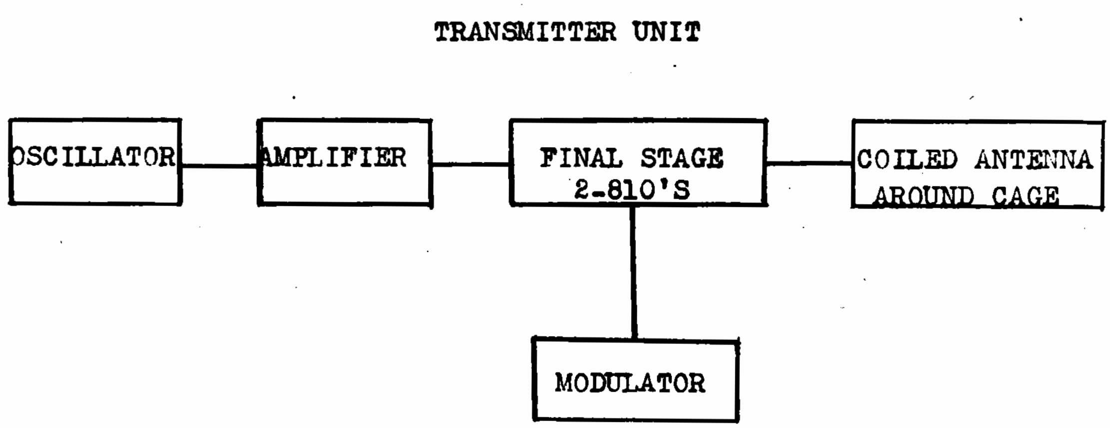
2. <u>The Flexible Lead</u>:

This is the type of lead now completely used and was made possible by the use of latex to strengthen the joints. This consists of two leads, each 12" long and consisting of twelve fine interwoven wires, encased closely in polyethylene tubing. One end is soldered to one platinum electrode in the electrode foot and strengthened by a 1½" long sleeve of closely fitting polyethylene tubing, joined to the foot to strengthen the joint. The other end was soldered to the screw end in the boss strengthened by two short investing sleeves of polyethylene tubing, the outer one large enough to fit over the screw. These joints were then strengthened by a coat of primer and subsequent layers of natural latex (40% water) fixed by immersion in a coagulant, made of methyl alcohol and 35% calcium nitrite. This was cured by baking in an oven at 37°C. for twenty-four hours. This type of lead has never caused failure of stimulation and produces very little tissue reaction.

II. Construction of a Suitable Transmitter and Primary Coil

The transmitter used was a modified Marconi PV - 500 transmitter delivering approximately 500 watts power at a frequency of 2.65 megacycles, arbitrarily chosen. This is schematically represented in Figure 34 on page 166 while the transmitter and modulator are shown in Figure 35 on page 167. This is modulated by a square wave generator calibrated to stimulate at 1, 3 and 10 milleseconds duration at frequencies from 2.5 to 70 per second. This modulator consisted essentially of two parallel 6L6's in the cathode circuit of the 810's with variable pulse shapes applied to the 6L6 grids. A bias control permitted sine wave modulation and square wave modulation, using a conventional flip-flop circuit and amplifier or "induction coil" modulation, where the secondary was wired in series with the bias supply. A metronome with mercury contacts acted as a stimulation interruptor for the induction coil, while a 1 revolution per minute motor driving a twelve contact wafer switch allowed variable square wave stimulation periods within each one minute cycle.

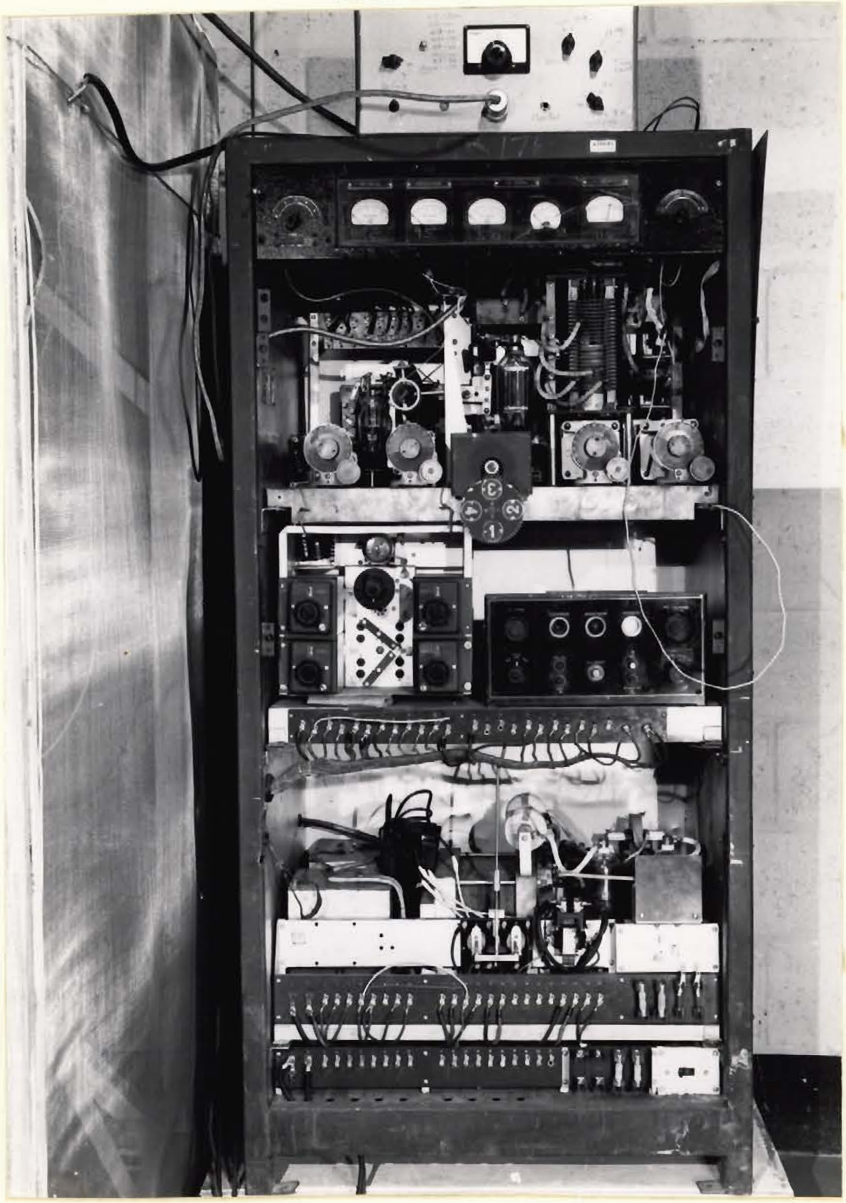
FIGURE 34



Transmitter Unit

Schematic representation of the parts used in the construction of the transmitter unit.

FIGURE 35



Transmitter and Modulator

These are described on Page 165.

An exciting coil having a rectangular outline was made by winding No. 14 copper wire about a wooden cage 36" long, 24" wide and 24" high. The wire was attached to the cage by porcelain insulators so that each turn was 29" across and stood 38" high. The coil consisted of five turns spaced four inches apart. The cage was in the high field concentration within the helix, the helix itself being part of a tuned output circuit. The whole coil was surrounded by suitably spaced copper screening which effectively prevented interference.

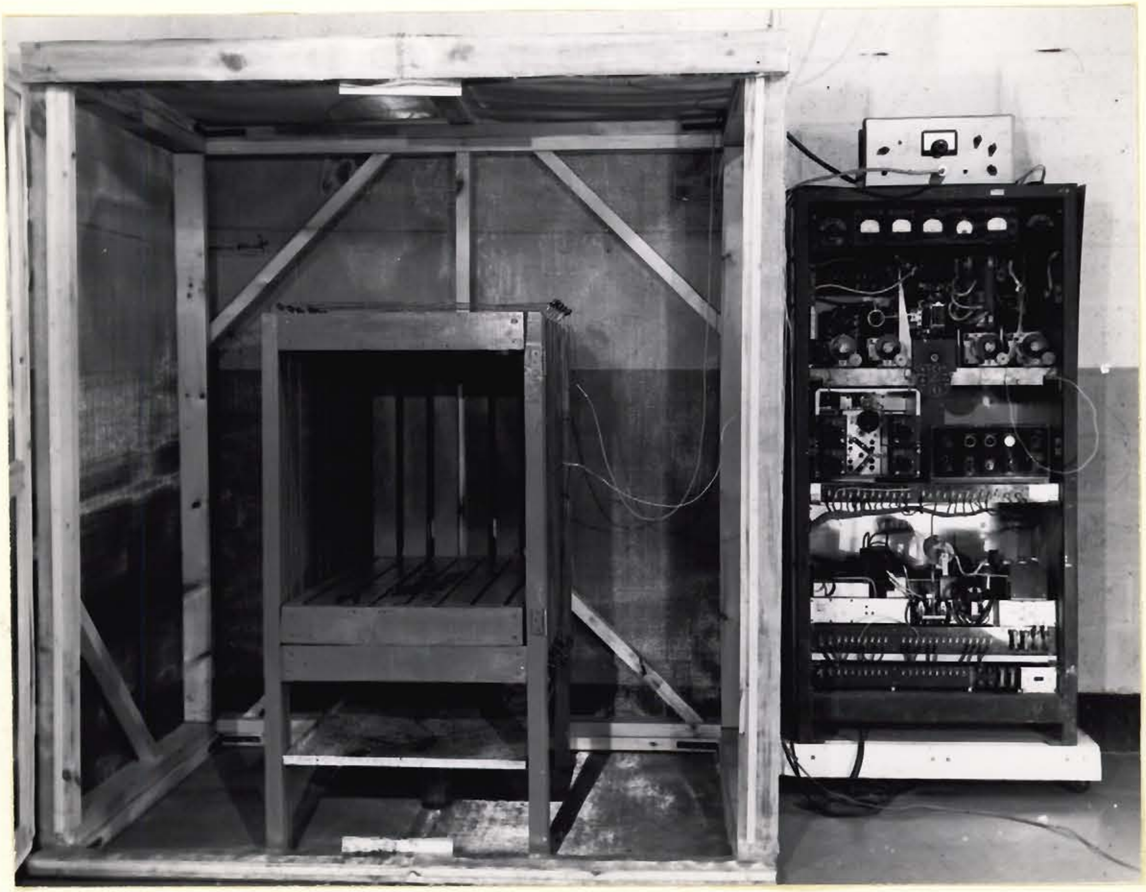
The cage, coil, screening, transmitter and modulator are shown in Figure 36 on page 169.

Mr. H. Millar constructed this type of transmitter and was responsible for its efficient service.

III. Construction of a Suitable Type of Electrode and Foot

It was decided at an early stage in the project to use platinum wire - No. 22 B and S, made by Johnson Matthey and Mallory Limited of Toronto and Montreal - for the electrodes, but the type of electrode to be used presented more difficulty. Three types of electrodes were used: as shown in

FIGURE 36



Complete Stimulation Unit

The transmitter and modulator are shown, as in Figure 35, with the addition of the cage surrounded by its primary coil and screening.

Figure 37 on page 171:

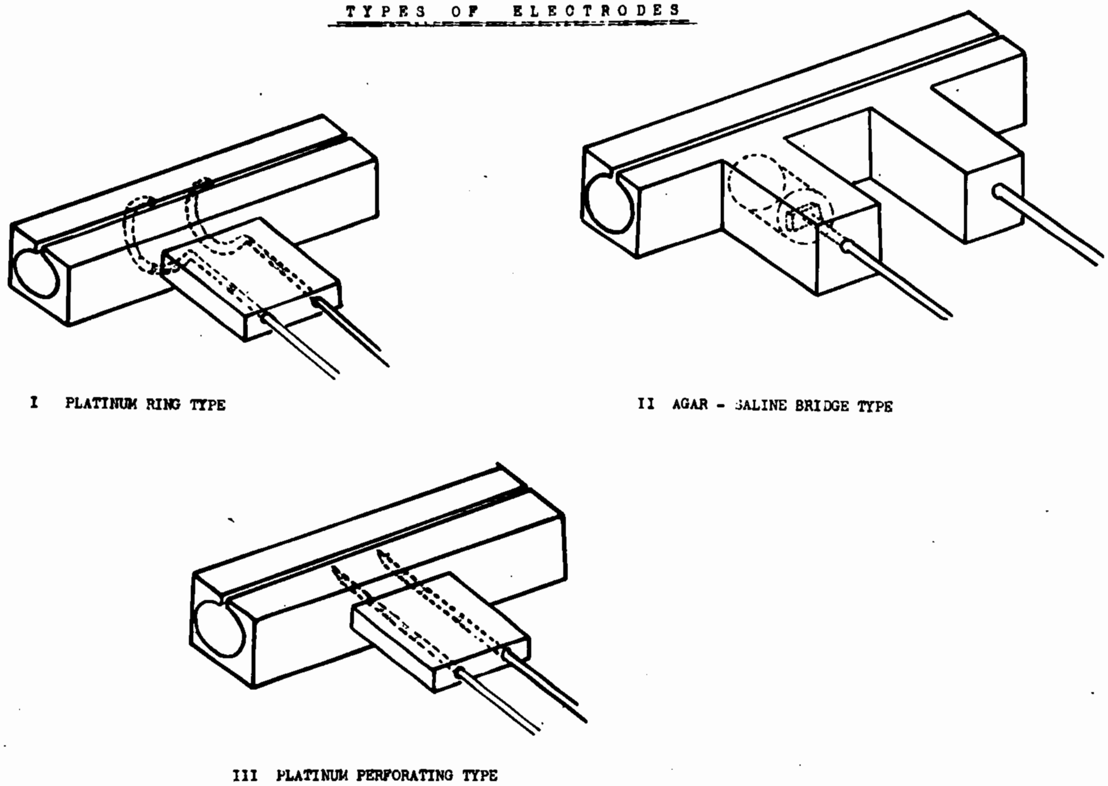
1. Perforating type.
2. Ring type.
3. Agar-saline bridge type.

1. The Perforating Type was the first used in any number of experiments. These bipolar electrodes are sharpened at the distal end to a point and the proximal ends are soldered to the wire leads as described above. The electrodes are placed 1/8" apart, at right angles to the longitudinal axis of the polyethylene foot, and do not quite reach the opposite side of the foot. The present foot is 1.25" long with an inside diameter of 2.5/16" and a wall diameter of 1.5/16". This wall is hinged at one side and open at the other to permit easy application to the vagus nerve. The foot is of dielectric material and satisfies the postulated criteria. This type of electrode necessarily damaged a varying number of nerve fibers and in some cases was blamed for necrosis of the nerve. Thus, although it seemed to effectively stimulate the nerve for limited periods of time, other types of electrodes were investigated.

2. The Ring Type was used for a short time during the transition from the perforating type to

FIGURE 37

TYPES OF ELECTRODES



Types of Electrodes

The construction of these is discussed in detail on pages 170, 172 and 173.

the agar-saline bridge type. It is similar to the perforating type except for the fact that the platinum is curved to form more than half of a ring, instead of being the needle type, and thus supports the nerve with less trauma.

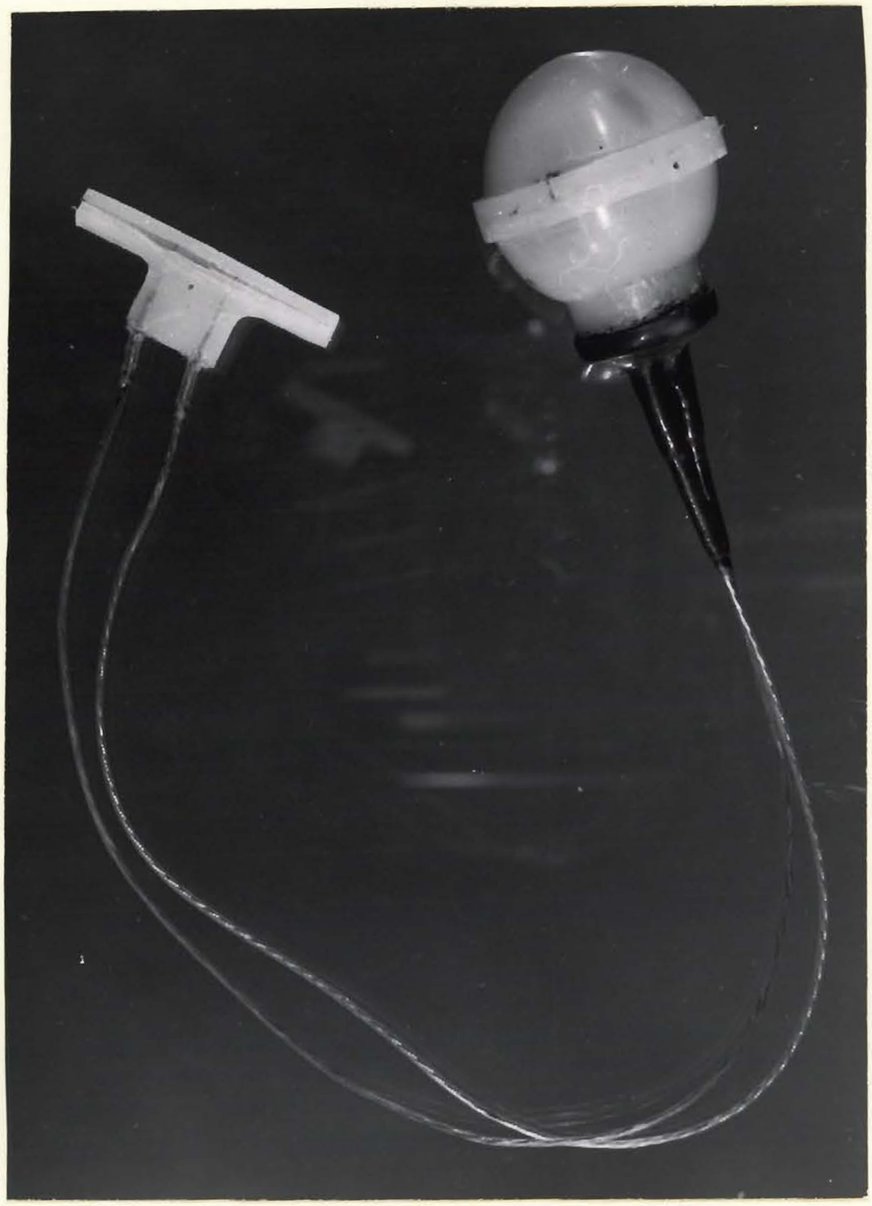
3. <u>The Agar-Saline Bridge Type</u> is the final type and is most effective, decreasing trauma and still allowing ready flow of current to the nerve. The foot was made as before with either a solid post leading to the actual cylinder, 11/16" wide, 7/16" long and 4/16" thick, or two separate smaller posts 4/16" wide, 7/16" long and 4/16" thick, but 3/16" apart as shown in Figure 37 on page 171. In each type two wells were drilled from the cylinder into the post, 2.5/16" wide and 4/16" long. The leads from the receiver were then threaded into the posts from the outer end, one into each well, and a flat spiral of No. 22 platinum wire was soldered to the lead at the bottom of each well. The leads were reinforced as above by short lengths of polyethylene tubing. A solution was then made consisting of 0.15 grams of powdered agar and 10 c.c. of normal saline and heated until complete mixing had occurred just before being used. At the operating table, using

aseptic techniques, this solution was injected by a small glass pipette into the wells, ensuring that no air entered the mixture. This solidified quickly and the excess was removed from the cylinder, thus leaving the two wells filled with the agar-saline preparation, forming a bridge to conduct the current between the platinum wire and the nerve in the cylinder. This method has been most effective and has caused least reaction by the nerve.

Photographs are shown of the two types of receiver equipment used. Figure 38 on page 174 shows the receiver arranged for direct current stimulation, with flexible leads reinforced by latex and with the perforating type of electrodes used in the earlier stages of the project. Figure 39 on page 175 shows the final type of apparatus used. Here there is the alternating current circuit with the 20 mfd. electrolytic condenser incorporated in the lead, the whole being reinforced by latex. The electrode foot has two posts and contains the agar-saline bridge type of electrode. Figure 40 on page 176 shows an enlargement of the receiver and casing shown in Figure 39.

Dr. R. Smith, who has been studying the effects of chronic remote cervical vagal stimulation on the heart, shared the problems presented in the construction of this receiver.

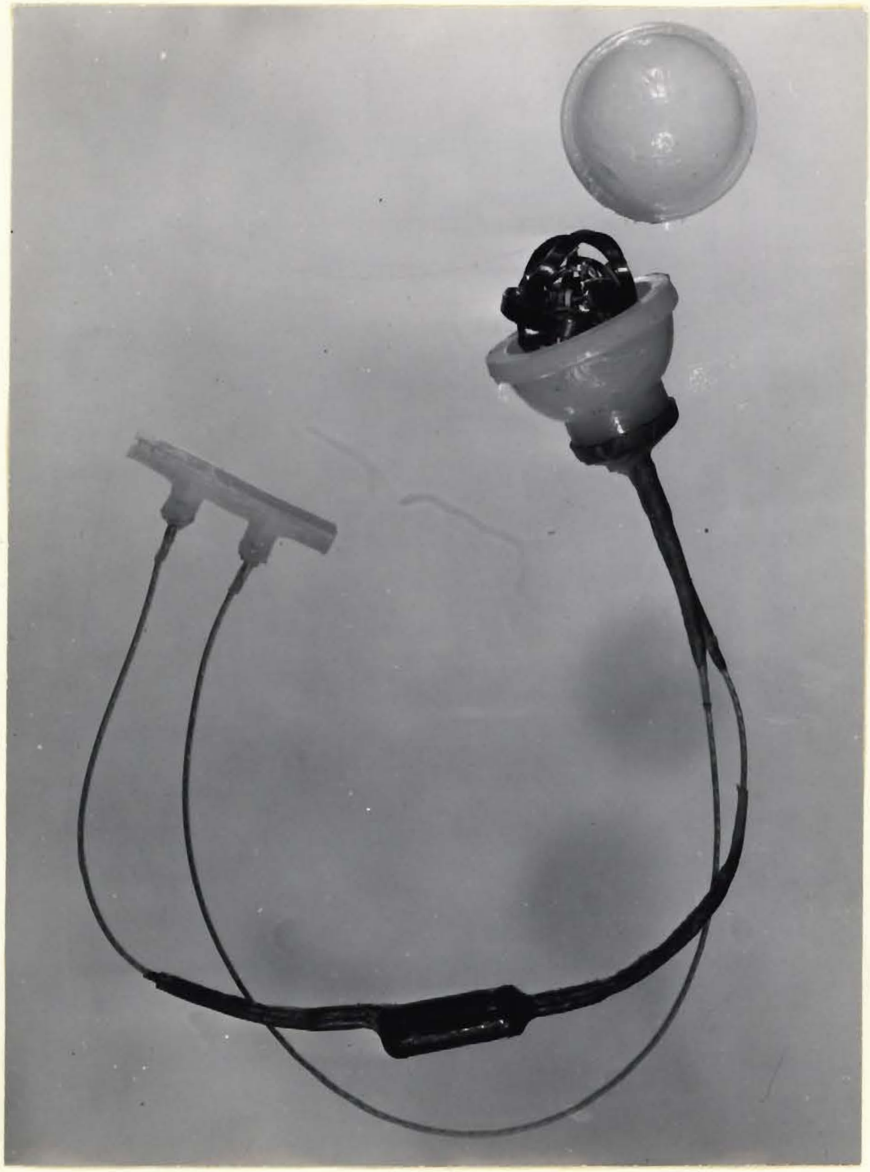
FIGURE 38



Direct Current Receiver

This shows the direct current receiver, with flexible leads, and perforating electrodes. The junction of the leads with the receiver casing is reinforced by latex.

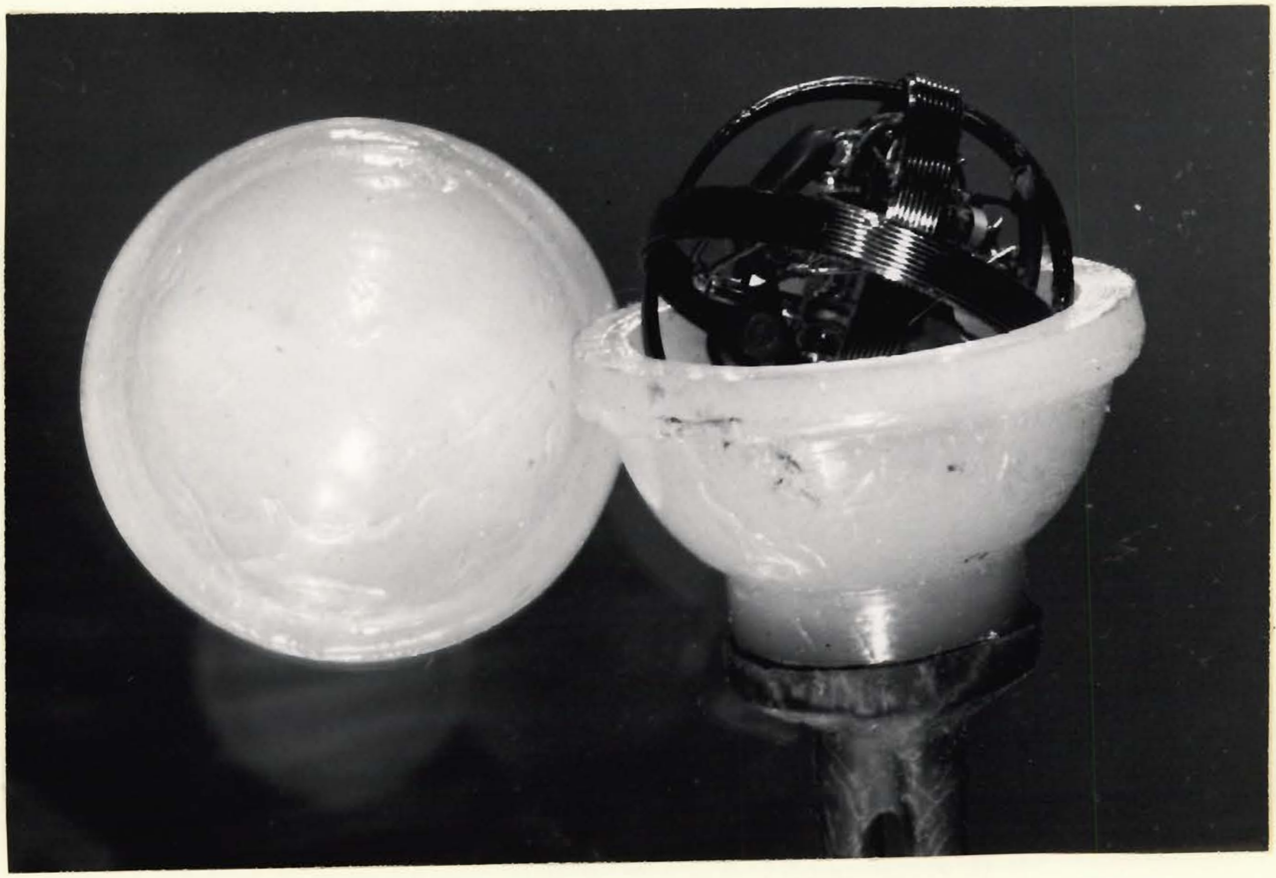
FIGURE 39



Alternating Current Receiver

This shows the alternating current receiver, with flexible leads, and agar-saline bridge electrodes. The 20 mfd. condenser and receiver junction with the leads are reinforced by latex.

FIGURE 40



Receiver and Casing

This is an enlargement of the receiver and casing shown in Figure 39, and shows the details of the receiver.

D. The Neurophysiological Properties of the Vagus

The neurophysiological properties of the vagus were most difficult to elucidate and presented many real problems in this research. By the use of the cross-sections of the vagus discussed in Chapter IV, Section D of this thesis, it was found that the vagus was made up essentially of very small fibers, lying in the range of 1 to 2 microns in the thoracic vagus and of varying sizes in the cervical vagus, ranging from 1 to 20 microns in diameter. In the thoracic vagus only a few larger fibers are myelinated while the great majority of the fine fibers are unmyelinated. In the cervical vagus once again great variety is seen: thick and heavily myelinated fibers, medium sized with a thinner coat of myelin and very fine, practically unmyelinated fibers.

A study of the spike potentials of the dog's vagus was also made, as will be discussed in Chapter VIII. It was found that the nerve is complex, comprising A, B and C fibers in the cervical segment; B, C and possibly A fibers in the upper thoracic segment; and B and C fibers in the lower thoracic segment. A strength-duration curve was made which indicated that the optimal stimulus should be 4 to

11 volts and the optimal pulse width should be 0.2 to 2.35 milleseconds.

The reaction of the nerve to chronic stimulation was also investigated. The value of different types of electrodes was studied as described in this chapter. The perforating type of electrode caused necrosis within two weeks in the majority of cases although one dog showed a grossly normal nerve after three weeks stimulation. The use of the agar-saline bridge type of electrode was much more effective and a cross-section of nerve stimulated for 121 hours is shown in Chapter IV, Section D. There is moderate surrounding fibrosis in the perineural area but the nerve fibers show no degeneration. Thus the nerve can apparently survive chronic stimulation.

The type of current used was alternating or direct, producing sine, square, and exponential types of waves. There was no change in gastric secretion with any of these, despite the use of a wide range of frequencies, pulse durations and intensity and the insertion of an interruptor in the stimulator. Thus all possible available forms of current were employed.

It was estimated in several acute experiments that the resistance of the vagus nerve was in the neighbourhood of 1000 ohms. This was considered in any interpretation of the intensity of stimulation.

E. Investigative Procedures Employed in this Project

I. Type of Fistula or Pouch to be Used:

It was decided to commence the experimental work by placing a Pavlov gastric fistula in each dog to be used. This fistula will be described in detail in the experimental chapter of this thesis but consists of a metal cannula inserted in the most dependent part of the stomach and sealed off by all layers of the abdominal wall. This cannula is closed by a rubber cork which can be removed easily when gastric secretion studies are carried out. It was also planned that a Pavlov or Dragstedt gastric pouch would be used if secretion was established by this method of stimulation. However, the ease of performing the Pavlov fistula and the lack of post-operative complications more than compensated for the regurgitation of duodenal contents and swallowing of saliva that occasionally complicated our experiments. Thus it was adequate for the studies carried out as we could be sure that the vagus nerves had not been traumatized and that the blood supply to the stomach was unimpaired after the initial operative procedure.

II. Analysis of Gastric Secretions:

The analysis of gastric secretions was necessarily of great importance, both for resting secretions and after vagal stimulation. The following aspects were tested in each case:

1. Appearance - this included the turbidity due to mucus or the discolouration due to bile. In the experiments in which saliva was swallowed, the frothy characteristic appearance of the upper level of the collected speciman was diagnostic.

2. pH - The pH was routinely checked by graded litmus paper and in at least half of each dog's samples, was checked on the pH meter.

3. Quantity - measured in c.c.'s for each period of the experiment.

4. Free and total audity - These were checked by the routine method[58] of adding Topfer's reagent to check free hydrochloric acid and then titrating with N/10 sodium hydroxide.

5. Pepsin - The peptic activity of the gastric juice was determined by the photoelectric method of

Riggs and Stadie[115]. This is a rapid, accurate method and avoids the delay and inaccuracies of using Mett's tubes. In principal, the method depends upon the use of a substrate consisting of a standardized, homogenized suspension of coagulated egg white. When acted upon by pepsin, the turbidity decreases with time. It is assumed that the amount of protein digested in unit time is proportional to this decrease in turbidity, and since turbidity can be measured by means of a photoelectric colorimeter, the peptic activity of the system can thus easily be determined. Since peptic hydrolysis of protein follows a mononuclear course, and since within limits its velocity constant is a function of the concentration of pepsin, it is convenient to express peptic activity as a velocity constant. Accurate measurement is possible down to a very low level of crystalline pepsin. The velocity constant is a curvilinear function of pepsin concentration and serves throughout the range encountered clinically as a convenient measure of the peptic activity of gastric samples. The authors found that determinations made on the highly active juice from Pavlov pouches showed so rapid a decrease in turbidity that it was necessary to use 1 c.c. of a 1 : 4 dilution. Under these circumstances the k values varied from 0.15 to 0.30. In view of the nature of our project and the possible high activity of the gastric juice, we used this latter method, since the same dilution in all specimens from a given source will give comparable results for that source.

III. <u>Training of Experimental Dogs</u>:

It is essential for any program of vagal stimulation that the experimental animal is satisfactorily conditioned before any interpretation of results is attempted. A set routine was followed in each dog. The animal was allowed to exercise for one hour and then at 9 a.m. brought to the room in which the stimulating apparatus was kept. The animal was placed in a Pavlov stand and the stomach was repeatedly lavaged by luke-warm tap water until it was completely empty. The dog was then placed in its cage inside the primary coil and resting secretions were collected, without stimulation, until five o'clock. This was repeated daily until it was determined that the normal rate of gastric secretion had been established. After this period of time, the lavage was carried out as before and resting secretions collected until the normal rate of secretion was reached.

Then stimulation was commenced and gastric secretions were collected at half hour or hour intervals, depending upon the type of stimulation used. Each day at five o'clock the animal was returned to his kennel and fed his only meal of the day. This method proved satisfactory and as will be seen in Chapter VIII, a normal resting secretion was regularly obtained.

In one dog, continuous stimulation was carried out for four days, the dog being exercised for one hour in the morning and fed at night as before, but otherwise remaining in its cage. In this case, gastric secretions were measured at fixed intervals during the day only.

CHAPTER VIII

EXPERIMENTAL PROCEDURES AND FINDINGS

On the basis of the review of previous work done on the subject of vagal stimulation and its effect on gastric secretion, and with the progressively improving methods used for stimulation in the course of the year, the experimental procedures used and the findings obtained will now be described. These are divided into four main groups and are further subdivided into complete descriptions of each experiment performed. In this way a more comprehensive review of the experimental work done may be presented. It is stressed that the major problem was the construction of a receiver and electrode which would be effective over a long period of time, with the least possible trauma to the vagus nerve.

A. A Study of the Spike Potentials of the Dog's Vagus

1. Introduction:

Since no precise reference to spike potential studies of the vagus nerve of the dog could be found in the literature, it was necessary to undertake several experiments directly toward this end.

This work comprised six experiments which were carried out in the Department of Physiology under the direction of Mr. V. Brooks and with the assistance of Dr. R. Smith and Mr. H. Millar.

Before prolonged stimulation could be carried out according to the physiological properties of the nerve fibers, it was essential to determine the types of fibers present in the vagus trunk and to calibrate the strength-duration curve of this nerve.

2. Purpose

The object of these experiments was two-fold:

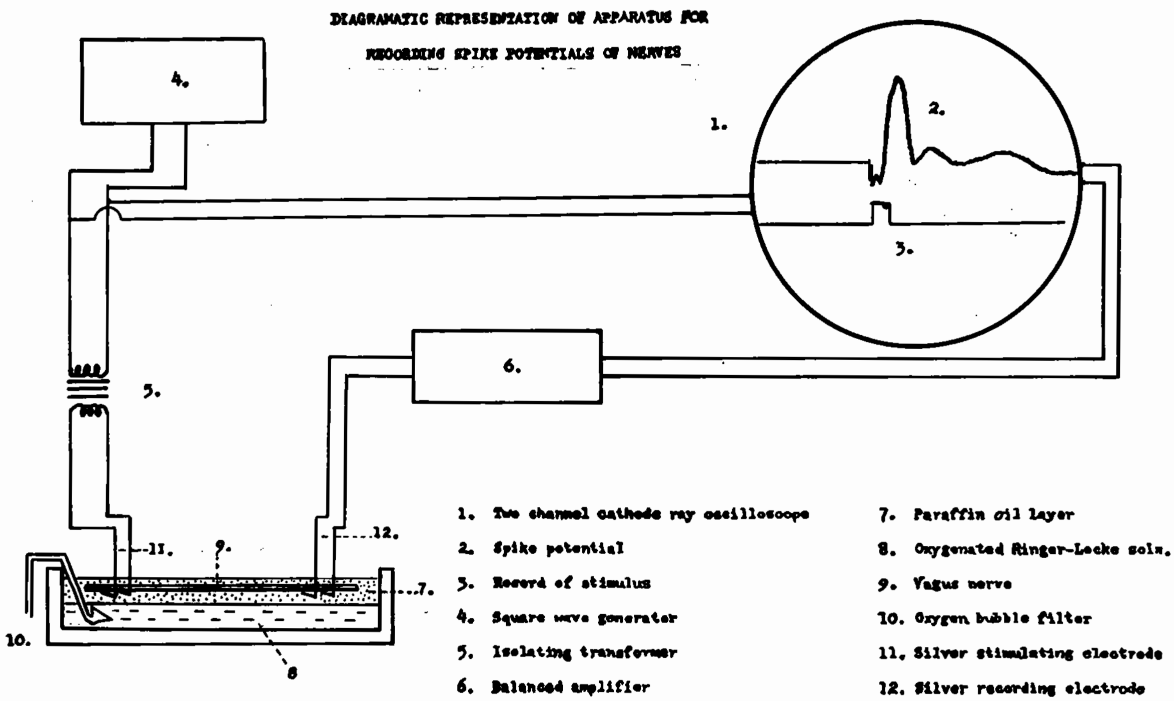
a. To determine the composition of the various segments of the dog's vagus nerve by recording the spike potentials of the various fiber groups.

b. To calculate strength-duration curves from each segment of the nerve which could be used to determine the optimal intensity and pulse duration with which to stimulate in chronic experiments.

3. Method:

a. Apparatus - the apparatus used is illustrated in Figure 41 on page 186. The stimuli were delivered by a battery operated square wave generator producing square waves which could be varied through

FIGURE 41



Spike Potential Study Apparatus

a range of frequency, intensity of 0 to 25 volts, and duration of 0.1 to 150 milleseconds. The output of the generator was conducted to the nerve through a high fidelity isolating transformer. The spike potentials of the nerve were picked up by silver recording electrodes and conducted into a high gain battery operated amplifier and then into one channel of a two beam Cossor oscilloscope. The other channel recorded the square wave stimulus as it was applied to the nerve. By means of this arrangement, it was possible to observe simultaneously the stimulus applied and the response or spike potential to be measured. A 35 millimeter camera was mounted so that photographs of the spike potential could be taken at any time. The shutter was synchronized with the stimulator and controlled by foot pedals.

Considerable care was taken to eliminate the ever present alternating current interference by the use of a shielded cage and good grounding. In spite of these precautions, A-C interference was troublesome throughout the experiments.

The fresh isolated nerve was placed in an oxygenated bath (5% CO_2) of Ringer-Locke solution maintained at 37^{o} Centigrade by a thermostat control. The nerve was allowed to rest on one set of stimulating electrodes and one set of recording electrodes. The electrodes were mounted on micrometer shafts to permit the spike potentials to be recorded through varying conduction distances. In order to reduce the degree of stimulus artefact, a layer of paraffin oil was floated on the surface of the Ringer-Locke solution. This thus reduced the artefact by means of its insulating properties around the electrodes.

b. Technique - Healthy dogs were anaethetized with intravenous nembutal. The chests were opened under intra-tracheal artificial respiration and segments of the vagus nerves were excised as rapidly and as atraumatically as possible, thus avoiding anoxia and injury to the nerve. Segments were taken from the lower thoracic, upper thoracic and cervical vagus nerves on both sides. These segments of nerves were placed immediately in oxygenated baths of Ringer-Locke solution maintained at 37^{o} centigrade prior to being placed in the electrode chamber.

4. Results:

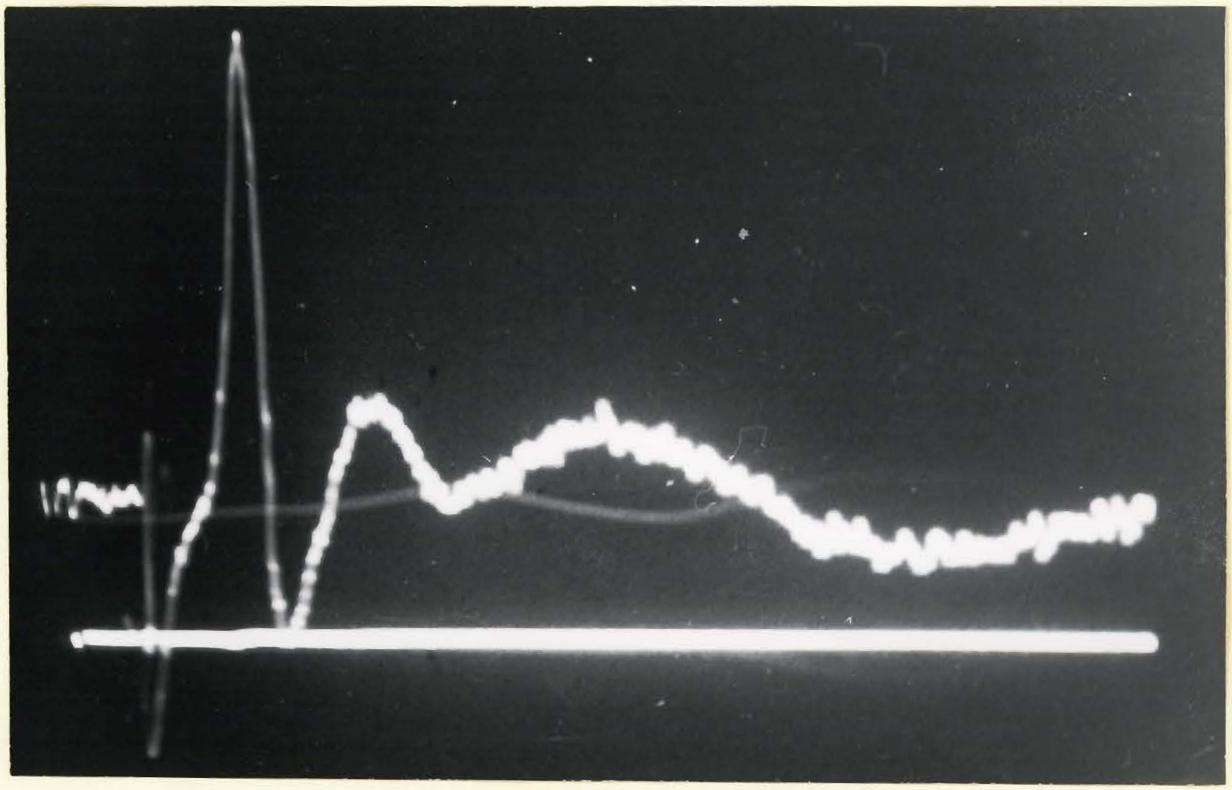
Considerable difficulty was experienced throughout all experiments due to either stimulus artefact or alternating current interference which tended to distort the spike potential. In spite of all precautions outlined above, these difficulties were never completely overcome.

In the lower thoracic segment, potentials were recorded which, according to their velocities, represented only C fibers. The conduction rates, when calculated were approximately 1.2 meters per second.

From the upper thoracic segment, three spike potentials were recorded as shown in Figure 42 on page 190. The conduction rates of these potentials indicated that they represented B and C fibers and possibly some A fibers. Similar potentials were observed from the cervical segment.

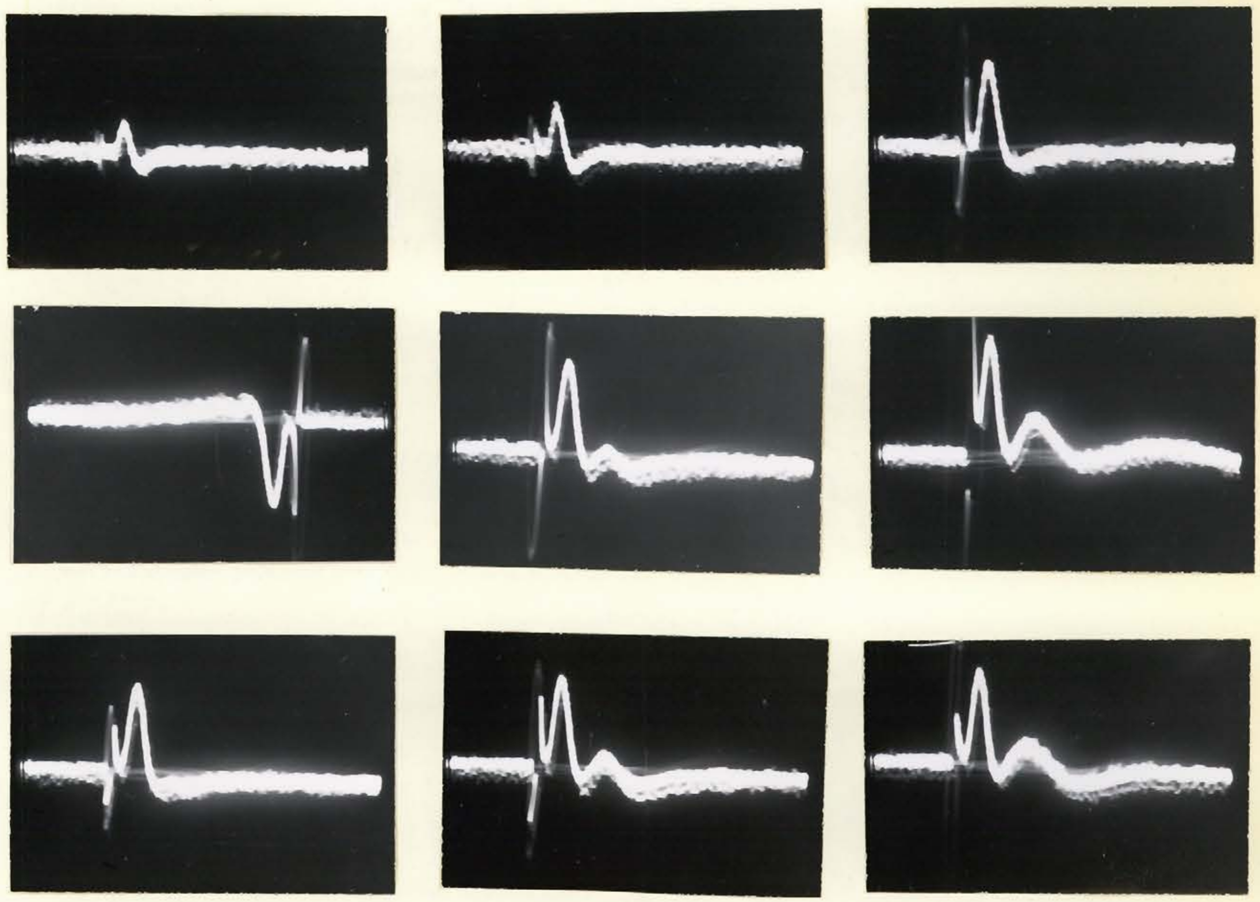
The accepted theory that various fibers are stimulated by different thresholds of stimuli is illustrated by Figure 43 on page 191 which shows two potentials representing B and C fibers respectively. The type of fiber represented was established by calculating the respective conduction velocities. When the intensity of the stimulus was gradually increased from a sub-threshold value of 0.2 volts through 1.18 volts, the potential from the

FIGURE 42



Upper Thoracic Vagus Spike Potentials

FIGURE 43



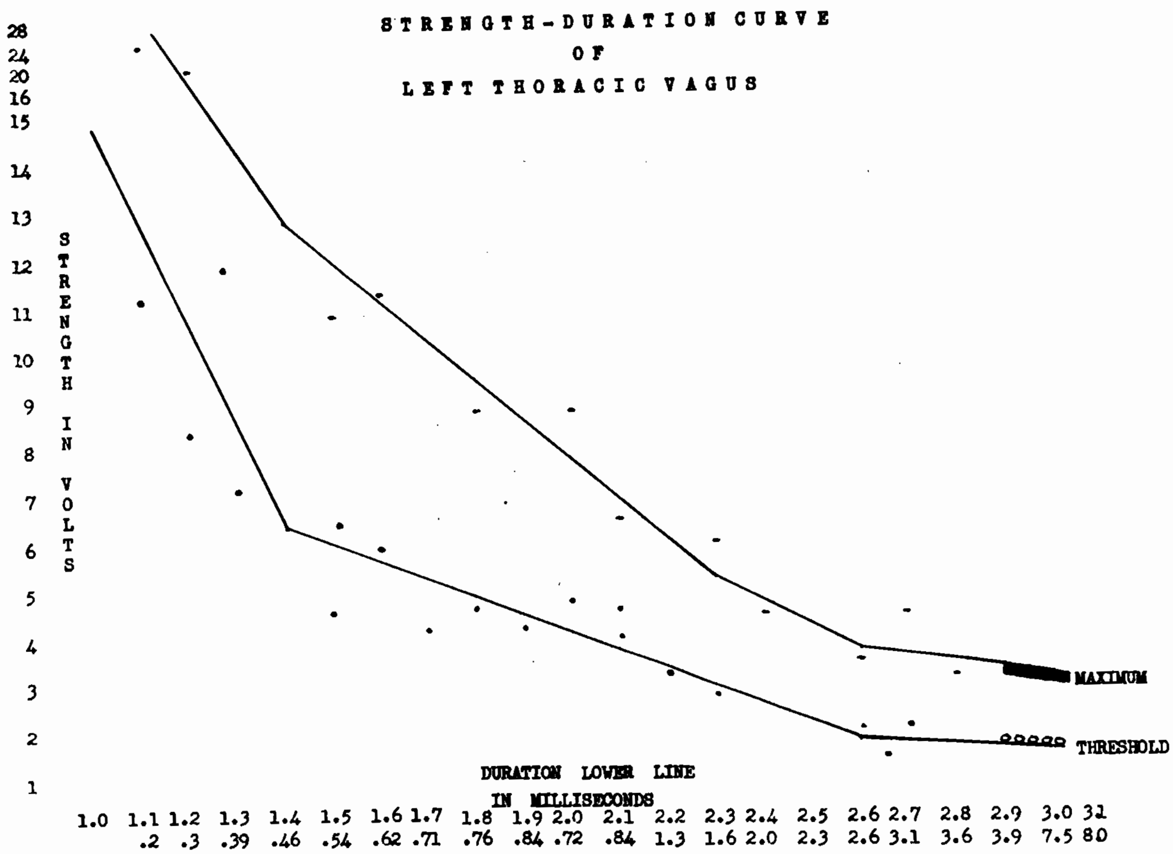
Varying Stimulation of B and C Fibers

B fibers appeared. As the stimulus was gradually increased in intensity through 4.5 volts to 7.7 volts, a second potential appeared which represented the C fibers. These recordings were taken on a 15 millisecond sweep.

A strength-duration curve was calculated for the upper thoracic segment of the dog's vagus and is illustrated in Figure 44 on page 193. This curve was carried out with an amplification of 3.0, a scope of 0.3 volts, a 500 millisecond sweep, a pulse width of 10 milliseconds and a scale unit of 50 microvolts. It indicated a threshold value of 1.5 to 15 volts for a duration of 0.2 to 7.5 milliseconds and a maximum intensity of 3.5 to 28 volts for a duration of 0.2 to 3.9 milliseconds. From this curve the optimal strength for nerve stimulation should be 4 to 11 volts and the optimal duration of stimulus should be 0.2 to 2.35 milliseconds.

In view of the doubt concerning the fragility of the vagus nerve, it was also decided to record spike potentials before and after stretching the isolated nerve. Two spike potentials, representing B and C fibers, were recorded from the left upper thoracic vagus. The nerve was then removed from the

FIGURE 44



Strength-Duration Curve

This shows the optimal strength of upper thoracic vagal stimulation should be 4 to 11 volts and the optimum duration of stimulus should be 0.2 to 2.35 milliseconds.

electrode chamber, stretched with moderate firmness, then replaced on the electrodes. It was noted that the spike potential of the B fibers had disappeared while that of the C fibers persisted. This was repeated and the same results were obtained. This experiment agrees with the work of Carlson[21, 22] of the University of Chicago who demonstrated that stretching a nerve would interfere with the conducting activity of that nerve, the C fibers being the last to be damaged .

B. The Influence of Vagal Stimulation on Gastric Motility

1. Introduction:

It is known that stimulation of the vagus nerve produces an increase in gastric motility. It was decided to carry out several acute experiments in which the cervical vagus would be stimulated by various methods and observations made of the effect on gastric motility. The cardiac effects are routinely obtained through vagal stimulation but the actual effect on the stomach could not be determined by analysis of the gastric secretions. It was thus thought that a study of the effects of vagal stimulation on gastric motility might give some indication whether the vagal fibers to the stomach were actually being stimulated.

2. Purpose:

The object of these experiments was to:

1. Acutely stimulate the cervical vagus of a dog and measure the changes in gastric motility.

2. Determine the efficiency of various methods of vagal stimulation used in our project.

3. Method:

a. Apparatus -

A Pavlov gastric fistula was prepared in the experimental animal about four days before the acute experiment. A thin rubber balloon was then used which on inflation just filled the stomach of the dog. This balloon was attached to a glass tube which fitted firmly in the rubber cork closing the fistula, projecting through on either side of the cork. The balloon was placed in the stomach and the cork closed the fistula. A rubber tube was then attached to the outside end of the glass tube and the balloon inflated just enough to fill the stomach without distension. This rubber tube was attached to a strain gauge pressure transducer connected to the strain gauge control unit. This in turn was led into the viso-cardiette which had had a direct current booster unit inserted to replace the alternating current unit in the viso-cardiette in order to stabilize the base-line and to increase the electrical intensity of the output. This was then led into the recording galvanometer and recorded on a moving drum.

Stimulation was carried out by means of square wave modulation on the alternating current circuit, induction coil modulation with the metronome set at twenty-five interruptions per minute and by the induction coil only with twenty-five interruptions per minute by the metronome. The capacity and wave forms of these various methods were determined by the Cossor oscilloscope.

b. Technique:

The dog was anaethetized with intravenous nembutal and the left cervical vagus dissected free for a distance of two inches from the left carotid sheath. The platinum contact or platinum ring type of electrode was then applied to the central end of the cut vagus nerve.

The animal was then placed in a canvas

sling with a hole cut to allow the cannula to pass through and the head was supported on a wooden stand.

The rubber balloon was inserted as indicated above, after gastric lavage had been performed, and connected to the recording unit.

Set periods of stimulation were then carried out and the results on gastric motility recorded on the moving drum.

4. Results:

Considerable difficulty was experienced in separating the record caused by respirations and the record caused by gastric motility. However, with each type of stimulation, there was a rise in pressure above the baseline and a change in the amplitude of the record, although the oscillations recorded were synonomous with the respirations. At one point, while under induction coil stimulation, the animal vomited and had a loose diarrheic stool. This stopped when stimulation was discontinued. It was noted that with stimulation, respirations increased in rate from 40 to 50 per minute to 80 or 90 per minute and became more shallow. The interpretation of these results is difficult and can not be aided by the inclusion of pictures of the record on the drum.

C. Acute Stimulation of the Cervical Vagus

1. Introduction:

In the course of our experiments of chronic stimulation of the vagus, it was necessary to carry out a series of acute experiments in which the cervical vagus nerve was stimulated under fixed conditions. As discussed in Chapter VI, the routine method of stimulation of the vagus is to cut the nerve as high as possible in the neck and to stimulate the central part of the distal segment by means of an induction coil and electrodes. Most investigators obtained an increase in gastric secretion by this method. It was thus deemed advisable to attempt to obtain gastric secretion by this classical method and then compare the results of our method of stimulation.

2. Purpose:

The object of these experiments was to acutely stimulate the cervical vagus by:

a. the classical nerve electrodes and induction coil with metronome interruptor.

b. the ring electrodes and direct-current receiver used in some of our chronic animals.

c. the ring electrodes and induction coil, fired through the transmitter to produce an exponential wave form. In this way, it could be determined if our method of stimulation would produce a change in gastric secretion in an acute experiment.

3. Method:

a. Apparatus - three types of equipment were used to produce stimulation:

i. An A.H. Baird of Edinburgh induction coil, with a metronome operating at twenty-two interruptions per minute of the induction coil current, carried to the nerve by leads to a pair of platinum contact electrodes in a bakelite foot.

ii. The transmitter unit and direct current circuit receiver with platinum wire ring electrodes as described in Chapter VII, Section C.

iii. Induction coil modulation with platinum ring electrodes. In order to attempt to produce the same wave form by use of the transmitter as produced by the induction coil, an ingenious apparatus was devised by Mr. H. Millar. The bias control used above permitted sine wave modulation and square wave modulation, using a conventional flip-flop circuit and amplifier. Induction-coil modulation was obtained by wiring the secondary in series with the bias supply. A metronome with mercury contacts acted as a stimulation interruptor for the induction coil.

With all these methods of stimulation, a Cossor oscilloscope recorded the voltage and wave forms applied to the nerve.

A canvas sling was used to support the anaesthetized dog in the upright position, with a hole in the sling through which the gastric secretions could be collected.

b. Technique:

Healthy, medium-sized dogs were anaethetized by ether induction and intravenous chloralose-urethane. The suggested mixture by weight comprised 3.6% chloralose, 36.4% urethane and 60% water. This averages a dose of .05 grams per kilogram of chloralose and .5 grams per kilogram of urethane - that is, a ratio of 1:10. This is then mixed with 20 c.c. of .9% saline, heated slightly to aid solution, and injected very slowly intravenously. The use of chloralose alone may produce spasm of the blood vessels, which would be unfavourable to gastric secretion, while urethane does not give such deep anaesthesia but it dilates the blood vessels. Thus this mixture induces good anaesthesia and probably has the least effect of any anaesthesia on gastric secretion.

In half the experiments performed the operative technique was similar to that used by Vineberg[128] in his experiments on vagal stimulation. The abdomen was shaved and an upper midline abdominal incision made without observing aseptic technique. The stomach was visualized and the pylorus doubly ligated. A Pavlov fistula was then made in the most dependent part of the stomach, and the cannula secured in position by two inverting purse string sutures and the abdominal wall was closed in two layers. A midline incision was then made in the neck, the oesophagus was ligated and both cervical vagi were dissected free from the carotid sheath and cut as high in the neck as possible.

In the remainder of the six experiments, a new technique was used, based on an article by Schachter[119], in which he observed that the operative trauma of the above procedure interfered with normal gastric secretion. He advised the preparation of a Pavlov fistula using aseptic technique in the dog four days before the acute experiment. Thus, on the day of the acute experiment, the abdomen was not opened and the neck dissection only was carried out, but without ligation of the oesophagus. He found in only a few experiments that regurgitation of duodenal contents or swallowing of saliva interfered with his results. In this way, a more normal physiological situation can be achieved in the experimental animal.

Both the above procedures were followed by the same type of nerve stimulation. The ring or contact platinum electrodes were applied to the central end of the distal segment of the cut vagus and stimulation carried out, the electrodes being moved more peripherally as the experiment progressed. Every effort was made to minimize trauma to the nerve, especially during the application of the electrodes. In most of our experiments, the left vagus only was stimulated. After application of the electrodes, the animal was placed in the canvas sling with the cannula protruding through a hole in the sling. The resting secretions were measured for one or two hours before stimulation was commenced.

The gastric analyses were made by the methods described in Chapter VII, Section E, samples being collected after adequate lavage of the stomach. The stimulation was thought to be functioning effectively when cardiac slowing could be produced.

4. Results:

a. Experiment 1:

The dog was anaesthetized and Vineberg's operative procedure carried out as described above. Table 1 on page 204 describes the results obtained by the various methods of stimulation.

b. Experiment 2:

The procedure of Experiment 1 was repeated but results were not contributory. No changes in gastric secretion were produced with square wave modulation nor by the use of the induction coil when used for a similar length of time as that effective in Experiment 1. However, both types of stimulation produced cardiac slowing from 160 per minute to 40 or 50 per minute, indicating vagal stimulation of the heart. After three hours of stimulation by both methods, grossly bloody, diarrheic stools escaped from the animal.

c. Experiment 3:

In this experiment, the technique was changed to Schachter's method described above. The fistula

TABLE I

EXPERIMENT I

Time in minutes	Amount in c.c's	Appearance	Free HCL	Total HCL	Pepsin(k)	pH
Resting Secretions						
0 - 60	8	viscous, bloody	not done because of blood in specimen			3.4
60 - 120	7	thin, bloody				
Induction coil with 22 interruptions per minute by the metronome producing 6 volts on the oscilloscope. Contact electrode on left vagus.						
140 - 160	7	bloody				
160 - 180	7.8	clearer	29	63	0.156	2.4
Electrode moved ½ centimeter peripherally						
180 - 200	13.5	coffee ground	62	93	0.164	1.6
200 - 220	18.5	coffee ground	72	98	0.179	1.4
Stimulation stopped. Resting secretions collected.						
220 - 240	4	coffee ground	84	106	0.160	1.2
Square wave modulation - frequency of 20 per second, 4 milliseconds duration, intensity of 7 volts.						
250 - 280	3	coffee ground	33	70	0.155	2.6

in this case had been made 58 days before. The results are listed in Table 2 on page 206.

d. Experiment 4:

Because of the interference from regurgitation of bile using Schachter's method in Experiment 3, Vineberg's operative technique was employed once more in this experiment. In this experiment no resting secretions were obtained in the two hour period before stimulation began. It was assured that the fistula was patent and that no mechanical obstruction could produce this unusual lack of secretion. Stimulation was carried out by the induction coil alone with 25 interruptions per minute by the metronome, producing 13 volts as recorded on the oscilloscope. Stimulation produced cardiac slowing from 150 to 50 per minute. Two hours of this stimulation failed to produce any gastric secretion. The animal died at this point. The unusual lack of gastric secretion was blamed on operative trauma as the cardiac effects proved the apparatus was functioning effectively.

e. Experiment 5:

Because of the complete absence of gastric

TABLE 2
EXPERIMENT III

Time in minutes	Amount in c.c's	Appearance	Free HCL	Total HCL	Pepsin(k)	pH
Resting Secretions						
0 - 60	1½	clear	0	110	N.S.Q.	N.S.Q.
Square wave modulation of right vagus, frequency of 20 per second, ½ millisecond duration, intensity of 8 volts. Produced cardiac slowing from 160 to 60 per minute.						
75 - 135	1½	clear	0	90	N.S.Q.	N.S.Q.
Induction coil modulation producing 6 volts and cardiac slowing from 160 to 60 per minute.						
140 - 200	7	trace of bile	0	22	0.010	6.33
200 - 260	6	bile ----				
Induction coil only with 25 interruptions per minute on the metronome, producing 14 volts on the oscilloscope. Produced cardiac slowing from 160 to 60 per min.						
260 - 280	17	bile ---				
280 - 300	6	trace of bile	25	60	0.205	2.5
Electrode placed on left vagus. No cardiac slowing observed.						
300 - 320	½	N.S.Q.	N.S.Q.	N.S.Q.	N.S.Q.	N.S.Q.
Electrode placed on right vagus as before. Produced cardiac slowing from 160 to 60 per minute.						
320 - 340	10½	bile -	0	30		4.09
340 - 360	5	trace of bile	15	38	0.133	3.3

secretion in Experiment 4, a return was made to Schachter's operative procedure. The dog used had had a Pavlov fistula prepared aseptically seven days before to avoid an abdominal operation as part of the acute experiment. In this experiment, the blood pressure was recorded by a cannula inserted in the right carotid artery, the cannula being attached to a mercury manometer by a rubber tube containing a solution of 3% sodium citrate. The results are listed in Table 3 on page 208.

f. Experiment 6:

This was the final acute experiment of this series and was carried out by Schachter's method. The Pavlov fistula had been prepared in the dog four days before the acute experiment. The results are listed in Table 4 on page 209.

D. Chronic Stimulation of the Vagus Nerve

1. Introduction:

The information listed on the previous pages concerning nerve physiology and the properties of the vagus nerve, as well as the acute experiments performed, have been a precursor to our attempt to

TABLE 3

EXPERIMENT V

Time in Minutes	Amount in c.c's	Appearance	Free HCL	Total HCL	Pepsin(k)	pH	B.P. MM,HG
Resting Secretions							
0 - 60	7	clear	0	22	.012	5.64	170
60 - 120	2	viscous					
120 - 210	2	viscous					
Induction coil method with 25 interruptions per minute on the metronome, producing 12 volts on the oscilloscope. Stimulation of left vagus produced cardiac slowing from 150 to 90 per minute.							
240 - 260	9	clear,mucus	45	77	.197	2.49	100
260 - 300	25	clear,mucus	90	120	.181	2.15	100
Induction coil modulation producing 14 volts. Stimulation of left vagus produced cardiac arrest with each impulse.							
300 - 320	14	clear,mucus	50	74		2.1	115
320 - 340	14	clear,mucus				2.3	
340 - 360	17	clear,mucus	20	62		2.6	

TABLE 4

EXPERIMENT VI

Time in minutes	Amount in c.c's	Appearance	Free HCL	Total HCL	Pepsin(k)	pH
Resting secretions						
0 - 30	2.5					
30 - 60	1.5	clear	60	79	.140	1.79
Induction coil modulation with 25 interruptions per minute on the metronome, producing 17 volts on the oscilloscope. Stimulation of left vagus slowed heart from 170 to 60 per minute.						
60 - 90	11.7					
90 - 120	70	bile --	94	105	.186	1.65
120 - 150	90	bile --	113	122	.260	1.60
150 - 180	85	bile --	138	151	.191	1.55
Stimulation stopped. Resting secretions						
180 - 210	28	bile --	110	114	.181	1.80

chronically stimulate the vagus. It was thought that if the increase in gastric secretion obtained in acute experiments could be chronically produced under controlled conditions, peptic ulcer formation might then occur. The problems faced have been clearly indicated in Sections B and C of Chapter VII and need not be repeated here. Despite all the methods used to produce peptic ulcers, no worker has yet been able to produce such an ulcer by stimulation of the vagus nerve. The clinical significance of this problem is emphasized by the operation of vagotomy as a treatment of peptic ulcer, the use of this procedure being based on studies of gastric secretion in gastric pouches before and after vagotomy. Although not one of the purposes of this project, the controlled production of peptic ulcer would then afford excellent opportunity of observing the efficiency of various medical and surgical forms of treatment.

2. Purpose:

The object of this project was:

a. to devise a successful method of producing chronic remote vagal stimulation by radiofrequency.

b. to produce an increase in gastric secretion in response to vagal stimulation.

c. to produce a peptic ulcer by chronic vagal stimulation.

3. Method:

a. Apparatus:

The technique of making the apparatus has been fully described in Section C of Chapter VII. As the project progressed, changes were made in the receiver circuit, the type of electrode and the wave form employed. These changes will be mentioned in the descriptions of the experiments carried out.

The animals selected for these procedures were healthy dogs, weighing ten to fourteen kilograms, supplied by the Department of Physiology. These dogs were kept under observation for three or four days before the Pavlov fistula was made and thus ensured to be well. Anaesthesia was produced by intravenous sodium nembutal, using a dosage of 1 grain per 5 pounds of body weight.

b. Technique:

i. Pavlov fistula: This procedure was carried out as a single operation approximately one week before application of the receiver to the vagus nerve. The dog was anaesthetized with intravenous nembutal and the abdomen shaved and prepared with alcohol and metaphen. An upper mid-line abdominal incision was made just large enough to deliver the greater curvature of the stomach. An incision was then made on the anterior gastric wall, just above and almost parallel to the most dependent portion of the greater curvature. This incision included all layers of the gastric wall and was just long enough to permit insertion of the gastric cannula. The outer rim of the Pavlov cannula is 12 millimeters wide, the diameter of the lumen is 20 millimeters and the circumference of the external rim is 52 millimeters. The internal rim is 8 millimeters wide and the circumference is 45 millimeters. The cannula is 41 millimeters long.

The stomach wall was then closed around the cannula by one heavy cotton purse string suture inserted before the gastric incision was made and quickly ligated around the cannula to reduce bleeding and to avoid the use of ligatures. A second inverting cotton purse string suture was placed if necessary. Figure 45 on page 213 shows this stage of the

FIGURE 45

Pavlov Fistula

The cannula has been inserted through the anterior gastric wall just above the greater curvature, held in position by two purse-string sutures.

operation. The abdominal wall was then closed in three layers, the peritoneum and fascial layers being sutured to the gastric wall around the cannula. A rubber cork was then inserted in the cannula and a thick gauze dressing rolled around the cannula below the outer flanges to cause the inner flange of the cannula to impinge firmly on the gastric and abdominal walls. No solid food was given for thirty-six hours post operatively. This proved to be a procedure with no mortality and very low morbidity, the chief complication being occasional seepage of gastric juice around the cannula with excoriation of the abdominal wall. On the second or third post-operative day, conditioning of the dog to the stimulation cage and collection of gastric secretions were commenced. This was continued, only being discontinued for a few days when the receiver was implanted.

ii. <u>Application of a Receiver to the Left Thoracic Vagus</u>: In the first series of chronic animals, the method described by Lafferty and Farrell of applying the receiver to the left thoracic vagus was employed. Using intravenous nembutal anaesthetic and the usual aseptic technique, the chest was opened through the left sixth intercostal spaces under intra-tracheal artificial respiration. Mechanical retractors permitted adequate exposure without cutting through a rib. The left pulmonary ligament was cut and the left lung packed off with moist towels, disclosing the left thoracic vagus and its large branch to the right vagus. Figure 46 on page 216 shows this exposure of the left thoracic vagus. The left vagus, above its branch to the right vagus, was carefully dissected free for $2\frac{1}{2}$" from its surrounding fascia without application of forceps to the nerve, with as little traction as possible and with adequate hemostasis.

The application of the receiver now varied depending upon the type of lead used. In the early series of dogs, a fixed lead was used as described in Chapter VII, Section C. The nerve was placed in the electrode foot and the foot was closed firmly around the nerve by two ligatures, one at each end of the foot. In order to obtain greater stability of the receiver and thus less traction on the nerve, these ligatures were sutured securely to the prevertebral fascia lateral to the descending aorta. The receiver casing was then held in a fairly solid position by one or two sutures through the polyethylene

FIGURE 46

Left Thoracic Vagus

The vagus, and its branch to the right vagus, are shown, being held on the stretch by a forcep applied to the fascia.

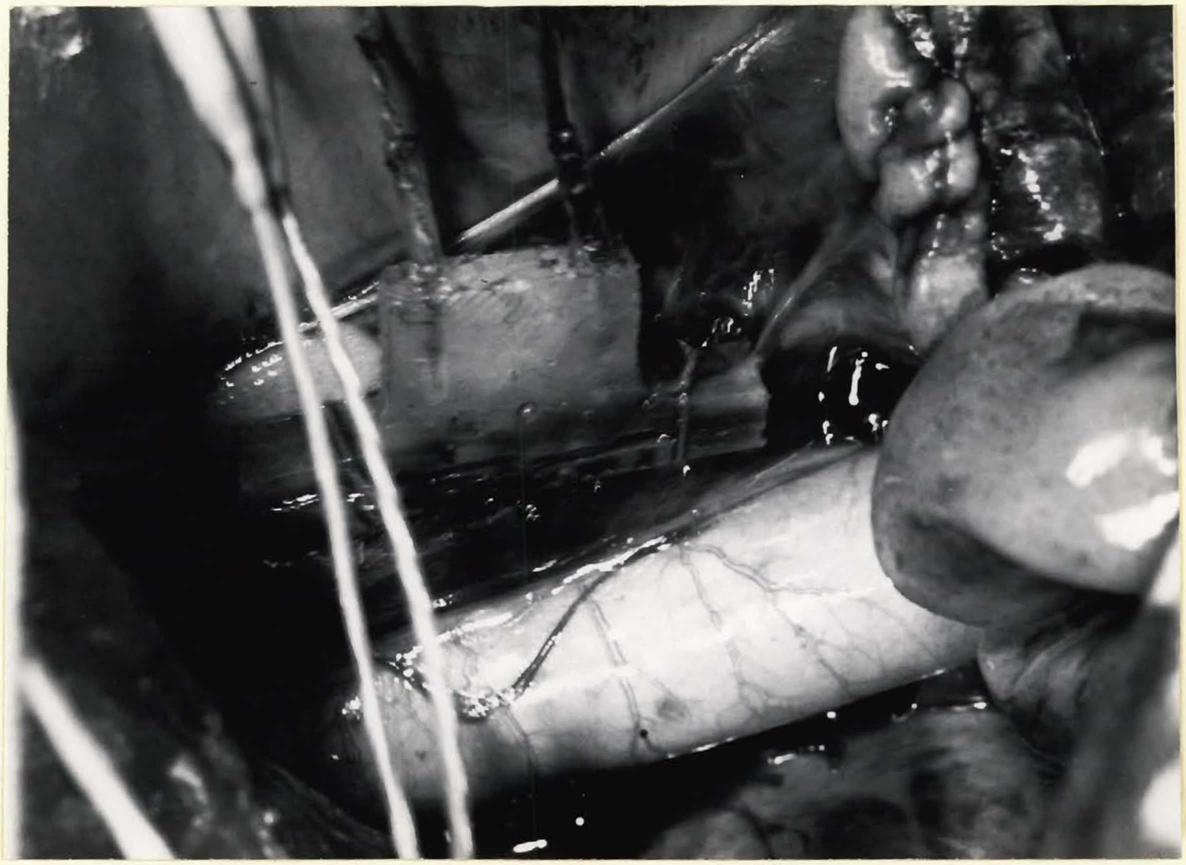
at the equator of the sphere, not communicating with the interior of the casing, and ligated around the 5th and 6th ribs or through the intrathoracic layer of fascia.

In the second series of dogs in which the flexible lead was used, as described in Chapter VII, Section C, the nerve was placed in the electrode foot and ligated as before but the foot was not secured to any surrounding tissue. This method of application to the thoracic vagus is shown in Figure 47 on page 218. The flexible lead was then placed superficial to the left lower lobe of the lung and the receiver casing, with sutures through the equator as before, was sutured to the intrathoracic fascia on the left deep aspect of the sternum. It was shown that the use of the flexible lead overcame most of the traction on the nerve and this method appeared quite effective.

The chest was then closed in a routine fashion without drains after inflating the lungs.

iii. Application of a Receiver to the Left Cervical Vagus: It was observed that application of the receiver to the thoracic vagus did not produce gastric secretion changes upon stimu-

FIGURE 47



Electrode Foot on the Left Thoracic Vagus

The agar-saline type of electrode has been placed on the vagus, the flexible leads being shown.

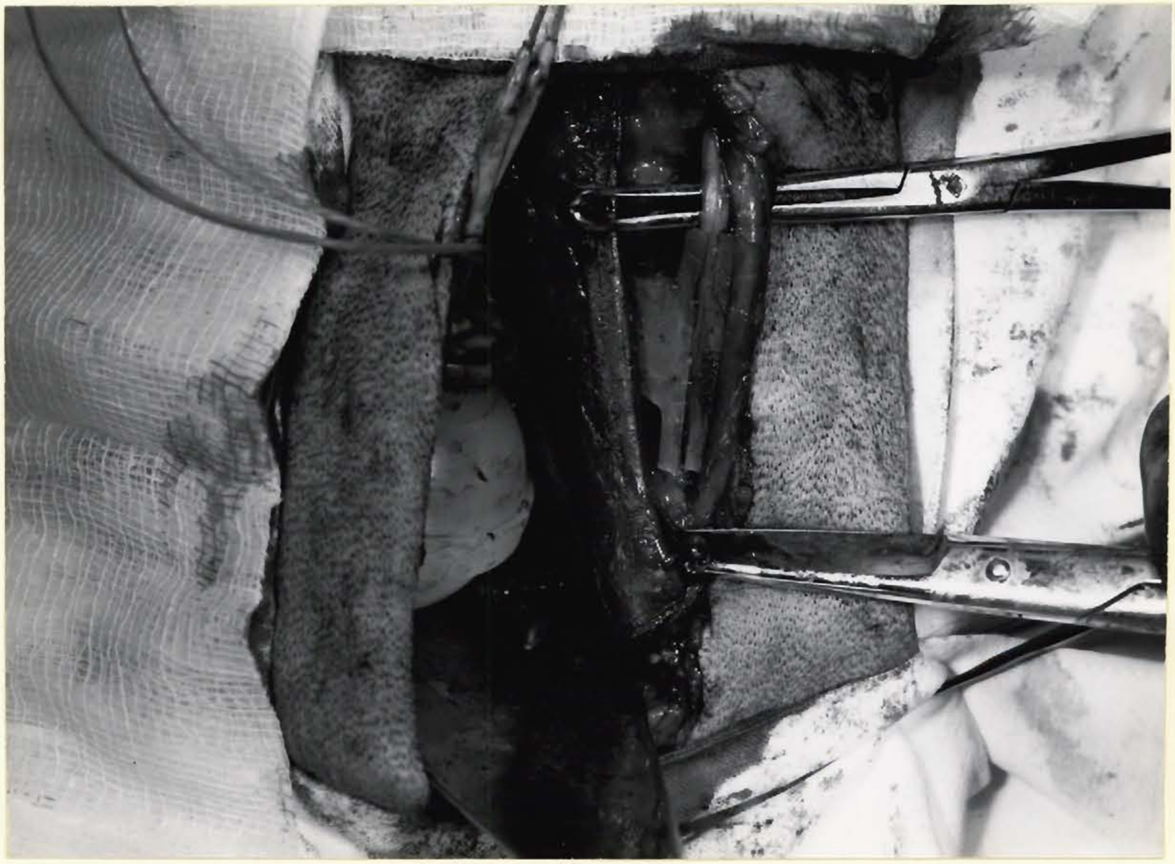
lation, due possibly to trauma to the nerve or to some inherent property of the thoracic vagus. It had been shown in acute experiments that stimulation of the cervical vagus produced increased gastric secretion and also slowing of the heart rate. It was thus decided to place the receiver on the left cervical vagus because satisfactory functioning of the receiver would be indicated by cardiac slowing and the operative procedure itself was easier.

Originally the neck was opened by a collar incision below the thyroid cartilage but post-operative collections of fluid in the subcutaneous area caused increased morbidity. This was largely overcome by the use of a vertical mid-line cervical incision. The trachea was exposed and the left carotid sheath visualized by blunt dissection between the sternomastoid and strap muscles. The left cervical vagus was then cleared from the surrounding sheath for $1\frac{1}{2}$" by sharp dissection. A fascial space large enough for the receiver was made by blunt dissection behind the right sternomastoid and sternohyoid muscles as inferiorly as possible. The receiver was then placed in this space, the flexible lead was carried under the strap muscles of each side and the electrode foot secured around the left cervical vagus by two ligatures. Figure 48 on page 221 shows the left cervical vagus contained in the electrode foot, with the left carotid artery on one side and the left strap muscles on the other. The receiver is in the right fascial space. This was so done that there was no traction on the nerve and thus no sutures were used to secure any part of the lead or receiver to the surrounding tissue. The muscles were then brought into apposition by sutures through the fascia and the skin closed. This is a relatively atraumatic procedure and can be done in less than half an hour.

iv. Left Thoracic Sympathectomy: In view of the lack of response of gastric secretion to vagal stimulation and because of the probable inhibitory effect of the sympathetic system, it was decided in the last series of dogs to also perform a thoracic sympathectory. Since this is a relatively major procedure in a dog, for experimental purposes only the left sympathetic chain was removed.

Under intravenous nembutal anaesthesia and intra-tracheal artificial respiration, the chest was opened through the left third intercostal space. The sympathetic chain was visualized lying along the

FIGURE 48



Electrode Foot on the Left Cervical Vagus

The agar-saline type of electrode has been placed on the vagus. The nerve and left common carotid artery are shown above the allis forcep. The receiver has been placed in the right fascial space and the flexible leads are deep to the strap muscles.

heads of the ribs and was dissected free from the stellate ganglion to the 4th or 5th interspace, both rami being cut. Part of the dissected sympathetic chain is shown in Figure 49 on page 223. This incision was then closed and the chest opened again through the left seventh interspace. The sympathetic chain was then excised from the fourth or fifth interspace above where the former procedure had stopped down to the level of the diaphragm. The splanchnic nerves and rami in this area were cut. Special care was taken not to cut the posterior intercostal vessels. This incision was then closed after inflating the lungs and no drains were used.

4. Results:

In all these animals gastric lavage was routinely carried out before collection of specimens of gastric secretion. For brevity, only representative results will be listed and not the results of each day's stimulation, which followed the system explained in Section E, Chapter VII.

a. Animal 1:

A fixed lead direct current type of receiver as described in Section C, Chapter VII, with

FIGURE 49

Left Thoracic Sympathetic Chain

A section of the sympathetic chain has been dissected free and is held on the stretch by forceps.

a tested capacity of twelve volts on the oscilloscope, and perforating electrodes, was applied to the left thoracic vagus by the described technique for this type of receiver.

On the fifth post-operative day, stimulation of six volts intensity produced coughing, salivation and restlessness, which were accepted as proofs of satisfactory stimulation. Resting secretions averaged 20 c.c.'s per hour with a pH of 3.

On the seventh post-operative day, stimulation of twelve volts intensity was necessary to produce the above signs which were moderate with a frequency of 10 per second and a pulse duration of 3 milliseconds but were marked - including attempts at vomiting - with a frequency of 40 per second and a pulse duration of 3 milliseconds.

On the eleventh post-operative day, the results shown in Table 5 on page 225 were obtained.

Stimulation in this animal produced marked salivation and restlessness, with pools of saliva on the floor at the end of each period of salivation. The 58 c.c. speciman consisted of 31.5 c.c's of clear secretion with a supernatant layer of thick, white mucoid material, possibly caused by swallowed

TABLE 5

ANIMAL 1

Time in minutes	Amount in c.c's	Appearance	pH
Resting secretions			
0 - 90	21	clear	1.5
90 - 120	13.5	clear	2
Stimulation by square wave modulation as before with an intensity of 12 volts, frequency of 20 per second and duration of 3 milliseconds.			
120 - 180	23.5	clear	1.5
180 - 240	x58	clear, mucus	7
240 - 300	21.5	clear	7
300 - 360	23	clear	7

saliva. No cardiac effects were ever observed.

Stimulation was carried out daily with very little changes in secretion. <u>On the thirteenth post-operative day</u>, no more signs of stimulation could be detected.

<u>On the fifteenth post-operative day</u>, the results shown in Table 6 on page 227 were obtained.

In view of the lack of results, the left cervical vagus of this animal was cut on the eighteenth post-operative day in order to see if afferent fibers might be interfering with the results of secretion. It is known that the distal part of a cut nerve will not function after four or five days.

<u>On the twenty-first post-operative day</u> - that is, three days after section of the cervical vagus - the results shown in Table 7 on page 228 were obtained.

<u>On the twenty-ninth post-operative day</u>, in view of the lack of change in gastric secretion, the receiver was removed. The receiver had no adhesions but the electrode foot was bound down by dense fibrous adhesions. On separation of these adhesions, the nerve in the foot was seen to be a necrotic mass.

TABLE 6

ANIMAL 1

Time in minutes	Amount in c.c's	Pepsin	pH
Resting secretions			
0 - 60	115(bile stained)		2.5
60 - 120	51	.132	1.15
Square wave modulation with an intensity of 12 volts, frequency of 30 per second and pulse duration of 3 milliseconds.			
120 - 180	22	.116	1.94
180 - 270	25	.112	1.95
Square wave modulation as above but with a frequency of 40 per second.			
270 - 330	19	.047	2.28
Resting secretion			
330 - 390	11		2.50

TABLE 7

ANIMAL 1

Time in minutes	Amount in c.c's	Pepsin	pH
Resting secretions			
0 - 60	44	.105	1.20
Square wave modulation with an intensity of 12 volts, frequency of 20 per second and a pulse duration of 12 milliseconds.			
60 - 120	12.5(bile stained preventing above determinations)		
Frequency changed to 30 per second			
120 - 180	4 (bile stained preventing above determinations)		
Frequency changed to 40 per second			
180 - 240	4 (bile stained preventing above determinations)		
Frequency changed to 50 per second and pulse duration to 5 milliseconds.			
240 - 300	5.5	.077	6.80

The capacity of the receiver on testing after removal was ten volts which indicated there was minimal loss of efficiency and that the receiver itself was effective after twenty-nine days in the animal. No pathological changes were observed in the gastro-intestinal tract.

b. Animal 2:

A fixed lead direct current type of receiver with a tested capacity of twelve volts with perforating electrodes was applied to the left thoracic vagus.

Stimulation of this dog for twenty-five days produced no appreciable changes in gastric secretion and no real signs of stimulation as seen in the salivation, restlessness and coughing of the previous dog. On removal of the receiver a similar picture was seen as before: The receiver still retained its original capacity, but the nerve in the electrode foot was necrotic and surrounded by fibrous adhesions. No pathological changes were observed in the gastro-intestinal tract.

c. Animal 3:

A flexible lead direct current type of receiver with a tested capacity of ten volts, with perforating electrodes, was applied to the left thoracic vagus.

This animal died on the second post-operative day before stimulation could be carried out. Autopsy showed massive pulmonary disease and empyema, which appeared to be of longer duration than two days.

d. Animal 4:

A flexible lead direct current type of receiver with a tested capacity of ten volts, with perforating electrodes, was applied to the left thoracic vagus.

This animal showed no response to stimulation at any time and was stimulated only six hours to see if there would be a response. In order to see whether the necrosis of the nerve was due to stimulation or compression by the electrode foot and damage by the perforating electrodes, this receiver was left in place without stimulation for forty-one days. On removal of the receiver, the usual fibrous adhesions were present around the electrode foot but the nerve itself, although showing the two small holes made by the perforating electrodes, was otherwise grossly normal. The receiver still retained its original capacity.

e. <u>Animal 5</u>:

A flexible lead, alternating current type of receiver, with a tested capacity of twelve volts, and with the agar-saline bridge type of electrode, was applied to the left thoracic vagus.

<u>On the fifth post-operative day</u>, stimulation of six volts intensity with a frequency of 20 per second and a pulse duration of 3 milliseconds, produced the usual signs of coughing, salivation and restlessness.

<u>On the tenth post-operative day</u>, the results shown in Table 8 on page 232 were obtained.

From the thirteenth post-operative day, no signs of stimulation were seen. Efforts were made daily to produce results until the sixteenth post-operative day when the receiver was removed. The receiver was still functioning normally and this nerve appeared grossly intact, although surrounded by the usual fibrous adhesions.

f. <u>Animal 6:</u>

A flexible lead, alternating current type of electrode, with a tested capacity of ten volts, and with the agar-saline bridge type of electrode, was applied to the left thoracic vagus.

<u>TABLE 8</u>

<u>ANIMAL 5</u>

Time in minutes	Amount in c.c's	Free HCL	Total HCL	Pepsin	pH
Resting secretions					
0 - 60	17				
60 - 120	25	20	35	.040	2.65
Square wave modulation with an intensity of ten volts, frequency of 20 per second and pulse duration of 1 millisecond.					
120 - 180	39	24	40	.032	2.40
180 - 240	28	18	39	.034	2.52

No response to stimulation was ever obtained and the receiver was removed on the tenth post-operative day and once again the receiver functioned normally and the nerve appeared grossly intact.

g. <u>Animal 7:</u>

A flexible lead, alternating current type of electrode, with a tested capacity of ten volts, and with the agar-saline bridge type of electrode, was applied to the left cervical vagus.

<u>On the third post-operative day</u> square wave modulation of two volts intensity, frequency of 20 per second and pulse duration of 1 millisecond produced coughing and restlessness. No cardiac effect could be accurately measured because of the coughing produced by stimulation. For the first time, the 1 revolution per minute timing motor driving a twelve contact wager switch was used, allowing square wave stimulation for twenty seconds only in each minute, thus giving the nerve an opportunity to recover from the stimulation.

<u>On the fifth post-operative day</u>, the results shown in Table 9 on page 234 were obtained.

<u>On the eleventh post-operative day</u>, for the first time induction coil modulation, as des-

TABLE 9

ANIMAL 7

Time in minutes	Amount in c.c's	Free HCL	Total HCL	Pepsin(k)	pH
Resting Secretions					
0 - 60	86(thin,turbid)				
60 - 120	25	118	130	.165	1.75
Square wave modulation with the timer on and an intensity of 8 volts, frequency of 20 per second and pulse duration of 1 millisecond.					
120 - 150	28	85	101	.208	1.82
150 - 180	21	77	91	.184	1.89
180 - 240	31				2.10
240 - 300	57				1.92
300 - 360	61	115	129	.192	1.62

cribed previously, was used for part of the stimulation period, and the results obtained are shown in Table 10 on page 236.

No excessive salivation was noted nor was any bile observed in the gastric secretion.

On the twelfth post-operative day, the animal was anaesthetized by intravenous nembutal and stimulation by the three above methods of stimulation produced cardiac slowing from 160 to 60 per minute. This effect can not be measured accurately on the unanaesthetized animal because of the coughing and restlessness produced by stimulation.

On the fourteenth post-operative day, the results shown in Table 11 on page 237 were obtained.

The animal still coughed on stimulation but as no changes in gastric secretion had yet been produced, on the fortieth post-operative day, a left thoracic sympathectomy as described before, was performed.

In order to ascertain the results of a longer period of stimulation this animal was stimulated for 96 hours by square wave modulation with an intensity of 8 volts, a frequency of 20 per

TABLE 10

ANIMAL 7

Time in minutes	Amount in c.c's	Free HCL	Total HCL	Pepsin(k)	pH
Resting Secretion					
0 - 60	40				
60 - 180	41	0	4		2.4
Square wave modulation with the timer on and an intensity of 12 volts, frequency of 20 per second and a pulse duration of 1 millisecond.					
180 - 210	0				
210 - 240	13(viscous)	0	11		3.8
Induction coil modulation with 25 interruptions per minute by the metronome and an intensity of 17 volts.					
240 - 255	6				
255 - 285	20	0	9	.046	6.57
285 - 315	60	0	5		
315 - 345	67(thick, mucoid)				6

TABLE 11

ANIMAL 7

Time in Minutes	Amount in c.c's	Free HCL	Total HCL	Pepsin(k)	pH
Resting Secretions					
0 - 30	15				
30 - 60	30				
60 - 90	39	85	95	.056	1.74
90 - 120	24	84	96	.054	1.71
120 - 150	19	91	103	.059	1.68
Induction coil modulation with 25 interruptions per minute by the metronome, intensity of 17 volts.					
150 - 180	20.5	79	95	.070	1.70
180 - 210	28	38	53	.047	1.45
210 - 240	19.5				
240 - 270	21	3	16	.019	3.61
270 - 300	18	8	24	.022	4.21

second and a pulse duration of 3 milliseconds. Sample gastric secretions for this period were not significant of vagal stimulation.

At the end of this time, 54 days after the original application of the receiver and after 121 hours of stimulation, autopsy was performed and the receiver removed. The receiver functioned effectively when tested and the nerve in the electrode foot appeared grossly and miscroscopically normal as shown in the figures on page 99 to page 101. Examination of the gastro-intestinal tract showed no signs of hyperemia nor ulceration:

h. Animal 8:

A left thoracic sympathectomy was performed and at the same time a flexible lead, alternating current type of receiver, with a tested capacity of twelve volts, and with the agar-saline bridge type of electrode, was applied to the left cervical vagus.

Before stimulation could be attempted, this animal died on the first post-operative day. Autopsy showed massive collapse of both lungs.

i. Animal 9:

A left thoracic sympathectomy was performed and at the same time a flexible lead, alternating current type of receiver, with a tested capacity of twelve volts, and with the agar-saline bridge type of electrode, was applied to the left cervical vagus.

Post-operatively a Horner's syndrome involving the left eye was present but no cough was elicited on stimulation. Cardiac effects were mildly present, slowing the heart from 160 to 120 per minute.

Stimulation in the next week produced no change in gastric secretion and no subjective signs of stimulation. This animal died on the tenth postoperative day. Autopsy showed moderate pulmonary disease. The receiver still functioned normally and the nerve in the electrode foot appeared grossly intact.

j. Animal 10:

A left thoracic sympathectomy was performed and at the same time a flexible lead, alternating current type of receiver, with a tested capacity of eight volts, and with the agar-saline bridge type of electrode, was applied to the left cervical vagus.

Stimulation of this animal produced no coughing, restlessness, cardiac slowing nor change in gastric secretion. This animal died on the tenth post-operative day, the autopsy showing signs of empyema and advanced pulmonary disease. The receiver was functioning normally and the nerve in the electrode foot appeared grossly intact.

CHAPTER IX

DISCUSSION OF RESULTS

The results obtained in all these experiments have been inconclusive. The chief purpose accomplished has been the construction of an apparatus which can function in the experimental animal for long periods of time and which can cause stimulation of the vagus nerve, as shown in the effects on the heart. The effects on gastric secretion still require more investigation and interpretation. The results will be discussed in the order in which the experimental procedures and findings have been described in the previous chapter.

A. A Study of the Spike Potentials of the Dog's Vagus

These results were very difficult to obtain, and even with the advice and aid of the Department of Physiology, the experiments carried out were not too satisfactory. In all cases stimulus artefact and alternating current interference distorted the appearance of the spike potential and rendered difficult the accurate measurement of results. Also, in two of the six experiments carried out, no spike potential could be elicited from the vagus nerves of five dogs studied, This was thought to be due to:

1. operative trauma in removing the nerve.

2. anoxia due to delay in removing the nerve or to improper oxygenation of the Ringer-Locke solution.

3. unsatisfactory action of the electrode chamber with its layer of paraffin oil over the Ringer-Locke solution. If the electrodes were not in the oil layer, electrolytic changes could occur, preventing the efficient functioning of the electrodes.

However, on the remaining four days, using the nerves from eight dogs, improved results were obtained. It was shown that the cervical vagus contained A, B and C fibers; that the upper thoracic vagus contained B and C, and possibly A fibers; and that the lower thoracic segment contained B and C fibers. These results agree with the study made of the cross-sections of the normal vagus nerve, as described in Section D, Chapter IV. The thoracic vagus shows B and C fibers by this method while the slides indicated that the majority of the fibers are fine and unmyelinated with only a few larger, myelinated fibers. In the cervical vagus, A, B and C fibers were found to be present, agreeing with the microscopic picture of a mixture of thick, heavily, myelinated fibers, medium sized fibers with thinner coats of myelin and some very fine unmyelinated fibers.

On the basis of this work, a strength-duration curve was calibrated for the upper thoracic portion of the vagus nerve. This showed that the optimum intensity of stimulus is approximately 4 to 11 volts with a pulse width of 0.2 to 2.35 milliseconds. This was in conflict with other opinions on the subject as many investigators using the induction coil method of vagal stimulation - with its necessary inaccuracies of calibration - believed that a strength of 1 to 2 milliamperes caused strong vagal stimulation. On the basis of the estimated resistance of 1000 ohms of the vagus, the optimal intensity would then be 4 to 11 milliamperes. These two values have been used consistently in our work, as has been shown in the previous chapter.

The fragility of the vagus nerve has been questioned and varying reports have been made. The manipulation required to apply the electrode foot to the vagus is as minimal as possible but the nerve necessarily is raised about 2 centimeters from its bed by indirect traction and there is some interference with its blood supply. The possibility therefore was considered that the lack of change in gastric secretion after stimulation was due to operative trauma interfering with the physiological properties of the nerve. This subject has been discussed by Jefferson, Phillips, Proffitt and Nicheles[78]. They considered the possibility that nervous shock from the handling and the sectioning of vagus fibers in vagotomy - even when all fibers are not cut - lead to atony of the stomach and loss of secretory function as well. They tested this in a series of controlled experiments in dogs. The left chest was opened and all visible vagus fibers were elevated gently with a smooth hook and separated for a distance of about 2 centimeters from the oesophagus. Following this, they were dropped and the incision was closed as usual. They did not believe that the manipulation of the vagus fibers destroyed their blood supply or their viability. In these animals, a profound dilatation of the stomach occurred just as seen following double vagotomy, lasting for five months after the operation when the animal was sacrificed. A large atonic stomach was found at autopsy and the vagus fibers were grossly intact. They also observed clinically and experimentally that in a number of incomplete vagotomies where the insulin test still indicated existence of vagus fibers to the stomach, atony of the stomach was present and the clinical results were good. They therefore believed that the beneficial effects which vagotomy may give are due to a profound nervous shock and atony of the stomach by which the ulcer pain disappears.

These results have not been proven experimentally by us but may be a factor in the lack of gastric secretion change in vagal stimulation, as the vagus is certainly manipulated as much in the application of the receiver. However, in the study of spike potentials, the upper thoracic vagus was stretched firmly after recording B and C fibers from it. On being stimulated again, the B spike potential had disappeared, while that of the C fibers persisted. This could not be tested for a sufficient length of time to determine if the C spike potential would also disappear. Also in the autopsies performed on the experimental animal after chronic vagal stimulation, atony of the stomach was not seen. A suitably exhaustive study of the spike potentials of the vagus nerve is probably necessary before any method of chronic vagal stimulation can be perfected. This study would require specialized knowledge and equipment that we do not possess at present in this laboratory.

B. The Influence of Vagal Stimulation on Gastric Motility

These results were complicated by the appearance on the recording drum of oscillations synonomous with respirations. Various efforts were made to control the rate of respiration without success. It was found that stimulation of the cervical vagus produced an increased amplitude of the oscillations and also an increased respiratory rate. Both of these findings ceased when stimulation was discontinued. Thus one must consider the possibility of the increased respiratory rate being directly due to vagal stimulation or as due to a secondary effect from increased gastric motility. These two possibilities could not be differentiated and no method of overcoming the problem was devised. Visualization of the stomach during vagal stimulation was attempted but the changes observed were not significant enough to conclude that gastric motility was directly increased by stimulation.

C. Acute Stimulation of the Cervical Vagus

In this series of experiments, an effort was made to determine if an increase in gastric secretion could be obtained by the classical induction coil method of stimulation used in this project. All methods of stimulation produced slowing of the heart rate.

Stimulation was carried out by the induction coil, with 22 interruptions per minute by the metronome, producing 6 volts as shown by the oscilloscope, using contact platinum electrodes on the left cervical vagus. Within an hour after stimulation, the gastric secretion collected in each 20 minute period doubled and then almost tripled the resting secretion obtained. There was a concomitant increase in free and total HCL, an increase in peptic activity and a fall to a more acid pH. When stimulation was stopped, there was an immediate decrease in the quantity of gastric secretion and a slightly slower fall in the free and total HCL and peptic activity and an increasingly less acid pH. The time lag before the increase in secretion was approximately forty minutes, slightly longer than that usually found. These results agree with the classical results obtained by this method[128].

Square wave modulation by our method, using the same intensity of stimulation and the same type of platinum contact electrodes, with varying frequencies and pulse durations, produced cardiac slowing but no change in gastric secretion.

Induction coil modulation with 25 interruptions per minute on the metronome, producing 17 volts on the oscilloscope, caused slowing of the heart as above but also caused a change in gastric secretion. This wave form was similar to that produced by the induction coil and was used in an attempt to duplicate the classical experiment of the induction coil method. These results were not as definitive as those caused by the induction coil method. The quantity of secretion was markedly increased within half an hour after stimulation and there was a moderate increase in the free and total HCL but the peptic activity and the pH showed no significant variations. After stimulation was discontinued there was a marked decrease in the amount of gastric secretion but the other values did not change appreciably. It will be thus seen that the induction coil modulation type of stimulation is not as efficient as the induction coil method above.

The lack of response to square wave modulation was difficult to explain as the same intensity of stimulus was used and cardiac slowing could be easily obtained. It is possible that the wave form itself is unsuitable for gastric stimulation but that it does suit the fibers to the heart. Varying frequencies and pulse durations did not produce any change in results.

A problem which complicates this type of procedure is whether to operate on the dog's stomach on the day of the operation and ligate both the oesophagus and pylorus, thus preventing the collection of other than gastric secretions. It was found more effective in this series of experiments to make the Pavlov fistula some days before the acute experiment and thus to leave the stomach undisturbed during the experiment. The more normal physiological state of the stomach thus obtained was more valuable then the occasional complication of regurgitation of bile and swallowing of saliva. However, regardless of what method is used, the use of anaesthesia and the operative trauma certainly do not provide a normal basis for the assessment of changes in gastric secretion due to vagal stimulation.

D. Chronic Stimulation of the Vagus Nerve

Although the effects of chronic vagal stimulation on gastric secretion and gastric morphology were not significant, the type of receiver used functioned well for periods up to 54 days in the intact animal. This receiver and its casing, as described in Chapter VII, can be fairly easily constructed within a few days and applied to any accessible nerve with the full expectation that stimulation will be produced. This has been proven in each of our experiments by the cardiac effects produced by cervical vagal stimulation. Thus this method of stimulation could doubtlessly be used on most nerves of the body when an accurately calibrated type of stimulation is required. The polythene and latex used in this construction do not cause unfavourable tissue reaction and they do make the casings resistant to all the actions of body fluids. Upon examination of the nerve after each type of electrode had been in position for relatively long periods of time, it was concluded that the agar-saline bridge type of electrode was the least traumatic and most efficient type of the electrodes tested. This is easily constructed and surrounds the nerve but does not cause actual pressure on it, as do the perforating and ring types of electrodes. The flexible lead decreases the traction on the nerve, allows application of the electrode in a much smaller area than that needed for the fixed lead receiver and has been shown to be strong and resistant to the actions of body bluids.

The types of currents and wave forms required have presented perplexing problems. As stated before square wave modulation, sine wave modulation and induction coil modulation were used, with first a direct current circuit and then an alternating current type of circuit in an effort to decrease polarization. A wide scale of stimulation could be used - a frequency of 2.5 to 70 per second, a pulse duration of 1, 3 or 10 milliseconds and an intensity up to 17 volts. On the basis of the tested 1000 ohms resistance of the vagus nerve, it has been estimated that each volt applied produces 1 milliampere of stimulation to the nerve. The timing motor was also used which allowed 20 seconds of stimulation in each minute. This was a further effort to decrease polarization and electrical damage to the nerve. The induction coil modulation produced a wave form exactly like that produced by the induction coil above, and each type was interrupted 22 or 25 times per minute by the metronome. Thus it can be appreciated that a wide variety of stimulation equipment was used and every effort made to overcome any error in the type of stimulation used, which could be causing the lack of change in gastric secretion.

If change in gastric secretion had been consistently obtained, the Pavlov gastric fistula would have been replaced by a Pavlov or Dragstedt type of gastric pouch, which necessarily gives a clearer picture of the changes in true gastric section. It was also planned to stimulate the animal for approximately eight hours a day - the usual daily time the average person spends in contact with his business environment - at the optimal intensity, frequency and duration of stimulation and to observe any changes in gastric morphology. Since the initial constant change in gastric secretion could not be obtained, this was not carried out. However, Dr. R. Smith, who studied the effects on the heart of the same type of stimulation of the cervical vagus, found no change in the gastro-intestinal tract at autopsy after stimulation for twenty-two hours a day for three weeks. This confirmed the necessity of producing some change in gastric secretion before attempting prolonged stimulation.

In most of the animals tested, stimulation was indicated by coughing, restlessness, salivation and in some cases, by actual vomiting. This was true for both the cervical and thoracic vagi but was more easily demonstrated in the cervical vagus. Also, in the cervical vagus, cardiac slowing could routinely be demonstrated although in some cases anaesthesia was required before this could be accurately measured, since coughing and restlessness due to the stimulation obscured the sound of the heart.

From the results listed in the previous chapter, the lack of any significant gastric response to stimulation can be seen. On several occasions, a moderate increase in gastric secretion - up to three or four times the resting amount - and thick, mucoid in appearance was seen, but there was no corresponding increase in free and total HCL and peptic activity as should be caused by vagal stimulation. In fact, in most of these cases, the pH rose from 2 or 3 to a level of 6 or 7 as the amount of secretion increased. Two possibilities were considered as causes of these unusual increases in secretions. The first and most likely is that the increase was due to swallowed saliva which also caused the change to a less acid pH. The other possibility is that this is the type of response Vineberg[127] described to weak vagal stimulation i.e. a more alkaline, mucoid type of secretion. No decision could definitely be made as to the cause of this response.

In most cases, the gastric secretions followed no constant response to stimulation - the gastric analysis showing no clear rise or fall in values. It has been shown in acute experiments that both the induction coil and induction coil modulation produced an increase in secretions. However, in chronic animals using the identical method of induction coil modulation, no similar change could be obtained. The only differences in these cases were that in the acute experiment, the vagus nerve had been cut and the central end of the distal segment was being stimulated in an anaesthetized animal.

Left thoracic sympathectomy produced no changes in any of the experimental animals in respect to their response to vagal stimulation. It had been thought that if inhibitory fibers in the sympathetic nerves could be excised, there would be more of a chance of obtaining gastric secretion from vagal stimulation.

In all these results, some unknown factor is playing an important part. The method used produces the typical cardiac response to vagal stimulation but can not produce the expected gastric response. Since the nerve is stimulated above both these organs, there must be some block or some change in physiological response of the nerve fibers between the heart and the stomach. It has been shown that most of the fibers in the thoracic vagus are unmyelinated whereas a good number of the cervical vagal fibers are myelinated. It is thus possible that these stronger, myelinated fibers carry the impulses to the heart but that the weaker, unmyelinated fibers are unable to carry the stimulated impulses to the stomach. The possibility of operative trauma or polarization interfering with normal nerve conduction must be considered, even though all efforts were taken to overcome these factors. In the acute experiments where change of gastric secretions has been produced, the nerve had been cut before stimulation. This is impossible to achieve in a chronic animal because of the speed of nerve degeneration but there is a possibility that afferent fibers produce a central change which inhibits gastric secretion.

Considerable emphasis has been placed on the electrical properties of the vagus nerve, as it is very probable that the best approach to this problem is through a detailed neurophyicological study of the isolated and intact vagus nerves.

It can be seen that the problem of chronic vagal stimulation, especially with respect to gastric secretion, is very complex and will require most thorough and exhaustive study in all its branches before success can be achieved.

CHAPTER X

SUMMARY AND CONCLUSIONS

1. The history of electrical stimulation has been reviewed, with particular emphasis on the role of radiofrequency.

2. The principles of nerve physiology have been briefly discussed, including the types of nerve fibers and spike potentials.

3. Gross and microscopic anatomy of the vagus nerve of the dog, with reference to some functional properties of the vagus fiber groups, have been described.

4. Cross-sections of the normal vagus nerve of the dog have been shown which indicate that the cervical vagus is divided into two bundles. One smaller bundle is composed of medium-sized fibers which are very uniform in diameter with a few larger myelinated fibers. The larger bundle shows great variety of fiber diameter - including thick, heavily myelinated fibers, medium-sized fibers with thinner coats of myelin and some very fine, practically unmyelinated

fibers. The thoracic vagus is composed almost exclusively of very fine, practically unmyelinated fibers 1 to 2 microns in diameter with only a few larger myelinated fibers.

5. Cross-sections of the cervical vagus nerve of a dog after 121 hours of stimulation show that there is a moderate perineural fibrosis but otherwise the nerve appears completely normal.

6. The physiology of gastric secretion, with reference to the roles of anaesthesia and the autonomic nervous system, has been discussed.

7. The methods previously employed to produce gastric secretion by vagal stimulation have been described, with reference to the experimental work done on vagotomy.

8. The construction of a receiver has been described which consists essentially of three interlocked tuned circuits, one in each plane, with crystal diodes as detectors, and a by-pass condenser as a radiofrequency filter. The final receiver of choice

had an alternating current circuit, made by introducing a 20 microfarad condenser in series with the electrodes to prevent flow of direct current though the nerve and a shunting resistor (4000 ohms) was introduced to allow the condenser to discharge after each pulse. This receiver had an estimated capacity of 25 to 30 volts.

9. The construction of a receiver casing has been described, made by the extrusion moulding of two polythene half-spheres, one being fitted with a hollow boss.

10. The construction of a fixed and flexible lead and the use of perforating, ring and agar-saline bridge types of platinum electrodes have been discussed. The final receiver was equipped with a flexible lead of many strands of wire, reinforced by polyethylene tubing and latex, and connected to the agar-saline bridge type of electrode in a polythene electrode foot.

11. Construction of a suitable transmitter and primary coil has been described to stimulate the receiver used in these experiments.

12. Studies of the spike potentials of the dog's vagus have been made. It was shown that the nerve is a complex one and that the cervical portion contains A, B and C fibers; the upper thoracic portion B and C, and possibly A, fibers; and the lower thoracic portion B and C fibers. A strength-duration curve of the upper thoracic vagus indicated that the optimal stimulus is approximately 4 to 11 volts with a pulse duration of 0.2 to 2.35 milliseconds. Stretching the upper thoracic segment showed that the B fibers were more readily damaged than the C fibers.

13. The influence of vagal stimulation on gastric motility has been studied but the influence of respiration interfered with the recording of gastric motility. With stimulation, there was a rise in pressure above the baseline of the recording drum, an increase in the amplitude of the record and an increase in respiratory rate from 40 to 50 per minute to 80 or 90 per minute.

14. Acute stimulation of the cut cervical vagus nerve has been carried out in six dogs. An increase in quantity, free and total HCL and peptic activity

of the secretions was obtained by stimulation with the induction coil method and by induction coil modulation of the transmitter. No response was obtained to square wave modulation stimulation at varying frequencies, intensities and pulse durations.

15. Chronic stimulation of the vagus nerve has been carried out in ten healthy dogs. The receiver still functioned effectively after a maximum of 54 days in the experimental animal. Necrosis of the nerve occurred in the electrode foot in some of the earlier animals but was overcome by the use of an agar-saline bridge type of electrode, alternating current circuit and the use of a timing motor in the transmitter unit. The receivers were first placed on the thoracic and then the cervical vagus nerves. In both cases, stimulation produced coughing, restlessness, salivation and occasionally vomiting while on the cervical vagus, stimulation produced marked slowing of the heart rate. No consistent changes were observed in gastric secretion and no pathological changes occurred in the gastrointestinal tract after a maximum of 121 hours of stimulation. Left thoracic sympathectomy produced no changes in the response of these dogs to stimulation.

16. A discussion of the results obtained has been presented. The possible reasons for the lack of response to gastric stimulation, when cardiac effects are so easily secured, have been discussed.

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