1968

Sympathetic Outflows from Cervical Spinal Cord in the Dog

Gary Gene Wiesman
Loyola University Chicago

Follow this and additional works at: https://ecommons.luc.edu/luc_theses

Part of the [Medicine and Health Sciences Commons](#)

Recommended Citation

Wiesman, Gary Gene, "Sympathetic Outflows from Cervical Spinal Cord in the Dog" (1968). *Master's Theses*. 2255.

https://ecommons.luc.edu/luc_theses/2255

This Thesis is brought to you for free and open access by the Theses and Dissertations at Loyola eCommons. It has been accepted for inclusion in Master's Theses by an authorized administrator of Loyola eCommons. For more information, please contact ecommons@luc.edu.

This work is licensed under a [Creative Commons Attribution-NonCommercial-No Derivative Works 3.0 License](#).
Copyright © 1968 Gary Gene Wiesman

**SYMPATHETIC OUTFLOWS FROM
CERVICAL SPINAL CORD IN
THE DOG**

**by
Gary Gene Wiesman**

**A Thesis Submitted to the Faculty of the Graduate School
of Loyola University in Partial Fulfillment of
the Requirements for the Degree of
Master of Science**

February

1968

BIOGRAPHY

Gary Gene Wiesman was born on October 7, 1942, in Granite City, Illinois.

He was graduated from the Granite City Senior High School in June, 1960. He attended Washington University, St. Louis, Missouri, and was awarded the Bachelor of Arts degree in June, 1963.

In September, 1963, he began his graduate studies in the Department of Anatomy, Loyola University, Stritch School of Medicine, Chicago, Illinois. He was the recipient of a Loyola University Graduate Student Teaching Assistantship in the Department of Anatomy, and his research was supported by an NIH Grant and Fellowship.

In September, 1964, he began studies in Loyola University, Stritch School of Medicine, and will receive his M.D. in June, 1968.

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to Dr. David S. Jones, Professor of Anatomy, and to Dr. Walter C. Randall, Chairman, Department of Physiology, for their guiding supervision and constructive criticism, patience, and enthusiastic support throughout this investigation.

My thanks are also due to Dr. Leslie A. Emmert, Assistant Professor of Anatomy, for his thoughtful criticisms and suggestions on technical problems.

I also would like to express my appreciation to Dr. Jose V. Kannankiul, Research Associate in Anatomy, for his encouragement and generosity of time, without whose counsel this undertaking would have been considerably more difficult.

Appreciation is also extended to Mrs. Lucia Smelte, Senior Technician, Department of Anatomy, for her advice concerning tissue preparation, and to Mr. John Maurer, Medical School Photographer, for his expert advice concerning photomicrography.

The study was supported in part by a National Institutes of Health research grant HE08682, and by grants administered by Dr. Walter C. Randall.

TABLE OF CONTENTS

	Page
I. INTRODUCTORY LITERATURE REVIEW	1
II. METHODS AND MATERIALS	25
III. RESULTS	31
I. Cardiovascular response to electrical stimulation of ventral roots of cervical cord	
II. Histological analysis of cervical anterior roots	
IV. DISCUSSION	48
V. SUMMARY AND CONCLUSION	52
VI. BIBLIOGRAPHY	53

LIST OF TABLES AND FIGURES

Table		Page
I.	TABULATION OF EFFECTS OF ELECTRICAL STIMULATION OF CERVICAL VENTRAL ROOTS C-5-C-8 ON HEART RATE, VOLUME PULSE, AND BLOOD PRESSURE IN THE DOG	41
II.	RESULTS OF DIRECT COUNTING FROM STAINED SECTIONS OF CERVICAL ROOTS FOR PREGANGLIONIC SYMPATHETIC FIBERS .	46

Figure

1.	ANIMAL NUMBER 9: RESULTS OF STIMULATION OF VENTRAL ROOTS OF CERVICAL NERVES C-5, C-6, C-7, AND C-8	33
2.	ANIMAL NUMBER 8: RESULTS OF THE STIMULATION OF VENTRAL ROOT OF THE EIGHTH CERVICAL NERVE AFTER TRANSECTION OF THE SPINAL CORD AT C-8-T-1	35
3.	ANIMAL NUMBER 9: RESULTS OF THE STIMULATION OF VENTRAL ROOT OF THE EIGHTH CERVICAL NERVE AFTER TRANSECTION OF THE SPINAL CORD AT C-8-T-1	36
4.	ANIMAL NUMBER 9: RESULTS OF STIMULATION OF VENTRAL ROOT OF C-6, C-7, AND C-8 CERVICAL NERVES	38
5.	ANIMAL NUMBER 13: RESULTS OF STIMULATION OF VENTRAL ROOT OF EIGHTH CERVICAL NERVE	39
6.	ANIMAL NUMBER 8: CROSS SECTION OF VENTRAL ROOT C-7 FROM EXPERIMENTAL ANIMAL OF FIGURE 1	43
7.	ANIMAL NUMBER 8: CROSS SECTION OF VENTRAL ROOT T-1 FROM EXPERIMENTAL ANIMAL OF FIGURE 1	45

INTRODUCTORY LITERATURE REVIEW

The earliest anatomical description of any part of the autonomic nervous system probably dates back to Galen's account of the nerve trunk lying along the necks of the ribs receiving fibers from thoracic and lumbar portions of the spinal cord. A decisive account of the anatomical and physiological relationships of the ANS was not pointed out until Gaskell (1886)²⁵ gave an account of the white communicating rami and their distribution. He pointed out that the efferent fibers in these rami arise in the spinal cord in cell columns which are interrupted by the development of the nerves to the limbs and that corresponding fibers also occur in certain of the cranial nerves. There exist also three outflows of medullated nerve fibers of small caliber, the bulbar, the thoraco-lumbar and the sacral, through which the peripherally located efferent ganglion cells are connected with the CNS. Gaskell (1889) also traced fibers in the rami communicantes and roots of the spinal nerves demonstrating the segmental nature of nerves to the periphery.²⁶ From the above observations Gaskell concluded that the autonomic outflow consists of a two neuron pathway system, the white

communicating rami containing the first part of the pathway and the rami communicantes the second part. In his later work, Gaskell (1916)²⁷ used the term "involuntary nervous system" to designate those efferent neurons located outside the CNS which supply fibers to involuntary structures.

Langley and Dickinson (1890)⁴⁴ discovered in the action of nicotine on the ganglia a new method of investigating the relationships of nerve fibers to peripheral ganglion cells. They demonstrated that nicotine injections stopped sweating and skin pallor produced by stimulation of rami coming from the cord, but the effect of stimulation of peripheral ramification of sympathetic chain were not affected by nicotine, therefore substantiating Gaskell's concept of a two neuron system in the ANS. The results obtained by the use of this method led Langley (1921)⁴⁶ to propose a new term, "autonomic nervous system", for the system of nerves in question.

When the term "autonomic" was introduced by Langley, it was well known that the thoracolumbar outflow through the sympathetic trunks supplies fibers to all parts of the body, while the cranial and sacral outflows supply fibers only to certain parts of the body. Also, functional effects induced by activation of thoracolumbar outflow, in general, were the opposite of those of the cranial and sacral outflows. Langley, therefore, regarded the thoracolumbar outflow as a system distinct from the rest of the autonomic nerves. Also, he regarded the thoracic

outflow supplying the eye as distinct from the cranial part of the outflow, which with the sacral outflow constitutes a system that innervates the alimentary canal and parts developmentally connected with it. He then divided the autonomic nervous system into tectal, bulbosacral and sympathetic system. Following the discovery that effects produced by adrenalin were similar to those produced by stimulation of sympathetic nerves, and that certain other drugs produced effects similar to those produced by stimulation of tectal and bulbosacral nerve, he grouped the tectal and bulbosacral autonomic nerves together as the "parasympathetic" system (1921).⁴⁶ Langley's classification of the nerves into autonomic, sympathetic and parasympathetic have been retained in present nomenclature.

By 1858, many anatomical and physiologic studies had been accomplished which lead to Claude Bernard's discovery of the vasomotor function of the sympathetic nerves to the blood vessels. These early studies of the vasomotor nerves gave rise to the concept of a universal vasoconstrictor action of sympathetic nerve fibers. Bernard also demonstrated the existence of vasodilator nerves by stimulating the chorda tympani resulting in dilatation of the arteries supplying the submandibular gland. On the basis of extensive physiological data, Bernard advanced the hypothesis that all sympathetic reflexes are mediated through the spinal cord and also demonstrated the existence of centers in the brain stem which on stimulation discharge impulses that are conducted

through sympathetic nerves. The search for higher centers which exert their influence through the autonomic nerves as well as interest in determining the nervous mechanisms operating in vasomotor and secretory control was thus initiated.

The anatomic distribution of peripheral sympathetics remained to be described for a thorough understanding of vasomotor control of blood vessels. The innervation of the blood vessels found by Bayliss and Bradford (1894)⁵ included efferent and afferent nerve fibers. The efferent nerves include both vasoconstrictor and vasodilator of which the former are chiefly sympathetic. The latter include both sympathetic and parasympathetic components. The existence of vasodilator fibers in sympathetic nerves has been demonstrated in experiments with animals and man. Lewis and Pickering (1932)⁴⁸ in experiments on human subjects demonstrated vasodilatation by heating the subject in a warming chamber arranged so that the head and hands were unexposed and consequent warming of the body resulted in warming of the hands. The vasodilatation involved in the warming of the hands, in these experiments, was effected through the sympathetic nerves as indicated by the fact that warming of the body did not elicit similar responses in hands deprived of their sympathetic innervation.

The action of the vasodilator nerves are studied on the premise that vasoconstriction and vasodilatation are mediated by different neurohormones. In general, the vasoconstrictor nerves

are adrenergic. The cholinergic nature of the vasodilator fibers in the muscles of the hind limb has been demonstrated in the cat²⁰ and in the dog²¹ by Folkow. According to Folkow, in the dog and cat the cutaneous nerves, except in limited areas, probably include no vasodilator fibers. A general distribution of vasodilator fibers in the cutaneous nerves in man has not been demonstrated. Constricting the cutaneous blood vessels with adrenalin, as reported by Barcroft et al (1947)³ did not materially affect the increase in blood flow in the forearm caused by warming the body. The increased flow, therefore, must have taken place in the muscles. This response was abolished by sympathetic denervation of the extremity. It must, therefore, be mediated through vasodilator reflexes.

Langley (1877)⁴³ did many "secretory" experiments in which he stimulated the "sympathetics" and the chorda tympani which led to vasomotor studies in the feet of dogs, cats, and rabbits, defining for the first time levels of vasomotor outflow. Later Bayliss and Bradford (1894)⁵ reported a study on the innervation of blood vessels using the fore and hind limbs of the dog. Using a volume plethysmograph to record vascular changes, he stimulated the peripheral ends of anterior and posterior roots of thoracic and lumbar nerves. Through this method he was able to define physiologic levels of vasomotor outflow tracts. However, since he stimulated both posterior and anterior roots he was probably also getting concomitant posterior root vasodilatation through

antidromic excitation. Bayliss also demonstrated pronounced reflex vasoconstriction of the hind limb upon stimulation of the central end of the sciatic nerve. Also, a pronounced pressor effect with passive dilatation of the hind limb during the excitation of the central end of the divided splanchnic nerves occurred; therefore, emphasizing the fact that one should be cautious in interpreting vasomotor phenomenon as a manifestation of direct efferent excitation of peripheral sympathetic outflow.

The embryologic development of the autonomic ganglia and neurons have been studied extensively. The primordia of the autonomic ganglia arise early in embryonic development and are related developmentally to the cerebrospinal nervous system. Most of the early investigators supported the theory that cells which make up the primordia of the ganglia of the sympathetic trunks and prevertebral plexuses are derived exclusively from the spinal ganglia and neural crests. Kuntz (1909a)³⁷ traced cells of medullary origin into the primordia of the sympathetic trunks via the ventral nerve roots and the communicating rami and advanced the opinion that the sympathetic ganglion cells are derived chiefly from the neural tube. He also demonstrated that autonomic ganglia bear the same histogenetic relationship to the cerebrospinal nervous system in all classes of vertebrates. The displacement of cells from the neural tube into the primordia of the autonomic ganglia both in the trunk and the head regions has been confirmed by numerous investigators. Jones (1937)³³ has demonstrated in

chicks that only cells of medullary origin become differentiated into autonomic ganglion cells and that those which become sympathetic and parasympathetic ganglion cells respectively are displaced along the efferent roots of the nerves that convey the corresponding preganglionic outflows. This point of view that the ganglion cells present chiefly or exclusively cells that are displaced from the neural tube along the paths of ventral spinal nerve roots has been supported particularly by Kuntz and Batson (1920)³⁸ and Brizzie and Kuntz (1950)⁹.

The primordia of the ganglia of the sympathetic trunks appear earliest in the caudal thoracic and the rostral abdominal regions. They are composed of aggregates of cells of neural origin lying along the dorsolateral aspects of the aorta. The segmental character of the sympathetic primordia gradually becomes apparent, as development advances, and the cell aggregates become connected by longitudinal fibers. The primordia of the sympathetic trunks arise somewhat later in the cervical than in the thoracic region. In humans the primordia of the sympathetic trunks grow cephalad in the cervical region both by the displacement of cells along the dorsal aspects of the descending aortae and by cell proliferation. When segmentation of these columns takes place, delimitation of the cervical sympathetic ganglia results. The question of development of autonomic ganglion from neural crest was studied by many experimenters and Sturdel (1952)⁶¹ traced cells from the neural tube along ventral nerve roots into

the sympathetic trunk primordia in chick embryos in which the neural crests had been completely excised in a series of segments. There was no evidence of neural crest derivatives in the segments in question. It was concluded that sympathetic trunks may arise in complete absence of cells derived from the neural crest or the spinal ganglia and that cells of neural origin in the sympathetic primordia must be derived exclusively from the neural tube along the ventral spinal nerve roots. The fact that such primordia do not arise in segments in which the portion of the mantle layer that gives rise to the intermediolateral cell column is destroyed, suggests that cells which enter the sympathetic primordia via the ventral nerve roots, under normal conditions, are derived mainly from this portion of the neural tube. Brizzie (1949)¹⁰ demonstrated that neuroglial cells in the sympathetic trunk ganglia are derived partly from the neural crest via the dorsal roots and in part from the neural tube via the ventral nerve roots and felt therefore that cells of neural crest origin that enter the sympathetic primordia can be accounted for without the assumption that any of them become differentiated into ganglion cells. Schaper (1897)⁵⁸ in a critical study of cell differentiation in the CNS, pointed out that the cells which arise by the mitotic division of the "germinal" cell in the ependymal layer do not all become neuroblasts. He described them as "indifferent" cells, some of which become differentiated into neurons and others into neuroglia. He also pointed out that in the higher vertebrae, many of

the indifferent cells retain their capacity for further differentiation by mitotic division and give rise to daughter cells of the same indifferent type which may become differentiated either into neurons or into supporting cells. It may be assumed, therefore, that many of the cells that become differentiated into neurons in the autonomic ganglion arise by the mitotic division of migrant cells either before or after they have become incorporated in the primordia of these ganglion. The neurons in the autonomic system, consequently, may be regarded as homologous with the neurons in the cerebrospinal nervous system. However, the question of origin of autonomic ganglion from neural crest or neural tube has not yet been settled.

The first reference to the possibility that chemical substances might be concerned in the transmission of excitation from the motor-nerve terminals to effector cells dates back to the turn of the century when Lweandowsky (1898)⁴⁷ and Langley (1901)⁴⁵ noted independently the similarity between the effects of injection of extracts of the adrenal gland and stimulation of sympathetic nerves. The brilliant researches of Otto Loewi (1921)⁴⁹ established the first real proof of the chemical mediation of nerve impulses by the peripheral release of specific chemical agents. He stimulated the vagus nerves of a perfused (donor) frog heart and allowed the perfusion fluid to come in contact with a second (recipient) frog heart used as a test object. A substance was liberated from the first organ that slowed the rate

of the second which he called "Vagusstoff" ("vagus substance", parasympathin) which was subsequently identified as acetylcholine. He also noted that an accelerator substance similar to epinephrine was liberated into the perfusion fluid when the action of the sympathetic fibers in the frog's vagus predominated over that of the inhibitory fibers. Many subsequent investigators established the fact that stimulation of craniosacral autonomic nerves to smooth and cardiac muscle and to exocrine glands resulted in the release of a chemical substance, acetylcholine, that acted upon effector cells to elicit their characteristic responses.

In addition to its role as the neurohumeral transmitter of all postganglionic parasympathetic fibers and a few postganglionic sympathetic fibers, such as those to the sweat glands (Dale and Feldberg, 1934)¹⁵ and the sympathetic vasodilator fibers (Evans, 1954)⁶², acetylcholine has been shown to have this transmitter function in three additional classes of nerves:

1) preganglionic fibers of both sympathetic (Perry and Talesnik, 1953)⁵¹ systems; 2) motor nerves to skeletal muscle (Dale et al, 1936)¹⁶, and 3) certain neurons within the CNS (Feldberg, 1950)¹⁹.

Just as there are biological indicators for the presence of acetylcholine, there are specific tests for detecting "sympathin", such as acceleration of the rate of the denervated heart, elevation of blood pressure, piloerection, inhibition of intestinal musculature and relaxation of the denervated non-pregnant

cat uterus. With such tests it has been repeatedly demonstrated that when postganglionic sympathetic nerves are stimulated there is released at the neuroeffector junctions a chemical substance that has sympathomimetic properties. For example, Bacq (1931)² found that stimulation of the cervical sympathetic nerves caused the mediator to appear in the aqueous humor of the eye. Barger and Dale (1910)⁴ noted that the effects of sympathetic nerve stimulation were more closely reproduced by the injection of sympathomimetic primary amines than by that of epinephrine or other secondary amines. Euler (1946)¹⁸ found that the sympathomimetic substance in highly purified extracts of sympathetic nerves and effector organs bore a strong resemblance to norepinephrine by all criteria used. He proposed that the sympathetic transmitter is norepinephrine and that sympathetic nerve stimulation on some occasions may, in addition, liberate small quantities of epinephrine itself.

With the advent of neurosurgical techniques, much interest was placed on the anatomical relationship of sympathetic outflow to its function. In order to insure permanent physiological results by sympathetic denervation, the surgical procedure must be anatomically complete and carried out in such a way that regeneration cannot take place. The question of vasomotor control was of prime importance in the rationale of sympathectomies in certain disease processes and much data on the vasomotor outflow system comes from experimental sympathetic stimulations done on

dogs and cats. In 1932, Burn¹³, upon stimulating the lumbar sympathetic chain of the dog while the hind limbs were perfused with defibrinated blood, found only a negligible response. However, with the addition of adrenaline to the perfusate, the response to sympathetic stimulation became significant and was primarily one of dilatation and occasionally constriction. He also observed often an initial vasodilatation converting to vasoconstriction. These vasodilator responses to the stimulation of the sympathetic chain in the dog were obtained without the use of adrenergic blocking agents, and suggested the existence of a generalized system of vasodilator fibers. These sympathetic vasodilator fibers were demonstrated by Bulbring and Burn¹² to be cholinergic and occurred in the muscles. The dilator effects were augmented by eserine and abolished by atropine. By volume plethysmographic recordings of the skinned hind limb of the dog they demonstrated a dual innervation of dog muscle, both constrictor and dilator. Folkow and co-workers²² in 1949 while working on the sympathetic vasomotor innervation of the skin of the dog found no evidence for the existence of vasodilator fibers to the skin of the dog hind limb. In these experiments only marked vasoconstriction was noted in the skin during sympathetic stimulation. Green³⁰, in a study of the autonomic control of blood flow in the hind paw of the dog, stimulated the lumbar sympathetic chain and via differential blockade of possible adrenergic and cholinergic mechanisms operating in the foot pad concluded that the paw skin

contains abundant alpha constrictor receptors, but very few if any adrenergic or cholinergic dilator receptors.

The question of autonomic outflow pathways in the vasomotor control of peripheral blood vessels has been of great interest to investigators primarily in the field of peripheral vascular surgery. Cox and Randall (1951)¹⁴ investigated pre- and postganglionic outflow of the sympathetic trunk to the foot pad of the dog using a photoelectric plethysmograph of Hertzman. In their investigation they demonstrated a great variability in the level of preganglionic inflow to the sympathetic trunk.

Surgical intervention has been carried out not only on lumbar sympathetics but on nearly all parts of the sympathetic system. Surgery involving the sympathetic trunk has usually consisted primarily of extirpation of one or more ganglia with the intervening internodes. Equally complete functional sympathetic denervation should be obtained by section of the preganglionic fibers alone, leaving the ganglion and the sympathetic roots intact. Preganglionic sympathectomy of this kind is used by various surgeons in order to avoid sensitization of the sympathetically denervated structures to adrenin in the circulating blood. However, contrary to current teaching, not all peripheral sympathetic conduction pathways traverse the sympathetic trunk. Wrote (1941)⁶⁴ in his description of communicating rami described groups of ganglion cells located in communicating rami and sympathetic roots in the lumbar region, and Skoog (1947)⁶⁰

described ganglia in the communicating rami of the cervical sympathetic trunk. Most of the ganglia reported have been located in relation to sympathetic roots and occasionally have been observed adjacent to spinal nerves near the origin of the communicating rami. Both Wreth and Skoog have suggested the possibility that in some instances ganglion cells located in relation to a spinal nerve may be synaptically related to preganglionic fibers that reach them through the ventral root of the nerve and in turn send their axons distalward in its peripheral rami. Such anatomical relationships of ganglionic and preganglionic neurons would result in peripheral sympathetic conduction pathways which do not traverse the sympathetic trunk. Therefore, conduction pathways formed in this manner are not interrupted by either extirpation of the corresponding segments of the sympathetic trunk or section of the communicating rami. Alexander et al (1949)¹ have observed aggregates of ganglion cells which may be called accessory sympathetic ganglion, located adjacent to spinal nerves or partially or completely embedded in nerve trunks, usually near the site of origin of the communicating ramus. Accessory ganglia have also been observed in communicating rami, usually near a sympathetic trunk ganglion, in sympathetic roots and in rami that interconnect spinal nerves. An accessory ganglion located in a sympathetic root probably receives only preganglionic fibers that pass through sympathetic trunks. The postganglionic fibers that arise in it join the spinal nerve. Conduction pathways formed in

this manner, since they traverse the sympathetic trunk, do not differ essentially from those with synaptic connections in sympathetic trunk ganglia. Therefore, the assumption that the accessory ganglia, which are intimately associated with spinal nerve trunks, are sufficiently numerous and of ample magnitude to account for a relatively large number of sympathetic conduction pathways not traversing the sympathetic trunk seems plausible. These accessory ganglia probably subserve much of the residual sympathetic activity that has been demonstrated in the corresponding dermatomes following extirpation of the sympathetic trunk.

In cervical sympathectomies the assumption was made that the first thoracic nerve played no significant part in the sympathetic innervation of the upper extremity. This assumption was supported by Gehegan et al (1942)²⁸ who found no preganglionic fibers for the hand in the ventral roots rostral to the first thoracic in monkeys and third thoracic in the cat. Ray et al (1943)³⁶ studied changes in cutaneous resistance during stimulation of ventral nerve roots of human subjects and reported preganglionic fibers for the hand present in the second to fifth thoracic nerves. Kuntz et al (1938)³⁹ found in stimulation of ventral roots of the first thoracic nerve vasoconstriction in the distal parts of the limb and activation of the sweat glands in the paw pads. However, the determination of preganglionic sympathetic outflow to the upper extremity remained

controversial. Sheehan and Pick (1943)⁵⁹ reported on the connections and constitution of the rami communicantes in the Rhesus monkey. In this study, four general types of sympathetic branches were differentiated according to their fiber content. Type I represents white rami, Types IIA and IIB, two varieties of grey rami, differing from each other according to the number of fine medullated fibers contained within each, and Type III which included the truly mixed rami. The fine myelinated fibers (under 3 micron diameter) which were present to some extent in all grey rami, occurred in particularly large numbers in the grey rami of C-8 and T-1 spinal nerves. Their abundance above the thoracolumbar outflow stimulated further research to determine if a similar arrangement existed in man. Kirgis and Kuntz (1942)³⁶ reviewed the topographical anatomy of the human sympathetic chain and primarily the cervical region but there had been few complete histological investigations reported as to the highest level of sympathetic outflow.

The first study of the constitution of the human sympathetic rami, following the discovery of the thoracolumbar outflow, was made by Harmon (1898-1900)³¹. Using the osmic acid stain on teased preparations he examined only the rami of C-5 to T-4 and T-11 to L-4 inclusively. In the rami of C-5 through C-8 and again at L-3 to L-5 (i.e., above and below the levels of sympathetic outflow) he observed myelinated fibers "under 4 microns in diameter" but never more than 30 at any one spinal

level. Juba (1930)³⁴ using Weigert stained material gave one of the few complete descriptions of the constitution of the various rami of a sympathetic branch examined in man. The wide distribution of medullated fibers, many of the smaller caliber, within the rami of the cervical and lower lumbar nerves was very evident. The rami of the upper cervical regions were noted to contain many finely myelinated fibers. Pick and Sheehan (1946)²⁹ studied the sympathetic rami in man and observed frequent occurrence of Type B fibers (grey rami heavily "peppered" throughout with small medullated fibers under 3 micron diameter and several hundred in number) in the rami of C-7 and C-8 and the vertebral nerve and again in the rami of L-4, L-5 and S-1, signifying the relative abundance at these levels of fine myelinated fibers (under 3 micron in diameter). The fact that a similar distribution of this type of grey rami exists in the cat and monkey, indicates some fundamental pattern, but what its significance may be is not yet clear. The nerves studied were distributed particularly to the blood vessels and sweat glands in the hand and foot, which are notoriously rich in their sympathetic innervation. They concluded that the number of finely myelinated fibers (under 3 microns in diameter) within grey rami have been greatly underestimated.

Regarding the upper extremity, in relation to residual sympathetic pathways, there are differences of opinion as to what constitutes a complete denervation. The role of the first

thoracic ventral root is unsettled and Geohegan and Aidor (1942)²⁹ have demonstrated that a functional reorganization may take place, resulting in sympathetic activity through roots that previously showed no evidence of activity. Van Buskirk (1941)⁶³ has described, in human embryos, sympathetic fibers which traverse as many as six segments via nerves in the vertebral canal and Kuntz and Dillon (1942)⁴⁰ found evidence of sympathetic activity after sympathectomy, which included stellate ganglionectomy, attributing residual activity to these fibers. Ray and Console (1948)⁵⁵, while studying residual pathways after sympathectomy concluded that after excision of the paravertebral ganglionated chain, regardless of the extent of the excision, residual sympathetic pathways which do not pass through any part of the paravertebral chain remain. After paravertebral sympathectomy these pathways become manifest only after a process of readjustment takes place. Therefore, an incomplete denervation may mimic complete denervation for a period of up to three months, with returning sympathetic activity within this period due to readjustment and later return due to some mechanism, or to invasion by fibers from adjacent undervated areas, or regeneration. These conclusions may account in part for the inconsistent and transient results of sympathectomy.

Alexander et al (1949)¹ in reviewing failures to achieve complete sympathetic denervation, particularly in the extremities, by extirpation of the appropriate segments of the sympathetic

trunks or by section of the white communicating rami of the appropriate segments, have also suggested the possible existence of peripheral sympathetic conduction pathways which do not traverse the sympathetic trunk. Such pathways could be postulated on the assumption that some embryonic nerve cells, in their displacement from the neural tube, have failed to reach the primordia of the sympathetic trunks and have been differentiated into ganglion cells in the ventral nerve roots, or adjacent to them, where they have become synaptically connected with preganglionic fibers which either do not extend into the sympathetic trunk or give off collaterals to the ganglion cells in question. In their examination of a series of human bodies, they observed small ganglia embedded in ventral nerve roots or adjacent to them, usually at the site of origin of a white communicating ramus and in communicating rami, particularly grey rami. Pick (1957)⁵² on reviewing the anatomy of sympathetic segments, further confirmed the idea that synapse of pre- and postganglionic fibers are not always confined to the ganglia of the sympathetic trunk, but it may be shifted, either distally forming an intermediary ganglia on the grey ramus or proximally onto the white ramus as suggested by Skoog (1947)⁶⁰.

Ehrlich and Alexander (1951)¹⁷ undertook further studies on the surgical implications of upper thoracic independent sympathetic pathways in order to obtain additional data relative to the occurrence of accessory sympathetic ganglions in man and their anatomic relationships to the lower cervical and thoracic spinal

nerves, and their significance in the sympathetic innervation of the upper extremity. They found frequent occurrence of accessory ganglion of which most, except those associated with grey communicating rami or sympathetic roots, are synaptically related to preganglionic fibers which reach them directly through the ventral nerve roots. Their axons in turn extending distalward in somatic rami of the spinal nerves forming sympathetic conduction pathways not traversing sympathetic trunk. Ray (1953)⁵⁷ upon reviewing the effectiveness of sympathectomy of the upper extremity concluded from the accumulating bulk of data reviewed, that the existence of sympathetic supply to the upper limb is so extensive that no practical operation, either preganglionic or postganglionic alone or in combination, can be relied upon to effect an absolutely complete denervation. In this article Ray refuted the theory that sensitization and regeneration accounted for return of sympathetic tone to denervated areas and confirmed the idea of accessory sympathetic ganglion.

Boyd and Monro (1949)⁶ stimulated by the observation of "sudomotor escape" areas, areas of sweating pattern following apparently complete sympathectomy, examined serially sectioned human embryos and fetuses in an attempt to locate sympathetic cells which would account for residual pathways to the sweat glands not removed in thoracolumbar sympathectomy. This investigation revealed the presence of numerous- and constantly present (though variable in number and situation)- autonomic cells and

ganglia in the course of the rami communicantes, some embedded in and some on the surface of the ventral primary rami of the lumbar nerves. Boyd (1957)⁷ elaborated further on the accessory sympathetic ganglia and felt for the most part they are to be found on the course of the rami communicantes and most common on the post-ganglionic or grey rami. However, the preganglionic connections of the cells of the accessory ganglion have not been definitely established. For the cervical accessory ganglia the preganglionic outflow is not conclusive but Boyd feels its likely that most of the preganglionic fibers pass up in the cervical chain. In the cervical region the accessory ganglia appear to be more widely dispersed and in several embryos, Boyd has found ganglionic aggregations in the lower cervical nerves as they pass between the scalene muscles on their way to the brachial plexus. Several courses for the preganglionic fibers to the accessory ganglion have been suggested. For the cells situated in spinal nerves containing a preganglionic outflow it is concluded that the preganglionic connection is restricted to that nerve. It was thought that all preganglionic supply to the cervical accessory ganglion reaches the cells of the ganglion via cervical paravertebral chain; but, the complications of the region introduced by the presence of the vertebral nerve, or nerves, by the presence of the ansa subclavia, by the frequent atypical arrangements of the sympathetic chain in front of the first ribs made an analysis of the cervical region difficult. Therefore, in the cervical region at least some of

the sympathetic fibers may escape in cervicothoracic sympathectomy.

Finally, the persistence of intact sympathetic cells with their postganglionic fibers doubtlessly explains many of the functional regenerative phenomenon following sympathectomies. The anatomical situation may be such that sectioned preganglionic fibers can readily extend into the region containing the intermediate ganglion. Also, Murray and Thompson (1957)⁵⁰ have suggested that functional reorganization, possibly by the development of collateral sprouts, can occur after sympathectomy. This raises the possibility that persisting intermediate ganglion may be of importance in providing the postganglionic pathway if functional contact with these cells is made by such collateral sprouts.

Kuntz et al (1956)⁴² studied the nerve fiber components of communicating rami and sympathetic roots in man. They described the histologic appearance of the sympathetic conduction pathways. The preganglionic components arising from the ventral spinal nerve roots reached the sympathetic trunk via a communicating ramus and histologically fell into the diameter range of less than 1-3.5 microns. The fibers within this diameter range were found to be arranged among larger, less densely aggregated fibers. These fibers were found as high as C-8. Histologic examination of serial longitudinal sections of sympathetic roots in 5 human cadavers revealed accessory ganglion cells frequently in those nerves that make up the brachial plexuses. Ganglion cells in sympathetic

roots or adjacent to them occur in small and large aggregates. Most of them are incorporated in compact ganglion. Most preganglionic fibers that make synaptic connections with accessory ganglion cells located in relation to sympathetic roots undoubtedly traverse these roots to points where the ganglion cells are located. Myelinated fibers within the preganglionic caliber range, however, occur in many sympathetic roots in the absence of accessory ganglion cells. In addition to sympathetic roots the sympathetic trunk gives off more slender segmental sympathetic rami which do not join the spinal nerves. In the cervical segments some of these rami join the plexus of the carotid and vertebral arteries contributing to their innervation. It is felt, that most of the preganglionic fibers that make synaptic connections with ganglion cells that are located in proximity to segmental nerves probably do not traverse sympathetic roots, but reach the cells in question through segmental rami from the sympathetic trunk.

Data relative to the rostral limit of the outflow of preganglionic fibers concerned with sympathetic innervation of the upper extremity are inconclusive. On the basis of digital vasomotor responses to stimulation of the ventral nerve roots in man, exposed during operative procedures, Bridges and Yohr (1955)⁸ concluded that in addition to lower thoracic nerve roots, the ventral roots of both the first thoracic and eighth cervical nerves are traversed by preganglionic fibers that are concerned

with the vasomotor innervation of the hand. Hoffman (1957)³² in an analysis of the sympathetic trunk and rami in cervical regions of man observed the occurrence of accessory ganglion cells throughout the entire cervical sympathetic trunk not previously reported. Also, he observed the presence of large numbers of myelinated fibers that do not exceed 3.5 microns in diameter in the vertebral nerve supporting the conclusion of some investigators that this nerve represents an accessory sympathetic trunk. On histologic section the large number of myelinated fibers in the diameter range of preganglionic fibers found in the vertebral nerve suggests that ganglion cells must be present in considerable abundance. Hoffman observed that the cervical and rostral thoracic sympathetic roots include myelinated fibers, most of which are less than 4 microns in diameter and are particularly abundant in the ventral roots of the sixth, seventh, and eighth cervical nerves. Hoffman then concluded that the rostral limit of the preganglionic outflow in man appears to be at least as high as the eighth cervical segment.

It is clear from the above review of literature that the upper limit of the preganglionic sympathetic outflow is not clearly understood.

METHODS AND MATERIALS

The following experiments were executed using 14 young mongrel dogs to determine if a preganglionic outflow from the cervical spinal cord could be demonstrated. Only young dogs were used whose average sizes ranged from 3-8 kilograms, and only those with tender, non-calloused white paws, because of the dependence on light reflection for recording of a volume pulse through a photoelectric plethysmograph. The dogs were first anesthetized with alpha-chloralose, the action of which induced anesthesia by central depression without a respiratory or cardiovascular depression under therapeutic dosage (1 cc/kg). A tracheotomy was done and a positive pressure respirator attached. The inner thigh was shaved, femoral artery was exposed and a catheter was then introduced into the artery and connected to a Statham pressure transducer and blood pressure recorded via a model 5 Grass polygraph.

Next, the back was shaved from the occipital protuberance to the level of the fourth thoracic spine and a midline incision made from the spine of the axis to the spine of the third thoracic vertebra. The muscle masses overlying the spines of the cervical vertebrae were separated using a soldering gun with a modified cutting head and all bleeders cauterized. The cervical

spines were stripped of their periosteal and ligamentous attachments exposing the lamina and articular processes as far as the third thoracic vertebrae. A laminectomy was done and the fifth cervical to the second thoracic spinal level were exposed. The front paw was then cleaned and a photoelectric plethysmograph was attached to the large central pad to measure cutaneous volume pulses. To attach the photoelectric detector to cutaneous areas, a thin layer of mineral oil is first placed on the face of the photocell, in order to increase light transmission and greatly improve the performance of the detector. The detector is then held firmly against the central pad by a flat, wide rubber band with a hole cut out which fits snugly around the detectors casing. Silver foil was then wrapped around the detector in order to avoid any interference from extraneous light sources. The miniaturised photoelectric plethysmograph designed by Rawson (1961)⁵⁴, was designed for recording, through capacitance coupling, pulsatile blood flow changes in a vascular bed.

Since we are interested in the pulsatile changes in the cutaneous vessels, mention should be made of experimentation with the plethysmograph by Rawson (1961)⁵⁴. In his research he demonstrated that although the illumination from the photoelectric cell penetrated the skin, the layers of fascia separating skin from muscle provided sufficient impedance to the light to prevent vascular changes in the muscle from affecting the cutaneous record. The changes recorded as pulsations are due to alterations in

absorption of light quantitatively related to increase and decrease in blood volume of the vascular bed with each pulse. Therefore, changes related to sympathetic vasoconstriction can be demonstrated by the changes as recorded from the front paw.

Next, muscular blockade was induced by Decamethonium, which acts to produce a paralysis of striated muscle by depolarization of the motor end plate without causing ganglionic paralysis. A unipolar electrode was attached to a Grass model 3C stimulator and grounded to a retractor. The stimulator was used to deliver a square wave pulse of predetermined strength, duration and frequency. The parameters for stimulating the anterior and posterior roots for optimum stimulation of sympathetic fibers has been determined on the basis of Bronk et al (1936)¹¹ who demonstrated a discharge rate of about 1 to 3 impulses per second in "resting" isolated sympathetic fibers and by Folkow (1952)²³ who demonstrated that a normal constrictor tone is maintained by an activity of only 1 to 2 impulses per second. The discharge of fibers at maximal physiological excitation from the vasomotor center was estimated not to exceed 10 impulses per second. Randall et al (1953)⁵³, while studying the vasomotor innervation of the dog's hind foot pad determined the optimum parameters for sympathetic trunk stimulation. These specific stimulating parameters were used in the present experimental procedures. The parameters used for stimulation were as follows: frequency of 10 cycles per second, duration of 5 milliseconds, and voltage

of 5 volts.

The segments of anterior and posterior roots were carefully lifted and a small cooper wire attached to a unipolar electrode was placed around the intact roots. Mineral oil was then used to flood the area to be stimulated, therefore avoiding any conduction through the medium. Spread of current, if it occurred, was detectable by a sharp drop in the stimulating potential observed on the oscilloscope. The oscilloscope was also used to check the frequency and duration of the stimulating pulse and the voltage settings were checked against a standard cell. Both the anterior and posterior roots were sequentially stimulated in the intact condition from the eighth cervical roots cephalad through the fifth cervical roots. Then each root was sectioned and their distal ends restimulated using the same parameters in order to rule out any reflex effects. Freeman et al (1950)²⁴ while studying afferent fibers in peripheral sympathetic nerves and Kuntz (1951)⁴¹ in reviewing the afferent innervation of peripheral blood vessels through sympathetic trunks, stated that the afferent fibers may reflexly elicit sympathetic efferent discharge. Kerslake (1951)³⁵ reported on the abolition of reflex vasodilatation of the hand, due to heating of the foot and leg, by sympathectomy of the heated limb. Therefore, by eliminating the possible reflex effects via the spinal cord or by conduction along the medium, an accurate interpretation of sympathetically elicited vasomotor activity of the front paw was observed. The

sympathetic responses were recorded on a model 5 Grass polygraph.

Immediately following stimulation, the anterior and posterior roots from the eighth cervical to the fifth cervical segment were removed for determination histologically of preganglionic sympathetic fibers. Each root was cut to about 12 mm lengths and placed in fixing solution. The sections were fixed in Descastro fixative, a combination of 6 gms chloral hydrate, 100 cc distilled water, 100 cc of 95% alcohol and 3 cc of nitric acid. Chloral hydrate was used as a fixing agent in either water or alcohol, but usually in water-alcohol mixture and has a specific application in the fixation of nervous tissue. In its action it causes rather marked shrinkage of cytoplasmic structures but gives rather good preservation of the general topographic character of the tissues fixed in it. Nitric acid is used as an auxiliary agent which enhances the effect of chloral hydrate in fixation of the tissue. The tissues were then placed in the cold at 4 degrees centigrade for 24 hours, allowing enough time for thorough penetration and reaction with the tissues. The tissue was then washed in distilled water via a dialyzer for 24 hours changing the water 3 times during the process. Following dialysis of soluble materials from the roots, the process of block staining was begun.

The tissue was placed in ammoniated alcohol (50 cc absolute alcohol and 3 drops of ammonium hydroxide) for 1 to 2 days, in order to remove the bulk of the myelin from the nervous tissue

and thereby facilitate penetration by the silver solutions. Next, the tissue was placed into 2% solution of silver nitrate in an oven at 37 degrees centigrade for 4 1/2 to 5 days. During this period the nervous tissue becomes impregnated with silver. In order that a metallic compound may be usable for staining, it must be readily convertible to an insoluble reaction product. The reduction of the silver nitrate is brought about by a reducing solution composed of 1 gm of pyrogalllic acid, 92 cc of water and 8 cc (37%) formaldehyde. The reduction process was carried out for 24 hours in the dark, since the effect of light during silvering is the impairment of the selectivity of the stain for neural elements. Tissue was then dehydrated by proper technique and embedded in 61 degrees paraffin which affords an excellent supporting medium to the interstices of the block of tissue for adequate sectioning. The sections were cut at 12 mu thickness and mounted on slides using egg albumin. Nerve fibers (axons) stained grey to black while connective tissue stained black. The sections were then examined for preganglionic sympathetic fibers using a diameter size of 3 microns or less as our criteria and a section from the second thoracic root as a standard. The roots were counted using a calibrated grid for measuring diameter size.

RESULTS

I. Cardiovascular response to electrical stimulation of ventral roots of cervical cord: In the course of this research a total of 14 dogs were employed. The first five experimental animals were subjected to a unilateral sympathectomy, using a transthoracic approach. The 4th rib was resected, the pleura incised and lungs retracted anteriorly. The stellate ganglion was dissected free and the ansa subclavius was dissected from around the subclavian artery as well as the branches arising cephalad from the stellate. The caudal cervical ganglion was dissected free and separated from the vago-sympathetic trunk. The sympathetic trunk was then dissected free as low as the fourth thoracic level and the sympathetic ganglia and trunk removed. The reasoning behind the sympathectomies was based on prior laminectomies in which vasoconstriction occurred in the foot pad of the dog during the surgery and did not reopen. Therefore, it was felt that sympathectomy before ventral root stimulation should permit recording of adequate volume pulses and facilitate recording of vasoconstrictive responses during root stimulation. However, in retrospect it seems that by removing sympathetic ganglion we were removing the most probable

source of synapses for preganglionic outflow. These animals were subsequently subjected to a laminectomy and the ventral roots of the cervical cord were then stimulated sequentially from the eighth cervical through the fifth cervical level. Because of the surgical mortality and lack of positive response to stimulation, the sympathectomies were discontinued. The following nine experimental animals were then operated on, doing direct ventral root stimulation following laminectomy without prior sympathectomies. In dogs number 8 and 9 transection of the cord at C-8 - T-1 was done in order to rule out central effects of electrical stimulation.

Nine dogs gave negative findings and of those exhibiting positive results, dog number 8 revealed the most definite positive findings. Results of stimulating the ventral roots of cervical nerves of dog number 8 are shown in Figure 1. The upper tracing is a record of femoral arterial pressure that shows relatively large oscillations synchronous with the positive pressure respirator. A rise in blood pressure (20-60 mm Hg systolic) and a marked increase in heart rate (60-100/min) during each stimulation may be noted. There was a gradation in response, the least being elicited by stimulation at the fifth cervical level and the greatest at the seventh and eighth cervical level. Onset of the response was prompt (within 3-5 seconds) and recovery was complete approximately 30-45 seconds after cessation of stimulation. Sectioning of the spinal cord between the eighth

FIGURE 1

Animal Number 8: Results of stimulation of ventral roots of cervical nerves C5, C6, C7, and C8. Upper tracing, blood pressure; middle tracing, cutaneous volume pulse; and bottom line, time of stimulation. Paper speed, 2 1/2 mm/sec.

cervical and first thoracic segment did not prevent the response.

The lower tracing of each pair of records shows the changes in pulse amplitude in the foot pad. Decrease in amplitude is due to vasoconstriction and decreased volume of blood flow through the foot pad during each pulse. The blood pressure response and augmentation of heart rate and vasoconstriction of cutaneous pulse can be regarded as indicative of preganglionic sympathetic outflow response to electrical stimulation in the ventral roots of the cervical nerves. The results in Figure 1 are these following stimulation of anterior roots after the anterior and posterior roots were sectioned. Sectioning of the spinal cord between the eighth cervical and first thoracic segment did not prevent the response.

The results following transection of cord at C-8-T-1 level can be seen in Figure 2 and 3. Figure 2 is from experimental animal number 8 in which prior to transection of the cord positive findings were recorded (Figure 1). Following transection, a definite augmentation of heart rate is apparent in the lower tracing, but without the concomitant rise in B.P. or peripheral vasoconstriction. Despite the lack of vasoconstrictive effect or B.P. rise, the fact that the augmentation of heart rate occurred, an effect considered sympathetic in origin, would seem to refute any possibility of retrograde stimulation of descending sympathetic fibers through the spinal cord. The recording represents stimulation of the distal end of the ventral root of C-8,

FIGURE 2

Animal Number 8: Results of the stimulation of ventral root of the eighth cervical nerve after transection of the spinal cord at C-8-T-1. Upper tracing, blood pressure and heart rate; middle tracing, cutaneous volume pulse; and bottom line, time of stimulation. Paper speed, 2 1/2 mm/sec.

C - 8

FIGURE 3

Animal Number 9: Results of the stimulation of ventral root of the eighth cervical nerve after transection of the spinal cord at C-8-T-1. Upper tracing, blood pressure and heart rate; middle tracing, cutaneous volume pulse; and bottom line, time of stimulation. Paper speed, 2 1/2 mm/sec.

at which time the distal root was held up away from any adjacent structures and then stimulated. Also, Figure 3 represents the same procedure, in which the distal end of C-8 of animal number 9 was stimulated following transection of the cord at C-8-T-1. In this stimulation, augmentation of H.R. was recorded as well as a rise in B.P. in the lower tracing. Again, no peripheral vasoconstrictive response was seen. These findings correlate well with those in Figure 4 in which similar cardiovascular findings occurred prior to transection of cord.

Figure 4 is from a 4 Kg female (animal number 9) in which the distal ends of previously cut ventral roots from C-6 through C-8 were stimulated and again demonstrated in the lower tracing the blood pressure rise (20-50 mm Hg systolic) and augmentation of heart rate (60-100/min) as noted in Figure 1. The lower tracing of each pair of records again demonstrated changes in the pulse amplitude in the foot pad as expected if sympathetic outflow is present.

Figure 5 is a single tracing during electrical stimulation of the eighth cervical roots of a 3 1/2 Kg male (animal number 13) in which only vasoconstriction was noted (lower tracing) without the concomitant changes in blood pressure and heart rate. The probable explanation for lack of blood pressure and heart rate changes lie in the work of Wreth (1941)⁶⁴ and Skoog (1947)⁶⁰ who described some accessory ganglia which may lie within or adjacent to spinal nerves. In these cases

FIGURE 4

Animal Number 9: Results of stimulation of ventral root of C6, C7, and C8 cervical nerves; upper tracing, blood pressure; middle tracing, blood flow; lower line, time of stimulation.

FIGURE 5

Animal Number 13: Results of stimulation of ventral root of eighth cervical nerve; upper line, time of stimulation; middle tracing, blood pressure; lower tracing, blood flow. Paper speed 2 1/2 mm/sec.

preganglionic fibers synapse directly with the accessory ganglia bypassing the cervical ganglia and continuing distalward along the course of the nerve. In this case only vasoconstriction would be the effect of stimulation; whereas, if the preganglionic fibers communicated with the cervical ganglia, outflow to the heart and subsequent augmentation of heart rate and blood flow would be exhibited.

Animal number 11, an 8 Kg female, and number 12, a 6 Kg female, also showed similar changes in blood flow, blood pressure, and heart rate as described in Figures 1-5.

Table I represents a tabulation of effects of electrical stimulation on heart rate, blood pressure and volume pulse in all 14 dogs stimulated.

Dogs number 1 through number 5 were first sympathectomized through a transthoracic approach and then subsequently operated on two days later and their ventral roots stimulated. Dogs number 1 through number 3 died prior to or during laminectomy procedure; dog number 4 died of anesthetic overdose; and dog number 5 was unable to tolerate laminectomy and died in shock during stimulation procedures. Because of the difficulties and poor tolerance of the experimental animals to two sequential major surgical procedures, it was decided that ventral root stimulation alone would be done taking precautions to assure that recording of specific ventral root stimulations could be verified (refer to discussion). Dogs number 6 and

TABLE I

TABULATION OF EFFECTS OF ELECTRICAL STIMULATION
OF CERVICAL VENTRAL ROOTS C-5-C-8
ON HEART RATE, VOLUME PULSE, AND BLOOD PRESSURE IN THE DOG

Animal number	Change in Heart Rate				Change in Volume Pulse				Change in Blood Pressure			
	C-5	6	7	8	C-5	6	7	8	C-5	6	7	8

Results in Sympathectomized Animals

1	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
2	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
3	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
4	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
5	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC

Results in Non-Sympathectomized Animals

6	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
7	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
8	+	+	+	+	+	+	+	+	+	+	+	+
9	NC	+	+	+	NC	+	+	+	NC	+	+	+
10	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
11	NC	+	+	+	NC	+	+	+	NC	+	+	+
12	NC	+	+	+	NC	+	+	+	NC	+	+	+
13	NC	NC	NC	NC	NC	NC	NC	+	NC	NC	NC	NC
14	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC

Results of Stimulation of the Eighth Ventral Root
After Transection of Cord Between C-8-T-1

8	+	+	+	+	NC	NC	NC	NC	NC	NC	NC	NC
9	+	+	+	+	NC	NC	NC	NC	+	+	+	+

number 7 were stimulated with no definite vasoconstriction response or blood pressure changes being recorded. Dogs number 10 and number 14 exhibited similar negative responses. The explanation for the lack of sympathetic response may be due to the difficulty in recording changes in the cutaneous pulse because of a too-calloused central pad. Also, noise within the electrical equipment did not afford a good standard pulse prior to stimulation. Also, since sympathetic outflow in the cervical roots are variable, the possibility exists that in the dogs with negative findings cervical sympathetic outflow was not present.

II. Histological analysis of cervical anterior roots:

Preganglionic sympathetic axons are small myelinated fibers from 1-3 microns in diameter. If such fibers are present in the ventral roots of cervical nerves, appropriately stained sections of these roots must reveal them. Figure 6 is a cross section of the seventh cervical ventral root of the dog which was stimulated to produce the functional responses shown in Figure 1 (C7 stimulation). The photomicrograph reveals numerous small myelinated fibers scattered among such larger and heavily myelinated motor axons. When measured on the 10 micron scale included in photograph, it is apparent that these small fibers are 1-3 microns in diameter and are, therefore, of the appropriate size to be classified as sympathetic preganglionic B fibers. Although at first glance the large motor

FIGURE 6

Animal Number 8: Cross section of ventral root C7 from experimental animal of Figure 1. A modified DeCastro technique was used. Scale, 10 microns between lines. Arrow indicates typical 3 micron diameter fibers which were found consistently in C5, C6, and C8 of the same experimental animal.

Individual variation in fiber numbers may account for the lack of positive findings.

From the preceding results one can see that the

fibers seem more numerous, an actual count reveals that small myelinated fibers outnumber large ones about 3:1. Similar small fibers were found scattered throughout the sections made from ventral roots of C5, C6, and C8 nerves. (Table II)

Figure 7 is a cross section of the first thoracic ventral root taken from the same animal as in Figure 6, and reveals the expected large population of small, thinly myelinated fibers. The photomicrograph was made at the same magnification as that in Figure 6, and comparison of the small fibers reveal identical size and staining characteristics.

Table II represents results of direct counting of pre-ganglionic sympathetic fibers in stained sections of five different animals. A grid calibrated to 3 microns was employed in measuring diameter size of nerve axons and an entire section of ventral root was counted at each cervical level. In comparison to T1, the ratio of small, thinly myelinated fibers (preganglionic sympathetic) in cervical to thoracic level was about 1:3, an adequate number to account for sympathetic responses recorded on stimulation.

Experimental animals with negative results physiologically were not stained and sectioned. In retrospect, these tissues should have been sectioned since the possibility of individual variation in fiber numbers may account for the lack of positive findings.

From the preceding results one can see that the

TABLE II

FIGURE 7

Animal Number 2: Cross section of ventral root T1 from experimental animal of Figure 1. Scale, 10 microns between lines. Arrow indicates typical 3 micron diameter preganglionic sympathetic fibers found consistently in the thoracic level of all dogs and identical to fibers found in Figure 6.

TABLE II

RESULTS OF DIRECT COUNTING FROM STAINED SECTIONS OF
CERVICAL ROOTS FOR PREGANGLIONIC SYMPATHETIC FIBERS

Animal Number	Level of Ventral Root	Number of 3 Micron or Less Fibers/Ventral Root	Average
8	T1	2950	
8	C5	1490	
9	C5	641	
11	C5	595	
12	C5	1312	1010
8	C6	939	
9	C6	1280	
11	C6	759	
12	C6	1536	1128
8	C7	847	
9	C7	1378	
11	C7	703	
12	C7	1331	1065
8	C8	774	
9	C8	764	
11	C8	867	
12	C8	1142	
13	C8	1556	1020

physiological results of stimulating the ventral roots of C5 through C8 in some cases results in vasoconstriction of cutaneous blood vessels. The anatomical findings of preganglionic sympathetic fibers correlate well with the physiological effect. The presence of preganglionic sympathetic fibers in C5 through C8 would bring up the question of presence of similar fibers in C1 through C4. Much work remains to be done in the investigation of cervical sympathetic outflow pathways, not only in the dog, but in other animals also.

DISCUSSION

Since the advent of autonomic neurosurgery, the question of aberrant pathways or accessory sympathetic ganglia has been brought up in an attempt to explain the lack of success in surgical sympathectomy for the relief of certain vasospastic diseases. Although past literature reveals extensive work pointing out physiologic evidence of sympathetic outflow as high as the eighth cervical levels and many authors have demonstrated preganglionic fibers in the communicating rami of sympathetic roots as high as the sixth cervical level, no experiments have been done in which morphologic and physiologic data, through stimulation of the cervical roots of dogs from C-5 through C-8, has been presented demonstrating preganglionic sympathetic outflow in the cervical roots of the dog.

The anatomical evidence for the presence of sympathetic vasomotor fibers in the cervical anterior roots were reported by Harmon⁴³ as far back as 1898. He described myelinated fibers under 4 micron in diameter in the sympathetic rami of C-5 through C-8 and Pick and Sheehan⁴⁵ observed many finely myelinated 3 micron diameter fibers within the grey rami of C-7 and C-8. In my results after sectioning the anterior roots of C-5, 6, 7, and 8

using a modified silver staining technique similar myelinated fibers smaller than 3 microns in diameter were identified, and by direct counting technique were found to be present in a ratio of about 3:1 in comparison to larger fibers. These fibers are histologically classified as preganglionic sympathetics and are identical to preganglionics found in the anterior roots at the thoracic levels of the same animals. These findings would seem to be in agreement with Kuntz et al⁵⁵ who noted the frequent occurrence of accessory ganglia cells in sympathetic roots or in close proximity therefore affording an explanation for the occurrence of preganglionic fibers in the same sympathetic roots. They described these myelinated fibers of less than 3.5 micron diameter in sympathetic roots as high as C-6. Also, by comparing actual counts of the total number of aggregated fibers in a ventral root and the fibers in the diameter range of preganglionic fibers in the communicating ramus arising from the nerve, there was a relatively close correspondence. Therefore, it appeared highly probable that the preganglionic fibers found in the communicating ramus traverse the ventral roots of the same nerve.

The above morphologic findings correlate well with the physiologic phase of the experiment. In the physiologic phase, ventral roots of C-5 through C-8 were exposed and stimulated. Upon stimulation of these ventral roots in several dog recordings of decreased cutaneous blood volume was used as a criteria for any vasoconstrictive effects. Folkow et al⁸ while studying

cutaneous blood flow in man demonstrated that vasoconstriction of the cutaneous blood vessels in man is purely sympathetic in origin. Also, using a photoelectric plethysmograph, Rawson⁶⁴, was able to record only vascular changes in the cutaneous blood vessels. Upon stimulation of the ventral roots of C-5 through C-8 in several dogs vasoconstriction was definitely recorded. In the light of Folkow et al⁸ the vasoconstrictive effects of stimulation can only be concluded as sympathetic activity. From the light of Rawson's experiments⁶⁴ we can also conclude that only cutaneous vasoconstriction and not muscular contraction was recorded. Bridges and Yahr⁵⁶ did similar studies on human subjects stimulating the anterior roots and recording digital vasoconstriction as high as the eighth cervical levels. No attempt was made to stimulate anterior roots any higher than C-8 and no other reports of such stimulation has been cited in the literature. The potentially complicating factor of muscle movement was obviated by the use of Decamethonium, a muscle relaxant. Control experiments were done in which the ventral roots were stimulated without Decamethonium and recordings were unreliable because of the excessive muscular activity of the dogs limb. When Decamethonium was administered a consistent recording of the volume pulse was maintained. The question of central effects was obviated by stimulating the ventral roots while intact and then cutting and stimulating the distal end. Similar recordings resulted from stimulation of intact or distal ends of

the anterior roots. The question of spread of the stimulus through the medium was avoided by flooding the area with mineral oil. Having taken these precautions, the results of the stimulation could only be via the roots stimulated.

Thus, we conclude that in the light of our research there are preganglionic efferent outflows above the classical level of T-1 in our experimental dogs. The significance of our findings can be understood in the light of numerous failures in attempted sympathectomies for vasospastic diseases. Our findings would account in some cases for the observation of sudomotor activity in patients with cervical transection of the spinal cord. Also, in stellate ganglionectomies our findings would account for the lack of a Horner's syndrome following this procedure.

SUMMARY AND CONCLUSION

The following conclusions pertinent to the study of cervical sympathetic outflow in the dog have been drawn:

1. Physiologic evidence of preganglionic sympathetic outflow via the ventral roots of C-5 through C-8 has been demonstrated by cutaneous vasoconstriction in some dogs.

2. Morphologic evidence of substantial numbers of preganglionic 3 micron diameter sympathetic fibers has been demonstrated by silver staining technique of cross sections of ventral roots from C-5 through C-8 of experimental dogs stimulated in (1).

3. The significance of our findings lies in the fact that many surgical procedures involving sympathectomy of the upper extremity by stellate ganglionectomy have failed; therefore, the anatomic outflow described above would account for many residual sympathetic activities in numerous surgical conditions.

BIBLIOGRAPHY

1. Alexander, W.F., A. Kuntz, W.P. Henderson, and E. Ehrlich. (1949) Sympathetic ganglion cells in ventral nerve roots. Their relation to sympathectomy. Science 109: 484.
2. Bacq, Z.M. (1931) Présence, dans l'humeur aqueuse du lapin, d'une substance sympathicomimétique après stimulation du sympathique cervical. C.V. Seane. Soc. Biol. 107: 1584.
3. Barcroft, H., W.McK. Bowman, and O.G. Edholm. (1947) Reflex vasodilatation in human skeletal muscle in response to heating the body. J. Physiol. 106: 271.
4. Barger, G. and H.H. Dale. (1910) Dale-Chemical structure and sympathemimetic action of amines. J. Physiol. (London) 41: 19.
5. Bayliss, W.M. and J.R. Bradford. (1894) The innervation of the vessels of the limbs. J. Physiol. 16: 10.
6. Boyd, J.D. and P.A.G. Monro. (1949) Partial retention of autonomic function after paravertebral sympathectomy. Lancet 2: 892.
7. Boyd, J.D. (1957) Intermediate sympathetic ganglion. British Med. Bull. 13: 207.
8. Bridges, T.J. and M.D. Yahr. (1955) Digital vasomotor responses following nerve root stimulation. Arch. Neuro. and Psychiat. 74: 534.
9. Brizze, K.R. and A. Kuntz. (1950) The histogenesis of sympathetic ganglion cells. J. Neuropath. and Exper. Neurol. 9: 164.
10. Brizze, K.R. (1951) Histogenesis of the supporting tissue in the spinal and sympathetic trunk ganglia in the chick. J. Comp. Neurol. 91: 129.

11. Bronk, D.W., L.K. Fergusin, R. Margaira, and D.Y. Solandt. (1936) The activity of the cardiac sympathetic centers. Am. J. Physiol. 117: 231.
12. Bulbring, E. and J.H. Burn. (1935) The sympathetic dilator fibers in the muscles of the cat and dog. J. Physiol. 83: 483.
13. Burn, J.H. (1932) The action of tyramine and ephedrine. J. Pharm. Expir. Therap. 46: 75.
14. Cox, J.W., W.C. Randall, W.F. Alexander, F.E. Franke, and A. B. Hertzman. (1951) A method for the study of cutaneous vascular responses. Bull. St. Louis Univ. Hosp. 4: 71.
15. Dale, H.H. and W. Feldberg. (1934) Chemical transmission of secretory impulses to sweat glands of cat. J. Physiol. (London) 82: 121.
16. Dale, H.H., W. Feldberg, and M. Vogt. (1950) Release of acetylcholine in the central nervous system. Med. Bull. 6: 312.
17. Ehrlich, E. and W.F. Alexander. (1951) Surgical implications of upper thoracic independent sympathetic pathways. Arch. Surgery 62: 609.
18. Euler, O.S. van. (1946) A specific sympathomimetic ergone in adrenergic nerve fibers (sympathin) and its relations to adrenaline and nor-adrenaline. Acta Physiol. Scand. 12: 73.
19. Feldberg, W. (1950) Role of acetylcholine in the central nervous system. British Med. Bull. 6: 312.
20. Folkow, B., K. Haeger, and B. Uvnas. (1948) Cholinergic fibers in the sympathetic outflow to the muscles of the hind limbs of the cat. Acta Physiol. Scand. 25: 49.
21. Folkow, B., J. Frost, K. Haeger, and B. Uvnas. (1949) The sympathetic vasomotor innervation of the skin of the dog. Acta Physiol. Scand. 16: 195.
22. Folkow, B., J. Frost, K. Haeger, and B. Uvnas. (1949) The sympathetic vasomotor innervation of the skin of the dog. Acta Physiol. Scand. 17: 195.

23. Folkow, B. (1952) Impulse frequency in sympathetic vasomotor fibers correlated to the release and elimination of the transmitter. Acta Physiol. Scand. 25: 49.
24. Freeman, L.W., H.B. Schumacker, Jr., and L.R. Radigan. (1950) A functional study of afferent fibers in peripheral sympathetic nerves. Surgery 28: 274.
25. Gaskell, W.H. (1886) On the structure, distribution and function of the nerves which innervate the visceral and vascular systems. J. Physiol. 7: 1.
26. Gaskell, W.H. (1889) On the relation between the structure, function, distribution and origin of the cranial nerves. J. Physiol. 10: 153.
27. Gaskell, W.H. (1916) The Involuntary Nervous System. Langman, Green and Company. London.
28. Gehegan, W.A., G.A. Wolfe, Jr., O.J. Aidor, K. Hare, and J. D. Hinsey. (1942) The spinal origin of the preganglionic fibers to the limbs in the cat and monkey. Am. J. Physiol. 135: 324.
29. Gehegan, W.A. and O.J. Aidor. (1942) Functional reorganization following preganglionectomy. Proc. Soc. Exp. Biol. (New York) 50: 365.
30. Green, H.D., W.B. Haward, and L.F. Kenan. (1956) Autonomic control of blood flow in hind paw of the dog. Am. J. Physiol. 187: 469.
31. Harmon, H.B. (1900) A study on the composition of the human sympathetic rami. J. Anat. Physiol. : 34.
32. Hoffman, H.H. (1957) An analysis of the sympathetic trunk and rami in the cervical and thoracic regions in man. Annals of Surgery 145: 94.
33. Jones, D.S. (1937) The origin of the sympathetic trunks in the chick embryo. Anat. Rec. 70: 45.
34. Juba, A. (1930) A histological description of the constitution of the various sympathetic rami of man. Z. ges. Anat. I. Z. Anat. EntwGesch. 92: 224.
35. Kerslake, D.McK. (1951) Abolition of nervous reflex vasodilatation by sympathectomy of the heated limb. J. Physiol. 113: 7.

36. Kirgis, H.D. and A. Kuntz. (1942) Inconstant sympathetic neural pathways. Their relation to sympathetic denervation of the upper extremity. Arch. Surg. 44: 95.
37. Kuntz, A. (1909a) A contribution to the histogenesis of the sympathetic nervous system. Anat. Rec. 3: 158.
38. Kuntz, A. and O.V. Batson. (1920) Experimental observations of the histogenesis of the sympathetic trunks in the chick. J. Comp. Neurol. 32: 335.
39. Kuntz, A., W.F. Alexander, and C.L. Furiolo. (1938) Complete sympathetic denervation of the upper extremity. Annals of Surgery 107: 25.
40. Kuntz, A. and J.B. Dillon. (1942) Preganglionic components of the first thoracic nerve. Their role in the sympathetic innervation of the upper extremity. Arch. Surg. (Chicago) 44: 772.
41. Kuntz, A. (1951) Afferent innervation of peripheral blood vessels through sympathetic trunks. South. Med. J. 44: 673.
42. Kuntz, A., H.H. Hoffman, and M.W. Jacobs. (1956) Nerve fiber components of communicating rami and sympathetic roots in man. Anat. Rec. 126: 29.
43. Langley, J.N. (1878) On the physiology of salivary secretion. J. Physiol. 1: 96.
44. Langley, J.N. and W.L. Dickinson. (1890) Pituri and nicotine. J. Physiol. 11: 265.
45. Langley, J.N. (1901) The difference of behavior of central and peripheral pilomotor nerve cells. J. Physiol. (London) 27: 224.
46. Langley, J.N. (1921) The Autonomic Nervous System. W. Haffer and Sons. Cambridge.
47. Lewandowsky, M. (1898) Ueber eine Wirkung des Nebennierenextractes auf das Auge. ZentBl. Physiol. 12: 599.
48. Lewis, T. and G.W. Pickering. (1931) Vasodilatation in the limbs in response to warming the body, with evidence for sympathetic vasodilator nerves in man. Heart 16: 33.

49. Loewi, O. (1921) Ueber humorale Uebertragbarkeit der Herzner-
venwirkung. I. Pfluger's Arch. ges. Physiol. 189: 239.
50. Murray, J.G. and J.W. Thompson. (1957) Collateral sprouting
in response to injury of the A.N.S., and its consequences.
British Med. Bull. 13: 213.
51. Perry, W.L.M. and J. Talesnik. (1953) The role of acetylcho-
line in synaptic transmission at parasympathetic ganglia.
J. Physiol. (London) 119: 455.
52. Pick, J. (1957) The identification of sympathetic segments.
Annals of Surgery 145: 3.
53. Randall, W.C., W.F. Alexander, J.W. Cox and A.B. Hertzman.
(1953) Functional analysis of the vasomotor innervation
of the dog's hind foot pad. Circ. Res. 1: 16.
54. Rawson, R.O. (1961) Apphotoelectric Plethysmographic Study
of the Sympathetic Vasomotor Outflow to the Hind Limb of
the Dog. Ph.D. Thesis. Loyola University, Chicago,
Illinois.
55. Ray, B.S. and A.D. Console. (1948) Residual pathways after
sympathectomy. J. Neurol. 5: 23.
56. Ray, B.S. and A.D. Console. (1949) Evaluation of total sym-
pathectomy. Annals of Surgery 130: 652.
57. Ray, B.S. (1953) Sympathectomy of the upper extremity-evalua-
tion of surgical methods. J. Neurol. 10: 624.
58. Schaper, A. (1897) Die fruhesten Differenzierungsvorgange im
Zentrolnervensystem, Kritische Studie und Versuch einer
Geschichte der Entwicklung nervoser Substanz. Arch.
Enter.-Mech. 5: 81.
59. Sheehan, D. and J. Pick. (1943) The rami communicantes in
the rhesus monkey. J. Anat. (London) 77: 125.
60. Skoog, T. (1947) Ganglia in the communicating rami of the
cervical sympathetic trunk. Lancet 2: 457.
61. Strudel, G. (1952) Contribution experimentale au probleme de
l'origine des ganglions rochidens et sympathiques chez
l'embryon de poulet. Compt. rend. Soc. Biol. 146: 105.
62. Uvnas, B. (1954) Sympathetic dilator outflow. Physiol. Rev.
34: 608.

63. Van Buskirk, C. (1941) Nerves in the vertebral canal. Their relation to the sympathetic innervation of the upper extremities. Arch. Surg. (Chicago) 43: 427.
64. Wrete, M. (1941) Die Entwicklung und Topographie der intermediären vegetativen Ganglien bei gewissen Versuchstieren. Ztsche. Zellforsch. u. mikr. Anat. 49: 503.

APPROVAL SHEET

The thesis submitted by Gary G. Wiesman has been read and approved by four members of the faculty of the Graduate School.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

January 8 1968
Date

Leslie H. Grunert
Signature of Advisor

Sympathetic Outflows from Cervical Spinal Cord in the Dog

Abstract. *In some dogs there are preganglionic fibers leaving the cervical spinal cord through the ventral roots of the lower cervical nerves. When these fibers are stimulated electrically in the anesthetized dog with skeletal muscle paralysis (induced by decamethonium), the effects are vasoconstriction in the front footpad, cardiac acceleration and augmentation, and a rise in arterial blood pressure.*

The sympathetic portion of the autonomic nervous system is often called "thoraco-lumbar" because preganglionic neurons have their cell bodies in the spinal cord from the first thoracic to the second or third lumbar levels. Axons of these preganglionic neurons leave the spinal cord through ventral roots of the thoracic and upper lumbar nerves and traverse the white communicating rami from spinal nerves to sympathetic trunks. If this anatomical description is correct, a person with a complete transection of the cervical spinal cord should have no sympathetic response to hypothalamic or other central nervous system activity. However, patients with cervical transections sometimes do have sweating responses which can be explained only by central nervous system influence over sympathetic outflow (1). Also, surgeons report that the sympathetic trunk can be severed above the first thoracic white communicating ramus and the inferior portion of the stellate ganglion removed without producing a Horner's syndrome, a condition in which there is a constricted pupil, dry and red face, and narrowed palpebral fissure due to loss of sympathetic innervation (2). This indicates that preganglionic axons reach the cervical sympathetic trunk superior to the first thoracic nerve.

The following experiments were performed on dogs to learn whether a sympathetic outflow from the cervical spinal cord could be demonstrated. Under α -chloralose anesthesia, the cervical spinal cord and the cervical dorsal and ventral roots were exposed. Muscular blockade was induced by decamethonium. Intact ventral roots, as well as the distal end of severed ventral roots, were stimulated before and after section of dorsal roots. Sympathetic responses were interpreted from records made on a model 5 Grass polygraph from a Statham pressure transducer connected to the femoral artery to

measure blood pressure, and a photoelectric plethysmograph (3) on the large central pad of the front foot to measure cutaneous volume pulses.

Results of stimulating the ventral roots of cervical nerves are shown in Fig. 1. The upper tracing of each pair is a record of femoral arterial pressure that shows relatively large oscillations synchronous with the positive pressure respirator. Note the rise in blood pressure (20 to 60 mm-Hg, systolic) and the marked increase in heart rate (60 to 100 beats per minute) during each stimulation period. There was a gradation in response, the least being elicited by stimulation at C5 (fifth cervical nerve) and the greatest

at C7 and C8. Onset of the response was prompt (within 3 to 5 seconds) and recovery was complete approximately 30 to 45 seconds after cessation of stimulation. These data are interpreted to mean that preganglionic sympathetic fibers are present in the ventral roots of the lower cervical nerves. Sectioning of the spinal cord between C8 and T1 (first thoracic nerve) did not prevent the response.

The lower tracing of each pair of records shows the changes in pulse amplitude in the footpad. Decrease in amplitude is due to vasoconstriction and decreased volume of blood flow through the footpad during each pulse. These data are also interpreted to in-

Fig. 1. Results of the stimulation of ventral roots of cervical nerves C5, C6, C7, and C8. Upper tracing, blood pressure; middle tracing, cutaneous volume pulse; and bottom line, time of stimulation. Paper speed, $2\frac{1}{2}$ mm/sec.