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A Photoelectric Plethysmographic Study of the Sympathetic Vasomotor Outflow to the Hind Limb of the Dog

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A PHOTOELECTRIC PLETHYSMOGRAPHIC STUDY OF THE SYMPATHETIC VASOMOTOR OUTFLOW TO THE HIND LIMB OF THE DOG

by

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BIOGRAPHY

Robert Orrin Rawson was born in East St. Louis, Illinois, on April 25, 1917, the younger of three children of Orrin G. and Mabel Casteel Rawson. He received his elementary education in Jefferson Grade School of East St. Louis and graduated from East St. Louis High School in 1935.

The same year he enrolled in Illinois State Normal University, and after two years attendance transferred to the College of Liberal Arts and Sciences of the University of Illinois at Urbana. He pursued a course of study with a major in Psychology and minors in Physics and Mathematics, and during his senior year was invited to engage in an undergraduate program of neurophysiological research under the guidance of Dr. L.A. Pennington of the Dept. of Psychology. He was awarded the degree of Bachelor of Science in 1940.

After graduation, Mr. Rawson was employed by the American Zinc Company of Illinois as a research metallurgist, and in 1942 was appointed production supervisor of a new electrolytic zinc plant of that company, working in this capacity until prior to the end of World War II.

During this tenure he was also employed as an announcer at a local radio station in St. Louis, and after the war resigned his position with the American Zinc Company to join the announcing staff of the St. Louis Post-Dispatch Radio Station KSD.

In 1946 he joined the announcing staff of Radio Station WIND, Chicago, simultaneously attending Actors Company of Chicago, a dramatic school.

In 1948 he entered the employ of the Midwest Division of the American Broadcasting Company, where he engaged in radio and television announcing, acting, and newscasting until 1955.

During his last year of employment with ABC, Mr. Rawson enrolled in a program of refresher studies at the University of Illinois, Navy Pier, in preparation for graduate study in Physiology.

In September, 1955, he began a program of study in the Department of Physiology at Loyola University, School of Medicine, leading to a degree of Doctor of Philosophy. While in the doctorate training program he engaged in part-time teaching as a faculty member at Columbia College, Pestalozzi-Froebel Teachers College, and the University of Illinois, Navy Pier, Chicago.

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CHAPTER I

GENERAL INTRODUCTION

A decisive functional anatomy of the autonomic nervous system was pointed out by Gaskell in 1889 (29). Using an osmic acid technique he traced fibers in the rami communicantes and roots of the spinal nerves and found that most of the non-medullated fibers passed along the segmental nerve to the periphery, and that a few traveled along the posterior root to end near blood vessels of the spinal cord without entering the cord itself. He concluded that non-medullated sympathetic fibers are always peripheral nerves. He later adopted the term "involuntary nervous system" to be applied to the autonomics, (31). Gaskell, a prolific investigator in this area, (25-27) showed that the CNS supplies efferents to the sympathetic chain through the white rami, and that these fibers leave the cord only in the thoracolumbar segments of the cord, (24,30). He concluded in 1885 that these efferents are the first part of a two neurone pathway to the periphery (28).

The work of Langley and Dickinson in 1889 (51) showed that nicotine injections stopped the sweating and skin pallor produced by stimulation of the rami coming from the cord, but that the effects of stimulation of the peripheral ramification of the

sympathetic chain were not affected by nicotine. This provided direct support for Gaskell's anatomical concept of a two neurone system in the autonomics.

Langley (58) regarded the thoracolumbar portion of the autonomics as distinct from the rest of the system. He divided the autonomic outflow into the tectal, bulbosacral, and "sympathetic" divisions. He later classified the tectal and bulbosacral portions as the "parasympathetic." These two terms, defining the two major divisions of the autonomic nervous system, are retained in current nomenclature.

Thus, by the end of the last century, the anatomical aspects of the autonomic nervous system were well defined, and a solid foundation was formed for functional studies in this area.

Prior to the 20th century however, functional studies of the vasomotor system had already begun with the work of Claude Bernard in 1851, who demonstrated that section of the cervical sympathetics caused dilatation of the vessels of the rabbit ear, and that stimulation of the peripheral cut end of the nerve caused constriction. He also demonstrated the existence of vasodilator nerves by finding an increased blood flow through the submaxillary gland upon stimulation of the chorda tympani. Since that time an enormous amount of research has been carried out to determine the nervous mechanisms operating in vasomotor and secretory control.

Undoubtedly the most outstanding early efforts in this direction were those of Langley. Langley's "secretory" experiments in 1877 (50) wherein he stimulated the "sympathetics" and the chorda tympani, led to vasomotor studies in the feet of dogs, cats and rabbits (53) in which he noted that vasomotor fibers to the foot issue only from L6, L7, S1, and possibly S2. In 1891 (52) he illustrated the arrangement of the lumbar and sacral nerves, and determined the ganglionic origin of sympathetic secretory fibers to the foot. In 1893 (54) he gave an elaborate description of the variation of the origin of secretory fibers from the cat's spinal cord, and showed that vasomotor fibers to the foot generally follow the same path as do the secretory fibers. In 1894 (55,56) he showed that in rabbits and cats there are a number of anatomical variations in the origin of the lumbosacral nerves. In a consecutive series of nerve roots, a certain number may pass out in either of two adjoining nerves. The greater the number which passed out the upper nerve, the more anterior was the origin of the nerve fibers which supplied any particular region of the body. Langley (57) showed that vasomotor fibers for the hind foot of the frog usually leave the cord in the 3rd to 7th spinal nerves. He reported that the origin of sympathetics for the frog foot is more extensive than in the mammal, and that the fibers run in the sciatic to the periphery, with no fibers taking the course of the blood vessels,

(which he stated is similar to the situation in the mammal). Later (59) he noted the phenomenon of vasodilatation in the cat hind foot pad which occasionally occured upon stimulation of the lumbar sympathetics. He saw flushing of the cat's foot after primary pallor when stimulated 45 to 60 secs. Atropine decreased or abolished the flushing, and he attributed the vasodilatation to metabolites set free by the action of sweat glands.

As a contemporary of Langley, Bayliss reported (3) vasomotor studies on the fore and hind limbs of the dog, recording vascular changes with the volume plethysmograph while stimulating the peripheral ends of the thoracic and lumbar anterior and posterior nerve roots. Bayliss found that vasoconstriction obtained in the hind limb with stimulation of roots from the 11th thoracic to the 3rd lumbar segments, with the 1st and 2nd lumbar roots showing the most marked effects. The roots at L4 usually showed a significant vasodilator action, and he attributed this to active vasodilator fibers. He stimulated both posterior and anterior roots (peripheral ends) because efferent vasoconstrictors had been supposedly demonstrated in them by Stricker (74). Unfortunately, in this series of experiments, he did not realize that while he was stimulating anterior root vasoconstrictors, he was also possibly getting concomitent posterior root vasodilatation through antidromic excitation.

Notable in this work of Bayliss was his demonstration of pronounced reflex vasoconstriction of the hind limb upon stimulating the central end of the sciatic. He also elicited a pronounced pressor effect with passive dilatation of the hind limb during excitation of the central end of the divided splanchnic. This emphasized caution in hastily interpreting vasomotor phenomena as a manifestation of direct efferent excitation of peripheral vessels. Bayliss made the incidental but significant comment that available instrumentation did not permit distinguishing between muscle and skin vasomotor activity.

In 1932 Burn (8) described the effect of stimulating the lumbar sympathetic chain of the dog while the hind limbs were perfused with defibrinated blood. He found only a negligible response to stimulation of the sympathetic chain. However, with the addition of adrenaline to the perfusate, the response to sympathetic stimulation became significant. This response was constrictor on occasions, but very often it was dilator. Powerful stimuli of short duration were almost always dilator, while prolonged stimulation was constrictor. He often observed an initial vasodilatation converting to vasoconstriction. When the stimulus was turned off and the vasoconstriction passed, a residual dilatation was again seen. Similar effects were not observed in cats. 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.

The existence of vasodilator fibers in the dog was also demonstrated by Schneider (72) who examined the blood flow through the femoral artery of dogs using a thermostromuhr. He found that when the abdominal sympathetic chain was stimulated there was an immediate increase in flow through the femoral artery, followed by a small but longer lasting decrease. When the stimulus was repeated several times, the dilator effect diminished.

In 1935 (6) Bülbring and Burn showed that the sympathetic dilatation previously described by Burn was cholinergic and occured in the muscles. The dilator effects were augmented by eserine and abolished by atropine. Constrictor effects were reve sed by administration of ergotoxine. Eserinized venous effluent collected during sympathetic stimulation caused contraction of leech muscle, demonstrating the presence of a cholinergic substance assumed to be the transmitter involved in dilatation. Bulbring and Burn also took volume-plethysmographic records of the skinned hind limb of the dog, and during stimulation of the corresponding sympathetic chain, observed constriction of the limb. This was converted to dilatation after eserine. The dilatation was reversed to constriction again following atropine. The initial constrictor effect of sympathetic stimulation was

also changed to dilatation by the injection of ergotoxine. This dilatation was augmented by eserine and abolished by atropine. Thus the evidence suggested a dual innervation of dog muscle, both constrictor and dilator.

Bülbring and Burn (7) stimulated the lumbar sympathetic chain while measuring the outflow of the saphenous vein in an attempt to determine the type of vasomotor innervation serving the skin. They observed an initial outflow acceleration which then gave way to a slower outflow. When they recorded both inflow and outflow, they found that sympathetic stimulation caused diminished inflow, but the effect on the outflow was an initial increase followed by a decrease. After adrenergic blockade there was no effect on either inflow or outflow. They interpreted these results to mean that the skin arterioles constricted and decreased inflow while the skin veins constricted and caused an initial increase in outflow. Thereafter the outflow decreased because of the diminished inflow. They suggested that the ergotoxine effect was to paralyze both the arterial and venous adrenergic innervation.

Rosenbleuth and Cannon in 1935 (70) studied the depressor effect obtained by stimulation of the abdominal sympathetic chain in dogs and cats given ergotoxine. They observed that the depressor effects obtained in some experiments was enhanced by eserine and more or less abolished by atropine. They concluded

that in addition to adrenergic dilator fibers, cholinergic vasodilators are present in the dog and cat.

In 1948 (17) Folkow and Uvnäs, using a drop flow integrator to measure blood flow to the cat hind limb, demonstrated that stimulation of the abdominal sympathetic chains resulted in pronounced vasodilatation in the presence of dibenamine or ergotamine. They interpreted this as an activation of vasodilator fibers to the limb vessels. The magnitude of the responses indicated that the dilator fibers were distributed to the muscles. This conclusion was based on comparable experiments using skinned legs. In another study (18), they concluded the vasodilator fibers to cat muscle were cholinergic in nature. In 1949, (19) Folkow and co-workers 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.

Folkow also investigated the discharge rate of the sympathetic nervous system (20), and he found that impulses appeared to discharge to the muscles at frequences of 1-2 per second during "resting" conditions, and rarely exceeded 6-8 impulses per second. By variations of the constrictor fiber activity within this discharge range the peripheral resistance could generally be changed some five to ten times.

Celander (14), by electrically stimulating the peripheral end of the divided lumbar trunk at L4, investigated the comparative vasoconstrictor intensity operating in the skin and muscle of the cat hind limb. The strongest cutaneous vasoconstriction observed with frequencies giving maximal effects (3-4v, 5ms, 10cps) corresponded to a more than a hundredfold increase of the peripheral resistance, at a perfusion pressure of about 130-150mm Hg. These large effects were in contrast to the smaller ones observed in the muscle which were about ten times less pronounced under the same stimulation parameters.

Folkow (22) in probing further into the functional aspects of autonomic innervation, has attempted to differentiate sympathetic fibers according to diameter and threshold. He concluded that fibers to A-V anastomoses in the cat foot pad, nictitating membrane and pupil have the largest diameter and the lowest threshold of fibers in this system. Those to skin vessels, and vasoconstrictors to muscles and tongue of the cat have a smaller diameter and a higher threshold, while vasodilator fibers to muscles have the smallest diameters and the highest thresholds.

In 1955, Youmans and Green (79), while stimulating the lumbar sympathetic chain at L3, L4 and L5 and measuring femoral blood flow, uncovered what they believed to be three types of vasoactive mechanisms in skeletal muscle which respond to autonomic stimulation. First, an adrenergic vasoconstrictor

(alpha receptor) which they noted as being mediated by norepinephrine. It was abolished by adrenergic blocking agents but not by atropine. Second, an adrenergic vasodilator mechanism, (beta receptor) whose effect was simulated by Isoprel and epinephrine but only slightly by norepinephrine. The pharmacologic effect was not blocked by atropine but was blocked by adrenergic blocking agents. Thirdly, another vasodilator mechanism, (gamma receptor), activated by electrical stimulation and methacholine. This mechanism, called cholinergic, was abolished by atropine and very high levels of adrenergic blocking drugs. In a later work Green (33) measured the blood flow in the hind paw of the dog through an electromagnetic flow meter while stimulating the lumbar sympathetic chain. With differential blockade of possible adrenergic and cholinergic mechanisms operating in the foot, he concluded that the paw skin contains abundant alpha constrictor receptors, but very few if any adrenergic or cholinergic dilator receptors.

Several investigators have attempted to learn more precisely the autonomic pathways involved in vasomotor activity in specific skin areas. In 1947, Patton (65) stimulated the abdominal sympathetic trunk in cats while recording electrical potential changes developed in the hind foot pad. He showed that the preganglionic distribution to the lumbar sympathetic trunk was limited to the twelfth thoracic through the fourth lumbar levels inclusive,

and that the postganglionic distribution was from the sixth lumbar through the second sacral vertebral levels.

Cox and Randall (15) investigated pre-and postganglionic outflow of the sympathetic trunk to the foot pad of the dog using a photoelectric plethysmograph of Hertzman. They found that the level of preganglionic inflow to the sympathetic trunk was quite variable. The highest level was at L1 or above, while the lowermost level was at L6. The postganglionic supply to the hind footpad was found to be less variable than the pre-ganglionic supply, the uppermost level observed being at L4, and the lowermost at S1.

Randall et al, (68) in a thorough study of the anatomical and functional correlation of the sympathetic innervation of the dog's hind footpad demonstrated a pronounced variability from animal to animal in the sympathetic innervation of this area. Vasoconstrictor responses to electrical stimulation of the sympathetic trunk were occasionally elicited at L2, some not above L4, and in a few not above L6.

The postganglionic outflow from the trunk to the footpad vessels was largely from L6 or L7 and frequently from S1.

The preganglionic inflow to the sympathetic trunk was, in some dogs, at L3 and above. In most animals, however, the preganglionics entering the trunk, as judged by successively increasing constrictor effects of electrical stimulation as the

electrodes were moved caudalward, were more and more in evidence in successive stimulations at L4, L5 and L6. Occasionally preganglionics entered as low as L7. In general preganglionic rami were found to enter the sympathetic trunk caudal to their level of exit from the spinal nerve, e.g., a ramus from spinal nerve L5 entered the trunk at L6, etc. The later experiments of Green (33) were in agreement with those of Randall in that constrictor responses were obtained consistently in the paw only if the sympathetic chain was stimulated below the last lumbar vertebra.

A comprehensive survey of the literature, and supplementary inspection of major reviews in the area (9, 21, 34, 45, 75), has revealed that in all of the studies made in the areas of autonomic anatomy and function, the sympathetic outflow for peripheral vasomotor activity has been mapped only with respect to general areas of effector nerve termination, with the exception of a very few localized areas such as the ear, and the animal foot pad.

It is clear that one direction for further elucidation of the problem of sympathetic outflow lies in localizing more specifically the terminations of sympathetic vasomotor fibers in relation to their origin in the sympathetic trunk. In a survey of the instrumentation available for this type of study, there appeared to be no adequate device available which could produce

records of localized vasomotor activity in animal tissue. It is therefore proposed that with the development of a suitable instrument, the sympathetic outflow to the skin and muscle of the hind limb of the dog may be studied by electrically stimulating the sympathetic trunk at various segmental levels, and directly observing the consequent vasomotor activity in specific skin and muscle areas.

This dissertation has outlined a review of the literature pertinent to the proposed study and now describes the approach to the problem in two parts. Part one reviews the instrumentation available prior to the study and describes the design, construction, and testing of a highly sensitive, miniaturized photoelectric plethysmograph. Part two outlines the experimental methods utilizing this instrument in the study, the results, and the conclusions drawn therefrom.

CHAPTER II

SURVEY OF INSTRUMENTATION

The literature reveals a variety of qualitative and quantitative procedures devised for the purpose of measuring flow of blood in the peripheral vascular bed. Very few, however, are applicable to the study of sympathetic innervation of cutaneous and muscle vessels, and none can be applied in investigations involving observations of separate changes occuring in the skin and muscle vessels of all areas of the body.

The classical volume plethysmograph, originated by Brodie and Russell (4), and developed by Hewlett and van Zwaluwenburg (46), is limited in its use to the extremeties, and does not differentiate between muscle and skin flow.

By means of various types of thermoelectric devices, skin surface temperatures may be taken, and related roughly to the state of the vascular bed underlying them. These devices, however, besides being limited almost exclusively to cutaneous study, are often subject to a considerable lag in response (11), so that accurate recording of pulsatile flow is difficult, if not impossible to obtain. The Hardy-Soderstrom radiometer

(36, 37) has been used for recording accurate measurements of skin temperature which have then been employed to calculate peripheral blood flow. The procedure is quite involved and again does not distinguish between cutaneous and muscle blood flow.

Calorimetric methods, first described by Stewart (73) in 1911, determine the rate of peripheral blood flow, but are not applicable to the study of sudden changes in the circulation, and are an index of cutaneous circulation only.

Isotope clearance techniques pioneered by Kety may be applied to peripheral circulation studies, but are obviously not suitable for examination of rapid changes in the smooth muscle of blood vessels.

The electrical strain gage plethysmograph of Whitney (78) which, although avoiding some of the disadvantages of the classical volume plethysmograph, still is not adaptable to all areas of the body, and has no more to offer in this respect than does its mechanical forerunner.

The impedance plethysmograph of Nyboer (64, 66, 76) is an electronic instrument which will measure and record small resistance changes in a segment of tissue. Changes in the volume of an electrical conductor will produce changes in total resistance, so that this instrument functions as a volume measuring device. However, the impedance plethysmograph, aside from

being of highly complicated circuitry, is useful only on the extremities.

Finally, a method of determining blood flow through the skin, based on the principle that the absorption of light by an illuminated tissue varies with its blood content, has been described by several investigators (32, 35, 40, 41, 60). The apparatus described by Hertzman and Dillon (42, 43) as a photoelectric plethysmograph, consists of two tubes, the open ends of which are directed toward and fixed just in contact with adjacent areas of skin. There is a prefocused flashlight bulb in one tube and an emission type photocell in the other. Light which is transmitted through the skin and is reflected back, falls on the photocell. With every pulse there is a change in the volume of blood in the skin, and a related change in the transmitted light is recorded on moving paper after being amplified through a d.c. amplifier. Calibration is obtained by simultaneous records on the fingers with the mechanical and photoelectric plethysmograph. The unit of blood flow measurement used by Hertzman is the "filter unit". The change in the response of the photocell is referred to a standard change produced by the interposition of a light filter made of clear glass. A pulse of one filter unit is one which produces a change in the photocell current exactly the same as that produced by the filter when it is placed in the path of the light

falling on the cell. The apparatus lends itself to prolonged observations on vascular reactions in sites where the other types of instruments could be used only with difficulty or not at all. Hertzman (39) applied the instrument to the nasal septum of man, the ear lobe, and to the skin of the forehead; sites of observation difficult or impossible to approach with the other instruments mentioned above.

The tracing which is recorded with the photoelectric plethysmograph is the volume pulse; this represents the difference between arterial inflow and venous outflow during a single cardiac cycle. According to Hertzman and Dillon (42) the amplitude of the volume pulse is, therefore, a measure of arterial inflow. Provided cardiac output and blood pressure remain constant, the magnitude of the volume pulse apparently depends upon the tone of the small arteries and arterioles and hence is an indication of arteriolar tone. However, as pointed out by Burton (10), variations in venous and capillary pressure may significantly alter the volume pulse without appreciably changing the rate of arterial inflow. He maintains, therefore, that differences in the height of the tracing do not necessarily reflect similar alterations in the arterial inflow.

The photoelectric plethysmograph also records changes in total blood in the tissue under observation. Since the total blood volume depends on the content of blood in the capillary

and venous bed, changes in these vessels could not represent alterations in arterial inflow, for Abramson and Ferris (1) have shown that veins can relax and contract independently of the arterioles, e.g., the volume of the forearm will decrease following a pinch stimulus without altering arterial inflow. Records of changes in tissue blood may then be just as much a reflection of changes in venous outflow as changes in arterial inflow.

The instrument, then, provides a record of the volume pulse and also an index of the total blood volume in a section of tissue under study, even though it may not differentiate between specific sections of the vascular bed undergoing changes in total blood volume. It can therefore be used to study the general vascular response of cutaneous blood vessels to various physiologic and pharmacologic stimuli, and would lend itself to the observation of rapid changes in tone of sympathetically innervated blood vessels in limited areas when the sympathetic trunk was electrically stimulated.

Unfortunately the instrument has many sources of error, and is inadequate in many ways for application to this particular study. A most important disadvantage is its insufficient sensitivity for observing vascular reactions on less vascularized skin areas such as the thigh. The apparatus, as described,

will not produce sufficient signal output to be amplified without simultaneously amplifying inherent circuit noise.

Another disadvantage is its cumbersome construction and method of physical application to the tissue which rigidly fixes it in space, thus allowing the skin under it to move with respect to the sensitive photocell. This introduces serious movement artifacts with each respiration or with even slight body tremor. The method of fixation also prevents a consistent application pressure to the tissue. Small variations in pressure transmitted to the tissue can significantly affect vascular resistance, thus alter volume pulse. Since the instrument is not attached to the tissue under study, it may also become separated from it, permitting light to be reflected from the surface of the tissue instead of going through it. The reflected light can be of such a magnitude as to saturate the photosensitive element and alter its response.

Of all the instruments and methods available to date which might be used to study rapid vascular reactions in the skin and muscle of the lower extremity of the dog, only the photoelectric plethysmograph showed any promise of providing a method for individual study of skin and muscle, and at the same time possessing an adequate frequency response for recording immediate

vascular reactions. It was only too clear, however, that the instrument would need radical revision in its design and construction before it could be adapted to the study of sympathetic innervation of the entire lower extremity.

CHAPTER III

DESIGN AND CONSTRUCTION OF A PHOTOELECTRIC PLETHYSMOGRAPH

It was decided that the problem of measuring qualitative changes in pulsatile blood flow in skin or muscle could be approached through the use of a photoelectric plethysmograph providing the instrument had sufficient sensitivity to changes in incident illumination to permit recording of small changes in pulse amplitude in all tissue areas to be observed. It was also evident that the instrument should be so designed as to be light in weight and to permit direct application to, and support by, the tissue areas studied. Satisfying this condition would enable the sensitive detector to move with the tissue. Movement with the tissue would provide a more consistent contact pressure. Variations in contact pressure due to respiratory movements or volume changes of the tissue alter tissue pressure, which is transmitted to the walls of all distensible vessels in the bed under study, thus altering total blood volume, and volume pulse.

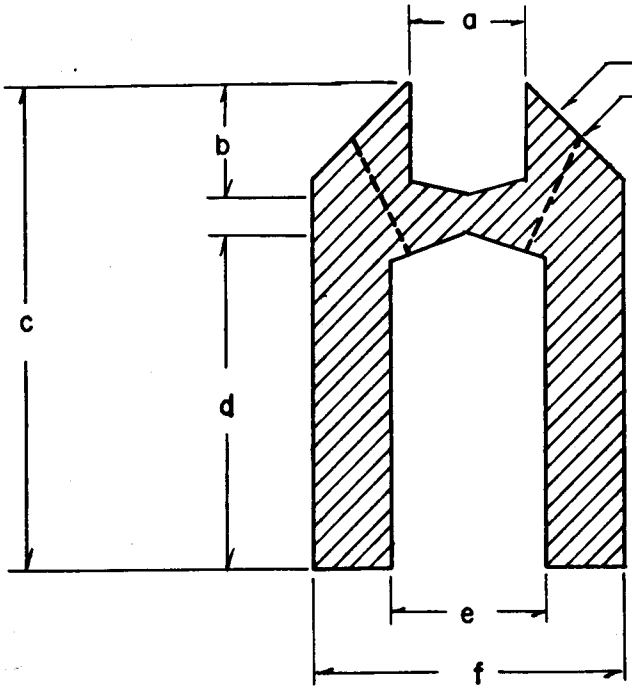
All mechanical movements of the subject would have less effect on the detector at its site of application of it were not rigidly fixed in space. Movements of the tissue can alter angles of light reflection, and hence incident light to the photocell. Minute movements, then can produce very disturbing artifacts when small light changes are greatly magnified through amplification of the photocell output.

The problem of differential absorption of oxygenated and reduced hemoglobin affecting the incident light reaching the photocell was considered, but since the immediate concern was one of measuring rapid changes in pulse amplitude due to electrical stimulation of the sympathetic nerves, the variable was ruled out as insignificant in this series, and reserved for consideration in future instrumental development. The problem was undertaken and is later discussed.

It was decided therefore that the range of spectral sensitivity of the photocell was only important in cases where relative changes in total blood in the tissue would be observed over periods of time in which significant changes in the state of the hemoglobin would obtain, and would not affect the validity of the vasomotor activity data recorded, because of immediate and pronounced effects that electrical stimulation of the sympathetic trunk is known to produce on the innervated vessels.

To satisfy the requirement of photocell sensitivity to small changes in incident light, a Cadmium selenide photocell, Clairex Cl-3, (Clairex Corp. N.Y.) was chosen, on the basis of its small dimensions and high sensitivity. The Clairex Cl-3 is a cadmium selenide photoconductor which, in common with all photoconductors, exhibits the property of altering its electrical resistance when radiant energy falls upon its photosensitive surface. Developed by E. Schwartz of Messrs. Hilger & Watts, Ltd., London, it consists of polycrystalline layer of treated cadmium selenide on one side of a ceramic wafer 3/16" in diameter by 1/16" thick. Indium electrodes cover part of the surface, leaving a sensitive area which is a rectangle 3/16 x 3/64 inches, with the electrodes along the length of the sensitive area. Its average light resistance at 2 foot-candles is 160 thousand ohms. It exhibits a light to dark current ratio greater than 10,000 after 2 foot-candles and 5 seconds in the dark. The Cl-3 photosensitive material is embedded at the end of a clear poly-ester plastic rod measuring $\frac{1}{8}$" in diameter and $\frac{1}{2}$" long with the leads emerging at the opposite end. Changes in incident light alter its electrical resistance inversely so that a decrease in light increases the photocell resistance, and the consequent change in voltage drop across the cell may be amplified and recorded.

The photocell was enclosed in a preformed cylindrical plexiglass light housing shown in Figure 1A.


a
bevel 45°
holes, .043",
for leads
b
c
d
e
f
(A)
R.O.RAWSON
STRITCH SCHOOL OF
MEDICINE
1959

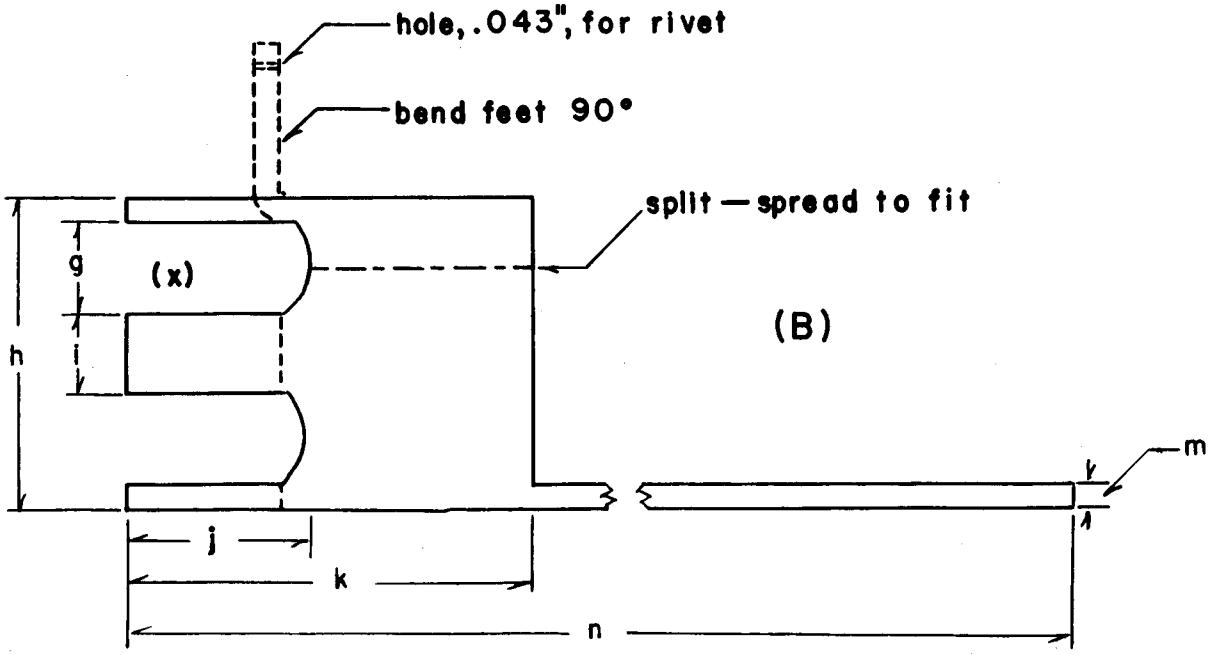

hole, .043", for rivet
bend feet 90°
split — spread to fit
g
(x)
h
(B)
m
j
k
n

FIGURE 1

SUGGESTED SPECIFICATIONS FOR PLETHYSMOGRAPH HOUSING AND METAL CARRIAGE

(A) Housing -- cut from a one inch piece of ½" plexiglass rod:

(a) 6/32" (d) 17/32"

(b) 7/32" (e) 0.25"

(c) 7/8" (f) 0.5"

Polish surfaces inside (a), at bottom (e-f), and beveled top, with tripoli and rouge.

Cement aluminum foil over all outer surfaces with Duco Cement. Coat with black enamel after hardening cement overnight.

Photocell inserts at (e), and leads project through holes bored above. Prevent light leaks in coating of photocell by recoating with black enamel; let dry.

Cover lower half of outside of housing with a piece of plastic friction tape to prevent metal carriage from scratching enamel and foil.

(B) Clip carriage -- milled from a two inch piece of ½" brass tubing with a 1/32" wall thickness:

(g) ¼" (j) 5/16" (n) 2"

(h) ½" (k) 5/8"

(i) 1/8" (m) 1/32

(x) - mill with side cutters to depth of 5/16"

Split remaining collar as indicated; spread and form to fit snugly around housing.

Feet, bent 90° to collar, are riveted to a one inch metal ring cut from thin gauge metal sheet. Ordinary straight bankers pins make satisfactory rivets.

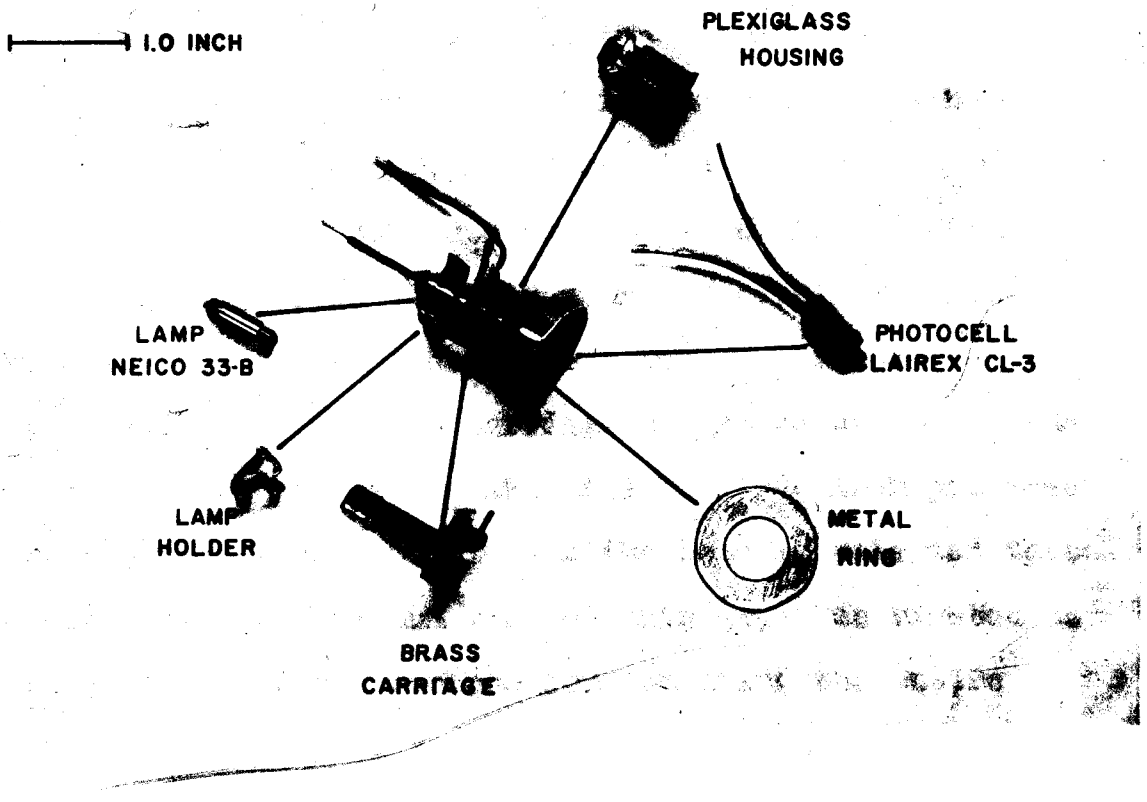
A small battery clip for holding lamp, and providing ground terminal, is bolted or riveted to upper end of upright post of metal carriage. Clip is bent to extend over lamp opening in housing (a).

A suggested alternative is to turn the plastic housing down to dimensions from a piece of 1" plexiglass rod, leaving a foot flange 1/8" thick and 1/4" wide. This eliminates the need for carriage feet and metal ring. Using this alternative, the depth of the collar would then be (k-j) or 5/16", and (n) would be approximately 1 5/8".

The photocell was cemented in place with vinyl cement, with its transparent face flush with the larger end of the plastic housing, and its leads extending through small holes in the 45 degree beveled top. A depression was bored at the top of the housing to accomodate the glass envelope of a small incandescent lamp, (Neico 33-B; National Electric Instrument Co., Elmhurst, N.Y.) The incandescent lamp was fitted to a metal clip riveted to a brass carriage milled according to the specifications shown in Figure 1B. The carriage then provided a support for accurately positioning the lamp in relation to the plastic housing. An aluminum ring of thin gage was riveted to the carriage to provide a surface for cementing the entire instrument to the skin, or to hold the instrument against muscle when skin flaps are approximated over the ring with suture clips.

The housing was covered with bright aluminum foil, then coated with black enamel to exclude extraneous light. Light from the lamp is reflected 90 degrees by the mirrored top of the housing and directed into the tissue under observation. Light returning from the tissue strikes the face of the photocell and alters its resistance according to the variations in intensity of the returning light. An exploded view of the completed detector is illustrated in Fig. 2A.

MINIATURE PHOTOELECTRIC
PLETHYSMOGRAPH



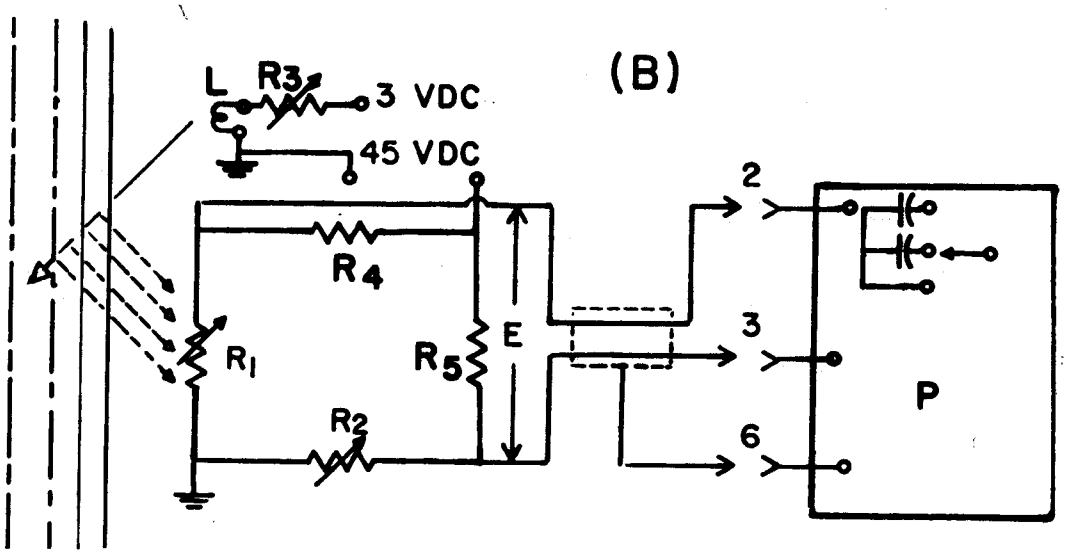


Figure 2.

The detector photocell is connected into a bridge circuit, Figure 2B, through a shielded lead of #26 stranded copper wire, and 1½ volts dc directed to the lamp by way of a second lead, the braided shield acting as a common ground. The circuit employs the photocell as one leg of a resistance bridge, balanced by a variable resistance approximately equal to the resistance of the photocell when it is operating under optimal illumination of the tissue at which there is minimum heating (see section on heating, page 58) but adequate sensitivity to light changes, reduces the photocell resistance to approximately 120 thousand ohms. Variations in photocell resistance produce changes in e.m.f. across the bridge, (E of Fig. 2B)

OPERATION CHARACTERISTICS OF THE DETECTOR

The maximum potential that may be applied to the detector photocell is limited by its 50 milliwatt power dissipation rating. Since the power consumption is a function of the current flow, and since the current is dependent upon the electrical resistance influenced by incident light, the level of illumination falling on the cell must be of such a magnitude as to prevent excessive current flow due to the applied potential. It was determined that the power consumption did not exceed the maximum rating when operating on 90 volts dc, with a constant illumination of one half the maximum available. Later it was

determined that 45 volts applied potential provided sufficient sensitivity (to pulsatile changes in incident light), and that since the photocell was insulated in a plexiglass housing which was subjected to additional heat from the incandescent light source, it would be advisable to operate the detector at the lower voltage to avoid possible overheating of the photosensitive unit.

The illumination intensity must also be considered from the standpoint of its effect on two other factors, maximum wavelength of emission and photocell saturation.

The light emission of an incandescent body is of a broad spectrum, but the wavelength at which its maximum emission occurs is a function of the absolute temperature of the emitting body. The relationship is expressed in the Displacement Law of Wien (38) which states that as the temperature of a radiating object increases, the wavelength of maximum radiation becomes shorter. This is represented by the formula:

$$L_{max}T = \text{constant}$$

where L_{max} is wavelength of maximum radiation, and T the absolute temperature. The fact that L_{max} is displaced with a change in T does not necessitate adjusting lamp voltage to an optimum value in order to insure that L_{max} is in a spectral band to which the photocell is maximally sensitive, i.e. 7250 Angstroms. To adjust lamp voltage until peak radiation lay in the

band at this wavelength would result in such a low temperature, that useful radiation energy would be seriously reduced. However, using a lamp voltage of 1½ volts provides sufficient filament heating to supply adequate radiation energy over a broad spectrum which encompasses the band of maximum spectral sensitivity of the Cl-3 photocell.

If, on the other hand, the lamp is energized with maximum operating voltage of 3 volts, its absolute temperature is considerably increased, thereby shifting its L_{max} into shorter visible wavelengths to which the Cl-3 is less sensitive.

When the lamp is operated at 3 volts the intensity of total radiation reaching the photocell is also increased. A limiting property of photosensitive materials is that they can become light saturated, and will undergo no further change with an increase in incident light. "Saturation" of a photosensitive element makes it insensitive to small light changes regardless of their wavelength. Saturation, or an approach to it, is therefore to be avoided by reducing the filament voltage, which concomitantly avoids over heating of the detector and tissue, at the same time shifting L_{max} toward the near infra-red wavelengths to which the cell is most sensitive.

CAPACITANCE COUPLING VS DIRECT COUPLING.

Changes in the potential difference across the bridge (Fig 2B) constitute the output of the detector circuit. Any change in the resistance of the photocell due to a change in its incident illumination will be reflected in a potential difference across the bridge output. The light returning from the skin is altered by two major variations in the cutaneous blood flow, the rapid change in tissue blood with each pulse, and the slower change in total tissue blood as the vascular bed drains or distends. There are then two major waveforms encountered when photoelectrically recording variations in tissue blood, a fast one and a relatively slow one. Neither of these waveforms is a pure sine wave, but each is made up of component frequencies. The major frequency components of these two waveforms, however, are different enough in magnitude to allow solution of a problem one encounters when attempting to amplify a small pulsatile change, when at the same time there may be a significant change occuring in the total tissue blood. Great amplification of the former would also amplify the latter, and over a period of several seconds the change in the total blood of the tissue may be several times that of the rapid pulsatile change in blood volume. Amplified fast waves (pulsatile) may

then be accompanied by an amplified slow wave (total blood) which may easily drive a recording device off scale. The fact that the major frequencies making up the two wave forms are different in magnitude, permits separating them and recording only the faster wave.

If the output of the balanced bridge circuit is fed into a dc amplifier, and the output of the amplifier used to drive a photokymograph or a pen writing oscillograph, any change in the total blood of the tissue will cause a steady dc voltage to appear across the bridge, driving the amplifier to change the base line. In a linear system the base line change will be proportional to the alteration in incident light to the photocell. The changing base line then reflects the change in total blood in the tissue. Superimposed on the base line is a record of the rapid alterations in incident light caused by the pulsatile variations in blood content. The combined signals then appear as the record shown in Figure 3A. If a capacitor of proper value is put in series with the bridge output (Fig 3B) the amplifier will no longer be affected by steady dc voltages at the input, and will only respond when the voltage impressed on the capacitor is changing.

This property of a capacitor is expressed in the formula $q/c = E$, (77) where q is a quantity of electricity (in coulombs)

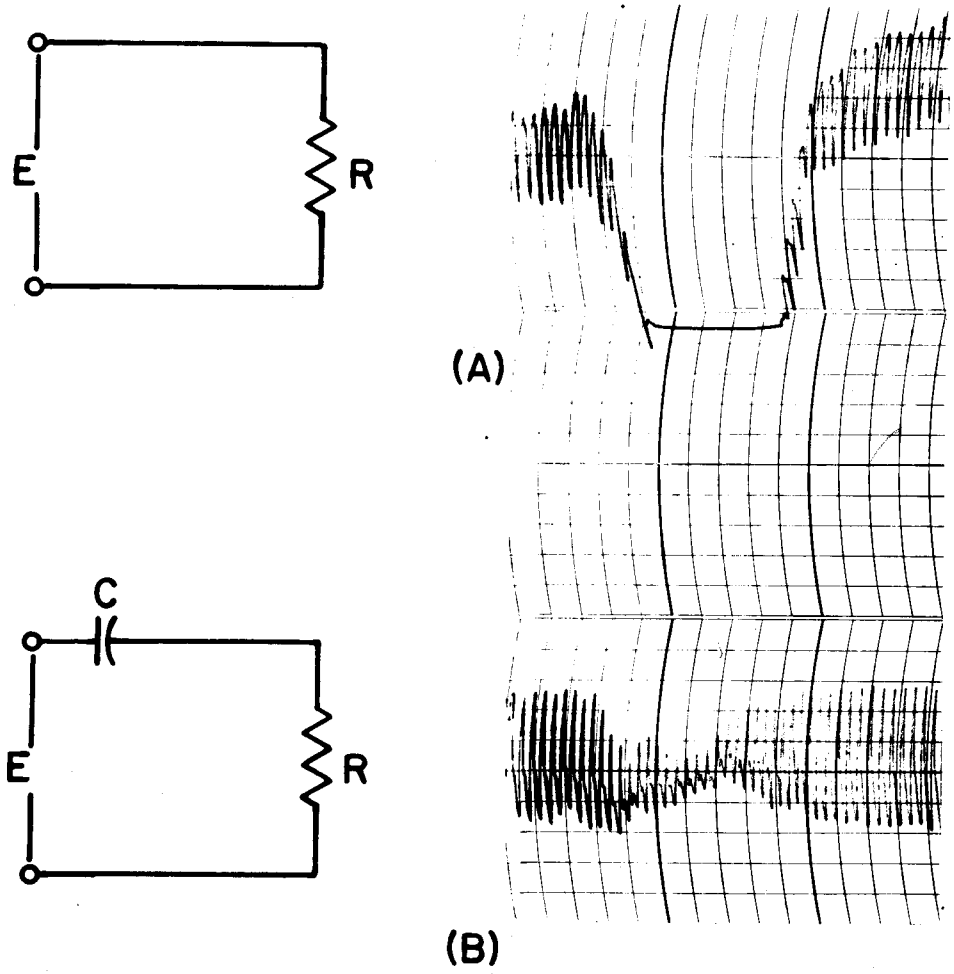


FIGURE 3. THE EFFECT OF CAPACITANCE COUPLING THE PHOTODETECTOR

called the "charge" consisting of an excess of electrons "piled up" on one side of the capacitor. C is the capacitance constant in farads, and E the impressed e.m.f. in volts. As E changes (as a consequence of changes in the photocell resistance in the bridge circuit,) the charge q also changes.

A "charge" of electrons on one side of the capacitor causes a flow of electrons away from the other side through the resistance R of the amplifier input. The electron flow is in proportion to the magnitude of q, and will continue as long as the charge is building up, or discharging. The rate of flow of these electrons (current) will also depend on the rate of change in q, which in turn is a function of E.

It is evident then that when C is constant, a steady voltage E will build up a maximum charge q, at which time a steady state will obtain, and current will cease to flow through the amplifier input resistance. If, however, E is constantly changing as it does as a consequence of pulsatile light changes, a proportionally changing current will flow through the input resistance R. From the above equation, as the charge on a capacitor increases, the voltage across it (which opposes the current flowing into it) also increases. The longer the current flows, the more opposition it meets, therefore a current which reverses frequently meets less opposition. The resistance to a flow of current which a capacitor imposes on a circuit therefore

decreases as the frequency of change increases. Likewise, as a capacitance is made larger in value, its resistance to current flow decreases, hence the larger the capacitor the closer it comes to allowing a steady dc current to flow through the circuit.

This property of a capacitance permits determining a value for a circuit component which will pass a changing current of a minimum frequency. Thus, the slow wave form due to change of total blood in the tissue may be largely eliminated from the total signal output of the bridge circuit, permitting only the rapidly changing pulse wave form to pass through. With such a scheme, the pulse wave form may be amplified many times, and, providing the capacitor is of the proper value, the base line "shift" due to slowly changing dc potentials will not appear on the record. The total excursion of the writing pen, then, is available for a pulsatile record without danger of losing the signal because of a permanent base line shift off scale, the base line remaining relatively stable as shown in Figure 3B.

CIRCUIT NOISE

It is not possible to record an infinitely small signal by simply employing infinitely large amplification. This is because there is generated in the signal source and in the

amplifier, random electrical disturbances which may be of such a magnitude as to obscure the signal.

Most modern commercial amplifiers have reduced noise inherent in their circuitry to such an extent that this factor presents no great disturbance in recording most biological potentials. It is the signal source which usually contributes significant random disturbance or noise.

Photoconductors are especially noisy, and the magnitude of noise generated may vary between detectors made of the same material. The noise is essentially made up of so called "thermal" noise which is a function of the absolute temperature and resistance of the element, and "current" noise which varies as the direct current passing through the element.

The pulse of the palmar surface of the thumb produces a potential difference across the bridge of from 40 to 80 millivolts. The voltage signal is large enough to provide an excellent signal to noise ratio, allowing amplification and recording of a smooth wave form. In other skin areas however, especially the abdomen, there appears to be considerably less pulsatile flow available for producing a good signal to noise ratio.

The inherent unfiltered noise of the C1-3 photocell, as measured on the amplification system of the Grass polygraph, amounts to a little less than 0.5 millivolt in some cells.

In some instances then, where the pulse signal is no more than 2 millivolts, the noise signal is 25% of the pulse signal, and the resulting recorded wave form is significantly distorted.

The problem then is to sufficiently amplify the wave form, while selectively filtering the higher frequencies which constitute the noise signal. This must be done without impinging on the higher frequencies found in the pulse wave.

It was discovered that by filtering out all the frequencies constituting the noise signal, that the amplitude of the pulse wave is significantly reduced. It is evident therefore that a compromise must be reached whereby sufficient noise is filtered from the total signal, while preserving a satisfactory amount of the pulse wave signal.

The Grass polygraph Model 5, equipped with a 5P1A preamplifier, provides an excellent amplifying and recording system which fulfills the requirements of the detector circuit as discussed above. Its response is essentially linear from 0 to 40 cps. Its preamplifier input selector provides either direct or capacitance coupling, and the filtering circuit of the driver amplifier permits the attenuation of unwanted noise signal frequencies. With the driver amplifier filter set at ½ amp 15, only ½ of the amplitude of a 15 cps signal is permitted to pass.

The higher the frequency above this value, the more it is attenuated, frequencies of 60 cps being completely attenuated. At the above filter setting, it has been found that most of the unwanted noise frequencies are eliminated from the signal without seriously reducing the amplitude of the pulse wave form.

FREQUENCY RESPONSE OF THE COMPLETE CIRCUIT.

The linearity of frequency response of the entire capacitance coupled system was determined by intercepting a source of incandescent light falling on the face of the photocell with a revolving perforated disc of constant speed. Using several discs with different numbers of perforations permitted changing the frequency of light activation of the photocell. The rate of rise and decline of each light pulse was identical, the perforation diameters being identical and the speed of rotation of the disc constant. The intensity of the light source was varied by changing its distance from the photocell, thus avoiding variations in spectral output of the light source which would obtain if its intensity were varied by altering its heating current.

As the intensity of the incident illumination became progressively less, the sensitivity of the photocell to each light pulse began to show evidence of decreased amplitude.

By simultaneously measuring the dc resistance of the cell (which is a measure of incident illumination), the sensitivity of the cell to each light pulse could be related to its incident illumination.

With the driver amplifier filter at ½ amp 60, the frequency response of the entire system is linear from 1 to 20 cps at any given level of illumination of the photocell. A decrease in the latter reduces photocell sensitivity to pulsatile light changes. However, the change in incident illumination produced by the two physiological extremes of complete blanching, to maximum distension of the human cutaneous vascular bed of the thumb by venous occlusion, reduces photocell sensitivity by only 5%. Under most experimental conditions therefore, the maximum error that could be expected in the recorded pulse would be negligible.

CIRCUIT TIME CONSTANT AND TRANSIENT BASE LINE SHIFTS.

When the photocell detector circuit is capacitance coupled to the Grass preamplifier through the input selector setting indicated as having a time constant of 0.1 second, and the detector is applied to a vascular area, the recorded pulse exhibits a relatively stable base line.

There are transient base line shifts however which reflects rapid alterations in total blood in the tissue. The magnitude of these transients are a function of the value of the coupling capacitor, and their duration is related to the time constant of the system.

The time constant of the input circuit (Figure 3B) is a function of the product of the resistance and capacitance, R and C. It may be defined as the time taken during capacitor discharge for the voltage across the capacitor to reach 63.2% of its final value, (the time taken for total discharge being theoretically infinite). Referring again to the equation $q/C=E$ when the capacitance is charged, E is maximum and hence will drive a current through R rapidly. As the charge is lost, q/C and hence E become less and the current (given by ohms law as E/R is also less. Thus the rate at which the charge q declines is rapid at first and becomes progressively slower; an exponential discharge. The time constant is expressed simply as $T = RC$, where T is in seconds, R is in megohms, and C in microfarads. (16).

When the base line of a pulse record undergoes a rapid transient change due to alteration of total blood in the tissue as a consequence of rapid vasoconstriction or vasodilatation, the delay of the transient in returning to the base line reflects the time required for this transient charge to dissipate from the capacitor in the circuit through the input

resistor. The shorter the time constant, then, the less time required for the transient base line change to disappear. Since the time constant is equal to RC, and since R is fixed, then the only recourse for shortening the time constant, thus producing a smoother pulse recording, is to decrease C.

As mentioned earlier, however, (page 35) the larger the value of the capacitance, the less its resistance to current flow becomes. Likewise, as its value decreases, the less it will be able to pass changing currents of lower frequency. Therefore, in attempting to attenuate the higher frequencies of the slower wave form which alter the base line, one may at the same time be attenuating the lower frequency components of the rapid pulse wave form. If vasomotor activity is intense, as it may be in electrical stimulation of sympathetic nerves, change in total blood in the tissue may be quite rapid, and important frequency components of both wave forms may overlap.

It is evident, therefore, that again a compromise must be reached whereby the desired pulse wave form is not sacrificed in attempting to attenuate the slow wave form. It is not objectionable to have a slight transient shift in base line accompanying a change in the pulsatile recording since it is, in effect, a confirmation of the validity of a recorded change in pulse amplitude. That is to say, when a decrease in

recorded pulse amplitude reflecting a vasoconstriction obtains, one would anticipate a decrease in total blood of the tissue. Hence if the base line drops momentarily at the onset of vasoconstriction, and then returns to normal during the vasomotor activity, this can be interpreted as a decrease in total blood, and confirms that vasoconstriction is taking place.

It will be noted later (Fig 10) that a slowly developing vasoconstriction produces no noticeable drop in the base line, whereas in Figure 11, when the vasoconstriction is complete in 3 to 4 seconds, there is a noticeable decreasing base line transient. The latter is interpreted as a rapid decrease in total blood, producing a wave form containing frequencies high enough to pass the capacitance coupling.

It is this momentary change in base line, or base line transient, which must be controlled by regulation of the time constant. If the time constant is too long owing to a large value of C, the more of the slower frequencies of the slow wave will come into the amplifier, thus driving the base line too far. The time for the transient to disappear will also increase. Likewise, the more rapid the total blood change, the more higher frequency components its wave form will contain, and hence the greater the magnitude of base line shift.

When the detector circuit output is fed to the Grass preamplifier with its time constant at 0.1 second, a small base line transient appears in the recording. When the time constant

is 0.8, a larger transient appears, (Fig 4 A&B). It will be noted that with the longer time constant there is a corresponding increase in pulse amplitude, owing to the larger input capacitance which allows more of the wave frequency components to pass.

Either record would be satisfactory under circumstances where the pulse signal is large, and the gain of the amplifier can be kept low. Under circumstances where the pulse signal is low, e.g. abdominal skin, the amplifier gain is purposely raised. Increased amplification of a small pulse signal also amplifies the higher frequencies of the slow wave form, and if the time constant is 0.8, the base line transient may be large enough to drive the recording pen off scale.

In all the experimental procedures employed in this investigation, it was found that a time constant of 0.1 second provided a satisfactory regulation of base line transients.

RECORDING TOTAL BLOOD IN THE TISSUE WITH THE PHOTOELECTRIC PLETHYSMOGRAPH.

The miniaturized photoelectric plethysmograph was designed for recording, through capacitance coupling, pulsatile blood flow changes in a vascular bed during electrical stimulation of the sympathetic trunk. The capacitance coupled system permits greater amplification of low signal pulse waves without

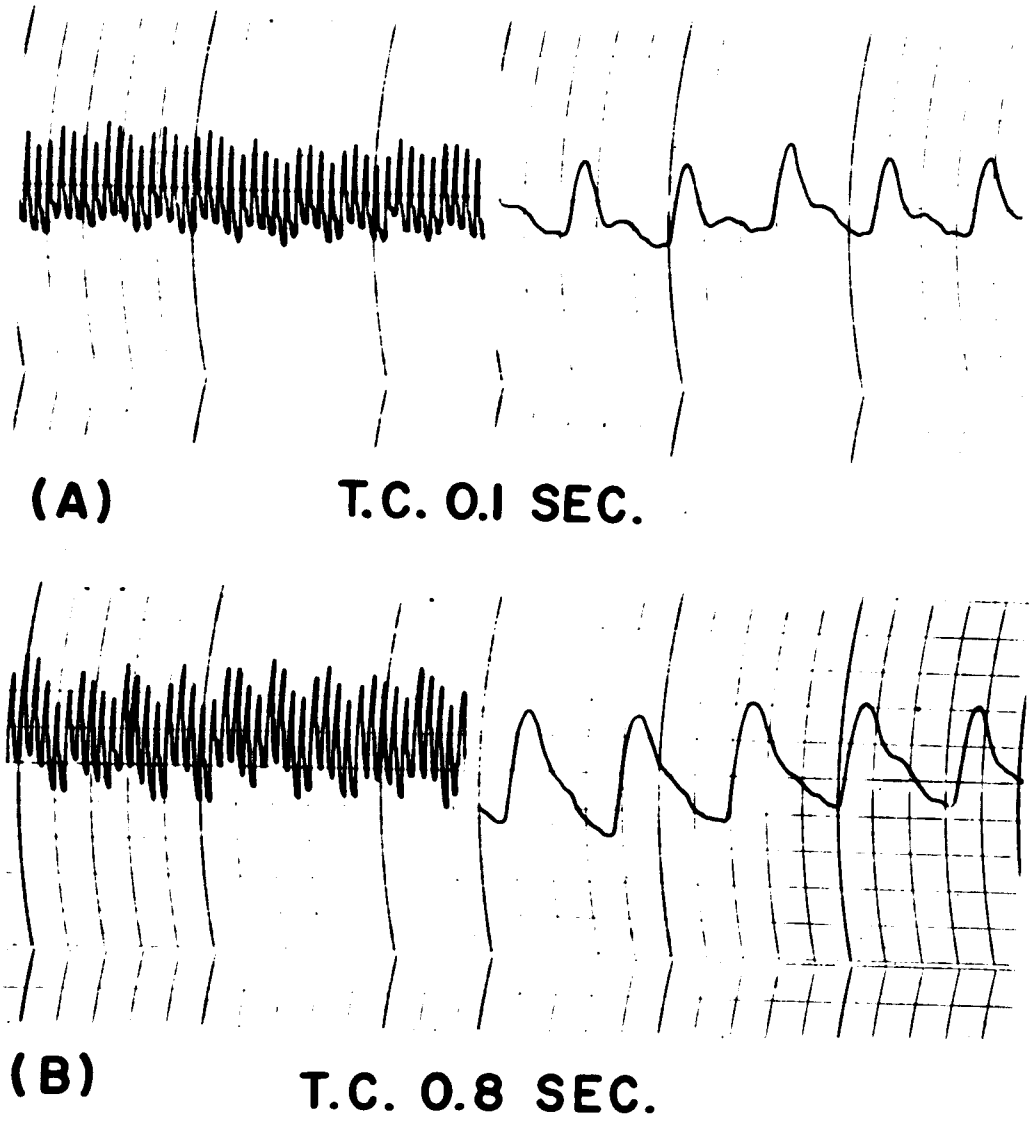


FIGURE 4. THE EFFECT OF CIRCUIT TIME CONSTANTS

without significant base line shift. The instrument is nevertheless applicable to the recording of rapid changes in total blood in a tissue when pulse waves are too small, or absent altogether. Such circumstances are found to exist in certain occlusive vascular diseases wherein the pulse wave is damped out before reaching the terminal vascular bed, or sometimes in the less vascularized areas of the thigh skin of animals. When this is the case, a record of the change in total blood in the tissue during sympathetic stimulation may be used to indicate the presence of innervation of the vessels involved.

Observation of rapid changes in total blood in the tissue is accomplished by directly coupling the bridge circuit to the preamplifier without the use of a series capacitor, and balancing the bridge until the voltage across it is zero.

Two characteristics of the Cl-3 photocell, however, make this procedure extremely limited in its application when using the circuitry and construction described.

One of the characteristics of the cell which can greatly influence the validity of a directly coupled recording is its high temperature coefficient of resistance, amounting to 0.6% per degree centigrade rise in temperature. This value is extremely high for an electronic component not designed to act as a temperature sensing device. Yet because it does perform in this manner, alterations in its resistance can occur with

changes in its temperature which could be interpreted as alterations in total blood.

Fortunately, however, the temperature of the plastic enclosed detector, which rises due to heat from the incandescent light source and from the warm tissue to which it is attached, does stabilize after a thirty minute warmup period. The resulting base line drift then becomes insignificant when recording rapid total blood changes. Figure 5 shows a thermal drift curve constructed for a representative Cl-3 photocell. It will be noted that even after only ten minutes warmup time, the line drift is only 1 millivolt or 10,000 ohms per minute. On the other hand, a rapid change in total blood in tissue such as is exhibited in the palmar surface of the thumb when the breath is held in deep inspiration, results in a 0.75 millivolt per second decrease in detector output, or a 7500 ohm per second decrease in photocell resistance due to decreasing tissue blood. In limited experimental circumstances, therefore, a direct coupled system can be used to advantage.

Completely stabilizing the detector unit to temperature drift is, however, quite feasible, and could be accomplished by balancing the photocell resistance change due to heating, with a temperature sensing component incorporated into the photocell.

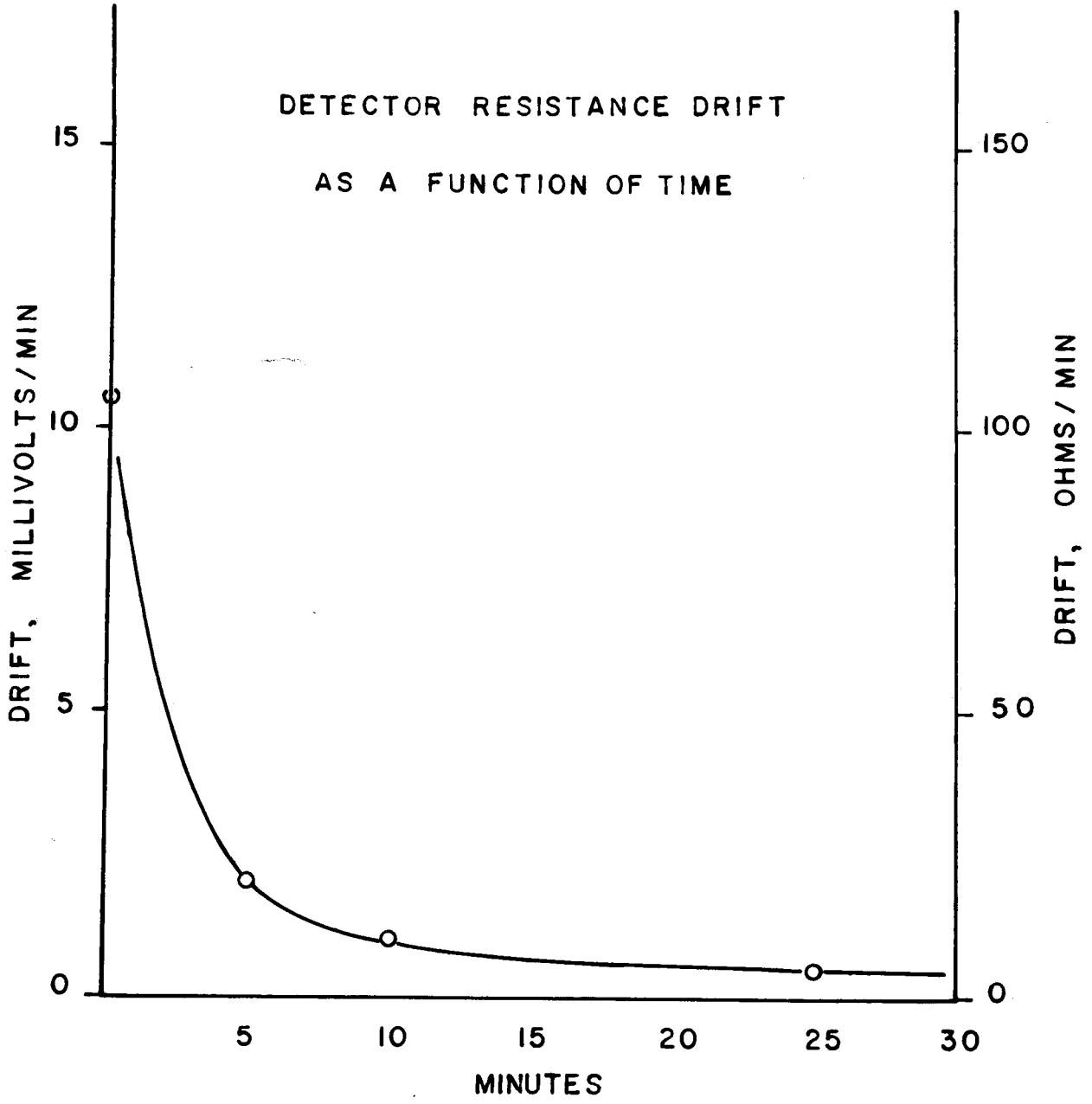

DETECTOR RESISTANCE DRIFT
AS A FUNCTION OF TIME
DRIFT, MILLIVOLTS/MIN
DRIFT, OHMS/MIN
15
10
5
0
150
100
50
0
5
10
15
20
25
30
MINUTES

Figure 5.

The second characteristic of the photocell which may complicate its use in a direct coupled system for observing total blood changes is its range of spectral sensitivity. The peak sensitivity of the Cl-3 to light is at 7350 Angstroms. As shown in Figure 6, this peak falls at a point where the transmission characteristics of HbO and HHb are widely divergent (47). Thus significant changes in oxygen saturation of the blood due to alteration in ventilation, pH changes and/or blood temperature variations, could all produce pronounced alteration of incident illumination, giving a false indication that total blood content of the tissue was changing. This is, in fact, the principle of photoelectric oximetry as worked out by Matthes (61) and developed in this country by Millikan (63).

The detector, then, is capable of operating as an oximeter. With a slight modification of the detector construction, however, this deficiency may be largely corrected. By covering the face of the photocell with an infra red filter (XRX 70; Polaroid Corp.) which cuts off its transmission sharply above 8000 Angstroms, only the lower end of the spectral range of sensitivity of the Cl-3 is utilized where it rapidly drops off below 8000 Angstroms. Although theoretically reducing the overall sensitivity of the unit, this places its peak sensitivity at approximately 8000 Angstroms, and the two transmission curves of hemoglobin cross at 8050 Angstroms.

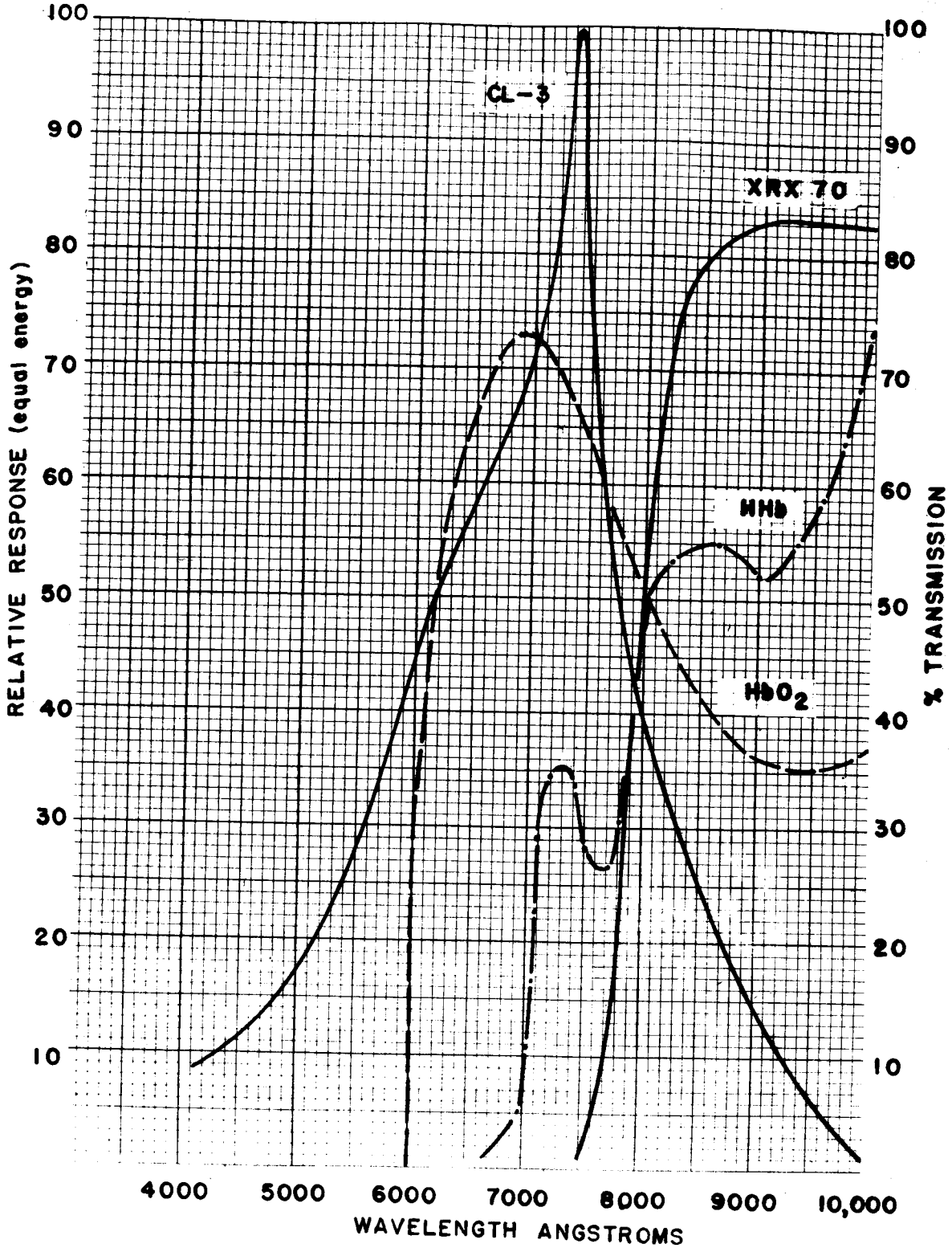

CL-3
XRX 70
HHb
HbO2
RELATIVE RESPONSE (equal energy)
% TRANSMISSION
100
90
80
70
60
50
40
30
20
10
4000
5000
6000
7000
8000
9000
10,000
WAVELENGTH ANGSTROMS

FIGURE 6

By shifting the peak sensitivity to the right on the infra-red scale, the maximum response is then further into the range of infra-red, where pigmented and non-pigmented skins become more and more equal in transparency as the wavelength of incident light becomes longer (38). The wavelength of light now being used to activate the photocell is less affected by darkly pigmented skin. A detector operating in longer light wavelengths requires less intense illumination to penetrate densely pigmented tissue, hence heating effects are decreased.

The interposition of an infra-red filter also removes wavelengths of light emitted by the hot filament (Wien's displacement law, page 30) which may tend to bring the photocell closer to saturation. Only useful wavelengths are then allowed to pass, and the Cl-3 is affected only by wavelengths to which it is most sensitive, saturation being minimized and sensitivity thus increased, in spite of the fact that the filter removes wavelengths to which the cell would respond. This explains the paradox that the photoelectric detector gives a pulsatile output of essentially the same magnitude, whether or not the photocell face is covered with the apparently "opaque" filter.

A later model of the photoelectric plethysmograph incorporating an infra-red filter as described, has been constructed and tested, using a modification of a procedure by Matthes for studying absorption characteristics of blood films. (62).

A glass chamber was constructed of two standard microscope slides. The slides were separated by two narrow glass strips 1 x 2 mm cemented along the outer edges of their two interfaces, and polyethlene tubing (0.125" O.D) was cemented to the open ends. This provided a glass chamber, which when filled with blood, created a uniform film 1 mm thick. On one side of the chamber was affixed a Cl-3 photocell, and through the other side was directed a beam of incandescent light. The beam could be intercepted by an XRX 70 filter. All extraneous light was eliminated by suitable covering of the components. 100 ml of heparinized dogs blood was then divided into two 50 ml portions, one saturated with O_2 by bubbling with the gas, while the other was bubbled with nitrogen until its color had deepened to the typical dark shade of venous blood. Oxygen content was estimated by the Roughton-Scholander micro-gasimetric technique (71).

After thoroughly flushing the blood chamber with nitrogen, the reduced blood sample was anaerobically transferred to the chamber. The dc resistance of the Cl-3 was measured and recorded on the Grass polygraph.

The chamber was then flushed with oxygen, and oxygenated blood transferred to it, followed by another resistance measurement. By repeating this procedure with an XRX 70 filter intercepting the light beam, data was collected which showed the relative effects of HHb and HbO on the resistance of the photocell both with and without an infra-red filter.

In table I., the photocell resistance in ohms is tabulated under the headings HHb and HbO. The presence or absence of the filter is indicated by a plus or minus sign respectively. As shown by the difference in light transmission between HbO and HHb without the filter in trials I and IV, a significant oximetric effect is possible in the detector when not covered by a filter. With a filter, however, this characteristic is largely eliminated.

TABLE I. EFFECT OF INFRA-RED FILTER ON PHOTOCELL RESPONSE.

Trial	Aver. photo cell resistance in ohms		XRX70 Filter	Aver. Difference in Transmission Hbo-HHB	
	HHB	Hbo		Ohms	%
I	147K	113K	-	-34K	-23
II	137K	147K	+	+ 10K	+ 7
III	175K	165K	+	- 10K	- 5.5
IV	188K	193K	+	+ 5K	+ 2
V	186K	129K	-	- 57K	-30

CHAPTER IV

ATTACHING THE INSTRUMENT, AND EVALUATING ITS PERFORMANCE

To attach the photoelectric detector to cutaneous areas, a thin layer of surgical appliance cement (Davol #262) is applied to the surface of the aluminum ring and also to a metal applicator having the same shape and area. The cement on the applicator is immediately transferred to the skin. After one or two minutes of air drying, the two layers of cement are approximated and the detector is held fast, where it remains firmly fixed until removed. A drop of mineral oil, previously placed on the face of the photocell, increases light transmission and greatly improves the performance of the detector.

To affix the detector to an animal muscle area, a one inch skin incision is made, taking care to avoid cutting the underlying muscle. Blunt dissection of the fascia separates the skin from the muscle in a circular area to accomodate the aluminum ring under the skin. After dissecting the fascia, the exposed muscle is bathed in mineral oil, and the detector ring inserted into the incision and held in place by approximating skin flaps over the ring with wound clips.

EVALUATION OF THE RECORDED PULSE WAVE FORM.

If the signal from a detector placed on the palmar surface of a finger be directly coupled to the preamplifier, its pulsatile wave form faithfully reproduces the volume pulse that is recorded simultaneously from a volume plethysmograph enclosing another finger of the same hand. Figure 7B shows the volume pulse of a human subject taken from the 5th digit enclosed in a digital volume plethysmograph coupled to a Statham pressure transducer, the system being filled with warm water. Figure 7A is a record of the signal from a photoelectric detector attached to the palmar surface of the thumb of the same hand, the circuit directly coupled to the amplifier. The amplitudes of the recorded pulses were made equal by adjusting the sensitivities of the amplifiers. It will be noted that the slope and duration of the major components of the pulse wave forms are qualitatively identical.

The wave form in Figure 7A is the "volume pulse" record which Hertzman and Dillon (42, 43) have correlated with the volume pulse of the skin of the finger measured by a mechanical plethysmograph. If a capacitor now be placed in series with the bridge output (Figure 2B page 27), the capacitance coupled system can be shown to respond qualitatively as the volume pulse.

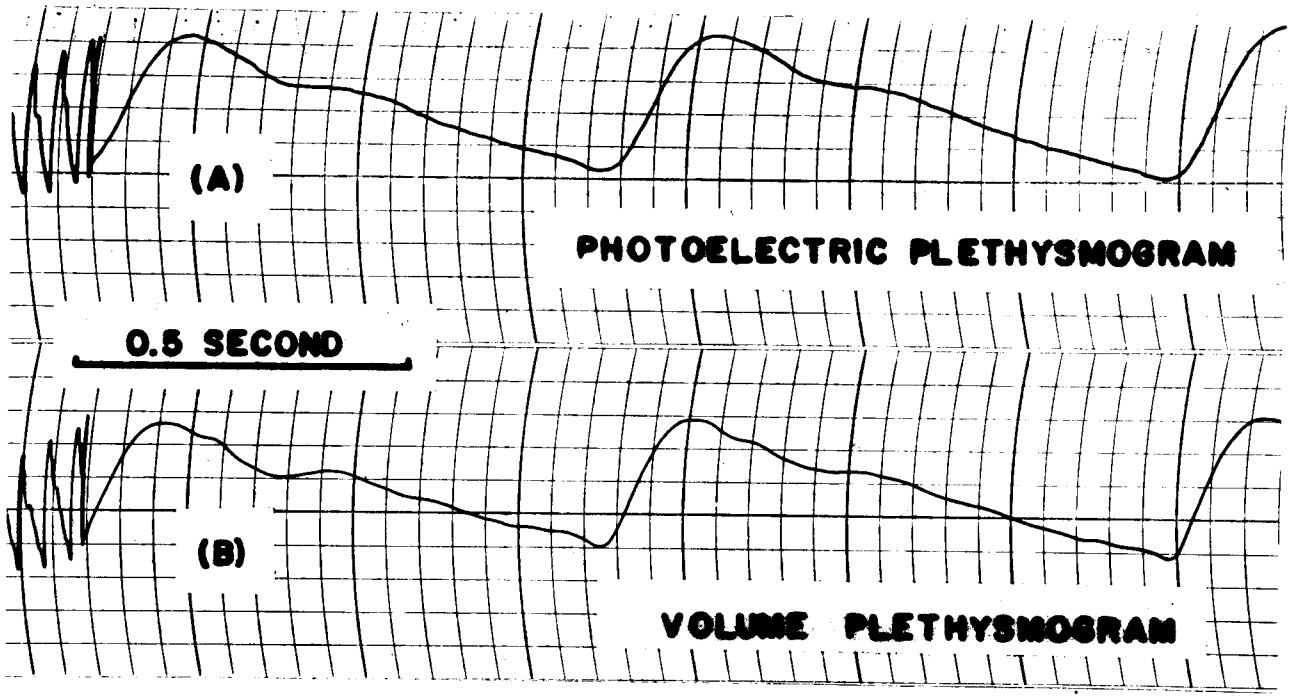


FIGURE 7. A COMPARISON OF PHOTOELECTRIC AND VOLUME PLETHYSMOGRAM WAVE FORMS.

If the first digit of one hand is enclosed in the digital volume plethysmograph, while on the palmar surface of the second digit is placed a capacitance coupled detector, simultaneous records may be taken showing variations in volume pulse and total blood in the first digit, and pulsatile blood flow patterns in the second.

This is illustrated in Figure 8A. It will be noted that as the pulse of the volume plethysmogram decreases, the total blood reflected in a dropping base line is also decreased, and as the volume pulse increases, the total blood increases. These are accepted interpretations of the variations in a volume plethysmogram.

It will be further noted that with each change in the pulse amplitude of the volume plethysmogram, there is a directly proportional change in the pulse amplitude of the photoelectric plethysmogram.

Again it is pointed out that the changes in the total blood of the tissue as reflected in the changing base line of the volume plethysmogram are largely eliminated in the capacitance coupled photoelectric plethysmogram, a factor which permits great amplification of the pulse signal without "going off scale".

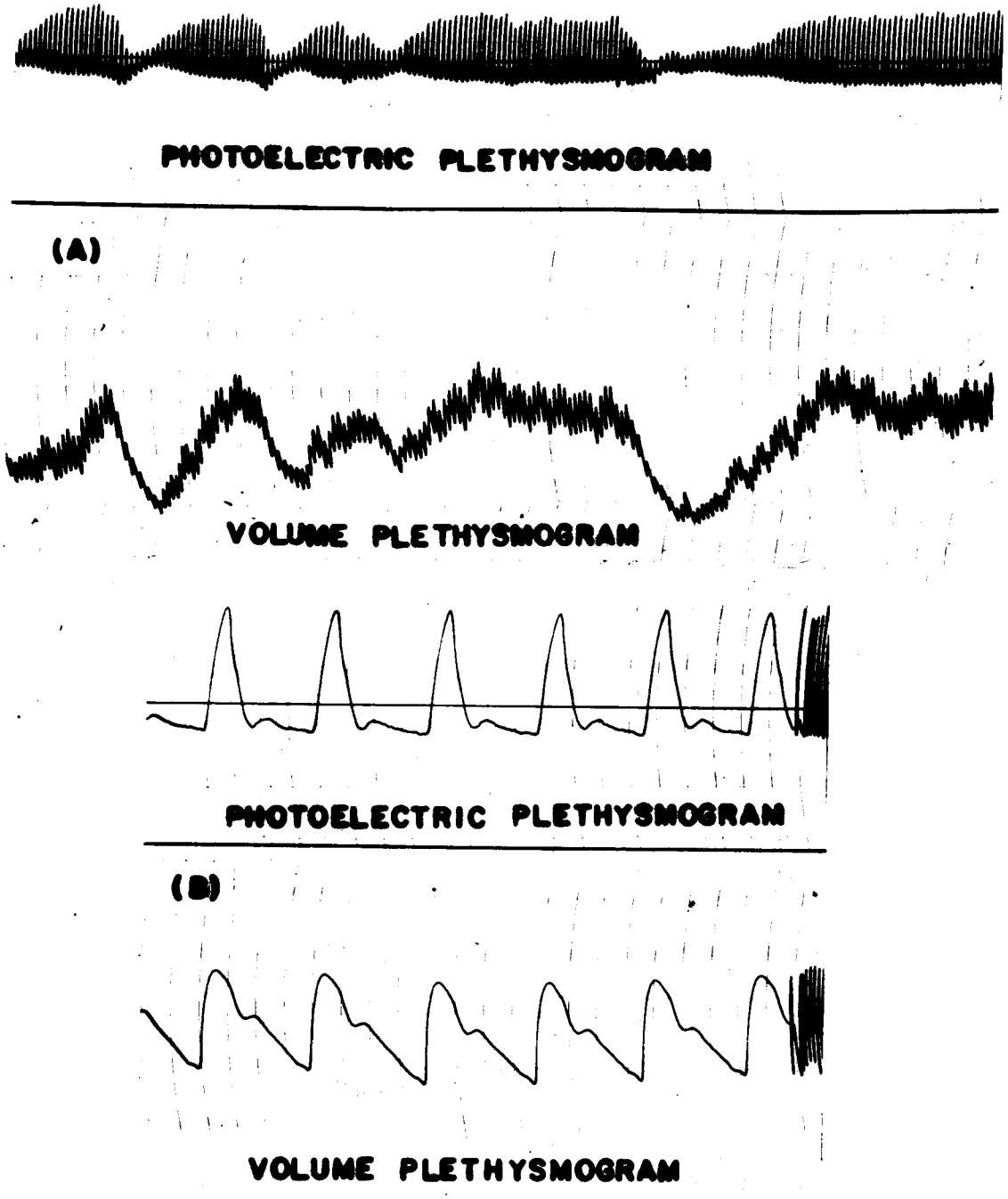


FIGURE 8. PHOTOELECTRIC (CAPACITANCE COUPLED) AND VOLUME PLETHYSMOGRAMS. (A) PAPER SPEED 2.5 MM/SEC. (B) 25 MM/SEC.

Figure 8A offers evidence of the ability of a capacitance coupled photoelectric plethysmograph to accurately reflect changes in the volume pulse.

The nature of a single pulse of the photoelectric plethysmogram may be studied in Figure 8B, wherein the same plethysmographic recordings of part A are separated by speeding up the graph paper. The pulse amplitudes were made approximately equal by adjusting the amplifier gains.

The character of the two pulses differ markedly. The lower wave form resembles the typical peripheral pressure pulse, with its ascending and descending limbs and dicrotic notch. The upper wave form has corresponding but altered components. The alterations in the photoelectric plethysmogram pulse result from capacitance coupling of the detector to the amplifier.

To explain the behavior of the upper wave form, we must refer again to the equation $q/C = E$. As the volume pulse ascends (blood filling the vascular bed), the photocell becomes darker, its resistance goes up, and the voltage drop (E) across it increases. The charge q increases with E and continues to do so until the volume pulse reaches a plateau, at which time the capacitor immediately begins to discharge, E decreasing toward zero potential at a rate governed by the

time constant of the system as previously described, (page 39). The immediate commencement of discharge accounts for the sharp peak of the photoelectric plethysmogram, whereas the volume plethysmogram (actual blood volume) peak is more rounded. If the volume pulse remained at its peak value, the photoelectric pulse would drop to zero signal and remain there. If, after total capacitor discharge, the volume pulse then descended, an opposite voltage would appear across the capacitor because the voltage change across the photocell would now be in the opposite direction (voltage drop E now <u>decreasing</u>) due to a decrease in resistance. In this case, the photoelectric pulse would then descend below the base line and return again to zero when the volume pulse reached another plateau. However, the ascending peak of the volume pulse is <u>not</u> maintained, but commences to descend even before the capacitor has completely discharged to zero signal. This response lag is again due to the time constant of the system. The photoelectric signal decreases but is immediately halted because of another plateau in the volume pulse, the dicrotic notch. Again the voltage E across the capacitor begins to reverse and return toward zero, but due to the response delay, does not reach zero before the volume pulse has again swung upward and then downward, causing E to follow its direction. The descending limb of the volume

pulse ends abruptly with the commencement of another ascending limb, and the slower responding capacitor charge (photoelectric pulse) follows.

This is the nature then of the single pulse of the capacitance coupled photoelectric plethysmogram. The slopes of its ascending and descending components being a function of the slopes of the corresponding components of the volume pulse, and at the same time dependent on the time constant of the circuit.

HEATING EFFECTS OF THE DETECTOR

Inasmuch as the photoelectric plethysmograph is a source of long wave radiation, and is covering live tissue which would otherwise be open to direct heat loss through convection, radiation, and, in the case of skin, evaporation, it may be justifiably asked, how much heating of the tissue obtains due to the use of the instrument?

With a thermocouple placed between the detector and the skin, temperature readings were made over extended periods of use. With the light source illuminating the tissue adjusted to the intensity recommended earlier (page 28), it was found that there is an increase in thermocouple temperature of 0.1 degree C after seven minutes of operation. The temperature

then stabilizes at this point, and unless ambient temperature changes, the thermocouple temperature remains unaltered. At the same time, no observable increase in cutaneous pulsatile flow was noted. It was concluded therefore that heating effects associated with the detector induce no observable effect on the pulsatile blood flow.

THE PURE CUTANEOUS PULSE

Since the detector illumination penetrates the tissue, it may be questioned whether the pulsatile wave form recorded from the cutaneous area is really due to blood changes in the skin, or whether some of the returning pulsatile light is due to changes in underlying muscle blood flow.

This question was investigated by making a skin incision in the thigh of the dog 2 to 3 inches from the site of application of the detector to the skin. After making a narrow tunnel separation of the skin from the muscle extending from the incision well under the locus of the detector, a piece of aluminum foil one-half inch square was inserted through the tunnel and placed under the detector between the skin and the muscle. This check was routinely performed several times, and in every instance the amplitude of the pulse record was unaffected by the insertion of the foil. It was concluded, that although the illumination may penetrate the skin, the layers of

fascia separating skin from muscle provide sufficient impedance to the light to prevent vascular changes in the muscle from affecting the cutaneous record. This is confirmed by the fact that it was necessary to dissect the fascia off the muscle before an adequate muscle recording could be obtained.

PHYSICAL CHANGES THAT ARE BEING RECORDED IN THE TISSUE

It is not known what portions of the vascular bed are actually causing a pulsation of light returning from the tissue.

Direct observation of capillaries by numerous investigators has failed to show any pulsatile flow in these vessels. Personal observations on human skin in the knee using a bright light source and viewing isolated capillary loops through a drop of mineral oil with a stero microscope have disclosed nothing but constant flow with periodic disappearance of the capillary. Upon observing vascular beds in the same manner in the abdominal skin of dogs, entire sections of terminal beds were visible including the terminal arterioles, and there was no indication that any of these vessels were pulsating. It is probable, however, that the pulsatile changes are due to alterations in absorption of light quantitatively related to the increase and decrease in blood volume of the vascular bed with each pulse.

The possibility existed that the pulse wave was a result of tissue movement by large pulsating vessels, hence a change in the incident light due to altering the angles of reflection from tissue.

Investigation of this question disclosed that when isotonic saline or clear plasma was substituted for blood perfusion of a vascular bed under pulsatile pressure, the recorded pulse wave disappeared. A perfusion system employing a Brewer automatic pipetting machine to generate a pulse pressure was used to force fluids through the vascular bed of the foot pad of the dog via a cannula in the femoral artery. Perfusion of heparinized whole blood produced a pronounced pulse recording. Without interrupting the fluid column, clear plasma was introduced into the pump inlet from another reservoir. In 3 to 4 seconds the pulse recording disappeared and the perfusate returning via the corresponding femoral vein and passing through polyethylene tubing was at first pink then faded to a clear plasma indicating that the vascular bed had been completely flushed with plasma. Immediately behind the plasma, a 2% solution of Methylene blue in saline was introduced, and in 3 to 4 seconds a pulse wave of large magnitude was recorded. In approximately 15 seconds, however, the pulse amplitude began to decrease and finally disappeared, even though the dye solution continued to return through the veins.

The disappearance of the pulse wave with the introduction of plasma perfusate, and its reappearance with Methylene blue solution (which is more opaque to red light than hemoglobin) is interpreted to mean that pulsatile changes in returning incident light are dependent on the presence of a light absorbing material in the fluid of the vascular bed. The subsequent disappearance of the pulse wave during methylene blue perfusion was attributed to the staining of the extravascular vluid and tissue by the dye which completely absorbed wavelengths of light to which the Cl-3 photocell is sensitive. Subsequent dissection of tissues of the foot pad showed them to be deeply stained with methylene blue.

CHAPTER V

CUTANEOUS AND MUSCLE VASCULAR RESPONSES TO ELECTRICAL STIMULATION OF THE LUMBAR SYMPATHETIC TRUNK IN THE DOG

METHODS

Twenty one dogs of both sexes averaging 12 kilograms in weight were anesthetized with an intraperitoneal injection of 32.5 mg/kg Nembutal sodium in saline. Animals with a minimum of skin pigmentation were selected to achieve maximum light transmission through the tissue. The hair of the right hind limb was clipped, shaved, and the skin thoroughly washed with soap and water. Photoelectric detectors were affixed to the large central foot pad, lateral calf and thigh skin areas, and one placed in direct contact with the gastrocnemius muscle of the same side through a one inch skin incision. Blood pressure was measured from the common carotid artery with a Statham P23AC pressure transducer coupled to a Sanborn Model 127 recorder.

300-500 ml of 3.5% PVP in saline was administered to each animal through slow i.v. drip during the course of each experiment to augment blood volume.

The abdominal cavity was completely exposed by a midline incision extending from the public symphysis to the xiphoid process, and the viscera deflected to the left and covered with cotton towels soaked in warm saline.

The right sympathetic trunk was carefully exposed from the level of the second lumbar vertebra to the pelvic brim, and loose cotton ligatures were carefully placed around interganglionic segments of the trunk, avoiding trauma to the individual rami. A sketch of the trunk, its ganglia and connecting rami was later correlated with the site of stimulation, location of the vertebral bodies, and the recorded vascular responses.

It was deemed imperative that, prior to any stimulation procedures, anesthesia of the roots of the cord be performed by subdural injection of 2 ml of 3% procaine at L1 through a spinal trocar. The presence of afferent fibers in the sympathetic trunk which may reflexly elicit sympathetic efferent discharge have been reported (23, 49). Reflex vasodilation in the hand elicited by heating the foot and leg was shown by Kerslake (48) to be abolished by lumbar sympathectomy, although the reflex could still be obtained on application of radiant heat to the body surface. The reduction of so called "cold" pain by sympathetic denervation of an extremity also indicates

the presence of sympathetic afferents which may be capable of eliciting reflex vasoconstriction. The segmental nature of some of these reflexes have been demonstrated by cutaneous vasoconstriction originating at isolated vertebral levels during urinary bladder distension (2). The elimination of such possible reflexes was obviously necessary if accurate interpretation of sympathetically elicited vasomotor activity in the hind limb was to be obtained.

The criterion for judging adequate block of reflex vasomotor activity was the direct observation of the release of vasomotor tone in the lower extremity as reflected in the increases in recorded pulse amplitude of the vessels under observation. Also, animals without spinal block served as controls in testing this procedure. In the controls, stimulation of the sympathetic trunk at the highest available level (L2) elicited pronounced vasoconstrictor activity in the vessels of the lower extremity (Table II).

Repetitive square wave stimuli of 3 volts, 5 milliseconds duration, and 10 impulses per second were delivered to the interganglionic segments through bipolar electrodes from a Simpson electronic stimulator. Stimulation voltage was monitored on a calibrated oscilloscope. The parameters for stimulating sympathetic fibers has been determined on the basis

of investigations of Bronk et al (5) who showed a discharge rate of about 1 to 3 impulses per second in "resting" isolated sympathetic fibers, and by Folkow (20) who showed 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. The specific stimulation parameters used in the present experimental procedures were based on those selected for sympathetic trunk stimulation by Randall and his associates (68).

The segment of the trunk was carefully lifted and its surface and adjacent tissue gently sponged to reduce current spread. Spread of current was detectable by the movement of neighboring skeletal muscles, or by a sharp drop in stimulating potential observed on the oscilloscope.

The records were analyzed by expressing the vascular response in terms of a pulse amplitude ratio. The amplitude of the volume pulses was measured to the nearest 0.5 mm before and at the end of each stimulation. The amplitude of the pulse is a function of the pulsatile blood change in the tissue, and preamplifier gain. In order to evaluate the relation between the pulse amplitude changes in each vascular bed, the pen excursion was converted to millivolts (the product of the pulse amplitude in centimeters times the mv/cm sensitivity

setting of the preamplifier). The mv ratio of the pulse amplitude before, to that at the end of stimulation, gave a value of relative change which may be interpreted as a measure of vasomotor activity in the tissue due to the stimulation of vasomotor fibers. A ratio of unity indicated no change in pulse amplitude while a ratio of less than unity, or more than unity, indicated an increase or decrease in pulse amplitude respectively.

The inherent noise in the complete system was measurable as a small, non-synchronous pen deflection. When the pulse record decreased to the noise level, its millivolt value was considered zero, and the change ratio thereby became infinity.

RESULTS

Figure 9 shows the effect on pulse amplitude observed in the muscle and skin of the lower extremity of one dog following procaine spinal anesthesia at L1. The release of prevailing vasoconstrictor tone in the skin vessels is evident. There appeared to be no vasoconstrictor tone in the muscle blood vessels at the time of anesthesia in this particular animal. Comparing final pulse amplitude in millivolts with those recorded before spinal block reveals a distinct gradient

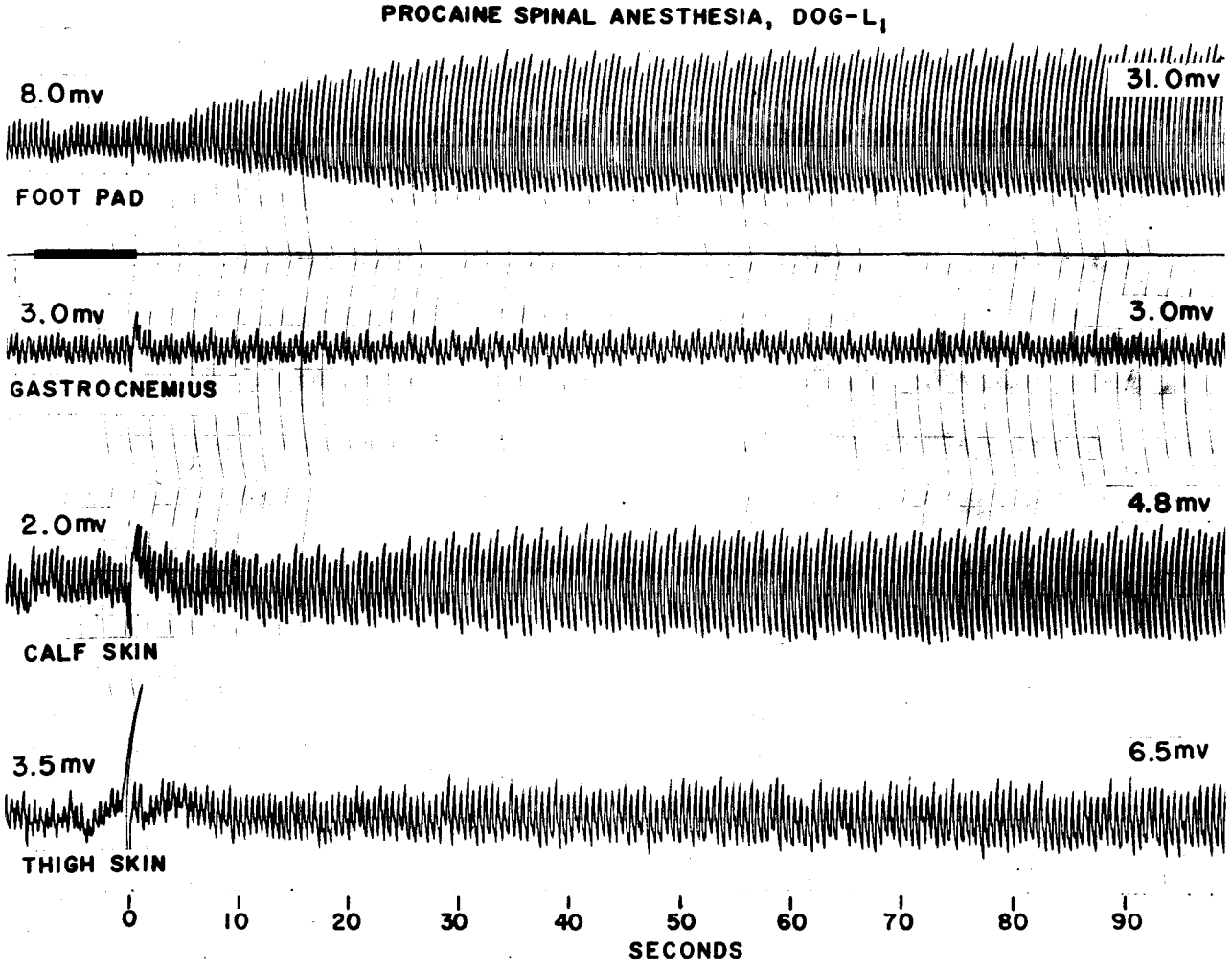

PROCAINE SPINAL ANESTHESIA, DOG-L1
8.0mv
31.0mv
FOOT PAD
3.0mv
3.0mv
GASTROCNEMIUS
2.0mv
4.8mv
CALF SKIN
3.5mv
6.5mv
THIGH SKIN
0
10
20
30
40
50
60
70
80
90
SECONDS

Figure 9.

in vasomotor tone decreasing from the foot pad to the thigh skin. It is evident that no significant change occurred in the gastrocnemius muscle.

Similar evidence of release of vasomotor tone was observed in the other animals of the series when spinal block was performed. Almost without exception the skin areas exhibited a pronounced increase in pulse amplitude, while the muscle pulse showed little or no increase.

It is believed that the procaine block provided adequate insurance against reflex vasomotor effects during lumbar trunk stimulation. In three control animals without spinal block, pronounced vasoconstriction was induced in the hind limb when the trunk was stimulated at the L2 level. The recorded vasomotor activity of the controls expressed in change ratios is tabulated in Table II. It will be noted that in every case a ratio of more than unity, indicating vasoconstriction, was elicited in two or more areas at this level, the activity in the foot pad being especially pronounced. As will be noted in later data, animals with spinal block exhibited no vasoconstrictor activity when stimulated at L2.

Figure 10 illustrates a pattern of vascular response in three cutaneous and a muscle bed in the hind extremity during electrical stimulation of the lumbar sympathetic trunk. Systemic blood pressure is shown at the top of the figure.

TABLE II. CHANGE RATIOS (SEE TEXT, PAGE 68) INDICATING PRONOUNCED VASOCONSTRICTION IN THREE CONTROL ANIMALS WITHOUT SPINAL BLOCK DURING SYMPATHETIC TRUNK STIMULATION AT L2 AND L3. T-THIGH SKIN; C-CALF SKIN; G-GASTROCNEMIUS MUSCLE; P-FOOTPAD.

Vertebral Level of Trunk Stimulation	Location of Pulse Detectors	ANIMAL NUMBER		
		1	2	3
L2	T	2.2	1.4	1.6
	C	1.4	1.7	1.2
	G	1.0	1.0	1.4
	P	1.0	29.0	11.0
L3	T	2.7	7.0	1.2
	C	1.1	4.1	1.7
	G	1.0	1.2	1.5
	P	1.2	inf.	19.0
L4	T	1.9	3.3	2.8
	C	1.3	2.5	2.4
	G	1.0	1.0	1.0
	P	2.1	inf.	inf.
L5	T	2.3	2.5	1.7
	C	1.2	2.1	2.0
	G	1.0	1.2	-
	P	5.4	inf.	inf.
L6	T	3.4	3.1	1.0
	C	1.4	3.3	1.4
	G	1.3	1.9	2.0
	P	13.0	inf.	inf.
L7	T	2.1	4.3	-
	C	1.0	2.5	-
	G	1.0	1.2	-
	P	1.0	inf.	-

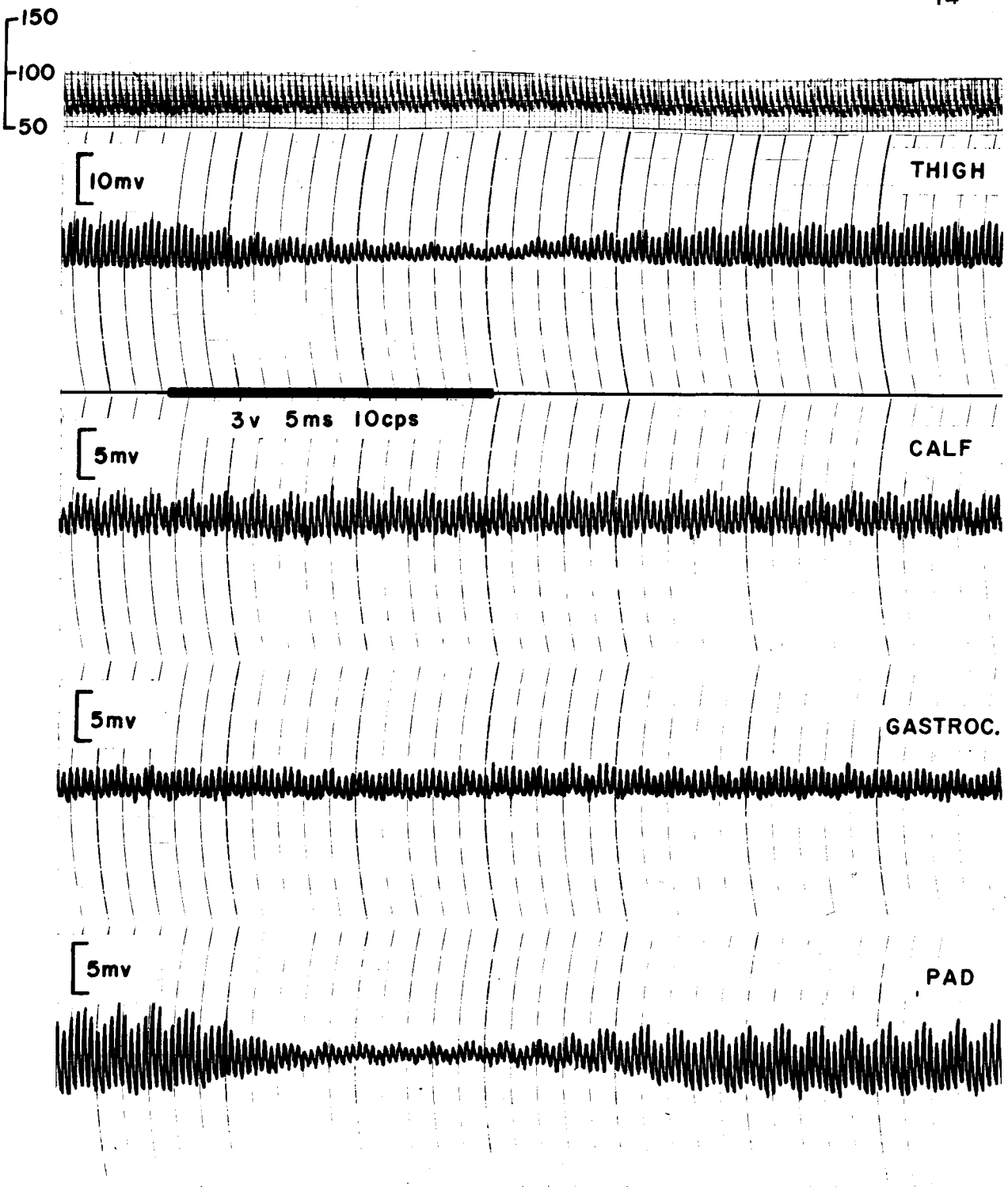

B.P.
mm Hg
150
100
50
10mv
THIGH
3v 5ms 10cps
5mv
CALF
5mv
GASTROC.
5mv
PAD
0
10
20
30
40
50
60
SECONDS

FIGURE 10

The stimulus was applied at L3 and induced a progressive decline in pulse amplitudes on both the thigh and foot pad with no significant alteration in calf skin or in gastrocnemius muscle. Constriction in thigh and pad skin attained moderate intensity at approximately the same time, and remained at this level until the stimulus was removed. Recovery of pulse amplitude began after four to five seconds, and gradually approached pre-stimulation values somewhat more promptly in thigh skin than in the foot pad. Expressed in terms of relative changes in pulse amplitudes, the mv ratio in thigh skin was 3.4 compared to a ratio of 4.4 in the foot pad before and during stimulation.

Figure 11A illustrates the simultaneous reactions of blood vessels in the same areas during stimulation at the L6-L7 interganglionic segment of a different animal. Although the cutaneous vessels all showed definite constrictor responses, the muscle pulse amplitude increased significantly during the stimulation and slowly returned to control levels during the recovery period. Atropine (0.3 mg/kg 1.v.) subsequently abolished the muscle dilatation, whereas skin vessel responses were unaffected.

Movement of the electrodes to a position immediately below the ganglion at L7 (Fig 11B) elicited more pronounced

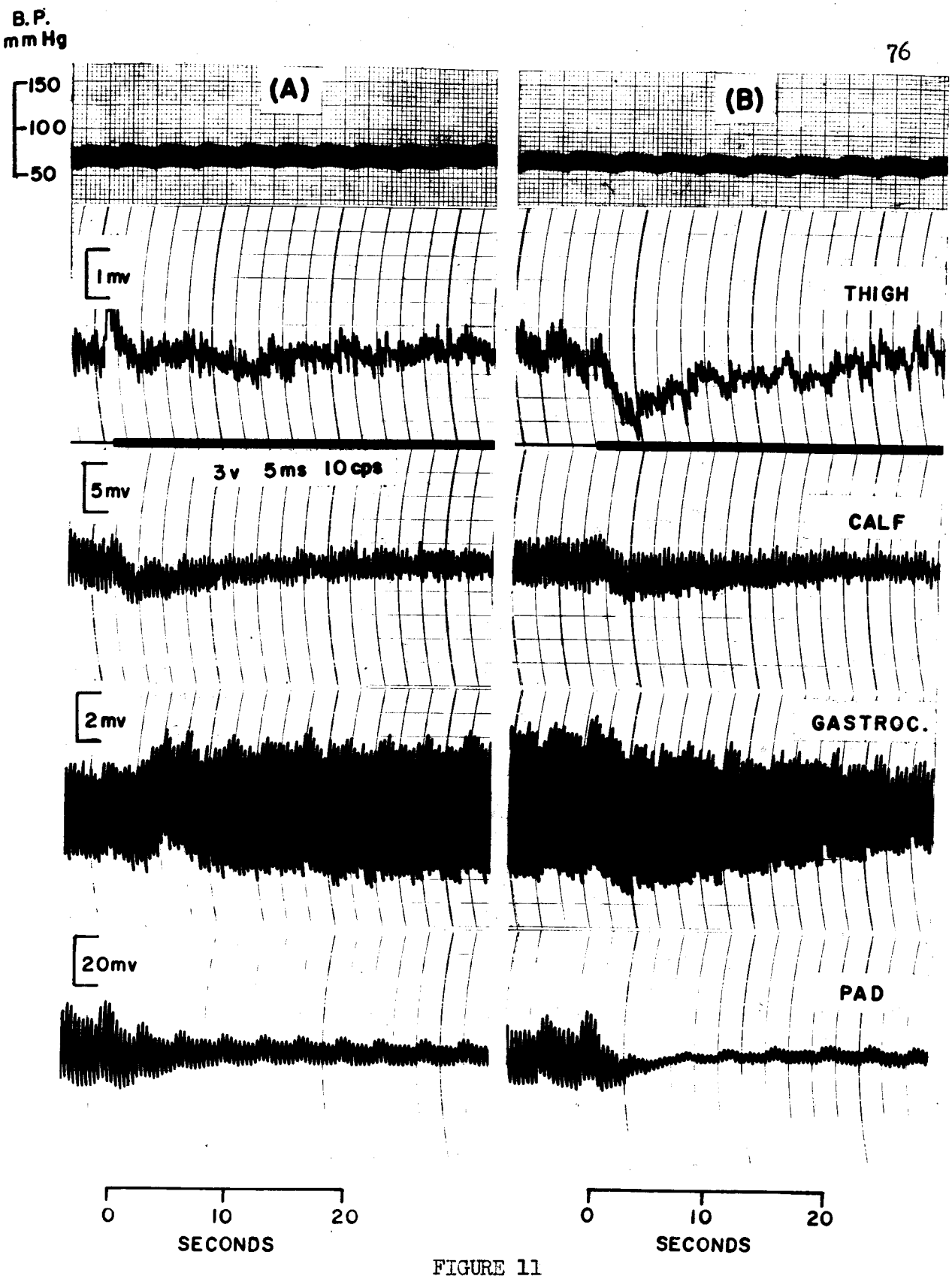

B.P.
mm Hg
150
100
50
(A)
(B)
1 mv
THIGH
3 v 5 ms 10 cps
5 mv
CALF
2 mv
GASTROC.
20 mv
PAD
0
10
20
SECONDS
0
10
20
SECONDS

FIGURE 11

constriction in the thigh and foot pad with little difference in response of calf skin vessels, when compared with Figure 11A. Muscle vessels, on the other hand, showed a definite constriction instead of dilation. It is probable, therefore, that vasoconstrictor fibers innervating the thigh and pad skin, as well as muscle vessels, entered the large ganglion at L7.

Although the most common response of muscle vessels to sympathetic trunk stimulation was constrictor, it was never profound, i.e., muscle pulses were never entirely obliterated during lumbar trunk stimulation, and generally showed relatively small change ratios. There was frequently no change in amplitudes of the gastrocnemius pulses even though cutaneous vessels in immediately adjacent skin showed simultaneous obliteration.

Finally, as in Figure 11A, significant increases in amplitude of muscle pulses were sometimes elicited in the absence of significant alterations in systemic blood pressure, and these have been interpreted as dilator responses.

Figure 12 is a composite from five separate stimulations of the lumbar trunk in a single animal and illustrates one common pattern of distribution of vasomotor fibers to the lower extremity. Arterial blood pressure is shown at the top of each set of responses with plethysmograms beneath.

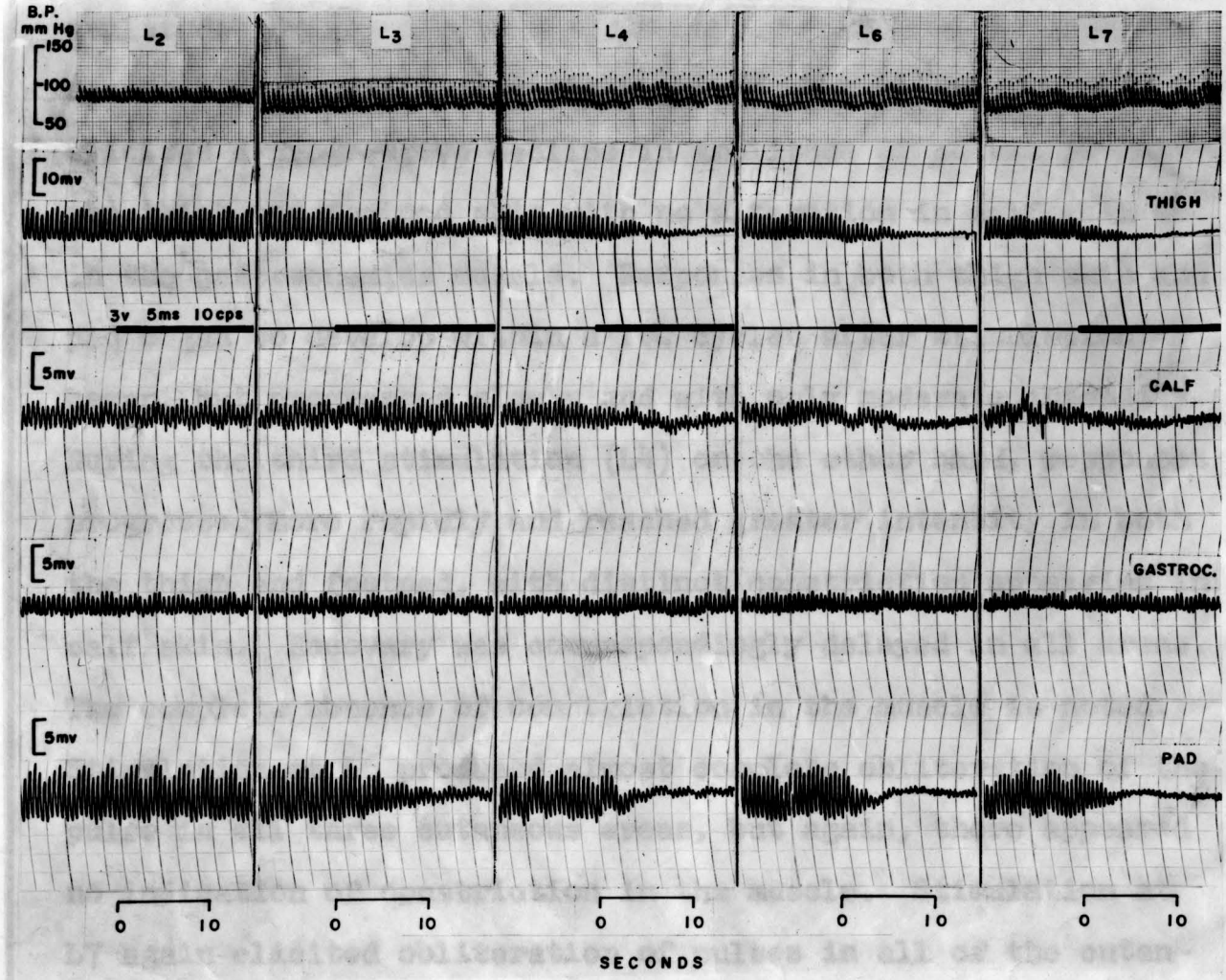

B.P.
mm Hg
150
100
50
L2
L3
L4
L6
L7
10mv
THIGH
3v 5ms 10cps
5mv
CALF
5mv
GASTROC.
5mv
PAD
0 10
0 10
0 10
0 10
0 10
SECONDS

Figure 12.

The vertebral level of stimulation is indicated at the top of the figure. Stimulation at L2 was applied immediately above a ganglion located at the superior border of L3 for a period of 26 seconds without inducing any detectable change in pulse amplitudes on any of the test areas. Movement of the electrodes to a position immediately caudal to this ganglion (L3) elicited a progressive decline in amplitude of pulses in both the thigh and footpad skin with no alteration in calf skin or in the gastrocnemius muscle. Responses in both thigh skin and pad began to develop within a few cycles after stimulation began, but progressed slowly and with only moderate intensity. During the third stimulation (L4) on the other hand, responses progressed more rapidly and reached greater intensity in both the thigh and footpad, with distinct constriction appearing in calf skin. Recovery was correspondingly delayed in all areas. The complete absence of constriction in the muscle is noted. Stimulation at L6 produced almost complete obliteration of the pulse in all three cutaneous areas, but again, there appeared no indication of constriction in the muscle. Stimulation at L7 again elicited obliteration of pulses in all of the cutaneous beds.

Thus, as the stimulating electrodes were moved successively caudad, a distinct pattern of vasomotor innervation of the hind extremity was revealed. There was no evidence of

constrictor fibers innervating any of the test areas in the lumbar trunk at the L2 level. Moderate constrictions in thigh and pad during the second stimulation is positive evidence of the entry of preganglionic fibers between L2 and L4. A more intense response during the third stimulation indicates the entry of additional fibers between L4 and L5, and still others were demonstrated between L6 and L7.

Blood pressure changes were not apparent during stimulation of the more superior portions of the trunk and were only slightly pressor when more inferior positions were excited. All of the above stimulation procedures were repeated at each separate location and the results shown in the figure thoroughly confirmed.

In Table III are shown pulse amplitude change ratios for eleven of the twenty-one animals. Results of stimulation at interganglionic segments extending from L2 through L7 are tabulated showing the change in ratio of pulse amplitude before, to that during stimulation. The table should be carefully examined for the following information: (1) comparative vertebral levels at which responses were elicited in different animals, (2) comparative levels at which responses were elicited in different areas of the same animal, (3) relative intensity of response in different areas, (4) comparison of responses in muscle and skin.

TABLE III

Vertebral Level of Trunk Stimulation	Location of Pulse Detectors	Animal Number										
		1	2	5	6	9	10	11	12	13	14	15
L_2	T	1	1.2	1	1	1	1	1		1	1.0	1
	C	1	1	1	1	1	1	1		1	1.0	1
	G	1	1	1	1	1	1	1		1.0	1.0	1
	P	1	1	1.3	1	1	1	1		1	1.0	1
L_3	T	1.3	1.3		4	1	1	1	1.8	1.6	3.4	1
	C	1.2	1		2.2	1	1	1	1.5		1.0	1
	G	1	1		1.3	1	1	1	1	1	1.0	1
	P	1	1		2.3	1	1	1		5	4.4	1
L_4	T		1.9	1	1.3	1.3	2	6.8	1.7		9.5	2.5
	C		1	4.8	2.0	1.4	3	11	1.5		3.0	1
	G		0.7	1	1.7	1	1.9	1.1			1.0	1
	P		1.2	1.7	3.5	1	9.3	inf	1.3		8.0	1.0
L_5	T	1	1.2	1	3.5	1.8	2	4.7	1	5	14.0	3.8
	C	2.3	1.1	2.3	2.5	2.0	4.3	10.0	1.5	3	6.5	2.1
	G	1	1	1	1.6	1.2	4.0	2.5	1.3	1.9	1.0	1.0
	P	8.5	1.3	2.3	10.0	5.4	8	inf	10.0	inf	6.4	1.8
L_6	T		1	1.2	3.3	2.0	2.3	5.3	1	10.0	3.3	1
	C	2.5	1	3.5	2.6	2.2	2.5	5.5	3.0	3.1	2.8	1.7
	G	1.8	1	1	1.9	0.7	3	1			1.0	1
	P	inf	5.7	4.2	14.0	5.0	7.5	3.6	5.3	28.5	9.5	34
L_7	T	1.5	1.5	2.0	2.5	2.3	1.8	2.5	1.0	inf	12.0	1
	C	7	1.5	3	2.3	1.8	2.9	2.6	1.3	2.2	5.6	1
	G	2.8	1.4	2	2	1.6	1.3	1		1.7	1.4	1
	P	inf	25.0	inf	6.0	9.0	inf	2.7	2.1	4.2	inf	inf

Numerical values are the change ratios (pulse in mv before stimulation/pulse in mv after stimulation) obtained for each area studied during electrical stimulation of sympathetic trunk at posterior borders of vertebral bodies from L_2 through L_7. Unity represents no change in pulse amplitude. A value of more than unity indicates vasoconstriction; less than unity vasodilatation; infinity—pulse disappears. T—thigh skin; C—calf skin; G—gastrocnemius muscle; P—footpad; right hind leg of dog. Stimulation parameters were constant (3.0 v., 5 msec., 10 cps) in all experiments.

(1) It is immediately evident that large variation exists from animal to animal in the levels at which vasomotor responses may be induced in any selected area. In no animals were marked responses elicited by excitation at the L2 vertebral level, and in a few, only relatively small changes were induced by stimulation above L5. In all animals there were significant alterations in the intensity of response as the electrodes were successively moved caudalward. This is interpreted to indicate the successive entry or exit of preganglionic or postganglionic vasomotor fibers respectively at the indicated ganglionic levels.

(2) In most of the animals no vasomotor response could be elicited in any of the test areas by stimulation at the highest available (L2) lumbar ganglion. There was evidence in five dogs that vasomotor fibers innervating the thigh entered the trunk at higher levels than those innervating the footpad. In one animal, on the other hand, vasoconstrictor responses were induced in the footpad at higher trunk levels than in the thigh. Clear evidence of the systematic entry of vasomotor fibers at progressively lower levels of the lumbar trunk which ultimately innervate blood vessels in successively lower portions of the extremity (thigh, calf and footpad, respectively) generally could not be demonstrated. In the majority of instances varying degrees of vasoconstriction in all of the skin

areas were initially and simultaneously recruited by stimulation between L3 and L5.

(3) Without exception, pulse amplitudes in calf and thigh skin were considerably smaller than those on the footpad following spinal block. That is, greater amplification was required to record pulses comparable to those in the footpad. This observation is consistent with the generalization that the footpad has a much greater vascular supply and is provided with a richer vasomotor innervation than the general cutaneous areas. Table III reveals that vasoconstrictor responses, elicited by electrical stimulation of the lumbar sympathetic trunk, are generally more intense in the skin of the footpad than in the other test areas. With few exceptions, when all of the test areas showed constriction, the ratio was largest in pad skin.'

(4) Without exception, changes in pulse amplitude were relatively slight on the surface of the gastrocnemius muscle. A ratio of unity on this area in the majority of animals when stimulated at L2 and L3 indicates little if any vasomotor innervation of the muscle at these levels of the trunk. Modest increases in the ratio at L4 through L7 indicates the entry of a few vasoconstrictor pathways in the more caudal portions. Occasional decreases in the ratio indicate actual

increase in amplitude of pulses during stimulation and must be inteppreted as vasodilator responses. This suggests the possibility of an alternative interpretation of the more modest responses, that the simultaneous excitation of constrictor and dilator pathways may partially cancel so that neither profound dilatation nor constriction results. It is also evident that in the absence of vasoconstrictor tone, modest increases in pulse amplitudes would accompany an elevation in systemic blood pressure, and give the false appearance of an active dilator response.

DISCUSSION

The known presence of afferent fibers in the sympathetic trunk (23, 49) made the use of spinal block anesthesia essential to prevent the complication of vasomotor reflexes during stimulation. The simultaneous and progressive increase in amplitude of volume pulses in all of the cutaneous areas immediately following administration of spinal anesthesia demonstrates the presence of significant (but varying amounts of) vascular tone in all of these vascular beds. The relatively larger dilation which regularly occurs in the footpad as compared with thigh and calf skin attests to the correspondingly

greater neurogenic tone in pad vessels. This conclusion is directly supported by a similarly greater constrictor response in pad skin when the sympathetic trunk was stimulated electrically. A relatively small increase in pulse amplitude in muscle beds during spinal anesthesia indicates correspondingly little vasoconstrictor tone.

Changing patterns of vasomotor response as the lumbar trunk was stimulated above and below each successive ganglion depends upon the presence or absence of vasomotor fibers which innervate each of the test areas. More intense vasoconstriction in a given area indicates the presence of more constrictor fibers under the electrodes. Wide variation in both intensity and distribution of vascular response (Table III) suggest very great differences in the anatomical distribution of pathways from the spinal cord, through the trunk, and to the ultimate neuromuscular terminations in the blood vessel walls. In a few animals, such vasomotor pathways were present in the trunk as high as L2, but in a majority, they entered between L3 and L5. Significant increments in the intensity of response upon stimulation between L6 and L7 provides strong evidence that preganglionic pathways may enter the trunk at much lower levels than conventionally taught. This is in confirmation of earlier studies of vascular responses in the dog (16, 68) and sudomotor responses in man (69).

There is some indication that vasomotor innervation of the more proximal portions of the extremity enters the trunk at somewhat higher vertebral levels than that which supplies more distal regions of the extremity. However, the fact that precisely the reverse relationship sometimes occurs, and the fact that vasoconstriction is frequently first and simultaneously elicited in all areas during stimulation at a single level makes this generalization untenable. It was impossible to predict before stimulation what pattern of vasomotor distribution to the hind extremity would be elicited in a given animal.

Blood pressure changes were minimal during lumbar trunk stimulation. Although there were occasional elevations of five to ten mm Hg during stimulation, they were considered too small to materially influence interpretation of the data. This suggests that a relatively small portion of the total peripheral resistance is mediated by the lumbar portion of the sympathetic trunk.

Although decreases in pulse amplitude recorded from the gastrocnemius were relatively small, they regularly occurred and furnish evidence that this muscle is supplied with at least a few constrictor fibers, coursing through the lumbar sympathetic trunk. An increase in amplitude during stimulation (generally accompanied by constriction in skin vessels)

is interpreted as a true vasodilator response. Such responses should not be surprising in view of the careful studies of Uvnäs and his collaborators (75). Uvnäs has produced muscle dilator reactions from hypothalmic stimulations, and the similar responses observed in our experiments probably represent stimulation of the efferent pathways for vasodilatation through the lumbar trunk. The abolition of this reaction by atropine further identifies the terminal endings as cholinergic.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The design and construction of a highly sensitive, miniaturized photoelectric plethysmograph have been described. The principles underlying the operation of this instrument, and the advantage of capacitance coupling have been thoroughly discussed. An evaluation of the instrument's performance has also been presented.

The following conclusions pertinent to the application of the instrument to the study of rapid vasomotor changes in the hind limb of the dog have been drawn:

1. The photoelectric plethysmograph described is the only instrument presently available which can separately record rapid vasomotor changes in skin and muscle of the hind limb of the dog.
2. The photoelectric detector, miniaturized to minimize its weight, significantly reduces movement artifacts by being attached to the tissue instead of being rigidly fixed in space.

3. Capacitance coupling permits maximum use of the detector's potential sensitivity, which is of a magnitude sufficient to detect vasomotor activity in the less vascularized cutaneous areas of the thigh.
4. The photoelectric detector is susceptible to an initial thermal drift, but after sufficient warmup, the temperature drift is insignificant.
5. It has been shown that unfiltered incident light from the vascular tissue results in the detector having oximetric characteristics, but that this factor is not a significant variable when studying rapid vasomotor activity during sympathetic nerve stimulation.
6. The affects of heat from the detector attached to tissue are negligible, and introduce no significant experimental error, during examination of rapid changes in the amplitude of the pulse wave recording.
7. The pulse wave that is recorded is derived from pulsatile changes in tissue blood content, and although the specific source of this pulsatile phenomenon in the vascular bed is not known, variations in recorded pulse amplitude during sympathetic stimulation reflect true vasomotor activity.

The vasomotor effects on four test areas of the dog hind limb during electrical stimulation of the lumbar sympathetic trunk have been described and discussed.

It is maintained that vasomotor response to electrical stimulation of the lumbar sympathetic trunk depends upon the presance or absence of vasomotor fibers innervating each of the test areas, and that the degree of vasomotor response depends upon the number of vasomotor fibers that are stimulated.

The presence of varying amounts of neurogenic tone in the skin and muscle vessels of the dog hind limb have been demonstrated, and evidence has been presented which confirms the existence of both vasoconstrictor and vasodilator fibers to the muscle. The increase in pulse amplitude in the muscle during trunk stimulation is interpreted as a true dilator response, mediated by a cholinergic mechanism.

The extent of vasoconstrictor innervation of the dog limb muscle cannot be estimated from the data since the presence of simultaneously activated vasodilators to the same area may mask the vasoconstrictor effects. The small vasoconstrictor activity exhibited in muscle as compared to that in skin, together with the evidence of very little vasoconstrictor tone release in muscle following spinal block suggests, however, that there is much less vasoconstrictor innervation of the muscle than there is of the skin.

The demonstration of a wide variation in the anatomical distribution of vasomotor fibers from the lumbar trunk to the blood vessels of the dog hind limb precludes forming a generalization as to the pattern of distribution of these fibers.

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APPROVAL SHEET

The dissertation submitted by Robert O. Rawson has been read and approved by five members of the faculty of the Graduate School.

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

The dissertation is therefore accepted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

Jan. 19, 1961
Date

W C Randall
Signature of Adviser