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CANINE CARDIAC DENERVATION: A STRUCTURAL, FUNCTIONAL, AND CHEMICAL STUDY

by

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INTRODUCTION

The research reported in this thesis is directed towards determining and correlating structural, functional, and chemical changes which may accompany various degrees of cardiac denervation. A detailed morphologic study of the normal cardiac innervation in the dog preceded this investigation. The cardiac nerves delineated for surgery are sympathetic, parasympathetic, and sensory. Functionally, these types of nerves each plays an important role in modifying and maintaining the proper conduction of impulses within the heart. Without these influences, changes are reported to occur in cardiac output (Harada and Willman, 1968) coronary flow, and oxygen and energy utilization (Barta, Breuer, Pappová, and Zlatoš; 1966b) and chemical composition (Jacobowitz, Cooper, and Barner; 1967). These factors may be instrumental in contributing to cardiac rejection in addition to autoimmune responses in heart transplant patients.

Basically, the chemicals norepinephrine and acetylcholine are products of cardiac nerves which stabilize the normal cardiac function; therefore each of these chemicals was quantitatively measured. Because individual cardiac nerves morphologically pass primarily to specific areas of the heart, assays were made from each chamber. In addition, in each chamber, changes in myocardial fiber, vascular, and neural structures were observed using histochemical and histologic stains. Increased catecholamines including norepinephrine have been incriminated in contributing to atherosclerotic blood vessel changes. Coronary arteries of the variously chronically denervated dogs were observed for arteriosclerotic and atherosclerotic changes.

Functional responses, as recorded with electrocardiogram, blood pressure, and heart rate tracings were, taken on animals presurgically, during surgery, immediately following surgery, and after months of chronic denervation. The pre-exsanguination functional recordings were correlated with chemical and structural parameters to find whether relationships existed between the various facets after cardiac denervation or partial denervation.

LITERATURE REVIEW

Differences in Cardiac Innervation

Innervation, though generally regarded as functionally important to the heart, is not consistent in type or extent between species and within individuals of a species. Parasympathetic, sympathetic, and afferent nerves link the heart to the central nervous system in mammals. According to Arey (1954) the human heart sets blood in motion during the fourth week when the embryo has 7 to 17 somites, but nerves first invaded the heart several weeks later. According to Truex, (1955), at birth preganglionic fibers of the vagus nerves had reached the heart, but postganglionic cells were undifferentiated and lacked stainable processes. Vogel, Jacobowitz, and Chidsey (1969) have demonstrated a completely developed adrenergic nerve supply to the calf heart at birth. No sympathetic fibers innervated the fish heart according to Conteaux and Laurent (1958). A recent study by Yamauchi and Burnstock (1968) however suggested an adrenergic supply to fish hearts. Hirsh, Jellinek, and Cooper (1964) have found nerve fibers and ganglion cells within the hagfish myocardium. However, no connection between the central nervous system and heart has been observed in the hagfish (Jensen, 1965). Adolph and Ferrari (1968) found that receptors for catecholamines are evidently present in the salamander heart long before sympathetic fibers invade the heart. They also reported that cholinergic nerve endings may function in the central nervous system before they do in the heart since acetylcholinesterase was first found in the salamander larvae at stage 36. Like isolated mammalian ventricles the hagfish's heart apparently responded to increased filling by

increasing its force of contraction. Randall (1968) indicated that the evolution of a vagal and sympathetic cardiac supply in vertebrates is primarily for more precise control of heart rate which can change cardiac output without changing stroke volume. Stroke volume changes were responsible for changes in cardiac output, and the regulation of heart rate was less precise in fish which may lack a sympathetic and sometimes a parasympathetic nerve supply.

The Extrinsic Cardiac Innervation

A recent study by McKibben and Getty (1968) delineated, in detail the gross extrinsic cardiac innervation in the dog and reviewed previous literature in this area. Extrinsic cardiac nerves were found to arise bilaterally from the vagi, recurrent laryngeal nerves, thoracic ganglia, cervicothoracic ganglia, and vertebral ganglia. Their course and distribution were described. Nerve cells gave rise to plexuses and endings in the heart wall. In the cat according to Nettleship (1936), from the nodose ganglia arose the fibers of the endocardial plexus; except in the apical portion; and the aortic and pulmonary artery plexuses. From the stellate ganglia arose the majority of the epicardial fiber network of the ventricles and the endocardial plexus near the apexes of the ventricles. Some myelinated fibers originated in the dorsal root ganglia and passed to the coronary vessels and others arose from the motor nucleus of the vagus and terminated about the epicardial ganglia.

The central nervous system's role in regulating the heart through the extrinsic cardiac nerves was reviewed by Mauck and Hockman (1967). The sympathetic nervous system's role in cardiac function in the dog was

studied by Mizeres (1955, 1957, 1958). He found that stimulation of the right ansa subclavia or second, third, and fourth thoracic rami communicantes elicited cardioacceleration while stimulation of the left counterparts resulted in augmentation and occasionally cardioacceleration.

Randall, Priola, and Pace (1967) have elicited a rise in systolic intraventricular pressures with or without cardiac acceleration by stimulation of the cervical vagosympathetic trunk of atrophinized dogs. The effects were abolished using an adrenergic blocking agent. This implicated the presence of adrenergic neurons in the cervical vagosympathetic trunk cranial to the vertebral ganglion in the dog. Using electrostimulation in the dog, Randall, Priola, and Ulmer (1963) concluded that sympathetic cardiac nerves from the right side go primarily to the atria and nodal tissue. The ventricular supply was reported as variable. Left sympathetic cardiac nerves were reported as the major supply to the ventricles with a lesser supply passing to the atria. According to Guyton (1966) sympathetic stimulation increases the rate of S-A nodal discharge, increases the excitability of all portions of the heart, and greatly increases the force of contraction of all cardiac musculature both atrial and ventricular. Both alpha-receptor vasoconstrictor and beta-receptor vasodilator sympathetic effects occur in cornary vessels, but coronary artery sympathetic cholinergic vasodilation has been shown by Feigl (1967) not to occur. Bohr (1967) found that small coronary arteries have little or no alpha receptor activity, only beta activity. Large coronary arteries had both alpha and beta adrenergic activity. Relaxation of contracted small coronary arteries resulted where adrenergic solutions were exposed to them. As coronary vessels increased in size alpha receptors increased.

When vessel size reached about 1.5 mm in diameter there was a predominance of contraction over relaxation. Adrenergic innervation of coronary vessels, even arterioles, did not penetrate the media, (Angelakos, King, and Uzgiris; 1968). Adrenergic nerves were found in close association with cardiac capillaries by these authors.

Jacobowitz, Cooper, and Barner (1967) reported that extrinsic cholinergic fibers appeared to terminate primarily in the left atrium and to a lesser extent in the right atrium. Small to moderate numbers were observed in the ventricles. The close apposition of adrenergic and cholinergic nerves was considered to take place in the terminal end net. Although Nonidez (1939) was unable to demonstrate parasympathetic fibers to the ventricles, Tcheng (1951) found both excitatory and inhibitory fibers to both the atria and ventricles though inhibitory fibers were scarce in the ventricles.

In electrophysiologic studies of the parasympathetic cardiac innervation, Mizeres (1955) found that threshold stimulation of the right cervical and thoracic vagus resulted in inhibition of the heart and a strong depression of the P wave. This represented atrial inhibition and suggested right vagal innervation of the S A node. Threshold stimulation of the left cervical and thoracic vagus by Mizeres (1957) resulted in disappearance of ventricular beats and the persistance of a few atrial beats. These nerves carried cardioinhibitory fibers to the left atrium and innervated the S A and A V nodal tissue according to Mizeres (1957). Vagal stimulation, after blocking sympathetic influences, resulted in distinct depression of both ventricular pressures (Pace, Randall, Wechsler, and Priola; 1968).

Stone, Bishop, and Dong (1967) comparing cardiac denervation in the dog by regional neural ablation and after blockage of cardiac sympathetic nerves with propranalol found differences in cardiac output. The former group changed ventricular output solely by change in stroke volume, the latter by changes in heart rate and stroke volume. The conclusion being that increased heart rate in beta blocked animals using propranalol was by a decreased parasympathetic activity.

The Intrinsic Cardiac Innervation

The intrinsic cardiac innervation has been investigated and previous literature in this area reviewed by Tcheng (1949, 1951) and Hirsch (1962, 1963). Hirsch (1963) histologically observed the intrinsic innervation of the hearts of fish, amphibians, reptiles, birds, and mammals and concluded that a common pattern prevails in the structure and distribution of these myocradial nerves. Postganglionic sympathetic, preganglionic parasympathetic, postganglionic parasympathetic, and sensory neurons, as well as cell bodies (ganglia) were found within the heart wall. Tcheng (1951) and Hirsch, Nigh, Kaye, and Cooper (1964) found that in addition to numerous subepicardial ganglia around the left and particularly right auricles, between the aorta and pulmonary artery, in the interauricular septum, and around the sinoauricular and auriculoventricular nodes, that intramural ganglia occurred deep within the myocardium. According to Tcheng (1951) parasympathetic postganglionic fibers arose from the intrinsic cardiac ganglia and sympathetic postganglionic fibers arose from ganglia of the sympathetic chain. Lawrentjew (1929) transected the vagi 3-4 cm below the nodose ganglion and found that degenerative changes

occurred in the distal segment four days after division, but nerve cells of the cardiac ganglia were not affected. Postganglionic parasympathetic fibers were distributed in great proportions to the surface of the conduction system, the nodal tissue, as well as to the coronary arteries and directly to the myocardium (Gerebtzoff, 1959).

No adrenergic ganglion cells were demonstrated in the heart by Ehinger, Falck, and Sporrong (1966), but small catecholamine containing non-nervous cells and mast cells were observed using fluorescence microscopy after paraformaldehyde treatment. Jacobowitz (1967) demonstrated adrenergic fibers synapsing with and depressing transmission of some cardiac ganglion cells. The cardiac ganglion cells did not show the catecholamine reaction but strained intensely for acetylcholinesterase. This further supported their cholinergic nature. It was also reported that some chromaffin cells in the confines of the cardiac ganglion received postganglionic parasympathetic innervation from cholinergic cells. A self-controlling feed back system involving the ganglion-chromaffin cell arrangement was suggested as a possible mechanism for the intrinsic regulation of cardiac contraction.

Sensory innervation of the dog's heart has been found from the epicardial and endocardial areas as well as from deep in the myocardium, (Tcheng, 1951). They were most plentiful in the right atrium, were present in the left atrium, and were rarely found in the ventricles. Sato (1954) found that sensory endings in the heart were rarely formed around small veins, but were found in large numbers along small arteries. No complex sensory terminations were found surrounding muscle fibers in the canine atria by either Sato (1954) or Miller and Kasahara (1964). The types of

cardiac sensory nerve endings have been classified by Miller and Kasahara (1964) as end-nets, complex unencapsulated endings, and encapsulated endings.

According to Kuntz (1949) impulses of pain of cardiac or coronary origin usually were conducted into the spinal cord on the left side. Myocardial ischemia, produced by coronary occlusion (Brown, 1967), probably was the stimulus for afferent feedback to increase activity of the heart. This response was through afferent nerves and was unaltered by vagotomy. Central sensory connections were by cell bodies located in the thoracic dorsal root ganglia or the nodose ganglia of the vagi (Nettleship, 1936). Milart and Welento (1967) transected the cervical sympathetic trunk in the sheep. Fifteen percent of the fibers caudal to the severing underwent change but eighty-five percent of the cranial fibers underwent change. In this species it is observed that fifteen percent of these fibers were afferent nerves with cell bodies located nasally of the cranial cervical ganglion. In the dog the nucleus ambiguus was the most reliable source of cardioinhibitory vagal efferents. The dorsal nucleus of the vagus and nuclei of the solitary tracts also suppressed heart rate, (Gunn, Sevelius, Puiggari, and Myers; 1968).

Cardiac Conduction

The question of whether conduction within the heart was neurogenic or myogenic was still disputed in the literature. Texts by Kuntz (1953), Mitchell (1953), and Botar (1966) gave general reviews of this area. Common to most authors was the feeling that the pacemaker was located in the area of the sino atrial node. Impulses passed from this node to the

atrioventricular node, were transmitted to the ventricles in the atrio-ventricular bundle, and were distributed to the ventricles through bundle branches. Whether nervous or modified myocardial fibers were individually or in combination responsible for distributing impulses through this conduction system was unsatisfactorily answered. According to Hirsch, Kaiser, and Cooper (1964, 1965a) intracardiac transmission of impulses during the past 50 years has been attributed to specialized myocardial conduction fibers. Purkinje cells, the specialized cardiac muscle fibers that constitute the impulse-conducting system according to the texts by Bloom and Fawcett (1968), Ham (1965), Copenhaver (1964), Elias and Pauly (1966) and Arey (1963), were found subendocardially, but did not penetrate into the depths of the myocardium, Tcheng (1951), Wensing (1965). Gerebtzoff (1959) reported that impulse transmission to myocardial fibers in man was by Purkinje fibers but in the turtle it was by postganglionic nerve fibers themselves. Carlson's (1905) finds supported neurogenic conduction in the King crab heart, since it's heart stopped beating when its nerve tissue was removed.

Merideth and Titus (1968) by histological sectioning in man found only three areas of direct fiber to fiber connection between the S A and A V nodes. The bulk of the cells forming these pathways were structurally indistinguishable from ordinary atrial muscle cells and were not the classic Purkinje-type fiber. Bishop and Cole (1967) in the horse suggested that both ordinary atrial fibers and specialized Purkinje-like fibers were active in conducting impulses from the sinus to the atrium and atrio-ventricular node. Nodal fibers were not seen directly connecting with large atrial Purkinje-like fibers.

Myocardial acetylcholinesterase histochemically was found by Isaacson and Boucek (1968) to be heavily localized in conduction tissue with a much lower staining intensity in the atria and ventricles. After producing myocardial ischemia by inducing ventricular fibrillation, acetylcholinesterase staining intensity in conduction tissue was slightly reduced. After electrical shock the intensity of staining was much less. The authors suggested that the rate of transmission through the A V node may be related to the acetylcholinestrase concentration in or on the cell membrane of the conducting tissue. Acetylcholinestrase may have been responsible for the delayed conduction in this area in the prenatal heart.

Dahlström, Fuxe, Mya-Tu, and Zetterström (1965) found a very sparse or no adrenergic innervation to Purkinje fibers. It was suggested that sympathetic stimulation might influence the rate of conduction in Purkinje cells or more likely these cells may act as pacemakers. Purkinje fibers were not considered as part of the contractile unit of the heart by these authors. Because of the apexes' abundant nerve supply and its being one of the first activated regions of the left ventricular wall, Tcheng (1951) in the dog, felt that the view of automation through the influence of nervous impulses was plausible. Wensing (1965) concluded that the generation and conduction in the heart was definitely neurogenic not myogenic and felt that conduction between the sinoatrial and atrioventricular nodes took place through nerve fibers rather than along muscle fibers. He further reported that every muscle fiber of the heart was in contact with nervous elements. Intraprotoplasmic penetration of myocardial fibers by autonomic nerve fibers was reported by Tcheng (1951). A dense network of autonomic interstitial cells (A I C) was described by Wensing (1965) as

releasing a neurosecretory product in the sinoatrial node inducing the generation of the impulse leading to contraction.

Cardiac Neurotransmission

Many substances, according to Gerebtzoff (1959) had definite effects at the synaptic level, but only the adrenalin-noradrenaline group and acetylcholine may be considered as chemical transmitters. The catecholamines according to Costa and Brodie (1964), acted on the target organ to produce chemical or mechanical energy or on adjacent neurons to produce more electrical impulses. Epinephrine discharged by the adrenal gland was regarded as an emergency substance liberated into the circulation and acting generally, while norepinephrine was considered the predominant transmitter substance, acting locally at or near the synaptic adrenergic nerve ending (Rushmer, 1956).

Young and Held (1968) with radioisotopes found that material in axons was obtained by transport of protein synthesized in the perikaryon to the fiber rather than by direct intra-axonal incorporation from the blood and that intra-axonal macromolecular constituents passed down the nerve fiber at varying rates. Norepinephrine was liberated at postganglionic sympathetic neuron synaptic junctions. The probable pathway for its biosynthesis in adrenergic nerves, according to Costa and Brodi (1964) was from tyrosine to D O P A to dopamine to norepinephrine. The rate of transport of amine in storage granules in adrenergic fibers, after their formation in the perikaryon, has been found to be 9-10 mm/per hour, with their life span about 70 days in the cat (Dahlström and Häggendal, 1966). The granules in the cell body turned over about every one to five hours

(Dahlström, 1966a). The concentration of noradrenaline at the adrenergic terminals was found to be about 300 times greater than in the cell body. Norepinephrine formed in nerves and stored either as readily mobile or reserve granular pools, diffused or was discharged onto receptor sites after nerve stimulation (Costa and Brodie, 1964). Burn and Rand (1959) have hypothesized that adrenergic nerve impulses liberated first acetylocholine at the terminals of sympathetic nerves. Acetylcholine in turn liberated norepinephrine. Jacobowitz (1965) demonstrated the presence of both catecholamines and acetylcholinesterase in individual nerve trunks and concluded that results after further investigation (Jacobowitz and Koelle, 1965) were consistant with the Burn and Rand hypothesis. Further study is necessary to ascertain whether acetylcholine and norepinephrine can be localized within the same neuron. Loffelholz (1967) by perfusion techniques concluded that release of noradrenaline from the sympathetic nerve endings, evoked by acetylcholine, was dominated by a calcium-sodium anatagonism. Calcium ions promoted and sodium ions inhibited noradrenaline release. According to Greene (1968), metabolism of circulating epinephrine and norepinephrine to normetanephrine and metanephrine was mediated by catechol O-methyl transferase (COMT) whereas tissue catecholamines were oxidized primarily by monoamine oxidase (M A O). M A O was not the only or most important means by which response to catecholamines was terminated. Greene (1968) included uptake and redistribution plus the action of other enzymes as collectively more important.

In attempting to understand the affects of catecholamines on the in situ heart Hoffman and Singer (1967), recognized several factors including

indirect affects, condition of responding cells, and unknown biochemical basis for electrophysiological effects, which limit the understanding of the affect of catecholamines on the heart.

Norepinephrine (NE) is the peripheral transmitter of the adrenergic nervous system, and epinephrine (E) is the major hormone released by the adrenals (Crout, 1968). According to Guyton (1966) the mechanism of action of the sympathetic nerve ending hormone norepinephrine on cardiac muscle fibers was not completely understood, but apparently it increased the permeability of myocardial fibers to sodium. This accelerated the onset of self excitation in the S A node increasing the heart rate. In the A V node, sympathetic stimulation resulting in increased sodium permeability made it easier for each fiber to excite the succeeding one. It was presumed that increased sodium permeability also increased calcium permeability which in turn excited the contractile mechanism of the muscle fiber. Cardiac and skeletal muscle contractile structure and behavior was very similar according to Huxley (1961). Cardiac muscle generally had more mitochondria which were associated with its ability to function continuously over a long period of time. Huxley (1961) further observed that cardiac muscle fibers were smaller in diameter and that a system of internal membranes (reticulum) which apparently relayed signals for contraction into the interior of skeletal muscle was sparse or lacking in cardiac muscle. Page (1968) found specialized calcium storage depots along the cytoplasmic surfaces of the plasma membrane lining the transverse tubules and also along the plasma membrane lining the longitudinal surfaces of the myocardial fibers. The latter site allowed action potentials to release calcium ions into the cytoplasm directly without intervening

electrical signals involving the transverse tubules. The findings of Katz (1967) supported the view that myocardial contractility can be modified by changes in the amount of calcium released during excitation-contraction coupling. He further demonstrated that exchange of intracellular K^+ with Na^+ could enhance myocardial contractility by stimulating actomyosin. Cardiac glucosides and catecholamines which greatly increase contractility did not significantly affect actomyosin.

Markova (1967) viewed nervous impulses as regulating myocardial energy metabolism. Useful cardiac action was reduced by sympathetic catecholamines and increased by parasympathetic acetylcholine. Acetylcholine also increased cardiac glycogen. Strubelt (1968) concluded that catecholamines produce an increase in basal metabolism which seemed to be a consequence of adenosine triphosphate utilizing metabolic processes induced by the glycogenolytic and lipolytic actions of catecholamines. Prominent actions of autonomic drugs included effects on carbohydrate metabolism, fat metabolism oxygen consumption, and electrolyte metabolism (Ellis, Kennedy, Eusebi, and Vincent; 1967). Norepinephrine had been shown to cause a transient release of stored potassium from smooth muscle (Jenkinson and Morton, 1965).

Gerebtzoff (1959) explained that in cholinergic neurons, choline acetylase transferred acetyl from coenzyme A to choline, using energy furnished by adenosinetriphosphate and creatinine phosphate. Acetylcholine was then stored as a precursor or in minute vesicles in the presynaptic axoplasm. With the action potential deflected downward, potassium ions diffused outward across the presynaptic membrane and the

transmitter was released, perhaps in response to the inward movement of calcium ions. The acetylcholine then bridged the roughly 200 A^{o} synaptic cleft to reach the subsynaptic membrane and generated a graded potential. It was then hydrolyzed by acetylcholinesterase. Acetylcholinesterase had been confirmed in preganglionic fibers in the dog, but was much lower than in synaptic regions (Gerebtzoff, 1959). He reported that postganglionic parasympathetic axoplasm was as high as the cell body cytoplasm in acetylcholinesterase activity. Gerebtzoff (1959) also reported that cholinesterases had been demonstrated in sensory endings and that acetylcholine may play the role of transmitter at the level of sensory receptors. Acetylcholine released by parasympathetic stimulation normally slowed and caused greater diastolic filling of the heart (Corday and Irving, 1961). This resulted in a larger stroke volume because of longer initial ventricular fiber length. According to Guyton (1966), acetylcholine secreted by vagal nerve endings decreased the rate of rhythm of the S A node and decreased the excitability of the AV junctional fibers between atrial musculature and the Purkinje system thereby slowed transmission of impulses into the ventricles. According to Mason, (1968), the significance of parasympathetic innervation of the ventricles has not been determined in regard to the regulation of contractile force. Few if any parasympathetic nerve fibers passed to the ventricular musculature according to Guyton (1966). Guyton (1966) described norepinephrine acting as an excitatory transmitter and acetylcholine as an inhibitory transmitter in cardiac muscle. When acetylcholine is released among the myocardial fibers the membrane potential becomes more negative, developing a state of hyperpolarization and heart rate slows. Potassium ions

selectively are more permeable to the muscle fibers. When norepinephrine is released along heart muscle fibers the membrane potential becomes less negative which makes the membrane more excitable and increases heart rate. Positive changed sodium ions are allowed to enter muscle fibers during sympathetic stimulation (Guyton, 1966). Known effects of parasympathetic nerves or of acetylcholine on energy metabolism according to Ellis, Kennedy, Eusebi, and Vincent (1967) was relatively limited, but it was known to inhibit epinephrine induced glycogenolysis (Vincent and Ellis, 1963).

Measurement of Neurotransmitters

Average normal norepinephrine values in the heart had been reported by several authors as listed for several species in Table 21. Dahlström, Fuxe, Mya-Tu, and Zetterström (1965) histochemically, and Shore, Cohn, Highman, and Maling (1958), and Hirsch, Jellinek, and Cooper (1964) biochemically, found no difference in the adrenergic innervation and noredrenaline concentration respectively between left and right auricles of the dog. Regional differences within chambers was emphasized by Dahlström, Fuxe, Mya-Tu, and Zetterström (1965). Shore, Cohn, Highman, and Maling (1958), however, found a 1.8:1 ratio between atrial and ventricular norepinephrine levels in the dog. They also found that removal of the epicardium and endocardium did not significantly alter chamber norepinephrine levels. Frolkis and Bogatska (1968) reports that heart muscle noradrenaline content falls with increasing age, but Vogel, Jacobowitz, and Chidsey (1969), in cattle, found little difference in norepinephrine concentrations of the cardiac chambers in control animals

between birth and 18 months of age, but levels were lower in the ventricles of animals 36 months old. Hökfelt (1951) found the noradrenaline content of the young rat heart to be lower than in the adult rat. The latter author found no diurnal variation in heart norepinephrine.

Levels of catecholamines may be altered in animals due to ingestion of certain foods. Kimura (1968) had localized catecholamines in several plants. Phillips and Elevitch (1966) reported that ingestion of bananas may increase urinary catecholamine levels. The choice of anesthetics can influence cardiac catecholamine levels. Paton and Gillis (1968) found that pentobarbital anesthesia alone led to reduced cardiac levels of catecholamines in cats. Reduced body temperature was also suggested by these authors as lowering cardiac catecholamine levels in anesthetized animals. Animals in shock were observed to have reduced levels of endogenous cardiac catecholamines, perhaps attributable to increased sympathetic nerve activity and decreased amine synthesis. Lin and Sturkie (1968) subjected chickens to cold ($0^{o}C$), or hot ($31^{o}C$) temperatures for up to 20 weeks. Norepinephrine levels were reported to increase in the right atrium of cold treated birds. Atrial and ventricular norepinephrine was reduced in the heat treated birds at 12 weeks, but no significant difference was present at 20 weeks. Pregnancy also was found to affect cardiac catecholamine levels. In both the guinea pig and rat, noradrenaline content of the heart was significantly decreased towards the end of pregnancy (Laes, Pekkarinen, Saarikoski, and Suramo; 1967). Despite all the evidence of cardiac catecholamine change under various circumstances Friedman, Bhagat, Lehr, and Hayashid (1969) report that cardiac catecholamine concentrations remain normally at a steady level, even in the

face of massive release as a result of vigorous sympathetic activity.

According to Phillips and Elevitch (1966), few would argue with the statement that fluorometry was the general technique of choice for catecholamine analysis. Various modifications of the trihydroxyindole procedure for fluorometric analysis of catecholamines as well as other methods were developed (Euler and Floding, 1955; Shore and Olin, 1958; Roston, 1958; Euler and Lishajko, 1959; Crout, 1961; Porter, Totaro, and Burcin; 1965; Engelman, Portnoy, and Lovenberg; 1968).

Myocardial norepinephrine was measured by Jacobowitz, Cooper, and Barner (1967) using the method of Hogan as reported by Porter, Totaro, and Burcin (1965). These procedures were not reported in the literature in detail, but were made available to me by A. F. Hogan of the Merck Institute for Therapeutic Research in West Point, Pennsylvania, 1968, and by D. Jacobowitz of the Department of Pharmacology at the University of Pennsylvania in Philadelphia, Pennsylvania, 1968, in personal communications. An automated trihydroxyindole method utilizing the Auto Analyzer and fluorometer to differentiate catecholamines was developed by Robinson and Watts (1965).

A historical review of catecholamine study was presented by Blaschko (1964). The formaldehyde fluorescence method for the histochemical demonstration of catecholamines was reviewed by Corrodi and Jonsson (1967) and Falck and Owman (1965). Histochemical demonstration of cardiac catecholamines during the period of 1961 to 1966 were listed by the Karolinska Institute (1967). Numerous other articles related to this subject have appeared in the literature including those by Angelakos

(1964); Falck (1964); Eränkö (1964, 1967); Hamberger and Norberg (1964); Hamberger, Malmfors, and Sachs (1965); Ehinger and Falck (1966); Spriggs, Lever, Rees, and Graham (1966); Angelakos and King (1967); Hamberger (1967); Laties, Lund and Jacobowitz (1967); Adams, Orton, and Zilkha (1968). A specificity test for catecholamine fluorescence was developed by Corrodi, Hillarp, and Jonsson (1964). Laties, Lund, and Jacobowitz (1967) improved and simplified the cardiac fluorometric demonstration of catecholamines using perfusion fixation with paraformaldehyde. This has been successful in rats and mice but to date has been unsuccessful in the dog heart according to D. Jacobowitz of the Department of Pharmocology at the University of Pennsylvania in Philadelphia, Pennsylvania, 1968, in personal communications.

Eränkö (1967) presented an excellent review of the development and uses of the histochemical demonstration of cholinesterases. Acetylcholinesterase (AChE) was found chiefly in nervous tissue and erythrocytes (Koelle, 1950). Numerous authors, reviewed by Jacobowitz and Koelle (1965) recognized that the presence of AChE in a neuron did not necessarily implicate the concomitant association and function of acetylcholine (ACh), nevertheless there were fairly good correlations between concentrations of AChE, ACh, and choline acetylase (ChAc) in various regions of the central and peripheral nervous system. A histochemical method for identifying acetylcholinesterase from nonspecific cholinesterases was described by Koelle (1950, 1951). Various improved and modified techniques have followed Koelle (1955), Holmstedt (1957), Ehinger and Falck (1966). Perhaps one of the best histochemical techniques developed is the El - Badawi and Schenk (1966) modification of the direct

coloring thiocholine method of Karnovsky and Roots (1964). Demonstration of cholinergic and adrenergic fibers histochemically in the same sections have been developed for tissues by El - Badawi and Schenk (1967) in the urinary bladder and genital tract, and by Ehinger and Falck (1965, 1966) in the iris of eye. Ellman, Courtney, Andres, and Featherstone (1961) combined the method of Koelle (1951) with a sulfhydral reagent studied earlier by Ellman (1958, 1959) and presented a new extremely sensitive method for quantitatively measuring acetylcholinesterase in blood or other tissues. Other quantitative methods have been developed by Potter (1967), Gall and Roth (1957), Giacobini and Holmstedt (1958), Garry and Routh (1965), Prince (1966), and McOsker and Daniel (1959). Cholinesterase determinations were adapted for quantitation of serum cholinesterase using automated equipment (Winter, 1960; Levine, Scheidt, and Nelson, 1966). Ellman, Courtney, Andres and Featherstone (1961) reported normal values of "tissue" acetylcholinesterase activity reported as the rate of Acetyl thiocholine hydrolysis in moles/liter per minute x 10-6 per gram of tissue. Values reported included muscle (thigh) 1.82, brain 10.31, and liver 1.07. Antopol, Glaubach, and Glick (1939); Ord and Thompson (1950); and Smirnov and Serbenyuk (1948) found greater atrial than ventricular concentrations of acetylcholinesterase. Frolkis and Bogatska (1968) explain that destruction of nerve endings and a reduction in acetylcholine production in these endings, with increasing age, leads to a weakening of nervous effects on the tissues. Cholinesterase activity in the atria and ventricle of 26-28 month old white rats fell to 70% and 60% respectively of the one month old rats.

Measurements of Cardiac Function

Electrocardiography is a tool for estimating cardiac function. Basic information for utilizing electrocardiography can be gained from numerous sources. Texts and articles by Marriott (1957); Clark, Szabuniewicz, and McCrady (1966a, b, 1967); McCrady, Clark, and Szabuniewicz (1966); Rubin (1968); Corday and Irving (1962); Detweiler, Hubben, Patterson (1960); and Fisher (1967) were particularly useful in understanding basic electrocardiology. Marriott (1957) discussed leads, rhythm and rate, amplitudes and intervals, arrhythmias, interpretation, and other facets of electrocardiography. Normal amplitudes and intervals observed in the dog by several authors, are reported by Detweiler, Patterson, Luginbühl, Rhodes, Buchanan, Knight, and Hill (1968); and Clark, Szabuniewicz, and McCrady (1966a). Lead two was considered the best lead for routine electrocardiography (Detweiler, Patterson, Luginbühl, Rhodes, Buchanan, Knight, and Hill; 1968). It was adequate for demonstrating heart rate and rhythm, and the duration, form, amplitude, and spacing of different electrocardiogram components (Clark, Szabuniewicz, and McCrady; 1967). In every electrocardiogram according to Marriott (1957), nine features should be examined. These include rhythm, rate, P wave, PR interval, QRS complex and interval, ST segment, T wave, U wave, and Q-T duration. A condensed explanation of these features, primarily taken from Marriott (1957) follows. The P wave represented the spread of electrical impulses through the atria, depolarizing them. When impulses passed through the atria in an unorthodox path this wave inverted as in ectopic atrial or AV nodal rhythm. Increased amplitude may indicate hypertrophy or dilitation of the atria and was found in mitral stenosis, and hypertension.

Increased P interval indicated left atrial enlargement or decreased atrial muscle. Notching was often present in mitral stenosis. Abnormally tall pointed P waves indicated right atrial strain while absence of the P waves occurred in some A-V nodal rhythms and in S A block. Duration of the P wave interval increased with the size of the heart according to Smith, Hamlin, and Crocker (1965).

The P - R interval from the beginning of the P wave to the beginning of the Q R S complex reflected the passage of impulses from the S A node to the ventricular muscle fibers. An abnormally prolonged interval indicated A - V block. Those P - R intervals in the dog which exceed 0.146 seconds at low cardiac rates and exceeds 0.13 seconds at medium and high rates and in which QRS equals or is above 0.07 second are probably abnormal according to Detweiler, Patterson, Luginbühl, Rhodes, Buchanan, Knight and Hill (1968). According to Wolff (1956) the P - R interval lengthens with age and is inversely related to heart rate.

The Q R S complex represented the period of impulse spread through the ventricles. To interpret this complex the duration, amplitude, and presence of Q waves, should be inspected in standard leads according to Marriott (1957). Twelve one-hundredth of a second or more duration indicated abnormal intraventricular conduction and usually reflected a bundle branch block or ventricular arrhythmia. If the amplitude was less than 5 mm it was considered abnormal and may indicate diffuse coronary disease, cardiac failure, pericardial effusion or widespread myocardial damage. It was difficult to assess the Q wave. Its amplitude may vary greatly but it should not exceed .03 seconds in width in man.

Observations of the S-T segment level in relation to the iso-electric T-P segment and to the shape of the S-T segment should be made according to Marriott (1957). It was sometimes elevated not more than 1 mm in normal leads and was never depressed more than 1/2 mm or so normally. According to Detweiler, Patterson, Luginbühl, Rhodes, Buchanan, Knight, and Hill (1968), in the dog, depression of the S - T function below -0.2 mV in lead II and elevation above .15 mV are abnormal. The T wave may be negative, positive, or biphasic. Abnormal amplitudes exceeded slightly over one-fourth the R amplitude.

The T wave represented repolarization of the ventricles. Its direction, shape, and height should be observed. It was normally upright, slightly rounded and slightly asymmetrial, and was usually not above 5 mm in height. Unusually tall waves suggested myocardial infarction, potassium intoxication, myocardial ischemia without infarction, or certain forms of ventricular strain. According to Greenspan, Wunsch, and Fisch (1965) in the dog, vagal stimulation decreases T wave amplitude when upright and increases negativity when the T wave is inverted.

The Q-T interval represented the duration of ventricular systole. Generally it should be less than half the length of the preceding R-R interval. It was lengthened in congestive heart failure, myocardial infarction, hypocalemia, and myocaditis. It was shortened in potassium intoxication.

A small U wave might follow the T wave and was most prominent in potassium deficiency. Its polarity was often reversed in myocardial ischemia and left ventricular strain, but its precise significance was uncertain.

Heart rates and blood pressure have also been used as meaningful parameters in evaluating cardiac function. The range of heart rates in dogs older than 6 months according to Lannek (1949) was 67 to 214 beats per minute with an average of 122 beats per minute. Average femoral artery blood pressure in pentobarbital anesthetized dogs using a capacitance manometer, according to Romagnoli (1953) was 99.6 mm Hg diastolic and 155.7 mm Hg systolic. The ranges were 75-121 mm Hg diastolic and 108-197 mm Hg systolic. Based on 1000 dogs (Katz, Skom, Wakerlin; 1957) the upper limit of mean femoral arterial pressure in the dog was given as 145 (135-155) mm Hg.

Marriott (1967); Clark, Szabuniewicz, and McCrady (1966a); and Corday and Irving (1961) gave excellent descriptions of variations from the normal rhythm of heart beats (arrhythmias). The following section primarily combined their descriptions. Arrhythmias were not always referred to in consistant ways by various authors. Scherf (1967) discussed some of the confusion in nomenclature. Arrhythmias were divided into supraventricular originating in the atria or atrioventricular node, and ventricular originating in the ventricles. Supraventricular arrhythmias showed normal QRS complexes while ventricular ones produced bizarre QRST complexes with prolonged QRS intervals. Four ventricular arrhythmias were generally described including ectopic beat, tachycardia, flutter, and filbrillation.

Ectopic beats, premature beat, or extrasystole were described by Marriott (1957) as synonymous terms. They were easily recognized by the distorted QRST complex, anticipation of the next normal beat, prolonged QRS interval, the S-T slope away from the main QRS deflection, and the

relatively long pause until the next cycle. The P wave was generally lost in the QRS complex. Ventricular tachycardia was merely a run of rapidly reported premature beats. It was generally a grave sign and often preceded ventricular fibrillation. Ventricular flutter was a rapid ventricular tachycardia giving a slightly modified pattern, but nothing was gained by separating it from ventricular tachycardia. Ventricular fibrillation was usually a terminal event. There was an absence of properly formed ventricular complexes.

Supraventricular arrhythmias may have originated from the sinoatrial node, atria, or atrioventricular node. Normal sinus nodal rhythms implied normal P wave. Above 100 impulses/min in man was sinus tachycardia and below 60 impulses/min was sinus bradycardia. Though variable, Detweiler, Patterson, Lugenbühl, Rhodes, Buchanan, Knight, and Hill (1968) listed 70 to 120 beats per minute as the normal heart rate in dogs. Sinus tachcardia was a common reaction to heart disease, fever, and heart failure. Sinus arrhythmia, a reflex respiratory phasic irregularity of normal impulses formation, may be a normal finding in the dog. Sinus arrest or stand-still resulted when the S-A node lost its ability to initiate impulses.

Atrial arrhythmias arose according to Marriott (1957) from an irritable focus in the atrium or from the A-V node. Normal QRS durations were present. The types of arrhythmias arising from the atria include ectopic beats, paroxysmal tachycardia, flutter, and fibrillation. All four probably arose from an ectopic focus in one of the atria. The ectopic atrial beat traveled an aberrent course and created a distorted, often inverted, P wave which was premature. Normal QRS-T sequences usually

followed. Premature beats were recognized as high, middle, or low in origin. A rapid run of premature atrial beats was called paroxysmal atrial tachycardia. An abnormal P wave preceded a normal QRS complex, but the rate was generally so fast that the aberrent P wave was often difficult to discern and may be merged with the T wave. Paroxysmal tachycardia was sometimes (rarely) recognized as high, middle, or low and was subdivided into types; without A-V block (1:1) and those with A-V block (2-8:1). Atrial flutter represented a faster discharge from the ectopic focus in the atrium than was present in atrial tachycardia. Atrial ventricular block occurred and the P wave became distorted. A saw tooth pattern was often seen with ventricular beats after every second to eight rapidly firing atrial wave. In atrial fibrillation atrial impulses no longer elicited regular ventricular responses. P waves were lost.

A-V nodal arrhythmias were of three types, premature beats, tachycardia, and nodal rhythm. Premature beats were diagnosable if the P wave was absent or followed the QRS complex. If the P preceded the QRS complex, the premature beat was indistinguishable from low atrial premature beat. Tachycardia was diagnosable for certain only if the P wave followed the QRS complex. If no P waves were visible, usually A-V nodal tachycardia could not be distinguished from atrial tachycardia. If the preceding P was visible, A-V nodal tachycardia could not be distinguished from low atrial tachycardia. Atrioventricular nodal rhythm occurred when the A-V node became the pacemaker of the heart. The P wave differed in shape and was usually inverted. Its position might vary as in premature beats and tachycardia. When the site of impulse formation wandered between the S-A, A-V, and intervening tissue the condition was known as a wandering or

shifting pacemaker.

Intra-atrial, sino-atrio, and atrio ventricular blocks prevented normal passage of impulses in the heart. Intra-atrial block occurred when impulses took longer than normal to activate the atria. A prolonged P wave (over .11 sec) resulted. There was a notching of the P wave. This condition was not uncommonly seen in coronary and mitral disease and in left ventricular hypertrophy. It probably represented atrial enlargement. Sino-atrial block was rather rare. In partial block infrequent suppression of the S A node impulse formation to occasional absence of entire P-QRS-T complex occurred. In complete block no impulse was formed in the S-A node. Two things happened. The A-V node took over as pacemaker or cardiac standstill persisted and the patient died. Electrocardiograms showed no P wave, an idioventricular rate 30-40/min, and a QRS interval normal to prolonged. This condition could be caused by increased vagal-tone, or coronary disease. Atrioventricular blocks were partial or complete according to Marriott (1957). A partial block resulted in a longer period for travel of impulse to the ventricles. The impulse was delayed in the A V conduction tissue as was reflected in a prolonged P-R interval. Dropped beats occurred but P waves were still present. A-V blocks were graded. First degree blocks showed prolonged P-R intervals without dropped beats. Up to every third beat was dropped in second degree blocks and up to every other beat was dropped in third degree blocks. In complete A-V block no impulses passed the A-V node or lower pacemakers took over resulting in an idioventricular rhythm. If the new pacemaker was in the ventricular muscle itself, the QRST cycle was bizarre with a prolonged

QRS interval resembling premature ventricular beat. If the new pacemaker was near or in the A-V node, the QRS interval and complex appeared normal. Isoproterenol, a sympathomimetic amine, was recommended by Ettinger (1969) for reducing or eliminating A-V block through action in increasing ventricular excitability and A-V impulse conduction. Massive doses however, could cause myocardial anoxia and necrosis (infarction) (Poupa, Prochazka, and Pelouch; 1968).

Some of the conditions which resulted in electrocardiogram changes which were found in conjunction with the present research follow. Myocardial infarction resulted in electrocardiogram changes. Tying a branch of a dog's coronary artery resulted in an inverted T wave due to simple ischemia. If the vessel was kept tied, within 1 or 2 minutes the S-T segment became elevated reflecting still reversible injury. Eventually QRS inverted to form a QS complex while the S-T came back to isoelectric line and T returned upright. A permanent QS complex reflected a pattern of necrosis (Marriott, 1957). According to Ebert, Allgood, and Sabiston (1968), following coronary occlusion or in cardiac infarction potassium was lost from the myocardium and arrhythmia associated with ectopic activity occurred. But, in catecholamine depleted hearts after cardiac denervation, early potassium loss and arrhythmias did not occur after coronary occlusion.

Experimental left coronary artery occlusion in the cat was followed by S-T segment elevation often followed by depression, flattening and inversion of the T wave and decreased amplitude of the P and QRS waves, (Brown, 1967). Jayaram and Arbulu (1968) suggested that beta adrenergic blocking drugs were of value in treating cardiac arrhythmias associated

with acute myocardial injury (as infarction) if pumping demands were maintained.

According to Marriott (1957), when observing an electrocardiogram be particularly alerted for the fresh appearance of Q waves or increasing prominence of pre-existing ones. This reflects necrosis. Also observe S T elevations and T wave inversions. Coronary insufficiency was suspected when T waves were flattened and inverted with or without S T depression. Left ventricular strain may also have been part of the picture. Mitral stenosis was the only valvular lesion with a specific pattern - the notched P in lead II. Hypokalemia, a deficit in potassium ions was characterized by a prolonged Q-T interval, a lower T wave and taller U wave, inversion of the T wave, and a sagging S-T sgement. Hyperkalemia was manifested on the electrocardiogram by tall thin T wave, later a longer P-R interval, progressing to a depressed S-T segment, and longer QRS intervals. Finally the wave disappeared and fibrillation of ventricle occurred.

Electrolyte imbalances of the body fluids influenced electrocardiograms in animals as shown by Surawicz (1967) and Clark, Szabuniewicz, and McCrady (1966a). Hyperpotassemia widened the QRS interval and elevated and peaked the T waves according to the latter authors. Hypopotassemia depressed S-T segments and lowered and widened T waves (Clark, Szabuniewicz, and McCrady; 1966a; and Surawicz; 1967).

Q-T interval shortening was additionally observed in hyperpotassemia by Surawicz (1967). He observed that advanced hyperpotassemia and dying hearts were almost identical in electrocardiogram change.

Hypocalcemia, a deficit of calcium ions was characterized by a prolonged Q-T interval, and normal T wave which may have inverted termirally.

Rubin (1968) summarized some additional acquired disturbances of the heart as follows. Dilatation, hypertrophy, ischemia, and necrosis will generally cause amplitude changes in the QRS complex. Ischemia and necrosis will also cause change in the S-T segment, and T wave changes. Atrio ventricular valvular insufficiences can result in atrial fibrillation due to extreme dilatation and irritation of the atrial myocardium. Obesity may result in decreased amplitudes in all complexes.

Neurotransmitters in Heart Disease

Rubin (1968) mentioned that disturbances to the sympathetic or vagus nerves due to chemical or mechanical influences may cause conduction disturbances. Catecholamines have been incriminated as contributing to heart failure and arteriosclerosis. Congestive heart failure according to Fisher (1967) was characterized by failure of the heart to maintain the circulation. In aging dogs, Luginbühl, Jones, and Detweiler (1965), found it can sometimes be associated with progressive fibrosis of A-V valves, focal myocardial fibrosis and necrosis, and intramural coronary arteriosclerosis.

Cardiac insufficiency following artificial constriction of the ascending aorta resulted in a drop of noradrenaline in rat cardiac tissue to almost half normal values in 16 days (Suramo, Saarikoski, Pekkarinen, and Olli; 1967). Part of the decrease was attributed to an increase in relative heart weight. After artificially inducing heart failure in cattle by right pulmonary artery ligation at 5,280 ft. elevation, the right

ventricular weight increased and norepinephrine levels fell in all chambers particularly in the right ventricle (Vogel, Jacobowitz, and Chidsey; 1969). Similarly, the catecholamine fluorescence histochemically was less demonstrable in heart failure. Recovery from heart failure, after returning animals to sea level, resulted in substantial reconstitution of the normal adrenergic nerve pattern in the heart within as early as 10 days after returning to sea level.

Fisher (1967) explained the pathogenesis of congestive heart failure and the roll of the autonomic nervous system as follows. A decreased cardiac output led to decreased systemic arterial pressure which caused sympathetic discharge and adrenaline release. These increased systemic catecholamines released from postganglionic endings or from the adrenal medulla increase cardiac work load, due also to peripheral vascular constriction, enhanced oxygen consumption, and had deleterious influences on myocardial carbohydrate and lipid metabolism (Tomomatsu, Ueba, Kondo, Oda, Ijiri, Kogame, Ito, and Yao; 1967). According to Fisher (1967) the sympathetic discharge brought cardiac reserves into action and there was a tachycardia and increased myocardial contractility. Cardiac dilatation occurred. Decreased output caused blood to be held back in the left atrium and then interfered with pulmonary flow resulting in pulmonary venous congestion. Decreased renal blood flow resulted in excessive sodium and water retention and renin was released. Renin acted upon angiotensinogen to produce angiotensin which in turn caused the release of aldosterone which further acted to retain sodium. The retained sodium also resulted in retained water causing an increased plasma volume particularly reflected in

increased venous pressure and distension. The venous congestion and decreased cardiac output interfered with liver functions of detoxification of aldosterone, and antidiuretic hormones. Further sodium and water retention occurred. Numerous functions were interfered with and tissue anoxia and death may have resulted.

Tomomatsu, Uebo, Kondo, Oda, Ijiri, Kogame, Ito, and Yao (1967) failed to establish a significant relationship between plasma concentration of catecholamines and various forms of heart disease. In congestive heart failure they found that administration of sympathetic blocking agents seemed to give unfavorable clinical results.

Poupa, Prochazka, and Pelouch (1968) found that repeated administration of adrenalin or nonadrenaline subcutaneously, increased heart glycogen. No correlation between heart glycogen concentration and resistance of the myocardium to anoxia was established. In isolated dog hearts Jedeikin and Buckley (1967) infused epinephrine which led to greater cardiac glycogen deplation than when epinephrine was not infused in fibrillating hearts. Coronary venous oxygen also tended to diminish. It was suggested that if epinephrine stimulated fibrillation further it could lead to glycogenolysis due to increased energy requirements, and or vascular constriction resulting in tissue hypoxia.

Arteriosclerosis as defined by Dahme (1965) included all chronic arterial metamorphosis which consisted of induration (hardening), loss of elasticity, and narrowing of the arterial lumen. Hyperplastic and degenerative change occurred in the intima and media. Atherosclerosis lesions according to Dahme (1965) were sclerotic (hardening) lesions consisting of tissue proliferation as well as atheromatous degeneration.

Atheromatosis degeneration was a fatty soft degeneration and necrosis.

A significant association between cigarette smoking and resulting increased catecholamines to the morbidity and mortality of coronary atherosclerosis has been pointed out by Kershbaum and Bellet (1966) and Kershbaum (1968). Catecholamines were liberated from the adrenal glands and extra-adrenal chromaffin tissue and were apparent mediators of increased free fatty acids. Changes in coronary and peripheral blood flow, blood pressure, cardiac output, and cardiac work were observed which could contribute to initiation and progression of atherosclerosis. Stimulation of the sympathetic nervous system and liberation of catecholamines may, according to Kershbaum and Bellet (1966), be factors in atherogenesis and clinical atherosclerosis because of their affect on lipid metabolism, on the thrombogenic mechanism, hypertension, and in myocardial tissue damage. The National Aeronautics and Space Administration recognizing the relationship between stress and catecholamine production and cardiovascular change, have been measuring urinary catecholamine levels in Project Mercury space pilots (Weil-Malherbe and Smith, 1968).

The phases of coronary atherosclerosis; intimal proliferation by smooth muscles, collagen, elastic and lipid materials; and focal necrosis with accumulation of extra cellular debris was investigated in man by Scott, Daoud, Wortman, Morrison, and Jarmolych (1966). A certain degree of thickness in the proliferation phase was reached before the second atheroma formation stage ensued. In man, Klassen and Sung (1968) observed that with age the intimia of cerebral blood vessels thickened and a homogeneous acellular subendothelial layer appeared. The internal elastic membrane also thickened until the third or fourth decade, then regressed.

The media with age became less cellular, more compact, and increased in collagen and elastic fibers. The adventitia was loose collagenous connective tissue in infancy and with age became more compact due to increased collagen. In man, Bloch (1969) observed that coronary vessels in their epicardial course showed severe atherosclerosis, but that when entering the myocardium the atherosclerosis was absent or minimal. If a given vessel re-entered the epicardium atherosclerosis reappeared. Atherosclerosis according to Bloch (1969) was almost universally absent in intramyocardial arteries. The physical environment may have influenced this difference; muscle dampening vibrations.

Detweiler, Hubben, and Patterson (1960) surveyed cardiovascular diseases in 1000 dogs and found 97 to have heart disease. After surveying 4,831 dogs over 11 percent were found to have heart disease (Detweiler, Patterson, Luginbühl, Rhodes, Buchanan, Knight and Hill; 1968). The most common types of heart disease were chronic valvular fibrosis resulting in insufficiency, sclerosis of deep coronary vessels, right ventricular dilatation and hypertrophy, and congenital heart disease (Detweiler and Patterson, 1965).

The coronary vessels have been studied by Pirie (1967) who reported that atherosclerosis leading to thrombosis and occlusion of the coronary arteries did not occur in dogs. Five types of lesions were described in coronary blood vessels of the dog. Sclerosis of the intramyocardial arteries was the most common lesion seen in coronary arteries of the dog by Pirie (1967), being more prevalent in the left ventricle. Deposits of hyaline material were found in branches of the coronary artery particularly in the intima. Disruption of the internal elastic membrane and loss of

smooth muscle cells occurred and myocardial degeneration and fibrosis ensued. Intimal plaques were sometimes found in large extra myocardial branches of the coronary arteries, but were never large enough to produce stenosis. Polyarteritis nodosa histologically showed fibrinoid necrosis of affected arterial walls surrounded by inflammatory cells, but was seen uncommonly in dogs. Xanthomatosis most commonly resembled other sclerosis of man but was rare in dogs. Dogs with atrophic thyroid glands or on high cholestrol diets showed xanthomatosis. Lipids accumulated in smooth muscle cells of the media and intimal cells as well as extracellularly. Thrombosis had occurred. Fibrinoid necrosis of small arterioles was sometimes observed in the myocardium associated with hypertension secondary to renal disease.

Detweiler, Patterson, Hubben, and Botts (1961) reported that coronary disease in the dog is confined almost entirely to small intramyocardial arteries. They observed no true atherosclerosis and found no pathological changes in superficial coronary arteries. It was suggested by these authors that small myocardial vessel disease (arteriosclerosis) resulted in myocardial damage due to interference with the blood supply. Endothelial cushions were found fairly commonly in older dogs by the above researchers. Fatty infiltration of these areas was not infrequent, but their relationship to myocardial disease was not determined. Roberts (1961) failed to find a single case of myocardial infaction in an autopsy study of 405 dogs. He reported that the dog seemed to be particularly resistant to the development of atherosclerosis even in old age and when consuming the diet of his master. In 800 dogs, Luginbühl, Jones, and Detweiler (1965), observed an absence or rarity of demonstrable fat in

sclerotic arteries. The first change in intramural coronary arteries was intimal thickening and fragmentation of the internal elastic membranes. Reticular fibers and sparce amounts of collagen were present in the intimal thickenings. An amorphous ground substance was present, staining with Alcian blue. Not uncommonly, a homogeneous translucent P A S positive material (hyalin), was observed in intramural left ventricular arteries on either side of the internal elastic membrane, between intimal smooth muscle cells, within the cytoplasm or beneath the endothelium. In extensive hyalinization the entire arterial wall was involved and intimal plaques were found. In two instances there was extensive hyalinization, vacuolated smooth muscle cells, and foam cells laden with fat. Extensive musculoelastic proliferation and hyaline degeneration may produce marked narrowing and occlusion of intramural coronary arteries. In extramural coronary arteries Luginbühl, Jones, and Detweiler (1965) observed only occasionally intimal plaques and thickenings. These were located primarily in the left anterior descending, left circumflex, and right coronary arteries. Age changes in the cardiovascular system have been extensively investigated; particularly the dog and hog, in the Department of Anatomy at Iowa State University and age changes of the cardiovascular system of the dog are well documented by Getty (1966a, b). He reported spontaneously occurring atherosclerotic plaque and/or intimal thickening in the coronary arteries of the dog. Under five years of age intimal thickening was characterized by increased amounts of elastic fibers but rarely connective tissue infiltration. In dogs ten years of age or older sclerotic lesions were present to one degree or another. Lipids did not seem to be concerned

in the pathogenesis of the lesion, at least in the young animal. Waters (1965) hypothesized that atherosclerotic lesions in the dog were basically an inflammatory reaction of vascular tissue to injury. The vascular permeability was increased transiently to plasma and then lipophagocytes, mononuclear, smooth muscle, and fibroblastic cells accumulated. Lipids were removed and the lesion progressed to a scar. The intimal scar enlarged and its nutrition became impaired. This resulted in central autolysis, more lipid deposition, vascularization, and eventually ulceration, thrombosis, calcification, or aneurysin.

Constantinides' (1968) findings indicated that the cells of the hyperplastic intima were derived primarily from proliferation of pre-existing endothelial cells, from proliferation of medial myocytes, and from the immigration of mostly mononuclear cells from the blood. Invasion by adventitial fibroblasts appeared to play a roll only in cases where the entire thickness of the media was destroyed allowing their fibroblasts to move through the injured area. Ground substance in connective tissue consisted of mucopolysaccharides and protein (Bertelsen, 1963). Mucopolysaccharides were present in the form of mucoproteins, mucoids, and glycoproteins. Mucoproteins consisted of complexes of acid mucopolysaccharides united with proteins whereas mucoids and glycoproteins contained carbohydrate groups bound to protein. Collagen was scanty in carbohydrates and gave a faint P A S stain in the media of blood vessels, according to Bertelsen (1963), but a positive reaction in the intima after mineralization of the media had commenced. Medial and intimal accumulations of acid mucopolysaccharides occurred stimultaneously and may

be related to low oxygen tension in vessel wall (Bertelsen, 1963). Fat may have been present in the intima in both proliferative and fibrotic stages of atherosclerosis, being partly intracellular and partly extracellular, but was very scant in the media, being located in the luminal part.

In producing myocardial changes by controlled artificial narrowing and occlusion of coronary arteries, Malinin, Stokes, Hardy, and Lumb (1968) found irregularity of P A S staining. They reported that fat stains proved of no value in earlier lesion. These authors found accumulation of glycogen droplets and appearance of lipid droplets in myocardial fibers surrounding infarcts in animals sacrificed at 72 hours after occulsion

Cardiac Denervation

At least five different surgical methods have been reported in the literature as having been employed for denervating the heart for various studies. These include:

1. Bilateral stellate and thoracic sympathetic ganglionectomy together with cervical vagal transection (Long, Truex, Friedmann, Olsen, and Phillips; 1958).
2. Regional neural ablation (Cooper, Gilbert, Bloodwell, and Crout; 1961).
3. Auto transplantation (Willman, Cooper, Cain, and Hanlon; 1962).
4. Transection of vagal cardiac nerves and extirpation of lower cervical and thoracic sympatheiic ganglia (Černý and Oláh; 1958).

5. Interruption of extrinsic cardiac nerves without thoracotomy (Tsakiris and Donald, 1968).

Structural, functional and chemical changes following these surgical procedures were reported as essentially no change to major changes.

Long, Truex, Friedmann, Olsen, and Phillips (1959) studied cardiac denervation in the dog by bilateral surgical removal of the stellate and upper eight thoracic ganglia of the sympathetic trunk and by bilateral vagotomy. No significant changes were found in heart rate or electrocardiogram recordings following bilateral sympathectomy. After bilateral vagotomy the heart rate was almost double but fell considerably in dogs surviving 10-15 days with electrolyte and dietary nursing. Special electrolytic and dietary supportive measures were necessary in bilaterally vagotomized dogs according to Long, Truex, Friedmann, Olsen, and Phillips (1958) and Jellinek, Kaye, Kaiser, and Cooper (1966). Markowitz, Archibald, and Downie (1964) recommended cauterizing the vocal cords or transecting the vagi, but sparing the right, recurrent laryngeal nerve. Esophageal and gastric dilatation, cardiospasms, and pylorospasms were sequelae of bilateral vagotomy according to Jellinek, Kaye, Kaiser, and Cooper (1966). Markowitz, Archibald, and Downie (1964) reported death in 2-3 days following bilateral vagotomy in the dog and associated it with pulmonary distress. Goldenberg, Buckingham, and Sommers (1967) discussed pulmonary alveolar lesions associated with bilateral vagotomy in the rat and reviewed other research on pulmonary changes related to bilateral vagotomy.

Goodall and Kirshner (1956) found that after extirpating the right cervical sympathetic ganglia, norepineprine levels fell to one-third normal levels. Extirpation of right thoracic, left cervical or left thoracic ganglia did not produce change in the cardiac norepinephrine levels. Right cervical and right thoracic ganglia influenced the noradrenaline content of the sheep heart according to Goodall and Kirshner (1956). In the guinea pig, Cervoni, Palazzolo, and Terry (1968) determined norepinephrine contents of various portions of the heart after unilateral and after bilateral stellate ganglionectomies. These results were summarized in Table 15. According to Cooper, Gilbert, Bloodwell, and Crout (1961) catecholamines still present in the hearts of animals subjected to bilateral thoracic sympathectomy probably did not represent storage of circulating endogenous amine, since the dogs with total extrinsic cardiac denervation showed no such effect. The source, they further reported was presently unknown and required further investigation. Levy (1966) extirpated the sympathetic trunk bilaterally from the stellate ganglion to the fifth thoracic ganglion and found little reduction in myocardial norepinephrine levels. Bilateral thoracic sympathectomy was reported by Hirsch, Kaiser, and Cooper (1965b) to markedly reduce fibers and fibrils in the perimysial plexus of the auricles and to a lesser degree in the ventricles. They further reported that bilateral vagotomy reduced in large amounts the perimysial plexus in both the atria and ventricles. According to these authors this supports the concept that the simple nerve-ganglion relationships of the heart was vagal. Jellinek, Kaye, Nigh, and Cooper (1964) extirpated the stellate and upper four thoracic ganglia but found that norepinephrine and epinephrine content in the heart was not

significantly changed. When the caudal cervical (vertebrial) ganglion was also bilaterally excised norepinephrine depletion was over 10 fold greater.

To study the effects of stresses and drugs and to better prepare for the management of the transplanted hearts, Cooper, Gilbert, Bloodwell, and Crout (1961) developed a method for denervating the heart called regional neural ablation. In this technique the adventitia was stripped from all vessels passing to and from the heart and the area of the cardiac plexus ventral to the aortic arch was denuded. Donald and Shepherd (1963) in cardiac denervated dogs which had been surgically denervated by regional neural ablation, found the dog's capacity to work unchanged as measured by oxygen consumption. Stroke volume together with heart rate increased during moderate exercise. In control dogs, heart rate changed predominately. In racing greyhounds, Donald, Ferguson, and Milburn (1968) found that the cardiostimulant action of both sympathetic nerves and circulating catecholamines was necessary for maximal performance. Removal of either, limited performance some, and removal of both severly limited performance. Regional ablation resulted in complete depletion of myocardial catecholamines in a minimum of 3 days according to Cooper, Gilbert, Bloodwell, and Crout (1961).

Jacobowitz, Cooper, and Barner (1967) indicated that it was apparent that cardiac denervation by mediastinal ablation might not be entirely complete. Catecholamine fibers could sometimes be identified microscopically when norepinephrine was not detectable within the limits of sensitivity of the chemical method of analysis. After mediastinal neural ablation in the cat, Jacobowitz, Cooper, and Barner (1967) found that the

number of A C H E staining nerves in the left atrium were reduced by 20-50% of control sections. This was attributed partially to removal of the cardiac ganglion of Wrisberg. Hirsch, Willman, Jellinek, and Cooper (1963) in the dog and rabbit, reported regression of intrinsic cardiac nerves and the destruction of their fibers and the fibrillar perimysial plexuses following total extrinsic denervation. Only postganglionic vagal efferents remained. Twelve to fourteen months postoperative, cardiac catecholamine levels had returned to normal and the perimysial plexus was restored.

The animals prepared by regional neural oblation did not exhibit gastronintestinal complications and weight loss which frequently prohibited survival or maintenance of condition following sympathetic ganglionectomy and cervical vagectomy according to Cooper, Gilbert, Bloodwell, and Crout (1961).

Cooper, Willman, Jellinek, and Hanlon (1962) utilized autotransplantation of the heart thus eliminating the denervation of and accompanying sequellae to other organs. It was found that cardiac catecholamine were depleted, supporting the hypothesis that catecholamines were depleted by denervation rather than by immunologic factors. A 68% immediate mortality rate was reported utilizing this surgical procedure. In addition, Napolitans, Cooper, Willman, and Hanlon (1964) reported that death was common during the second and third week after autotransplantation though myocardial fibers showed no alterations in fine structure at this time when observed with the electron microscope. The research of Potter, Cooper, Willman, and Wolfe (1965) supported these findings. The results of the

study by Willman, Cooper, and Hanlon (1964) supported the view that cardiac homotransplants would be capable of adapting to support life in the noninnervated state while connections with the central nervous system were being re-established if immunochemical factors were not deterrents.

Leandri (1967) reported histologic changes following autotransplants and homografts (from another dog) of the canine heart. Polymorphonuclear cells, lymphocytes, monocytes, and plasma cells were concentrated in the perivascular spaces particularly subendocardially and subepicardially in homografts. Within the myocardium these perivascular collections appeared as nodules. This cellular infiltration was present in 8 and 9 day post-operative animals. Immunosuppressive therapy seemed to suppress the infiltration. Medial and subintimal infiltration of coronary arteries led to narrowing of vessel lumina, and was a source of thrombosis and damaged myocardium. In both autotransplants and homotransplants necrosis including loss of striation, vacuolization, cytoplasmic homogenization, and cytoplasmic lysis were observed. Fibriotic changes were seen in the subendocardial, subepicardial and perivascular spaces. Dong, Fowkes, Hurley, Hancock, and Pillsbury (1964) eighteen to twenty-three months after autotransplantation, found that the atrial pacemakers governed cardiac rhythm, that there was vagal and sympathetic reinnervation of the heart, and that there was no evidence of congestive heart failure. The major aspects of cardiac control and myocardial performance were viewed as normal.

Barta, Bózner, Černý, and Mrena (1966) and Barta, Breuer, Pappová and Zlatoš (1966) utilized the surgical cardiac denervation method proposed by Černý and Oláh (1958) to study changes in the denervated heart. This

surgical procedure included removal of cardiac nerves which arose from the recurrent laryngeal nerves and vagosympathetic trunks and then passed to the pretacheal plexus. The caudal cervical (vertebral ganglion) and stellate ganglion together with the linking ansa subclavia were extirpated bilaterally. The vagi were not transected, thereby eliminating esophageal and gastric dilatation, cardiospasms and pylorospasms which were sequelae of bilateral vagotomy (Jellinek, Kaye, Kaiser, Cooper; 1966). Sequelae 14-18 days following the Černý and Oláh (1958) technique for cardiac denervation, according to Barta, Breuer, Pappová, and Zlatoš (1966) included a decreased mean blood pressure, decreased coronary blood flow, and an increased coronary vascular resistance. The heart had decreased oxygen and energy requirements, decreased work, and decreased efficiency. A small number of nuclei were found in the right auricle and enlarged mitochondria were seen in some places in the myocardium. Jellinek, Kaye, Nigh, and Cooper (1964) following bilateral extirpation of the thoracic sympathetic trunk and caudial cervical (vertebral) ganglia, a similar procedure, found that glycogen and carbohydrate substance accumulated in the myocardium and that the total cardiac fat and fatty acid content was significantly greater. Phospholipids, nitrogen, ubiquinone, and lipase activity were not significantly different than in control dogs. Barta, Breuer, Pappová, and Zlatoš (1966) recognized that catecholamines stimulate cardiac metabolism and oxygen consumption and that their depletion after the Černý and Oláh (1958) denervation technique may play an important roll in decreased usage of oxygen and energy substances. Another change observed by Barta, Breuer, Pappová, and Zlatoš (1966) 14-18 days after denervation of the heart, included QQ interval change from .88 $\pm$ 0.24 to 1.04 $\pm$ 0.18 seconds.

Bartŏs, Černý and Kratochvil (1962) suggested this may be the result of liponeogenesis from carbohydrate substances. Accumulations of lipidic droplets in the myocardium were then verified histologically and with the electron microscope. These authors concluded that metabolic and hemodynamic disorders occurring after surgical denervation should be considered as serious if not decisive in the causes of cardiac failure occurring 2-3 weeks after autotransplantation.

Tsakiris and Donald (1968) based their surgery on the assumption that bilateral stellate (cervicothoracic) ganglion extirpation and bilateral vagal transection will eliminate central connections to the heart. Bilateral thoracotomy was employed to place snares around the cervicothoracic ganglia. Rami communicantes between the seventh and eighth cervical together with the vertebral nerve and any thoracic cardiac nerves arising from thoracic sympathetic ganglia were transected. Bilateral cervical vagotomy together with disruption of cervicothoracic ganglion, via the implanted snare, was accomplished 10 days later without entering the pleural cavity. The heart was not depleted of catecholamine. Heart rates increases from 96 beats/minute before to 111 beats/minute after denervation. Randall, Priola, and Ulmer (1963) have found regeneration of preganglionic sympathetic fibers after stellectomy, but cell bodies of postganglionic fibers were not found in the original site of the removed stellate ganglion. Regenerated fibers were not observed passing to the heart and it was concluded that these fibers could not account for maintenance or recovery of normal cardiac responses to stimulation. Randall, Priola, and Ulmer (1963) reported that no information, to their knowledge,

existed regarding specific sprouting of postganglionic fibers within the myocardium from intact nerves on the side opposite the sympathectomized side. Regeneration time may be influenced by environmental factors. Carp kept at high temperatures showed faster cardiac parasympathetic regeneration than did carp kept at lower temperatures (Gas-Baby, Laffont, and Labat; 1967). Malmfors and Olson (1967) transplanted slices of the heart auricle into the anterior chamber of the eye. Adrenergic iris nerves were able to reinnervate these autologous, homologous, or heterologous grafts after vascular contacts were made. Intact sympathetic adrenergic neurons could easily extend their innervation areas.

Kuntz (1949) related that resection of pulmonary parasymathetic nerves had a rational basis in patients with bronchial asthma since broncho-constrictor fibers were parasympathetic. Complete or partial resection of the vagi was also used in treatment of gastric and duodenal ulcers to decrease production of acids according to Kuntz (1949). Extirpation of the stellate ganglion or its rami communicantes has been used for relief of cardiac or coronary pain (Kuntz, 1949); and blockage of the stellate ganglion has been used in certain psychiatric patients (Taraba, 1968); and catecholamine action has been reported to be associated with migrane (Adams, Orton, and Zilkha; 1968).

MATERIALS AND METHODS

In developing techniques for the various facets of this research project and in collecting systematic data, fifty dogs and one cat were utilized. Twenty-nine animals underwent surgery. The other twenty-three were sacrificed for the development and standardization of qualitative and quantitative techniques for measuring norepinephrine and acetylcholinesterase in heart tissue. Table 1 summarizes the animals used and their utilization. This study includes surgical, functional, biochemical, histochemical, and histological facets, each of which will be presented separately to best include the complex materials and methods of each area.

Surgery

Materials

Well equipped Biomedical Engineering surgical suite and preparation room

Gowns, gloves, hats, masks and drapes

Standard instrument tray plus bone forceps, Gelpi retractors, ten inch thoracic thumb forceps, nine inch metzenbaum scissors, bulldog vascular clamps, and a bone saw

Ohio closed system gas machine - Ohio Chemical and Surgical Equipment Co., Madison, Wisconsin

Bennet Assister, model BA-2 - Bennett Respiration Product Inc., Los Angeles, California

Endotracheal catheter

Five and twelve cubic centimeter syringes with twenty gauge one inch needle

Thiamylal sodium 4%

Clipper #40 head

Vetafil - Dr. S. Jackson, 7801 Woodmont Ave., Washington, D.C.

Methoxyflurane

Germicidal liquid

Tincture of zepherine

Methods

Of the twenty-nine animals selected for surgery, thirteen were chosen for comparative chronic studies. Five additional animals were controls. The remainder represent animals utilized for developing surgical techniques and in acute studies.

Techniques for complete and partial denervation of the dog heart were based on previous morphologic dissections (McKibben and Getty, 1968).

All surgical dogs had their water and food withdrawn six to twelve hours preoperatively. Each was anesthetized using 4% thiamylal sodium intravenously to effect, and maintained in a surgical plane of anesthesia with methoxyflurane. The latter was administered with a closed system gas machine connected to an endotrachial catheter. Controlled respiration was maintained during intrathoracic surgery with the aid of a Bennett assister. No other drugs were administered during the presurgical or surgical periods. Penicillin and dihydrostreptomycin in aqueous suspension, were administered intramuscularly at 10,000 units and 12.5 milligrams per pound respectively per day for two to five days postsurgically.

For surgery, all dogs were positioned in dorsal recumbency. All were clipped, washed with germicidal liquid, and sprayed with tincture of zepherine. Sterile precautions were taken in surgical scrubs, and during donning of gowns and gloves as well as in draping the dogs and throughout surgery.

Generally the first surgical procedure was cannulation of the left femoral artery for measurement of arterial blood pressure.

In those dogs requiring intrathoracic surgery, the skin was incised midventrally from a point about four inches cranial to the level of the manubrium sterni, to an area just caudal to the level of the xiphoid process. The incision was extended through the facia and muscle attachments on the ventral surface of the sternebrae. Hemostasis was employed when needed. The combined use of a bone saw and bone cutting forceps was preferred over a mallet and chisel for splitting the sternebrae. Upon entering the plural cavity, the Bennett assister was utilized to maintain respiration until the cavity was closed. Gelpi retractors aided proper visualization of structure deep within the thorax. Nine inch Metzenbaum dissecting scissors and ten inch thoracic tissue forceps were useful in deep dissections. Internal thoracic arteries and veins were usually ligated for better exposure. Intrathoracic dissections generally proceeded from deep to superficial structures to minimize the effect of hemorrhage in the surgical field. After the first few dogs, it was decided to change from stainless steel to vetafil for apposition of the transected sternebrae. No complications were experienced and the vetafil was easier to use. At the time of thoracic closure, the lungs were inflated to expel as much air from the pleural cavity as possible. Only twice was a thoracic tap to remove further air and fluid felt to be necessary. During closure of the incision sites, mercerized cotton thread was used subcutaneously and vetafil was employed to close the skin.

Table 2 outlines the procedures employed for the surgical patients. Further explanations of additional surgical procedures employed are

considered with the individual dog's surgical descriptions.

Control dogs 1, 2, 3, 4 and 5 Control animals were anesthetized and surgically their femoral arteries were cannulated for recording arterial blood pressure and for exsanguination.

Dogs 6 and 7 The first five thoracic ganglia caudal to the cervicothoracic ganglia were bilaterally isolated and their cardiac nerves transected. Likewise the cervicothoracic and vertebral ganglia were then exposed bilaterally and their cardiac branches transected beginning with the more caudal ones and proceeding cranially. When removing vagal cardiac nerves bilaterally, particular care was taken to remove left caudal vagal cardiac nerves. These often escape casual observation. The left recurrent laryngeal nerve, which gives branches to the heart from within the cardiac plexus, was transected at its origin from the left vagus nerve. Right recurrent cardiac nerves were transected, after isolating the right recurrent laryngeal nerve, without injury to the latter nerve.

Dog 20 The regional neural ablation technique developed by Cooper, Gilbert, Bloodwell, and Crout (1961) was employed. Surgery was prolonged and difficulty was experienced in maintaining proper oxygen and anesthetic levels. The difficulty was apparently associated with an adaptation to the Ohio gas machine which allowed direct measurement of respiration on the Grass polygraph.

Dog 8 Cardiac branches of the right recurrent laryngeal nerve and right vertebral ganglion were transected. The sympathetic trunk from the right sixth thoracic through right cervicothoracic ganglion was isolated and extirpated. The right vagus was transected caudal to the right recurrent laryngeal nerve. Recording difficulties were experienced with

arterial blood pressure.

Dog 9 Cardiac nerves originating from the left vagus nerve were transected as was the left recurrent laryngeal nerve near its origin from the left vagus. The left sympathetic trunk from the vertebral ganglion through seventh thoracic ganglion was extirpated.

Dogs 13, 14, and 15 The thoracic contribution to the extrinsic cardiac innervation was interrupted to various degrees. In dog 13, only sympathetic ganglia between the cervicothoracic and eight thoracic ganglia were bilaterally removed. Central communications remained intact to the cervicothoracic ganglia from cervical rami communicantes bilaterally, and from the first three left thoracic rami and first two right thoracic rami communicantes. The cervical rami were the only central communications to the cervicothoracic ganglia left intact in dog 15. Thoracic rami communicantes were transected bilaterally from the first through seventh ramus. In dog 14, the cervicothoracic, as well as the thoracic ganglia between the cervicothoracic ganglion and the sixth thoracic ganglion was bilaterally extirpated.

Dogs 16 and 19 Exposure of the vertebral ganglia bilaterally, and their separation from the vagi, required careful dissection. Each vagus nerve was isolated along the caudal portion of the accompanying carotid artery and was followed caudally to reach the vertebral ganglion on each side. These ganglia lay between the brachiocephalic veins and subclavian arteries in a poorly accessible area. Further, they are bound closely to the vagi and require careful dissection to separate them from the vagi without injury to the latter.

Dog 12 Exposure of the left vertebral ganglion was similar to the surgical approach used for dogs 16 and 19. The left caudolateral vertebral cardiac nerve was then identified and transected.

Dog 17 The cervical sympathetic trunk was bilaterally transected cranial to the vertebral ganglion. Initially the vagosympathetic trunk was isolated just cranial to the vertebral ganglion in the thorax. The vertebral ganglion was carefully separated from the vagus bilaterally and the sympathetic trunk extending cranially from the vertebral ganglion was bilaterally transected.

Dog 10 A one inch right paramedian incision approximately five inches in length and extending cranial-caudal equidistance from the level of the occipito-atlantal junction was made on the ventral surface of the neck and jaw. The incision was carried dorsally between the sternocephalicus muscle and muscles of the pharynx and larynx. The mandibular salivary gland and lymph nodes were reflected laterally. Retractors were useful in facilitating exposure in the deeper dissection. The right carotid artery and vagosympathetic trunk was isolated medial to the sternocephalicus and followed to the origin of the internal carotid artery. The right vagus and right sympathetic trunk is tightly fused in this area, but separation is possible at the level of origin of the right cranial laryngeal nerve from the right vagus. The sympathetic trunk, passing cranially, passes medial to the right cranial laryngeal nerve to reach the right cranial cervical ganglion. A branch may extend between the right cranial cervical ganglion and right vagus at the level of the former. After separating the sympathetic and vagal trunks at this level, the vagus was transected caudal to the origin of the right cranial laryngeal nerves.

The sympathetic trunk was left intact. The surgical area described was very vascular and nerve branches were abundant in the area. Therefore, considerable care must be taken during surgery.

Dog 18 An incision approximately four inches long was made through the ventral midcervical skin about one inch right paramedian. The incision was carried medial to the sternocephalicus muscle by blunt dissection to reach the right common carotid artery and right vago-sympathetic trunk. The whole right vagosympathetic trunk was transected since the vagus and sympathetic divisions are not discretely separate in this area in the dog.

Dog 11 The left recurrent laryngeal nerve gives branches to the heart as it passes through the cardiac plexus. The nerve therefore was transected at its origin from the left vagus nerve rather than injuring other cardiac nerves while transecting recurrent cardiac nerves within the cardiac plexus. Exposure of the right recurrent laryngeal nerve was similar to that for exposing the nearby vertebral ganglion in dogs 16 and 19. The right recurrent cardiac nerves were isolated and transected near their origins from the right recurrent laryngeal nerves as the latter passed around the right subclavian artery.

Dogs 21, 22, 23, 24, and 26 This surgery bilaterally was similar to that described for dog 10 unilaterally. Both vagi were transected.

Dog 25 The individual vagal cardiac nerves were transected near their origin from the vagi. Both cranial vagal and caudal vagal cardiac nerves were transected. Sympathetic cardiac nerves associated with the vagi were separated from the vagi as much as was possible.

Dog 27 The vagi were isolated in the thorax. The right vagus was sectioned just caudal to the origin of the right recurrent laryngeal nerve. The left vagus was separated from the left vertebral ganglion and was transected at this level.

Dog 28 On 6-17-68 the right vagus nerve was transected just caudal to the origin of the right recurrent laryngeal nerve. One month later, 7-15-68, the left vagus was transected just caudal to the origin of the left cranial laryngeal nerve.

Cat 29 Both right and left vagosympathetic trunks were transected in the mid-cervical region similar to the method employed unilaterally in dog 18.

Dogs utilized in the chronic study following surgical recovery were caged in animal quarters where they were maintained on dry dog food and water. No contagious disease problems ensued except in dog 11 which developed distemper. Two female dogs whelped following surgery, but none were pregnant at the time of tissue harvest.

Functional

Materials

Biomedical Engineering facilities

Grass model 5 polygraph - Grass Instruments, Quincy, Massachusetts

Statham P 23 A c and P 23 D transducers - Statham Co., Hato Rey, Puerto Rico

Vascular catheter

Needle and Backhaus clamp electrodes and leads

Surgical materials

Methods

Electrocardiogram and aortic blood pressure recordings were taken on the dogs which underwent surgery. In each a standard bipolar lead II electrocardiogram was recorded using electrodes attached to the brachium of the right forelimb and stifle of the left hindlimb. A ground electrode was attached to the right hindlimb. Comparative recordings were taken at a paper speed of 50 millimeters per second. Sensitivity of electrocardiograms was standardized at 1 mV per centimeter, in chronic denervated dogs and control dogs, however, prior to the chronic animal harvest some variability may be present since recordings were adjusted to best visualize wave forms. Blood pressure measurements were recorded on the polygraph through the transducer which was attached to a fluid filled intravascular catheter inserted into the right femoral artery and threaded into the descending aorta. The polygraph sensitivity for recording aortic pressure was standardized at 100 millimeters of mercury per centimeter deflection.

Analysis of functional data was programmed by the Iowa State University Computation Center. An analysis of variance (ANOU) test was utilized to measure whether significant differences existed between the mean recorded values of chronic denervated dogs 6 through 17 during presurgical, surgical, acute postsurgical, and chronic postsurgical periods. A product moment correlation matrix was also statistically employed to analyze what relationships were present between the various functional parameters and chemical and structural parameters in dogs 2 through 18.

Harvest and Storage

Materials

Biostat-Cryenco-liquid nitrogen tank, model 11 with cannister and probe - Cryogenic Engineering Company, 4955 Bannock Street, Denver, Colorado 80216

4 liter thermos

Stainless steel milk dipper

Torsion balance model DL+2-1 - The Torsion Balance Company, Clifton, New Jersey

Ultra low temperature freezer

Styrofoam chest

Mincer and mincing board

Foil and magic marker

Beakers - 1000 ml., ringstand, and clamps

Quart jars, four and eight ounce bottles

Liquid nitrogen

10% buffered neutral formalin

2 - methylbutane

Surgical materials previously listed

Physiologic materials previously listed

Methods

The dogs were anesthetized and prepared for surgery as described in the surgical methods section. Physiologic data was recorded as described in the physiology methods section.

After receiving adequate recordings, the animals were exsanguinated. Periodically 50 mm/sec recordings were obtained during this phase to observe changes in electrocardiograms. Generally about the time cardiac

ventricular fibrillation occurred, the chest was opened and the heart quickly removed. After blotting to remove excess blood, representative samples were excised from each chamber (Figure 1). Atrial chambers were removed intact. Four, half centimeter square pieces were excised from the ventral portion of each atrium for histochemical studies of catecholamines and acetylcholinesterase and for the histologic staining procedures. The pieces for histochemical study were immediately wrapped in foil and placed in 2-methylbutane suspended in a thermos of liquid nitrogen. They were then stored in liquid nitrogen in the Biostat-cryenco until transferred to the ultra low temperature freezer. Excised squares for histologic staining procedures were placed in 10% buffered neutral formalin. The same procedure was followed in collecting ventricular tissues except that approximately one centimeter square pieces were excised from the dorsal ventricular walls. Generally it was attempted to include at least one blood vessel in the square. Tissue not being processed was stored in a styrofoam chest which contained ice until processed. After completing the harvest of each chamber for histochemical and histologic study, the remainder of the atrial chamber or ventricular tissue sample was finely minced and mixed, weighed to 100 mg samples, packaged in foil, and placed in 2-methylbutane suspended in a thermos of liquid nitrogen. Samples were stored in liquid nitrogen in the Biostat-Cryenco until transferred to the ultra low temperature freezer for storage at $-70^{\circ}C$. Mincing was accomplished with closely spaced razor blades embedded in a paraffin wax block.

Chemical Assay

Materials

Norepinepherine assay

Virtis "23" homogenizer - Virtis Co., Gardiner, New York

Lab-Line test tube super-mixer - Lab-Line Instruments Inc., Melrose Park, Illinois

International 4-place clinical centrifuge - International Equipment Co., Needham Heights, Massachusetts

Ultra low temperature freezer

Beckman expandomatic Ph meter - Beckman Instruments Inc., Richmond, California

Drying oven - Despatch Oven Co., Minneapolis, Minnesota

Biostat-Cryenco liquid nitrogen tank

Fluorometer-Turner model 111 - G. K. Turner Associates, Palo Alto, California

385 primary narrow pass filter (combination of Corning 0-51 and 7-51 filters)

485 secondary sharp cut filter

High sensitivity sample holder

Microcuvette sample holder 0.25 ml sample volume

Microcuvettes, round, Borosilicate glass

Analytical balance Sartorius 2463 - Sartorius, Westbury, New York

Torsion balance - model D L T 2 - 1

Heavy duty bunsen burner

12 ml graduated centrifuge tubes

Volumetric pipets 10, 5, 1, and 1/10 ml

Micropipets 20 lambda (microliters) disposable

Bottles, 4, 6, 8, 16 ounce

Erlenmeyer flasks 125, 500 ml

Volumetric flasks 10, 110, 250, 500 ml

Beakers 50, 150, 250, 500, 1000 ml

Propipettes

Parafilm "M" laboratory film

Clock - interval timer

Distilled water

Glacial acetic acid

Concentrated hydrochloric acid

N - Butanol

Iodine

Potassium iodide

Versene

Sodium hydroxide

Sodium phosphate monobasic

Sodium phosphate dibasic

Sodium sufite

L - Noradrenaline Bitartrate Hydrate

Acetylcholinesterase assay All materials used in norepinephrine assay were also used in the acetylcholinesterase assay except the chemicals, fluorometer and its accessories, and the centrifuge and its accessories. In addition the following equipment was necessary.

Bausch and Lomb Spectronic 20 Spectrophotometer - Bausch and Lomb Co., Rochester, New York

Test tubes - B L 4 inch long - 11.66 mm diameter

Sodium phosphate - monobasic and dibasic

5:5 Dithiobis - 2 - nitrobenzoic acid (D T N B)

Acetylthiocholine iodide

Glutathione

Sodium Bicarbonate

Methods

Norepinephrine assay The method utilized was modified from the Jacobowitz modification of the Hogan method which Dr. Jacobowitz of the Department of Pharmacology at the University of Pennsylvania in Philadelphia shared with me in personal communications in 1968. Tissue removed from the ultra low temperature freezer was placed in the liquid nitrogen Biostat-Cryenco tank, where for convenience it was stored until homogenization.

One hundred mg minced frozen samples were placed directly from the liquid nitrogen into the Tenbroek grinder. The grinder contained 5 cc N-Butanol and was cooled in an ice water bath. Tissue should not be permitted to thaw between harvest and the time it is placed into cooled butanol, since the freezing process lyses cells thereby permitting monoamine oxidase (MO) and catechol-O-methyl transferase (COMT) to degrade norepinephrine at room temperature. The grinding procedure was accomplished using a Virtis grinder modified with a cork of an appropriate size to rotate the pistle of a Tenbroek grinder. Grinding generally was accomplished in less than five minutes. Three different samples were taken from each chamber of each heart and processed according to Table 12. Solutions used in this assay were as follows:

0.1 M phosphate buffer - pH 6.5

Versene pH 6 - 6.5 by adding 5 N sodium hydioxide, then add water to make 4% disodium - E D T A (Versene)

Iodine - 4-8 g potassium iodide + 0.25g iodine - qs. 100 ml water

Alkaline sulfite - 0.625g sodium sulfite in 5 ml water plus 20 ml

5 N sodium hydroxide made fresh daily

5 N acetic acid - 71 ml glacial acetic acid qs. 250 ml water

5 N sodium hydroxide - 50g NaOH - qs. 250 ml water

.01 N Hydrochloric acid - 0.5 ml - 36.5% concentrated hydrochloric acid - qs. 500 ml water

Standards The Stock L noradrenaline was 50ugm/ml in .01 N HCL. A 25ugm/ml solution was utilized for delivering 0.5ugm in a 20 lambda pipette for internal standards; and .05, .10, .50, 1.0, and 2.0 ugm noradrenaline was delivered per 20 lambda pipette from 2.5, 5, 25, 50, and 100 ugm/ml L noradrenaline standards respectively for standard curve (Graph 2). These are reported as half sample values in Table 15, since the readings were made on 2 ml Butanol extract portions as was the case with tissue readings.

Calculations Raw data for calculations is listed in Tables 13, 14, 16, 17, and 18. In Table 19, Equations 1 and 2 convert the fluorescent transmission information into micrograms of norepinephrine per gram of tissue. The actual recovery after adding known quantities of norepinephrine to the samples, is found in Equation 3. This is divided by the calculated recovery to find the percent recover (Equation 4). The ugm/gm noradrenaline calculated in Equation 3 is then corrected to simulate 100% recovery in Equation 5.

All readings were made on the fluorometer using microcuvettes with the high sensitvity holder. A 385 mu primary and a 485 mu secondary filter was utilized for best results.

Acetylcholinesterase The method utilized was a modification of the Ellman, Courtney, Andres, and Featherstone method (1961).

Tissue removed from the ultra low temperature freezer was placed in the liquid nitrogen Biostat-Cryenco tank, where for convenience it was stored until homogenization. Each 100 mg tissue sample was homogenized in 5 ml phosphate buffer (pH 8), using the modified Virtis-Tenbroek homogenizer combination. The homogenizer was suspended in cool water to prevent heating during the homogenizing process. The homogenate was assayed at room temperature according to the procedure in Table 23. The substrate acetylthiocholine iodide was added and mixed by bubbling in the test tube containing the other reagents while it was in the sample holder of the colorimeter. Solutions used in this assay were as follows:

Phosphate buffer solutions pH 7 and 8 prepared using mono and dibasic sodium phosphate in distilled water

Substrate prepared as 0.075 molar solution; 21.67 mg acetylthiocholine iodide per milliliter of distilled water

(Fresh solution prepared weekly)

Reagent prepared as a 0.01 molar solution 39.6 mg 5.5' dithiobis (2-nitrobenzoic acid) dissolved in 10 ml of 0.1 molar phosphate buffer pH 7; then add 15 mg of sodium bicarbonate

Standards Glutathione was used to provide the thiol group for the standard curve (Fowler and McKenzie, 1967) (Graph 1). In standardization, an extinction coefficient of the nitrothiobenzoate ion using the colorimeter was found. This was accomplished using stock

solutions of 1, 3, 5, 7, and 10 u moles of glutathione per millimeter of distilled water. Twenty lambda of each stock solution was added to a solution of 100 microliters DTNB in 3.0 ml of a 0.1 m phosphate buffer pH 8. Change in absorbance was recorded each minute over a six minute period. The mean change occurring in the last 5 minutes was recorded for each standard concentration. According to Ellman (1959) and Robert Angelici of the Chemistry Department at Iowa State University, during personal communications, the extinction coefficient is calculated as explained in Table 27.

A summary of data utilized in this process is found in Table 26. From this data the extinction coefficient for nitrothiobenzoate was set at 14,500. Having established this value, tissue levels of acetylcholinesterase can now be measured. Acetylcholinesterase hydrolyzes acetylthiocholine on a 1:1 ratio resulting in thiocholine which reacts with DTNB as did glutathione. Knowing the extinction coefficient we can now determine the unknown original concentration of acetylcholinestrase in tissues using the formula similar to that suggested by Ellman, Courtney, Andres, and Featherstone (1961) (Table 27).

Average optical density colorimetric changes and calculated moles hydrolized are listed for each chamber of each dog in Table 24 and 25. Each sample in each chamber was run in duplicate and averaged to the value in Tables 24. All samples were read in the spectronic 20 colorimeter at 412mu wavelength.

Histochemical

Norepinephrine

Materials

Cryostat, International - Harris model C T - International Equipment Co., Needham Heights, Massachusetts

Chucks and embedding compound - Tissue-Tek - Ames Co., Elkhart, Indiana

Sectioning knife, slides, and cover slips

1 and 2 liter desiccators with plates

Fluorescent microscope with B G - 12 primary, and a secondary BV filter

Biostat-Cryenco liquid nitrogen tank

Fume hood

Precision Thelco model 16 oven - Precision Scientific Co., Chicago, Illinois

Phosphorus pentoxide

Paraformaldehyde powder

Sulfuric acid

Method The fluorometric demonstration of catecholamines in ganglia, the nictitating membrane, and the vas deferens has been accomplished by Jacobowitz and Koelle (1965), and Spriggs, Lever, Rees, and Graham (1966), without the aid of freeze dry and associated apparatus described by Falck and Owman (1965). No freeze dry equipment was available to me, and Dr. Jacobowitz of the Department of Pharmacology, University of Pennsylvania, in Philadelphia during 1968, in personal communications wrote "The Falck and Hillarp method is difficult, especially for an inexperienced student working alone on a Ph.D. thesis problem. Too much

time is involved in setting this up and gathering equipment and experience." Because of these factors it was felt that the methods used successfully without freeze dry equipment might be adapted to heart tissue therefore efforts were expanded in developing this technique. Basically this involved cutting cryostat sections of tissues, at 14-16 u which had been immediately frozen at harvest and not allowed to thaw. The sections were placed on slides and immediately transferred to a desiccator with phosphorus pentoxide for 1.5 hours. Following drying, slides were removed and placed in another desiccator with previously hydrated paraformaldehyde powder. This was incubated at 80°C for 1 hour.

The histochemical reaction expected is a condensation of the catecholamine with formaldehyde gas to form a dihydroisoquinaline derivative which when excited with ultraviolet light, fluoresces. This can be observed using fluorescent microscopy.

Personal communications were exchanged with Spriggs of the Department of Materia Medica and Pharmacology at the University of Wales in Great Britian in 1968, after great difficulty was experienced with repeatibility using the above technique on heart tissue. Several of Spriggs' suggestions in altering dehydrating and incubation procedures were tried with no further success resulting. Jacobowitz in further communications during 1968 wrote "Cryostat sections of the heart, unless freeze-dried, never work!" I was however able to observe fluorescence in cardiac muscle without freeze dry apparatus (Figures 36-41), but not repeatedly. Perfection of this technique could be a fruitful area of further study.

Acetylcholinesterase

Materials

Cryostat - International-Harris model C T

Chucks and embedding compound - Tissue-Tek - Ames, Co., Elkhart, Indiana

Sectioning knife, slides and cover slips

Biostat-Cryenco liquid nitrogen tank

Light microscope

Chemicals and solutions listed by El-Badawi and Schenk (1966).

Methods The tissues were removed from the liquid nitrogen tank, unwrapped, and placed directly into the freezing tissue-tek on the chuck within the cryostat. Sections were cut at 12-16u.

Initial efforts using the El-Badawi's and Schenk (1966) modification of the Karnovsky-Roots (1964) method for demonstrating acetylcholinesterase were not satisfactory for demonstrating intracardiac nerves. Modifications resulted in quite acceptable results. Initially, incubation of slides at 37°C with the acetylthiocholine iodide, sodium citrate, cupric and sulfate, tetraisopropylpyrophosphoramide (Iso OMPA), and potassium ferricyamide was done on a slide warming table by adding the incubation medium to the slides under petri dishes on the warmer. The small quantities lost by evaporation were replenished periodically. It was later found that slides incubated in Coplin jars placed in a paraffin oven maintained at 37°C provided more consistent results. In the incubation procedure Karnovsky and Roots (1964) reported that thiocholine split from acetylthiocholine by acetylcholinesterase in the nerves is believed to reduce ferricyonide to ferrocyanide preferentially. The ferricyanide

combines with copper ions to form insoluble copper ferrocyanide. The copper ions in the incubation medium are complexed with citrate to prevent formations of copper ferricyanide. Iso OMPA is a blocking agent used to prevent nonspecific cholinesterases from splitting acetylthiocholine which would result in false positive staining. Following incubation sections were rinsed in distilled water, counterstained for 15 seconds in Eosine, dehydrated in 95% and absolute alcohol 15 seconds each, cleared in 1:1 absolute alcohol-xylene mixture and two changes of xylene for 30 seconds each, and mounted in Permount. Acetylcholinesterase active sites were identified by brownish black cupric ferrocyanide deposits.

Optimal incubation times varied with the heart chambers and animals therefore, 3 hour, 8 hour, and 19 hour incubation times were used. Many epicardial nerves are stained at the three hour period. Those of myocardial blood vessels particularly in the ventricles are better stained after 8 hours and after 19 hours, intramyocardial twigs may be discerned though nuclei and portions of myocardial cells also may be stained in the atria.

Histologic Stains

Materials

Microtome and knife

Slide boxes

Slide warmer table

Lipshaw tissue float

Autotechnicon model 2 A - The Technicon Co., Chauncey, New York

75 x 25 mm slides

24 x 40 mm and 24 x 50 mm cover glasses

Gelatin

Permount mounting medium - Fisher Scientific Co., Fair Lawn, New Jersey

Ethanol 95%

Absolute ethanol

Chloroform

Paraplast

Staining dishes and racks

Methods

Those tissues fixed in buffered neutral 10% formalin and stained with Verhoff's-Van Giesen's, Alcian Periodic Schiff, and Harris Hemotoxylin and Eosin were dehydrated, cleared, and embedded in paraplast using the auto-technicon. The following schedule was followed.

Process and Chemical Changes in Order	Duration in Hours
Dehydration	
Ethanol 70%	0.5
Ethanol 80%	1.0
Ethanol 95%	1.0
Ethanol 95%	1.0
Ethanol Absolute	1.0
Ethanol Absolute	1.0
Ethanol Absolute	1.0
Clearing	
Chloroform No. 1	1.0
Chloroform No. 2	1.0
Embedding	
Paraplast No. 1	2.0
Paraplast No. 2	2.0

Tissues fixed in buffered neutral 10% formalin to be stained with Oil Red O were embedded using tissue-tek and cut on the cryostat for staining. Oil Red O sections were placed directly onto slides, stained, and mounted in Farrant's medium. Tissues for other stains were cut at 5u thickness on a rotary microtome, floated onto slides, stained, and cover glassed using mounting medium.

Staining procedures used were as follows:

Hemotoxylin and Eosin

Chemical Changes in Order	Duration
Xylene	5 min
Xylene	5 min
Absolute Ethanol	2 min
95% ethanol	2 min
Tap water	2 min
Harris Hematoxylin	15 min
Tap water	rinse
Acid alcohol	1 dip
Tap water	rinse
Distilled water	rinse
Lithium carbonate (1%)	6 dips
Distilled water	rinse
0.5% eosin with acetic acid	2 min
95% ethanol	1 min
95% ethanol	1 min
Absolute ethanol	1 min
Absolute ethanol	1 min
1:1 absolute ethanol:xylene	30 sec
Xylene	2 min
Xylene	10 min
Mount	

Verhoeff's-Van Gieson's Stain

Chemical Changes in Order	Duration
Xylene	5 min
Xylene	5 min
Absolute ethanol	2 min
Absolute ethanol	2 min
95% ethanol	2 min
70% ethanol	2 min
Tap water	5 min
Verhoeff's elastic stain (until black)	15 min
Tap water	rinse
2% $FeCl_2$ (until only elastic fibers and nuclei are stained)	12 sec
Distilled water	rinse
Van Gieson's stain	30-60 sec
Distilled water	rinse
95% ethanol	rinse
Absolute ethanol	1 min
Absolute ethanol	1 min
1:1 absolute ethanol:xylene	30 sec
Xylene	2 min
Xylene	10 min
Mount	

Alcine Blue Periodic Schiff's Stain (PAS)

Chemical Changes in Order	Duration
Xylene	5 min
Xylene	5 min
Absolute ethanol	3 min
95% ethanol	3 min
70% ethanol	3 min
50% ethanol	3 min
Distilled water	rinse
Alcian Blue (0.2%)	30 min
Running tap water	2 min
Distilled water	rinse
0.5% periodic acid	10 min
Distilled water	rinse
Schiff's reagent	25 min
Sulfurous acid rinse	2 min
Sulfurous acid rinse	2 min
Sulfurous acid rinse	2 min
Running tap water	5 min
Weigert-Lillie alum hemotoxylin	5 min
Running tap water	2 min
95% ethanol	1 min
95% ethanol	1 min
Absolute ethanol	1 min
Absolute ethanol	1 min

Alcine Blue Periodic Schiff's Stain (PAS) (Cont.)

Chemical Changes in Order	Duration
1:1 absolute ethanol:xylene	30 sec
Xylene	2 min
Xylene	10 min
Mount	

Oil Red O

Chemical Changes in Order	Duration
Frozen sections 16 u - place on gelatin coated slide	
Alcohol - Tween 80 without dye - agitate	10 min
Oil Red O stain	Overnight
Differentiate in alcohol - Tween	rinse quickly
Distilled water	rinse
Mayer hemotoxylin	2-3 min
Distilled water	rinse
Scott's solution	blue
Tap water	5 min
Mount in Farrant's medium and ring cover-glass for permanaency	

Histologic sections were photographed using a Leitz Ortholux microscope equipped with 4, 10, 25, 40, and 100 power plano objectives. For bright field observations a 0.9 numerical operature condensor was used. During fluorescent observations for catecholamines in heart tissue a D1.20 numerical operature dark field condensor was fitted to the Leitz scope. A HBO 200 watt high pressure mercury vapor lamp provided the light and a BG-12 3 mm thick primary exciting filter was used together with a secondary barrier filter. Photography was conveniently accomplished using an Orthomat fully automated camera adapted to the Leitz microscope. Most histologic sections were initially studied using a Dynazoom Bausch and Lomb binocular microscope fitted with 3.5, 10, 43, and 97 power acromatic objectives.

For measuring myocardial fiber diameters and vascular laminae, a micrometer was adapted to one ocular of the Dynazoom Bausch and Lomb

binocular light microscope. The microscope was calibrated. One hundred units were found per 0.1 mm (100m) at one hundred times magnification, therefore 1 unit = 1 u. At 430 magnification, 42 units per 0.010 mm or 427 units per 0.10 mm (100u) were observed. This converted to 1 unit = .234u. At 970x magnification 970 units per 0.10 mm resulted in 1 unit equaling .103u. Myocardial fiber diameter measurement was most easily accomplished using routine hemotoxylin and eosin sections. Ten cross-sectional fibers showing nuclei were measured at random for each chamber of each animal. In measuring blood vessel dimensions, the Verhoeff's-Van Gieson's elastic stain was most useful. Indications of fiber length according to Munnell (1967) can be estimated by counting in focus nuclei per given area in longitudinal sections of tissue.

These were counted in each chamber of each dog using a 5 mm^2 grid placed in the ocular so that it is in the same focal plane as the section. Five such areas were measured in each chamber and averaged.

RESULTS AND DISCUSSION

For continuity the results and discussion will be presented in surgical, functional, chemical, histochemical, and histologic chapters.

Heart Surgery

Table 2 summarizes the surgical procedures performed on twenty-nine animals during the present study. Of these animals, thirteen different cardiac denervated dogs and five control dogs were used in chronic studies (dogs 1-18). Two animals were used in preliminary surgical technique development (animals 20 and 29), and the remainder were used in acute denervation studies (dogs 19, 21, 22, 23, 24, 25, 26, 27, 28). Prior dissections of cadavers in both the thoracic and cervical areas facilitated the surgical procedures which followed. Satisfactory surgical results were verified in animals by post mortem examination.

Paramount in results of the chronic surgical procedures was the development of a relatively uncomplicated surgical method for total extrinsic cardiac denervation which involved minimal affects on other body organs. The method used in the present study was based on the anatomical dissection of the extrinsic cardiac nerves in a previous study by this investigator (McKibben and Getty, 1968). The method selectively removed only the extrinsic cardiac nerves, leaving the ganglia of origin or vagi, and other visceral nerves intact as much as possible. Alteration of function associated with denervation of other organs was minimized, resulting in more accurate measurements than in bilateral ganglionectomy and bilateral vagotomy. The method of cardiac denervation called regional

neural ablation developed by Cooper, Gilbert, Bloodwell, and Crow (1961) also was advantageous in this respect, but may not always be complete (Jacobowitz, Cooper, and Barner; 1967); was difficult; and resulted in high mortality. Of the forty animals subjected to regional neural ablation by Cooper, Gilbert, Bloodwell, and Crow (1961) only fifteen survived. Autotransplantation of the heart though very useful in studying changes associated with heart transplants, presented limited value in studying the effect of extrinsic cardiac denervation since alterations of the vascular system associated with the surgery also influenced results. The method described in this thesis was believed to be an improvement over previously reported methods and resulted in depletion of cardiac norepinephrine as measured chemically. In this technique cardiac nerves arising from the thoracic, cervicothoracic, and vertebral ganglia as well as those from the vagi and right recurrent laryngeal nerve were transected near their origins. The left recurrent laryngeal nerve, which contributed branches to the heart, was transected near its origin since the cardiac branches themselves were not accessible without disrupting other structures. The various partial cardiac denervations were modifications of the total denervation technique or of the acute bilateral vagotomy technique. Descriptions of each technique used were presented in the surgical section of the materials and methods portion of this thesis. Methoxyfluorane was chosen as the anesthetic of choice for the surgical procedures since it was a relatively safe, short acting anesthetic and no reference to alteration of the cardiac norepinephrine levels was found in the literature. Pentobarbital sodium anesthesia used by some investigators has been shown to depress the measured values of cardiac norepinephrine by Paton and

Gillis (1968). No externally adverse sequellae followed surgery of these chronically denervated dogs except dog 10. They maintained their weight, were alert, and had no disease problems except in dog 11 which developed distemper. Dogs 10 and 15 whelped after surgery, but no dogs were pregnant at or near the time of tissue harvest. This was important since Laes, Pekkarinen, Saarikoski, and Suramo (1967) found that norepinephrine levels in the heart decreased towards the end of pregnancy. Dog 10 following right vagotomy just below the nodose ganglion showed most of the signs of the bilaterally vagectomized dogs which will be described later in the results and discussion. After becoming very emaciated and then whelping, she started regaining condition and externally appeared normal within about 2-3 months after surgery and at tissue harvest. Dog 18 did not show adverse signs following right midcervical vagotomy.

In the acute denervation studies, dog 19 after recovering from surgery and while awaiting the proper interval for tissue harvest was inadvertently sacrificed by another research group. Dog 25 developed respiratory difficulties during surgery, which were apparently associated with a device attached to the gas machine to measure respirations. Further, the technician was completely involved with trying to keep the dog anesthetized and meaningful recordings were not obtained.

Those acutely studied animals subjected to bilateral vagotomy presented unique results. Initially to establish that bilateral vagotomy was compatible with life, the vagus nerve was bilaterally sectioned in a cat. This animal recovered from surgery and was kept for several months. The first dog subjected to bilateral vagotomy (dog 21) died two days following

surgery of unexplained causes. In the next three dogs which underwent bilateral vagotomies, (dogs 22, 23, and 24) extenuating circumstances shrouded the problems associated with the bilateral vagotomy. In one a forgotten assignment to withhold food and resultant aspiration of stomach contents occurred. In another dog, a substitute dog was utilized and apomorphine was given to empty stomach contents. Violent vomition followed recovery from surgery and in the post mortem examination the stomach was found ruptured through the wall of the thoracic esophagus. A third dog was lost after showing respiratory distress during surgery. His temperature rose to over 108^{o}F during surgery followed by death the following day. After the next bilaterally vagectomized dog, number 26, expired, careful scrutiny was made of mechanical, surgical and postoperative procedures. A species difference was suspected in the roll of, and necessity of the vagus in maintaining life. Markowitz, Archibald, and Downie (1964) revealed possibilities of pulmonary complications associated with bilateral vagotomy and it was suggested that the right recurrent laryngeal nerve be left intact in bilateral vagotomized dogs.

Sequelae noted in retrospect on the previous bilaterally vagectomized dogs included intense thirst, great quantities of mucous formation with drooling, and violent vomition of food and water within a few seconds to minutes after eating or drinking. The animals became dehydrated extremely rapidly and died within 2 to 3 days without supportive therapy. Atropine seemed to have little effect in stopping these manifestations. In the last two surgical procedures for bilateral vagotomy (dogs 27 and 28) the right recurrent laryngeal nerve was left intact. Both were maintained

beyond the time when pulmonary factors were reported to cause death. In dog 28, the left vagus was transected just below the nodose ganglion one month after transecting the right vagus caudal to the origin of the right recurrent laryngeal nerve. During the intervening month the dog appeared normal. Following the transection of the left vagus, however, he exhibited the same sequelae as previously vagectomized dogs. Therapy included regular injections of atropine in dosages between 1/100 and 1/25 grain. This did not result in any change in sequelae. Tranquilization using trifluprómazine hydrochloride and intestinal protectants appeared to make the dog more comfortable but vomition persisted immediately after eating or drinking. Two and one-half percent dextrose in half strength saline was administered intravenously and subcutaneously and helped maintain the animal until 10 days postsurgically. In agreement with Jellinek, Kaye, Kaiser, and Cooper (1966), it was concluded after post mortem examinations of these dogs that megaesophagus, cardiac and pyloric sphincter contraction, and gastric dilatation were the sequelae responsible for the metabolic changes leading to death. Pulmonary changes were apparently not instrumental in the pathogenesis except where inspiration of food and water into the lungs occurred.

Heart Function

Functional studies were conducted to find whether acute changes occurred in functional data, associated with extrinsic cardiac denervation; what were the effects of denervation on functional parameters after denervation of long standing; and what correlations existed between functional, chemical, and structural findings. Functional data including

electrocardiograms, blood pressure and heart rate recordings were taken on all surgical animals except numbers 20 and 29 which were used in preliminary surgical methods. Animal position was standardized in dorsal recumbency, for surgical reasons, but was not the most commonly utilized position for electrocardiogram recordings reported by other authors. This position apparently had some affects on electrocardiogram recordings. Unfortunately, according to Smith, Hamlin, and Crocker (1965) no studies exist which relate relative or absolute variation in heart position to variations in the electrocardiograms of domestic animals.

Recordings of all animals from number 6 through 28 with the exception of dogs 20, 21, and 25 were utilized in discerning what acute functional changes accompanied various types of extrinsic cardiac denervation. Functional recordings of dogs 6-17 were additionally useful in chronic denervation studies. Dogs 2 through 18 were included in the study of the correlation between functional chemical and structural parameters. Recordings of dogs 19, 22, 23, 24, 26, 27, and 28 were useful in analysis of acute studies and representative recordings of these animals can be found in Figures 29, 30, 31, 32, 33, 34, and 35. In dogs 1, 21, and 25 functional data was of questionable usefulness because of recording difficilties hence limited use was made of their recordings. Representative functional recordings of dogs 2-18 were useful in both acute and chronic studies and were included in Figures 2-28.

Data from electrocardiogram, blood pressure, and heart rate recordings during the presurgical, surgical, acute postsurgical, and chronic postsurgical periods were studied and representative mean values reported in

Tables 3, 4, 5, 6, and 7. Analysis of variance (ANOV) tests were statistically employed to determine whether significant differences occurred in these mean recordings. Because of a lack of replication of experimental units, the three way interaction was considered negligible and that source of variation was used as the source of error to test the other hypotheses. The ANOV test was a very powerful statistical test, according to Popham (1967), by which conclusions could be drawn about mean differences through the process of analyzing variances. This test was very useful in the present study. As with any living dynamic system however, familiarity with the parameters measured may prevent one from making wrong conclusions from statistical analyses. The hypotheses and results of the ANOV statistical analyses were listed in Tables 8, 9, 10, and 11. When all periods were analyzed, results indicated a highly significant difference between animals of the study in electrocardiogram wave amplitudes, in intervals, and in heart rate. Elimination of the postsurgical period gave a better analysis of acute changes. When the postsurgical period was omitted from the analysis highly significant differences between animals in the remaining three periods were reported for electrocardiogram amplitude and interval, and for pulse pressure. When the presurgical period was eliminated in the analysis thereby reducing the bias of this period, highly significant differences were recorded in electrocardiogram amplitudes and intervals, heart rate, and sytolic blood pressure. In considering <u>all</u> animals, significant or highly significant differences between presurgical, surgical, acute postsurgical, and chronic postsurgical periods were present in electrocardiogram intervals, diastolic blood pressure, and pulse pressure. If the chronic postsurgical period was omitted from the analysis, no significant or highly

significant difference between periods was observed while if the pre-surgical period was omitted, electrocardiogram intervals, distolic blood pressure, and pulse pressures were significantly or highly significantly different. This was interpreted as indicating that the chronic denervated animals' electrocardiogram intervals, diastolic blood pressure, and pulse pressures were changed by the surgical procedures. No significant electrocardiogram changes in amplitude between periods were recorded, and differences observed should not be misinterpreted as obvious changes due to denervation procedures since in the initial presurgical period amplitudes were adjusted to allow better analysis of wave forms. This resulted in amplitude changes due to slight mechanical sensitivity changes, which though comparable in the same animal over the first three periods, were not necessarily comparable between animals nor between the first three periods and the chronic period. Electrocardiogram interval, blood pressure, and heart rate recordings were standardized throughout the four periods. Also, all the chronic postsurgical periods recordings were standardized for use in the correlation study. Interaction data in electrocardiogram amplitude and interval studies was carefully interpreted. Because the amplitudes and intervals of the various waves were so greatly different within each animal in a given period, a tremendous F value resulted in comparing these waves. This was an expected difference, but when interpreting interactions the influence of the high F value should be considered. Interactions when considering all periods, statistically showed significant or highly significant difference in the recorded amplitude values between given periods and specific types of denervation, and between various wave

amplitudes and specific types of denervation. When the chronic post-surgical period was omitted the interaction between periods and specific types of denervation were not significant.

Interactions were highly significantly different between the various intervals (P, P-R, QRS, and QT) of specific dogs. Interactions were significantly different between the recorded intervals within given periods when the presurgical data was omitted, but not when all data was included or when the chronic postsurgical data was omitted.

These statistical findings stimulated the further inquiry into which factors within the individual animals were responsible for significant or nonsignificant statistical functional results and whether correlations between chemical, structural, and functional data were present. Therefore, functional data recorded just prior to exsanguination in dogs 2-18, and reflecting cardiovascular functions just prior to death, were included in a correlation study. The data was tested using a simple correlation method to analyze whether relationships between functional parameters and chemical and structural parameters existed. The results listed by the computer were reported in Table 36. The null hypothesis states that there is no relationship between the various components at the .05 or .01 levels of significance. Table 37 lists the combinations which rejected the null hypothesis and accepted the alternative hypothesis that a significant or highly significant relationship existed between the specifically observed values. These initial group statistical analyses gave indications of certain expected and unexpected changes and lack of change. The correlation matrix was viewed realizing the possibility of unassociated biologic

pairings statistically which really had no scientific relationships. The correlation matrix, Tables 36 and 37 included the following highly significant functional correlations.

Highly significant positive relationships between the amplitudes of P and Q waves, P and S waves, and Q and S waves were found. It is not surprising to see such a correlation between strengths of depolarization. The durations of atrial and ventricular actions as represented by P-R interval and Q-T interval respectively, were found to be highly significantly positively related also. These two as expected were both negatively highly related to heart rate. This was in agreement with findings by Wolff (1956) that the P-R interval was inversely related to the heart rate. Another highly significant positive functional correlation was the R amplitude with pulse pressure, indicating that the strength of ventricular contraction influenced the pulse pressure. Why the Q amplitude was apparently related to the thickness of the adventitia in proportion to its intima in the right coronary artery was uncertain and perhaps associated with chance.

At the 0.05 level of significance further functional relationships were observed. The P amplitude's positive relationship to the right coronary artery adventitia to intima ratio was unexplained. The amplitudes of the R and S waves were significantly negatively correlated with the acetylcholinesterase levels in the left ventricle and the latter wave was negatively correlated with the right atrial acetylcholinesterase also. Acetylcholinesterase activity was used as an indicator of acetylcholine content of tissues. The present findings suggested that with increased

levels of left ventricular and right atrial acetylcholinesterase and or acetylcholine, suppression of the force of ventricular contraction resulted. Mason (1968) indicated that the significance of parasympathetic action in regulating contractile force of the ventricles was not determined. But, stimulation of the left vagus nerve depressed ventricular beats and stimulation of the right vagus inhibited atrial P wave amplitude (Mizeres, 1955, 57). According to Dukes (1955) acetylcholine, released at the terminals of vagal cardiac nerves, didn't affect ventricular contractility. If this is accepted then the acetylcholinesterase would appear to be responsible for decreased amplitude of ventricular contractility.

A negative correlation between the Q-T interval and acetylcholinesterase content in the left ventricle was also found in this study. This suggested that an increased concentration of either acetylcholinesterase or reflected acetylcholine was associated with a shortening of the ventricular systole. Sippel (1955) hypothesized that cholinesterases participated in cardiac conduction and facilitated the rapid conduction of heart waves. Present research would support this hypothesis, but according to Isaacson and Boucek (1968), acetylcholinesterase was heavily concentrated in cardiac conduction tissue and was responsible for delaying conduction. Burn and Rand (1959) hypothesized that acetylcholine liberated at nerve terminals may be responsible for norepinephrine liberation. Norepinephrine decreases conduction time and increases myocardial excitability. This possibly could explain the inverse relationship between, in this case, acetylcholine and the Q-T interval. Blood pressure was greatly reduced by vagal stimulation (Dukes, 1955). This was supported in present findings

by the significant negative correlation between the pulse pressure and the acetylcholinesterase content of the left ventricle. Increased parasympathetic activity increased acetylcholine which decreased cardiac output and lowered pulse pressure.

The final negative significant correlation with functional recordings was between the Q-T interval and myocardial fiber diameter of the right ventricle. In skeletal muscle the thicker white muscle fibers contracted faster than thin red muscle fibers. The right ventricular measurements suggested a similar relationship in cardiac muscle, but similar relationships did not occur in other chambers. Also, the right ventricle contained the lowest norepinephrine content; the neurotransmitter usually associated with decreasing the duration of ventricular systole. A significant negative correlation between norepinephrine and the Q-T interval was not found, but there was a negative correlation between norepinephrine and the Q-T interval in all four chambers.

The functional results, considered in the overall statistical analyses presented to this point, have been on a group basis. Many differences within specifically denervated animals will be considered in the following paragraphs to better understand the group changes and the effects of the specific denervations on individual animals.

Dogs 1-5 controls refer to Tables 3 and 7 and Figures 2, 3, 4, and 5

Pre-exsanguination and exsanguination functional data was recorded. Control 1 electrocardiogram data was not included since leads were switched in changing mechanical equipment at the time of surgery. The ages of the control animals were purposely varied to give a wider spectrum of changes

which might be significant due to age. Despite this factor electrocardiogram recordings were very similar. The P wave had a greater amplitude in dog 2 than in the other dogs, but was within normal limits. Heart rates of the control dogs varied from 126 to 164 beats per minutes; mean sytolic blood pressure varied between 110 and 170 mm Hg; diastolic blood pressure varied between 65 and 130 mm Hg; and pulse pressure was between 40 and 70 mm Hg. Figure 5 showed the effect of heart block associated with methoxyflurane anesthesia. This effect did not persist for extended periods of time. Early effects of exsanguination including depressed atrial and ventricular function were recorded in Figure 2. When exsanguination was further progressed, in Figure 4, the T wave amplitude and the Q-T interval increased. In Figure 3 ventricular ectopic beats took over with atrial contributions not visible.

Dog 6 complete cardiac denervation - refer to Tables 3, 4, 5, 6, and 7 and Figure 6

Amplitude changes were observed in the P wave. It decreased during and immediately following surgery and became biphasic. The weakened contraction was accompanied by increased rate of atrial conduction. The atrial P wave returned to normal amplitude and configuration in the chronic postsurgical period. Little or no Q and S waves were observed in any of the four periods. T waves were inverted or biphasic in all periods and their amplitudes were low. The R amplitude was greatest during the postsurgical period. Both QRS and Q-T intervals became of longer duration during surgical and acute postsurgical periods, but returned to near presurgical levels in chronic postsurgical recordings, perhaps being related

to the return of norepinephrine. Heart rate decreased during surgical and acute postsurgical periods, but returned to presurgical levels in the chronic postsurgical period. Both systolic and diastolic aortic blood pressures fell greatly during the surgical and acute postsurgical periods and remained at these lower levels in the chronic postsurgical recordings. Pulse pressure responded in the opposite way.

Dog 7 refer to Tables 3, 4, 5, 6, and 7 and Figures 7 and 8

A decreased force of atrial contraction in the chronic postsurgical period was accompanied by a longer period required for transmission of impulses from the SA node to the ventricles. Perhaps the lack of sympathetic innervation decreased the excitability of the atria. The strength of atrial and ventricular contraction (P and R wave amplitudes) decreased slightly during the course of surgery. Decreased amplitudes were present in all chronic postsurgical waves except the Q wave. The T wave was decreased proportionately to the greatest degree in the chronic postsurgical period and became diphasic instead of inverted. Interval lengthening in the acute postsurgical periods was present in the QRS complex but it returned to presurgical levels in the chronic postsurgical period. Heart rate was slightly lower in the acute postsurgical period and fell considerably during the chronic postsurgical period. Aortic systolic and diastolic blood pressures increased slightly in the surgical and acute postsurgical periods but dropped greatly in the chronic postsurgical period. The pulse pressure responded in the opposite way. During the exsanguination recording, the amplitude of all waves decreased except the T wave. It increased in negative amplitude and was of

longer duration.

Dog 8 right cardiac nerve transections - refer to Tables 3, 4, 5, 6, and 7 and Figures 8 and 9

Amplitude recordings of the P wave decreased during surgical and acute postsurgical periods, but returned to presurgical levels in the chronic postsurgical period. The T wave was of much greater amplitude and width in the chronic postsurgical period. Other amplitude values were about the same. The P interval was reduced during surgery and the Q-T interval lengthened. The P-R interval was reduced in the acute post-surgical period. In other periods, intervals were about the same. The heart rate was almost halved between the presurgical and surgical periods. The rate recovered to about 3/4 the presurgical value in the acute and chronic postsurgical periods. The femoral vein was cannulated by the assistant surgeon instead of the femoral artery, hence, aortic blood pressure recordings were lost except in the chronic postsurgical period. At this latter period values were similar to those in chronic totally denervated dog hearts. During exsanguination the amplitude of the R wave fell proportionately more than the P wave as blood pressure approached zero. The T wave almost disappeared. Later blood pressure rose, the R wave decreased further in amplitude, and P and T waves were much greater in amplitude, the latter reaching over 0.3mV. This type recording persisted for several minutes before blood pressure returned to zero. T waves stayed positive in all recordings.

Dog 9 - left cardiac nerve transections - refer to Tables 3, 4, 5, 6, and 7 and Figures 10 and 11

Functional recordings in dog 9 varied considerably over the four periods. P wave amplitude increased during and following surgery, but was within normal values in the postsurgical recording. The Q amplitude was essentially unchanged. Ventricular contractile force as reflected in the R wave progressively greatly increased over the presurgical, surgical and acute postsurgical periods, but returned to lower levels in the chronic period. A relatively deep S wave in the presurgical and surgical period became an indistinct wave in the latter two periods. The T wave amplitude in presurgical and surgical periods was near normal upper limits, according to standards reported by Clark, Szabunicwicz, and McCrady (1966). During the acute postsurgical period this wave increased to above normal limits in amplitude, but in the chronic postsurgical period 192 days later, the T wave was normal; being below original presurgical levels. The only relatively large changed interval was the Q-T interval which was reduced after the presurgical period and remained at that normal lower level. A slightly lower QRS interval accompanied this change in the chronic postsurgical period. Heart rate changes were greatly elevated in the acute postsurgical and chronic postsurgical periods over the previous two periods. Systolic and diastolic blood pressure conversely, decreased greatly during the acute and chronic postsurgical periods. Pulse pressure remained relatively unchanged. Morphological and stimulation studies, McKibben and Getty (1968) and Mizeres (1955, 57), respectively, indicated that right heart nerves passed in larger numbers

to the right atrium and SA node which regulated heart rate. These would be intact in this dog. The left heart nerves passed more to the ventricles and left atrium thereby influencing more the force of the heart's contraction. The present data supported morphologic and stimulation studies.

During exsanguination (Figure 11), with the systolic blood pressure reaching zero, giant ventricular contractions following long periods of filling were recorded. The P waves preceded the ventricular contractions and were of normal amplitude though biphasic. The T wave was greatly increased in amplitude and was inverted as it had been in other recordings. During later stages, the P wave became slightly elevated, the ventricular contractions were depressed in amplitude, and the T wave was greatly elevated in a positive direction. The T wave became taller than the R wave, and intervals all became lengthened during exsanguination as anoxia slowed the conduction then contraction processes.

Dog 10 - right vagotomy at the cranial laryngeal nerve - refers to Tables 3, 4, 5, 6, and 7 and Figures 13 and 14

Unusual electrocardiogram recordings were observed for dog 10. The P amplitude was comparatively large in relationship to other waves during the presurgical, surgical, and acute postsurgical periods. It progressively increased in amplitude over these periods and at the time of chronic postsurgical recordings was abnormally elevated. The Q wave, proportionately high in presurgical and surgical periods, was lower in acute postsurgical periods, but greatly elevated at the chronic postsurgical recording. These changes indicated atrial strain and together with the slightly lengthened P-R interval implied that some atrial block may have occurred.

With the knotched P, mitral stenosis would be suspected. The P wave also became diphasic. The possibility of mitral stenosis was investigated on the fixed specimen and although the mitral valves were thickened and slightly nodular, it was felt that they probably were not primarily responsible for the electrocardiogram changes. Marriott (1957) warned that increased prominence of the Q wave may reflect necrosis. Though vascular changes were observed in this dog no myocardial necrosis was observed. The R wave increased progressively in amplitude between periods and the S wave deepened in the chronic postsurgical period. These wave changes together with a changed T wave which was knotched suggested ischemia or necrosis of the ventricular myocardium according to Rubin (1968).

Intervals were not greatly different between periods except the Q-T interval which progressively decreased in length. This change had been observed by Surawicz (1967), as reflecting hyperpotassemia and also dying hearts. Other changes associated with hyperpotassemia were not found in recordings of this dog. Ventricular strain and myocardial infarction, elicit some of the amplitude changes seen in this dog's recording, and have lengthened Q-T intervals. The conduction route rather than duration of waves seemed to be altered in the ventricles of dog 10. Heart rate was essentially unchanged by the right vagotomy, but blood pressure rose during the acute postsurgical period, then fell below presurgical levels at the time of tissue harvest over nine months later.

During exsanguination, Figure 14, the atrial stain was even further evident. The conduction path was altered to a more normal pattern within

the ventricles, and the T wave inverted, probably associated with ischemia.

Dog 11 - recurrent cardiac nerves were transected bilaterally - refer to Tables 3, 4, 5, 6, and 7 and Figures 15 and 16

Electrocardiogram amplitude changes included a gradual increase in atrial and ventricular contraction intensity in the acute postsurgical period. These amplitudes were much lower at tissue harvest. The increased amplitude suggested increased strain on the atria and ventricle due to surgical intervention itself or less parasympathetic influence on the heart. The T wave also increased in amplitude during this period suggesting movement toward potassium intoxication or ischemia without infarction or possibly ventricular strain according to Marriott (1967). The T amplitude decreased in the chronic postsurgical recording.

Two large interval changes occurred. The Q-T interval lengthened considerably during surgery, but returned to somewhat lower levels in the acute and chronic postsurgical period though not to within the normal limits which were present presurgically. This would suggest prolonged conduction and contraction time in the ventricles which is sometimes associated with congestive heart failure, myocardial infarction, hypokalemia, and myocarditis according to Marriott (1967).

The other interval change was the P-R intervals lengthening in the chronic postsurgical period. This reflects poorer conduction rates in the atria. It would appear that both atrial and ventricular contraction and repolarization cycles have been slowed by cholinergic recurrent cardiac nerve transections. This supported overall results in this study and the hypothesis of Sippel (1955). It should also however be remembered that

this dog was in the acute phases of canine distemper at harvest and that this may have altered the functional as well as other data. Heart rates were slowed similar to sympathectomized dogs, after recurrent cardiac nerves were transected. Blood pressure values also dropped slightly. Metophane heart block was evident in the initial pre-exsanguination recordings, but this was transient.

Dogs12 - transection of the left caudolateral vertebral cardiac nerve - Tables 3, 4, 5, 6, and 7 and Figures 17 and 18

Though only one of numerous heart nerves, the left caudolateral vertebral cardiac nerve was large and morphologically appeared to be instrumental in supplying sympathetic innervation particularly to the left atrium and ventricle through to the other two chambers as well. No amplitude changes other than slight atrial and ventricular strain were recorded during the chronic postsurgical period. In the chronic postsurgical period all amplitude values were within limits which are considered normal, but the T wave was slightly elevated over previous recordings. The intervals were not changed radically either. The Q-T interval which shortened during the surgical and acute postsurgical periods, lengthened slightly in the chronic postsurgical period. The QRS complex also lengthened slightly in the chronic postsurgical period. Heart rate interestingly increased slightly as did blood pressure though diastolic pressure fell in the chronic postsurgical period resulting in a large pulse pressure for this period. According to Mizeres (1958) the left caudolateral vertebral cardiac nerve carries many of the augmentor fibers to the heart. Cardioacceleration is generally not associated with left

cardiac nerves in the dog. Further study of the roll of this nerve in altering cardiovascular function is required. During exsanguination, with systolic pressure near zero, Figure 18, the atrial and ventricular contractions were weaker, the S-T segment deepened. The T wave, unlike that in most other animals didn't invert and fell in amplitude.

Dog 13 - bilateral extirpation of the thoracic sympathetic ganglia between the eighth thoracic and the cerviocothoracic ganglion - Tables 3, 4, 5, 6, and 7 and Figures 17 and 18

Thoracic cardiac nerves have morphologically been found to be few in number and size. The findings of this study were that they may play an important roll in cardiac function. After their ablation, the R and S amplitudes both increased considerably. The R amplitude was below presurgical levels in the chronic postsurgical period and the S amplitude was zero. This was accompanied by considerable drop in the QRS interval in the chronic postsurgical period though other intervals remained constant. The heart rate increased progressively with the periods, while the blood pressure decreased. Pulse pressure was slightly decreased in the chronic postsurgical period. It was unknown why the R and S amplitudes should increase in the acute postsurgical period, but perhaps the initial heart action had been depressed by anesthesia. The thoracic sensory feedback mechanism having been altered may have resulted in this change too. Increased body fat or poor electrical connections may be responsible for pre-exsanguination amplitude depression. The QRS interval was faster, indicating increased ventricular systole.

Not reflected in the figures was the fact that during surgery while manipulating the left thoracic sympathetic trunk, blood pressure averaged 200 mm Hg to 225 mm Hg systolic and 150 mm Hg to 170 mm Hg diastolic. After removal of the left trunk blood pressure fell to 110 mm Hg systolic and 75 mm Hg diastolic, but rose again during manipulation of the right trunk to 220 mm Hg systolic and 150 mm Hg diastolic. After the right trunk was removed blood pressure stabilized at about 175 mm Hg systolic and 120 mm Hg diastolic. Blood pressure in the chronic period averaged 150 mm Hg systolic and 105 mm Hg diastolic. This changed vascular pressure was essentially what would be expected. During exsanguination the atrial and ventricular amplitudes continued to fall and the heart beat slowed but the high amplitude compensatory ventricular beats did not occur.

Dog 14 - bilateral extirpation of the cerviocothoracic through first five thoracic ganglia - refer to Tables 3, 4, 5, 6, and 7 and Figures 21 and 22

This procedure removed all postganglionic fibers arising from the cerviocothoracic or thoracic ganglia and all the sensory neurons returning to the thoracic spinal cord. The ventricular contractions weakened in the acute postsurgical period as was seen by the reduced R amplitude. This strength returned in the chronic postsurgical period. In this latter period a deep S wave also developed which was within normal limits. The P and QRS intervals were shortened as in dog 13 in the chronic post-surgical period indicating rapid atrial and ventricular contractions. The heart rate however was actually slowed perhaps under the greater cholinergic influence. Systolic blood pressure which fell during the acute postsurgical period was about the same as the presurgical value. Diastolic

blood pressure did not completely return to presurgical levels and a larger pulse pressure in the chronic postsurgical period resulted. Surgical dissection of the right sympathetic trunk resulted in elevation of systolic blood pressure to 180 mm Hg and diastolic blood pressure to 110 mm Hg. This fell to 45 mm Hg systolic and 25 mm Hg diastolic pressure after transection of the right trunk but recuperated to 160 mm Hg systolic and 60 mm Hg diastolic. After transection of the left sympathetic trunk blood pressure again fell to 65 mm Hg systolic and 35 mm Hg diastolic before finally stabilizing at about 100 mm Hg systolic and 70 mm Hg diastolic. During exsanguination P waves disappeared and ectopic ventricular beats persisted. The T wave became diphasic but didn't become elevated.

Dog 15 - bilateral transection of the thoracic sympathetic rami communicates - refer to Tables 3, 4, 5, 6, and 7 and Figures 23 and 24

This surgical procedure provided an opportunity to observe cardiac function with sympathetic postganglionic fibers intact while eliminating the sensory cardiac nerves which return to the thoracic spinal cord.

No large amplitude changes occurred. During and immediately following surgery the P-R and Q-T intervals were somewhat prolonged indicating slower atrial and ventricular conduction and contractions. These results support those reported by Brown (1967), indicating that afferent feedback to increase the activity of the heart, was through these thoracic cardiac nerves. P-R and Q-T intervals returned to near presurgical values in the chronic postsurgical period. Intramural fibrosis of a ventricular vessel was observed in this dog (Figures 102-107). This might have resulted in

the T wave becoming inverted in the acute postsurgical period in electrocardiogram recordings, but the T wave was not inverted in the chronic postsurgical period and the QRS complex was not changed. When the T and QRS changes occurred it suggested ischemia or infarction (Marriott, 1957). Heart rate decreased greatly during the surgical and acute postsurgical periods as might be expected with P-R and Q-T interval lengthenings. The heart rate in the chronic postsurgical period did not quite return to presurgical rates. Blood pressure recordings during surgery were elevated during dissection of the rami communicantes and thoracic ganglia. After their transection the systolic and diastolic blood pressure fell slightly below presurgical levels. Systolic pressure recovered to presurgical levels by the chronic postsurgical recording, but diastolic pressure remained near acute postsurgical levels. The pulse pressure therefore was elevated. T waves were slightly elevated and atrial and ventricular weakening was recorded early in the exsanguination recording (Figure 24).

Dog 16 - bilateral vertebral ganglion extirpation - refer to Tables 3, 4, 5, 6, and 7 and Figures 25 and 26

This animal, following surgery, was almost completely devoid of cardiac norepinephrine as measured chemically. This asserts that almost all the postganglionic sympathetic neurons arise here and that other partial denervations of the cervicothoracic and thoracic cardiac nerves involved primarily cardiac preganglionic fibers and sensory fibers or postganglionic fibers passing to other organs. These findings are in agreement with Farr, Wacksman, and Grupp (1968) who by physiological stimulation concluded that the major site of ganglionic synapse of cardiac sympathetic nerve fibers

is in the caudal cervical (vertebral) ganglion.

Unfortunately the presurgical, surgical, and acute postsurgical electrocardiogram recordings on this dog were strange. In the chronic postsurgical recording, difficulty in cannulating the femoral artery was experienced. Some blood was lost, therefore, the blood pressure recordings of this period cannot be considered as completely valid. Little amplitude change in waves was recorded during the presurgical, surgical, and acute postsurgical periods. The T wave was elevated in all three periods. In the chronic postsurgical period this returned to normal amplitude. Intervals were essentially the same during the presurgical, surgical, and acute postsurgical periods, but all were shortened in the postsurgical period. In the first three periods the QRS and Q-T intervals were elevated above normal levels. Heart rate decreased slightly during the surgical and acute postsurgical periods, but returned to presurgical values in the chronic postsurgical period. Blood pressure fell considerably between consecutive periods, however, pulse pressure remained relatively constant.

During exsanguination the P wave increased and R wave decreased. This was an unusual combination of increased atrial response and decreased ventricular response to stress and anoxia. The T wave inverted and became smaller.

Dog 17 - bilateral cervical sympathetic trunk transection cranial to the vertebral ganglia - refer to Tables 3, 4, 5, 6, and 7 and Figure 27

No sympathetic cardiac nerves morphologically were demonstrated as passing to the heart in the dog from the sympathetic trunk cranial to the

vertebral ganglion. No changes therefore would be expected in cardiac function. Amplitude values of P and R waves were progressively depressed during the first three periods. This may be the result of anesthesia, time, and surgical intervention weakening the atrial and ventricular contractions or it may be related to stimulation of the vagi during dissection to separate the sympathetic trunks from the vagi. The initial presurgical recording was above the one millivolt per centimeter calibration, therefore comparison with the amplitude of the chronic postsurgical period was not valid. The various intervals were about the same between periods. The Q-T interval was slightly lengthened. Heart rate was slower during and immediately following surgery, but returned to near the presurgical rate in the chronic postsurgical period. Blood pressure increased considerably during and immediately postsurgically, but returned to near presurgical pressures in the chronic postsurgical period. Pulse pressure varied and was elevated during surgery. It seems strange that this surgery would increase blood pressure so drastically while decreasing the heart rate and depressing the atrial and ventricle outputs. The sympathetic nerves of this segment of the sympathetic trunk must have a great influence on vascular constriction.

Dog 18 - right midcervical vagosympathetic trunk transection - refer to Tables 6 and 7 and Figure 28

To implement the cervical sympathetic and vagal transections, the vagosympathetic trunk was transected. This should have augmented the results of dog 10 and 17 and made chronic postsurgical data comparable between these three animals. All amplitude and interval recordings were

within normal limits in dog 18. The P, Q, and S amplitude changes of dog 10 were not present in dog 18. It was suspected that dog 10, in its old age, did not presurgically have a normal recording and that changes in that dog should be considered with this in mind. Heart rate in dog 18 was not as high as in either dog 10 or 17. Since presurgical recordings were not available, no comparative changes could be ascertained. Systolic and diastolic blood pressure and pulse pressure in dog 18 was between those of dogs 10 and 17. During exsanguination atrial and ventricular force was depressed initially, then later were occurring almost simultaneously with what appeared to be an elevated T wave.

Dog 19 - bilateral vertebral ganglion extirpation - refer to Figure 29

As explained in the results of dog 16 which also underwent bilateral vertebral ganglion extirpation, the postganglionic sympathetic cardiac neurons appeared to arise primarily from those ganglia. The decline in blood pressure between presurgical, surgical, and acute postsurgical recordings supported findings in dog 16. Initially the blood pressure was about 200 mm Hg systolic and 150 mm Hg diastolic, but was 160 mm Hg systolic over 155 mm Hg diastolic just prior to extirpation of the right vertebral ganglion. After extirpating the right vertebral ganglion, systolic pressure fell to 140 mm Hg systolic and 125 mm Hg diastolic. It rose about 10 mm Hg in each before extirpation of the left vertebral ganglion then returned to 140 mm Hg systolic and 130 mm Hg diastolic. This fell to 115 mm Hg systolic and 100 mm Hg diastolic in the acute postsurgical period. The decreased pulse pressure was attributed to a blood clot in the cannula.

Dog 22 - bilateral vagotomy - refer to Figure 30

Changes in blood pressure recordings were observed in this dog which was bilaterally vagectomized just caudal to the origin of the anterior laryngeal nerve. Initially blood pressure was 150 mm Hg systolic and 50 mm Hg diastolic and the heart rate was 150 beats per minute. Immediately prior to transection of the left vagus nerve the systolic blood pressure was 103 mm Hg, the diastolic pressure was 65 mm Hg, and the heart rate 140 beats per minute. Immediately prior to the transection of the right vagus nerve the blood pressure had increased to 210 mm Hg systolic, 125 mm Hg diastolic, and the heart rate was 135 beats per minute. Following transection of the right vagus in the acute postsurgical period systolic blood pressure was 210 mm Hg and diastolic 125 mm Hg, while heart rate was 150 beats per minute. It was observed that blood pressure increased following vagotomy and heart rate was increased slightly also.

Dog 23 - bilateral vagotomy - refer to Figure 31

In dog 23 recordings illustrated decreased blood pressure amplitude over an extended surgical period. This was increased by transections of the vagi. The markers during the surgical periods indi ated first right vagal transection and then left vagal transection in the following recording. Immediate increases in blood pressure resulted, though during the course of the surgical procedure systolic blood pressure fell from 200 mm Hg initially to 175 mm Hg, and diastolic blood pressure rose from 110 mm Hg initially to 140 mm Hg at the acute postsurgical period.

Dog 24 - bilateral vagotomy - refer to Figure 32

Bilateral vagotomy was again surgically accomplished just distal to the origin of the cranial laryngeal nerve bilaterally. Initial blood

pressure recordings were 200 mm Hg systolic and 110 mm Hg diastolic. After almost two hours of surgery blood pressure read 190 mm Hg systolic and 125 mm Hg diastolic. After right vagotomy, but before left vagotomy, systolic pressure fluctuated between 210 and 235 mm Hg and diastolic pressure was 150 to 155 mm Hg. After left vagotomy it was about 225 mm Hg systolic and 175 mm Hg diastolic and stabilized near 210 mm Hg systolic and 160 mm Hg diastolic pressure in the acute postsurgical period. Even with prolonged surgery of almost 3 hours, the blood pressure in this dog exceeded the initial pressure.

Dog 26 - bilateral vagotomy - refer to Figure 33

The vagus was transected bilaterally just distal to the origin of the cranial laryngeal nerves. Initially, systolic blood pressure was 190 mm Hg, diastolic blood pressure was 125 mm Hg, and heart rate was 120 beats per minute. Just prior to transecting of the right vagus, systolic pressure was 150 mm Hg; diastolic pressure was 115 mm Hg and the heart rate was 160 beats per minute. About 15 minutes after transection of the right vagus, systolic blood pressure was 160 mm Hg and diastolic blood pressure was 125 mm Hg while heart rate had increased to 180 beats per minute. After transecting the left vagus, systolic blood pressure rose to 180 mm Hg and diastolic blood pressure was 130 mm Hg. The heart rate remained at 180 beats per minute. In the late acute postsurgical period heart rate fell to 150-160 beats per minute. Systolic blood pressure was 190 mm Hg and diastolic pressure 145 mm Hg. These results supported the stimulation findings of Mizeres (1955, 1957) in the dog that the right vagus primarily affected the SA node area hence heart rate, while the left vagus affected

the other three chambers to a greater degree resulting in alteration of cardiac output.

Dog 27 - bilateral vagotomy - refer to Figure 34

Transection of the vagi in the thorax was performed caudal to the right recurrent laryngeal nerve and just cranial to the left vertebral ganglion. Initial blood pressure recordings showed 165 mm Hg systolic and 130 mm Hg diastolic pressure, with a heart rate between 150 and 165 beats per minute. After transecting the right vagus, systolic blood pressure rose from 175 mm Hg to 195 mm Hg and diastolic blood pressure rose from 150 to 155 mm Hg. Heart rate was 150 beats per minute. Following transection of the left vagus, in the acute postsurgical period, systolic blood pressure was 180 Hg and diastolic blood pressure was 150 mm Hg. The heart rate was 160 beats per minute. This dog showed moderate increases in blood pressure and little increase in heart rate when bilaterally vagectomized in the thorax. The amplitude of the ventricular contraction was also greater in the acute postsurgical period.

Dog 28 - two stage bilateral vagotomy - refer to Figure 35

The right vagus was initially transected in the thorax. One month later the left vagus was transected just distal to the origin of the anterior laryngeal nerve. Initially the systolic blood pressure was 110 mm Hg and the diastolic blood pressure was 70 mm Hg. Heart rate was 140 beats per minute. In the acute postsurgical period systolic blood pressure rose to 150 mm Hg systolic and 100 mm Hg diastolic pressure, with heart rate remaining about 140 beats per minute. Just prior to the second stage of

surgery, the systolic blood pressure was 180 mm Hg and the diastolic pressure was 105 mm Hg. Heart rate was 100 beats per minute. Following left vagotomy blood pressure rose very slightly from 165 mm Hg systolic and 90 mm Hg diastolic, to 180 mm Hg systolic and 110 mm Hg diastolic. Heart rate in the acute postsurgical period was 132 beats per minute. These results indicated that unilateral right vagotomy results in prolonged increase in blood pressure though heart rate in this animal decreased. After transection of the remaining left vagus little change occurred.

Functional group tendencies

In considering the surgical animals to evaluate functional trends, relationships were observed between groups of animals with different types of extrinsic cardiac denervation. Although atrial strain was observed in several dogs during the surgical and acute postsurgical periods, no pattern could be found between the degree or type of extrinsic cardiac denervation and the atrial function as manifested in the P amplitude and interval, and the P-R interval. Only the unilateral right vagotomized dog 10 showed abnormally elevated P amplitude in the chronic postsurgical period and the dog with bilateral recurrent cardiac nerve transection had a prolonged P-R interval. These results together with the ventricular contractions in the vagotomized dog would be consistant with the observation of Guyton (1966) that the effect of parasympathetic stimulation on the heart was to decrease the force of the atrial beat, slow the heart rate, and constrict coronary arteries. Cholinergic action resulted in an overall decrease in heart activity and was progressive in action as was shown by infusion of acetylcholine into the heart in progressively increased concentrations.

This was shown by Blumenthal, Wang, Markee, and Wang (1968) to initially cause coronary vasodilatation, then decreased myocardial force, and finally in highest concentration caused slowing of the heart rate. Sippel (1955) hypothesized that cholinesterases had the opposite affects. The suppression of vagal activity in the present research would support decreased inhibition of myocardial contraction by acetylcholine, and decreased facilitation of conduction time by cholinesterases. In the present study the force of ventricular contraction appeared altered by the type of denervation. Those animals which underwent removal of portions of the parasympathetic nervous system had increased contractile forces following surgery. Lack of parasympathetic nerves resulted in sympathetic nerves influencing contractions. According to Guyton (1966) sympathetic stimulation increased the cardiac volume output at each filling and sympathetic inhibition decreases this output. Cardiac output after complete extrinsic cardiac denervation in the dog was shown to respond slower and at a lower level to treadmill exercise by Harada and Willman (1968). Under the conditions of the present study the amplitudes and intervals of the heart contractions were not greatly altered in complete extrinsic cardiac denervations and in most sympathectomized dogs in the chronic postsurgical period, though ventricular conduction was slower in the acute postsurgical period in completely denervated dogs. An exception was in bilateral thoracic and cervicothoracic sympathectomy dog 14 where force of ventricular contractions decreased, but normal amplitudes were present in the chronic postsurgical period. In the dog where thoracic or preganglionic thoracic contributions alone were transected the ventricular contractile force was

low in the chronic postsurgical period. Perhaps a sensory feedback mechanism had been interfered with.

The duration of the spread of impulses over the ventricles (QRS interval) appeared not to be altered in a pattern which unequivocably characterized the type of denervation. The conduction was slowed immediately following surgery in the completely extrinsically denervated dogs, but had returned to more rapid values in the chronic postsurgical period. The conduction time was very rapid yet not very strong in the dog which underwent thoracic sympathectomy. In this dog the duration of ventricular systole was normal and essentially unchanged. A fibrosed vessel was present in the myocardium, and surrounding tissue was undergoing degeneration. Normal, but rapid conduction in the ventricles also was observed in the chronic postsurgical period in dog 16 which had both vertebral ganglia removed resulting in almost complete depletion of cardiac norepinephrine. This seemed almost contrary to the expected results since norepinephrine reportedly facilitated conduction and excitation of the fibers. Perhaps acetylcholinesterase, not depleted by extrinsic cardiac denervation facilitated the rapid conduction. The amplitude of ventricular contraction was low in this dog perhaps as a result of domination by cholinergic neurons. Abnormally elevated recordings of Q amplitudes were observed in dog 10 and abnormally elevated S waves were present in dogs 10 and 14. Though difficult to interpret they may be associated with abnormal conduction paths in the ventricular myocardium. Ventricular strain, myocardial ischemia (anoxia) or hyperpotassemia was evident in several dogs prior, during, and immediately following surgery as recorded in elevated T waves.

Only dog 8 in which left intrinsic cardiac denervation was performed, showed this change at the chronic postsurgical period. This dog had not shown this elevated T wave previously and considering other waves, I would suspect the same factors of ventricular strain or anoxia were involved. The total period of ventricular systole Q-T interval was quite stable in the various dogs except during surgery. Prolonged Q-T intervals were observed in several animals during surgery presumably due to the surgical intervention and manipulation of thoracic structures.

Since hearts without or partially without extrinsic cardiac nerves, it was considered, might react differently during ischemia produced by exsanguination, recordings were taken at harvest to substantiate or reject this possiblity. It was found that the hearts reacted in similar ways. In pooling the recordings of control dogs, exsanguination appeared to electrocardiologically show first a decreased amplitude of the P and T waves and inversion of the T wave. Heart rate slowed (Figure 2). Later the P wave became elevated as did the T wave and the intervals were lengthened (Figure 4). Finally no atrial beats were observed and very large slow ectopic ventricular beats started (Figure 3). In dogs 7, 8, and 9 (Figures 8, 10, and 12) with complete or unilateral extrinsic cardiac nerve transection the exsanguination recordings were very similar. The very large contractions in dog 9 (Figure 12) preceded a phase in which the P wave was almost as large and the T wave was larger than the R wave. This elevated T wave was associated with ischemia by Marriott (1957), and present recordings supported his observations. In Figure 14 abnormal conduction in the heart was affected by ischemia apparently resulting in a

trend to return towards more normal routes of conduction in this crisis. Other variations of responses are observed in Figures 18, 20, 22, 24, 26, and 28. No patterns were established to correspond with particular responses by differently denervated hearts, but the variety of responses to the ischemic crisis are interesting.

According to Mizeres (1955, 1958) right sympathetic extrinsic cardiac nerves accelerated heart rate and right vagal extrinsic cardiac nerves depressed the heart rate. In dogs with complete extrinsic cardiac denervation, heart rate, in the present study, fell following presurgical periods. Where regeneration of cardiac nerves was occurring (dog 6) the rate was again normal in the chronic postsurgical period. Transection of all right cardiac nerves (dog 8) resulted in a considerable drop in heart rate, but heart rate elevated when left heart nerves alone were transected (dog 9). Transection of the cervicothoracic ganglion and rami communicantes associated with it appeared to play a large roll in regulating heart rate. In agreement with Brouha, Cannon, and Dill (1936) heart rate decreased after extirpation of the thoracic and cervicothoracic ganglia bilaterally. Parasympathetic nerve transections did not provide reliable heart rate results in the chronic study since one dog was sick (dog 11), another showed abnormal cardiac recordings (dog 10), and a third had incomplete recordings (dog 18). In the acute studies on bilaterally vagectomized dogs it was observed that increased heart rate occurred immediately following vagotomy. The contractile force usually increased also. In the dog held one month following right vagotomy, however, the heart rate was slower than initially. In considering all vagotomized dogs in the present

study, results were found to be similar to those of Kjellberg, Rudhe, and Sjostrand (1952), who blocked the vagi resulting in increased heart rate, decreased duration of systolic contraction, raised arterial blood pressure, and increased degree of contraction in the left ventricle.

Mean femoral blood pressure normally ranged between 135 and 155 mm Hg according to Katz, Skom, and Wakerlin (1957). Femoral systolic blood pressure ranged from 108 to 198 mm Hg and diastolic from 75 to 121 mm Hg according to Romagnoli (1953). Mean aortic pressure in the present study in the presurgical period ranged from 88 mm Hg, in the 3 month old dog, to 181 mm Hg and averaged 132 mm Hg. Sympathectomized dogs showed lower mean aortic blood pressure after nerve transections and bilateral vagotomy resulted in increased mean aortic blood pressure. In sympathectomized dogs the lowered blood pressure persisted in the chronic postsurgical period. In the chronic postsurgical period, the dogs with complete or near complete cardiac norepinephrine depletion (dogs 6, 7, and 16) averaged only 88 mm Hg mean aortic blood pressure; down 46 mm Hg. In all three dogs in which the cervicothoracic and or thoracic ganglia or rami communicantes were transected, mean chronic postsurgical blood pressure fell 16% below presurgical means. These results were very similar to those of Grimson, Wilson, and Phemister in 1937. They found that mean blood pressure fell an average of 26 mm Hg following sympathectomy of the stellate through sacral sympathetic trunk bilaterally. They also found that the average time for return of presurgical blood pressure was five and three-quarters months with a range of two to nine months. In the three similar dogs (13, 14, and 15) of the present study, measured six to nearly 10 months following

surgery, the systolic pressure was near presurgical pressure, but diastolic pressure was still depressed. Though recordings in the acute postsurgical period of the right vagotomized dog (10) showed an elevated mean blood pressure, mean blood pressure was lower at harvest in this dog. One month after right vagotomy in dog 28 mean blood pressure was elevated. In the acute studies on bilaterally vagotomized dogs, blood pressure was elevated in the acute postsurgical period in all dogs. Normally the rate and rhythm of the heart was reported as stable and normal in the dog under methoxyfluorane but blood pressure falls progressively as the depth of anesthesia was increased (Jones, Jones, Stockton, and Tigert; 1962). This finding was generally supported in the present research.

Heart Chemistry

Hearts from thirteen dogs (dogs 6-18) chronically extrinsically denervated to various degrees together with hearts from five control dogs were used in chemical analyses for cardiac norepinephrine and acetylcholinesterase.

Norepinephrine

Norepinephrine content was directly related to the sympathetic innervation of the heart. Analysis of variance statistical analyses of the mean norepinephrine content of the myocardial chambers in animals extrinsically denervated in different ways showed that highly significant differences in mean values occurred between animals and between chambers (Table 22). This finding was in agreement with Angelakos (1965). The mean norepinephrine concentration in ugm/gm when considering all dogs

studied, was 2.39 ± 0.40 for the right atrium, 1.69 ±0.27 for the left atrium, 1.01 ± 0.14 for the left ventricle, and 0.82 ±0.12 for the right ventricle. Mean norepinephrine levels of control animals were considerably higher at 4.19 ± 0.14 in the right atrium, 2.17 ± 0.46 in the left atrium, 1.47 ± 0.46 in the left ventricle, and 1.22 ± 0.34 in the right ventricle. The present findings support findings by Chidsey, Harrison, and Braunwald (1962) in the dog, that left atrial concentrations of norepinephrine are normally lower than right atrial concentrations. It appeared, when considering other animals which were not subjected to sympathectomy, (dogs 10, 11, 17, and 18), that the right atrial concentrations in the control animals were slightly elevated. Norepinephrine concentrations between animals varied from 0.00 to 6.25 ugm/gm in the right atrium, 0.00 to 4.68 in the left atrium, 0.00 to 2.00 in the left ventricle, and 0.00 to 1.63 in the right ventricle (Table 20). After 286 days following total cardiac denervation, the norepinephrine content of the heart was found to be returning in dog 6. Higher concentrations were present in the atria than in the ventricles and slightly greater amounts were returned to the left side in comparison with the right. In the second completely denervated heart, dog 7, measurable amounts of norepinephrine had not returned to the heart 92 days following complete cardiac denervation. In unilateral extrinsic cardiac nerve transections in dogs 8 (right side) and 9 (left side) norepinephrine levels were lower than control mean values in every chamber after 202 and 192 days postsurgery. Left chamber values were slightly higher in norepinephrine content than right chamber values in dog 8. This supported morphologic distribution patterns of sympathetic nerves (McKibben and Getty, 1968) and stimulation studies by Mizeres (1958).

Somewhat less difference was noted between depressed norepinephrine concentration in different chambers in dog 9 when comparing chambers with control levels. Dog 10 did not involve sympathetic transection but the right vagus was transected just distal to the nodose ganglion. Norepinephrine levels were above mean control levels in all chambers except the right atrium. This being one of the older dogs in the study, it might have been expected, according to Frolkis (1968), that norepinephrine levels would be below average. Dog 11 was visibly ill with distemper at harvest. Although sympathetic nerves were not transected, norepinephrine levels were reduced to about half the mean control levels in all chambers. Cardiac norepinephrine levels have been shown to be reduced by increased external environmental temperatures by Lin and Sturkie (1968). The present finding suggested that increased internal environmental temperature may have decreased cardiac norepinephrine. In dog 12 only one large cardiac nerve was isolated and transected, this being the left caudolateral vertebral cardiac nerve. This was often the largest of five or six nerves which arose from the vertebral ganglia and passed to all chambers of the heart. Extirpation of both vertebral ganglia resulted in 96 to 100% depletion of norepinephrine in the heart chambers, therefore, the 36 to 83% of control levels of norepinephrine in dog 12, did not appear as low as passing observations would suggest. Elevated right atrial control values contributed to the lower percentage. Morphologic dissections indicated that thoracic ganglia contributed minor sympathetic supplies to the heart. A relatively small drop in cardiac norepinephrine in all chambers followed

removal of these ganglia in dog 13, supporting the former findings. When the cervicothoracic ganglion was also extirpated, however (dog 14), cardiac norepinephrine values were reduced almost 1/3. Because this was 293 days postsurgery some return of original norepinephrine levels in the heart was probable. The drop in norepinephrine content would appear to be less if the control right atrium level was adjusted. It appeared however that some reduction occurred in all chambers. The norepinephrine in the guinea pig heart was almost depleted by bilateral stellate ganglion removal according to Cervoni, Palazzolo, and Terry (1968). Levy (1966) found little reduction and Jellinek, Kaye, Nigh, and Cooper (1964) reported no significant change in norepinephrine content of the heart following bilateral removal of the cervicothoracic and thoracic ganglia. Dog 15 was unique in that theoretically only preganglionic fibers were transected bilaterally. If no degeneration effects were transferred to postganglionic neurons, the norepinephrine levels should have remained at normal levels. This was observed in the left chambers in the present study, but values on the right side were reduced about one-third from control mean values. According to Rehn (1958) after preganglionic denervation noradrenaline content of organs innervated by associated postganglionic fibers was not altered. Interesting results were found after bilateral vertebral ganglion extirpation of dog 16. Ventricular norepinephrine contents measured 0 ugm/gm and atrial concentrations were only 3 to 4% of control mean values. This is considerably greater depletion than Goodall and Kirshner (1956) reported. Extirpation of the right cervical sympathetic ganglion reduced norepinephrine levels by 2/3 in the dog according to

Goodall and Kirshner (1956), while extirpation of right thoracic, left cervical, or left thoracic ganglia did not produce change in the cardiac norepinephrine levels. Sympathetic cardiac nerves originating cranial to the vertebral ganglia were generally not considered to supply the heart. The norepinephrine levels found in dog 17 following bilateral sympathectomy cranial to the vertebral ganglia, gave supportive evidence to this view.

In dog 18 the right cervical vagosympathetic trunk was transected. The norepinephrine contents of the chambers were near control mean values, except in the left atrium where the concentration was greatly elevated. Both right vagotomized dogs 10 and 18 showed this elevation. Interestingly, in the left atrium the acetylcholinesterase which reflected acetylcholine levels of both these dogs was lower than in control dogs. The correlation matrix, though indicating that a negative relationship existed between norepinephrine and acetylcholinesterase, was not significant at the .05 level. I would suspect that a very high negative correlation would not occur in normal nondenervated dog hearts where both neurotransmitters were working in conjunction with each other.

Significant correlations between norepinephrine and other functional, chemical, and structural parameters were found in the correlation matrix (Table 36) and are summarized in Table 37. At the significant or highly significant levels all the chambers were positively correlated with one another in norepinephrine content. Only two additionally significant correlations were present with norepinephrine, these being left and right ventricular norepinephrine correlated positively with right ventricular

nuclei per five $5mm^2$ fields. The other correlation combinations between norepinephrine and the nuclei per five $5mm^2$ fields were also positive; most being just under the .05 significance level. This trend would indicate that increased norepinephrine content is associated with shorter cardiac muscle fibers.

Acetylcholinesterase

Acetylcholinesterase content reflected also the acetylcholine content in tissue. This was generally associated with parasympathetic nerve endings, sensory nerves, and possibly sympathetic nerves. But according to Sippel (1955) the exact function of cholinesterase in the heart remains problematic.

Analysis of variance statistical analyses of the mean acetylcholinesterase content of the myocardial chambers in animals denervated in different ways revealed highly significant differences between mean values of various animals and between chambers of the heart (Table 22). The mean acetylcholinesterase concentration values times 10^{-6} moles hydrolized per minute, when considering all dogs studied, was 0.995 ± 0.04 for the left ventricle, 4.57 ± 0.30 for the left atrium, 1.232 ± 0.09 for the right ventricle, and 2.964 ± 0.18 for the right atrium. Mean control animal acetylcholinesterase levels times 10^{-6} moles hydrolized per minute were 1.09 ± 0.10 for the left ventricle, 4.792 ± 1.2 for the left atrium, 1.339 ± 0.16 for the right ventricle, and $2.931 \pm .41$ for the right atrium. Present findings that the highest mean acetylcholinesterase concentrations were in the left atrium, then right atrium, right ventricle, and left ventricle respectively, in the dog were in agreement with the observations

of Antopol, Glaubach, and Glick (1939) in the rabbit. Mean acetylcholinesterase concentrations in 10^{-6} moles hydrolized per minute varied in the left ventricle from 0.650 to 1.193, in the left atrium from 3.183 to 7.55, in the right atrium from 1.672 to 4.420, and in the right ventricle from 0.678 to 1.632 (Table 25). In the total cardiac denervated dogs acetylcholinesterase levels were not alike. In dog 6, denervated for over 9 months, acetylcholinesterase levels were slightly above mean control levels in all chambers. In dog 7 in which the denervation had been of shorter duration, the levels of acetylcholinesterase were less in all chambers than in control animals, measuring 79, 66, 74, and 98% of control means for the left ventricle, left atrium, right ventricle, and right atrium respectively. These quantitative results were not fully in agreement with histological results of Jacobowitz, Cooper, and Barner (1967). In the cat, they found a 20-25% reduction of acetylcholinesterase staining fibers in the left atrium, slightly reduced numbers in the right atrium, and little change in the ventricles after complete extrinsic cardiac denervation by regional neural ablation. Present results suggested that though adrenergic cardiac innervation was not completely re-established in dog 7 three months following surgery, cholinergic levels were re-established nine months following complete extrinsic cardiac denervation. In dog 8 atrial levels of acetylcholinesterase were elevated while ventricular levels were slightly lower. In dog 9 atrial and right ventricular levels were lower and left ventricular levels normal. These findings suggested that right extrinsic cardiac denervation resulted in slightly greater acetylcholine reduction in the ventricles. Left extrinsic

cardiac denervation primarily reduced atrial levels, however, right vagotomy resulted in depression in all chambers except the right atrium of dog 18. It was suspected that the renervation process because of a shorter intervening distance, was almost completed in dog 8, was beginning in the low cervical vagotomized dog (18), and had not reached the heart in dog 10 which was vagotomized in the cranial cervical region.

Sensory innervation in the dog heart was plentiful in the right atrium, present in the left atrium, and rare in the ventricles according to Tscheng (1951) and sensory pain fibers are conducted primarily into the left side of the spinal cord according to Kuntz (1949). Therefore if the left thoracic sensory nerves were removed the right atrium then left atrium and finally the ventricular acetylcholine levels quantitatively should be affected in that order of severity. In dogs 14 and 15 sensory connections to the thoracic spinal cord were completely eliminated. Right atrial concentrations of acetylcholinesterase were decreased together with the left atrial, and in dog 14, the ventricular concentration.

Dog 10, over nine months following high right vagotomy failed still to approach mean acetylcholinesterase control values being 69, 77, 50, and 57% of left ventricular, left atrial, right ventricular, and right atrial control values respectively. Dog 11 showed lower levels than controls in the left chambers following bilateral recurrent cardiac nerve transections. Ventricular levels were lower in dog 12 while atrial levels were near normal or above. All concentrations of acetylcholinesterase in the chambers of dog 13, in which thoracic sympathetic ganglia caudal to the cervicothoracic ganglia were removed, were very close to the control mean values.

Acetylcholinesterase levels fell considerably after extirpation of the thoracic and cervicothoracic sympathetic ganglia. The acetylcholinesterase level of all chambers was elevated above control concentrations in dog 16 in which bilaterally the vertebral ganglion was separated from the vagus and extirpated. Separation from the vagus and transection of the sympathetic trunk cranial from the vertebral ganglion yielded similar data in dog 17. Perhaps fibrotic changes following surgery encompassed and continually stimulated the vagi. Transection of the right vagosympathetic trunk was performed in the midcervical region in dog 18. Ventricular levels of acetylcholinesterase in this dog were reduced to about 60% of control mean concentrations. The left atrial concentration of acetylcholinesterase was also about 1/4 lower but right atrial levels were elevated above control mean values indicating perhaps that renervation of the heart had started.

In the correlation matrix, acetylcholinesterase chemical correlations with other parameters which were significant, included the negative association between acetycholinesterase or acetylcholine and the R and S amplitudes and the Q-T interval. As discussed previously parasympathetic stimulation has an overall depressing effect on heart activity according to Guyton (1966). If parasympathetic activity were low the R and S amplitudes under the influence of the sympathetics would be elevated. A less popular hypothesis was that if acetylcholine was necessary for liberation of norepinephrine, then the ventricular depression would perhaps evolve around acetylcholinesterase liberation by parasympathetic neurons as being the depressive force. But, Sippel (1955) hypothesized that

specific cholinesterase participated in cardiac conduction and non-specific cholinesterase facilitated rapid conduction of the heart wave. Isaacson and Boucek (1968) found that acetylcholinesterase, very highly concentrated in conduction tissue, may be responsible for delaying conduction. There was a significant negative correlation found between acetylcholinesterase and the Q-T interval of ventricular contraction in the present study. Combining the Isaacson and Boucek (1968) results with present findings would suggest that acetylcholine rather than acetylcholinesterase was in a negative correlation with the Q-T interval, thereby facilitating faster ventricular systole. The other hypothesis which was in accord with Sippel (1955) would suggest that the cholinesterase itself contributed to the rapid ventricular conduction. Though the exact mechanism of action requires further study, it was found that the cholinergic neurons played a significant roll in regulating conduction and contraction in the ventricles. According to Mason (1968) this had not been previously determined.

Guyton (1966) described parasympathetic lowering of blood pulse pressure. This was compatible with the significantly negative relationship between acetylcholinesterase in the left ventricle and pulse pressure in the present study. It was difficult to explain the negative significant relationship of acetylcholinesterase levels in the ventricles, to the ratio of thickness of left coronary adventitia to intima and to the ratio of right coronary media to intima unless changed pulse pressure was involved. For example, if the cholinergic concentration was low in the ventricles, therefore, the pulse pressure high, the adventitia and media of the coronaries according to these correlations would be expected to thicken.

Very little correlation was present between acetylcholinesterase levels and fiber length as represented by nuclear counts of five $5mm^2$ fields, except a positive one with the right ventricle and with the left atrium. Perhaps if right ventricular action was suppressed by high levels of parasympathetic actions over prolonged periods, the fibers of left atrium might atrophy, however, similar significant relationships between acetylcholinesterase and fiber length in other chambers were not present. The acetylcholinesterase concentration in these chambers was negative in correlation with age, being significantly so with the right atrium. This would generally support previous findings by Frolkis (1968) in rats. Positive correlations existed between all chambers in their acetylcholinesterase contents. A highly significant relationship existed between the ventricles. This would seem necessary for synchronization of contraction under the influence of this chemical.

Heart Structure

In thirteen chronically denervated and five control dogs, tissues were harvested and fixed in 10% formalin for histologic studies or immediately frozen in liquid nitrogen and stored for histochemical studies. No macroscopic abnormalities were observed in the structure of the hearts harvested.

Histochemical

Histochemically both acetylcholinesterase content and catecholamines were studied, however, results in the latter technique without special freeze dry equipment were not of a consistent enough nature for comparative

study.

Acetylcholinesterase Acetylcholinesterase positive neurons were found in all chambers of all animals studied. Acetylcholinesterase staining of intrinsic cardiac nerves was not observed to be drastically different between the variously denervated dogs. Apparently the acetylcholinesterase staining nerves, remaining following complete extrinsic cardiac denervation (Figures 47, 62, and 64) were primarily postganglionic parasympathetic neurons. Jacobowitz, Cooper, and Barner (1967) after regional neural ablation, to cause extrinsic cardiac denervation in the cat, reported that the number of acetylcholinesterase staining nerves in the left atrium was reduced by 20-25%, slightly reduced in the right atrium, and not changed in the ventricles. They thought perhaps extirpation of the cardiac ganglion of Wrisberg in their surgical technique might be responsible for the decrease. Cholinergic nerves in all animals were particularly prevalent in the epicardium (Figure 59). These nerves were often associated with the blood vessels, both in the epicardium (Figures 60, 62, and 63) and in the myocardium (Figures 61 and 97). In blood vessels the nerves stained most intensely in the adventitia (Figures 53 and 65), but penetrated also into the media (Figure 65). They were found also among myocardial fibers (Figures 46, 48, 49, and 50) and were observed terminating on the myocardial fibers (Figures 52 and 53). In addition, ganglia (Figure 51) were found in the epicardium. They stained less easily than the fibers around them as was illustrated in the differences between 3 hour, 8 hour, and 20 hour incubation sequences in Figures 57, 58, and 59. This suggested that the acetylcholinesterase

and associated acetylcholine were not completely formed within the cell body by precursors or that those ganglia may not be cholinergic ganglia. The former possibility was in agreement with Koelle (1968) that the synthesis of acetylcholine appeared to take place almost exclusively at the terminal of the axon. Variability in the staining intensity of myocardial fibers was observed also. Figures 54, 55, and 56 illustrate typical progressive staining in the atrial myocardium with increased duration of incubation. This change did not occur in the 3 month old dog in the study (Figures 57, 58, and 59), which may have indicated age differences in the content, form, or stainability of acetylcholinesterase. The ventricular myocardial fibers did not stain in any animals and intramyocardial right ventricular nerve fibers stained weakly in all animals. This weak intensity of staining was compatible with quantitative values found in this study.

Norepinephrine Figures 36-45 illustrated fluorescence and autofluorescence which was accomplished, without freeze dry apparatus, on ganglion and heart tissue. Diffusion was a problem and the fluorescence weakened and disappeared within about 30 minutes. Therefore myocardial photography generally required high speed film. Further development of this technique will be necessary for reliable consistant results.

Histologic observations

Histologically, the general structural parameters as well as specific dimensional, and special metabolic processes were observed in the myocardium. Useful in this portion of the study of dogs 1 through 18 were specific stains as hemotoxylin and eosine, Verhoff's-Van Giesen's

Periodic Schiff, and Oil Red O stains.

Of particular interest in the histologic study was whether the reduction or loss of extrinsic cardiac nerves resulted in structural modifications. Many questions were raised. In the myocardium did changes occur in myocardial fiber diameter and length? Was the metabolism of the myocardium as reflected by glycogen and fat content altered proportionately? Are the coronary blood vessels altered structurally and in components? According to Guyton (1966) removal of sympathetic nerves to vessels resulted immediately in almost maximal vasodilation of the vessel. Over a period of days or weeks the intrinsic tone in the smooth muscle was increased, usually almost restoring normal vasoconstriction. Does the conductive Purkinje system remain intact following denervation? Support for answers to these questions follows, but further in depth study of each type denervation is required.

In the control dogs, it was suspected that age differences played a roll in the structural observations found. Purposely, a very young and a very old dog were included in this group to reflect age changes. Of the 18 dogs studied however 15 were 5 years or less in age. Structural changes in myocardial fiber diameter and length were observed (Tables 29 and 30). Highly significant differences existed between myocardial fiber diameters between chambers and between animals. A highly significant difference in fiber length was also present between animals and a significant difference was found between fiber lengths of the four heart chambers (Table 31). Age was a factor in the highly significant differences between animals as was revealed by significant negative correlations between age

and nuclei/5 fields and fiber diameter in the left atrium. The age difference was particularly noticeable between the young dog 4 and the other dogs (Tables 29 and 30); compare Figures 48 and 120. Munnell (1967) in the beagle dog, observed age changes in diameter and length of right ventricular myocardial fibers. In dogs over six months of age he found myocardial fiber diameter remained relatively constant, averaging 14.5 ± 0.24 u, and length decreased slightly with age after six months; the mean nuclear count being 31.03 ± 1.23 for dogs over 6 months. The mean myocardial fiber diameter of the cardiac chambers in the present study considering all 18 dogs, was 15.25 ± 0.40 u for the left ventricle, 13.69 ± 0.55 u for the left atrium, 15.72 ± 0.58 u for the right ventricle, and 12.30 ± 0.42 u for the right atrium. Mean control fiber diameters, excluding dog 4 due to age, were 16.9 ± 1.02 u for the left ventricle, 14.9 ± 2.65 u for the left atrium, 16.9 ± 1.73 u for the right ventricle, and 14.4 ± 1.70 u for the right atrium. The dogs used in this study were much larger than those used by Munnell (1967). The small difference in right ventricular myocardial fiber diameter may be associated with the size difference according to Harrison, Ashman, and Larson (1932).

The mean nuclei per five $5mm^2$ fields, reflecting fiber lengths of the chambers numbered 38 ± 8.11 in the left ventricle, 42 ± 3.5 in the left atrium, 40 ± 6.18 in the right ventricle, and 48 ± 5.33 in the right atrium. In control animals excluding dog 4 because of age, mean nuclei per five $5mm^2$ fields numbered 28 ± 7 in the left ventricle, 46 ± 6 in the left atrium, 31 ± 6 in the right ventricle, and 47 ± 13 in the right atrium. The correlation matrix (Table 37) included the strength and direction of

relationships between fiber diameters and nuclei per five $5mm^2$ fields representing length. Negative correlations which were significant or highly significant were present for 13 of the 16 combinations between these two measurements suggesting strongly that in animals with greater fiber diameter, the fibers may also be longer as represented by fewer nuclei per given area. It should also be considered however that increased mass due to increased diameter alone may result in significantly fewer nuclei per area.

Eighteen days after denervation of the heart by the Černý and Oláh technique, Barta and Sapakova (1968) found a decrease in myocardial fiber diameter of 20.9% in the left ventricle and 17.4% in the right ventricle. The nuclei were observed to shrink. In the present investigation the totally denervated and unilaterally chronically denervated dogs (6, 7, 8, and 9) averaged slightly below normal in nuclei and near or below normal in fiber diameter, being positively related rather than the negative generally expected. Of these dogs, two had a lower heart rate. Harrison, Ashman, and Larson (1932) related myocardial fiber diameter directly to the size of the animal and inversely to the heart rate. A negative correlation between fiber diameter and heart rate was also found in 3 chambers in the present study, but the correlation was not at a significant level statistically. Though other cardiac denervations of various types may have affected myocardial diameter and length, it was difficult to pinpoint these with certainty.

Other structural and metabolic myocardial parameters which were studied were the amount of fat in the myocardium and the relative histologic

representation of glycogen in the chambers. Norepinephrine played a role in increasing metabolism of carbohydrates and in mobilizing fats. Lack of the adrenergic innervation resulted in glycogen and fat accumulation in the myocardium according to Jellinek, Kaye, High, and Cooper (1964). Figure 116 shows small lipid droplets in the myocardium of dog 1. Repeated administration of noradrenaline subcutaneously was found to increase heart glycogen (Poupa, Prochazka, and Pelouch; 1968) while infusion of epinephrine in isolated dog hearts by Jedeikin and Buckley (1967) led to depletion of cardiac glycogen. Present histologic results support the accumulation of cardiac glycogen after extrinsic cardiac denervation. In the Alcian Blue-PAS stain, dog 6 totally chronically denervated for over 9 months and with noradrenaline returning, showed no increase in glycogen staining in the myocardium. Dog 7 which still had norepinephrine depletion, showed heavier stores of glycogen in all chambers than dog 6 but did not appear to have greater amount than the control dogs: compare Figures 114 and 115 with Figures 106 and 120. Dog 8, which lacked right heart nerves, stained very well in all chambers but particularly in the left ones. Staining was just average to slightly below in the right chambers of dog 9 which had the left heart nerves removed.

In dog 10 both the glycogen content and intramyocardial fat were abundant in all chambers. This dog had received limited exercise since surgery 293 days previous, was seven years of age, and in good flesh at harvest. She was not obese.

Glycogen staining in dog 16 which also had near catecholamine depletion of the heart showed excellent glycogen staining. All other dogs showed

normal staining of myocardial glycogen. Purkinje cells in all dogs were higher than nonconducting myocardial cells in glycogen (Figures 117, 118, and 119) and in no dogs were decreased levels of glycogen observed in Purkinje cells.

No relationship to degree of cardiac innervation and lipid content of myocardium was established. Dogs 3 and 10, which were older, had higher levels. Other animals were similar to each other in lipid content. No perivascular collections of white blood cells and no loss of striations, which accompany autotransplantation according to Leandri (1967), were found in the complete extrinsic cardiac denervated dogs of the present study (Figures 112 and 115).

Increased catecholamine levels have been incriminated as contributing to atherosclerotic changes in man. The adrenergic, catecholamines releasing, cardiac nerves help regulate vascular tone in response to myocardial needs for metabolic products. Without the direct innervation, the arterial tone may be altered and this in turn may be reflected in structural changes. Eighteen dogs including five control and thirteen other dogs, whose extrinsic cardiac innervation had been altered to various degrees, were used in observing whether coronary arterial structural changes might ensue following interruption of cardiac nerve supplies. Measurements of intimal, medial, and adventitial thickness, because the vessels varied in size, were reported in ratio of medial and of adventitial thickness to the intimal thickness. A highly significant difference was found between the mean ratios of the media to intima and the mean ratio of the adventitia to the intima in the coronary arteries. A highly significant

difference also existed in the mean medial and adventitial ratios to intima of the coronary arteries between animals. When considering the interaction between differences in the ratios of the coronary arteries, with the different individual animals, a highly significant difference was found (Table 35). A significant difference was found between measurements of the right and left coronary arteries. These findings, showing differences in coronary artery measurements between animals and arteries within the same animal, lead to the interesting speculation that these changes were associated with the degree or type of denervation.

Besides dimensional measurements, special stains were employed to help ascertain whether changes in content were associated with these dimensional changes. Using Verhoeff's-Van Giesen's stain and subjectively estimating collagen content, it was found statistically that no significant difference in the medial collagen content between the right and left coronary arteries occurred. A highly significant difference was observed in medial collagen content between the various animals studied (Table 35). Estimations of coronary acid-mucopolysaccharide neutral mucopolysaccharides and lipid material were also made and included in Table 34. These results and others were considered for the various animals and their suspected relationship to the degree of denervation were presented. In control dog one, observations were similar in both right and left coronary arteries. No atherosclerotic changes were observed. The intima at its thickest portion when observed using the Verhoeff's-Van Giesen's stain, was about 5.9 u. No separations in the internal elastic membrane were present. Small amounts of collagen were located subintimally and between smooth muscle cells of

the media, but no hyaline deposits were observed. A few small streaks of intercellular fat deposition were present between smooth muscle cells of the media (Figures 84 and 85), but these were not observed in epicardial vessels of the other chambers (Figure 108). Little elastic tissue was present in the media. The adventitia was composed primarily of collagenous connective tissue. Some fat was also localized in this portion of the vessel (Figure 85). Observations in both left and right coronary arteries were similar in dog 2. The intima was 5.6 u and 5.1 u in its widest place in left and right coronary arteries respectively. The internal elastic membrane was single but frayed more than in dog 1. A greater accumulation of ground substance acid mucopolysaccharides was found subintimally and interspersed between medial muscle fibers than in dog 1 (Figure 66). The collagen content as stained using the Verhoeff's-Van Giesen's stain was also slightly greater than in dog 1 (Figure 67). Elastic fibers in the media were thicker and more numerous. No hyaline nor fat deposits were observed (Figures 66 and 86).

The intima of the oldest dog in the study, dog 3, was up to 13.3 u thick at its widest point in the left coronary artery, but only about 5.1 u in the right coronary artery. No plaques were present but sclerosis appeared more progressed in the left vessel. The internal elastic membrane was frayed in places giving the appearance of more than one internal elastic membrane. Smooth muscle cells of the media were few while collagen comprised much of the medial wall (Figure 69). Elastic fibers, where present in the coronary arteries, followed tortuous courses. Although collagen and acid mucopolysaccharide ground substance were present both

subintimally and throughout the media, the largest accumulations were near the adventitia where cords extended into the media. This was observed in smaller epicardial arteries also (Figures 100 and 101). Deeply staining PAS positive areas in the media indicated hyaline deposits (Figure 68). Fat deposits accompanied other medial changes in cardiac vessels (Figure 109). In the adventitia collagenous connective tissue was quite dense but contained some fat. In control 4, the very young dog, left coronary intimal thickness reached 9.4 u and right coronary intimal thickness reached 4.7 u. No atherosclerotic plaques were observed. The internal elastic membrane was single with little fragmenting. Very sparce amounts of acid mucopolysaccharides were present subintimally and in the rest of the media. Collagen was present in small amounts in the media primarily invading from the adventitia, but was rarely observed subintimally. No medial fat was present and it was sparce in the adventitia (Figure 88). The adventitia was composed primarily of collagenous fibers and was thicker than the media. No atherosclerotic plaques were observed in the coronary arteries of dog 5. At its thickest the intima of the right and of the left coronary arteries measured to 8 u and 4.7 u respectively. The internal elastic membrane was single with little fraying. Medial collagen and acid mucopolysaccharides ground substance were moderate. Small areas of PAS positive material was present subintimally. Elastic fibers of the media were thin and sparce. The adventitia was composed mostly of collagenous connective tissue and contained some fat. No intimal plaques were present in dog 6 which had complete extrinsic cardiac denervation over 9 months previously. The left coronary artery intima measured 4.7 u

in thickness and the right one 6.6 u at their thickest points. The internal elastic membrane was single and without fraying. Moderate medial collagen and little acid mucopolysaccharide ground substance or elastic fibers were present in the media and no hyaline deposits were observed in the left coronary artery. A moderate amount of collagen, acid mucopolysaccharide, and elastic fibers were observed in the media of the right coronary artery. Little fat was present in the media (Figure 89). Collagenous connective tissue made up the bulk of the adventitia. In both coronary vessels the vessel wall was relatively thin in comparison to the luminal diameter.

No atherosclerotic changes were noted in the coronary arteries of dog 7 which underwent complete extrinsic cardiac denervation 3 months previously. The intima in the left coronary artery reached 10.5 u and in the right coronary 8.0 u at their greatest thickness. The internal elastic membrane was split in one area, but fraying was rare. Splitting of the internal elastic membrane was observed in an epicardial vessel of the right atrium (Figures 112 and 113). Adventitial collagen invasion of the media was prevalent with elastic fibers tangled in the septae (Figure 112). Acid mucopolysaccharide concentrations in the coronary arteries were moderate to high throughout the media, with large subintimal collections particularly in the right coronary artery (Figures 82 and 83). Some fat was present in the media and adventitia; the latter being primarily composed of collagen. One very thick walled artery was observed in the left ventricle (Figure 111). Coronary vessels were free from intimal plaques in the dog unilaterally extrinsically denervated on the right side (dog 8). The

thickness of the intima measured 4.2 u in the left and 6.6 u in the right coronary artery. The internal elastic membrane was single and not frayed. Medial collagen concentrations were moderate and acid mucopolysaccharides were moderate to heavy in amounts (Figure 81). Elastic fibers in the media were average in number, thin, and orderly in arrangement. Sparce medial fat was present. No adventitial differences were observed. One epicardial vessel showing odd proliferative changes was observed in the left ventricle (Figure 110). In dog 9 (left extrinsic cardiac denervation) the intima measured at its thickest 7.0 u in the left coronary artery and 5.9 u in the right one. The internal elastic membrane was not split or fragmented. Considerable collagen was present in the media together with moderate to heavy amounts of acid mucopolysaccharides and moderate elastic tissue (Figures 79 and 80). Little fat was accumulated in the media. Small deposits of hyaline were present in the media (Figure 119). The adventitia was similar to other dogs. Little hint of these changes was evident with the hematoxylin and eosin stain (Figure 78). The intima of dog 10 (right cervical vagotomy) at its thickest position measured 6.3 u in the left and 6.6 u in the right coronary arteries. The internal elastic membrane had some fraying, but was single (Figure 75). Medial collagen and acid mucopolysaccharide accumulations were heavy (Figures 74 and 75). Some PAS positive accumulations were present (Figure 74). Elastic fibers were numerous and often disassociated in relationship to smooth muscle cells of the media. Fat was sparce in the media, but more frequent in the adventitia. Figures 90, 91, 92, 93, 94, 95, and 96 illustrated modifications in blood vessels occurring at points of great stress; the areas of branching.

Figure 90 showed the right coronary artery branching. Acid mucopolysacchrides (Figures 90, 92, and 94), collagen and elastic fibers (Figures 91, 93, and 95), and fat (Figure 96) accumulated where vessels branched. The internal elastic membrane frayed and smooth muscle cells almost disappeared in these muscular cushions (Figure 93). The age of this dog probably contributed to the changes observed. The intima at its thickest part measured 5.9 u and 10.5 u respectively in the left and right coronary arteries of dog 11 (bilateral recurrent laryngeal nerve transection). The internal elastic membrane was not split, but thick lamina of elastic tissue lay close to it. In the media, elastic tissue was plentiful; collagen and acid mucopolysaccharides moderate in amounts; and fat was sparce. The adventitia resembled that of other dogs. Left and right coronary artery intimal measurements at their thickest observed portions were 7.3 u and 6.3 u respectively in dog 12 (left caudolateral vertebral cardiac nerve transection). The internal elastic membrane was not split. Moderate accumulations of collagen and acid mucopolysaccharides and sparce amounts of elastic fibers and fat were observed in the media of the coronary arteries. The adventitia was similar to preceding animals. Largest intimal thickness in the left and right coronary arteries of dog 13 (thoracic ganglia extirpation) were 7.7 u and 7.0 u respectively. The internal elastic membrane was not split. Medial elastic and collagenous fibers and acid mucopolysaccharide ground substance were moderate and in bands (Figure 98 and 99) and fat was not present. Adventitial morphology was similar to that of other animals. Dimensions of the thickest portions of the intima in dog 14 (extirpation of thoracic and cervicothoracic

ganglia) were 7.0 u for each of the coronary arteries. The internal elastic membrane was not split. Elastic tissue was plentiful in the media (Figure 73). Acid mucopolysaccharide and collagenous fibers were in greater amounts in the left than right coronary artery media and subintimal area. Subintimal areas appeared foamy, (Figure 72) but were apparently not fat laden (Figure 87). The adventitia resembled that of other dogs. In dog 15 (thoracic rami communicantes transection) the maximum intimal thickness found in the left and right coronary arteries was 9.1 u and 8.2 u respectively. No splitting of the internal elastic membrane was found. The media contained moderate or less amounts of elastic fibers, collagen, and acid mucopolysaccharides, but no fat deposits were observed. The adventitia was similar in composition to that of other dogs. An intramural vessel undergoing fibrosis was observed in the left ventricle (Figures 102, 103, 104, 105, 106, and 107). Tissue changes were evident adjacent to the vessel. In dog 16 (bilateral vertebral ganglion extirpation) maximal intimal thickness of the left and right coronary arteries respectively were 6.1 u and 6.3 u. The internal elastic membrane was not split and little fraying of the membrane occurred. The media contained moderate levels of elastic tissue, collagen, and acid mucopolysaccharides (Figures 76 and 77). Fat was rare in the media. Adventitial morphology was similar to other dogs. Maximal intimal thicknesses of left and right coronary arteries in dog 17 (cervical vagosympathetic trunk transection) were 5.6 u and 7.3 u. The internal elastic membrane was not split except in one small area and fraying was minimal. Elastic fibers, collagen fibers, and acid mucopolysaccharides in the media were less than in most dogs studied

(Figures 70 and 71). Fat was rare in the media. The adventitia was similar to that in other animals. The intimal thickness at its greatest thickness in the left and right coronary arteries in dog 18 (right cervical vagotomy) was 7.3 u and 4.7 u respectively. The internal elastic membrane appeared split for a short distance in the right coronary artery. Little to moderate elastic tissue, collagen, and acid mucopolysaccharide content was present in the media. Fat was rare in the media. The adventitia resembled that of other animals.

In considering these results, it was readily recognized that in the older dogs studied (dogs 3, 9, and 10) there are sclerotic age changes including increased medial collagen, acid mucopolysaccharide ground substance, and lipid material as in man (Klassen and Sung, 1968). Elastic fibers of the media in dogs (3, 9, 10) were often aberrant in course, thicker, and more numerous. Small accumulations of PAS positive material intercellularly in the media suggested that hyalinization of the vessels was occurring. The proportion of media and adventitia to intima and the adventitial composition was not particularly different than in other animals. In the youngest dog, number four, the media and adventitia were much thinner than in other mature animals. Collagen and acid mucopolysaccharide concentrations were much lower than in adult dogs and as in most dogs observed the former appeared to be invading from the adventitia. Elastic fibers, compared with other medial contents, were high proportionately.

Of the surgically denervated dogs, the totally denervated dogs all had relatively low ratios of media and adventitia to intima. A consistant pattern in other partially denervated animals was not present. Collagen

and acid mucopolysaccharide levels in the coronary artery media were generally greater in animals which had received sympathetic denervation than in control or vagectomized dogs. Bertelsen (1963) relates increased medial and intimal accumulation of acid mucopolysaccharides to low oxygen tension in the vessel wall. Perhaps the lowered mean arterial blood pressure in the sympathectomized hearts contributed to lower oxygen tension and accumulation of the acid mucopolysaccharides. Lipid material was sparce in the vessels of all animals with the exception of dog 3, the oldest of the dogs studied. Atherosclerotic intimal plaques which interferred with circulation were not present in any of the animals. This was different than in man (Bloch, 1969), but consistent with usual findings according to Detweiler, Patterson, Hubben, and Botts (1961); Getty (1966a, 1966b); and Pirie (1967) in the dog heart. However the extent of internal sclerosis and plaquation is dependent upon age (Getty, 1966).

Normal dimensional features of blood vessels summarized by Gofman and Young (1963) for medium sized arteries showed the intima from 2.48 to 7.36 u, the internal elastic membrane from 4.40 to 6.78 u, the media 104 to 211.5 u, and the adventitia 20.9 to 9.1 u. They point out that there is evidence that intra-arterial pressure accelerates atherosclerosis development, even in the dog. The dimensions reported in the present study were very similar though the adventitia was occasionally somewhat thicker. The intima varied in thickness within and between animals, but only in dogs 3, 7, and 11 were thicknesses of over 10 u found. Surgical procedures were not related in these three dogs. Arterial changes observed were almost entirely in the media. These changes appeared to begin not only in the subintimal area,

but at least as importantly, as invasive septa from the adventitia and as build up of materials originating in the media itself.

CONCLUSIONS

1. A method for complete extrinsic cardiac denervation with minimal effects on other viscera was described and successfully employed.
2. Following bilateral vagotomy a cat was maintained without special supportive measures for several months. Bilaterally vagectomized dogs required electrolyte and metabolic supportive therapy to counteract the sequellae of megaesophagus, cardiac and pyloric sphincter contraction, and gastric dilitation which contributed to death of the dog within three days without supportive therapy.
3. Animals in the chronic postsurgical period showed electrocardiogram interval, diastolic blood pressure, and pulse pressure changes which were significantly or highly significantly different than in previous periods when analyzed statistically.
4. Significant or highly significant differences were observed in electrocardiogram wave amplitudes and intervals and in heart rate, systolic blood pressure, and pulse pressure between animals studied.
5. Interaction between both amplitude and interval waves and the specific type of denervation was highly statistically significant, but questionably valid since F values between amplitude and interval waves were so greatly elevated.
6. Strengths of contraction and durations of depolarization in the atria were highly significantly positively correlated in the animals studied.
7. The strengths and duration of atrial depolarization were highly significantly correlated with ventricular strengths and durations of depolarization. Both were highly significantly negatively related to

heart rate.

8. A significant negative correlation between the force of ventricular contraction and the left ventricular acetylcholinesterase and/or acetylcholine was found suggesting suppression of ventricular contractile force by the parasympathetic nervous system.
9. Increased ventricular contractile force accompanied extrinsic cardiac denervation of the parasympathetic nervous system.
10. Transection of thoracic rami communicantes or thoracic ganglia, interrupt the sensory feedback mechanism responsible for sympathetic stimulation of myocardial contraction.
11. The time required for spread of impulses in the ventricular myocardium (QRS interval) and ventricular systole (Q-T interval) were significantly negatively correlated with acetylcholinesterase and or acetylcholine content of the left ventricle.
12. Complete extrinsic cardiac denervation and sympathectomies appeared to cause little amplitude or interval change in chronic postsurgical recordings. The ventricular conduction was slowed slightly in complete extrinsic cardiac denervation during acute postsurgical recordings.
13. Ventricular strain, ischemia, or hyperpotassemia were suspected in some dogs during or immediately following surgery, but this could not be related to specific types of surgery.
14. During exsanguination electrocardiogram recordings were observed but no unique pattern characteristic of various denervation types was reflected in this stressed, ischemic procedure.

15. Mean aortic blood pressure on 17 dogs in this study, presurgically, averaged 133 mm Hg.
16. Transection of each vagus nerve resulted in an increased mean aortic blood pressure.
17. Mean aortic blood pressure dropped in sympathectomized dogs from presurgical values and persisted in the chronic postsurgical period.
18. Complete extrinsic cardiac denervation resulted in decreased heart rate in the acute postsurgical period. The rate was normal just prior to harvest in the dog denervated 9 months earlier, but was slow in the dog denervated for only 3 months. Unilateral right extrinsic cardiac denervation resulted in a slowed heart rate while in left extrinsic cardiac denervation the heart rate increased.
19. Mean blood pressure fell about 16% following isolation of the thoracic spinal cord segments from the heart.
20. A modification of previously described methods for norepinephrine assay in heart tissue was described.
21. The cardiac chamber walls were highly significantly different in norepinephrine content. The greatest quantities were localized in the right atrium, then left atrium, followed by the left ventricle, and finally the right ventricle.
22. Complete extrinsic cardiac nerve transections affected both the norepinephrine and acetylcholinesterase contents of the heart.
23. Complete sympathectomy by transection of extrinsic cardiac nerves resulted in cardiac norepinephrine depletion. This innervation was restored about 1/3 in the atria and 1/6-1/9 in the ventricles 9 months

postsurgical, but was not returned in chemically measurable amounts before 3 months postsurgically. It appears that reinnervation proceeds at a more rapid rate in the atria than in the ventricles.

24. The almost exclusive site of origin for the norepinephrine containing postganglionic fibers appeared to be the vertebral ganglia since extirpation of these ganglia resulted in near depletion of cardiac norepinephrine.

25. Homolateral extrinsic cardiac denervation depressed norepinephrine values in all chamber walls but right denervation showed a greated tendency to affect the right chamber walls and left denervations to to affect the left atrium and both ventricles.

26. Thoracic ganglia appeared to contribute very few postganglionic sympathetic fibers to the heart, but the cervicothoracic ganglia may have contributed up to one-third of the sympathetic supply. Since bilateral vertebral ganglion extirpation resulted in almost complete depletion of cardiac norepinephrine, the effect of cervicothoracic ganglion extirpation suggested some type of influence by the latter ganglion through the former ganglion.

27. The sympathetic trunk cranial to the vertebral ganglia appeared not to contribute to the sympathetic innervation of the heart.

28. Decreased norepinephrine levels in one dog appeared to be associated with illness accompanied by a fever.

29. Age, though reported to be a factor in reducing cardiac norepinephrine in rats and cattle, appeared not to influence norepinephrine levels in this group of dogs.

30. A negative correlation was found between norepinephrine and acetylcholinesterase, but not at a significant level.

31. Acetylcholinesterase activity was reported for the heart chamber walls. The left atrium contained the highest activity, followed by the right atrium, right ventricle, and left ventricle respectively.

32. Statistically a positive correlation existed between acetylcholinesterase contents of the heart chamber walls.

33. Acetylcholinesterase concentration also reflecting acetylcholine concentration, and both being associated primarily with postganglionic parasympathetic neurons, but also preganglionic parasympathetic, sympathetic preganglionic, and postganglionic and sensory neurons, appeared to be altered by cardiac denervation. Since postganglionic neurons were not disturbed as would be the case in regional neural ablation, changes in sensory, preganglionic, and sympathetic acetylcholinesterase, and or acetylcholine would be the neuronal types reflecting acetylcholinesterease or acetylcholine concentration. These had lower concentrations of transmitter substance than did postganglionic parasympathetic neurons.

34. The acetylcholinesterase and or acetylcholine content of the dog with complete cardiac denervation only three months previous showed decreased levels in all chamber walls. The other completely extrinsically denervated heart, from dog 6, showed elevated values. This might indicate that the cholinergic innervation returns to the heart sooner than adrenergic innervation.

35. Homolateral extrinsic cardiac nerve denervation did not establish firm relationships in delineating the cholinergic nerve distribution in the heart. After right extrinsic cardiac denervation; atrial aceylcholinesterase was normal to elevated in the atria and near normal in the ventricles, suggesting that renervation was essentially completed. Both atrial and the right ventricular concentrations of acetylcholinesterase were still depressed in the left extrinsic cardiac denervated dog. In the right vagotomized dogs acetylcholinesterase levels were below control levels in all chambers except in the right atrium of dog 18, which might have reflected that renervation was beginning in this dog.
36. Lowering of acetylcholinesterase levels associated with destroyed sensory neurons and sympathetic neurons, which entered the thoracic spinal cord, were present in the dogs subjected to destruction of thoracic and cervicothoracic ganglia.
37. Evidence of a negative correlation between age and cardiac acetylcholinesterase was found, except in the left atrium.
38. A modification of previously described methods for histochemical staining of nerve acetylcholinesterase was adapted to cardiac tissue.
39. Histochemically, cardiac nerves stain more rapidly and more intensely in the atria than in the ventricles.
40. After prolonged periods of incubation, atrial myocardial fibers stain but ventricular fibers remain unstained suggesting greater intramyocardial fiber acetylcholinesterase in atrial fibers compared to ventricular fibers. Quantitative chemical findings supported histochemical findings.

41. In the 3 month old dog atrial myocardial tissue did not stain for acetylcholinesterase, though cardiac nerves did, after incubation periods which resulted in deep myocardial staining in adult dogs.
42. Acetylcholinesterase containing neurons innervate cardiac muscle fibers directly.
43. Acetylcholinesterase staining neurons penetrate the width of the media of coronary vessels from the adventitia.
44. Consistant differences in intensity of acetylcholinesterase histochemical staining between various extrinsically denervated hearts was not found.
45. Paraformaldehyde condensation fluorescence of cardiac catecholamines was accomplished without freeze dry apparatus.
46. Highly significant differences were statistically found in myocardial fiber diameter and myocardial length between the dogs in the chronic extrinsic cardiac denervation study.
47. Highly significant differences were statistically found in myocardial fiber diameter and length between chambers of the heart.
48. Highly significant negative correlation was found between fiber diameter and nuclei per five $5mm^2$ fields which was used to indicate fiber length.
49. Complete and unilaterally extrinsically denervated hearts averaged slightly below control dog values in both fiber diameter and length.
50. Age was a highly significant factor in differences in myocardial fiber length and diameter between dogs.

51. Myocardial glycogen concentrations of dogs on which total extrinsic cardiac denervation or sympathectomies were performed were equal to or exceeded concentrations in control dogs.
52. No changes in the glycogen content resulting from extrinsic cardiac denervations, were observed in Purkinje fibers of the myocardium.
53. Fat content of the myocardium and epicardium was different between animals, but no correlation between accumulations of fat and the type denervation could be ascertained.
54. The ratio of media and of adventitia to intima in coronary arteries was similar to slightly less in dogs' hearts nearly depleted of norepinephrine.
55. No intimal plaques were observed histologically in any of the dogs. Changes in the coronary arteries were almost exclusively found in the subintimal area and throughout the media.
56. Collagen in the media appeared to arise not only in the subintimal area, but perhaps more importantly in invasive septa from the adventitia and from within the media itself.
57. Acid mucopolysaccharide ground substance besides being distributed throughout the media was particularly prevalent in the subintimal area in dogs which were undergoing sclerotic changes.
58. Coronary arteries of older dogs contained greater amount of collagen, acid mucopolysaccharides, and hyaline than those of younger dogs.
59. Muscular cushions at the sight of coronary artery branching in older dogs contained large quantities of acid mucopolysaccharides and collagen. The elastic fibers were plentiful, some lipid material was

present, and very small diameter smooth muscle cells were present near the luminal side of the media.

60. Collagen, acid mucopolysaccharide, and hyaline content in coronary arteries of dogs with near or complete norepinephrine depletion generally exceeded that of animals of similar ages whose hearts were not depleted of norepinephrine.

61. This is an initial study with limited numbers of animals within each of the measured parameters due to the complexity of each phase therefore further expansion of the research is anticipated to provide further understanding of the functional, chemical, and structural relationships between the heart and its innervation.

SUMMARY

Following extrinsic cardiac denervation to different extents, heart function, chemistry, and structure were studied to ascertain what changes accompanied specific denervations in dogs and whether correlation existed between the various parameters measured. A surgical technique utilizing anatomical descriptions of extrinsic cardiac nerves was employed to effectively completely or partially extrinsically denervate the heart. Highly significant functional differences were found in electrocardiogram amplitudes and intervals, heart rates, and blood pressures between the animals studied. Some differences accompanied specific types of extrinsic cardiac denervation. Statistically significant correlations between functional and the chemical and structural results were discussed. Modifications of previously described methods for quantitating norepinephrine and acetylcholinesterase were reported. Highly significant statistical differences were found between chambers of the heart and between animals for both acetylcholinesterase and norepinephrine. Differences related to specific denervations and correlations with other parameters were discussed. Histochemically and histologically structural changes in the myocardial fiber diameter and length, fat content, glycogen content, nervous supply, and vascular morphology were investigated. Highly significant statistical differences were found between coronary arterial layers and components and in muscle fiber lengths and diameters. These were related to specific animals and correlated with other parameters.

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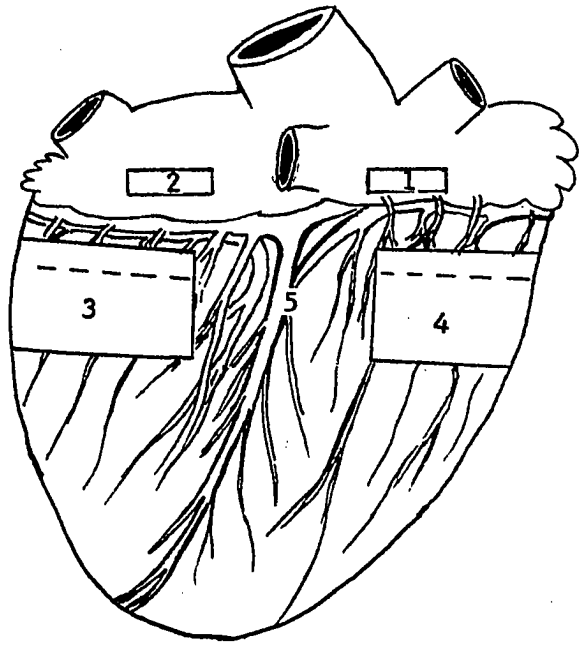
APPENDIX A. ILLUSTRATION OF AREAS OF HEART TISSUE EXCISION

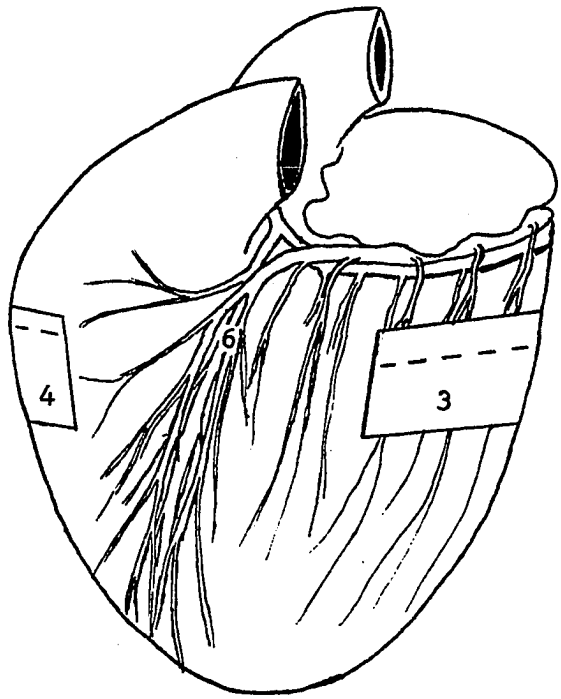
FIGURE 1. Areas of tissue excision for histochemical, histologic, and chemical studies of the dog heart.

1 = right atrial sample
2 = left atrial sample
3 = left ventricular sample
4 = right ventricular sample
5 = region at right coronary artery structural study
6 = region at left coronary artery structural study

Chemical samples in the ventricles were obtained below the dotted lines.



A. Right lateral view



B. Left lateral view

APPENDIX B. TABLES

TABLE 1. Dogs utilized in various phases of the research

Dog	Date sacrificed	Use	Dog	Dog sacrificed	Use
01	10-10-68	C,S,F,C_{NE},C_{AChE},HC,SS	27	4-16-68	S,F
02	11- 6-68	C,S,F,C_{NE},C_{AChE},HC,SS	28	7-25-68	S,F,
03	12-12-68	C,S,F,C_{NE},C_{AChE},HC,SS	29	Summer'68	S
04	1- 9-69	C,S,F,C_{NE},C_{AChE},HC,SS	30	1- 5-68	T_{CNE}
05	1- 5-69	C,S,F,C_{NE},C_{AChE},HC,SS	31	1-16-68	T_{CNE}
06	1- 9-69	S,F,C_{NE},C_{AChE},HC,SS	32	1-30-68	T_{CNE}
07	11- 7-68	S,F,C_{NE},C_{AChE},HC,SS	33	2- 6-68	T_{CNE}
08	11-13-68	S,F,C_{NE},C_{AChE},HC,SS	34	2-13-68	T_{CNE}
09	1- 9-69	S,F,C_{NE},C_{AChE},HC,SS	35	4- 2-68	T_h
10	10-24-68	S,F,C_{NE},C_{AChE},HC,SS	36	4-10-68	T_h,T_{CNE}
11	1-10-69	S,F,C_{NE},C_{AChE},HC,SS	37	4-10-68	T_h,T_{HC}
12	10-17-68	S,F,C_{NE},C_{AChE},HC,SS	38	4-12-68	T_h,T_{HC}
13	12-13-68	S,F,C_{NE},C_{AChE},HC,SS	39	4-12-68	T_h,T_{HC}
14	10-31-68	S,F,C_{NE},C_{AChE},HC,SS	40	4-12-68	T_h,T_{HC}
15	11-13-68	S,F,C_{NE},C_{AChE},HC,SS	41	6-13-68	T_h
16	11-14-68	S,F,C_{NE},C_{AChE},HC,SS	42	6-13-68	T_h
17	12-12-68	S,F,C_{NE},C_{AChE},HC,SS	43	7-16-68	T_{CAChE}
18	11- 7-68	S,F,C_{NE},C_{AChE},HC,SS	44	8- 1-68	T_{CAChE}
19	7- 1-68	S,F	45	8-19-68	S,F,T_{CAChE},T_{CNE},T_{HC}
20	2-16-68	S,F	46	8-23-68	T_{CNE}
21	1-17-68	S,F	47	8-26-68	T_{HC},T_h,T_{CNE},S,F,T_{CAChE}
22	1-19-68	S,F	48	8-30-68	T_{CNE},T_{CAChE},T_{HC}
23	1-28-68	S,F	49	9- 4-68	T_{HC}
24	3-16-68	S,F	50	9- 6-68	T_{HC}
25	3-21-68	S,F	51	9-19-68	T_{HC}
26	4- 7-68	S,F			

TABLE 1 (Continued)

Dog	Date sacrificed	Use	Dog	Dog sacrificed	Use

S = surgery

C = control

F = functional data

C_{NE} = chemical data - norepinephrine

C_{AChE} = chemical data - acetylcholinesterase

HC = histochemical data

SS = specific histologic tissue stains

T_h = technique development in harvesting

T_{CNE} = technique development chemically for tissue - norepinephrine

T_{CACh} = technique development chemically for tissue - acetylcholinesterase

T_{HC} = techinique development histochemically for tissue - norepinephrine and acetylcholinesterase

TABLE 2. Dogs submitted to surgery

Animal	Sex	Age years	Weight pounds	Surgery	Date of surgery	Date of termination	Interval in days
01	F	1	26	Arterial cannulation	10-10-68	10-10-68	0
02	F	1	20	Arterial cannulation	11- 6-68	11- 6-68	0
03	M	10	28	Arterial cannulation	12-12-68	12-12-68	0
04	M	.25	14	Arterial cannulation	1- 9-69	1- 9-69	0
05	F	2	28	Arterial cannulation	1-10-69	1-10-69	0
06	M	2	54	Complete extrinsic cardiac denervation	3-28-68	1- 9-69	287
07	M	2	45	Complete extrinsic cardiac denervation	8- 7-68	11- 7-68	92
08	M	4	37	Right extrinsic cardiac nerves transected	4-25-68	11-13-68	202
09	M	6	51	Left extrinsic cardiac nerves transected	7- 1-68	1- 9-69	192
10	F	7	60	Right vagotomy at cranial laryngeal nerve	1- 4-68	10-24-68	293
11	M	2	25	Bilateral recurrent cardiac nerves transection	7-22-68	1-10-69	172
12	M	5	50	Left caudolateral vertebral cardiac nerve transection	7-29-68	10-17-68	80
13	M	2.5	46	Bilateral extirpation of thoracic sympathetic ganglia between T_8 and cerviothoracic ganglion	4-18-68	12-13-68	239
14	F	5	45	Bilateral extirpation of the cervicothoracic through first five thoracic ganglia	1-11-68	10-31-68	293
15	F	2	43	Bilateral transection of thoracic sympathetic rami communicantes	5-16-68	11-13-68	181

16	M	1	31	Bilateral vertebral ganglion extirpation	6-24-68	11-14-68	143
17	M	1	23	Bilateral cervical sympathetic trunk transection cranial to vertebral ganglia	7- 8-68	12-12-68	157
18	M	2	45	Right midcervical vago-sympathetic trunk transection	5- 7-68	11- 7-68	181
19	F	2	50	Bilateral vertebral ganglion extirpation	2- 1-68	7- 1-68	150
20	M	3	45	Complete extrinsic cardiac denervation	2-15-68	2-16-68	1
21	F	2	35	Bilateral vagotomy	1-15-68	1-17-68	2
22	F	2	30	Bilateral vagotomy	1-18-68	1-19-68	1
23	F	1	35	Bilateral vagotomy	1-25-68	1-28-68	3
24	M	1	35	Bilateral vagotomy	3-15-68	3-16-68	1
25	F	3	40	Bilateral vagal cardiac nerves transected	3-21-68	3-21-68	0
26	F	2	35	Bilateral vagotomy	4- 5-68	4- 7-68	2
27	M	2	45	Bilateral vagotomy	4-11-68	4-16-68	5
28	F	2	35	Bilateral vagotomy	6-17-68 7-15-68	7-25-68	10
29	F	1	8	Bilateral vagotomy	12-13-67	Summer '68	200 Approx.

TABLE 3. Mean electrocardiogram recording values during the presurgical period

	Amplitude in mV					Interval in seconds			
Dog	P	Q	R	S	T	P	P-R	QRS	Q-T
02	0.45	0.00	0.95	0.00	0.20	0.07	0.12	0.04	0.28
03	0.13	0.00	0.83	0.00	0.23	0.07	0.10	0.09	0.25
02	0.20	0.00	0.95	0.00	0.23	0.07	0.10	0.06	0.23
05	0.25	0.00	1.15	0.00	0.10	0.07	0.08	0.08	0.20
06	0.20	0.00	0.70	0.00	0.10	0.08	0.09	0.07	0.17
07	0.28	0.04	1.60	0.35	0.55	0.08	0.09	0.09	0.31
08	0.20	0.00	1.20	0.10	0.20	0.07	0.09	0.06	0.20
09	0.45	0.00	1.40	0.18	0.55	0.08	0.11	0.09	0.30
10	0.18	0.10	0.55	0.10	0.15	0.09	0.10	0.07	0.30
11	0.40	0.00	1.40	0.00	0.25	0.10	0.11	0.08	0.24
12	0.45	0.00	1.60	0.20	0.23	0.10	0.10	0.08	0.36
13	0.20	0.00	0.80	0.30	0.10	0.07	0.12	0.10	0.26
14	0.20	0.00	2.10	0.05	0.30	0.09	0.10	0.09	0.32
15	0.10	0.00	1.00	0.05	0.03	0.09	0.10	0.10	0.24
16	0.20	0.00	0.80	0.00	0.60	0.10	0.10	0.08	0.32
17	0.50	0.00	2.60	0.05	0.00	0.10	0.10	0.12	0.20

TABLE 4. Mean electrocardiogram recording values during the surgical period

	Amplitude in mV					Interval in seconds			
Dog	P	Q	R	S	T	P	P-R	QRS	Q-T
06	0.20	0.00	0.80	0.00	0.10	0.07	0.08	0.08	0.20
07	0.20	0.00	1.50	0.20	0.55	0.08	0.09	0.10	0.28
08	0.10	0.00	1.30	0.05	0.20	0.04	0.11	0.04	0.39
09	0.55	0.05	1.50	0.20	0.50	0.10	0.11	0.09	0.24
10	0.20	0.15	0.65	0.10	0.13	0.09	0.11	0.06	0.28
11	0.35	0.00	1.80	0.00	0.35	0.10	0.11	0.08	0.43
12	0.55	0.00	1.80	0.20	0.20	0.09	0.10	0.08	0.28
13	0.20	0.05	0.60	0.20	0.10	0.06	0.13	0.09	0.24
14	0.15	0.00	2.00	0.03	0.10	0.08	0.11	0.07	0.28
15	0.15	0.00	1.30	0.00	0.30	0.10	0.13	0.10	0.38
16	0.20	0.00	0.90	0.00	0.80	0.10	0.10	0.10	0.36
17	0.20	0.00	1.20	0.05	0.10	0.09	0.09	0.12	0.29

TABLE 5. Mean electrocardiogram recordings during the acute postsurgical period

	Amplitude of mV					Interval in seconds			
Dog	P	Q	R	S	T	P	P-R	QRS	Q-T
06	0.10	0.00	0.80	0.00	0.10	0.06	0.07	0.12	0.22
07	0.23	0.00	1.70	0.23	0.40	0.08	0.09	0.12	0.32
08	0.10	0.00	1.20	0.10	0.15	0.05	0.07	0.05	0.26
09	0.70	0.00	1.80	0.05	1.20	0.08	0.09	0.09	0.25
10	0.23	0.05	0.70	0.10	0.18	0.09	0.11	0.06	0.23
11	0.50	0.00	1.90	0.00	0.58	0.10	0.11	0.08	0.34
12	0.50	0.00	1.70	0.20	0.10	0.10	0.10	0.08	0.28
13	0.20	0.00	1.10	0.50	0.15	0.08	0.13	0.09	0.26
14	0.20	0.05	1.40	0.00	0.20	0.10	0.11	0.09	0.32
15	0.18	0.00	1.00	0.00	0.20	0.09	0.12	0.10	0.31
16	0.25	0.00	0.90	0.00	0.60	0.10	0.10	0.10	0.34
17	0.25	0.00	1.10	0.05	0.05	0.10	0.10	0.10	0.26

TABLE 6. Mean electrocardiogram recordings during chronic postsurgical period

	Amplitude of mV					Interval in seconds			
Dog	P	Q	R	S	T	P	P-R	QRS	Q-T
06	0.25	0.05	1.50	0.00	0.09	0.08	0.09	0.08	0.19
07	0.15	0.05	1.20	0.00	0.05	0.08	0.11	0.08	0.32
08	0.25	0.00	1.10	0.00	0.45	0.08	0.11	0.06	0.25
09	0.25	0.05	1.10	0.00	0.20	0.07	0.10	0.07	0.25
10	0.90	0.80	1.10	0.50	0.25	0.07	0.09	0.07	0.21
11	0.20	0.05	1.50	0.05	0.15	0.06	0.14	0.06	0.30
12	0.20	0.00	1.20	0.00	0.30	0.10	0.10	0.10	0.31
13	0.13	0.00	0.60	0.00	0.15	0.07	0.11	0.03	0.26
14	0.20	0.05	2.10	0.40	0.20	0.05	0.12	0.06	0.32
15	0.15	0.00	0.90	0.00	0.05	0.07	0.11	0.08	0.25
16	0.30	0.00	0.70	0.03	0.25	0.08	0.09	0.04	0.26
17	0.15	0.00	1.10	0.10	0.10	0.08	0.09	0.08	0.24
18	0.20	0.05	1.40	0.00	0.18	0.07	0.10	0.10	0.26

TABLE 7. Mean systolic blood pressure, diastolic blood pressure, pulse pressure, and heart rate recordings during the presurgical, surgical, acute postsurgical, and chronic postsurgical periods

Dog	Mean heart rate Beats per minute				Mean systolic blood pressure in mm Hg		
	Pre-surgical	Surgical	Acute post-surgical	Chronic post-surgical	Pre-surgical	Surgical	Acute post-surgical
01	135				140		
02	132				170		
03	126				125		
04	164				110		
05	156				170		
06	160	150	150	162	170	155	125
07	143	144	135	087	130	133	145
08	180	096	132	138			
09	132	120	190	178	200	225	170
10	166	151	156	166	155	153	165
11	132	096	096	096	105	105	100
12	108	120	120	132	138	133	145
13	120	100	132	136	210	163	175
14	114	108	108	096	155	160	100
15	144	096	096	126	178	200	160
16	132	120	120	132	143	130	125
17	156	120	120	144	158	225	190
18				132			

TABLE 7 (Continued)

Dog	Mean diastolic blood pressure in mm Hg					Mean pulse pressure in mm Hg			
	Chronic post-surgical	Pre-surgical	Surgical	Acute post-surgical	Chronic post-surgical	Pre-surgical	Surgical	Acute post-surgical	Chronic post-surgical
01		080				060			
02		130				040			
03		080				045			
04		065				045			
05		100				070			
06	135	148	125	095	093	022	030	030	042
07	110	105	105	115	060	025	028	030	050
08	120				080				040
09	120	145	185	125	068	055	040	055	052
10	095	123	120	135	050	032	033	030	045
11	110	095	095	090	040	020	010	010	070
12	190	108	118	118	100	030	015	027	090
13	150	153	122	120	105	057	041	055	045
14	160	100	100	070	073	055	060	030	087
15	175	128	140	110	115	050	060	050	060
16	080	108	100	100	050	035	030	025	030
17	175	121	125	140	115	037	100	050	060
18	153				098				055

TABLE 8. ANOV analysis hypotheses and statistical significance of electrocardiogram mean amplitude values differences

Null hypotheses (Ho):

Ho_A - There will be no difference between the four periods (presurgical, surgical, acute post-surgical, and chronic postsurgical) in their recorded electrocardiogram amplitude values.

Ho_B - There will be no difference between the amplitudes of the P, Q, R, S, and T deflections on the electrocardiogram.

Ho_C - There will be no difference between the various animals specifically denervated as related to the recorded electrocardiogram amplitudes values.

Ho_{AB} - There will be no interaction between given periods and amplitude readings as recorded using the electrocardiogram.

Ho_{AC} - There will be no interaction between given periods and type denervation.

Ho_{BC} - There will be no interaction between amplitude readings and type denervation.

E.C.G. ANOV Analysis

Amplitude				Required Significant F Value	Highly significant F Value	Calculated F Value	Relationship of Ho
Ho_A	1	F	3.132	2.67	3.93	0.73	N.S.
	2	F	2.88	3.10	4.85	0.14	N.S.
	3	F	2.88	3.10	4.85	1.13	N.S.
Ho_B	1	F	4.132	2.44	3.45	466.36	* *
	2	F	4.88	2.47	3.53	415.71	* *
	3	F	4.88	2.47	3.53	407.58	* *

Ho_C						
	1	F	11.132	1.86	2.38	7.53	* *
	2	F	11.88	1.90	2.45	10.64	* *
	3	F	11.88	1.90	2.45	6.75	* *
Ho_{AB}						
	1	F	8.132	2.00	2.63	0.84	N.S.
	2	F	8.88	2.04	2.71	0.25	N.S.
	3	F	8.88	2.04	2.71	1.23	N.S.
Ho_{AC}						
	1	F	33.132	1.54	1.56	3.07	* *
	2	F	22.88	1.66	2.04	1.59	N.S.
	3	F	22.88	1.66	2.04	4.11	* *
Ho_{BC}						
	1	F	44.132	1.46	1.70	6.16	* *
	2	F	44.88	1.51	1.78	6.35	* *
	3	F	44.88	1.51	1.78	5.14	* *

A = periods of measurement
B = amplitudes
C = type denervations
N.S. = not significant
* = significant
** = highly significantly
1 = data of all periods
2 = data with chronic postsurgical data omitted
3 = data with presurgical data omitted

TABLE 9. ANOV analysis hypothesis and the statistical significance of electrocardiogram mean interval value differences

Null hypotheses: Ho

Ho_A - There will be no difference between the four periods (presurgical, surgical, acute post-surgical, and chronic postsurgical) in their recorded electrocardiogram interval values.

Ho_B - There will be no difference between the lengths of the P, P-R, QRS, and Q-T intervals recorded on the electrocardiogram.

Ho_C - There will be no difference between the intervals of the different animals.

Ho_{AB} - There will be no interaction between given periods and intervals.

Ho_{AC} - There will be no interaction between given periods and type denervations.

Ho_{BC} - There will be no interaction between interval readings and type denervations.

E.C.G. ANOV Analysis

Interval - Set 2			Required Significant F Value	Highly significant F Value	Calculated F Value	Relationship of Ho
Ho_A						
	1 F	3.99	2.70	3.98	4.21	* *
	2 F	2.66	3.14	4.95	1.32	N.S.
	3 F	2.66	3.14	4.95	7.81	* *
Ho_B						
	1 F	3.99	2.70	3.98	757.14	* *
	2 F	3.66	2.75	4.10	487.00	* *
	3 F	3.66	2.75	4.10	744.71	* *
Ho_C						
	1 F	11.99	1.88	2.43	4.77	* *
	2 F	11.66	1.94	2.54	3.78	* *
	3 F	11.66	1.94	2.54	5.00	* *

Ho_{AB}						
	1	F	9.99	1.97	2.59	1.87	N.S.
	2	F	6.66	2.24	3.09	1.56	N.S.
	3	F	6.66	2.24	3.09	2.57	*
Ho_{AC}						
	1	F	33.99	1.57	1.89	1.21	N.S.
	2	F	22.66	1.71	2.14	1.07	N.S.
	3	F	22.66	1.71	2.14	1.50	N.S.
Ho_{BC}						
	1	F	33.99	1.57	1.89	2.88	* *
	2	F	33.66	1.64	1.97	2.09	* *
	3	F	33.66	1.64	1.97	3.38	* *

A = period
B = intervals
C = type denervations
1 = data of all periods
2 = data with chronic postsurgical data omitted
3 = date with presurgical data omitted

TABLE 10. ANOV analysis hypotheses and the statistical significance of mean heart rate value differences

Null hypotheses: Ho

Ho_A - There will be no difference between the periods in recorded heart rates.

Ho_B - There will be no difference between the type denervations relative to heart rate.

				ANOV			
				Required Significant F Value	Highly significant F Value	Calculated F Value	Relationship of Ho
Ho_A							
	1	F	3.33	2.89	4.44	2.90	N.S.
	2	F	2.22	3.44	5.72	4.24	N.S.
	3	F	2.22	3.44	5.72	2.26	N.S.
Ho_B							
	1	F	11.33	2.09	2.84	3.95	* *
	2	F	11.22	2.26	3.18	2.64	*
	3	F	11.22	2.26	3.18	4.50	* *

A = periods
B = type denervations
1 = date of all periods
2 = data with chronic postsurgical date omitted
3 = data with presurgical data omitted

TABLE 11. ANOV analysis hypotheses and the statistical significance of mean systolic, diastolic, and pulse pressure value differences

Mean systolic blood pressure null hypotheses: Ho
(presurgical data omitted)

Ho_A - There will be no difference between the mean systolic blood pressures of the time periods.

Ho_B - There will be no difference between the mean aortic systolic blood pressures of the different types of cardiac denervated animals.

		ANOV Required Significant F Value	Highly significant F Value	Calculated F Value	Relationship of Ho
Ho_A	F 2.20	3.49	5.85	2.67	N.S.
Ho_B	F 10.20	2.35	3.37	3.41	* *

Mean diastolic blood pressure null hypotheses: Ho
(presurgical data omitted)

Ho_A - There will be no difference between the mean aortic diastolic blood pressures of the time periods.

Ho_B - There will be no difference between the mean aortic diastolic blood pressures of the different types of cardiac denervated animals.

		ANOV Required Significant F Value	Highly significant F Value	Calculated F Value	Relationship of Ho
Ho_A	F 2.20	3.49	5.85	13.49	* *

Ho_B	F	10.20	2.35	3.37	2.59	*

Mean aortic pulse pressure null hypotheses: Ho

Ho_A - There will be no difference between the mean aortic pulse pressures of the time periods.

Ho_B - There will be no difference between the mean aortic pulse pressures of the different animals.

ANOV

				Required Significant F Value	Highly significant F Value	Calculated F Value	Relationship of Ho
Ho_A							
	1	F	3.30	2.92	4.51	4.15	*
	2	F	2.20	3.49	5.85	0.43	N.S.
	3	F	2.20	3.49	5.85	4.28	*
Ho_B							
	1	F	10.30	2.16	2.98	2.41	*
	2	F	10.20	2.35	3.37	4.47	* *
	3	F	10.20	2.35	3.37	1.59	N.S.

A = periods
B = type denervations
1 = data of all periods
2 = data with chronic postsurgical data omitted
3 = data with presurgical data omitted

TABLE 12. Method for norepinephrine assay for each chamber of each dog

Tissue sample I		Tissue sample II		Tissue sample III	
Homogenize 100 mg tissue in 5 ml Butanol and 0.75 ml 0.01 N HCl		Homogenize 100 mg tissue in 5 ml Butanol		Homogenize 100 mg tissue in 5 ml Butanol and 0.75 ml 0.01 N HCl	
		Divide homogenate equally into			
		Sample 2	Internal standard		
		Add 0.37 ml 0.01 N HCl	Add 0.35 ml 0.01 N HCl and 0.02 ml of 25μ gl/ml L-noradrenaline		
		Vortex until water phase is absorbed	Vortex until water phase is absorbed		
Centrifuge 13 minutes Sample 1a	Sample 1b	Centrifuge 13 minutes	Centrifuge 13 minutes	Centrifuge 13 minutes Sample 3	Sample blank
Add 2 ml Butanol extract to 3 ml 0.1 M Phoshate Buffer	Add 2 ml Butanol extract to 3 ml 0.1 M Phosphate Buffer	Add 2 ml Butanol extract to 3 ml 0.1 M Phosphate Buffer	Add 2 ml Butanol extract to 3 ml 0.1 M Phosphate Buffer	Add 2 ml Butanol extract to 3 ml 0.1 M Phosphate Buffer	Add 2 ml Butanol extract to 3 ml 0.1 M Phosphate Buffer
Vortex 20 sec.	Vortex 20 sec.	Vortex 20 sec.	Vortex 20 sec.	Vortex 20 sec.	Vortex 20 sec.
Centrifuge 13 minutes	Centrifuge 13 minutes	Centrifuge 13 minutes	Centrifuge 13 minutes	Centrifuge 13 minutes	Centrifuge 13 minutes
Discard Butanol layer	Discard Butanol layer	Discard Butanol layer	Discard Butanol layer	Discard Butanol layer	Discard Butanol layer

Add 1.0 ml phosphate layer to 0.25 ml Versene	Add 1.0 ml phosphate layer to 0.25 ml Versene	Add 1.0 ml phosphate layer to 0.25 ml Versene	Add 1.0 ml phosphate layer to 0.25 ml Versene	Add 1.0 ml phosphate layer to 0.25 ml Versene	Add 1.0 ml phosphate layer to 0.25 ml Versene
Add 0.20 ml Iodine	Add 0.20 ml Iodine	Add 0.20 ml Iodine	Add 0.20 ml Iodine	Add 0.20 ml Iodine	Add 0.25 ml alkaline sulfite
Wait exactly 2 minutes	Wait exactly 2 minutes	Wait exactly 2 minutes	Wait exactly 2 minutes	Wait exactly 2 minutes	Wait exactly 2 minutes
Add 0.25 ml alkaline sulfite	Add 0.25 ml alkaline sulfite	Add 0.25 ml alkaline sulfite	Add 0.25 ml alkaline sulfite	Add 0.25 ml alkaline sulfite	Add 0.20 ml Iodine
Wait exactly 2 minutes	Wait exactly 2 minutes	Wait exactly 2 minutes	Wait exactly 2 minutes	Wait exactly 2 minutes	Wait exactly 2 minutes
Add 0.30 ml 5N acetic acid	Add 0.30 ml 5N acetic acid	Add 0.30 ml 5N acetic acid	Add 0.30 ml 5N acetic acid	Add 0.30 ml 5N acetic acid	Add 0.30 ml 5N acetic acid
Boil 5 minutes	Boil 5 minutes	Boil 5 minutes	Boil 5 minutes	Boil 5 minutes	Boil 5 minutes
Cool in ice bath 1 minute	Cool in ice bath 1 minute	Cool in ice bath 1 minute	Cool in ice bath 1 minute	Cool in ice bath 1 minute	Cool in ice bath 1 minute
Read N.E. at 385/485	Read N.E. at 385/485	Read N.E. at 385/485	Read N.E. at 385/485	Read N.E. at 385/485	Read N.E. at 385/485

TABLE 13. Average half sample fluorometer readings in % transmission from norepinephrine of tissue samples (each sample run in duplication)

Dog		Left Ventricle		Left Atrium		Right Ventricle		Right Atrium	
		10x	30x	10x	30x	10x	30x	10x	30x
1									
	1	8	18.5	12	28	7	15	14.5	36.5
	2	8	20.0	9.75	23.5	6	14	13	31.75
	3	7.5	19.75	9.25	21.75	6.5	15.5	14.5	35.5
	Av.	7.83	19.42	10.33	24.42	6.5	14.83	14	34.58
2									
	1	10	23	14.5	35.25	11	25	23.5	56
	2	10	24.75	14.75	34	8.5	21.5	18.5	43
	3	8.75	21.5	12.5	28.5	10	22	20.75	50
	Av.	9.58	23.08	13.92	32.58	9.83	22.83	20.92	49.67
3									
	1	7.75	17.75	9	20.25	9.25	20.25	13.25	30.25
	2	7.50	17.5	9	20.25	9.25	21.75	11.00	25.5
	3	8.00	20.25	8	19.75	9.75	22.5	11.25	26.0
	Av.	7.75	18.5	8.67	20.08	9.42	21.5	11.83	27.25
4									
	1	13	30.5	12.5	30	11.25	25.25	20.5	49.5
	2	11	26.25	14.25	34.5	11.5	27.25	14.5	35.5
	3	12	28.0	18.5	44.0	10	23.5	17.0	40.5
	Av.	12	28.25	15.08	36.17	10.92	25.33	17.33	41.83

TABLE 13 (Continued)

Dog		Left Ventricle		Left Atrium		Right Ventricle		Right Atrium	
		10x	30x	10x	30x	10x	30x	10x	30x
5									
	1	7	18	8.5	20	9.25	22.0	11.25	26.25
	2	7	17	10	24	10.5	25.0	10.5	25.5
	3	8	20.	8.75	21.5	8.75	19.5	12.5	29.5
	Av.	7.33	18.33	9.08	21.83	9.50	22.17	11.42	27.08
6									
	1	4.25	11.25	6.25	16	5	11.5	6.75	16
	2	6	14.0	7.00	16.5	6	14.5	6.5	15
	3	5	12.75	6.00	15.75	4.75	12	5.5	14
	Av.	5.08	12.67	6.42	16.08	5.25	12.67	6.58	15
7									
	1	4.5	9	3.5	8	4	9	2.25	6
	2	4.5	9	3.5	8	3.5	8	3	8
	3	4.5	9.5	3.5	8	4.5	9.5	3.5	8
	Av.	4.5	9.17	3.5	8	4.0	8.8	2.92	7.33
8									
	1	8	21	9	23	7.75	18.25	8.5	20
	2	8	18.75	8	19.75	7.5	17.25	8.25	20.75
	3	6.75	16	7	16	7.0	18.25	7.5	18
	Av.	7.58	18.58	8	19.58	7.42	17.92	8.08	19.58
9									
	1	6	14.5	7.75	18.75	6	15	9.5	22.5
	2	5	15	8.25	19.25	8.75	19.25	9.75	21.75
	3	5.75	14.25	8.00	19.5	6.75	18	12.0	26.5
	Av.	5.58	14.58	8.00	19.17	7.17	17.42	10.42	23.58

TABLE 13 (Continued)

Dog		Left Ventricle		Left Atrium		Right Ventricle		Right Atrium	
		10x	30x	10x	30x	10x	30x	10x	30x
10									
	1	6.15	14.25	10	22.5	5.75	14.5	9	21
	2	7.75	17.15	9.75	22.5	6.75	15.5	10	24
	3	6.25	14.5	12	26	7.15	19.0	11	26.5
	Av.	6.72	15.3	10.58	23.67	6.55	16.33	10	23.83
11									
	1	7	16.25	7.75	18	7	16	11	25.25
	2	6.5	16.5	8	17.75	7.75	17.25	12.75	31.5
	3	6.75	15	9	21.5	7.25	16.75	12.5	28.25
	Av.	6.75	15.92	8.25	19.08	7.33	16.67	12.08	28.33
12									
	1	5.15	12.25	8.75	20.25	6	14	6.5	15
	2	5	12.0	6.65	16.25	5.75	13.25	8.0	18.25
	3	5	12.0	10.0	24.25	6.5	15.75	9.5	22.5
	Av.	5.05	12.08	8.47	20.25	6.08	14.33	8.0	18.58
13									
	1	9	21.5	11	24.75	9.25	20.5	15.75	36
	2	11.75	27.5	11.75	27.5	7.75	19.5	15.25	37.75
	3	9.75	22.0	13.5	31	8.75	19.75	17.0	39
	Av.	10.17	23.67	12.08	27.75	8.58	19.92	16	37.58
14									
	1	6.5	15.5	6.5	15	4.5	9.75	7.75	19
	2	7.25	17.25	7.0	18.25	7.75	17.75	10.5	24.25
	3	7.75	18	6.0	13.25	6.0	15.00	6.5	15.5
	Av.	7.17	16.92	6.5	15.5	6.08	14.17	8.25	19.58

TABLE 13 (Continued)

Dog		Left Ventricle		Left Atrium		Right Ventricle		Right Atrium	
		10x	30x	10x	30x	10x	30x	10x	30x
15									
	1	8.5	19.5	10.75	23.75	8.25	19	12	28
	2	10.0	24.5	12.0	29	9.25	21.5	11.25	26
	3	8.75	21.5	10.5	26	9.0	21	13.5	33
	Av.	9.08	21.83	11.08	26.25	8.83	20.5	12.25	29.0
16									
	1	4.5	10.5	4.25	8.75	5.5	11.5	5.25	12.25
	2	5.0	11.0	4.5	11.00	3.5	8.0	5.5	14.00
	3	4.5	10.75	4.0	9.00	5.0	11.5	4.5	10.25
	Av.	4.67	10.75	4.25	9.58	4.67	10.33	5.08	12.17
17									
	1	10.5	25.5	9.5	22.5	8.75	21.5	14.25	33
	2	10	25.0	11.5	28	10.75	25.5	13.75	34
	3	11.5	26.75	13	29.5	9.0	23.25	15.75	37.75
	Av.	10.67	25.75	11.33	26.67	9.5	23.5	14.58	34.92
18									
	1	6.25	18	15.5	37.5	7.75	18	14.25	35.5
	2	6.0	16	19	46	8.0	21.25	11.5	29
	3	7.75	17.75	18	42.5	8.5	22	12.5	30
	Av.	6.67	17.25	17.5	42.0	8.08	20.42	12.75	31.5

TABLE 14. Average half sample fluorometer readings in % transmission from norepinephrine of tissue sample blank

Dog	Left Ventricle		Left Atrium		Right Ventricle		Right Atrium	
	10x	30x	10x	30x	10x	30x	10x	30x
1[a]	5	14	5	12	6	14	5	12
2	4.5	11	4.5	10	6	15	5.5	13
3	4.5	13	4	9	6	13	4	9
4	5	13	4	9.5	5	10.5	5	10.5
5	6	13	4.5	10	6	13.5	5	11
6	4	10.5	4.5	9.5	5	11.5	4	9
7	4.5	9.5	3.5	8.0	4.5	10	3.5	8
8	5	10.0	4	9	5	12	4.5	10.5
9	4	8.5	5	10.5	5	12	5.5	11.5
10[a]	7	16	5	10	6	12	6	15
11	5	10	4.5	10	5	11	4.5	10
12[a]	6	14	6	14	9	20	7	17
13	7	15	5	11.5	6	14	4	10
14[a]	6.5	15	5	10.5	5	13.5	4	9
15	4.5	11	5	11	5	14	5	11
16	5	11	4	9	5	12	5	10
17	7	15	4.5	10	5	13	3	8
18	4	9	4	10	3	8	3	8

[a]Full sample rather than half sample values.

TABLE 15. Average half sample fluorometer readings of L noradrenaline standard assays in % transmission

Concentration of L noradrenaline added in 20 lambda amounts to butanol for assay in ugm/ml	Actual L noradrenaline added to butanol for assay in ugm		Fluorometer readings 3x	10x	30x
2.5	.05	Standard	2	4	10.25
		Blank	1	2	5
		Net	1	2	4.75
5.0	.10	Standard	3.5	6.25	15
		Blank	2.0	2.5	5
		Net	1.5	3.65	10
25	.50	Standard	9	19.75	46
		Blank	1	3	5
		Net	8	16.75	41
50	1.00	Standard	18	40	93
		Blank	2	4	8
		Net	16	36	85
100	2.0	Standard	31	70.5	Over 100
		Blank	1.5	3.5	
		Net	29.5	67	

TABLE 16. Average fluorometer % transmission readings of 0.5 ugm L noradrenaline external standard and external standard blanks taken periodically during the assay periods

Date assured	10x fluorometer setting		30x fluorometer setting	
	External standard	External standard blank	External standard	External standard blank
10-11-68	42.0	3.0	101	8.0
10-11-68	36.0	2.0	87	5.5
10-11-68	45.5	4.0	113	8.0
10-25-68	43.0	5.0	106	15.0
11- 1-68	45.0	---	113	----
11- 8-68	44.0	3.0	110	8.0
11-16-68	38.5	3.0	93	7.0
12-16-68	37.0	5.5	93	14.0
1- 15-69	40.0	4.0	99.0	9.0
1- 18-69	40.0	4.0	99.0	9.0
1- 21-69	39.0	3.0	96.5	7.0
Av.	41.0	3.65	101.0	9.1

TABLE 17. Average half sample fluorometer readings in % transmission from norepinephrine of internal standard samples

Dog	Left Ventricle		Left Atrium		Right Ventricle		Right Atrium	
	10x	30x	10x	30x	10x	30x	10x	30x
1	44	100+	46	100+	30	70	52.5	100+
2	48	100+	50	100+	47	100+	53.5	100+
3	37.5	90.5	39	93	41.5	100+	47.5	100+
4	41.5	100+	53	100+	47	100+	47	100+
5	43	100+	43	100+	49	100+	42	100+
6	42	100+	41.5	99	40	94	42	100+
7	42	100+	37	86	43.5	100+	39	92
8	41	100+	42	100+	42	100+	40	100+
9	39.5	97	45	100+	43	100+	49.5	100+
10	44	100+	48	100+	38	98.5	42	100+
11	42	100+	50	100+	44	100+	51	100+
12	38	90	45	100+	35.5	85	41	99.5
13	42	100+	42	100+	34	80	42	100+
14	42.5	100+	46	100+	52	100+	47	100+
15	39	95	39	100+	42	100+	42	100+
16	40	98	41	102	36	86	39.5	98
17	39	98	48	100+	44	100+	48	100+
18	40	100+	42	100+	47	100+	42	100+

TABLE 18. Percent recovery of norepinephrine from tissue samples

% recovery 30x

	Mean ugm NE found	ugm NE added	ugm calculated recovery	ugm actual recovery	% recovery
Dog 1					
LV	.136596	.5	.636596	.522088	82.0
LA	.20260	.5	.70260	.548862	78.1
RV	.08617	.5	.58617	.321285	54.8
RA	.31444	.5	.81444	.635876	78.1
Dog 2					
LV	.06646	.5	.56646	.582329	102.8
LA	.124218	.5	.624218	.6222489	99.7
RV	.043087	.5	.543087	.548862	101.1
RA	.201686	.5	.701686	.642570	
Dog 3					
LV	.030253	.5	.530253	.441767	83.3
LA	.0609626	.5	.5609626	.46854	83.5
RV	.046754	.5	.546754	.475234	86.9
RA	.244310	.5	.744310	.582329	78.2
Dog 4					
LV	.083885	.5	.583885	.488621	83.7
LA	.1466813	.5	.6466813	.655957	101.4
RV	.0815897	.5	.5815897	.562248	96.7
RA	.17235	.5	.67235	.562248	83.6
Dog 5					
LV	.03116	.5	.53116	.495314	93.3
LA	.065088	.5	.565088	.515394	91.2
RV	.047673	.5	.547673	.575635	105.1
RA	.088465	.5	.588465	.495314	84.2

TABLE 18 (Continued)

% recovery 30x

	Mean ugm NE found	ugm NE added	ugm calculated recovery	ugm actual recovery	% recovery
Dog 6					
LV	.0119196	.5	.5119196	.508701	99.4
LA	.036194	.5	.536194	.508701	94.9
RV	.006419	.5	.506419	.46854	92.5
RA	.0330	.5	.533	.508701	95.4
Dog 7					
LV	.0	.5	.5	.5020	100.4
LA	.0	.5	.5	.4485	89.7
RV	.0	.5	.5	.52209	104.4
RA	.0	.5	.5	.475234	95.0
Dog 8					
LV	.047211	.5	.547211	.481927	88.1
LA	.058212	.5	.558212	.508701	91.1
RV	.032546	.5	.532546	.495314	93.0
RA	.049961	.5	.549961	.475234	86.4
Dog 9					
LV	.033459	.5	.533459	.475234	89.1
LA	.047673	.5	.547673	.535475	97.8
RV	.029796	.5	.529796	.508701	96.0
RA	.066463	.5	.566463	.589022	104.0
Dog 10					
LV	.13531	.5	.613531	.49531	80.7
LA	.20535	.5	.70535	.57563	81.6
RV	.11367	.5	.61367	.42838	69.8
RA	.17968	.5	.67968	.48192	70.9

TABLE 18 (Continued)

% recovery 30x

	Mean ugm NE found	ugm NE added	ugm calculated recovery	ugm actual recovery	% recovery
Dog 11					
LV	.032526	.5	.53255	.49531	93.0
LA	.049963	.5	.549963	.60910	110.8
RV	.031169	.5	.531169	.522209	98.3
RA	.100843	.5	.600843	.62249	103.6
Dog 12					
LV	.05591	.5	.55591	.44176	79.5
LA	.14576	.5	.64576	.52208	80.8
RV	.04767	.5	.54767	.35475	64.8
RA	.11092	.5	.61092	.45515	74.5
Dog 13					
LV	.04767	.5	.54767	.46854	85.6
LA	.08938	.5	.58938	.46854	79.5
RV	.03254	.5	.53254	.37483	70.4
RA	.15172	.5	.65172	.5087	78.1
Dog 14					
LV	.10084	.5	.60084	.4812	80.2
LA	.11276	.5	.61276	.54886	89.6
RV	.08159	.5	.58159	.62918	108.2
RA	.16592	.5	.66592	.57563	86.4
Dog 15					
LV	.59589	.5	.559589	.46185	82.5
LA	.083883	.5	.583883	.45515	78.0
RV	.035753	.5	.535753	.49531	92.5
RA	.09901	.5	.59901	.49531	82.7

TABLE 18 (Continued)

% recovery 30x

	Mean ugm NE found	ugm NE added	ugm calculated recovery	ugm actual recovery	% recovery
Dog 16					
LV	.0	.5	.5	.53547	107.1
LA	.0032	.5	.5032	.54886	109.1
RV	.0	.5	.5	.48192	96.4
RA	.01832	.5	.51832	.52878	102.0
Dog 17					
LV	.05915	.5	.55915	.42838	76.6
LA	.091675	.5	.59168	.58233	98.4
RV	.057755	.5	.557755	.52209	93.6
RA	.148058	.5	.64806	.60241	93.0
Dog 18					
LV	.045379	.5	.545379	.48193	88.4
LA	.176017	.5	.676017	.50870	75.2
RV	.0683	.5	.5683	.58902	103.6
RA	.12926	.6	.62926	.52209	83.0

TABLE 19. Formulae for the determination of norepinephrine in heart tissue samples

1. Total μgmN.E. 100 mg $S = 2\left[\frac{\mu gmStd}{\%TStd} - \%\ T_{Std\ b} \times (\%\ T_S - T_{Sb})\right]$

2. Total μgm N.E./gm = Total μgm/100 mg S X 10

3. I. S.$r_{\mu gm\ N.E./100\ mg}$ $I.S. = \frac{\mu gmStd}{\%T_{Std}} - \%\ T_{Std\ b} \times \%T_{I.S.} - \%T_{Sb}$

4. % N.E. recovery $= \frac{I.S.\ r_{\mu gm\ N.E./100\ mg\ I.S.}}{N.E.\ \mu gm/100\ mg\ S + N.E.\ \mu gm\ added}$

5. Corrected μgm N.E./gm $= \frac{Total\ \mu gm\ N.E./gm}{\%\ N.E.\ recovery}$

Total μgm N.E./100 mg S	= The total micrograms of norepinephrine per 100 milligrams of sample
μgm Std	= Micrograms of standard
$\%\ T_{Std}$	= Percent fluorescent transmission by the standard
$\%\ T_{Std\ b}$	= Percent fluorescent transmission by the standard blank
$\%\ T_S$	= Percent fluorescent transmission by the 100 milligram sample
$\%T_{Sb}$	= Percent fluorescent transmission by the 100 milligram sample blank
2	= Dilution factor from dividing samples and standards into two fractions for better recovery
Total μgm N.E./gm	= Total micrograms norepinephrine per gram of tissue
I.S.$r_{\mu gm\ N.E./100\ mg\ I.S.}$	= Recovery of noadrenaline in micrograms per 100 milligram internal standard sample
$\%\ T_{I.S.}$	= Percent fluorescent transmission by the 100 milligram internal standard sample

TABLE 19 (Continued)

Total μgm N.E./100 mgs	= μgm norepinephrine found per 100 mg of sample
N.E. μgm added	= μgm L noradrenaline added to the internal standard
% N.E. recovery	= Percent noradrenaline recovery
I.S.	= Internal standard

TABLE 20. Mean chemical concentration of norepinephrine in cardiac chambers expressed in micrograms per gram of tissue

Dog	Left ventricle	Left atrium	Right ventricle	Right atrium
01	1.666	2.594	1.572	4.026
02	1.293	2.492	0.852	4.433
03	1.726	1.460	1.076	6.248
04	2.004	2.893	1.687	4.123
05	0.668	1.427	0.907	2.101
06	0.240	0.763	0.139	0.692
07	0.000	0.000	0.000	0.000
08	1.072	1.278	0.700	1.157
09	0.751	0.975	0.621	1.278
10	1.677	2.517	1.629	2.534
11	0.700	0.902	0.634	1.947
12	0.703	1.804	0.736	1.489
13	1.114	2.249	0.924	3.885
14	1.257	1.258	0.754	1.920
15	1.445	2.151	0.773	2.394
16	0.000	0.059	0.000	0.180
17	1.544	1.863	1.234	3.184
18	1.027	4.681	1.319	3.113

TABLE 21. Reported quantities of cardiac norepinephrine in gm/gm

Chamber	Potter, Cooper Willman, and Wolfe (1965)	Angelakos (1965)	Shore, Cohn, Highman, and Maling (1958)	Goodall and Kershner (1956)		Hirsch, Willman, Jellinek, and Cooper (1963)	
	10 dogs	6 dogs	Dog	Dog	Sheep	Dog	Rabbit
RA	2.36	1.06-2.23	auricle 2.4 - atrium minus auricle 2.7			2.64	1.56
RV	0.75	.26- .69	various areas of ventrides 1.3 - 1.4			0.85	2.02
LA	1.42	.94-1.91	auricle 2.8 - atrium minus auricle 2.3			1.46	1.56
LV	0.66	.29- .66	epi and endo cardium removal 1.3 - 1.8			0.73	2.13
IVS	0.88					1.03	1.93
Whole heart				.543	1.22		

TABLE 21 (Continued)

Chamber	Shore and Olin (1958)	Angelakos, Fuxe, and Torchiana (1963)		Cervani, Palazzolo and Terry (1968)			
	5 rabbits	Rabbit	Guinea pig	Guinea pig			
				normal	bilateral stellate ganglion removal	right stellate ganglion removal	left stellate ganglion removal
RA		2.81-4.52	4.63	2.89	.18	.50	2.25
RV		3.03-3.24	2.21	1.43	.12	.56	.44
LA		1.53-1.85	3.11	1.82	.04	1.60	.35
LV		1.75-1.95	1.96	2.08	.07	2.09	.40
IVS				.96	.08	.50	.23
Whole heart	1.4-2.6	2.03-2.16					

TABLE 21 (Continued)

Chamber	Laes, Pekkarinen, Saarikoski, and Suramo (1967)		Robinson and Watts (1965)	Landsberg and Axelrod (1968)	Porter, Totaro, and Leiby (1967)	
	Guinea pig	Rat	Rat	Rat	Rat	Mouse
RA						
RV						
LA						
LV						
IVS						
	Preg-nant	Preg-nant				
Whole heart	2.01 1.69	0.64 .44	1.01	1.02	.477	.510

TABLE 21 (Continued)

Chamber	Jacobowitz, Cooper, and Barner (1967)	Vogel, Jacobowitz, and Chidsey (1969)	
	5 cats	Cattle 1 day to 18 mo.	36 mo.
RA	0.43-2.25	1.46	1.18
RV	1.67-2.04	1.44	0.59
LA	0.45-1.37	1.53	1.34
LV	1.01-2.37	1.11	0.72
IVS			
Whole heart			

TABLE 22. ANOV analysis hypotheses and the statistical significance of mean quantitative chemical level differences

Acetylcholinesterase null hypotheses: Ho

Ho_A - There will be no difference between the mean content of acetylcholinesterase in the four chambers of the heart.

Ho_B - There will be no difference between the types of denervated animals in acetylcholinesterase content in the heart.

ANOV

		Required Significant F Value	Highly significant F Value	Calculated F Value	Relationship of Ho
Ho_A	F 3.51	2.79	4.20	132.80	* *
Ho_B	F 17.51	1.84	2.38	2.42	* *

Norepinephrine null hypothesis

Ho_A - There will be no difference between the mean content of norepinephrine in the four chambers of the heart.

Ho_B - There will be no difference between the types of denervated animals in norepinephrine content in the heart.

ANOV

		Required Significant F Value	Highly significant F Value	Calculated F Value	Relationship of Ho
Ho_A	F 3.51	2.79	4.20	18.44	* *
Ho_B	F 17.51	1.84	2.38	5.54	* *

A = chambers of the heart
B = type of denervations

TABLE 23. Colorimetric assay of acetylcholinesterase

0.2 ml of 20 mg 1 ml tissue homogenate in phosphate buffer pH8 was added to 2.8 ml phosphate buffer pH 8.
100 ml DTNB solution was added and the absorbance measured at 412 mm.
The absorbance was readjusted to zero and 20 lambda of acetylthiocholine iodide solution added.
Changes in absorbance were recorded each minute for six minutes.
The mean change during the last 5 minutes was used for calculating acetylcholinesterase activity.

TABLE 24. Heart tissue acetylcholinesterase recovery - 412 mu, 1.1 I.D. 100 mg samples

Dog	Sample	Left ventricle Average O.D. change Per minute	Left atrium Average O.D. change Per minute	Right ventricle Average O.D. change Per minute	Right atrium Average O.D. change Per minute
1	1	.0335	.153	.036	.1550
	2	.0345	.212	.0525	.1175
	3	.0312	.227	.051	.138
2	1	.0510	.110	.0490	.107
	2	.0360	.150	.0375	.095
	3	.0435	.131	.0325	.100
3	1	.0495	.242	.054	.103
	2	.0390	.216	.066	.086
	3	.0375	.307	.044	.083
4	1	.038	.136	.048	.088
	2	.046	.139	.064	.155
	3	.0435	.113	.061	.087
5	1	.043	.164	.053	.090
	2	.042	.182	.046	.120
	3	.040	.190	.052	.110
6	1	.041	.182	.058	.100
	2	.049	.244	.057	.126
	3	.041	.133	.062	.126
7	1	.031	.108	.0355	.097
	2	.033	.139	.0400	.112
	3	.032	.108	.0355	.108
8	1	.0410	.177	.046	.157
	2	.0365	.203	.0385	.149
	3	.0400	.238	.038	.119
9	1	.040	.124	.040	.113
	2	.039	.176	.041	.068
	3	.044	.165	.038	.102
10	1	.0290	.108	.0214	.0615
	2	.0269	.204	.0282	.0470
	3	.0280	.102	.0260	.0780

TABLE 24 (Continued)

Dog	Sample	Left ventricle Average O.D. change Per minute	Left atrium Average O.D. change Per minute	Right ventricle Average O.D. change Per minute	Right atrium Average O.D. change Per minute
11	1	.032	.157	.067	.140
	2	.031	.113	.062	.130
	3	.035	.103	.049	.124
12	1	.028	.196	.0332	.0985
	2	.0285	.2275	.0237	.1030
	3	.0287	.117	.0255	.0868
13	1	.038	.162	.049	.133
	2	.039	.196	.049	.112
	3	.039	.169	.047	.126
14	1	.021	.175	.030	.0795
	2	.0325	.14	.0285	.069
	3	.023	.138	.0295	.078
	Av.		.151	.0293	
15	1	.041	.141	.069	.0815
	2	.039	.152	.059	.072
	3	.037	.150	.053	.0835
	Av.		.148		.079
16	1	.042	.322	.051	.151
	2	.051	.214	.062	.146
	3	.040	.306	.069	.196
	Av.	.044			
17	1	.044	.156	.060	.162
	2	.036	.202	.064	.125
	3	.048	.228	.056	.141
18	1	.0255	.128	.028	.103
	2	.023	.149	.033	.133
	3	.024	.136	.0265	.159
	Av.	.024	.138	.029	

TABLE 25. Mean chemical contration of acetylcholinesterase in cardiac chambers, expressed in value times 10^{-6} moles hydrolized per minute

Dog	Left ventricle	Left atrium	Right ventricle	Right atrium
01	0.888	5.308	1.251	3.680
02	1.170	3.506	1.070	2.708
03	1.130	6.860	1.471	2.439
04	1.143	3.479	1.551	2.959
05	1.121	4.806	1.354	2.869
06	1.175	5.012	1.587	3.156
07	0.861	3.183	0.995	2.842
08	1.054	5.541	1.098	3.811
09	1.103	4.170	1.067	2.538
10	0.752	3.712	0.678	1.672
11	0.879	3.345	1.596	3.533
12	0.764	4.846	0.739	2.585
13	1.040	4.725	1.300	3.327
14	0.687	4.062	0.789	2.031
15	1.049	3.972	1.623	2.125
16	1.193	7.550	1.632	4.420
17	1.148	5.254	1.614	3.838
18	0.650	3.703	0.785	3.542

TABLE 26. Calculations for nitrothiobenzoate extinction coefficient using the Spectronic 20 colorimeter

Basic equation: $\epsilon l = \frac{A}{co/D}$

Glutathione standard in u m/ml	Co 20 lambda of standard (Glutathione) in um added	D total dilution in ml	Co/D (X10-5)	A absorbance per minute	$\epsilon l_{1.1}$	ϵ
1	.02	3.12	.641	.102	15,913	14,466
3	.06	3.12	1.932	.321	16,692	15,175
5	.10	3.12	3.205	.513	16,006	14,551
7	.14	3.12	4.487	.718	16,002	14,547
10	.20	3.12	6.410	1.020	15,913	14,466

TABLE 27. Calculations utilized in determining quantiatively the acetylcholinesterase content of tissue samples

A. The extinction coefficient for nitrothiobenzoate

$$\left[Co = \frac{A}{\epsilon 1} D\right] = \left[\epsilon 1 = \frac{A}{co/D}\right]$$

Co = original concentration of standard

A = absorbance/minute at 412 mu

ϵ = extinction coefficient

D = dilution factor

1 = light path length

B. Tissue acetylcholinesterase expressed in moles substrate hydrolized per minute per gram of tissue

$$R = \frac{\Delta A}{1.45.(104)} \times (1/400/3120)^{Co} = 5.38\ (10^{-4})\frac{\Delta A}{Co}$$

R = rate, in moles substrate hydolized per minute per gram of tissue

Δ A = change in absorbance per minute

Co = original concentration of tissue (mg/ml)

1/400/3120 = dilution factors and unit change to liters

400 = .4 ml homogenate added
3120 = 3.12 ml total volume

$1.45\ (10^4)$ = extinction coefficient for nitrothiobenzoate

TABLE 28. Histochemical staining of myocardial neural elements for acetylcholinesterase

Animal	Staining incubation time in hours	Left ventricle	Left atrium	Right ventricle	Right atrium
1	2	-	+	-	+
2	3	+	++	+	++
3	3	-	-	-	-
	8	-	++	-	-
	19	+	+++	+	+++
4	3	+	+	-	0
	8	++	+++	-	0
	19	+++	+++	+	+
5	3	-	++	-	-
	8	+	++	-	+++
	19	+++	+++	+	+++
6	5	++	++	+	++
7	3	+	++	+	+++
8	3	-	+	-	+
	6½	++	N	+	0
9	5	-	-	+	++
	8	+	++	-	N
	19	+	++	+	N
10	3	+	+	-	+
	8	0	N	-	N
	19	0	N	+	N
11	3	+	+	++	-
	8	+++	+++	+++	+++
	19	+++	+++	+++	+++
12	2	+	+	+	+
13	3	+	+	-	-
	8	+++	+++	-	++
	19	N	+++	++	+++

TABLE 28 (Continued)

Animal	Staining incubation time in hours	Left ventricle	Left atrium	Right ventricle	Right atrium
14	2	+	+	+	+
	3	++	++	+	++
15	8	+++	+++	+	+++
	19	+++	+++	++	+++
	3	-	+	-	++
16	6	++	++	++	++
	3	-	+	+	+++
17	8	++	++	++	+++
	19	+++	+++	+++	+++
18	3	+	++	+	+

- = no AChE staining observed
+ = very little AChE staining observed
++ = moderate AChE staining observed
+++ = considerable AChE staining observed
N = not incubated
0 = no nerve trunks observed in section

TABLE 29. Mean myocardial fiber diameter in microns

Dog	Left atrium	Left ventricle	Right atrium	Right ventricle
01	11.5	18.3	13.8	17.3
02	13.3	14.3	11.9	14.0
03	17.3	17.3	16.1	15.0
04	9.1	10.8	9.4	11.9
05	17.8	17.8	15.9	21.8
06	13.8	14.3	14.0	17.1
07	15.0	15.7	14.0	13.8
08	12.4	14.7	11.9	15.9
09	12.2	15.7	12.2	14.3
10	16.4	13.8	13.8	17.6
11	15.4	16.4	12.2	15.2
12	12.9	14.5	12.6	12.6
13	13.8	16.6	12.6	18.5
14	15.0	16.1	14.0	16.1
15	12.6	14.5	14.0	14.3
16	11.2	16.6	11.5	17.3
17	11.2	15.7	13.8	17.3
18	13.3	14.5	10.8	14.5

TABLE 30. Mean nuclei per five $5mm^2$ fields in cardiac chambers

Dog	Left atrium	Left ventricle	Right atrium	Right ventricle
01	56	23	39	23
02	40	31	66	40
03	40	38	53	33
04	92	164	120	134
05	46	20	31	28
06	40	24	32	22
07	38	28	42	28
08	44	24	36	32
09	29	19	34	41
10	26	33	33	31
11	53	35	61	45
12	32	24	46	31
13	39	24	46	28
14	28	28	26	31
15	53	45	61	49
16	44	50	47	22
17	38	30	37	38
18	46	44	59	43

TABLE 31. ANOV analysis hypotheses and the statistical significance of mean myocardial fiber diameter and length difference

Mean myocardial fiber diameter null hypotheses: Ho

Ho_A - There will be no difference between the mean myocardial fiber diameters in the heart chambers.

Ho_B - There will be no difference between the mean myocardial fiber diameters of the different animals.

ANOV

			Required Significant F Value	Highly significant F Value	Calculated F Value	Relationship of Ho
Ho_A	F	3.51	3.79	4.20	17.03	* *
Ho_B	F	17.51	1.84	2.38	5.43	* *

Nuclei per five $5mm^2$ fields reflecting fiber length null hypotheses: Ho

Ho_A - There will be no difference between the mean nuclei per five $5mm^2$ fields in the heart chambers.

Ho_B - There will be no difference between the mean nuclei per five $5mm^2$ fields of the different animals.

ANOV

			Required Significant F Value	Highly significant F Value	Calculated F Value	Relationship of Ho
Ho_A	F	3.51	2.79	4.20	3.39	*
Ho_B	F	17.51	1.84	2.38	16.82	* *

A = chambers of the heart
B = type denervations

TABLE 32. Estimated content of myocardial lipids and glycogen using Alcian Blue - P.A.S., and Oil Red O stains

Dog	Glycogen				Lipid material			
	Left ventricle	Left atrium	Right ventricle	Right atrium	Left ventricle	Left atrium	Right ventricle	Right atrium
01	+ to ++	+ to ++	+ to ++	+	+	+	+	+
02	++	++	++	++	0	+	0	+
03	+ to ++	++	+ to ++	++	++	+	++	++
04	+ to ++	++ to +++	++	++	0	+	0	0
05	++	++ to +++	++ to +++	++ to +++	+ to ++	+	++	+ to ++
06	++	++	++	++	+	++	+ to ++	++
07	++ to +++	++ to +++	++ to +++	++ to +++	0	+	+	+
08	++ to +++	++ to +++	++	++	0	+	+	++
09	++	++ to +++	+ to ++	++	+	+	+	++
10	++ to +++	++ to +++	++ to +++	++ to +++	++	++	++	++
11	++	++	++	++ to +++	+	0	+	+
12	++	++	++	++ to +++	0	+	0	0
13	++	++	++	++	0	+	++	+
14	++	++	++	++	+	+	+	+
15	++	++	+ to ++	++	0	+	0	+
16	++ to +++	++	++ to +++	++ to +++	0	0	0	++
17	+ to ++	++	+ to ++	++	0	0	0	+
18	+ to ++	++ to +++	++	+ to ++	+	0	0	+

0 = none
+ = very little
++ = moderate
+++ = considerable

TABLE 33. Coronary artery dimensions expressed in ratio of media and of adventitia to intima

Dog	Left coronary artery		Right coronary artery	
	Media	Adventita	Media	Adventita
01	38	24	21	13
02	33	17	24	17
03	29	21	32	21
04	09	10	06	10
05	33	14	13	12
06	25	15	18	18
07	28	14	39	33
08	37	13	32	16
09	55	20	36	20
10	28	13	50	40
11	28	15	24	15
12	44	41	22	11
13	33	14	50	29
14	44	20	18	09
15	15	09	19	12
16	34	21	25	19
17	34	11	25	20
18	43	33	47	20

TABLE 34. Specific histologic staining of coronary arteries for medial collagen, acid mucopolysaccharides, neutral mucopolysaccharides, and lipid material

Dog	Mean estimated collagen using Verhueff's Van Geisen stain		*Mean estimated acid mucopoly-saccharides using Alcine Blue- P.A.S. stain		*Mean estimated lipid material using Oil Red O stain	
	Left coronary	Right coronary	Left coronary	Right coronary	Left coronary	Right coronary
01	10	10	+	+	+	0
02	20	25	++	++	+	+
03	70	50	+++	++	++	+
04	25	15	+	0	0	0
05	35	40	++	++	0	0
06	30	40	+ to ++	++ to +++	0	+
07	50	50	++	+++	0	+
08	25	35	+++	+++	+	+
09	65	50	+++	++	+	+
10	60	65	+++	+++	+	+
11	35	35	++	++	+	+
12	40	40	++	++	+	+
13	40	40	+++	++	0	0
14	40	30	++	+	0 to +	0 to +
15	45	40	+++	+++	0	0
16	30	40	+++	+	0	+
17	35	35	++	+	+	0
18	25	20	++	+	+	0

* 0 = none
\+ = very little
++ = moderate
+++ considerable

TABLE 35. ANOV analysis hypotheses and the statistical significance of mean coronary artery measurement differences

Ratio of coronary artery medial and adventitial thickness to coronary artery intima null hypotheses: Ho

Ho_A - There will be no difference between the mean ratio of coronary artery media and adventitia to coronary artery intima between the right coronary artery and the left coronary artery.

Ho_B - There will be no difference between the mean medial and mean adventitial ratios of thicknesses of the two coronary arteries.

Ho_C - There will be no difference between the mean ratio of coronary artery media and adventitia to coronary intima of the different animals.

Ho_{AC} - There will be no interaction between the mean ratio of coronary artery media and adventitia to coronary intima of the right and left coronary arteries and the ratio of the media and adventitia to the intima in different animals.

ANOV

			Required Significant F Value	Highly significant F Value	Calculated F Value	Relationship of Ho
Ho_A	F	10.34	2.12	2.89	2.71	*
Ho_B	F	2.34	3.28	5.29	42.39	* *
Ho_C	F	17.34	1.94	2.57	5.99	* *
Ho_{AC}	F	17.34	1.94	2.57	4.66	* *

Coronary artery % collogen in media null hypotheses: Ho

Ho_A - There will be no difference between the mean % collogen in the right and left coronary arteries.

Ho_D - There will be no difference between the mean % collogen in the coronary arteries of the different animals.

ANOV

			Required Significant F Value	Highly significant F Value	Calculated F Value	Relationship of Ho
Ho_A	F	1.17	4.45	8.40	0.31	N.S.
Ho_D	F	17.17	2.28	3.25	10.83	* *

A = components of the vessels
B = different arteries
C & D = type denervation
AC = interaction between periods

TABLE 36. Simple correlation coefficient matrix between functional parameters just prior to exsanguination, myocardial chemical levels, myocardial, myocardial structural observations, and age

	1	2	3	4	5	6	7	8	9	10
1	1.0000									
2	0.8948	1.0000								
3	-0.0336	0.0615	1.0000							
4	0.6584	0.7748	0.4360	1.0000						
5	0.2599	0.1239	-0.0871	0.1178	1.0000					
6	-0.0595	-0.1168	-0.3741	-0.4161	0.2125	1.0000				
7	-0.2016	-0.1997	0.2886	-0.0032	0.0378	-0.4417	1.0000			
8	-0.1617	0.0349	0.3048	-0.0784	-0.1565	0.3347	-0.3336	1.0000		
9	-0.3159	-0.2805	0.2913	0.0166	0.0721	-0.0586	0.6988	-0.0735	1.0000	
10	0.3723	0.2731	-0.3345	-0.0287	0.1720	0.2188	-0.6752	0.0297	-0.8157	1.0000
11	-0.3232	-0.3696	0.1691	-0.1592	-0.2516	0.1282	0.0017	0.3186	0.1202	-0.0604
12	-0.2387	-0.3819	-0.1819	-0.3406	-0.2272	0.2149	-0.1383	0.1222	-0.1332	0.1412
13	-0.2546	-0.1204	0.6140	0.2240	-0.1334	-0.0875	0.2200	0.4282	0.4426	-0.3389
14	-0.0192	-0.0652	0.2885	0.0907	0.3010	0.0639	-0.0111	0.1011	0.3182	0.0095
15	-0.1075	-0.1279	0.1971	-0.1090	0.3165	0.3869	-0.0533	0.4387	0.3980	-0.1128
16	0.3057	0.4612	-0.2154	0.2019	0.1069	0.0178	0.0221	-0.0334	0.0851	-0.0281
17	0.5117	0.6624	-0.2961	0.3247	-0.1038	0.0807	-0.1678	-0.0930	-0.1156	0.0631
18	-0.2699	-0.2210	-0.0009	-0.0630	-0.2109	-0.1595	0.0101	-0.0427	0.1631	-0.3458
19	0.2214	0.3299	0.2429	0.3025	-0.1955	-0.3024	0.0315	0.2271	-0.0151	-0.2588
20	0.1848	0.1823	-0.0409	0.2223	-0.1919	-0.1685	-0.4233	-0.2022	-0.4948	0.2026
21	-0.0153	0.1178	0.1143	0.1986	-0.3808	-0.0733	-0.2420	0.3423	-0.1572	-0.1415
22	-0.0676	-0.0731	-0.1912	-0.1009	0.0889	-0.1025	-0.0402	-0.1151	-0.1522	0.1991
23	-0.2878	-0.3211	-0.2286	-0.4027	-0.0374	-0.0927	0.0723	-0.1108	-0.1990	0.1037
24	-0.1092	-0.1084	-0.1163	-0.1373	0.0597	-0.1659	0.0866	-0.0575	-0.1145	0.2385
25	-0.1530	-0.2173	-0.3088	-0.3372	0.0351	-0.0682	0.2130	-0.1314	0.0119	0.0542
26	0.1810	0.2360	-0.1358	0.3102	0.1925	-0.3350	0.0477	0.0097	-0.2083	0.2446
27	0.1602	0.1688	-0.0555	0.0623	0.0807	-0.1329	-0.0544	0.2009	-0.1827	0.2604
28	0.3086	0.3827	-0.0981	0.3287	0.1851	-0.2412	-0.1412	0.1375	-0.3125	0.3851
29	-0.0258	-0.0314	-0.3177	-0.0328	0.0359	-0.2939	0.0728	-0.0276	-0.1442	0.1141
30	-0.1815	-0.3944	-0.5841	-0.5152	-0.0916	0.1577	-0.2865	-0.3585	-0.4997	0.4235
31	-0.1717	-0.2372	-0.3826	-0.1847	0.3288	0.3297	-0.4441	-0.0591	-0.2308	0.1313
32	-0.4167	-0.4404	-0.3637	-0.4619	-0.3555	-0.0328	-0.0606	-0.2141	-0.3616	0.0775
33	-0.3693	-0.4712	-0.2279	-0.4994	0.1329	0.2812	-0.0532	-0.2563	-0.0347	-0.0556
34	0.2279	0.3788	0.0598	0.3638	0.3695	-0.0713	-0.0632	0.3101	0.0255	0.0678

TABLE 36 (Continued)

	11	12	13	14	15	16	17	18	19	20
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11	1.0000									
12	0.8594	1.0000								
13	0.5984	0.1047	1.0000							
14	0.1870	0.0381	0.3041	1.0000						
15	0.2695	0.0759	0.4052	0.6044	1.0000					
16	-0.2454	-0.1159	-0.2957	0.3640	0.1593	1.0000				
17	-0.4462	-0.2608	-0.4592	0.0065	-0.1761	0.8207	1.0000			
18	0.0788	-0.0160	0.1783	0.4292	0.0763	0.1907	0.0845	1.0000		
19	0.0377	-0.1103	0.2461	0.1126	0.0287	0.2480	0.2963	0.5996	1.0000	
20	0.0297	0.0713	-0.0540	0.1007	-0.3029	0.1034	0.2003	0.5990	0.4759	1.0000
21	0.2542	0.1563	0.2496	0.0158	-0.1536	-0.0043	0.1599	0.6298	0.7648	0.5140
22	-0.2815	-0.2141	-0.2122	-0.6205	-0.2136	-0.4143	-0.2612	-0.6888	-0.5410	-0.4444
23	-0.1658	-0.0791	-0.1984	-0.7045	-0.3226	-0.5126	-0.4107	-0.5252	-0.4643	-0.3141
24	-0.1231	-0.1082	-0.0699	-0.5333	-0.2393	-0.4202	-0.3318	-0.7194	-0.5358	-0.5178
25	-0.1231	-0.0217	-0.2054	-0.6081	-0.1126	-0.3366	-0.2864	-0.6355	-0.4950	-0.5897
26	0.1980	0.2321	0.0213	-0.3064	-0.2436	-0.0400	-0.0724	-0.3999	-0.0945	-0.1873
27	0.3597	0.4218	0.0387	-0.0662	0.2186	0.2212	-0.0253	-0.4673	-0.1511	-0.1872
28	0.1638	0.1526	0.0795	-0.1934	-0.0686	0.0933	0.0421	-0.4217	-0.0379	-0.0620
29	0.2442	0.3695	-0.1040	-0.2407	-0.0600	0.0483	-0.0272	-0.0784	0.1179	-0.1094
30	-0.0993	0.2366	-0.5641	-0.3211	-0.4951	-0.3917	-0.1700	0.0621	-0.2942	0.1940
31	-0.1659	-0.0162	-0.2973	0.1370	0.1456	-0.0573	-0.0795	0.4457	-0.0266	0.3380
32	-0.1355	0.0334	-0.3160	-0.5649	-0.5279	-0.4785	-0.2814	0.1256	-0.2463	0.1732
33	-0.2132	0.0419	-0.3490	0.0613	0.0117	-0.0162	-0.1116	0.1649	-0.4280	0.1724
34	-0.1216	-0.2317	0.1266	0.3449	0.2773	0.3545	0.2533	0.2607	0.5166	-0.0322

TABLE 36 (Continued)

	21	22	23	24	25	26	27	28	29	30
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
21	1.0000									
22	-0.5670	1.0000								
23	-0.4987	0.8840	1.0000							
24	-0.5716	0.9401	0.8664	1.0000						
25	-0.6135	0.8921	0.8848	0.8934	1.0000					
26	-0.0524	0.4397	0.2934	0.5421	0.4290	1.0000				
27	-0.3622	0.3207	0.2589	0.4016	0.4251	0.6233	1.0000			
28	-0.1621	0.4473	0.2983	0.5441	0.4117	0.8823	0.7916	1.0000		
29	0.0647	0.3005	0.2506	0.3570	0.4511	0.7946	0.5803	0.7002	1.0000	
30	0.0498	0.1778	0.3204	0.1437	0.1877	-0.0026	-0.3174	-0.1820	0.1613	1.0000
31	0.2434	-0.1600	-0.1614	-0.3653	-0.2689	-0.1652	-0.3613	-0.2698	0.0151	0.4602
32	0.0616	0.2950	0.5264	0.2296	0.3080	-0.211	-0.3012	-0.1840	0.1121	0.7576
33	-0.4088	0.0517	0.2524	-0.0566	0.0754	-0.4034	-0.1473	-0.3055	-0.2299	0.3435
34	0.4679	-0.3191	-0.5141	-0.2951	-0.3687	0.2612	-0.0762	0.1703	0.2580	-0.2534

TABLE 36 (Continued)

	31	32	33	34
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				
29				
30				
31	1.0000			
32	0.3729	1.0000		
33	0.4297	0.4520	1.0000	
34	0.2563	-0.3939	-0.5491	1.0000

TABLE 36 (Continued)

1 = P amplitude
2 = Q amplitude
3 = R amplitude
4 = S amplitude
5 = T amplitude
6 = P interval
7 = P-R interval
8 = QRS interval
9 = QT interval
10 = Heart rate
11 = Systolic blood pressure
12 = Diastolic blood pressure
13 = Pulse pressure
14 = Left coronary artery medial thickness
15 = Left coronary artery adventitial thickness
16 = Right coronary artery medial thickness
17 = Right coronary artery adventitial thickness
18 = Fiber diameter left ventricle
19 = Fiber diameter left atrium
20 = Fiber diameter right ventricle
21 = Fiber diameter right atrium
22 = Nuclei per five $5mm^2$ areas of left ventricle
23 = Nuclei per five $5mm^2$ areas of left atrium
24 = Nuclei per five $5mm^2$ areas of right ventricle
25 = Nuclei per five $5mm^2$ areas of right atrium
26 = Norepinephrine level of left ventricle
27 = Norepinephrine level of left atrium
28 = Norepinephrine level of right ventricle
29 = Norepinephrine level of right atrium
30 = Acetylcholinesterase level of left ventricle
31 = Acetylcholinesterase level of left atrium
32 = Acetylcholinesterase level of right ventricle
33 = Acetylcholinesterase level of right atrium
34 = Age of animal

TABLE 37. Simple correlation coefficient matrix components which are significantly or highly significantly related

Direction of relationship	.05 (.482 r) Related components (In addition to those recognized as significant and listed at the .01 level as being highly significant)		Direction of relationship	.01 (.606 r) Related components	
+	P amplitude	Right coronary artery adventitia	+	P amplitude	Q amplitude
			+	P amplitude	S amplitude
N	R amplitude	Acetylcholinesterase left ventricle			
N	S amplitude	Acetylcholinesterase left ventricle	+	Q amplitude	S amplitude
N	QT interval	Acetylcholinesterase left ventricle	+	Q amplitude	Right coronary artery adventitia
N	Pulse pressure	Acetylcholinesterase left ventricle	+	R amplitude	Pulse pressure
N	Left coronary artery adventitia	Acetylcholinesterase left ventricle	+	P-R interval	QT interval
N	S amplitude	Acetylcholinesterase right atrium	N	P-R interval	Heart rate
N	Q-T interval	Myocardial fiber diameter right ventricle	+	Systolic blood pressure	Diastolic blood pressure

TABLE 37 (Continued)

Direction of relationship	.05 (.482 r) Related components (In addition to those recognized as significant and listed at the .01 level as being highly significant)		Direction of relationship	.01 (.606 r) Related components	
+	Systolic blood pressure	Pulse pressure	N	Q-T interval	Heart rate
N	Nuclei/5 fields right ventricle	Left coronary artery media	+	Right coronary artery adventitia	Right coronary artery media
N	Nuclei/5 fields right ventricle	Myocardial fiber diameter left atrium	N	Nuclei/5 fields left ventricle	Left coronary artery media
N	Nuclei/5 fields right ventricle	Myocardial fiber diameter right ventricle	N	Nuclei/5 fields left atrium	Left coronary artery media
N	Nuclei/5 fields right ventricle	Myocardial fiber diameter right atrium	N	Nuclei/5 fields left ventricle	Myocardial fiber diameter left ventricle
+	Nuclei/5 fields right ventricle	Norepinephrine left ventricle	N	Nuclei/5 fields right ventricle	Myocardial fiber diameter left ventricle

TABLE 37 (Continued)

Direction of relationship	.05 (.482 r) Related components (In addition to those recognized as significant and listed at the .01 level as being highly significant)		Direction of relationship	.01 (.606 r) Related components	
+	Nuclei/5 fields right ventricle	Norepinephrine right ventricle	N	Nuclei/5 fields right atrium	Myocardial fiber diameter left ventricle
N	Nuclei/5 fields left atrium	Myocardial fiber diameter left ventricle	N	Nuclei/5 fields right atrium	Myocardial fiber diameter right atrium
N	Nuclei/5 fields left atrium	Right coronary artery media	+	Nuclei/5 fields left ventricle	Nuclei/5 fields left atrium
N	Nuclei/5 fields right atrium	Myocardial fiber diameter left atrium	+	Nuclei/5 fields left ventricle	Nuclei/5 fields right ventricle
N	Nuclei/5 fields right atrium	Myocardial fiber diameter right ventricle	+	Nuclei/5 fields left ventricle	Nuclei/5 fields right atrium
N	Nuclei/5 fields left ventricle	Myocardial fiber diameter right atrium	+	Nuclei/5 fields left atrium	Nuclei/5 fields right ventricle
N	Nuclei/5 fields left atrium	Myocardial fiber diameter right atrium	+	Nuclei/5 fields left atrium	Nuclei/5 fields right atrium

TABLE 37 (Continued)

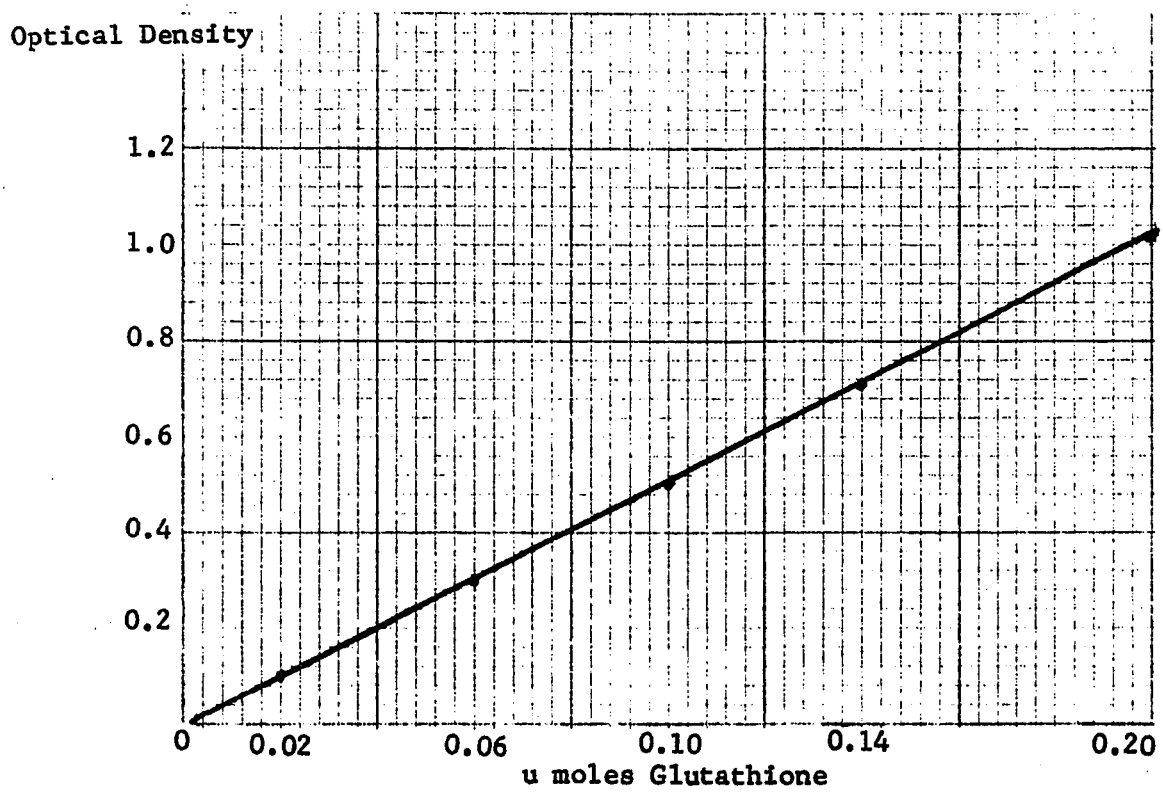
Direction of relationship	.05 (.482 r) Related components (In addition to those recognized as significant and listed at the .01 level as being highly significant		Direction of relationship	.01 (.606 r) Related components	
+	Myocardial fiber diameter left atrium	Myocardial fiber diameter left ventricle	+	Nuclei/5 fields right ventricle	Nuclei/5 fields right atrium
+	Myocardial fiber diameter right ventricle	Myocardial fiber diameter left ventricle	+	Myocardial fiber diameter right atrium	Myocardial fiber diameter left ventricle
+	Myocardial fiber diameter right ventricle	Myocardial fiber diameter right atrium	+	Myocardial fiber left atrium	Myocardial fiber diameter right atrium
N	Acetylcholin-esterase right ventricle	Right coronary artery media	+	Norepinephrine left ventricle	Norepinephrine left atrium
N	Acetylcholin-esterase right ventricle	Left coronary artery adventitia	+	Norepinephrine left ventricle	Norepinephrine right ventricle
+	Acetylcholin-esterase right ventricle	Nuclei/5 fields left atrium	+	Norepinephrine left atrium	Norepinephrine right ventricle

TABLE 37 (Continued)

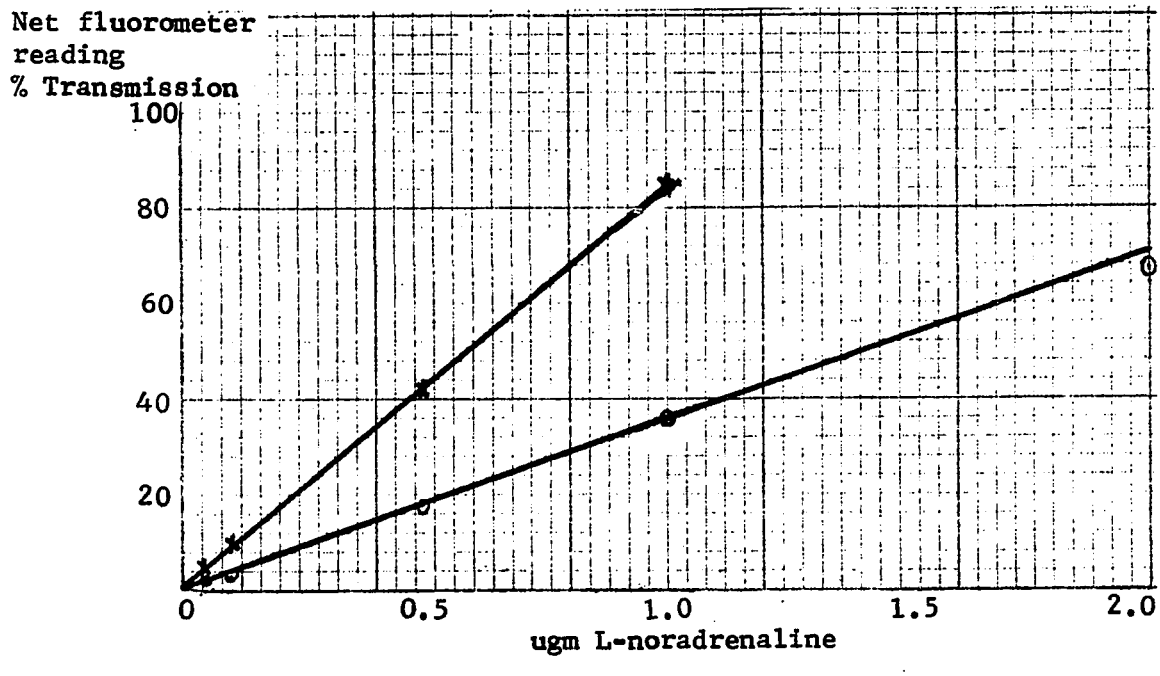
Direction of relationship	.05 (.482 r) Related components (In addition to those recognized as significant and listed at the .01 level as being highly significant)		Direction of relationship	.01 (.606 r) Related components	
N	Acetylcholinesterase right atrium	Age	+	Norepinephrine left ventricle	Norepinephrine right atrium
N	Nuclei/5 fields left atrium	Age	+	Norepinephrine right ventricle	Norepinephrine right atrium
+	Myocardial fiber diameter left atrium	Age	+	Acetylcholinesterase right ventricle	Acetylcholinesterase left ventricle
+	Norepinephrine left atrium	Norepinephrine right atrium	N	Nuclei/5 fields right atrium	Left coronary artery media
+	Left coronary artery adventitia	Left coronary artery media			
+	Nuclei/5 fields left ventricle	Myocardial fiber diameter left atrium			

+ = positive relationship
N = negative

APPENDIX C. GRAPHS



GRAPH 1 Glutathione standard curve



x = 30X range selector
o = 10X range selector

GRAPH 2 L-noradrenaline standard curve

APPENDIX D. ELECTROCARDIOGRAMS

FIGURE 2. Dog 2: electrocardiogram and blood pressure recordings just prior to tissue harvest. Note early depression of atrial and ventricular force in the early pre-exsanguination period.

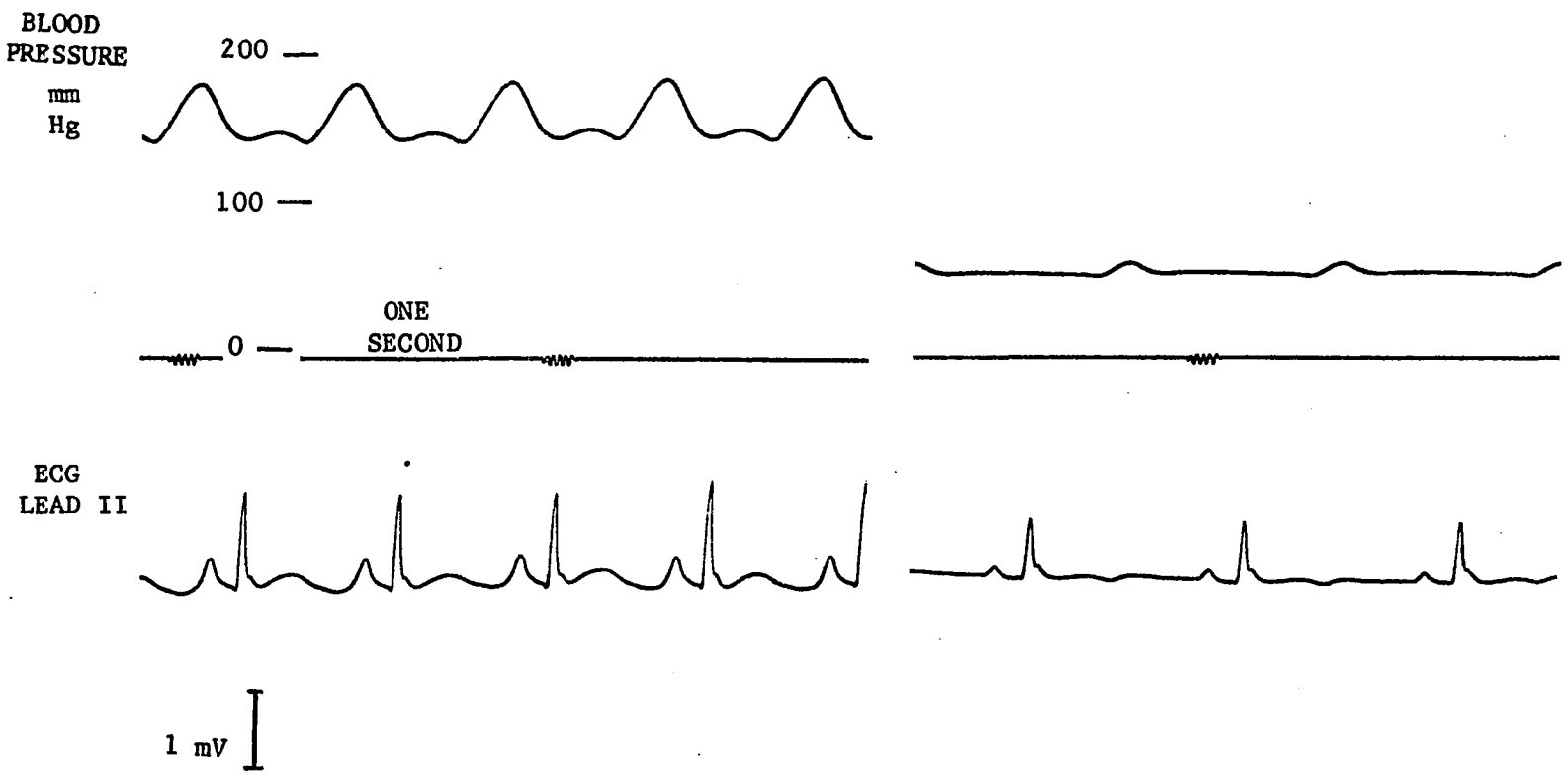

BLOOD
PRESSURE
mm
Hg
200
100
0
ONE
SECOND
ECG
LEAD II
1 mV
PRE-EXSANGUINATION
EXSANGUINATION

FIGURE 3. Dog 3: electrocardiogram and blood pressure recordings just prior to tissue harvest. Ventricular ectopic beats have taken over regulation of heart beats in this dog following loss of nearly 700 ml of blood.

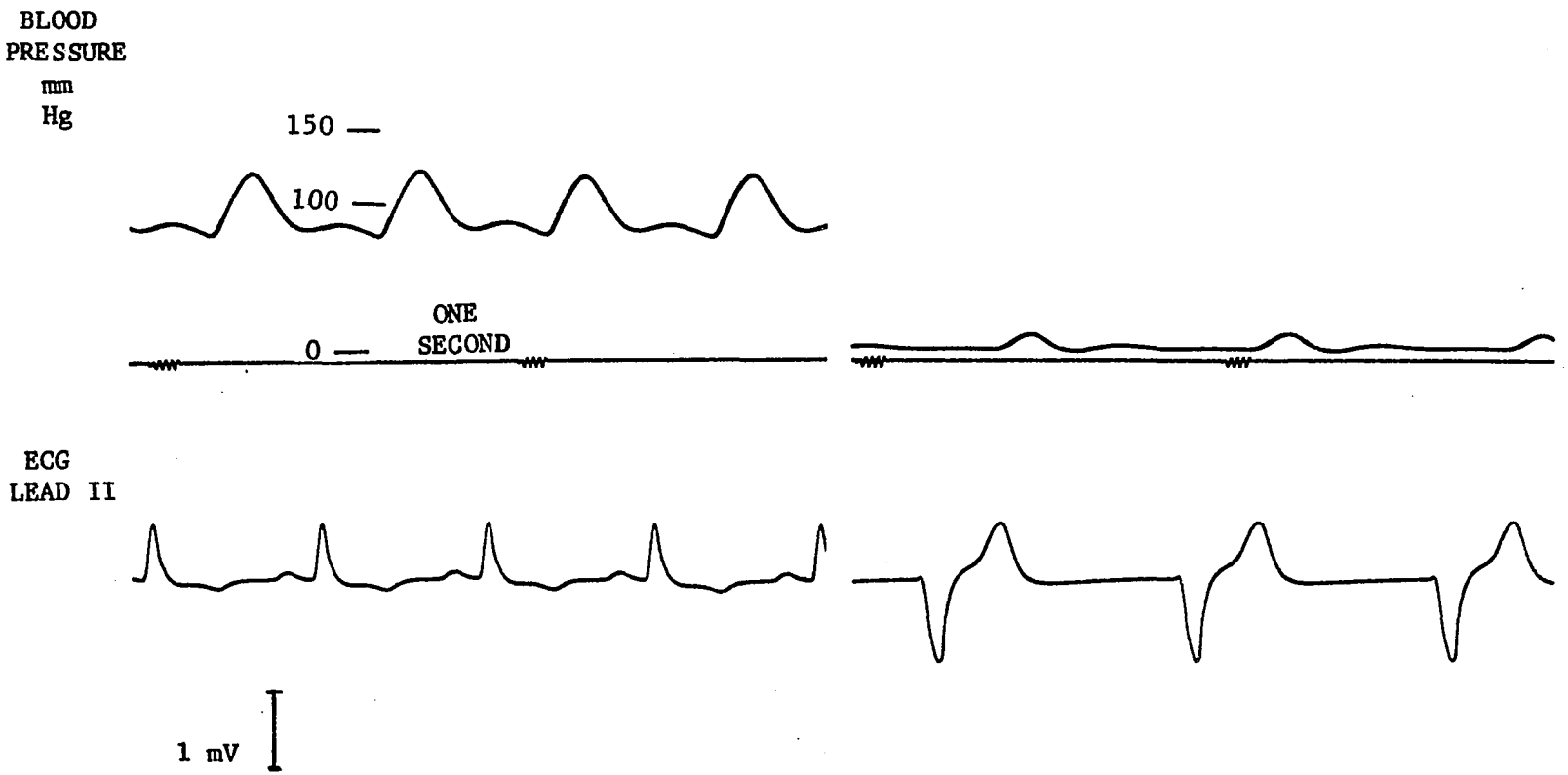

BLOOD
PRESSURE
mm
Hg
150
100
0
ONE
SECOND
ECG
LEAD II
1 mV
PRE-EXSANGUINATION
EXSANGUINATION

FIGURE 4. Dog 4: electrocardiogram and blood pressure recordings just prior to tissue harvest. Note increased T wave amplitude and Q-T interval associated with ischemia in the exsanguination period.

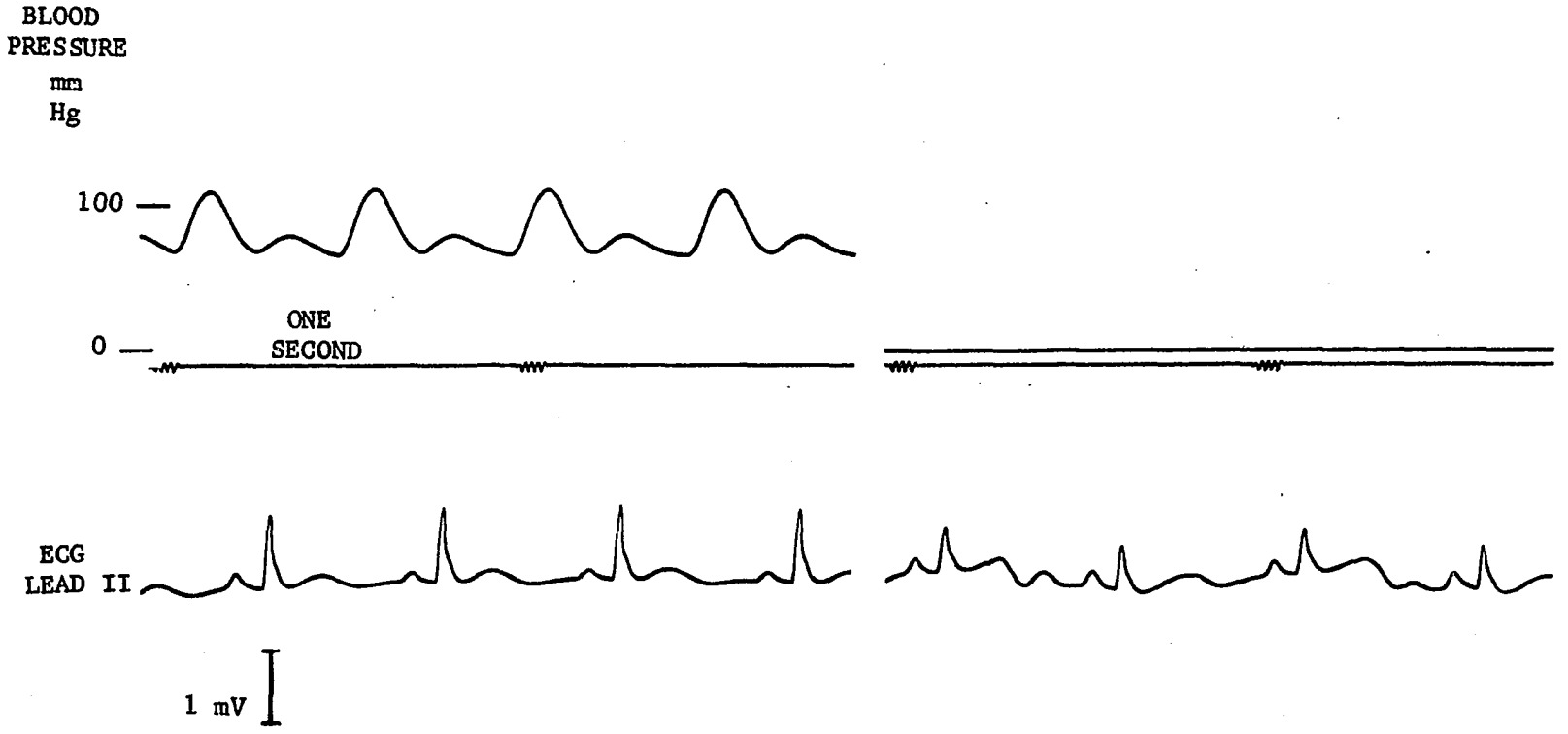
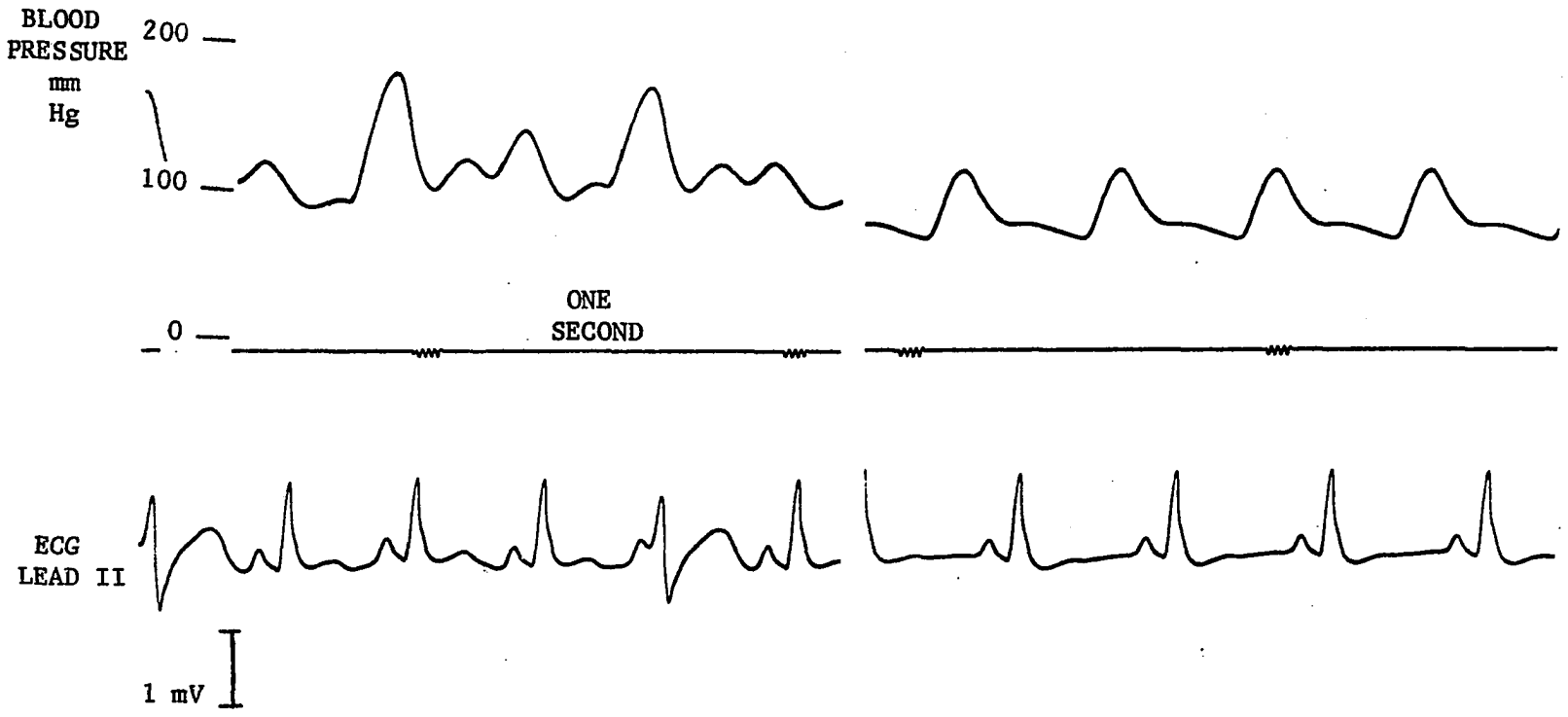

BLOOD
PRESSURE
mm
Hg
100
0
ONE
SECOND
ECG
LEAD II
1 mV
PRE-EXSANGUINATION
EXSANGUINATION

FIGURE 5. Dog 5: electrocardiogram and blood pressure recordings just prior to tissue harvest. Note the heart block associated with methoxyflurane anesthesia initially.



PRE-EXSANGUINATION

PRE-EXSANGUINATION

FIGURE 6. Dog 6: presurgical, surgical, acute postsurgical and chronic postsurgical electrocardiogram and blood pressure recording. Note blood pressure changes, and QRS and QT intervals particularly.

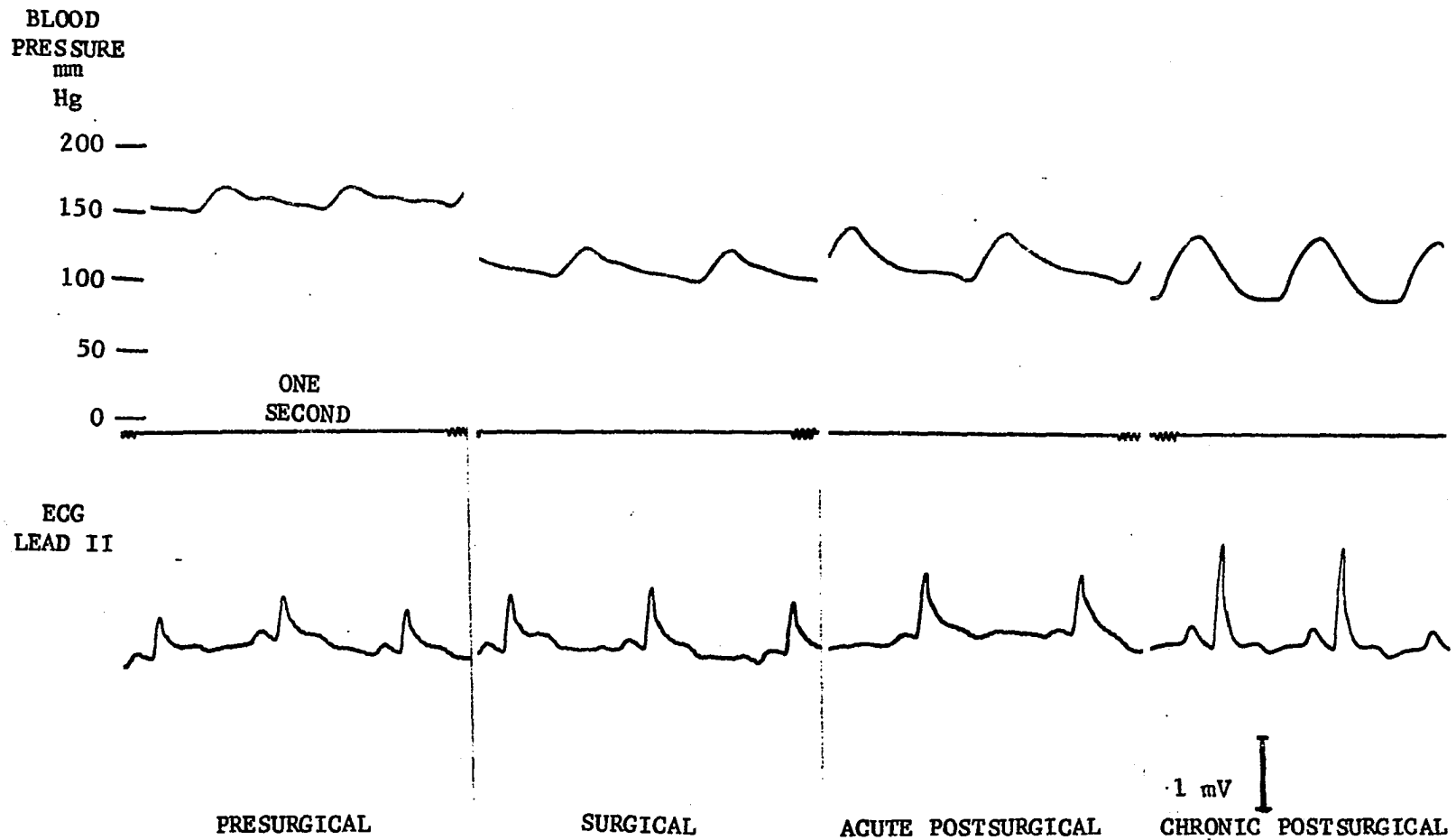

BLOOD
PRESSURE
mm
Hg
200
150
100
50
0
ONE
SECOND
ECG
LEAD II
1 mV
PRESURGICAL
SURGICAL
ACUTE POSTSURGICAL
CHRONIC POSTSURGICAL

FIGURE 7. Dog 7: Presurgical, surgical and acute postsurgical electrocardiogram and blood pressure recordings. Amplitudes and intervals of atrial and ventricular depolarization are slightly lowered and lengthened in progressive periods.

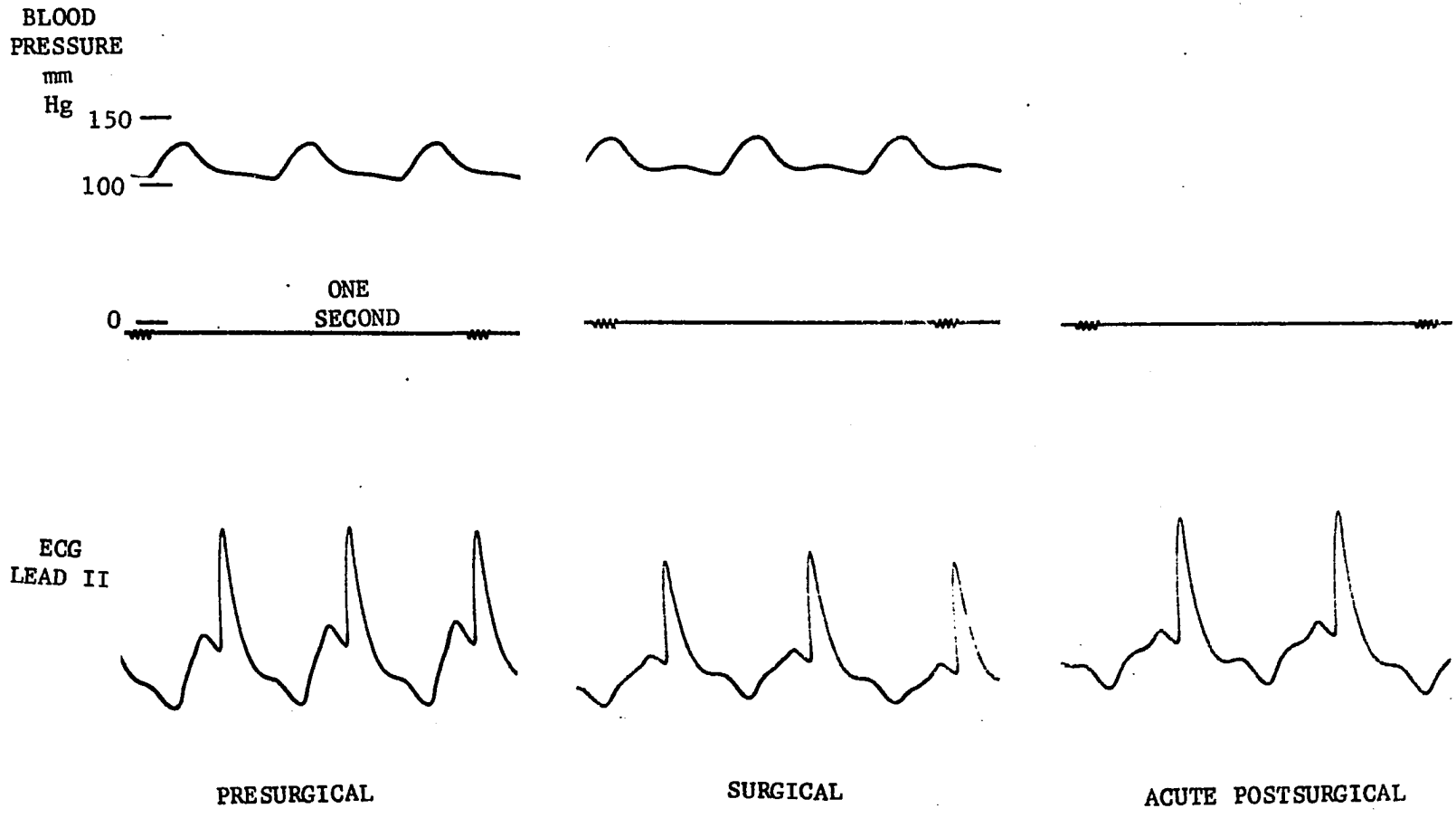

BLOOD
PRESSURE
mm
Hg
150
100
0
ONE
SECOND
ECG
LEAD II
PRESURGICAL
SURGICAL
ACUTE POSTSURGICAL

FIGURE 8. Dog 7: chronic postsurgical, pre-exsanguination, and exsanguination electrocardiogram and blood pressure recordings. Note particularly the lower heart rate and blood pressure than in previous periods. Also in the exsanguination period the T wave change is compatible with ischemia.

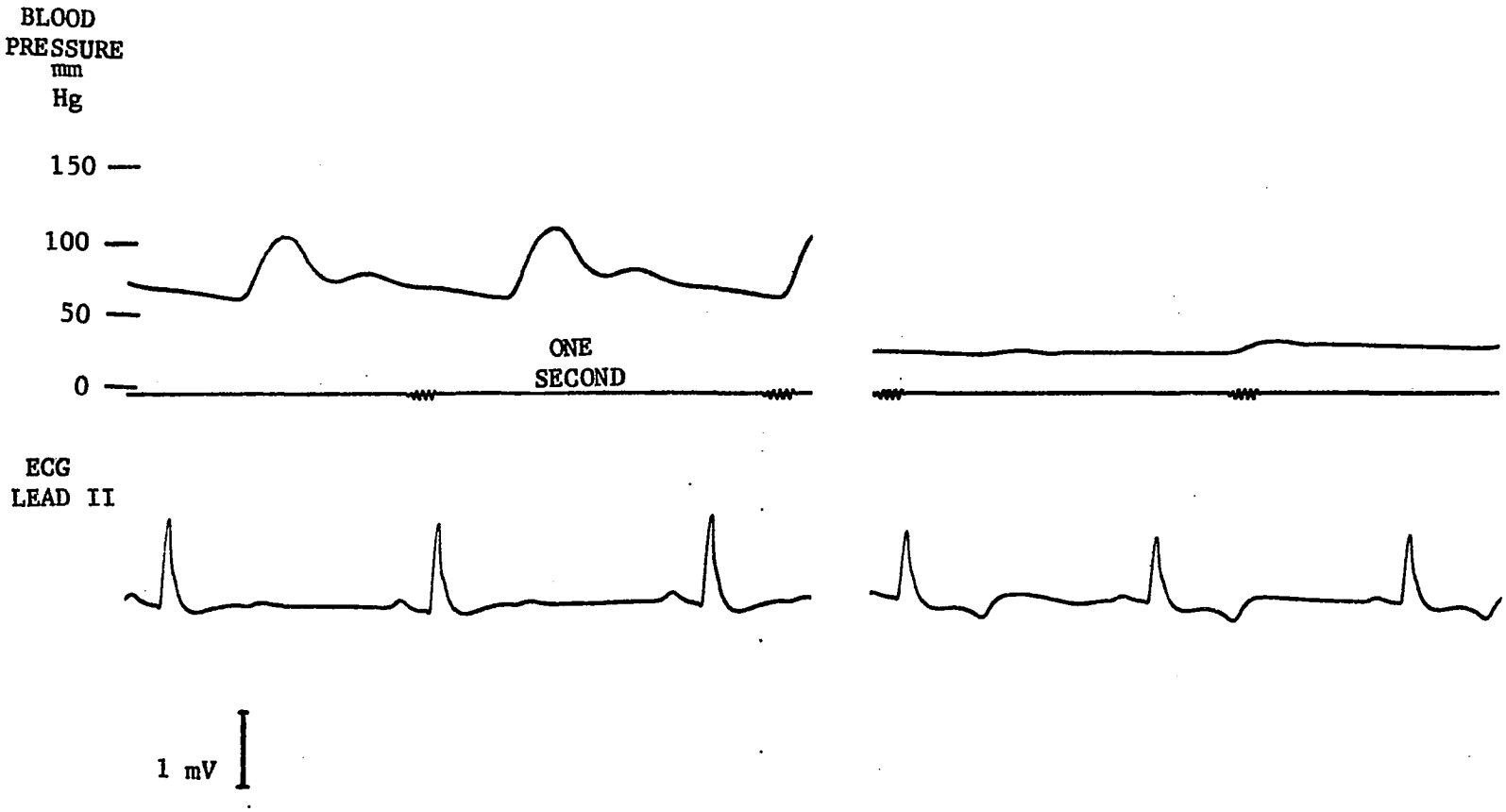

BLOOD
PRESSURE
mm
Hg
150
100
50
0
ONE
SECOND
ECG
LEAD II
1 mV
PRE-EXSANGUINATION
EXSANGUINATION

FIGURE 9. Dog 8: Presurgical, surgical, and acute postsurgical electrocardiogram recordings. Heart rate changes were particularly evident. The duration of ventricular sytole lengthened, associated to a great extent with widened interval and increased amplitude of the T wave.

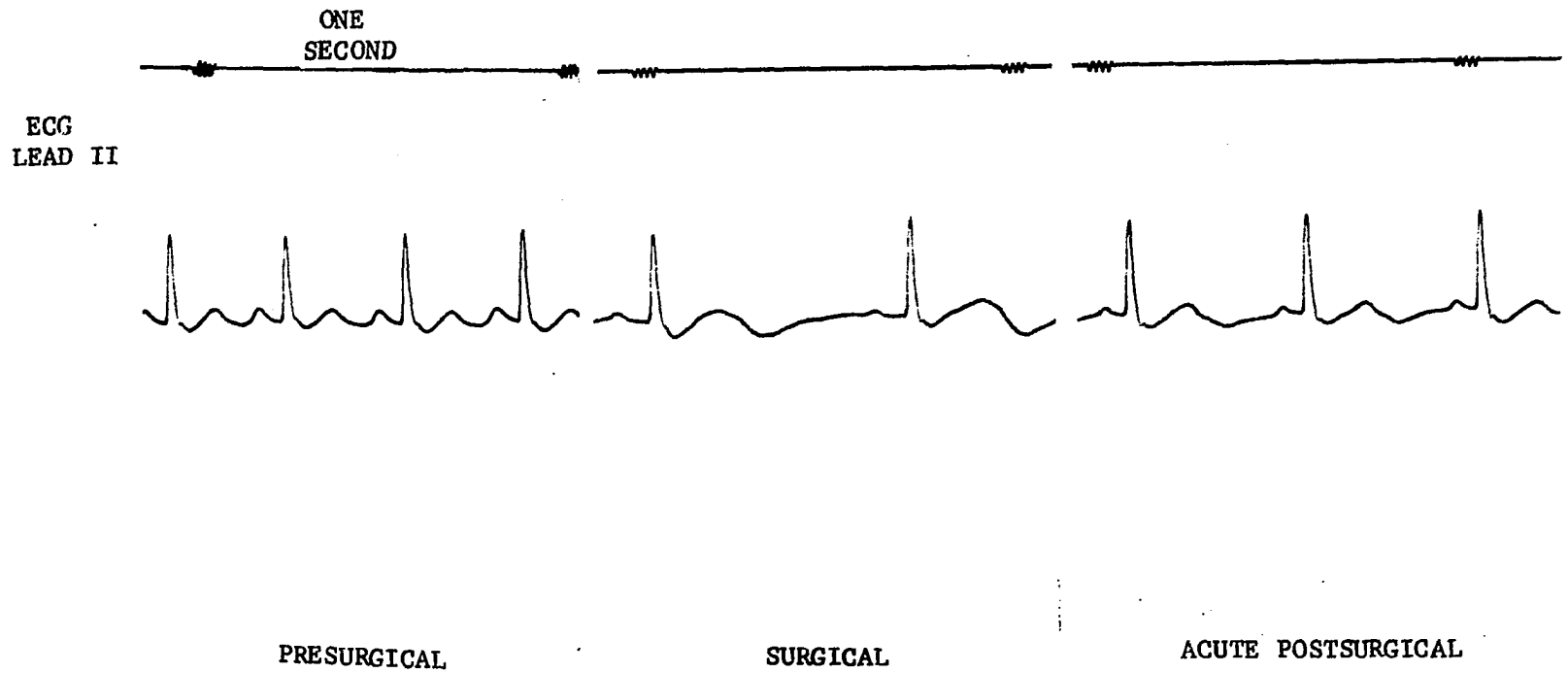

ONE
SECOND
ECG
LEAD II
PRESURGICAL
SURGICAL
ACUTE POSTSURGICAL

FIGURE 10. Dog 8: chronic postsurgical, pre-exsanguination, and exsanguination electrocardiogram and blood pressure recordings. Observe the persistance of the elevated and lengthened T wave which together with the P wave became almost greater in amplitude than the R wave during exsanguination.

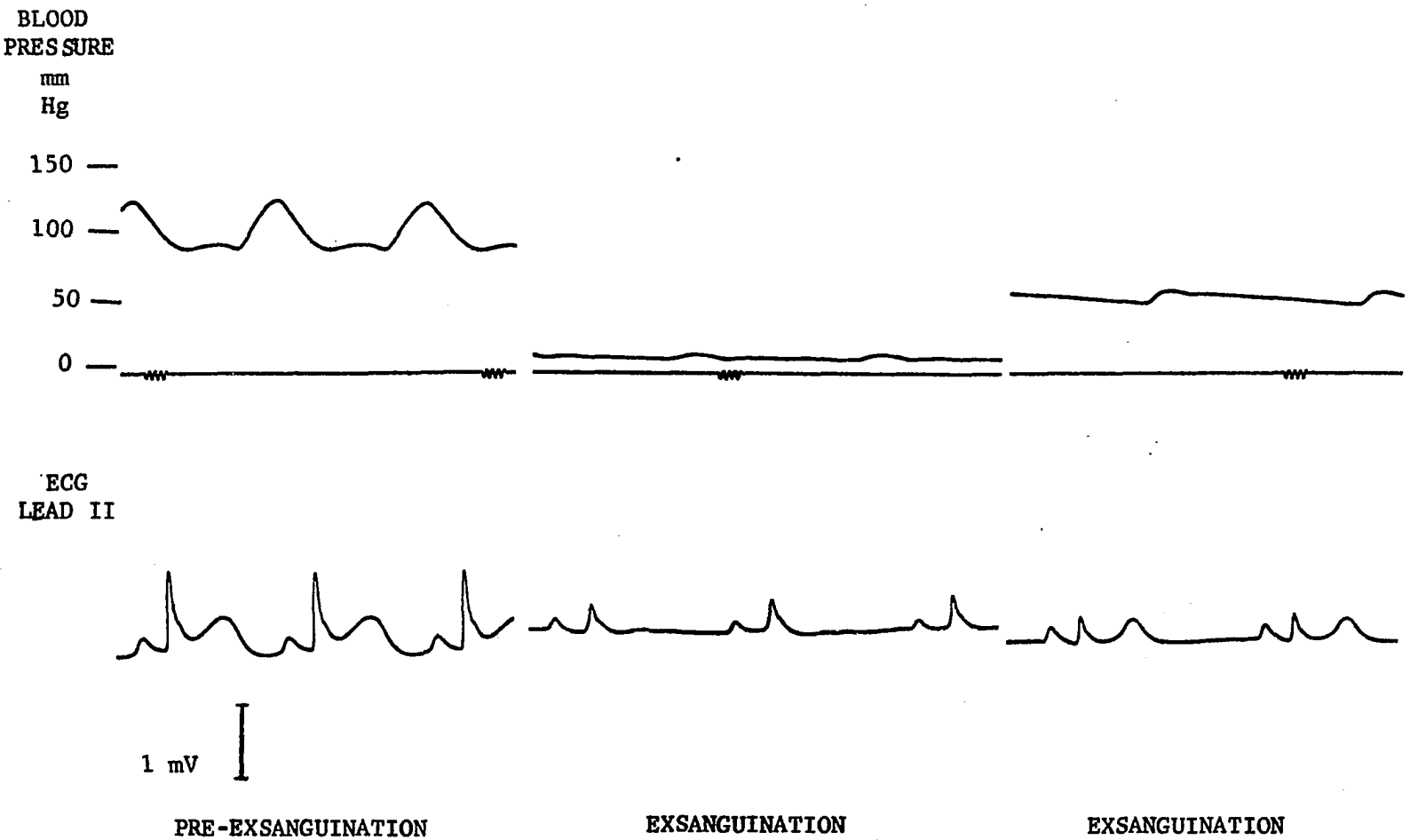

BLOOD
PRESSURE
mm
Hg
150
100
50
0
ECG
LEAD II
1 mV
PRE-EXSANGUINATION
EXSANGUINATION
EXSANGUINATION

FIGURE 11. Dog 9: presurgical, surgical, and acute postsurgical electrocardiogram and blood pressure recordings. Note the increased heart rate in the latter period and the increased blood pressure during dissection of the left sympathetic trunk during surgery. The atrial and ventricular contractions became progressively stronger.

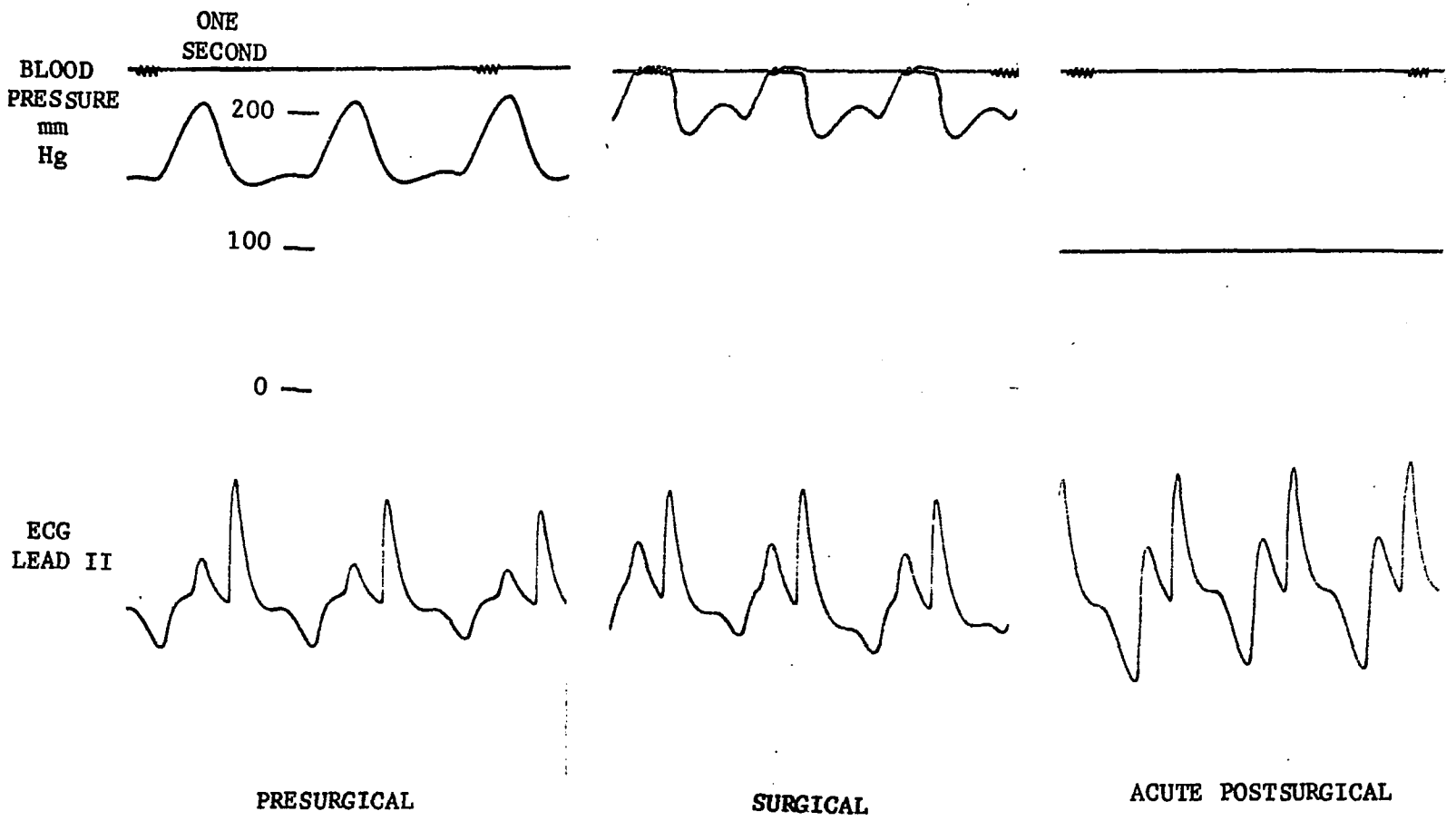
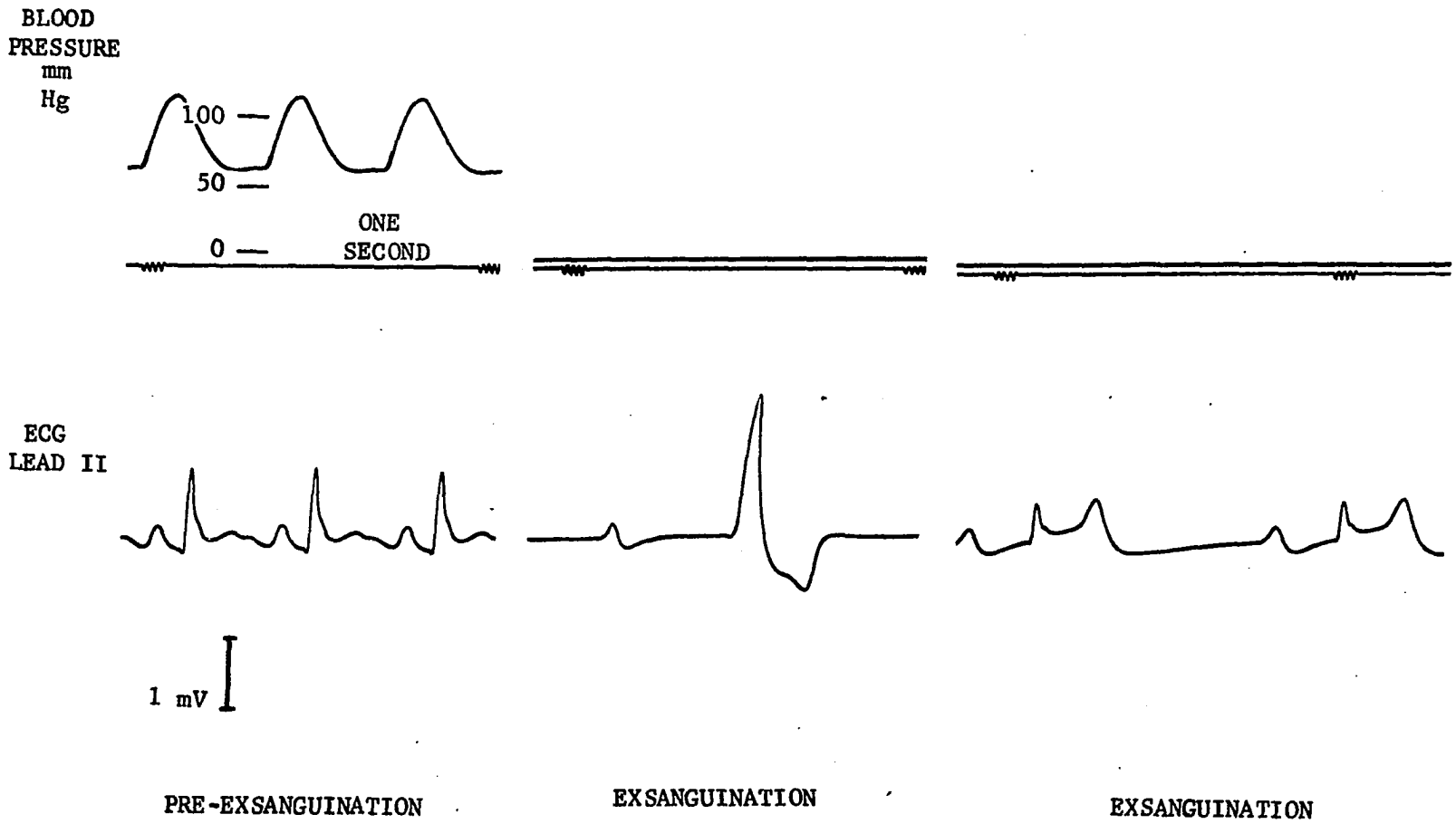

ONE
SECOND
BLOOD
PRESSURE
mm
Hg
200
100
0
ECG
LEAD II
PRESURGICAL
SURGICAL
ACUTE POSTSURGICAL

FIGURE 12. Dog 9: chronic postsurgical, pre-exsanguination, and exsanguination electrocardiogram and blood pressure recordings. Heart rate under the influence the right sympathetic nerves is still elevated over the presurgical rate. Blood pressure is much lower. Electrocardiogram recording values are normal. Large ectopic ventricular beats predominate in the first exsanguination recording, and the T wave becomes higher than the R wave in a later recording.


BLOOD
PRESSURE
mm
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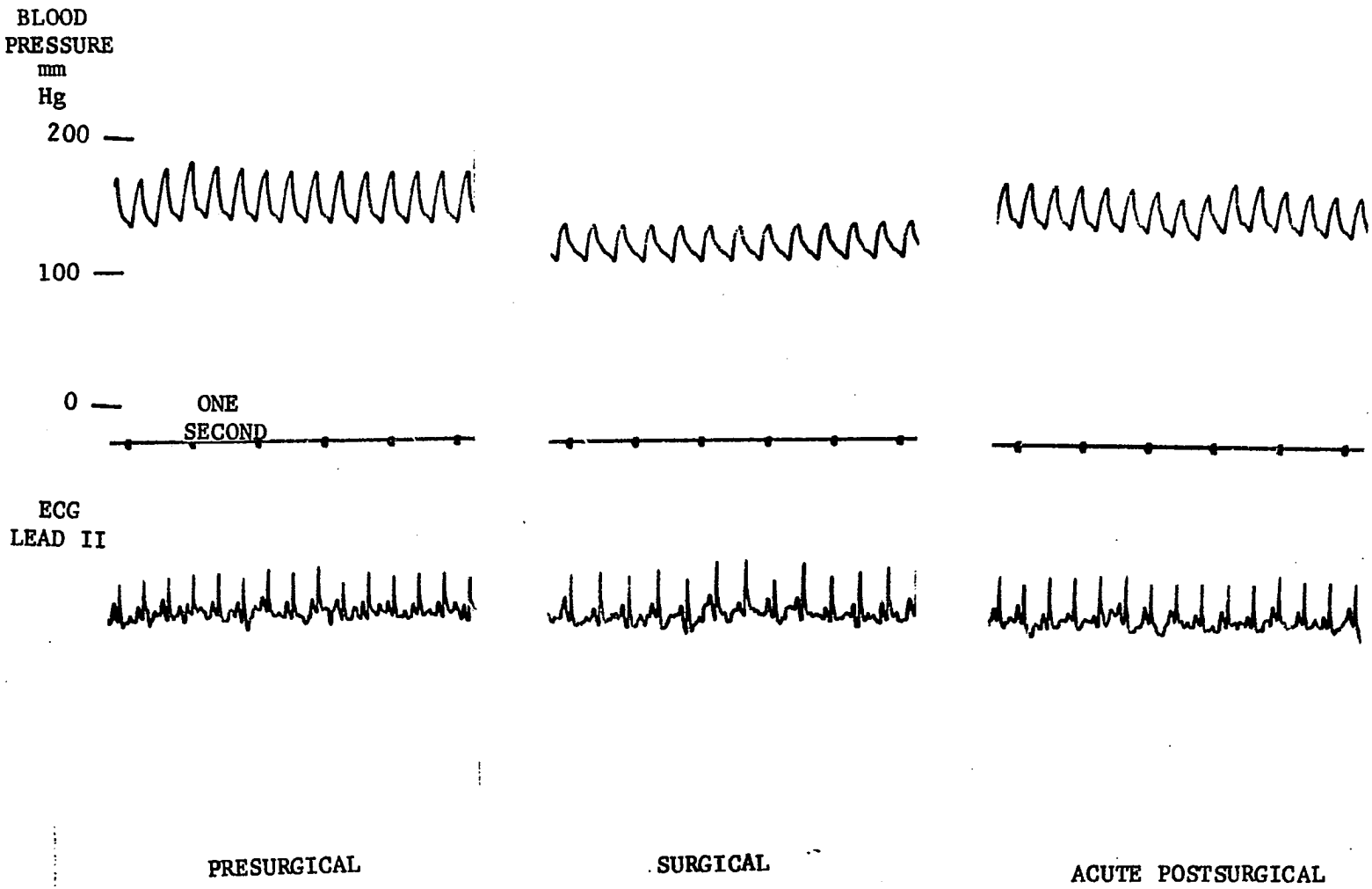
50
0
ONE
SECOND
ECG
LEAD II
1 mV
PRE-EXSANGUINATION
EXSANGUINATION
EXSANGUINATION

FIGURE 13. Dog 10: presurgical, surgical, and acute postsurgical electrocardiogram and blood pressure recordings. Paper speed was faster, but elevation of the P wave can be observed.


BLOOD
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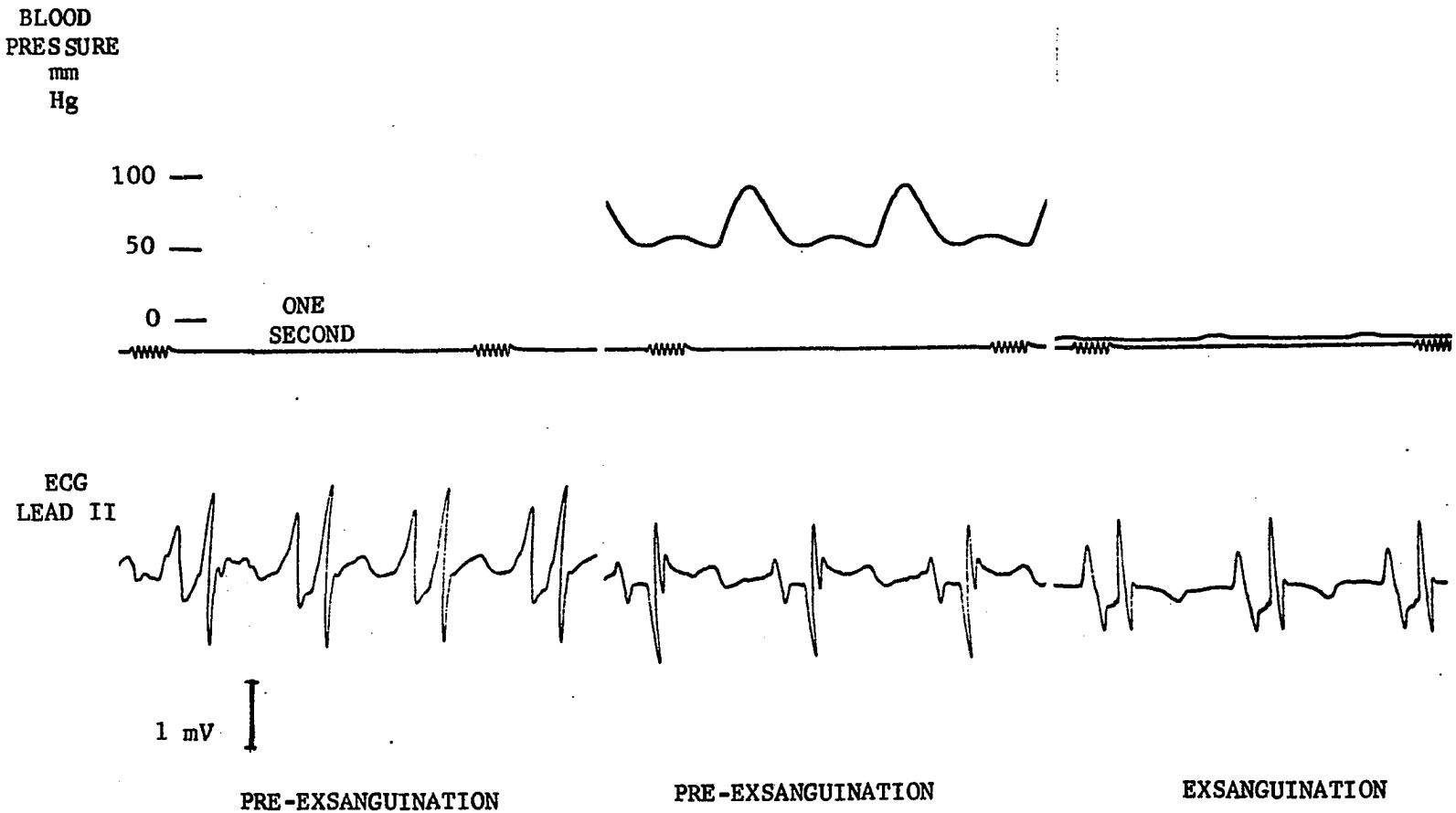
ONE
SECOND
ECG
LEAD II
PRESURGICAL
SURGICAL
ACUTE POSTSURGICAL

FIGURE 14. Dog 10: chronic postsurgical, pre-exsanguination, and exsanguination electrocardiogram and blood pressure recordings. Heart rate is essentially unchanged, but blood pressure is lower than in previous periods. Abnormally strong atrial contractions are present together with altered ventricular conduction. In the second pre-exsanguination recording note the knotched biphasic P wave. During exsanguination some tendency towards returning to more normal ventricular conduction occurred. The T wave was inverted.


BLOOD
PRESSURE
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Hg
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50
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ONE
SECOND
ECG
LEAD II
1 mV
PRE-EXSANGUINATION
PRE-EXSANGUINATION
EXSANGUINATION

FIGURE 15. Dog 11: presurgical, surgical, and acute postsurgical electrocardiogram and blood pressure recordings. Observe particularly the increased amplitude and interval of the T wave which represents repolarization of the ventricles.

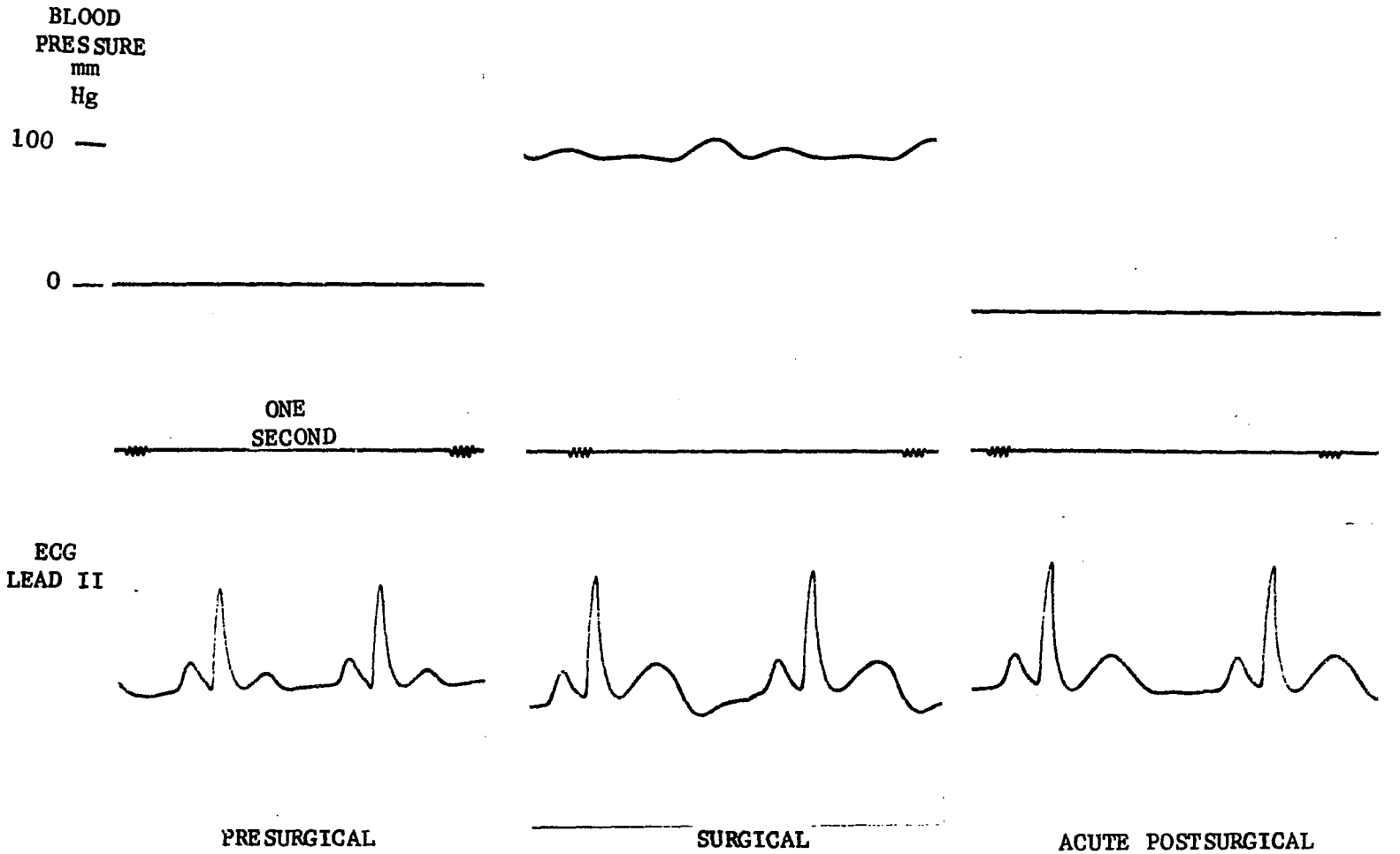
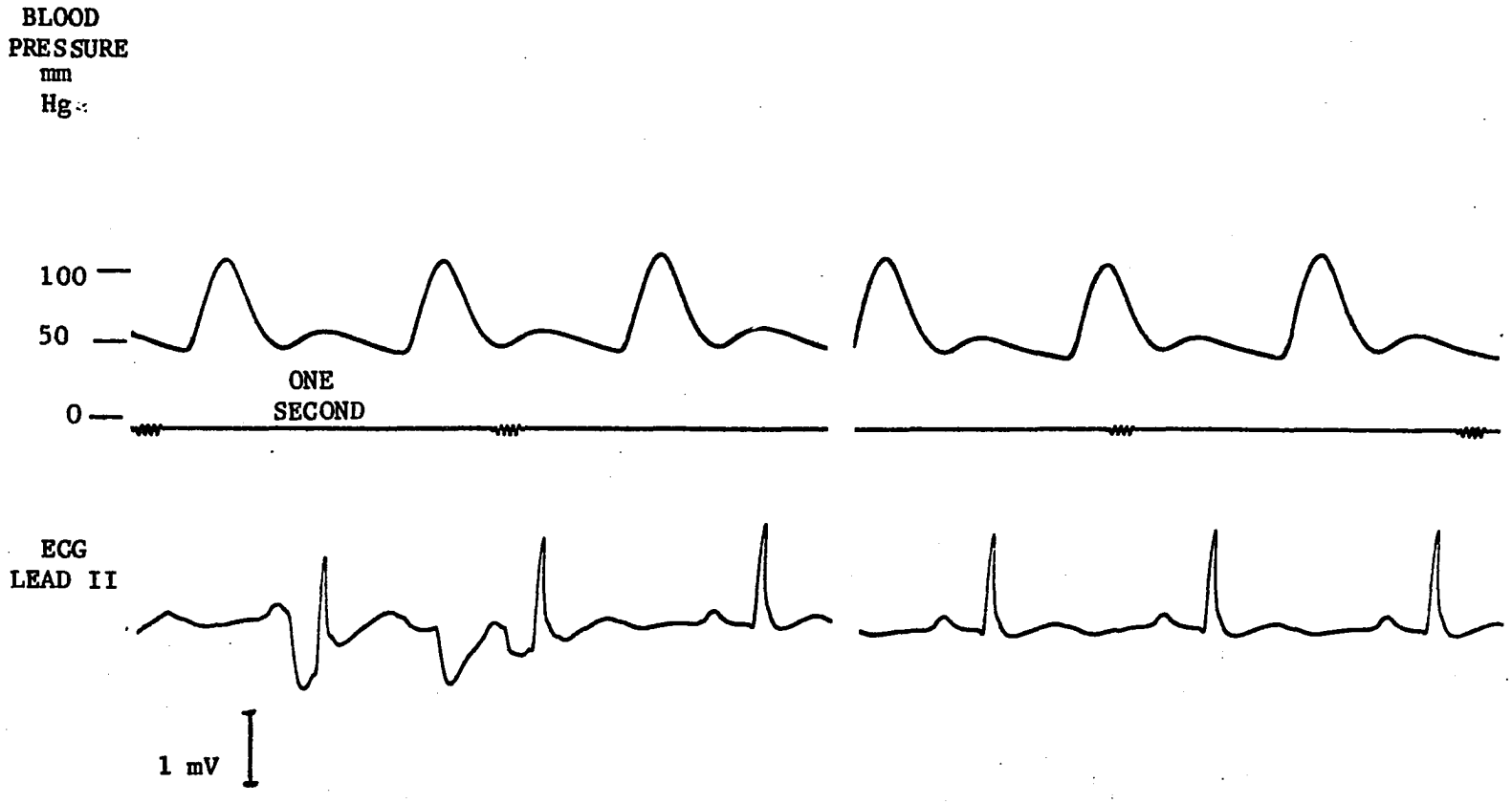

BLOOD
PRESSURE
mm
Hg
100
0
ONE
SECOND
ECG
LEAD II
PRESURGICAL
SURGICAL
ACUTE POSTSURGICAL

FIGURE 16. Dog 11: chronic postsurgical, pre-exsanguination electrocardiogram, and blood pressure recordings. Observe the methoxyflurane block in the initial recording which was absent from the later recordings.


BLOOD
PRESSURE
mm
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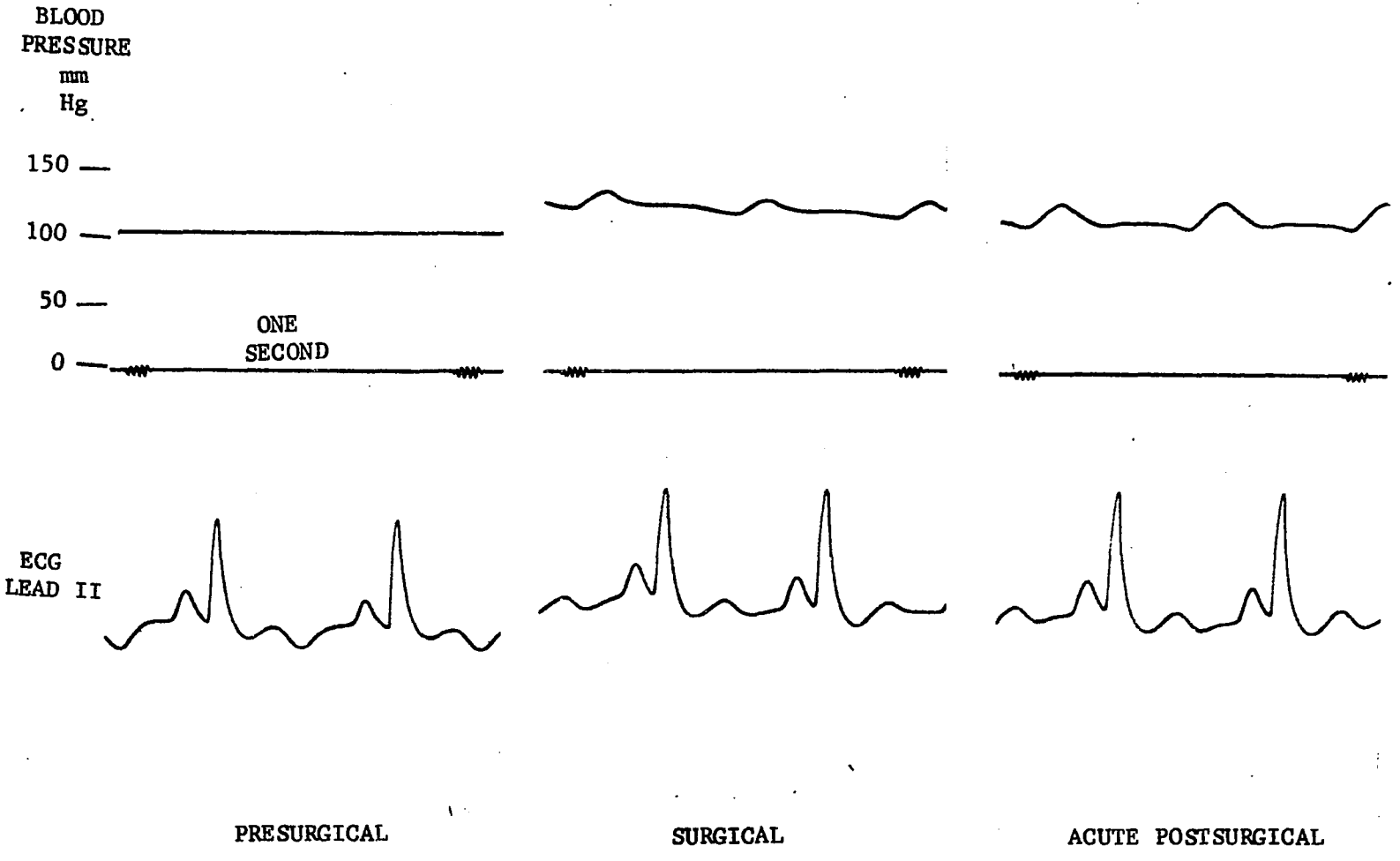
100
50
0
ONE
SECOND
ECG
LEAD II
1 mV
PRE-EXSANGUINATION
PRE-EXSANGUINATION

FIGURE 17. Dog 12: presurgical, surgical, and acute postsurgical electrocardiogram and blood pressure recordings. Heart rate and blood pressure increased.


BLOOD
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ONE
SECOND
ECG
LEAD II
PRESURGICAL
SURGICAL
ACUTE POSTSURGICAL

FIGURE 18. Dog 12: chronic postsurgical, pre-exsanguination, and exsanguination electrocardiogram and blood pressure recordings. Heart rate and blood pressure is further elevated. Note that the T wave during exsanguination did not increase in amplitude nor did it invert.

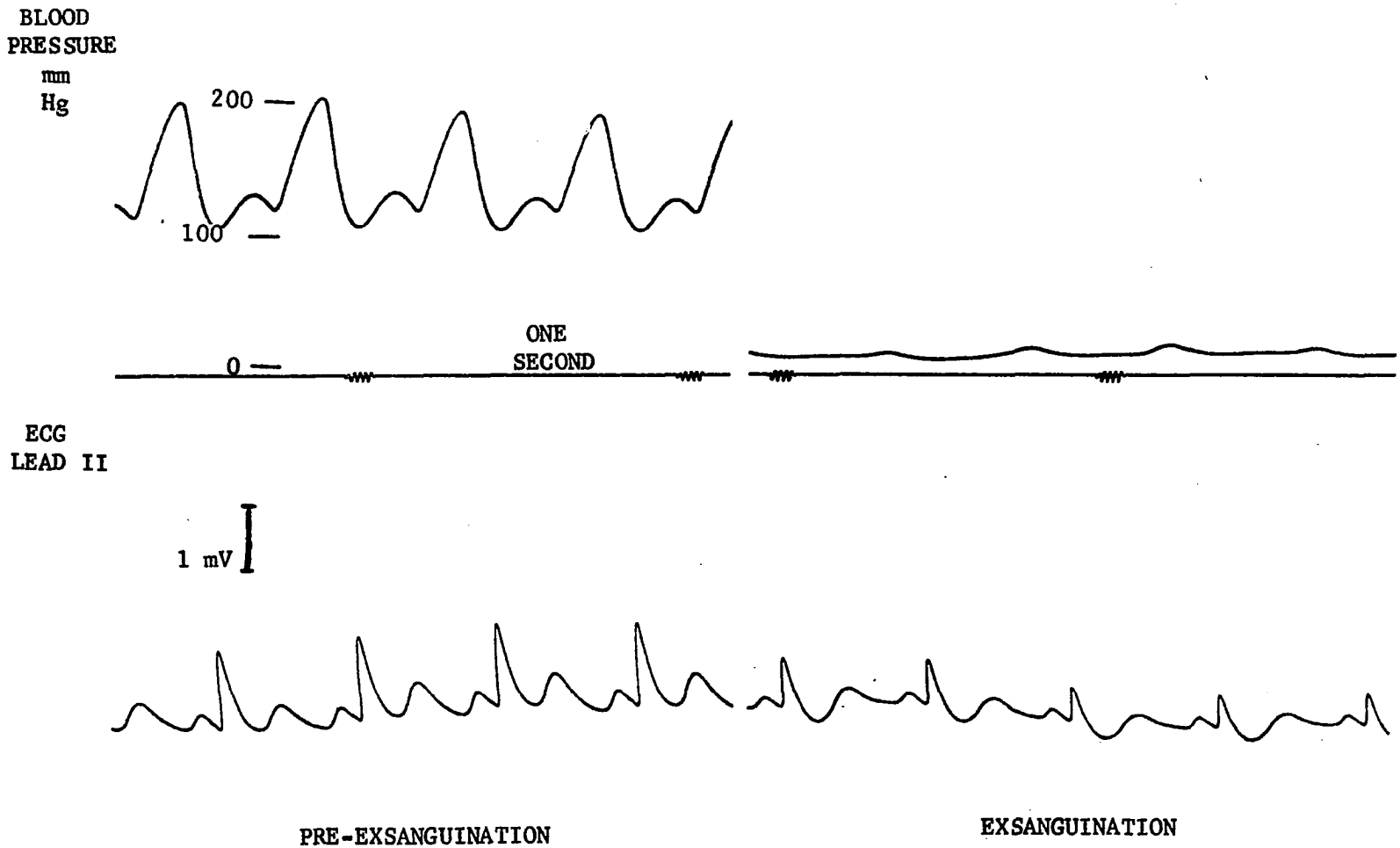

BLOOD
PRESSURE
mm
Hg
200
100
0
ONE
SECOND
ECG
LEAD II
1 mV
PRE-EXSANGUINATION
EXSANGUINATION

FIGURE 19. Dog 13: presurgical, surgical, and acute postsurgical electrocardiogram and blood pressure recordings. Note the R and S wave amplitude changes. The blood pressure drop during the surgical period followed removal of the left thoracic sympathetic trunk.

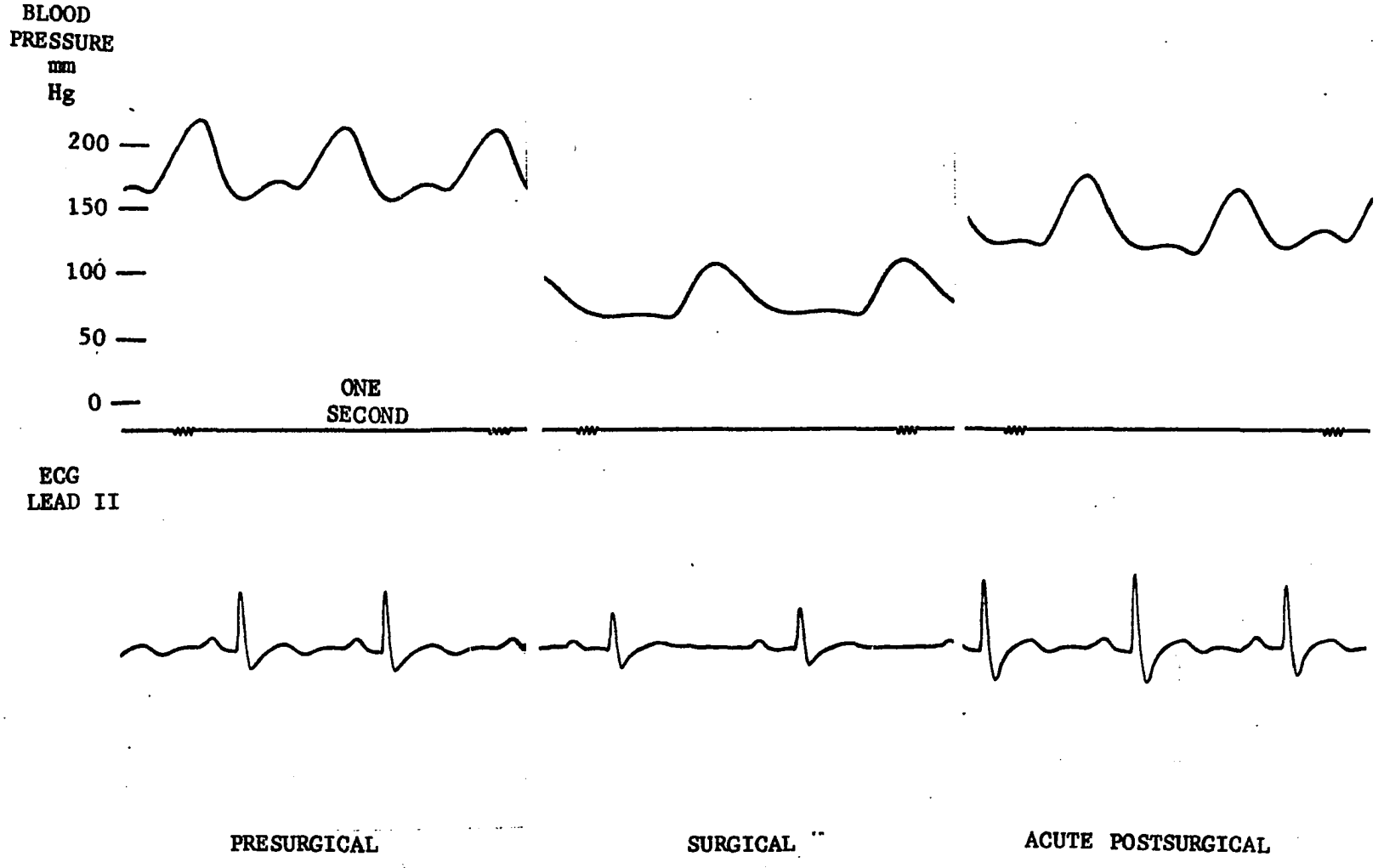

BLOOD
PRESSURE
mm
Hg
200
150
100
50
0
ONE
SECOND
ECG
LEAD II
PRESURGICAL
SURGICAL
ACUTE POSTSURGICAL

FIGURE 20. Dog 13: chronic postsurgical, pre-exsanguination, and exsanguination electrocardiogram and blood pressure recordings. The blood pressure is lowered and strength of atrial and ventricular contraction decreased from previous periods. Note that during exsanguination heart contractions were further depressed.

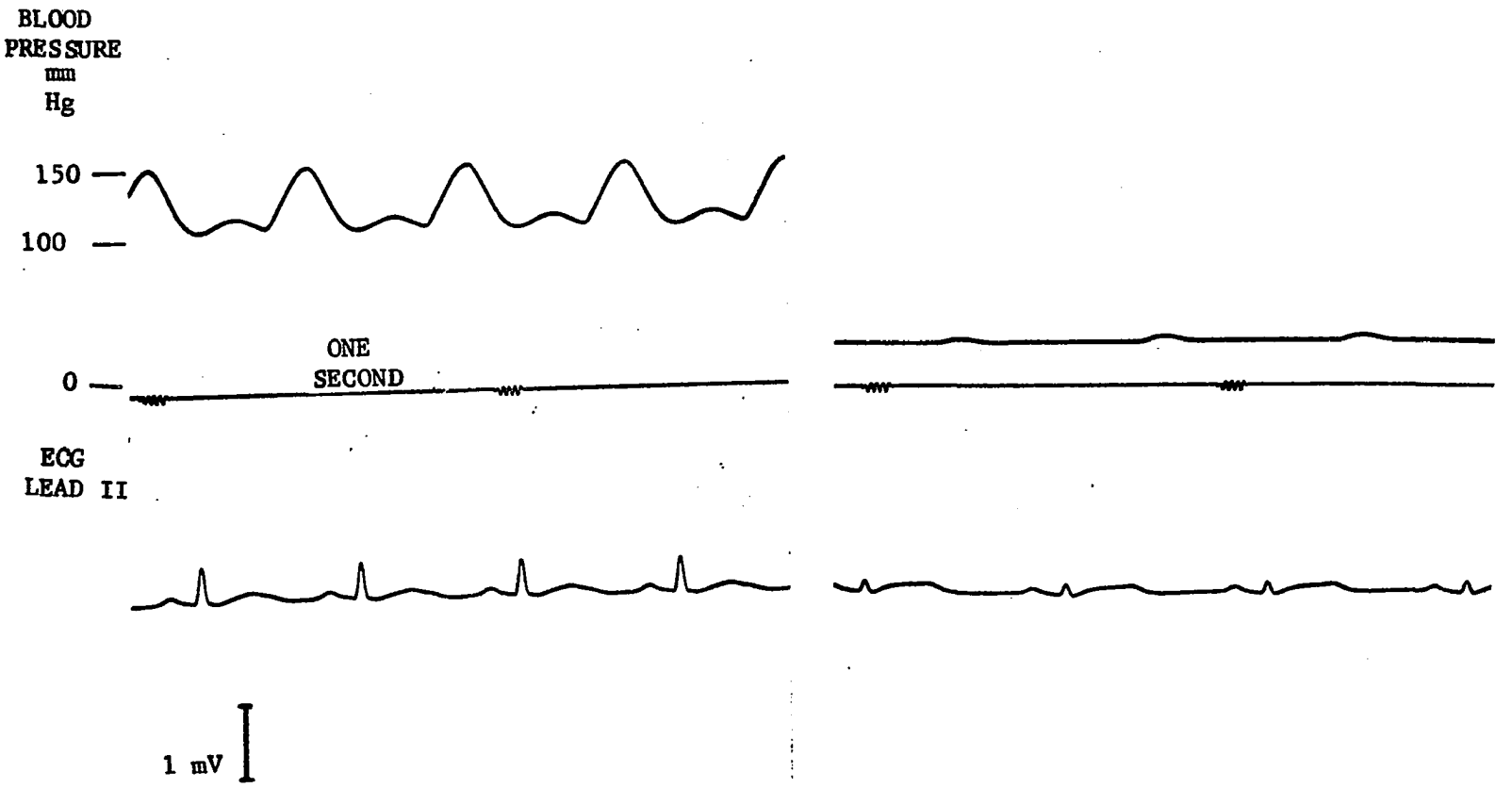

BLOOD
PRESSURE
mm
Hg
150
100
0
ONE
SECOND
ECG
LEAD II
1 mV
PRE-EXSANGUINATION
EXSANGUINATION

FIGURE 21. Dog 14: presurgical, surgical, and acute postsurgical electrocardiogram and blood pressure recordings. The strength of ventricular contraction is decreased following removal of the right thoracic and cervicothoracic ganglia in the surgical recording and further decreased following removal of similar ganglia on the left side, as observed in the acute postsurgical recording. Extracardiac interference produced small undulating waves on the recording.

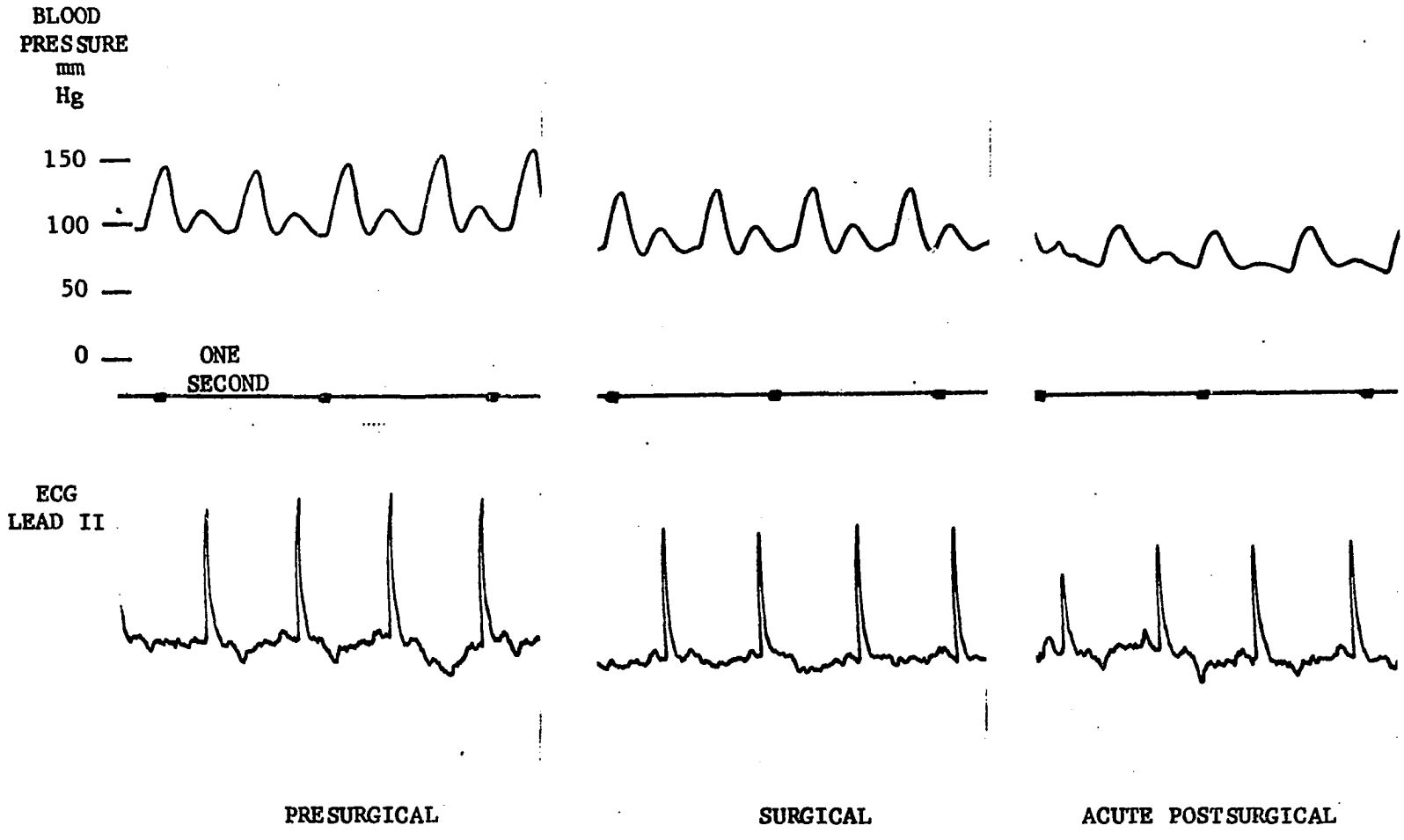

BLOOD
PRESSURE
mm
Hg
150
100
50
0
ONE
SECOND
ECG
LEAD II
PRESURGICAL
SURGICAL
ACUTE POSTSURGICAL

FIGURE 22. Dog 14: chronic postsurgical, pre-exsanguination, and exsanguination electrocardiogram and blood pressure recordings. Heart rate is slower than in previous periods. P waves disappeared and ectopic ventricular beats persisted during exsanguination.

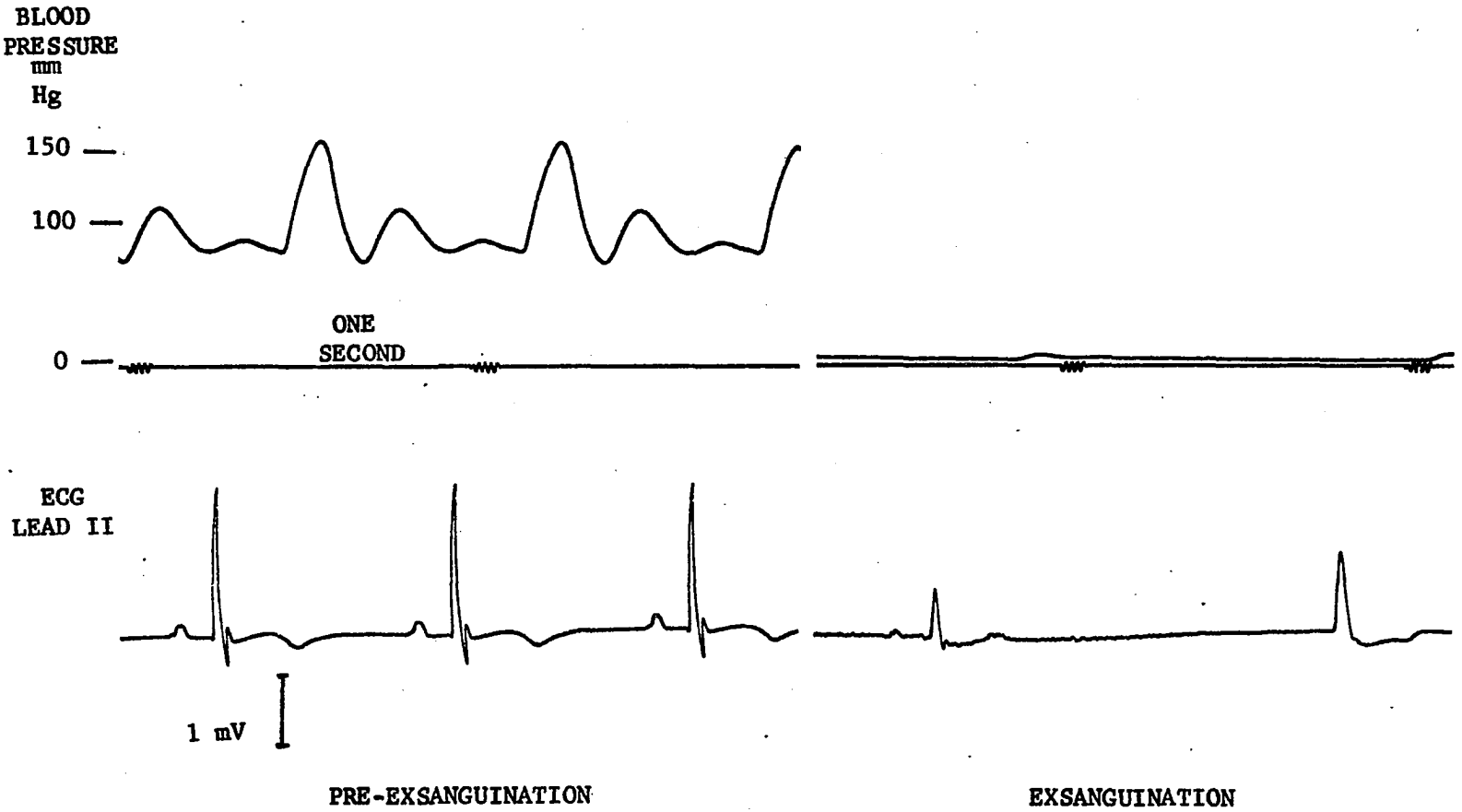

BLOOD
PRESSURE
mm
Hg
150
100
0
ONE
SECOND
ECG
LEAD II
1 mV
PRE-EXSANGUINATION
EXSANGUINATION

FIGURE 23. Dog 15: presurgical, surgical, and acute postsurgical electrocardiogram and blood pressure recordings. Heart rate and blood pressure both decreased during and following surgery. Note the inversion and increased amplitude of the T wave following the presurgical period.

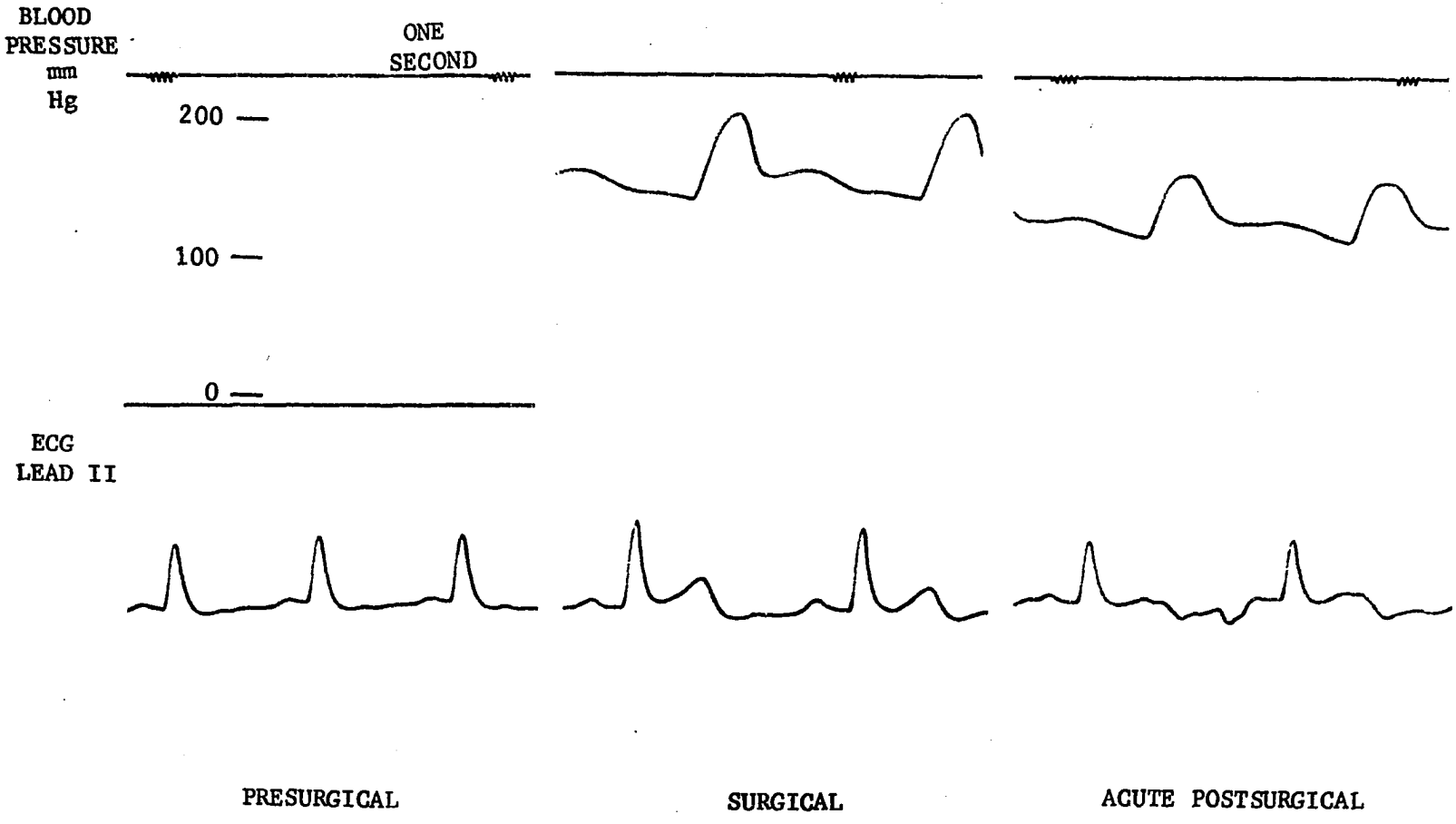

BLOOD
PRESSURE
mm
Hg
ONE
SECOND
200
100
0
ECG
LEAD II
PRESURGICAL
SURGICAL
ACUTE POSTSURGICAL

FIGURE 24. Dog 15: chronic postsurgical, pre-exsanguination, and exsanguination electrocardiogram and blood pressure recordings. The force of ventricular contraction is low. Note the beginning elevation of the T wave early in the exsanguination period.

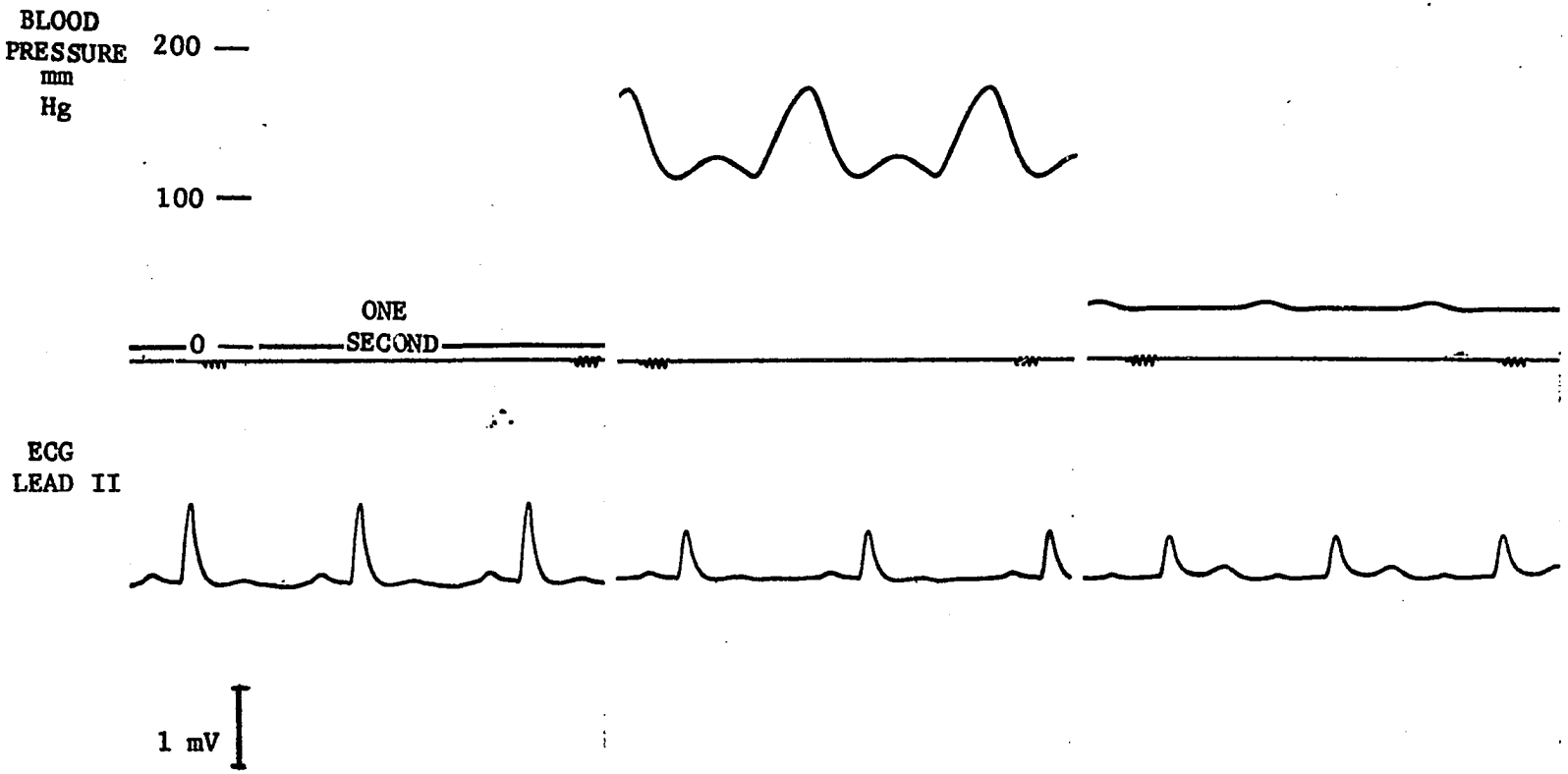

BLOOD
PRESSURE
mm
Hg
200
100
ONE
SECOND
0
ECG
LEAD II
1 mV
CHRONIC POSTSURGICAL
PRE-EXSANGUINATION
EXSANGUINATION

FIGURE 25. Dog 16: presurgical, surgical, and acute postsurgical electrocardiogram and blood pressure recordings. Electrocardiogram recordings were unusual suggesting affects of positioning, mixing of leads, faulty recording, or animal peculiarity.

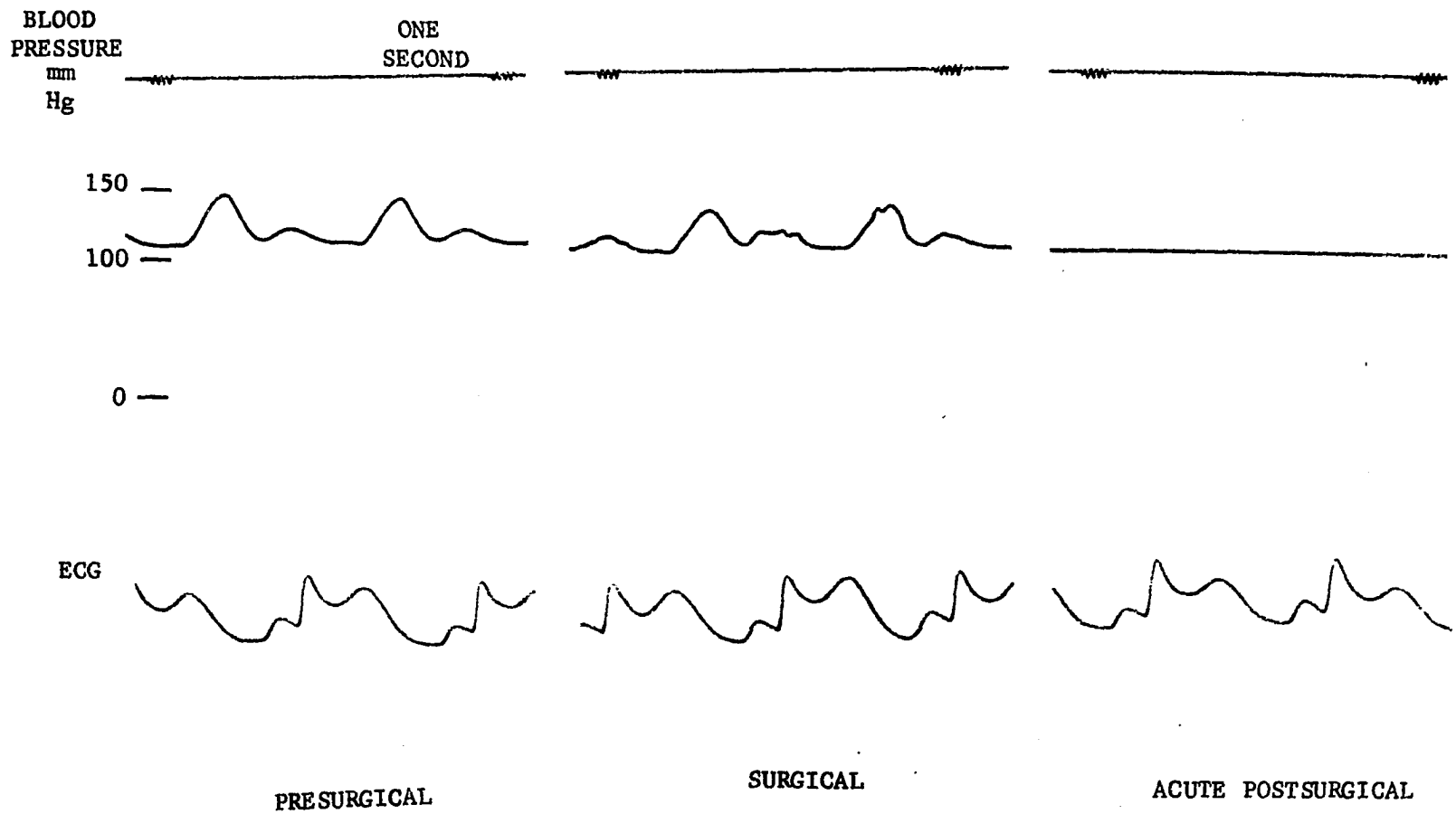

BLOOD
PRESSURE
mm
Hg
ONE
SECOND
150
100
0
ECG
PRESURGICAL
SURGICAL
ACUTE POSTSURGICAL

FIGURE 26. Dog 16: chronic postsurgical pre-exsanguination electrocardiogram and blood pressure recordings. P and T waves appear elevated in comparison to the R wave. Pre-exsanguination blood pressure recordings may be slightly higher than is recorded since limited blood loss was experienced. Note the increased P and decreased R amplitude during exsanguination.

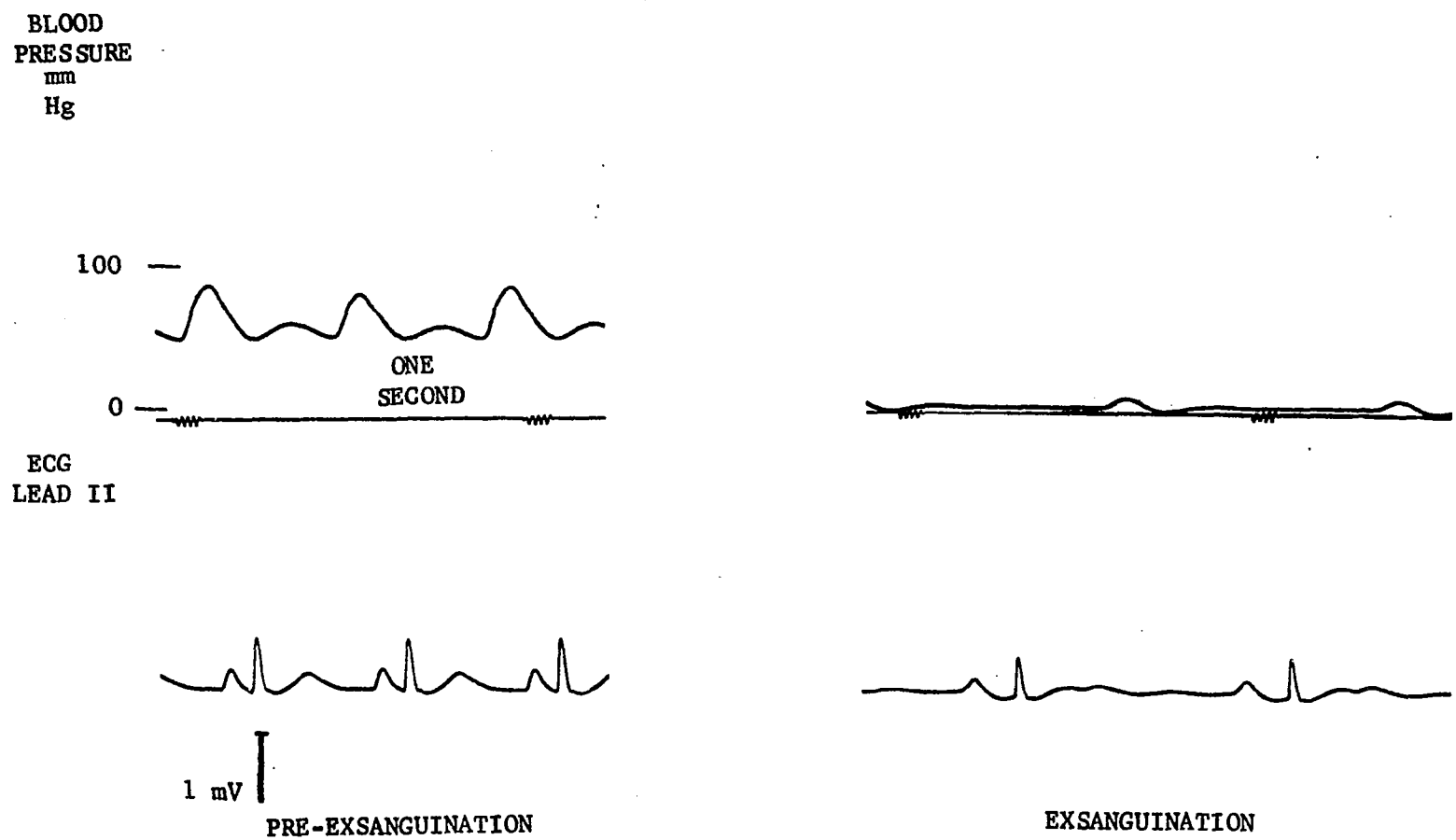

BLOOD
PRESSURE
mm
Hg
100
0
ONE
SECOND
ECG
LEAD II
1 mV
PRE-EXSANGUINATION
EXSANGUINATION

FIGURE 27. Dog 17: presurgical, surgical, acute postsurgical, and chorinic postsurgical electrocardiogram and blood pressure recordings. Note the depression of the amplitude of heart contractions and heart rate during and immediately following surgery which may partially be associated with stimulation of the vagi while dissecting the sympathetic trunks from the vagi. Also note the blood pressure changes.

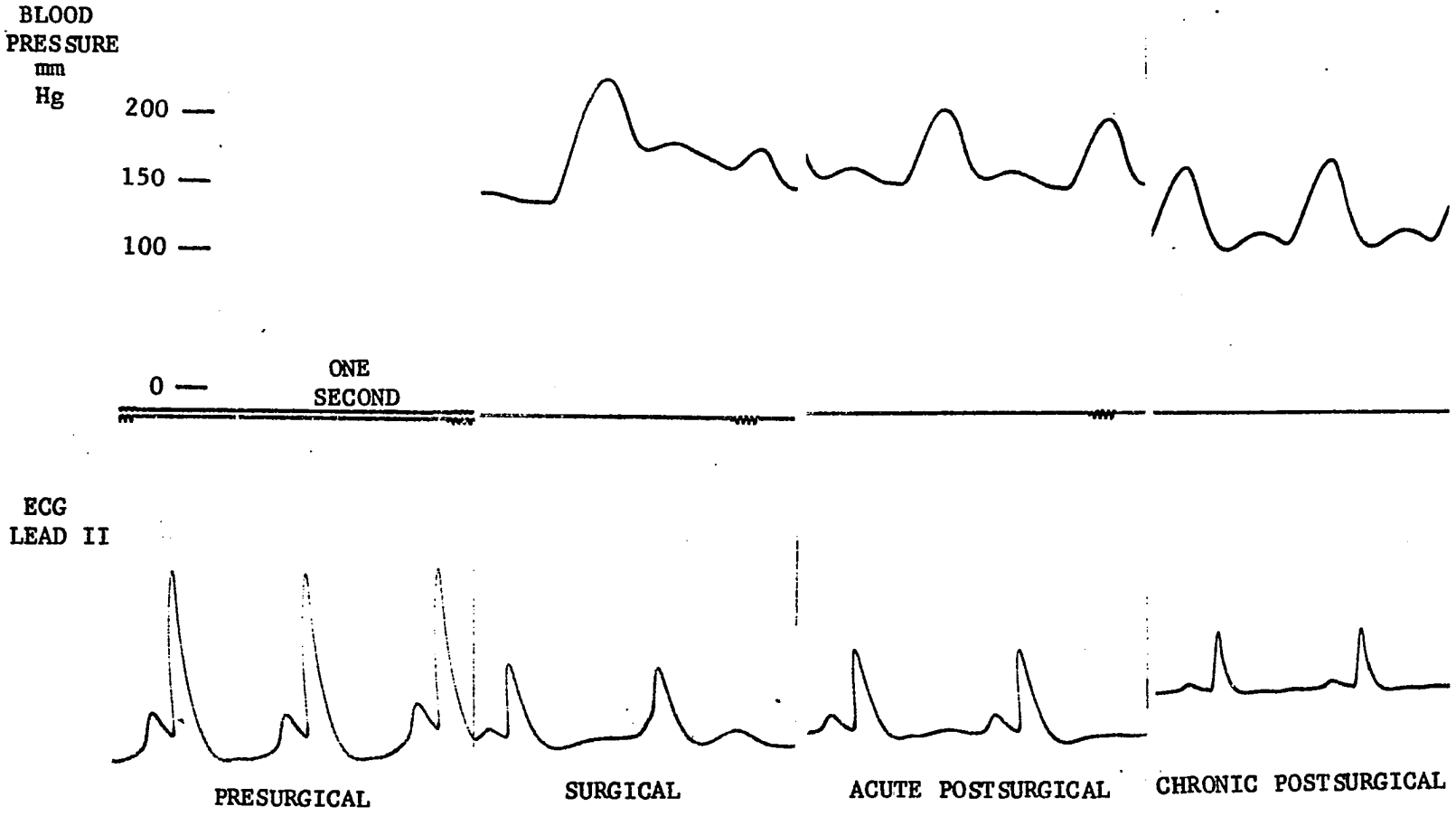

BLOOD
PRESSURE
mm
Hg
200
150
100
0
ONE
SECOND
ECG
LEAD II
PRESURGICAL
SURGICAL
ACUTE POSTSURGICAL
CHRONIC POSTSURGICAL

FIGURE 28. Dog 18: chronic postsurgical, pre-exsanguination, and exsanguination electrocardiogram and blood pressure recordings. Note the faulty repolarization of the ventricle late in the exsanguination period.

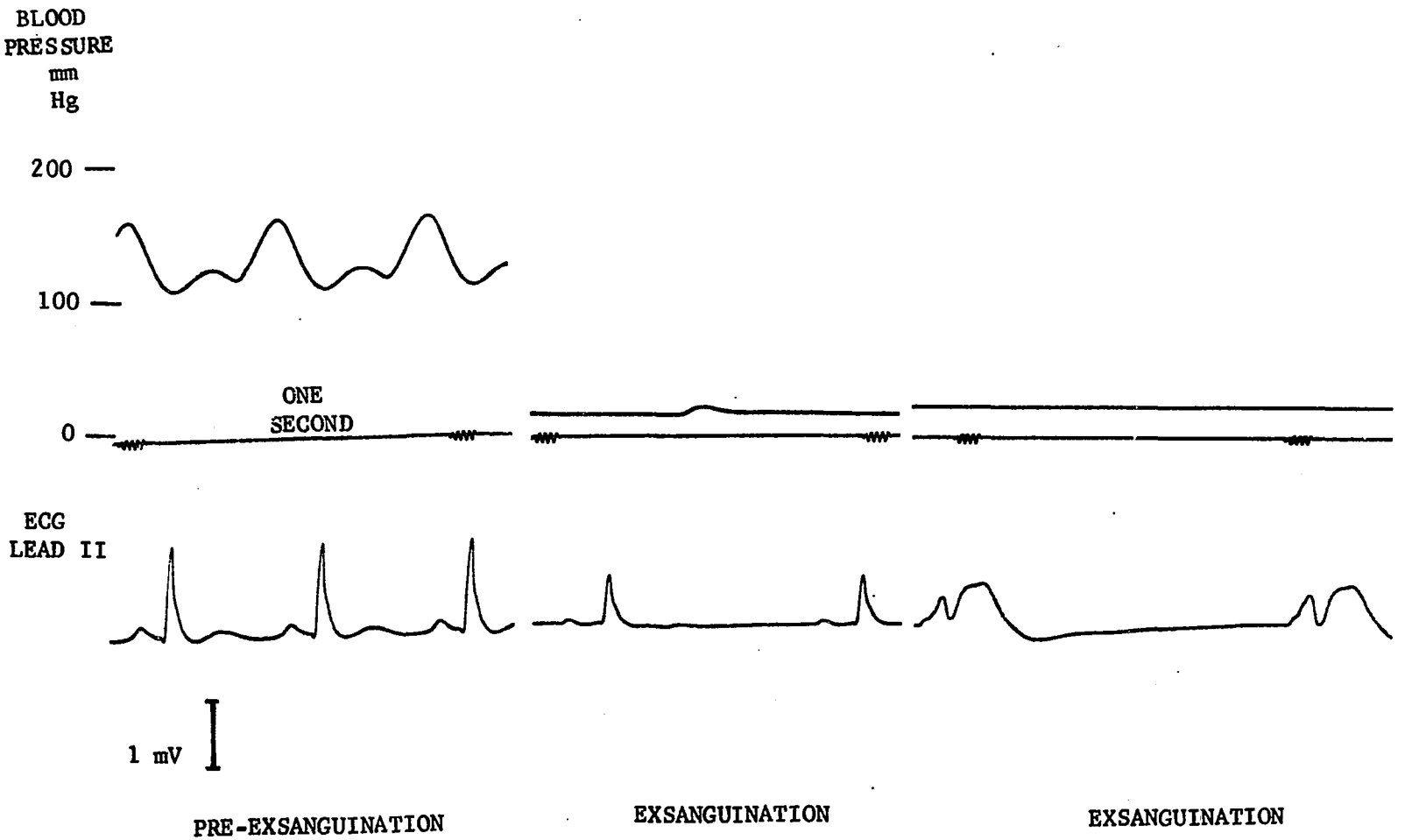

BLOOD
PRESSURE
mm
Hg
200
100
0
ONE
SECOND
ECG
LEAD II
1 mV
PRE-EXSANGUINATION
EXSANGUINATION
EXSANGUINATION

FIGURE 29. Dog 19: presurgical, surgical, and acute postsurgical electrocardiogram and blood pressure recordings. Note the decreased blood pressure in the acute postsurgical period.

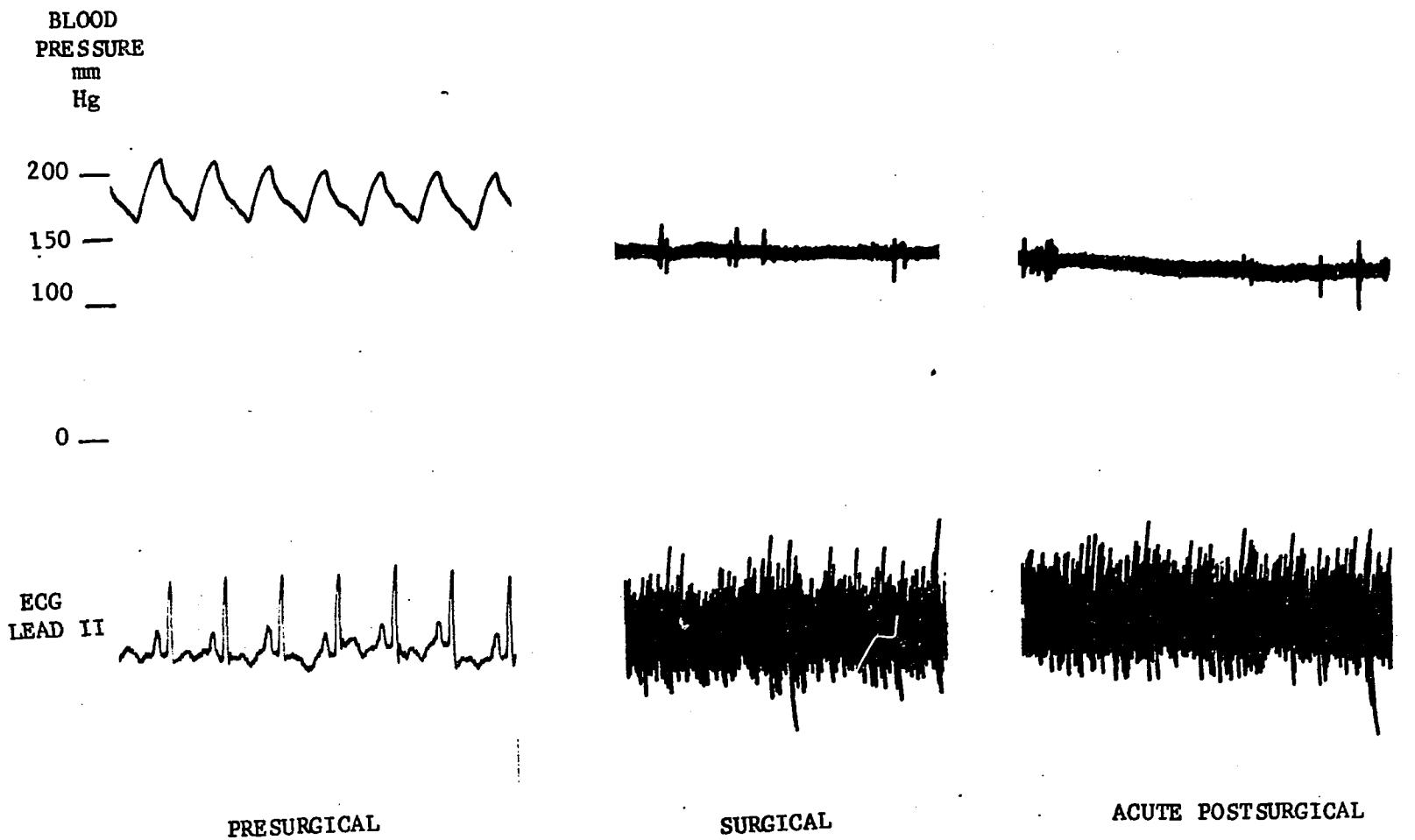

BLOOD
PRESSURE
mm
Hg
200
150
100
0
ECG
LEAD II
PRESURGICAL
SURGICAL
ACUTE POSTSURGICAL

FIGURE 30. Dog 22: presurgical, surgical, and acute postsurgical electrocardiogram and blood pressure recordings. The increase in blood pressure following left vagotomy is observed in the surgical recording. The acute postsurgical recording reflects blood pressure increase after bilateral vagotomy.

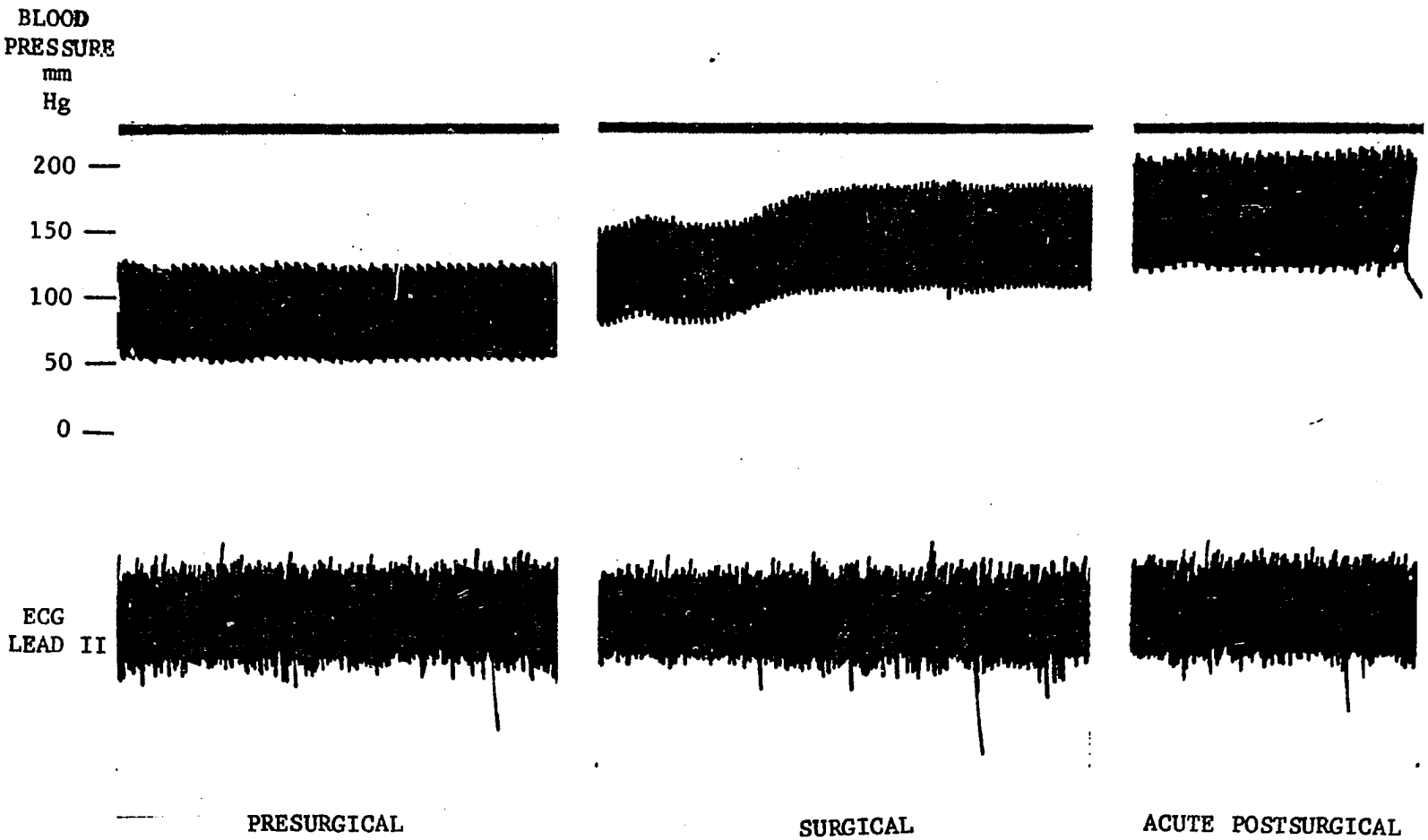

BLOOD
PRESSURE
mm
Hg
200
150
100
50
0
ECG
LEAD II
PRESURGICAL
SURGICAL
ACUTE POSTSURGICAL

FIGURE 31. Dog 23: presurgical, and surgical electrocardiogram and blood pressure recordings. During the surgical periods blood pressure increased following transection of the right and left vagi as indicated by the first and second markers respectively.

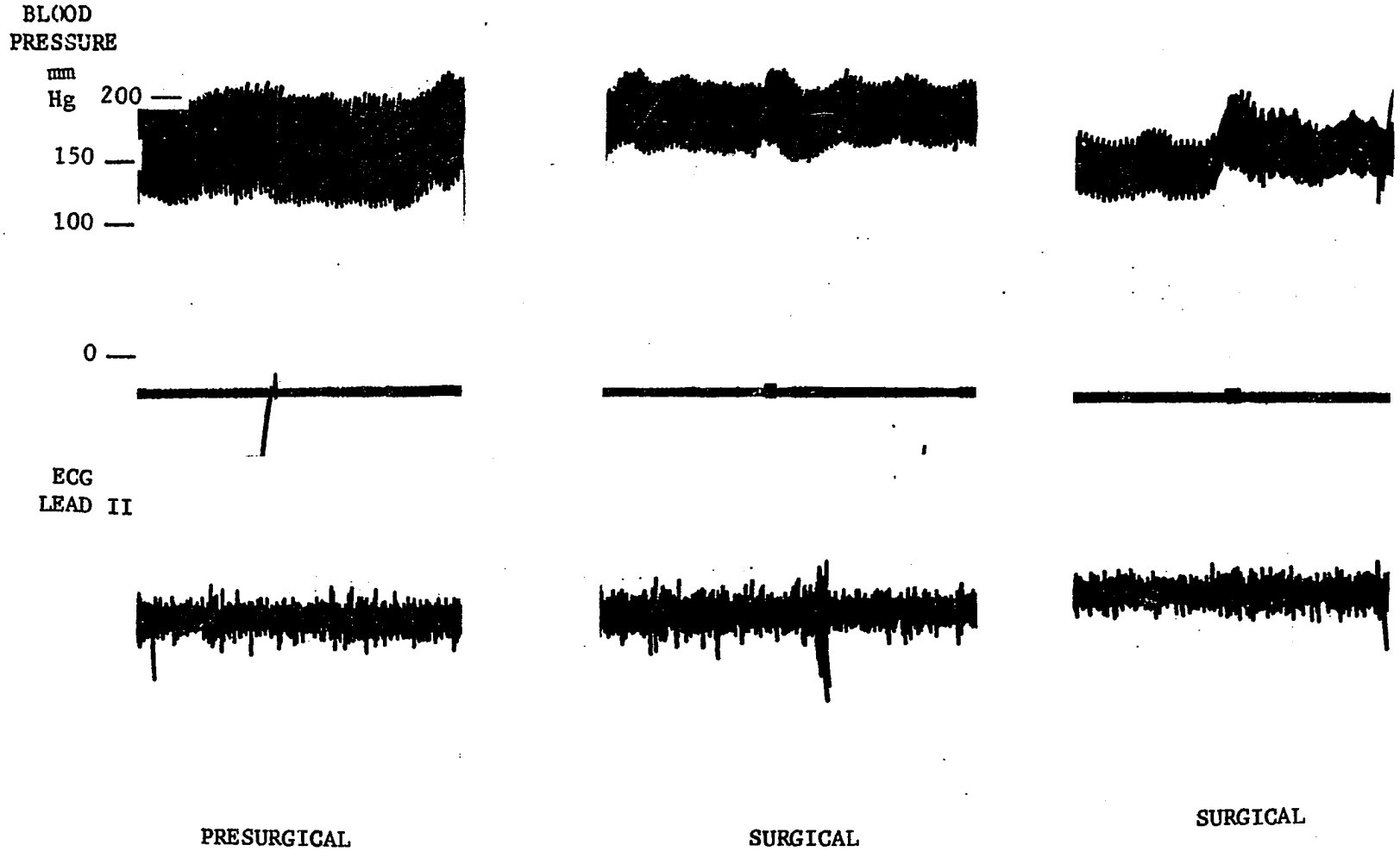

BLOOD
PRESSURE
mm
Hg
200
150
100
0
ECG
LEAD II
PRESURGICAL
SURGICAL
SURGICAL

FIGURE 32. Dog 24: presurgical, surgical, and acute postsurgical electrocardiogram and blood pressure recordings. The first surgical recording reflects the change in blood pressure associated with right vagotomy. In the second surgical recording the pressure stabilized and in the third surgical recording the left vagus was transected.

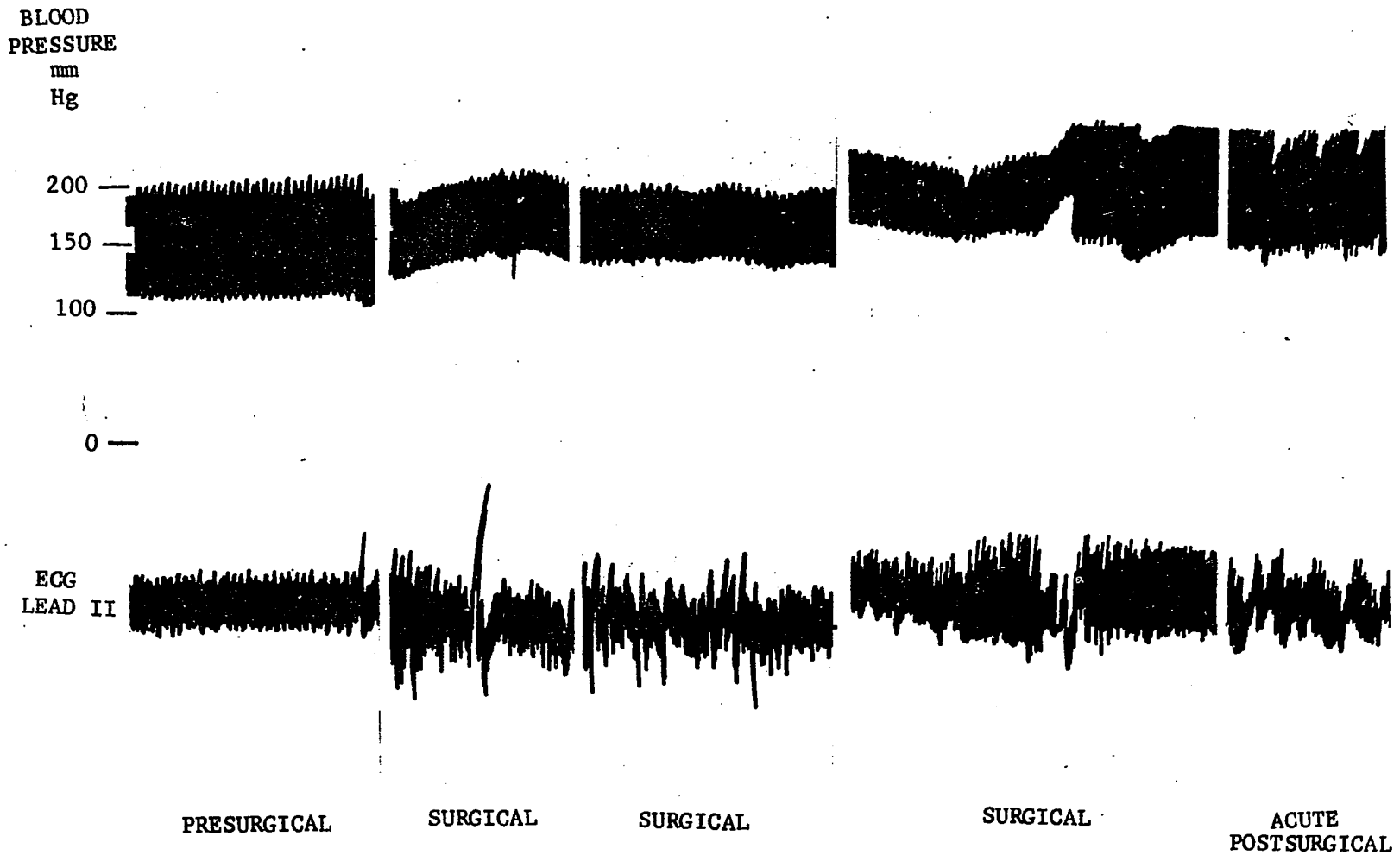

BLOOD
PRESSURE
mm
Hg
200
150
100
0
ECG
LEAD II
PRESURGICAL
SURGICAL
SURGICAL
SURGICAL
ACUTE
POSTSURGICAL

FIGURE 33. Dog 26: presurgical, surgical, and acute postsurgical electrocardiogram and blood pressure recordings. Note the increased force of heart contractions and increased blood pressure following bilateral vagotomy. In the first surgical recording the right vagus was transected, and in the second surgical period the left vagus was transected.

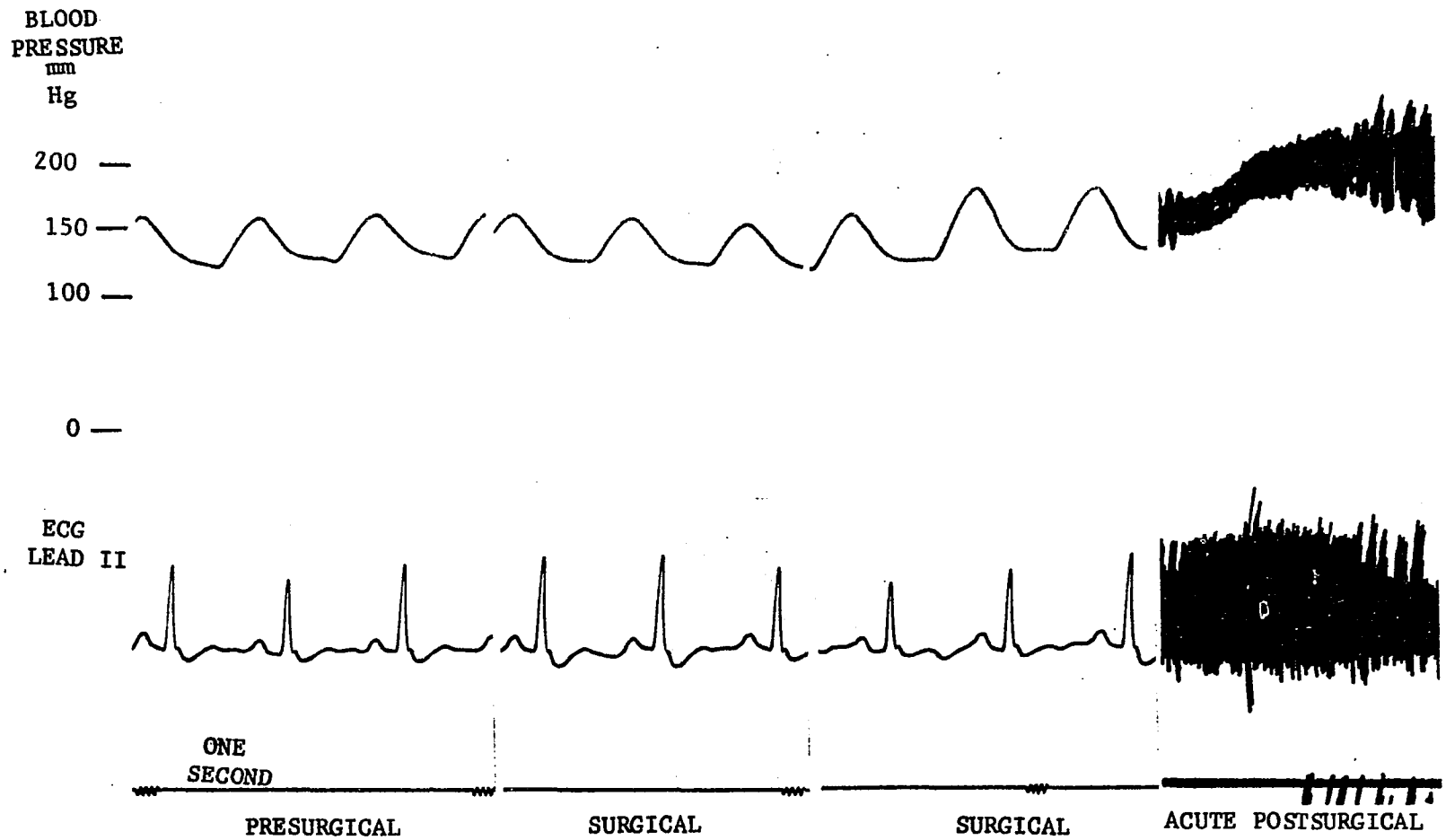

BLOOD
PRESSURE
mm
Hg
200
150
100
0
ECG
LEAD II
ONE
SECOND
PRESURGICAL
SURGICAL
SURGICAL
ACUTE POSTSURGICAL

FIGURE 34. Dog 27: presurgical, surgical, and acute postsurgical electrocardiogram and blood pressure recordings. Note the progressive increase in blood pressure and force of ventricular contraction. The surgical recording was taken following transection of the right vagus.

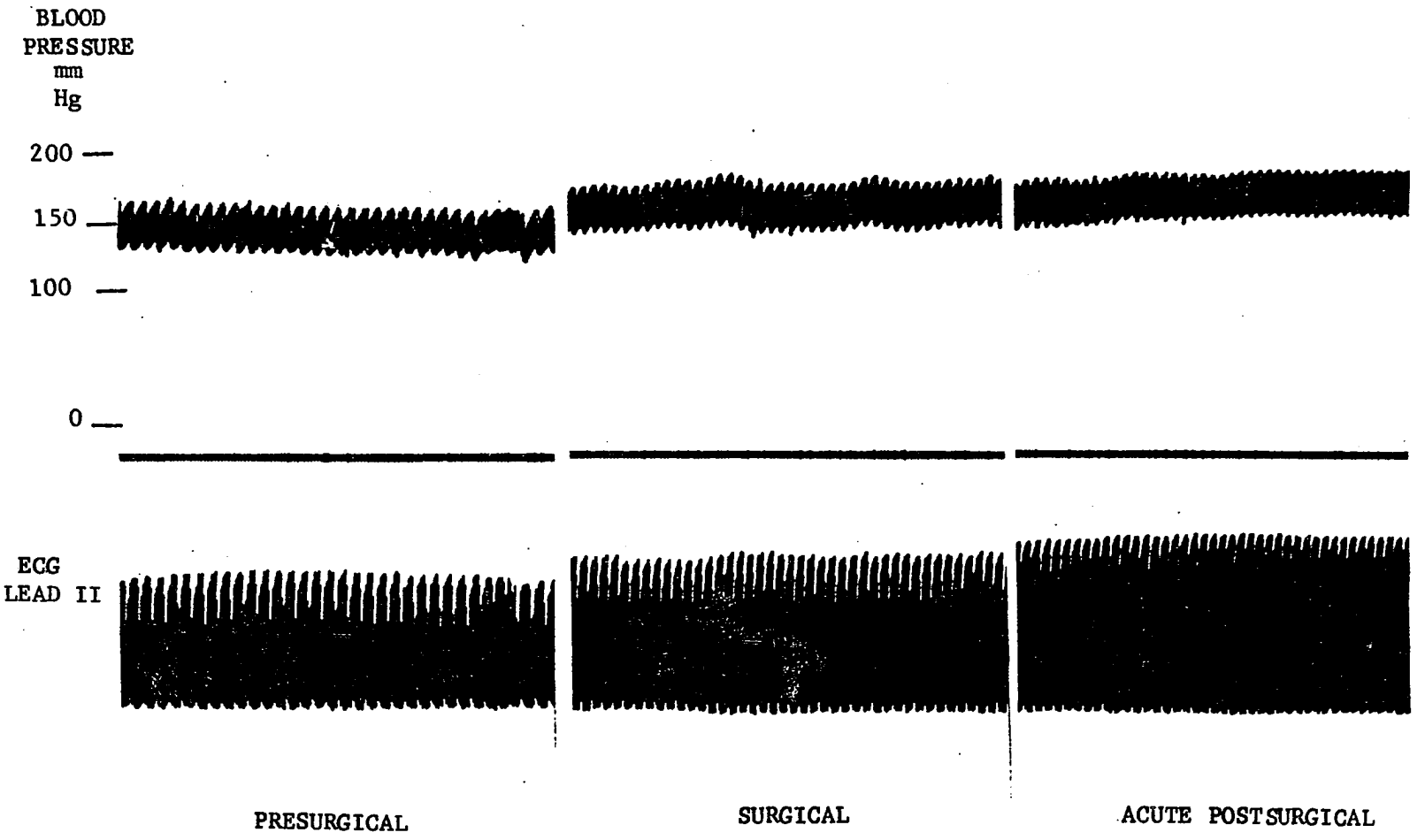
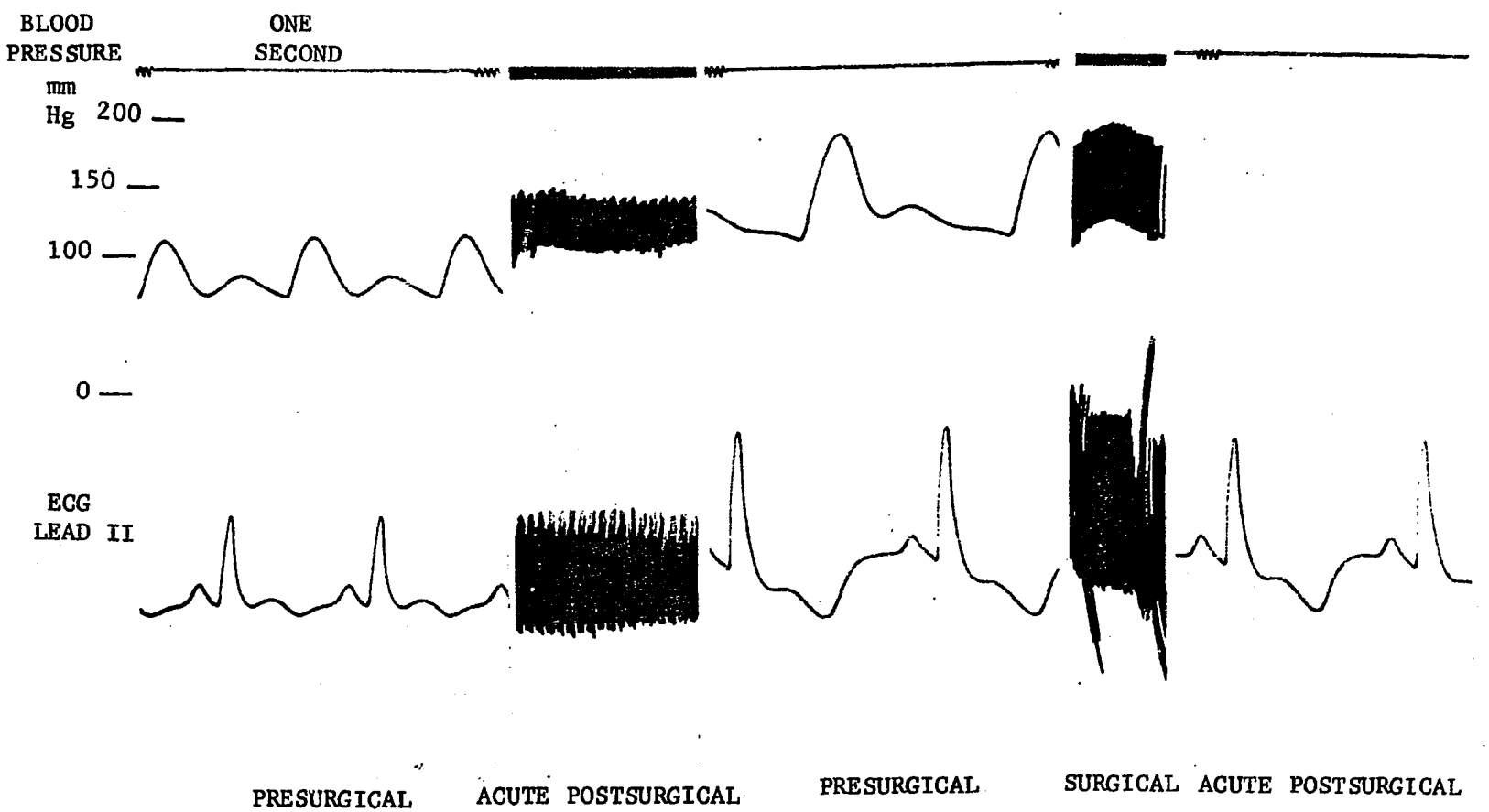

BLOOD
PRESSURE
mm
Hg
200
150
100
0
ECG
LEAD II
PRESURGICAL
SURGICAL
ACUTE POSTSURGICAL

FIGURE 35. Dog 28: presurgical, surgical, and acute postsurgical electrocardiogram and blood pressure recordings associated with two stages of surgery. Note the increased blood pressure following transection of the right vagus represented in the acute postsurgical recording to the left. In the second presurgical period the aortic blood pressure was still elevated. Following transection of the left vagus in the second surgical period blood pressure rose slightly.


BLOOD PRESSURE
mm Hg
200
150
100
0
ONE SECOND
ECG LEAD II
PRESURGICAL
ACUTE POSTSURGICAL
PRESURGICAL
SURGICAL
ACUTE POSTSURGICAL

APPENDIX E. PHOTOGRAPHS

FIGURE 36
Catecholamine fluorescence in the cervicothoracic ganglion. Some diffusion has occurred, but cytons and their nuclei are outlined. 250X. 50 ASA.

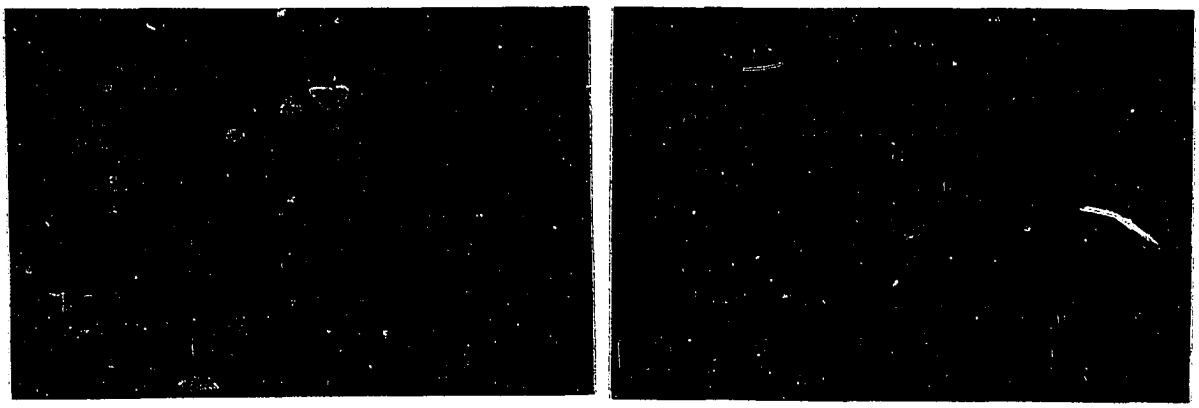
FIGURE 37
Catecholamine fluorescent nerves and autofluorescent lipofuscin pigments, bright green lines, and yellow dots respectively in the myocardium. 50 ASA.

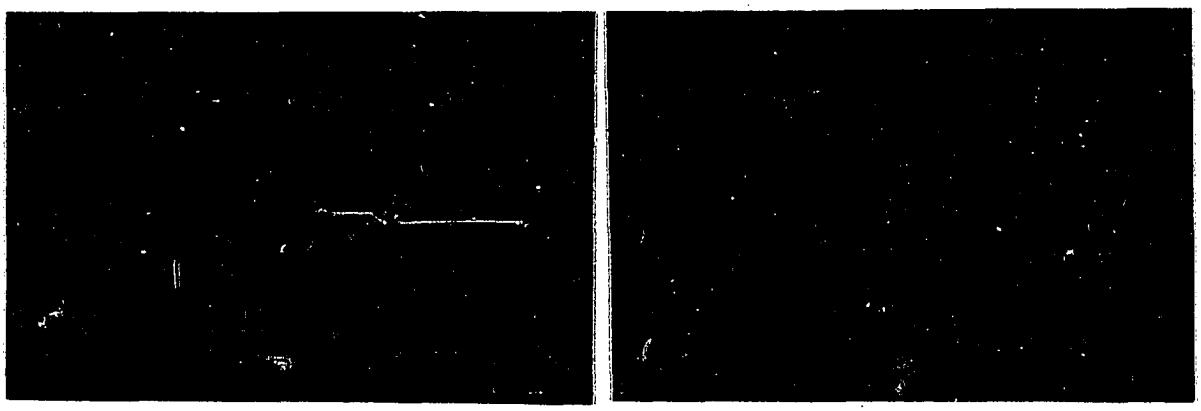
FIGURE 38
Catecholamine fluorescent nerves and autofluorescent lipofuscin, bright green and yellow respectively in the myocardium. 200ASA.

FIGURE 39
Autofluorescence of connective tissue in the myocardium; dull green lines. 500 ASA.

FIGURE 40
Catecholamine fluorescent nerves and autofluorescent lipofuscin pigment in the myocardium. 500 ASA

FIGURE 41
Diffusion of cardiac catecholamines. However, some neurons are still fluorescent (bright green lines), and autofluorescent pigments (yellow) are also present. 500 ASA.





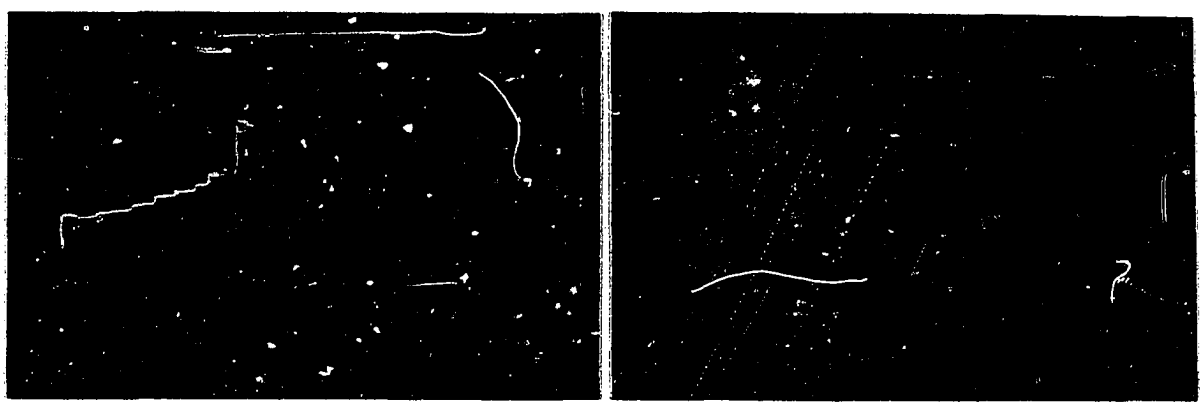


FIGURE 42
Catecholamine fluorescence in the cervicothoracic ganglion. Low power photograph showing well outlinted green cell bodies and some cell processes of adrenergic neurons. 200 ASA.

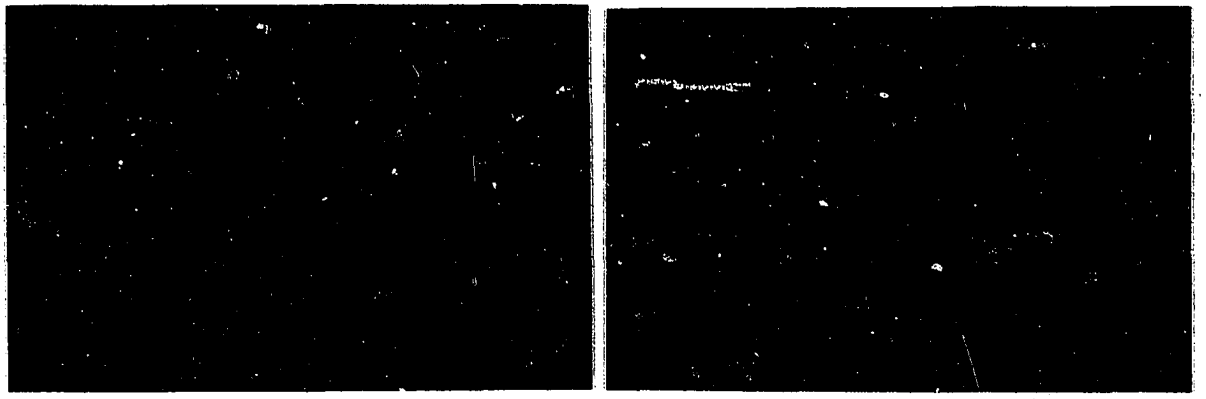
FIGURE 43
Catecholamine fluorescence in the cervicothoracic ganglion. Yellow green cell bodies were highly fluorescent. 250X. 200 ASA.

FIGURE 44
Catecholamine fluorescence of cardiac nerves was green. Diffusion also is present as reflected in the green background. 500 ASA.

FIGURE 45
Catecholamine fluorescence of cardiac nerves was green. Smaller yellow autofluorescent lipofuscin pigments were also observed. 200 ASA.

FIGURE 46
Acetylcholinesterase positive staining myocardial nerve in the left atrium following right cervical vagotomy in dog 18. Three hours incubation. 250X. 500 ASA.

FIGURE 47
Left ventricular epicardial cardiac nerve staining positively for acetylcholinesterase following complete extrinsic cardiac denervation in dog 6. Five hours incubation. 250X. 50 ASA.



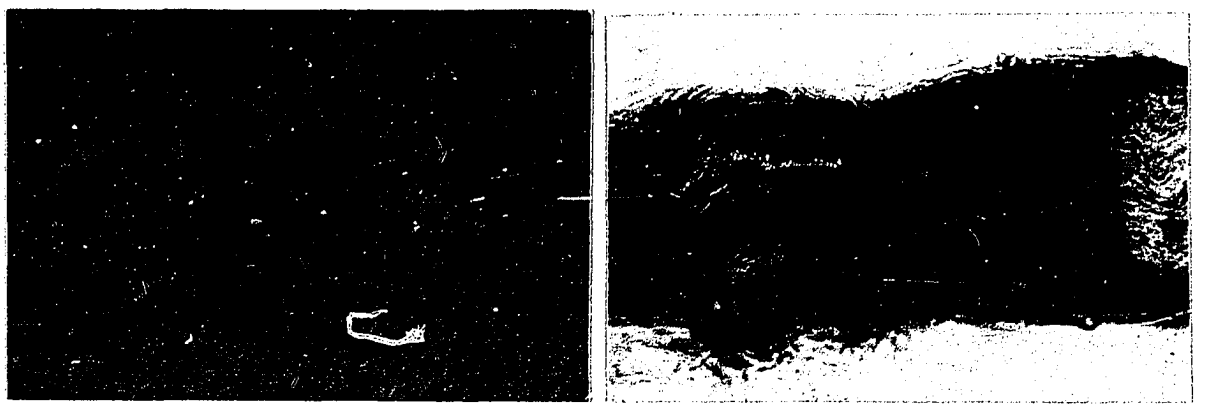


FIGURE 48

Acetylcholinesterase positive staining of left atrial intramyocardial nerves following bilateral recurrent laryngeal cardiac nerve transection dog 11. The nuclei of the cardiac muscle fibers were also stained after 8 hours of incubation. 250X. 50 ASA.

FIGURE 49

Acetylcholinesterase positive staining of left atrial intramyocardial nerve following bilateral recurrent laryngeal cardiac nerve transection dog 11 after 19 hours of incubation. Note the increased myocardial staining too. 400X. 50 ASA.

FIGURE 50

Acetylcholinesterase positive staining of right atrial intramyocardial nerves following transection of the thoracic rami communicantes, dog 15. Observe the lack of myocardial fiber staining after 19 hours of incubation. 1000X. 50 ASA.

FIGURE 51

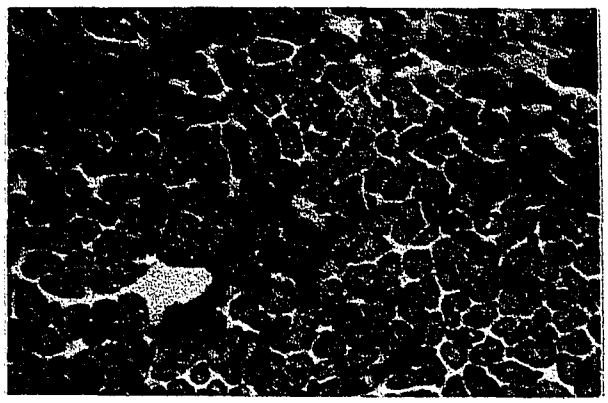
Acetylcholinesterase positive staining ganglion in the epicardium of the left atrium of dog 15. The cell bodies show less staining than surrounding processes after 19 hours of incubation. 250X. 50 ASA.

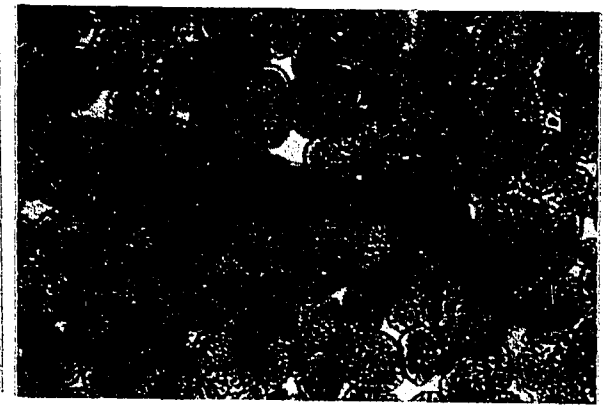
FIGURE 52

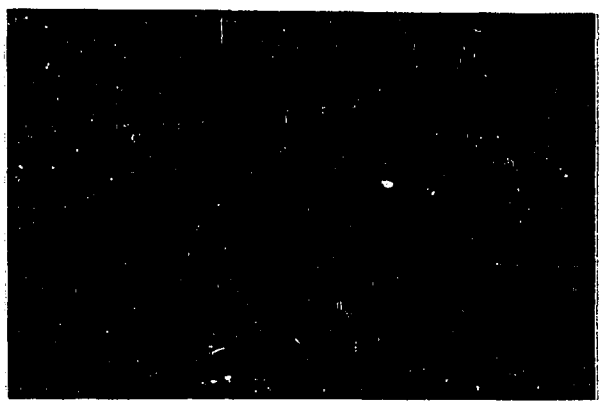
Acetylcholinesterase positive nerves appears to be branching into free nerve endings on the myocardial fibers in the right lower quadrent: dog 13, bilateral thoracic ganglia removal after 19 hours of incubation. 1000X. 50 ASA

FIGURE 53

Acetylcholinesterase positive staining nerve ending showing an interesting relationship to the myocardial fiber nucleus in the center of the photograph. Dog 13 after 19 hours of incubation. 1000X. 50 ASA.







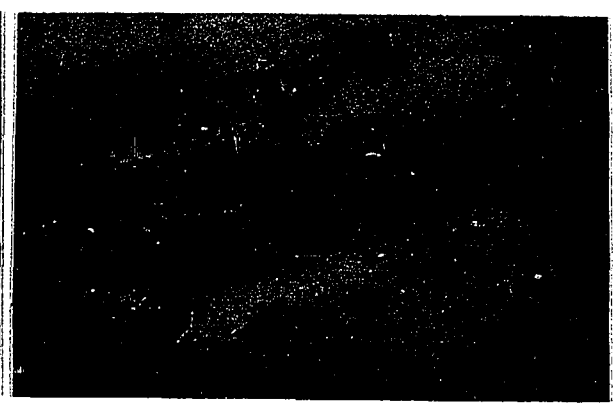


FIGURE 54
Acetylcholinesterase positive staining of the nerves and left atrial myocardium of dog 17 after bilateral cervical sympathetic trunk transection was poorly developed following 3 hours of incubation. 40X. 50 ASA.

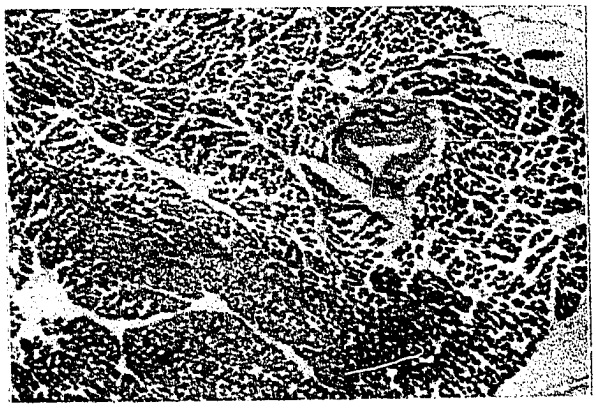
FIGURE 55
Acetylcholinesterase positive staining of vascular and myocardial nerves and of the myocardium was well deliniated in dog 17 after 8 hours of incubation. 40X. 50 ASA.

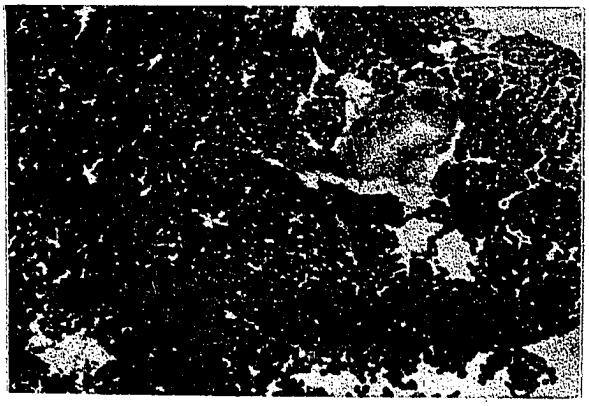
FIGURE 56
Acetylcholinesterase positive staining was intense in the same tissue as in Figures 54 and 55, after 20 hours of incubation. 40X. 50 ASA.

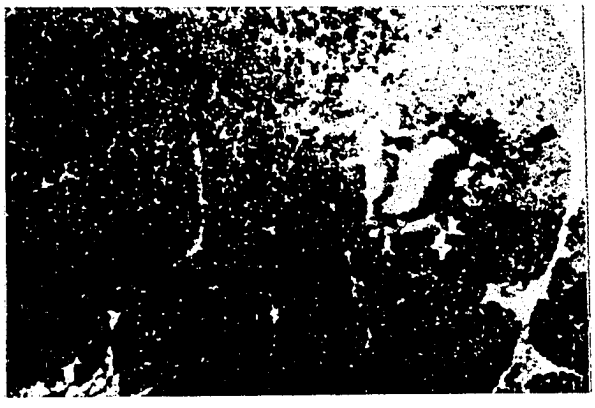
FIGURE 57
Little positive acetylcholinesterase was observed in the left atrium of control dog 4 following 3 hours of incubation. 25X. 50 ASA.

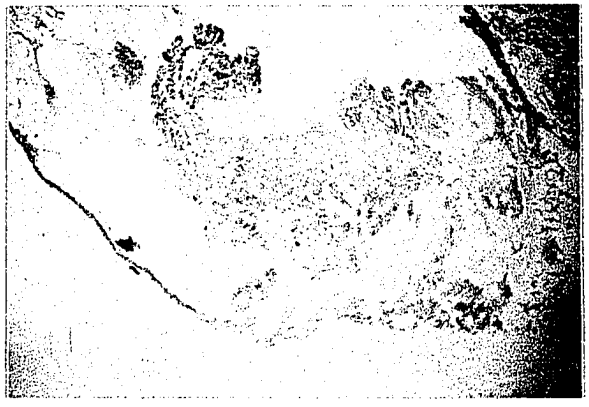
FIGURE 58
After 8 hours of incubation acetylcholinesterase positive staining nerves and ganglia are readily observed. Note the unstained cell bodies of cholinergic neurons in the two ganglia in the left half of the photo. 25X. 50 ASA.

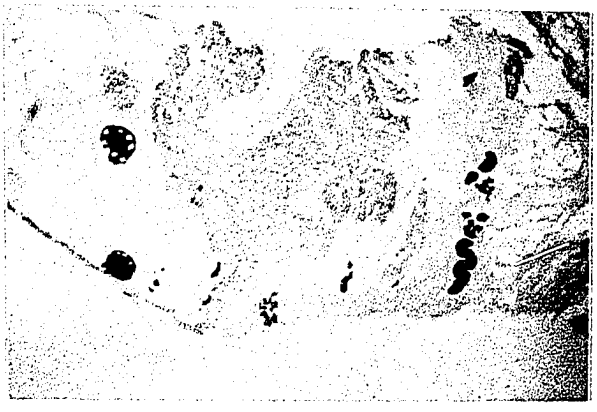
FIGURE 59
Following 20 hours of incubation acetylcholinesterase positive staining nerves were stained even more. Even the cell bodies in the ganglia appeared stained. Large nerve trunks which have not stained are seen at the left extremity of the photo. Cardiac muscle fibers remain unstained. 25X. 50 ASA.











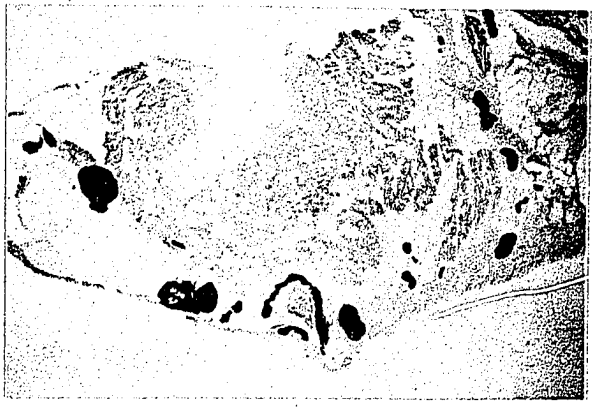


FIGURE 60

Acetylcholinesterase positive staining of nerves to an artery in the epicardium of the left ventricle of dog 13. The ventricular myocardium remained unstained after 19 hours of incubation. Nerves extended into the ventricle in septae usually containing blood vessles. 25X. 50 ASA.

FIGURE 61

Acetylcholinesterase positive stain of nerves associated with an intramural blood vessel in the left atrium of control dog 1. 250X. 50 ASA.

FIGURE 62

Acetylcholinesterase positive staining epicardial and myocardial nerves in the right atrium of dog 7 whose heart was completely extrinsically denervated 3 months previously. 40X. 500 ASA.

FIGURE 63

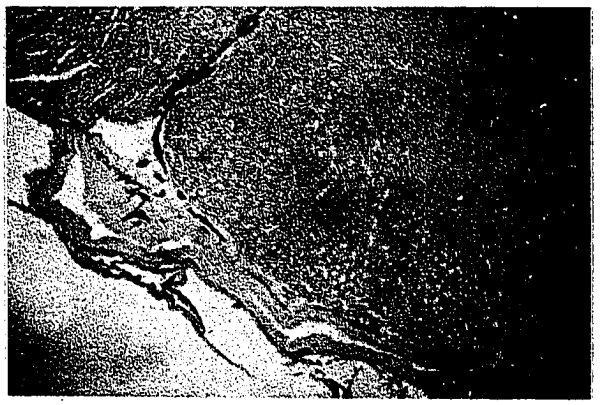
Acetylcholinesterase positive staining of an epicardial blood vessel of the right atrium in dog 13 after nineteen hours of incubation. Note the concentration of neurons in the adventitia. 40X. 50 ASA.

FIGURE 64

Acetylcholinesterase positive staining of cardiac nerves in the right atrium three months following complete extrinsic cardiac denervation in dog 7. 100X. 500 ASA.

FIGURE 65

Acetylcholinesterase positive staining nerves extending through the adventitia into the depths of the media in this left ventricular artery. The clear area in the left lower quadrent is the lumen of the vessel. This section was incubated for 19 hours. 400X. 50 ASA.



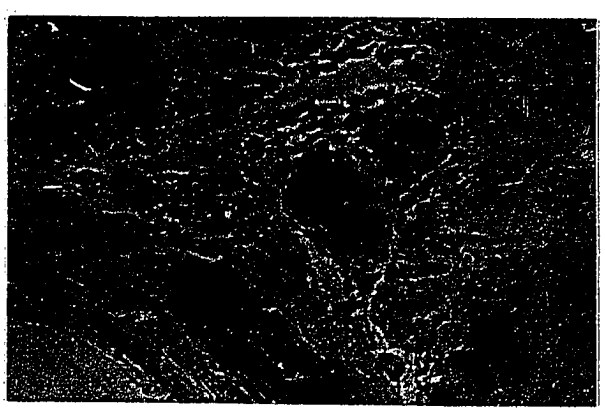


FIGURE 66

The left coronary artery of control dog 2 showing little acid mucopolysaccharide ground substance in the media, though small deposits are noted subintimally. No haline deposits are present. Alcian - PAS stain. 250X. 50 ASA.

FIGURE 67

The left coronary artery of control dog 2 shows little collagen in the media while the adventitia is composed primarily of collagenous fibers. Verhoeff's-Van Giesen's stain. 250X. 50 ASA

FIGURE 68

The left coronary artery of control dog 3, 10 years old, shows deposition of blue staining acid mucopolysaccharide and PAS positive red hyaline deposition in the media. Alcian-PAS stain. 250X. 50 ASA.

FIGURE 69

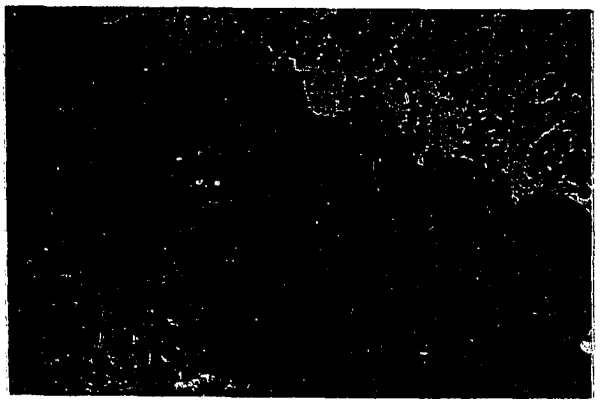
The left coronary artery of control dog 3. Note the few number of smooth muscle cells and great quantities of collagen deposition, in pink, within the media. Verhoeff's-Van Giesen's stain. 250X. 50 ASA.

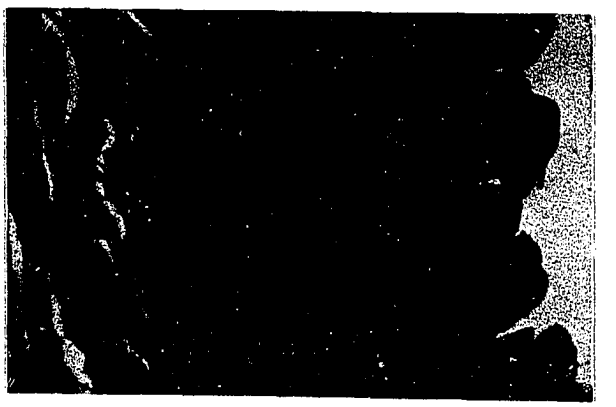
FIGURE 70

The right coronary artery of dog 17, which had the sympathetic trunk cranial to the vertebral ganglion transected bilaterally. Little sclerotic change is observed. Acid mucopolysaccharide deposits are limited and smooth muscle cells predominate in the media. Alcian-PAS stain. 250X. 50 ASA.

FIGURE 71

The right coronary artery of dog 17 shows very sparce collagen deposition and elastic fibers in the media. Verhoeff's-Van Giesen's stain. 250X. 50 ASA.





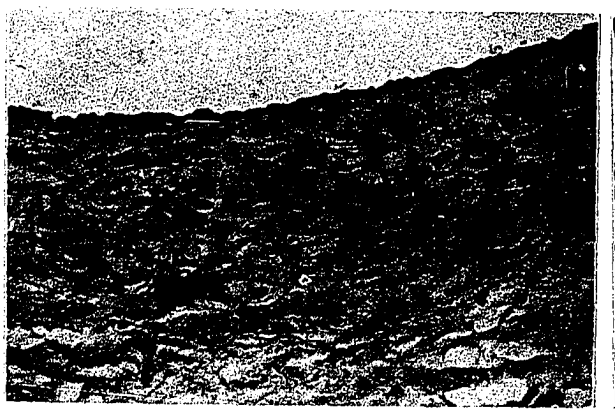


FIGURE 72

The left coronary artery of dog 14 following bilateral extirpation of the thoracic and cervicothoracic ganglia. Observe particularly the subintimal deposition of acid mucopolysaccharides and PAS positive material as well as medial depositions. Alcian-PAS stain. 250X. 50 ASA.

FIGURE 73

The left coronary artery of dog 14. Medial collagen and elastic tissue is abundant. Verhoeff's-Van Giesen's stain. 250X. 50 ASA.

FIGURE 74

The left coronary artery of dog 10, 7 years of age, showing medial sclerotic changes. Considerable acid mucopolysaccharide deposition (blue) and PAS positive material (red) is seen. Alcian-PAS stain. 250X. 50 ASA.

FIGURE 75

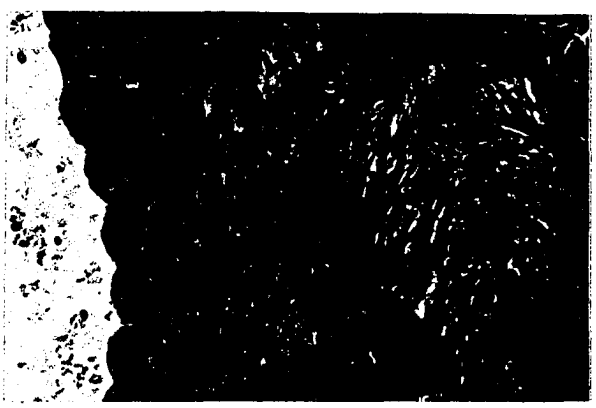
The left coronary artery of dog 10 in which the right vagus nerve was transected. Abundant deposition of collagen (pink) can be observed together with a large number of elastic fibers (black). Smooth muscle cells are sparce. Verhoeff's-Van Giesen's stain. 250X. 50 ASA.

FIGURE 76

The left coronary artery of dog 16 which had both vertebral ganglia removed. Acid mucopolysaccharide is abundant both in the subintimal area and throughout the media. Alcian-PAS stain. 250X. 50 ASA.

FIGURE 77

Left coronary artery of dog 16. Note moderate amounts of collagen on elastic fibers. Verhoeff's-Van Giesen's stain. 250X. 50 ASA.



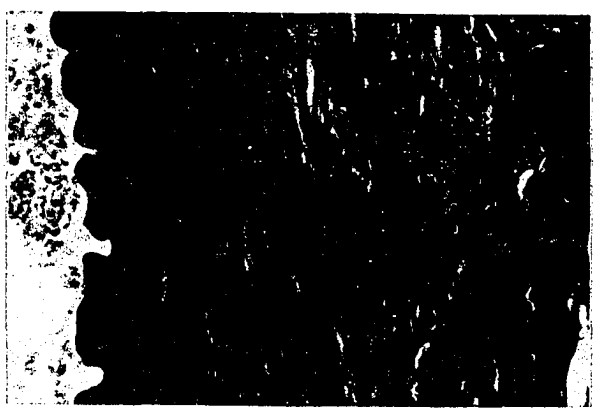


FIGURE 78

The left coronary artery of dog 9 which surgically had the left extrinsic cardiac nerves transected. This section stained with routine hematoxyline and eosin stains gives little indication of the changes observed in the next two figures. 250X. 50 ASA.

FIGURE 79

The same area of the left coronary artery of dog 9 showing considerable acid mucopolysaccharide ground substance and PAS positive hyaline deposition in the media. Alcian-PAS stain. 250X. 50 ASA.

FIGURE 80

The same area of the left coronary artery of dog 9 showing increased medial collagen. Verhoeff's-Van Giesen's stain. 250X. 50 ASA.

FIGURE 81

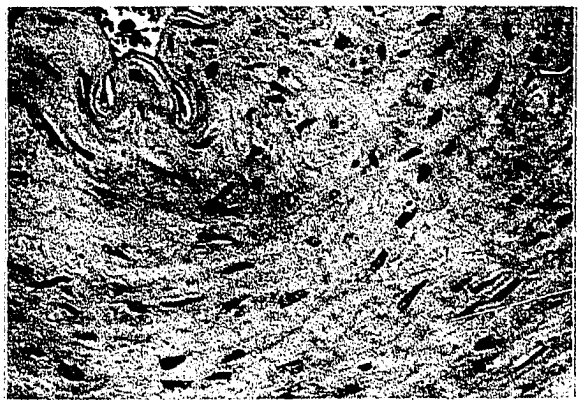
The left coronary artery of dog 8 which surgically had the right extrinsic cardiac nerves transected. Observe the increased acid mucopolysaccharide deposition, longitudinal orientation of many smooth muscle cells, and sparce deposition of PAS positive material in the media. Alcian-PAS stain. 250X. 50 ASA.

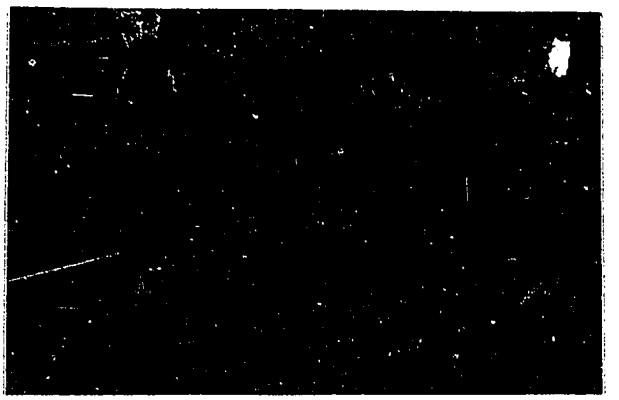
FIGURE 82

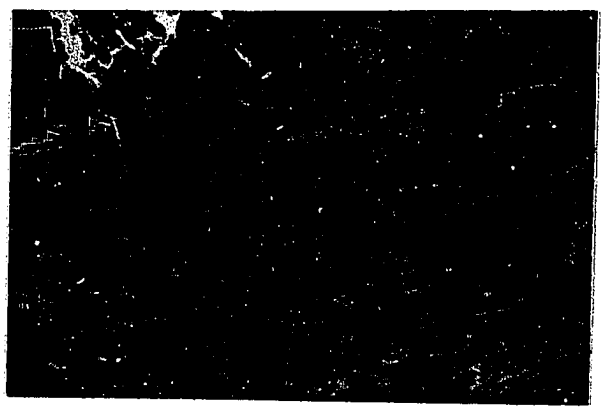
The right coronary artery of dog 7 which had complete extrinsic cardiac denervation 3 months previously. Observe the increased deposition of acid mucopolysaccharides subintimally and throughout the media. Alcian-PAS stain. 250X. 50 ASA.

FIGURE 83

Enlarged photograph taken from the same area as in Figure 82 illustrating the subintimal deposition of acid mucopolysaccharide.







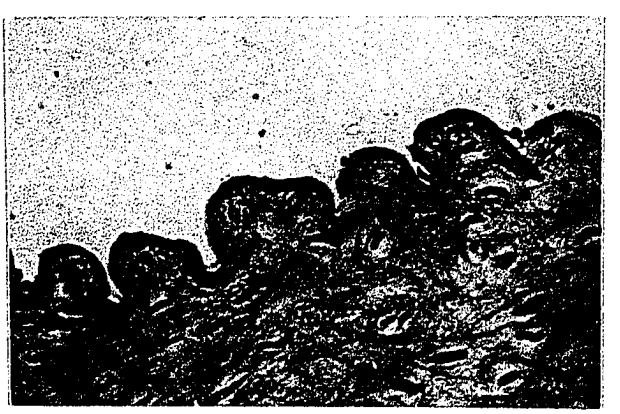


FIGURE 84

The right coronary artery of control dog 1 shows a faint orange area suggestive of lipid deposition in the media. Oil Red O stain. 400X. 50 ASA.

FIGURE 85

A lower magnification of the same artery as in Figure 84 showing the area of suggestive fat deposition in the media at the top of the photo. Oil Red O stain. 100X. 50 ASA.

FIGURE 86

The right coronary artery of control dog 2 shows no deposition of fat in the media but lipid droplets were present in the adventitia. Oil Red O stain. 100X. 50 ASA.

FIGURE 87

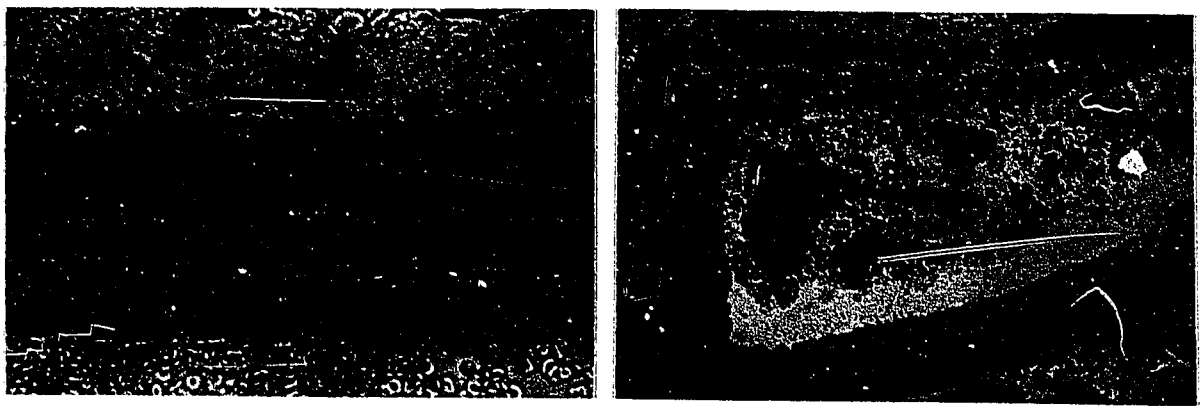
The right coronary artery of dog 14 which surgically had the cervicothoracic and thoracic ganglia bilaterally extirpated. This section shows lipid droplets in the media and in the lumen of the vessel. Most of these droplets focused at a level different than the tissue and were not considered as true fat deposits in the in vivo tissues. Oil Red O stain. 400X. 50 ASA.

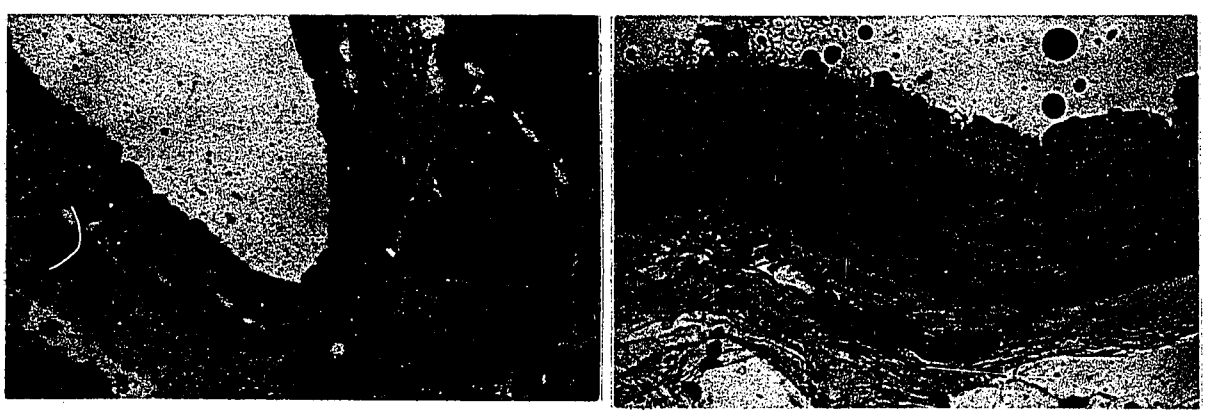
FIGURE 88

The right coronary artery of control dog 4 illustrating that no fat was observed in the media. Oil Red O stain. 250X. 50 ASA.

FIGURE 89

The right coronary artery of dog 6, nine months following complete extrinsic cardiac denervation showed adventitial fat and areas suggesting medial lipid deposits near the adventitia. Some of these medial deposits were stain artifacts. Oil Red O stain. 100X. 50 ASA.





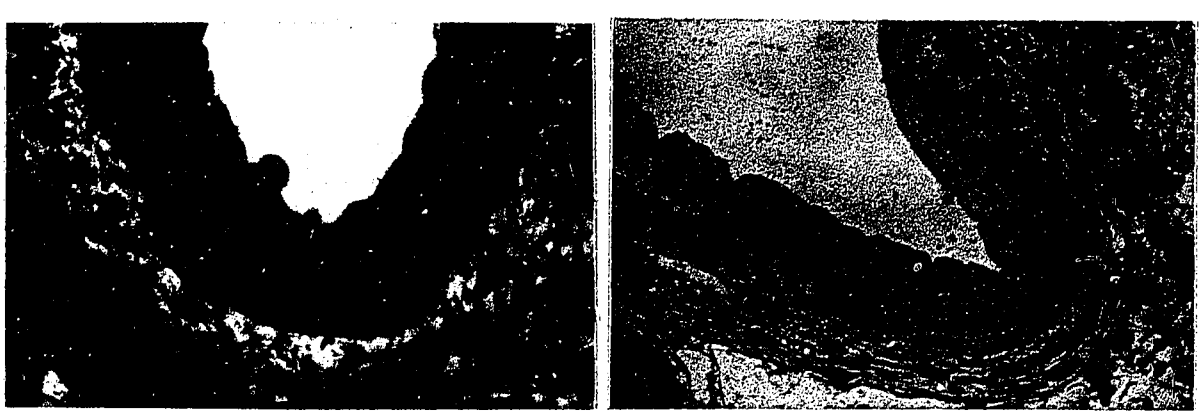


FIGURE 90

Branching of the right coronary artery in dog 9 illustrates the abrupt change in vascular components. Alcian-PAS stain. 40X. 50 ASA.

FIGURE 91

Greater magnification of the branching observed in Figure 90 showing increased collagen and elastic tissue at the stressed areas (cushions) where branching occurs. Verhoeff's-Van Giesen's stain. 100X. 50 ASA.

FIGURE 92

Increased magnification of the upper vascular wall at the arterial branching in dog 9 showing great accumulations of acid mucopolysaccharide ground substance, and reorientation of smooth muscle fibers. Alcian-PAS 250X. 50 ASA.

FIGURE 93

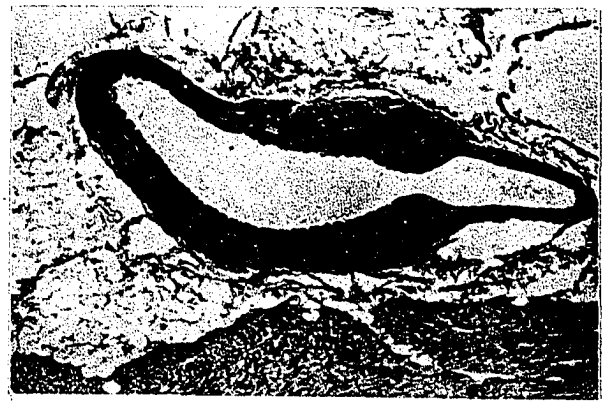
This is a similar area and magnification to that in Figure 92, showing the collagen and elastic fiber concentrations in this area. Verhoeff's-Van Giesen's stain. 250X. 50 ASA.

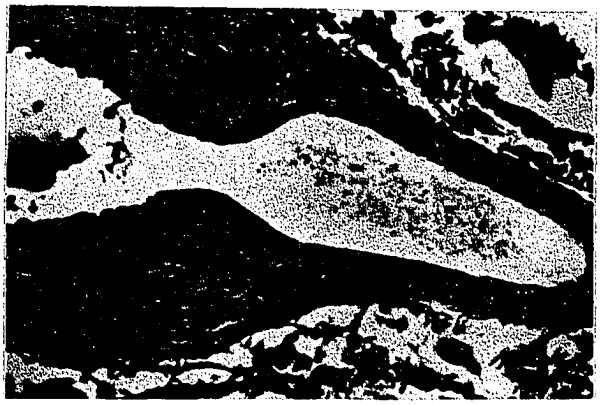
FIGURE 94

In this increased magnification of Figure 92, layering of the subintimal tissue appears to be present and some smooth muscle cells are very small in diameter. Alcian-PAS stain. 400X. 50 ASA.

FIGURE 95

This photograph of the same area seen in Figure 94, illustrates the fraying of elastic fibers from the internal elastic membrane, small smooth muscle cells near the lumen, and increased collagen. Verhoeff's-Van Giesen's stain. 400X. 50 ASA.





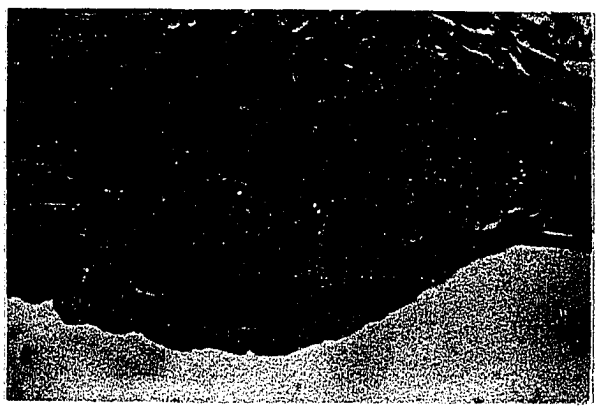


FIGURE 96

Branching of the left coronary artery in dog 10 shows fat accumulation (orange) in the cushion, accompanying the changes noted in Figures 90, 91, 92, 93, 94 and 95. The lumen of the vessel is toward the bottom of the photo and the branch of the coronary artery continues off the right side of the photo. Considerable orange staining lipid material is located in the perivascular space and in the lumen. This latter material did not reflect in vivo morphology. Oil Red O stain. 100X. 50 ASA.

FIGURE 97

This photo though staining lightly shows that dark staining acetylcholinesterase positive nerves are still present in this coronary vessel of the left atrium, after right vagotomy in dog 10. Three hours ACHE stain. 400X. 500 ASA.

FIGURE 98

Thick sheets of acid mucopolysaccharides (blue) are observed throughout the media of the left coronary artery in dog 13 in which the thoracic ganglia were bilaterally removed. Alcian-PAS stain. 400X. 50 ASA.

FIGURE 99

This photo illustrates collagen occupying some of the same areas as the acid mucopolysaccharides of Figure 98. They appear to be extending in fascicles originating from the adventitia at the top. Verhoeff's-Van Giesen's stain. 400X. 50 ASA.

FIGURE 100

Vascular changes are not limited to the main coronary arteries, as is seen in this photograph of an epicardial vessel in the left ventricle of the 10 year old control dog 3. Acid mucopolysaccharides (blue) have accumulated throughout the media and smooth muscle cells are sparce. PAS positive material is present (red). Alcian-PAS stain. 400X. 50 ASA.

FIGURE 101

An epicardial vessel of the right atrium in dog 3 also shows sclerosis. Large bands of acid mucopolysaccharides appear to be penetrating the media from the adventitia. Deposits of PAS positive material are also prevalent in the media. Alcian-PAS stain. 400X. 50 ASA.

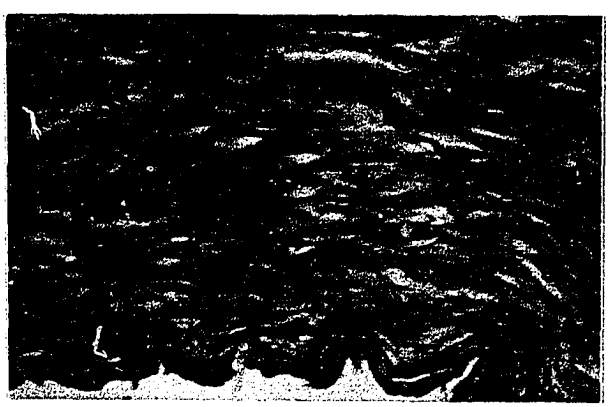


FIGURE 102

Deep within the myocardium of dog 15 in which the thoracic rami communicantes were transected, this fibrosed vessel was found together with the surrounding myocardial degeneration. Hemotoxyline and Eosine stain. 40X. 50 ASA.

FIGURE 103

Enlarged view of the vessel seen in Figure 102. Hemotoxyline and Eosine stain. 250X. 50 ASA.

FIGURE 104

The same vessel and magnification as in Figure 102, showing collagen fibrosis (red) of the vessel and surrounding tissue. Verhoeff's-Van Giesen's stain. 40X. 50 ASA.

FIGURE 105

Enlarged view of the vessel showing collagenous and elastic fiber fibrosis of the artery. No perivascular invasion by leucocytes is present. Verhoeff's-Van Giesen's stain. 250X. 50 ASA.

FIGURE 106

View of the same vessel using the Alcian-PAS stains. The wall of the vessel was composed of acid mucopolysaccharides. More intense PAS positive staining surrounded the vessel in the myocardium. 40 X. 50 ASA.

FIGURE 107

Alcian-PAS stain of the same vessel illustrating the sparce number of nuclei and the accumulated acid mucopolysaccharide in the media. The intima appears intact. 250X. 50 ASA.

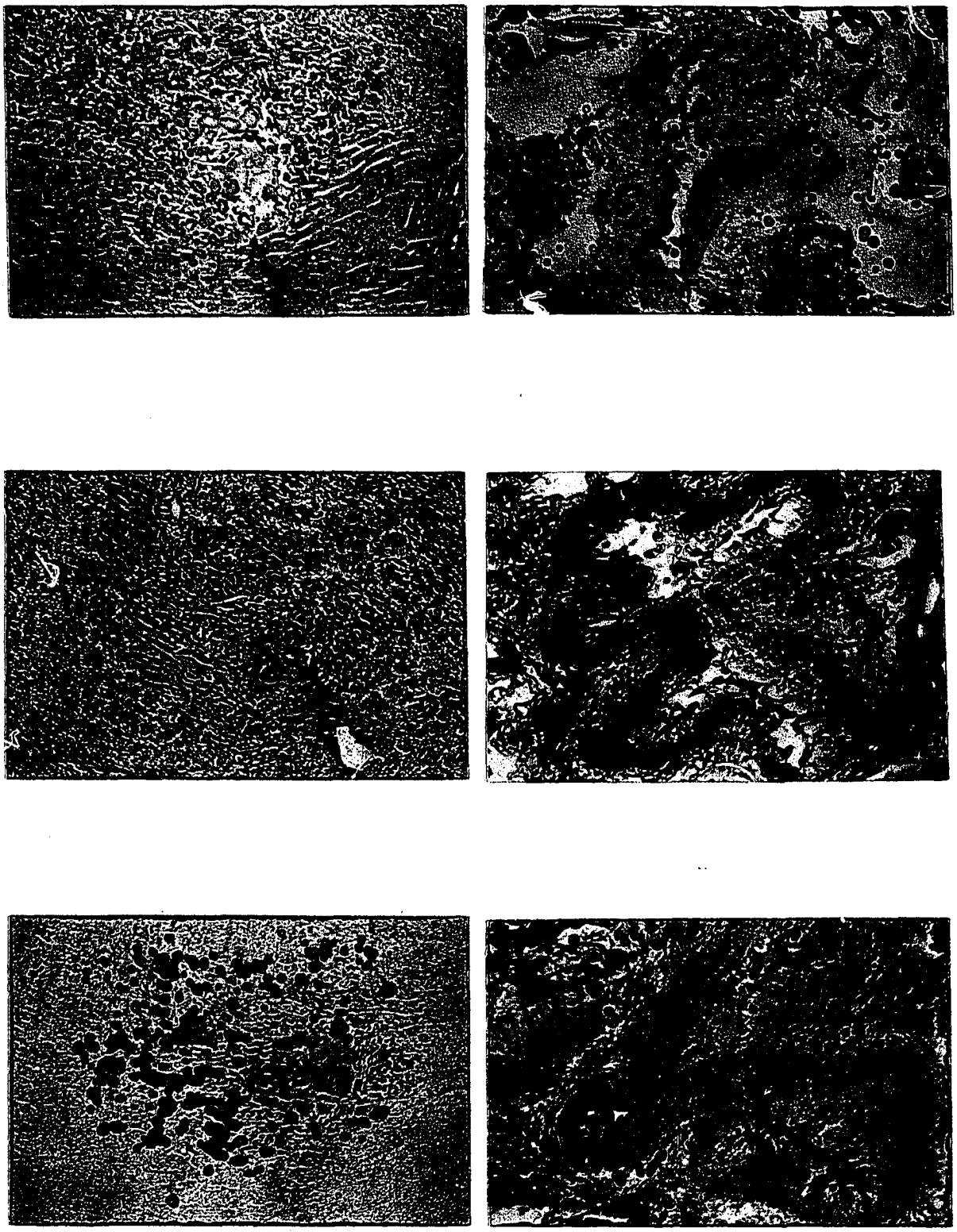


FIGURE 108

This epicardial artery of the left ventricle in control dog 1 contained in medial fat. A muscular cushion extends into the lumen from the bottom of the vessel. Oil Red O stain. 100X. 50 ASA.

FIGURE 109

Proliferative sclerotic changes have nearly occluded the lumen of this deep myocardial vessel in the 10 year old control dog 3. Lipid deposits (red) are accumulating in the media as well as the adventitia. Oil Red O stain. 100X. 50 ASA.

FIGURE 110

The luminar surface of this vessel, located in the left ventricle of dog 8 which had its right extrinsic cardiac nerves transected, was irregular with protrusions into the lumen which were undermined by vascular channels in the media. Acid mucopolysaccharide ground substance predominated in the wall of the vessel. Alcian-PAS stain. 40X. 50 ASA.

FIGURE 111

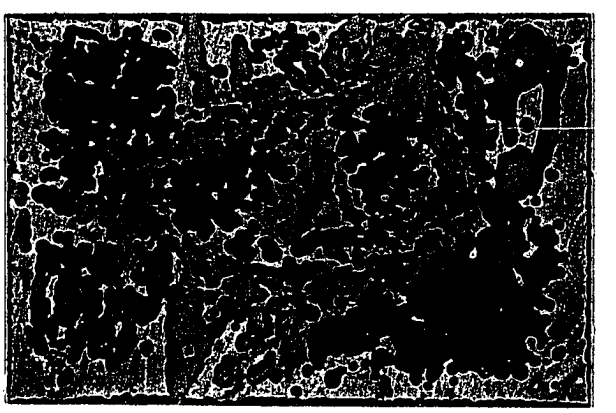
This vessel with greatly thickened walls which almost occluded the lumen was found in the left ventricular myocardium of dog 7 which surgically had its extrinsic cardiac nerves transected 3 months previously. Alcian-PAS. 40X. 50 ASA.

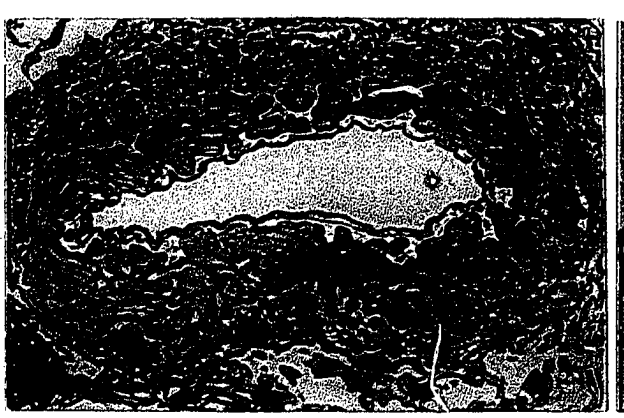
FIGURE 112

Dog 7 also showed vascular change in this right atrial vessel. The internal elastic membrane was double, collagen was invading the media from the adventitia and all muscle fibers were not oriented in the same direction. Verhoeff's-Van Giesen's stain. 100X. 50 ASA.

FIGURE 113

This photograph better demonstrates the double internal elastic membrane of Figure 112. Verhoeff's-Van Giesen's stain. 400X. 50 ASA.





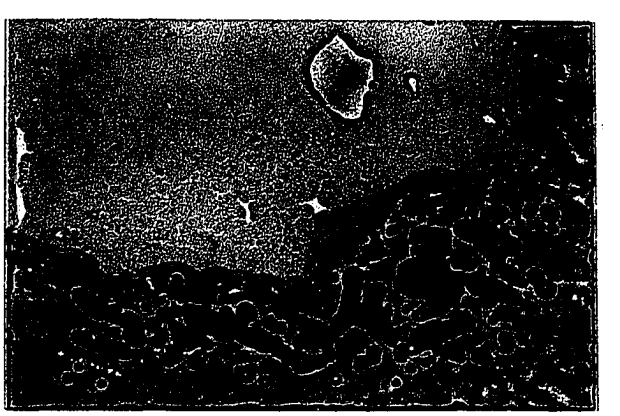


FIGURE 114
Tissue glycogen (red) content of this left ventricular muscle was average to slightly elevated in dog 6 in which the extrinsic cardiac nerves were transected. Alcian-PAS stain. 100X. 50 ASA.

FIGURE 115
Tissue glycogen content was similar in longitudinal section in dog 6. Myocardial fiber striations and intercalated discs are easily observed. Alcian-PAS stain. 100X. 50 ASA.

FIGURE 116
Lipid droplets (orange) in the left ventricular myocardium of control dog 1 are illustrated in this photo. They were often patchy areas and were felt generally to be droplets which migrated and stained during the tissue processing. Oil Red O. 400X. 50 ASA.

FIGURE 117
Large red staining Purkinje fibers were richer in glycogn than other myocardial fibers. The Purkinje cells occupy the top half of this photo. Note the size relationship between Purkinje cells and small right ventricular myocardial cells in this 3 month old dog 4. Alcian-PAS stain. 250X. 50 ASA.

FIGURE 118
The size differential between Purkinje fibers on the left, and left ventricular myocardial fibers on the right side of the photo, is not very great. The glycogen content, however, is still higher in Purkinje fibers in this 10 year old control dog. Alcian-PAS stain. 400X. 50 ASA.

FIGURE 119
Purkinje fibers in longitudinal section in this photo were apparently not affected by left extrinsic cardiac denervation (dog 9). Alcian-PAS stain. 100X. 50 ASA.

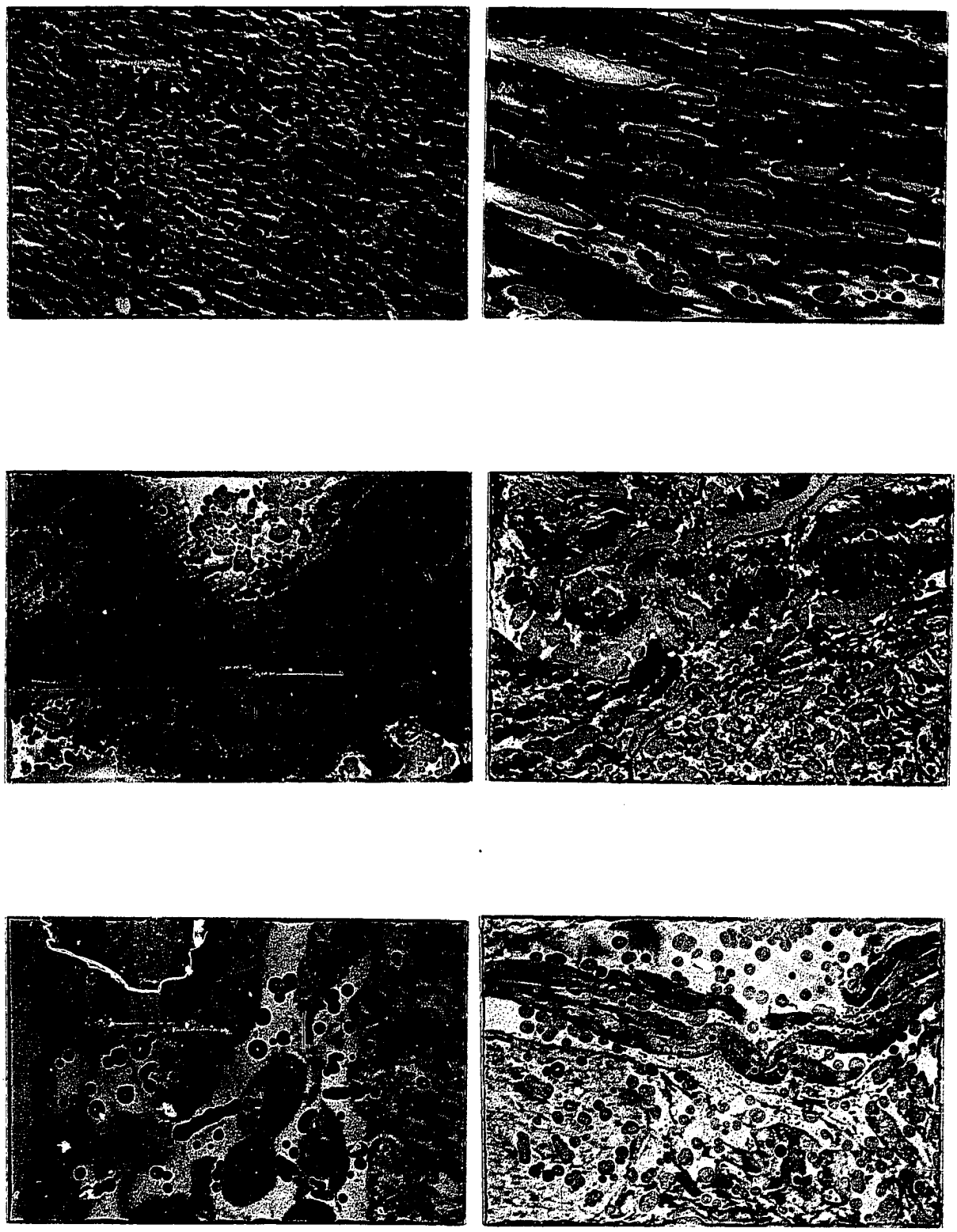


FIGURE 120

The diameter of the myocardial cells is very small in this three month old dog 4. Note also the acid mucopolysaccharide staining endocardium to the right side of the photo. Alcian-PAS. 250X. 50 ASA.

FIGURE 121

This Virtis homogenizer (stainless steel) was combined with a ten Broeck grinder (glass extending into sink) to obtain satisfactory tissue homogenates.

FIGURE 122

Some glassware which was utilized for chemical analyses included the 20 lambda pipetts in the container in the background, 0.1, 1.0, and 5.0 ml pipetts in the middle, Ten Broeck tissue grinders (tube and pestle), small fluorometry micro-cuvettes in the foreground, graduated centrifuge tubes and spectrophotometer tubes in the background. The white pad in the foreground contains parafilm which is useful in covering glassware when mixing.

FIGURE 123

This photograph shows some of the other equipment used in chemical analyses. The white instrument on the left is the spectrophotometer. The fluorometer is on the right. A timer, centrifuge, and mixer are in the center along with other smaller equipment.