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# Serum enzyme levels and electrocardiographic changes in dogs with surgically-induced cardiac alterations.

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# SERUM ENZYME LEVELS AND ELECTROCARDIOGRAPHIC CHANGES IN DOGS WITH SURGICALLY-INDUCED CARDIAC ALTERATIONS



by

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# I. INTRODUCTION

Today, small animal pets are receiving increasingly better care. This leads to greater longevity. The treatment of geriatric problems by the small animal clinician has become an important phase of practice, and this phase will continue to grow. Many of these problems are centered around cardiac and liver pathologies, and the means now available for diagnosing these abnormalities in dogs often fail to give unequivocal information. Therefore, treatment is often based on intuition and past experience with no guarantee that the treatment is specific for the condition presented.

As our knowledge of diseasescontinues to increase, methods to diagnose them must improve. Some of the problems that faced the veterinarian in recent years have been resolved; however, very little progress has been made in the area of diagnosis of cardiac problems. The electrocardiograph has been used as a diagnostic aid in veterinary medicine yet most of the information, which can be derived from it, is empirical since much of the interpretation of the canine electrocardiogram has been based on studies from the human electrocardiogram.

In the past few years great advances have been made in enzyme chemistry. Many serum enzymes have been investigated and a few of them have been shown to be of value as diagnostic aids in human medicine. It appears that the

concentrations of some of these same enzymes in blood serum may be used by the veterinary profession as diagnostic aids. The ones which have proved to be most useful in human medicine in diagnosing certain abnormalities of the myocardium and which have given some indication of responding in the same manner in dogs have been serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic (SGPT), and serum lactic dehydrogenase (SLDH).

The primary objectives of this investigation were to 1) establish a normal set of values for serum enzyme levels of glutamic oxalacetic transaminase, glutamic pyruvic transaminase, and lactic dehydrogenase in dogs; 2) to establish the accuracy of a clinical assay test commonly used by research workers; 3) to examine the usefulness of these serum enzymes as diagnostic indexes of myocardial necrosis, valvular insufficiency and epicarditis; 4) to determine what constitutes a normal canine electrocardiogram; and 5) to define any electrocardiographic changes which may be of importance in diagnosing these pathologic conditions and correlating them with the changes observed in serum enzymes levels.

# II. REVIEW OF LITERATURE

## A. Enzymes

In the past ten years there has been an explosive increase in interest in the enzymes of blood and other tissues. Many laboratories have become concerned with this intriguing field with considerable volumes of data being accumulated. Following the demonstration of the normal range of activity of certain enzymes in the serum of animals and man, a sizable experience has accrued attesting to the experimental as well as the clinical implications of alterations in the serum activity in pathological conditions of certain tissues and organ systems. The major part of this work was done in human medicine and several reviews are available which describe the clinical significance of these enzymes as well as some of the work done experimentally with dogs (15, 46, 57, 60, 101, 143, 161). Even though much of the early work was done with dogs, the emphasis was soon placed on the importance of these enzymes in human medicine and animal data were either forgotten or disregarded. Very little work has actually been done by the veterinarian in the study of these enzymes and their possible clinical significance in diagnosis. Early studies were merely pilot studies and results were used mainly for comparison with those found in human serum levels. The early work in veterinary medicine has been done largely by Cornelius et al. (31, 32,33) of California.

Many methods have been devised for measuring these serum enzyme levels. The first methods were based on chromatography (78, 133), later spectrophotometry and colorimetry (17, 22, 64, 65, 76, 77, 107, 128, 134, 149, 150, 154, 163) then fluorimetry (87, 88). Both macro and micro determinations are possible. There are available for the clinician today simple, inexpensive, clinical methods (14, 34, 145).

Clinicians, impressed by the ease of frequent, adequate sampling of blood and the elegance of spectrophotometric enzyme assay, have done a great deal of the work on the investigation of enzyme alterations. In only a few cases, chiefly with cellular enzymes, have any clinical studies been directed toward an understanding of the mechanisms of enzyme alterations in disease conditions. Only in the past two or three years have factors such as the movement of enzymes across cell membranes, the origin and excretion of enzymes of serum, the relationship to stress, the influence of diet, and the characteristics of these enzymes come under investigation. With such studies came progress in the study of basic physiologic and pathologic processes as well as in the clinical areas.

Enzymes are organic catalysts produced as a result of cellular activity but independent of the presence of living cells in their operation. The name "enzyme" (from the Greek, "in leaven"; "in yeast") is associated with the process of

fermentation. Enzymes act on bio-chemically important substances. One essential difference must be stressed between inorganic catalysts such as platinum, which catalyzes many chemical reactions, and organic enzymes such as SGOT, SGPT, and SLDH which are extremely specific. It is well known that most chemical reactions do not proceed entirely in one direction. Several reactions of the substrate are possible, depending upon the nature of the enzyme. Because of its specificity, the enzyme catalyzes only one of these reactions.

A chemical reaction which is reversible will attain a state of equilibrium when the velocity of the forward reaction is equal to the velocity of the reverse reaction. The factors which influence the equilibrium state are temperature, pressure, concentration of the reacting substances and the presence of a catalyst. The catalyst speeds up both the forward and backward reaction and thus allows the system to attain a state of equilibrium in a shorter time. A catalyst will not make a reaction proceed unless that reaction can proceed of its own accord, no matter how slowly.

The chemical properties of enzymes, as well as their stability and solubility characteristics, are closely related to those of proteins. As more and more enzymes have been isolated in a crystalline form and have proved to be single components, it has become more certain that enzymes are indeed proteins. Recently, it has been found that certain

enzymes are composed of component enzymes termed isozymes.

So far, no enzyme has been isolated which is not a protein. It is also true, however, that in many instances for an enzyme to be active some co-factor of a non-protein nature is required. These inorganic ions may be attached to the enzyme protein. Such ions include $Ca^{++}$, $Mg^{++}$, $Mn^{++}$, or $Zn^{++}$.

The broad category of enzymes can be broken down into a classification on the basis of several criteria. In this research interest has been placed on a particular group of enzymes, termed transferases, and more specifically on a group of enzymes designated as transaminases.

The role of the transaminase reaction in metabolic transformations is twofold (62). First, it provides a machanism for the interconversion of amino acids via keto acids. Thus one can visualize the synthesis of an important amino acid like glutamic acid from alpha-ketoglutaric acid, which arises as the result of the oxidation of carbohydrates. In this way, an interrelationship between glutamic acid, aspartic acid, alanine and the tricarboxylic acid cycle intermediates is established. Secondly, transamination provides a mechanism for the oxidation of many L-amino acids, according to the following reactions:

L-Amino acid + α-ketoglutaric acid > α-keto acid
+ L-glutamic acid

L-Glutamic acid + 1/2 $O_2$ α-ketoglutaric acid + $NH_3$

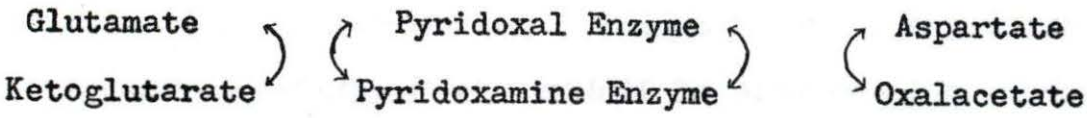
Glutamic acid dehydrogenase, the enzyme involved in the last reaction is capable of linking the metabolism of amino acids and carbohydrates without the need for oxidative deamination, which, in the mammal, is not quantitatively important, and can be forgotten as far as this discussion is concerned (62). In addition, transamination is important in the biosynthesis of many amino acids.

There is evidence that separate transaminases exist for the separate reactions (23). These purified enzymes are stimulated by the addition of pyridoxal phosphate (vitamin $B_6$), for when an animal is placed on a diet deficient in this vitamin, the transaminase content of tissues decreases (62). In addition, the amine form of vitamin $B_6$, pyridoxamine phosphate, is also a stimulant for these enzymes. This has led to the hypothesis that the enzymatic activity involves the interconversion between the two co-enzyme factors. Meister (103) has suggested the following formulation of the reaction:

Pyridoximine phosphate and enzyme $\longrightarrow$ enzyme-pyridoxamine phosphate

or (pyridoxal phosphate) (enzyme-pyridoxal phosphate)

Enzyme-pyridoxamine phosphate and keto acid = enzyme-pyridoxal phosphate and amino acid. Jenkins (74) reports that there is no doubt that the over-all reaction is as follows:




Indications for the existence of a coenzyme activator for the apo-transaminases were supported by the evidence from experimental studies of vitamin $B_6$ deficiency. Deficiency of vitamin $B_6$ was associated with lowered levels of transaminase activity, and the addition of pyridoxal phosphate to the tissue or cell preparation resulted in at least partial restoration of enzyme activity (8, 12, 91, 140). Recent studies have confirmed earlier work on the activation of transaminase by pryidoxal phosphate, and have shown as well that many of the newly discovered transaminases also require this coenzyme (103).

In 1955 Marsh et al. (100) reported that in both man and monkeys, increasing the intake of pyridoxal resulted in a significant increase in the blood level of the transaminase and of vitamin $B_6$. Reductions in the pyridoxal intake were followed by a lowering of the blood concentration of both factors. The changes in the level of transaminase were often more gradual, however, than were those of vitamin $B_6$. The time required to reduce the levels to a minimum after withdrawal of all pyridoxine or to the initial levels after the supplemental dosage was discontinued seemed to be dependent to some extent upon the duration of the period of the increased intake.

In 1959 Jenkins and co-workers (75) showed that glutamic aspartic transaminase contains two residues of tightly bound

pyridoxal phosphate per molecule. Their data support the theory that the "pyridoxal" form is more stable than the "pyridoxamine" form of this enzyme.

Ranke et al. (126) reported on the relationship of vitamin $B_6$ and transaminases in 1960. They found that there is a mild state of vitamin $B_6$ deficiency among healthy, old adults. It was also found that these older individuals had a lower SGOT than young individuals, regardless of which groups of old and young subjects were compared. Daily oral administration of 15 mg. of vitamin $B_6$ to the elderly individuals elevated the SGOT content. The addition of pyridoxal phosphate to the sera in vitro elevated the SGOT content of the sera of old individuals.

It is unlikely that the observed differences in SGOT can be explained solely by differences in dietary vitamin $B_6$ intake, since this study was done on three groups of young subjects and three groups of old subjects on a variety of diets. It does not seem that differences in the SGOT content with age are primarily due to a progressive decrease of dietary content of vitamin $B_6$. On the other hand, the SGOT levels can be raised if the intake is sufficiently large. The in vitro experiment showed that the decrease in SGOT activity in the aged is due primarily to the decrease in the coenzyme, pyridoxal phosphate, and not to the protein moiety or the apoenzyme of the SGOT molecule. The possibility of regression of leukocyte

pyridoxal phosphate content with age has been suggested by others and this could possibly be the factor involved.

In 1950 Cammarata and Cohen (24) showed that at least 25 amino acids participate in the transamination reaction. The reaction was catalyzed by aqueous extracts of pig heart, liver, and kidney (later many more tissues have been used). Each transamination reaction appeared to be due to a different transaminase. They found that the reaction was accelerated by pyridoxal phosphate and slightly inhibited by ammonia.

The variety of amino and keto acids capable of participating in transamination makes it necessary to distinguish the different effects. Accordingly, it has become customary to distinguish the enzymes responsible for transamination reactions in one of several ways. The enzymes which catalyze reversible transamination reactions are designated by a term referring to both amino acids concerned, e.g., glutamate-aspartate transaminase. Another method of designating the enzymes is to name them by a term describing the favored products of the equilibrium reaction, e.g., glutamic oxalacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT). The latter method of designation has been used in the clinical literature.

Although it now appears that at least several distinct transaminases exist in each of the tissues that have thus far been studied, final characterization of the individual catalytic systems is yet to be achieved. However, the chemical

separation and purification of the GPT and GOT of heart muscle have been reported by several groups (23, 55, 75). Both of these enzymes have also been separated from each other electrophoretically, using as an enzyme source, serum obtained from patients with hepatitis (161). It would thus appear that at least GPT and GOT are separable, distinct, and individual catalytic systems.

Transamination was first suggested as a possibility by Nadham in 1927 and later demonstrated in pigeon breast muscle in 1937 by Braunstein and Kritzmann (19). They found that when L-glutamic acid and pyruvic acid were incubated with chopped liver or muscle α-ketoglutaric acid and alanine were produced, the amino group of the glutamic acid being transferred to pyruvic acid. Transamination, thus, involves the transferral of an amino group ($NH_2$). As was mentioned earlier many amino acids can take part in this reaction, glutamic acid is especially active in this respect, and it is with this amino acid that this review is especially concerned. The two transaminases which have been found to have clinical usefulness are GOT and GPT, both of which have glutamic acid as part of the substrate upon which they exert their action. L-aspartic acid may replace glutamic acid, but the reaction is much slower.

The actual reactions are as follows:

1. L-Glutamic acid + oxalacetic acid
   ↓ ↑
   α ketoglutamic acid + L-aspartic acid

2. L-Glutamic acid + pyruvic acid
   ↓ ↑
   α ketoglutamic acid + L-alanine

According to Cohen and co-workers (30), reaction 1 proceeds to the right at the fastest rate in all tissues studied. The reaction to the left proceeds only 1/2 to 1/3 as rapidly. Reaction 2 is very slow when compared with reaction 1.

These two systems are widespread in higher plants, many microorganisms, and in the blood and tissues of animals and appear to be a function of tissues in general; the following descending order of concentration has been reported in man: heart muscle, skeletal muscle, brain, liver, kidney, testes, lung, and spleen (60).

Table 1. Average distribution of enzymes in human tissues (units/gm. wet tissue)

| | GOT | GPT | LDH |
|---|---|---|---|
| Heart | 155,000 | 7,130 | 240,000 |
| Liver | 142,400 | 43,800 | 390,000 |
| Skeletal Muscle | 99,300 | 4,750 | 600,000 |
| Kidney | 90,900 | 19,300 | 640,000 |
| Pancreas | 28,300 | 1,950 | 150,000 |
| Spleen | 13,600 | 1,210 | 140,000 |
| Lung | 10,000 | 668 | 25,000 |
| Serum | 20 | 15 | 400 |
| | (141) | (141) | (162) |

In 1959 Cornelius et al. (32) reported on various tissue activities of GOT and GPT in normal horses, cattle, calves, pigs, dogs, and fowls. They found considerable GOT activity in almost all tissue analyzed in each species, but high GPT activity was found only in the canine liver. After the administration of carbon tetrachloride and during subsequent hepatic necrosis, significant elevations in serum GOT activity were found in the horse, cow, pig and dog, but marked elevations of serum GPT activity were confined to the dog. Normal ranges of SGOT activity have been reported as 5 to 40 units, of SGPT as 5 to 40 units, and of SLDH as 100 to 600 units (132).

In the reaction shown above, pyruvic acid and oxalacetic acids, products of carbohydrate metabolism, are transferred into amino acids, thus showing how the body is able to synthesize some of its amino acids. The reverse reaction indicates how the more complex glutamic acid might be produced from alanine or aspartic acid and another intermediate in carbohydrate metabolism, alpha-ketoglutaric acid.

Since transamination is a rapid process and concerned chiefly with those compounds which play key roles in intermediary metabolism, Cohen (29) suggests that it represents a "shuttle" mechanism in tissue respiration "Whereby certain key protein and carbohydrate intermediates are rapidly interconverted". The demonstration of transamination affords further

evidence for the highly dynamic character of protein-metabolism. Amino groups are being continually exchanged between nitrogenous and non-nitrogenous compounds, and amino acids thereby synthesized. Transamination may be important from another standpoint. The non-nitrogenous compounds formed in these two-way reactions - pyruvic acid, alpha-ketoglutaric acid and oxalacetic acid - are concerned in oxidation-reduction systems.

Under normal conditions the abundant transaminases are confined, almost exclusively, within tissue cells and only very small amounts are found in the circulation. It is this relatively small "baseline" serum level which first suggested and has permitted ready detection of increased serum values resulting from the destruction of tissue and the assumed release of enzyme from the large tissue stores. However, whether or not all the serum increase comes from the particular tissue involved or if some other mechanism is involved is not known. It is relatively certain that most of it comes from the damaged tissue as much work has been done showing the great decrease of enzyme content in the pathologic tissue compared with the normal (4, 82, 89, 104, 116, 131, 132). Hamolsky and Kaplan (60) reported that the clinical findings of LaDue, Wroblewski and Karmen (85) have been strongly supported by experimental studies of myocardial damage by several workers using various techniques, revealing excellent correlations of

increased serum levels of enzyme activity with tissue damage. Significant elevations of SGOT, although short-lived, have been found with experimentally induced infarcts of less than 1 gm. of myocardium. There was a suggestive correlation between the size of the infarct and the height and duration of increased serum enzyme activity. Enzyme levels decrease rapidly in infarcted muscle as compared with normal muscle. In almost every instance, serum enzyme levels were not increased in the presence of significant myocardial ischemia without histologic necrosis. They further report that SGPT is frequently normal in the face of elevated SGOT in the less extensive infarctions, but may rise in the presence of large infarcts. Although it has been stated and generally believed that SGPT is not elevated until SGOT values of 150-200 units are reached, Rowell and Smith (130) found 14 episodes of elevation in human subjects of SGPT with SGOT values below 150 units.

There appears to be a highly effective mechanism for the rapid elimination or degradation of the enzyme from the serum. Dunn (40) reported on this in 1958. He found that following intravenous injection three-fourths of the injected enzyme disappeared from the blood within six hours. The remaining portion was cleared within 20 to 72 hours depending upon the amount injected. The rapid disappearance phase was due to a diffusion of the enzyme into the interstitial fluid. Equilibrium of serum and lymph GOT was reached within six to

eight hours and marked the end of the rapid disappearance phase. A diffusion of GOT from blood to interstitial fluid was also found following myocardial infarction and hepatocellular necrosis. In no case was any GOT found in the spinal-fluid or urine. He felt that no inactivation occurred in the kidney. The exact mechanism of destruction and elimination still remains obscure.

Recently it has been suggested by Van Rymenant and coworkers (155) that there is evidence that the elevated transaminase levels, found in patients with cancer, may decrease to normal following the administration of large quantities of protein. This opens the door for a vast amount of research upon protein levels and qualities and their effect upon serum transaminases.

Recently, a study was made of the effect of physical exercise on the serum GOT and GPT in humans (139). It was demonstrated that physical exercise of a moderately strenuous nature (running slowly, chin ups, swimming, and rowing) regularly raises the level of SGOT (in most instances to levels above the upper limit of normal). The SGPT, although showing a slight rise, tended to remain within normal limits. In other work involving rats, it was reported that there was a 2- to 6-fold increase in the serum GOT, GPT and LDH levels after exercise (7). This was much greater than that reported in man, but may be due to the longer period of exercise. In this study in rats, pathologic studies revealed transient

fatty changes in skeletal muscle fibers, heart, liver, kidney, and adrenal cortex; some rats also showed a few foci of inflammation and necrosis in the muscles. Highman and associates have published several papers relating to the stress of hypoxia and the effect of autonomic drugs upon serum enzyme activities (67, 68, 69). Gollnick and Hearn (54) reported that LDH activity was increased in the heart ventriclar muscle but remained unchanged in skeletal muscle following excercise in rats.

LDH refers to a group of enzymes which catalyze the reversible oxidation-reduction reaction between lactate and pyruvate, involving the conversion of the reduced and oxidized forms of the pyridine nucleotide (DPNH and DPN).

$$\text{Lactate} + \text{DPN}^{+} \underset{\longleftarrow}{\xrightarrow{\text{LDH}}} \text{Pyruvate} + \text{DPNH} + \text{H}^{+}$$

This reaction is a critically important step in the carbohydrate metabolic cycle and accordingly, the enzyme, a zinc-containing compound, is widespread, having been found in virtually every vertebrate and invertebrate species tested, in almost every mammalian tissue studied, and in many microorganisms (60). It is also present in body fluids such as urine, serum, serous effusion, and cerebrospinal-fluid (162).

In the presence of the enzyme, lactate is oxidized to pyruvate while DPN is reduced to DPNH; conversely, the enzyme will also catalyze the reverse reaction in which pyruvate is

reduced to lactate while the reduced DPNH is oxidized to DPN. Thus, measurement of enzyme activity can be carried out in either direction depending on the initial substrates employed and other factors, and different clinics have adopted one or the other method.

Meister (102) emphasizes the fact that LDH catalyzes the reduction of several different alpha-keto and alpha-gamma-diketo acids. The kinetics of LDH-catalyzed reactions have been explored in several reports (58, 113, 142, 151, 159).

Under normal conditions these enzymes are confined almost entirely within the tissue cells although a small amount is normally found in the blood and an even smaller amount in the cerebrospinal-fluid. Wroblewski (162) reported on the activity of human tissues in the following order from most to least: kidney, skeletal muscle, liver, heart, pancreas, spleen, brain, and lung (see Table 1). Unfortunately no corresponding figures are available for the various species of domestic animals.

LDH activity varies in body fluids in response to pathological processes in tissues bathed by these fluids.(162). Necrosis of tissue results in the release of intracellular LDH, which finds access into the extracellular fluid compartment, resulting in an increase in SLDH. In experimentally produced myocardial infarction in dogs, Wroblewski (162) reports that SLDH increases in proportion to the amount of necrotic myocardial tissue.

The determination of SLDH requires certain precautions in the handling of blood specimens. Erythrocytes are rich in LDH, and the slightest hemolysis can significantly elevate serum levels in a specimen. The erythrocytes also contain transaminases, but in lesser amounts, however, hemolysis is to be avoided (46). Hsieh and Blumenthol (72) reported that serum taken from clotted blood after one hour at room temperature showed a 25 percent increase in LDH as a result of leakage from the erythrocytes. They state that it is imperative to separate serum within thirty minutes after blood is drawn, unless refrigerated promptly. Blood can be stored for 24 hours with no increase in SLDH if kept at 4°C. Plasma from oxalated or citrated blood should not be used for serum enzyme determinations.(81, 114).

LDH is a relatively stable enzyme. Erickson and Morales (46) found that serum could be stored for at least a week at 4°C. and could be frozen for periods up to a month with negligible loss of LDH. King (81) found no change in serums stored for three weeks at 0 to 4°C., and Wroblewski and LaDue (167) reported no loss of activity in serum stored at room temperature for 96 hours. Sigma Chemical Company (145) recommends that undiluted serum be used within three days when stored at 4°C. or within 10 days when frozen when using their clinical reagents.

The transaminases are probably more stable than SLDH. Wroblewski (161) reports that storing serum at room temperature for 24 hours or at 4°C. for five days does not significantly alter transaminase activity. It has been reported that frozen samples remain stable for long periods of up to at least four months.[1]

Hess (66) discussed some of the possible mechanisms involved in maintenance of normal serum levels as well as the pathological serum levels of "DPN-dependent" enzymes. He states that the physiological disintegration of erythrocytes and other blood cells that lead to their normal end state might play a role in the maintenance of normal enzyme concentration, as may also that of the intestinal mucosa, whose high cellular turnover is indicated by its high mitotic rate. He thinks that there is insufficient evidence to support either of these possibilities; however, the former seems more likely. The cellular turnover rate of muscle and nervous tissue is minimal and may be neglected; in addition he has as yet been unable to determine any arteriovenous differences in enzyme activity either in normal persons or in tumor patients, which indicates the participation of either of these tissues, the possible physiological internal secretion of enzymes is a

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[1]Buck, W. Animal Disease Eradication Division, National Animal Disease Laboritories, Ames, Iowa. Serum enzyme stability. Personal Communication. 1960.

highly interesting aspect of the matter, but evidence for this is lacking at present.

Since these enzymes are also found in the cerebrospinal-fluid and since there have been several reports on the possible diagnostic significance of these enzymes in pathological conditions of the central nervous system it is of interest to discuss briefly the controversy over the existence of a "blood-brain" barrier. Its presence with respect to GOT and LDH has been mentioned in a few reports, usually when an elevation of the enzymes in the serum was not reflected in the spinal fluids. Wroblewski et al. (164) reported that with LDH there was no relationship between serum and spinal-fluid activity and that each varied independently of the other, presumably because of a blood-brain barrier. Wakim and Fleisher (156) gave more definite evidence of a barrier in dogs with experimental cerebral infarction, in which marked elevations of spinal-fluid transaminase was associated with only minimal change of serum levels. Conversely, high serum levels resulting from acute hepatic necrosis were not reflected in spinal-fluid. In 1957 Lieberman and co-workers (93) however, reported serum transaminase elevations in 43 percent of patients who had suffered recent cerebrovascular accidents, and in whom myocardial infarction was ruled out by serial ECG's and absence of other clinical signs. Shortly thereafter, Fleisher, Wakim and Goldstein (50) reported moderate elevations of transaminase activity in both serum and spinal-

fluid in a study of cerebrovascular disease in human beings. This differed from the results of their previous work with dogs. Green et al. (55), on the other hand, observed no elevations of transaminase in the serum after cerebral infarction, but did find moderate increased activity in the spinal-fluid in 7 of 11 patients. Later in 1957 Lieberman and his group did more work on this problem (92). They reported cases of striking dissociation between levels of enzyme activity in simultaneously drawn specimens of blood and spinal-fluid which lend support to the hypothesis that a "blood-cerebrospinal-fluid barrier" is operative so far as GOT is concerned. In the cases in which elevation occured in both serum and spinal-fluid, the serum level was always greater than that of the spinal-fluid. There may be species differences which are yet to be discovered, but it is probably safe to assume that there is a blood-brain barrier in all animals for these three enzymes. However, work to support this hypothesis is in order.

Recently, it has been found that certain enzymes are composed of component enzymes termed isozymes. In 1959 Markert and Moller (97) proposed the term isozymes for the different molecular forms in which protein can exist with the same enzymatic behavior. Beef heart LDH was first separated into two such isozymes or catalytic active components by electrophoresis (112). Since that time five LDH isozymes have

been identified in rabbit and human tissues; typical patterns were observed for each tissue (165). Markert and Moller (97) also found characteristic isozyme patterns for each tissue and species, and observed that the pattern for a single tissue changed during embryological differentiation. Other properties of these LDH isozymes have been studied by various workers.

Fleisher et al. (48) reported the separation of GOT from crude extracts of heart and liver into two fractions by electrophoresis. They could not show the presence of more than one component with GPT. In 1961, Augustinsson and Erne (11) reported the existence of isozymes in serum GOT and GPT from man, cows, horses, and pigs. For all species of animals studied, both GPT and GOT activities were found in the alpha and beta globulin fractions of the serum.

The existence of these fractions of enzyme activity in mammalian sera may be of importance in clinical studies. If the heterogenity of these enzymes is actually due to different molecular forms, these may differ in relative concentration from one disease to another; e.g., in myocardial disease and hepatic disorders. In addition, it may well be that the various forms have different cellular origins. It follows then that such isozyme determinations would be much more specific as to the tissue and organ involved.

The three enzymes are expressed as units per ml. of serum per minute. Karmen (77) defined SGOT activity as: 1

unit equals a decrease in optical density (O. D.) of 0.001 under standardized conditions. The SGPT has been defined similarly. There has been more controversy regarding SLDH; however, enzyme activity is usually expressed in terms of units. A unit of LDH is defined as an increase in O. D. of 0.001 per minute per ml. of serum (60).

The spectrophotometric method of measuring SGOT was devised in which the GOT reaction is coupled to the oxidation of reduced diphosphopyridine nucleotide (DPNH) by oxaloacetate in the presence of an excess of purified water dehydrogenase. The oxidation of DPNH, and thereby the transamination reaction, is followed by measuring the decrease in light absorption at 340 mμ. at which wavelength the reduced pyridine nucleotides have an absorption peak. The spectrophotometric measurement of SGPT is accomplished by utilizing a technique analogous to that described for SGOT. The GPT reaction is coupled to the reduction of pyruvate to lactate by DPNH in the presence of an added excess of purified LDH. The colorimetric methods are generally based upon measuring the amount of hydrazone present, which is highly colored.

The determination of LDH is facilitated by the spectral properties of the coenzyme as discussed by Erickson and Morales (46). DPNH has a maximum light absorption at a wave length of 340 mμ., whereas DPN absorption at this wave length is negligible. LDH can be determined spectrophotometrically by measurement of the rate at which DPNH is

oxidized or reduced, depending on whether the reaction is studied from the pyruvate or the lactate side. With the reagents, pyruvate and DPNH or lactate and DPN, in excess, the measured rate of reaction is proportional to the concentration of LDH in the sample assayed. In the colorimetric method of Sigma (145) pyruvic acid reacts with 2,4-dinitrophenylhydrazine to form an intensely colored hydrazone which has a high O. D. over the broad wavelength range of 400 to 500 mμ. Lactic acid, beta-DPN and beta-DPNH do not contribute a significant O. D. at this wavelength. Therefore, by formation of the same hydrazone O. D., one can measure the varying O. D.'s resulting from the conversion of part of the pyruvic acid to lactic acid due to LDH activity.

Determination of SGOT, SGPT, and SLDH activities have been used extensively in human medicine to aid in the diagnosis of myocardial infarctions and hepatic disease (9, 26, 55, 63, 79, 82, 83, 85, 105, 108, 109, 124, 155, 166, 168). Although SGOT and SGPT activities are elevated in both of these conditions, SGOT elevations more sensitively reflect myocardial infarctions and chronic liver disease, whereas elevations in SGPT activity are more constant in acute liver disease (20, 26, 63, 161, 167). This is also apparently true in laboratory animals and dogs (20, 31, 35, 49, 60, 101, 161). There is very little information relating SLDH changes to pathology in dogs, however, there are a few reports

relating to laboratory animals (16, 51, 70, 73, 96). It is reported that SLDH is a highly valuable indicator of myocardial necrosis in humans, failure to observe elevations on serial testing is an important negative finding, and it affords the added advantage over SGOT of a more persistent abnormality (up to 10 to 14 days after infarction)(60). The results on dogs from the small amount of work done indicates that SLDH is increased following myocardial infarction but not to as relatively as high level as SGOT (116, 127, 132).

Several groups of workers produced various types of cardiac, liver and pulmonary pathology experimentally in dogs (2, 3, 4, 18, 62, 53, 83, 84, 89, 116, 127, 131, 132, 144, 150). Their work indicated that myocardial infarction and liver necrosis resulted in elevation of all three enzymes. However, serum levels remained near normal in pericarditis, myocardial ischemia, valvular insufficiency and pulmonary infarction. One clinical report from California indicates that serum transaminase determinations can be of considerable aid in diagnosing liver pathology (170). Cornelius reports (31) that SGPT levels are used as the test of choice in liver function in hepatocellular necrosis in the dog and cat.

Detweiler in his early work on canine cardiology reported that the incidence of cardiovascular disease among dogs is considerably higher than is generally realized (37).

He further states that there is little understanding of the degree and character of cardiovascular involvement and that myocarditis and myocardial degeneration generally go unrecognized, except at necropsey.

Since then it has been reported by workers at Pennsylvania (38, 39) that the incidence of heart disease in dogs of all ages selected at random is about 15 percent, it appears that more work should be carried out on the value of these serum enzymes in the diagnosis of various pathological conditions occurring in this species.

## B. Electrocardiography

Volumes of descriptive literature are available on the configuration, timing, and lead systems relevant to electrocardiograms of man and animals. Although many workers have made significant contributions to the study of the electrocardiogram, two are especially noteworthy both for the merit of this work and for their influence on the approach taken by others in this field as it relates to the human subject. As described by Abildskov (1), the early history of electrocardiography is dominated by the work of Sir Thomas Lewis. In a very short time, use was made of his observations to form an important part of the clinical evaluations of patients. Wilson played a similar role in the history of electrocardiography in later years (1). His contributions may be summarized as the application of mathematics and basic principles

of physics to this study, and resulted in bringing order to a field which would otherwise consist of a bewildering collection of empiric data.

During the past few years the techniques employed in human clinical electrocardiography have undergone relatively little change, although a new type of recording, the spatial vectorcardiogram, has been developed recently. The 1943 recommendations of the American Heart Association's committee on standardization of electrocardiographic nomenclature and precordial leads have been widely adopted, and both the range of usefulness and the limitations of the electrocardiogram as currently used have been well defined. The technique of spatial vectorcardiography is still in its infancy and new discoveries about it are still occurring and hence it is not so clearly defined.

The boundaries and guidelines which are so well provided for the human electrocardiogram have not been so clearly defined for the canine electrocardiogram. Although volumes of literature are available, on canine electrocardiography, most of the information is empirical with much of the nomenclature and guidelines being borrowed from those set down for the human electrocardiogram. Much of this literature is worthless in the light of newer information, especially since the major differences between the human and canine anatomy have been recognized. However, the basic electrical phenomena occurring

within the dog's heart and the human heart are very similar and this does furnish some basis for the exchange of information between the two species physiologically speaking. Work cited in the following review is on dogs unless otherwise stated.

The structure of clinical electrocardiography has been built on limited knowledge of the sequence in which electrical events involve various portions of the heart. The general path of activation in the atria was relatively easily defined because these structures are thin and for most purposes the spread of electrical events through them may be considered as surface phenomenon (1). Ashworth and Nahum (10) describe an auricular electrocardiogram showing a QRS wave, an ST segment, and a T wave. It is a well known concept that the impulse starts at the S-A (sino-atrial) node located in the right atrium. The innervation to and of the dog's heart was described and discussed by Tcheng (152). Puech et al. (125) described the septal auricular activation as occurring from above downward, and from back forward. They reported, contrary to the classical concept, that the posterior-inferior region of the left auricle, near the entrance of the pulmonary veins, was found to be the last zone of activation. They found the conduction rate of the right atrial body and its appendage range from 487 to 1,000 mm/sec. In reference to the P wave, in most cases, sinus activity takes place from

.005 to .015 seconds before inscription of the P-wave. The end of the right auricular activity never goes beyond the apex of the P-wave. The left auricular activity encompasses most of the ascending segment of P, to the middle or lower third of the descending portion of the wave.

The impulse travels from the auricles tothe ventricles through the A-V node also terms the bundle of His. Alanis and Guillermo (6) reported on the propagation of impulses through this structure and the connection to the Purkinje cells of the ventricles. There is much controversey about the sequences of electrical events within the ventricles. The events of activation on the surface, unlike those of the auricles, cannot be presumed to indicate the same as that in the underlying muscle, because of the thickness of the muscle mass and the finding of various types of nervous tissue there.

The classical study by Lewis and Rothschild (90) indicated that activation began high on the septal wall. Later Sodi-Pallares et al. (148) and Burchell and associates (21) found evidence indicating that the apex of the septum was excited before the base. Harris' (61) findings generally confirmed those of Lewis, whereas the results reported earlier by Nahum and co-workers (110) were similar to that of the groups of Sodi-Pallares and Burchell.

More recently Scher et al. (135, 136, 137, 138) and Durrer et al. (42, 43, 44, 45) have investigated this

phenomenon independently using similar techniques. Both groups confirmed earlier studies indicating that the general sequence of activation of the left ventricular wall proceeds from apical toward basal regions. Scher's et al. studies, which included study of the intraventricular septum, also confirmed the finding that the left side of the septum was the earliest portion of the ventricular muscle to be activated. His results indicated that activation begins in the mid portion of the left septal endocardium. Results of the two groups differed with regard to the manner of excitation in the subendocardial regions. Both found a very rapid activation of the endocardial surface of the ventricular wall as did Harris (61). Studies by Scher's group, however, indicated a spread of activation through the remainder of the ventricular wall in an endocardial to epicardial fashion. Harris also reported this and that the spread through the wall was much slower than the activation of the entire endocardium. The results of the work by Durrer and associates seem to indicate myocardial extensions of the Purkinje system so that activation in the inner layers may be progressing toward the endocardium as well as the epicardial surfaces. In this respect the findings of Durrer are similar to those of Prinzmetal and associates (122).

In 1961 Durrer et al. (41) reported that the question of intramural extension and the degree of this extension of the

Purkinje network is still not solved. They have looked for evidence of such a network in the left ventricular wall for years, but never found it. Previous reports gave only indirect evidence for its presence. However, they did find such an intramural extension in goats as has been reported by other workers (106). It again must be pointed out that there is danger in over extrapolation from one species to another.

In the outer layers of the ventricular wall the excitatory process progresses with nearly constant velocity, approximately 50 cm. per second, toward the epicardial surface (41). The ventricular septum is activated from both sides (137). No functional boundary between the portion supplied by the right and left bundle can be demonstrated. (137). The basal regions of the septum are activated latest in the cardiac cycle; therefore, the excitation wave in the ventricular septum progresses in an apico-basal direction (41).

Pruitt, Essex and Burchell (123) related the speed of activation to direction of the muscle fibers. They stated that endocardial activation is rapid, not because of the Purkinje fibers, but because the subendocardial bonds of the myocardial muscle form a network through which excitation can move rapidly along the axis of the fibers. Spread across the septum in bundle branch block and across the free wall of the left ventricle in the normally activated heart is slow because excitation is moving through fibers in which the long axis is perpendicular to the advancing wave of excitation.

Hamlin and Smith (59) have summarized the data and explained the series of events as follows: After the excitatory impulse traverses the atrioventricular nodal conduction system, it is quickly carried by the Purkinje fibers to all areas of the endocardium and subendocardium at a velocity of 2.5 meters per second. The Purkinje system affords a method whereby almost all areas of the ventricles are stimulated and therefore contract simultaneously. Activity represented electrocardiographically occurs only when the wave of excitation traverses muscle by conduction from fiber to fiber. The electrocardiograph is silent during the time when the Purkinje system alone is carrying the impulse. The first area activated is the interventricular septum. After this septal activation, the impulse spreads by fiber-to-fiber conduction through the ventricular myocardium from subendocardial terminations of the Purkinje system to the epicardial surfaces. This activation occurs simultaneously through both ventricles. However, the peripheral electrocardiogram, which represents the resultant of the simultaneous activities of both ventricles, is recorded as if the front were moving only from the left ventricular endocardium to the left ventricular epicardium because the left ventricle is 4 to 6 times as thick as the right. Finally, the ventricular activation process is terminated when the base of the heart is activated in an apicobasilar direction.

Despite the conflicts of these reports it is apparent that much of the electrical impulse phenomenon of the heart is fairly well understood and that it is from these facts that clinical interpretations can be made. However, it is obvious that when a detailed order of activation is defined, the electrocardiogram will give a much more realistic picture of the location of the lesion involved in cardiac pathology.

The repolarization pathway has presented even more difficulties in its study. It cannot be investigated with the methods applied to the analysis of depolarization because opening the thorax changes the T wave. The literature is notably lacking in explanations for this phenomenon. Although there are several papers in the available English literature, only one seems worthy of mention at this time. Studies by Reynolds and VanderArk (129) show that in general the order in which local ventricular muscle units recover excitability is correlated with the epicardial T-waves as recorded in direct unipolar leads. The effective refractory period was determined at the surface and in the deeper layers of the dog heart and was correlated with the surface T-waves. A small delay in recovery of cardiac excitability appeared at the surface when T-waves were negative and in the deeper layers when T-waves were positive. They further showed that both surface and deeper layers in a limited area normally complete recovery within a short space of time, thus suggesting

that a majority of cardiac fibers recover during the same period of time. Since only slight changes in the rate of recovery at the surface or in the deeper layers are apparently necessary to alter the polarity of the T-wave, this partly explains the lability of the T-wave. However, it is apparent that this is not a complete explanation of the origin and direction of the T-wave. This phenomenon awaits further study and clarification.

Abildskov (1) has gone into a brief discussion of the origin of bioelectric potentials. It is beyond the scope of this paper to delve into the vast amount of research in this area of biochemistry and biophysics. However, as he so aptly points out, considerable clinical usefulness of the electrocardiogram has been achieved in spite of only meager knowledge of the processes which give rise to the electric phenomenon of the heart. Hamlin and Smith (59) state that the heart is an electric generator within the thorax and that an electrocardiogram is a tracing of variations in body potential which has been generated by the heart and transmitted through the body volume to a surface point.

In this same paper they point out the variation in the anatomy of the dog and man and the danger of over-extrapolation from species to species.

Eyster, Meek, and Goldberg (47) reported on the relationship between the electrical and mechanical events of the dog heart. They concluded that different local regions of the

dog's heart start contracting at different time instants and that the occurrence of mechanical activity is coincident with or separated by a brief interval from the occurrence of maximum flow of electric current and maximum time rate of change of current, resulting from a potential gradient established between neighboring regions in which the potential is respectively above and below the potential of the resting muscle.

At the present time the author knows of no textbook which is primarily concerned with canine electrocardiography. However, for a basic understanding of the principles underlying this science the reader is referred to texts by Wolff (160) and Marriott (99).

While position during recording and methods of placement of electrodes are well established for human electrocardiography there is much controversy in canine electrocardiography. The general concensus seems to be that dogs should be placed in lateral recumbency on their right side (10, 38, 86, 94, 95, 146, 153, 157). However, there is evidence that the supine position is best (71, 115, 121). In preliminary studies by the author, the sternal position was found to give good reproduceability. Wallace in his treatise on technique of canine electrocardiography states that having dogs on their right sides is the technique of choice (157). Katz and co-workers (80) reported on positioning in 1934, stating that there were variations in the electrocardiographic

tracings with dogs placed on their right sides, but they felt this was due to normal variations in the position of the heart. They found changes in amplitude and even direction of all complexes, especially the T-wave. They found these same changes when animals were recorded in the normal standing position. Lannek in his classical work also preferred the right side (86). Lumbard and Witham (95) varied positions on 50 dogs and found variations in all positions from dog to dog. The effect of changing of position varied in a random way. Peterson et al. (121) used the supine position because of the variabilities previously reported from the right lateral position. They reported that variations were much less in the supine positions than those reported in the lateral position. Horwitz, Spanier, and Wiggers (71) using the supine position reported that variations on the same dog taken at different times were as great as among different dogs. Newton, Ellis, and Zaremski (115) recently described a technique in which they reported that virtually no contour changes in serial electrocardiograms occurred on anesthetized dogs in the supine position, and it is therefore suitable for chronic experiments. Because of the random changes in the recording Lumbard and Witham (95) stressed the fact that it is important to have the dog in the same position in chronic experiments to insure correct interpretation of serial recordings. Tsagan (153) recently reported that changes in ECG following myocardial infarction were most marked when the

dog was on its right side with the right front leg forward and the left back. He reported that the position of the front limbs altered the Q and T-waves, and had a considerable effect on the ST interval. It is therefore of importance to maintain the front legs in a fixed position when recording the electrocardiogram.

Litvak, Siderides, and Vineberg (94) summarized extreme variation of the electrocardiogram of the dog as compared with the human as being due mainly to positional change. The heart of the dog lies more centrally in the thorax and assumes a more vertical position than does the human heart. The absence of the mediastinum as compared with the human being, makes the heart easily susceptible to positional changes. Consequently, pneumothorax, paralysis of the diaphragm, abdominal distention, and even variations in heart rate tend to displace and rotate the heart producing electrocardiographic changes.

The clinical approach to position seems to favor having dogs positioned on their right sides[1] (38, 86, 157).

The use of needle electrodes, wires, plate electrodes, and clambs of various types has been described in the literature (38, 80, 86, 94, 115, 121). Clipping of hair or leaving it in place does not effect the recording (120, 121).

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[1]Baker, D. L. Professor Medicine and Surgery. Iowa State University, Ames, Iowa. Positioning dogs for clinical ECG recordings. Private communication. 1962.

The use of anesthetics and tranquilizers has been described as having little effect upon the electrocardiogram (153, 157). The use of morphine is to be avoided because it increases vagal tone (157). Cannard et al. (25) discussed the electrocardiogram during anesthesia in the human.

The placement of electrodes upon the animal body has been quite clearly defined in regard to limb leads (146, 157). However, there is some discrepancy among various workers as to where precordial lead electrodes should be placed or even if they should be used at all (38, 86, 95, 115, 146, 157).

Several papers have reported on the configuration of the electrocardiogram of normal dogs. The results have varied from one group of workers to another, partly because of different techniques, but probably more important because of the normal variation in the canine electrocardiogram. In 1949, Lannek (86) published a detailed statistical analysis of the normal canine electrocardiogram, together with observations on a series of animals showing electrocardiographic abnormalities. This work served as a cornerstone of clinical canine electrocardiography in the following years. Petersen et al. (121) reported on the electrocardiographic tracings in normal beagle dogs in 1951. Following this there were papers by Grollman and co-workers in 1952 (56), Horwitz, Spanier and Wiggers in 1953 (71), Soave in 1954 (146) and Lombard and Witham in 1955 (95). The following tables summarize findings:

Table 2. Heart rate of normal dogs

| No. of dogs | Breed | Position | Range | Ave. | Author |
|---|---|---|---|---|---|
| 32 | Beagle | Supine | 90-180 | 138 | (121) |
| 50 | Indeterminate | Right side | 62-158 | 100 | (95) |
| 30 | Mongrel | Supine | 72-162 | 121 | (71) |
| 50 | Indeterminate | Right side | 80-160 | | (146) |

Lannek (86) reported that sex differences are not probable in electrocardiographic tracings. He also found that breed differences are improbable except for the amplitude of the T-wave on certain breeds (collie, dachbrache, dachshound, greyhound, harrier, rottweiler and setter) being greater. He did find significant differences in certain parts of the complex based upon age difference. He also reported a significant difference in heart rate among dogs less than six months and those over but no additional differences with increased age.

The time duration of the various segments and the amplitude of the various waves as reported by other workers are shown in Table 3 and 4. In preliminary investigation the author found considerable differences in amplitudes from day to day and from position to position and it is thought that the relative rather than the absolute amplitude is of more significance. Lannek states that the R amplitudes are in themselves of no great diagnostic value, they become

important on low voltage and on calculating the negative T-wave as a percentage of the R amplitude. He also states that the variations in amplitudes of the tracings, measured at four-five hour intervals during one day are considerable.

Table 3. Time duration (seconds) of various segments of ECG complex as reported by others

| P | P-R | QRS | QT | Ref. |
|---|---|---|---|---|
| Average | .106 | 1046 | .19 | (121) |
| Range | .08-.12 | .04-.06 | .14-.24 | |
| Average | .10 | .05 | .26 | (95) |
| Range | .08-.15 | .04-.06 | .20-.38 | |
| Average .07 | .12 | .06 | .21 | (71) |
| Range .04-.12 | .10-.15 | .04-.08 | .16-.28 | |
| Average .06 | .106 | .045[b] | .187 | (86)[a] |
| Range .02-.10 | .06-.17 | .03-.06 | .14-.25 | |
| Range .04-.08 | .08-.10 | .03-.06 | .16-.21 | (146) |
| Range | .06-.18 | .03-.08 | | (56) |

[a]Longest interval in standard leads I, II, III, and aVF.

[b]Medium weight breeds.

Lannek (86) reports the P wave is often isoelectric in lead I and the amplitudes are never high. In leads II and III, P is in nearly every case positive on inspiration but may become negative in lead III on expiration. A P-wave which remains negative throughout should be regarded as an abnormality in all leads with the exception of lead I.

There has been much discussion of the presence and significance of Q-waves in the canine electrocardiogram. So much

Table 4. Amplitudes of the various components of the dog ECG as reported by others (1 cm. = 1 mv.)[a]

| Q | | R | | S | | T | | Ref. |
|---|---|---|---|---|---|---|---|---|
| **Lead I** | | | | | | | | |
| 0 | (0-.2) | .35 | (.15-.70) | 0 | (0) | 0 | (-.1-.1) | (121) |
| .10 | (.00-.60) | .3 | (.00-1.00) | | | -.03 | (-.30-.15) | (95) |
| .16 | (0-1.15) | 1.05 | (.20-2.22) | .09 | (0-.80) | .05 | (-.36-.63) | (71) |
| .03 | (0-.6) | .12 | (0-.35) | .01 | (0-.2) | -.005 | (-.1-.7) | (56) |
| .77 | | 1.11 | | .45 | | | | (86)[b] |
| .14 | | .7 | | .06 | | .05 | | (146)[c] |
| **Lead II** | | | | | | | | |
| 0 | (0-.20) | 1.8 | (1.0-2.7) | .25 | (0-1.0) | .3 | (.05-.8) | (121) |
| .24 | (0-.90) | 1.14 | (.25-2.30) | .10 | (0-1.50) | -.07 | (-.45-1.00) | (95) |
| .17 | (0-.89) | 1.77 | (.30-3.09) | .09 | (.0-.79) | .18 | (-.27-.74) | (71) |
| .04 | (0-.10) | 1.08 | (.10-2.3) | .14 | (0-.40) | .15 | (-.10-.60) | (56) |
| 1.02 | ( | 2.52 | | .55 | | | | (86)[b] |
| .21 | | 1.7 | | .09 | | .14 | | (146)[c] |
| **Lead III** | | | | | | | | |
| .1 | (.1-.2) | 1.5 | (.9-2.5) | .4 | (0-1.4) | .3 | (.1-1.0) | (121) |
| .12 | (0-.45) | .76 | (.05-1.35) | .14 | (0-2.5) | -.03 | (-.25-.70) | (95) |
| .13 | (0-.44) | 1.11 | (.14-2.43) | .23 | (0-1.48) | .13 | (-.26-.81) | (71) |
| .02 | (0-.35) | .92 | (.1-1.95) | .16 | (0-.6) | .16 | (-1.0-.6) | (56) |
| .62 | | 2.09 | | .71 | | | | (86)[b] |
| .12 | | 1.00 | | .18 | | .15 | | (146)[c] |
| **Lead aVF** | | | | | | | | |
| 0 | | .67 | (.5-1.3) | .05 | (0-.25) | .11 | (-.1-.4) | (56) |
| .17 | (0-.7) | 1.35 | (.1-2.52) | .17 | (0-.67) | .13 | (-.25-.74) | (71) |

[a]Average and range.

[b]Upper limits of normal amplitude.

[c]Average only.

importance has been attached to this complex presumably because of its diagnostic importance in human electrocardiography in myocardial infarction. Lombard and Witham (95) reported that Q-waves appeared in all three limb leads in 60 percent of the records, in 32 percent in leads II and III and in the other 8 percent they occurred in one or more leads or were not present at all. They found the Q/R ratios in lead I never exceeded 2.5:1. However, they found by changing position of the dog from its right side to its back, belly or left side they could cause a reduction in size or a disappearance of the Q-wave. They further reported significant day-to-day variations in leads I and II, but not in lead III. Horwitz et al. (71) found a Q-wave in approximately 1/3 of their tracings in lead I and in 82 percent this was followed by an inverted T-wave. They reported that Lalick had found this occurring in 78 percent of his records. However, Lombard and Witham (95) couldnot confirm these results.

Probably one of the most baffling aspects of canine electrocardiography is the extreme variability of the T-wave. This is reported and discussed in almost all the papers dealing with the normal canine electrocardiogram. Without going into a detailed discussion it will suffice to say that this variability is apparently normal and until the underlying phenomenon which causes this particular complex is better understood this will, in all probability, stand. Horwitz

et al. (71) did find that in 84 percent of the cases in which an S-wave was recorded in lead III of 1 mm. or more, an upright T-wave followed. They reported Lalick had recorded this same occurrence in 75 percent of his tracings. Petersen's group (121) found on serial recordings that in no instance did a positive T-wave in leads II, III, and aVF become negative. As mentioned earlier Lannek (86) found a deeper negative T-wave in leads II and III of certain breeds. He noted that this might be attributed to the temperament of the breeds involved.

Bellet, Gazes, and Steiger (13) reported typical T-wave patterns observed in acute infarction and in those dogs with small infarcts involving chiefly the endocardial portion of the left ventricle. In the former, observed in transmural infarcts, the T-waves were characterized by inversion and an increase in amplitude. The latter showed inversion and a diminuation in amplitude which remained small. It should be remembered that they had control electrocardiograms to compare with before pathology occurred.

Another important segment deviation occurrence which has been studied extensively is the ST segment. This also has been important in human electrocardiography. In preliminary studies the author found it to be the most reliable of any change in the recording for detecting cardiac pathology. Petersen (121) found that there was sometimes depression or elevation of the ST segment up to 1 mm. in dogs in the

supine position, chiefly in leads II, III, and aVF. Grollman et al. (56) found no deviation in lead I but up to 2 mm. up or down in leads II, III, and aVF in 18 normal dogs in the supine position. Litvak, Siderides, and Vineberg (94) found only a slight ST elevation in lead I in one of 12 normal dogs. Lombard and Witham (95) make the comment that the merging of the terminal part of the QRS complex with the ST-T segment is so characteristic a picture of the dog electrocardiogram when taken under anesthesia at a paper speed of 50 mm/second that it must be considered normal. They found it occurring to some degree in all leads. They further found that in the limb leads, 22 percent of the records showed no S-T deviation. 30 percent up to .09 mv. and 48 percent between .10 and .20 mv. Other authors reporting S-T deviations of this order of magnitude have attributed the phenomenon to a variety of experimental causes or as a "normal" occurrence. They found that on day-to-day recordings that the shift was most noticeable in lead II. Nahum, Hoff, and Kisch (111) reported from their experimental work that elevations of the R-T in any lead denotes damage to the surface of the left ventricle, while depression of the S-T indicates damage to the right ventricle, however, this appears to be an over simplification of the situation. Lannek (86) states that the shifting of the ST segment towards the negative side, is clinically, by far the most important factor. He states that the ST segment may be described as a straight line or, seen from above, a

concave or convex curve. The straight and the concave forms are the most common, whereas the convex type is seen less often in normal cases but is relatively common in myocardial lesions. He further states that most of the deviations from the normal picture are to be found in the ST segment and the T-wave, in the form of depression of the ST, decrease of the T amplitude and shifting of the T/R relation (negative T expressed as a percentage of the amplitude of R in the same complex) towards higher values. The depressions of the ST segment are usually small although in many instances significant. The cause is probably currents of injury.

Durrer et al. (41) found that all epicardial complexes show S-T elevations from an ischemic area when recording directly through an open thorax. Detweiler, Hubben, and Patterson (38) found early myocardial degeneration and arterial thickenings upon necropsy of three hearts of dogs showing an RS-T segment deviation of .2 mv. or greater in several leads. Tsagan (153) describes in great detail the changes occurring in the ST segment following experimental myocardial infarction. Zao, Yen, and Herrmann (169) studied the effects of oxygen gradient upon the ST segment. The ECG revealed a positive correlation between the ST segment elevation and the oxygen gradient, i.e., the ST segment increased in height with an increasing oxygen gradient, and decreased in height with a decreasing oxygen gradient. They discussed the possibilities involved.

The question of arrhythmias is often brought up in the dog. Because of the normal sinus arrhythmia of the dog's heart it is often difficult to detect the abnormal arrhythmias without the use of the electrocardiograph. Almost any type of arrhythmia, other than the normally occurring sinus arrhythmia can be detected by the electrocardiograph and can be considered as indicating cardiac pathology (86).

Ventricular extrasystoles (ectopic beats) are rare in dogs and available evidence (36, 37) indicates that myocardial lesions are usually present when this arrhythmia is encountered under clinical conditions in dogs which have received no drugs or toxins known to cause ventricular premature beats. Lannek (86) also states that ventricular extrasystoles and block are regarded as significantly abnormal features, not appearing in the normal animal. Patterson and co-workers (120) concur with this opinion.

The knowledge in this basic field of electrocardiography is advancing rapidly and as more information is obtained the increased understanding of the physiology involved will aid in the clinical application of this knowledge. Thus, treatment will be based increasingly on facts instead of empiricism.

# III. METHODS AND MATERIALS

## A. Procurement and Care of Normal Dogs

A total of 50 normal, mongrel dogs of all ages and both sexes were used in this study. As dogs were obtained by the Department of Physiology and Pharmacology, they were given a complete physical examination as described by Detweiler (38) which included observation of the condition of the animal, palpation, and auscultation of lungs and heart. Dogs which appeared to have some abnormal characteristics were discarded at this point. Those dogs which appeared normal then had a complete hematological examination including hematocrit and hemoglobin determination, total erythrocyte count, total leukocyte count, and a differential leukocyte count. Dogs having hematologic values within normal ranges were kept for surgery, provided the presurgical electrocardiogram appeared to be normal. Table 5 shows the results of the blood studies of the normal dogs. A vaccination program[1] over a five-week period prepared the animals for further experimental use. The animals were vaccinated with 1 cc. of an antigen[2] intradermally at 7-day intervals for five weeks. A serum sample

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[1]Booth, R. R. 3920 E. Jackson Blvd., Elkhart, Indiana. Distemper-hepatitis Immunization. Personal Communication. 1960.

[2]Virogen D-H: Pitman-Moore Co., Indianapolis, Indiana.

was collected from all dogs at this point and again prior to any surgical procedure for enzyme determination (see Table 6).

The cages in which the dogs were housed were made of galvanized iron. They were cleaned twice daily. Water and dry commercial dog food[1] containing 24 percent protein and 6 percent fat were fed ad libitum to the experimental dogs.

Dogs were divided into three groups as to age for pre-surgical ECG recordings. Dogs in group I were 4 to 12 months of age; group II, one year to five years; and group III, over five years. Estimation of age was based entirely upon appearance and characteristics of teeth.

## B. Blood Serum Enzymes

SGOT was measured by the method of Hergt and Langin (64)(see appendix) which is a slight modification of Karmen's original procedure (77). SGOT, SGPT, and SLDH were measured by the clinical Sigma methods (14, 45). The Sigma method of SGOT determination was compared with the research method of Hergt and Langin (see Table 7). All determinations were made using the Beckman Model B spectrophotometer.[2] All blood samples collected for serum were allowed to clot, immediately centrifuged, the serum removed with pipettes, placed in 15 ml.

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[1]General Mills, Minneapolis, Minnesota.

[2]Beckman Instrument Co., Instruments Division, 2500 Fullerton Road., Fullerton, California.

test tubes, and sealed with waxed corks. These samples were frozen immediately and sixteen samples were run at a time at various time intervals later. In preliminary trials it was found that very little loss in activity from freezing occurred; however, it has been reported that repeated freezing and thawing will decrease enzyme activity.[1] SLDH samples were not stored in the freezer for longer than ten days.

## C. Electrocardiography

Electrocardiograms were taken with the Sanborn Visocardiette Model 51 electrocardiograph.[2] Needle electrodes were placed subcutaneously as the dog was placed on its sternum and belly, front limbs extended straight forward with head resting between them, and hind legs directed to the rear. The standard limb leads I, II, III were recorded plus augmented unipolar limb lead aVF. The needles were placed in a position adjacent to the lateral epicondyle of the femur and radius. (Shown in Figure 10, Appendix). A small area of hair was kept clipped at these points so as to insure placing the electrodes in the same position each time a recording was made. A recording was taken prior to surgery and if the ECG appeared to be normal, surgery was performed as described later. Several recordings were made at various intervals

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[1]Buck, W. Animal Disease and Eradication Division, National Animal Disease Laboratories, Storage of Serum-Enzymes, Personal Communications, Ames, Iowa. 1960.

[2]Sanborn Co., Medical Division, Waltham, Mass.

following surgery to monitor any changes in the electrical phenomenon of the heart. All recordings were made while the dogs were anesthetized with sodium pentobarbital (prior to and immediately following surgery) or while they were tranquilized with promazine hydrochloride[1] (later serial studies).

Methods of measuring the electrocardiogram were quite similar to those used by Lannek (86). The isoelectric line was established as being that segment of the cycle extending from the end of the T-wave to beginning of the P-wave. Segments for measuring, if possible, were selected where the base line runs parallel to the paper and where the lower contour of the preceding and succeeding T-P segment and the engraved line have formed a straight line.

Errors due to measuring have been minimized through multiple determinations. Lannek (86) found that the error of measuring varies with the technical quality of the tracings. He found that 99.7 percent of the error of measurement for amplitudes may be considered to lie within the limits $\pm$ 0.48 mm., and that for durations within $\pm$ 0.012 seconds.

All amplitudes and durations were measured with dividers and then calculated from a known set of reference points. In those cases of an odd time interval it was rounded off to the

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[1]Promazine Hydrochloride - Fort Dodge Laboratories, Inc., Fort Dodge, Iowa.

nearest even interval, i.e., the duration has been measured with an exactitude of 0.01 seconds. The amplitude for upward deflections has, as is customary, been measured to the upper contour of the base line, for downward deflections to the lower contour of the base line. The durations of deflections have been measured from the point where the convex or concave curvature leaves the contour of the isoelectric line to the point where this curve rejoins said contour.

Measurement of the S-T junction displacement and voltages of the QRS deflections are referred to the level at which the first QRS deflection takes off from the P-R segment. Ratios are given as they occurred unless one of the amplitudes was 0.5 mm. or less in which case the ratio was calculated so that all ratios could be compared to 1. P-R interval was measured from the beginning of the P-wave to the earliest component of the QRS complex in the lead in which it was longest. The QRS interval was measured from the point where the first deflection of the QRS group departs from the base line to the point at which the last deflection comes to rest at the S-T junction. This was measured on several groups and an everage taken for each recording. The Q-T interval was measured from the beginning of the QRS complex to the end of the T-wave in that lead in which the end points were most clearly defined, and in which the largest value was found.

A 3-channel physiograph[1] was used to record the phonocardiogram and to monitor any changes in the heart sounds of those dogs used in the valvular insufficiency part of this project. Phonocardiograms of dogs were recorded once or twice presurgically and at various intervals following surgery. In addition all dogs were examined periodically by ausultation and palpation to determine the course of their condition.

## D. Surgery

### 1. General considerations

Three types of cardiac pathology were produced by surgical methods. In each case other dogs were sham-operated performing the same procedures except for production of the actual pathological process. A total of fifty animals had surgery performed; however, nine of these died during surgery or shortly after before all phases of the project could be completed.

All materials which were used in the surgical procedures were sterilized by one of two methods. Steam sterilization under pressure was used for surgical instruments, gloves, linens, vetafil[2] and silicone rubber tubing[3]. Chemical

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[1]E and M Instrument Co., 5815 Sidney St., Houston, Texas.

[2]Fort Dodge Laboratories, Synthetic nonabsorbable suturing. Fort Dodge, Iowa.

[3]Silatube - Ronthor Reiss Corp., Ronsil Products Division, Little Falls, New Jersey.

disinfection by immersion in aqueous 0.02 percent chlorhexidine[1] provided the means for disinfecting materials with low heat tolerance and instruments with sharp cutting edges which become dulled by repeated autoclaving.

Anesthetization of the dogs used in surgical procedures was accomplished by the intravenous administration of sodium pentobarbital solution given to effect. The dose was approximately 30 milligrams per kilogram body weight. Solid food was withheld for at least 12 hours prior to surgery. Hair over and adjacent to the surgical field was removed by using an electric clipper with a No. 40 blade. The skin was then prepared for surgery by repeated scrubbings with a 25 percent aqueous germicidal detergent which contained 0.6 percent benzethonium chloride[2]. The lather was removed with cotton after each scrubbing. An endotracheal catheter with cuff was inserted into the trachea and the cuff was inflated.

The tube was then taped to the upper jaw to prevent accidental dislodgment. The catheter was attached to an artificial respirator apparatus[3] if thoracic surgery was to be performed and the oxygen pressure was set for 11 to 14 centimeters of water.

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[1]Nolvasan - Fort Dodge Laboratories, Fort Dodge, Iowa.

[2]Germicidal Detergent. Parke, Davis and Co., Detroit, Michigan.

[3]PR-3 Prothoracic Respirator with breathing bag. Professional Veterinary Service, 819 SW 12th Ave., Miami, Florida.

The hands and arms of the surgeon were repeatedly scrubbed with the 25 percent aqueous germicidal detergent and then rinsed with tap water. After completing the scrubbing, the surgeon dried his hands and arms with a sterile towel and donned a sterile, full-length operating gown. He then put on dry, sterile surgical gloves.

The animal was then draped with sterile cloths leaving the surgical field exposed. Adjacent areas of hair and skin were securely covered.

There are four principles of good surgical techniques which, if observed, enhance the chance for successful results. These principles are devoted to minimizing undesirable sequalae to tissue injury (27).

These principles are first, aseptic technique, or preventing the introduction of infectious organisms into the field of surgery. This is accomplished by using sterile equipment and avoiding contamination. The second is atraumatic technique, or minimizing unnecessary devitalization of tissue. The third requirement is hemostasis or the control of hemorrhage to prevent shock. Finally, the last requirement is careful closure of exposed tissues. This is achieved by accurate apposition of divided parts and by meticulous suturing technique.

2. Myocardial infarction

The production of experimental myocardial infarction was accomplished in two stages. Preliminary trials by the author and Cholvin[1] indicated that the procedure described below is very satisfactory.

The procedure was made on 14 dogs and consisted of two stages. The first stage involved thoracic surgery. The thorax was entered from the left side at the fourth or fifth intercostal space depending on whether the anterior or posterior left descending coronary artery was to be ligated. An incision was made through the skin from 2 to 3 cm. above the point of the scapula, depending on the size of the dog, extending ventrally to within 2 to 3 cm. of the ventral midline. The incision was continued through the subcutaneous tissue and the muscular tissue: latissimus dorsi, superficial pectoral, serratus ventralis, and intercostal muscles. The parietal pleura was carefully penetrated with scissors and cut the full length of the incision. The ribs were spread by self-retaining rib retractors and the lungs were packed off with sterile-saline-soaked or blood-soaked-swabs. This procedure was followed in all thoracic surgery. The pericardial sac was incised parallel to and approximately 1 cm. ventral to

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[1]Cholvin, N. R. N. I. H. Research Fellow - Department of Physiology and Pharmacology, College of Veterinary Medicine, Iowa State University, Ames, Iowa. Now Department of Medicine and Surgery, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan.

the phrenic nerve and fixed to the body wall by means of two or three strategically located stay sutures. A piece of vetafil ligature was then placed around either the anterior or posterior left descending coronary artery or some large branch of the anterior artery at various levels. One throw was placed in the ligature thus enclosing the artery in a loose knot. The ends of the ligature were then threaded through two, 3-5 inch pieces of silicone rubber tubing. This material was used because of the report that it caused very little tissue reaction (27). The pericardial sac was sutured with vetafil incorporating the tips of the silicone tubing within the sacs. These were fixed in place by a purse-string suture around the tubes and through the sac. The pleura and ribs were brought together and sutured with interrupted vetafil ligatures. The silicone tubes with the vetafil ligature within were incorporated within the body wall, one arising near the top of the rib and the other near the bottom of the ribs. The tubes were sutured in place at the junction of the ribs with a purse-string suture. The ends of the vetafil ligatures were brought through the extending end of the silicone rubber tube with a straight needle and tied leaving a loop of excess vetafil. The pleura and muscle layers were brought into apposition and sutured with continuous catgut. The tubes were laid down upon the muscles lying subcutaneously and one stay suture affixed. The subcutaneous tissues

were sutured with interrupted catgut and the skin closed with simple interrupted vetafil sutures. The same suturing techniques were used throughout each surgical procedure with respect to closing the pericardium, thorax, muscles, subcutaneous tissues, and skin.

In preliminary work the vetafil ligatures were brought out together to lie subcutaneously. This often resulted in the vetafil becoming adhered to tissues so that the ligature could not be tightened down and the angle for tightening the ligature was very poor and occasionally resulted in rupturing the artery.

The animal was allowed to recover for six to eight days at which time it was again anesthetized and prepared for surgery. A small incision was made over each of the silicone tubes and they were brought to the surface. The vetafil ligatures were isolated, pulled taut to ligate the vessel and the ends tied together tightly. The subcutaneous tissues were sutured as were the incisions in the skin. In some cases the silicone tubes were removed; in others, they were left in place. The vetafil was cut as close to the skin as possible at top and bottom 20 to 30 minutes later.

The first post surgical ECG was taken at one hour and the second at two hours. Further recordings were made at various intervals. Serum samples were collected every 6 to 10 hours for three to four days starting at 10 to 15 hours post-

surgery and then at various intervals until the dog was sacrified. Hematological studies were made at various intervals commencing at 4 days to one week post surgery.

Eight sham-operated animals were handled in exactly the same manner except the vetafil ligature was merely passed under the artery with no knot and the amount of tension placed on the ligature was not as great as that put on the dogs in which an infarction was later produced.

3. Epicarditis

The production of epicarditis was also accomplished in two stages in six dogs. In this case the thorax was entered at the right fourth intercostal space in the region of the cardiac notch. The first step involved the placing of a silicone rubber tube into the pericardial sac through a very small incision in the sac. The tube was sutured in place within the sac with a purse-string vetafil suture. The tube was then brought to lie subcutaneously in the same manner as that described for the infarction dogs.

Six to ten days later the tube was isolated while the dog was under anesthesia and 10 ml. of a sterile talc suspension were deposited within the pericardial sac. The end of the tube was closed with a piece of rubber tubing and a vetafil ligature. The tube was fixed in place subcutaneously and the tissues sutured. ECG recordings were taken at

various intervals following surgery as were serum and blood samples. Three dogs were sham-operated in precisely the same manner except for the deposition of sterile talc.

4. Valvular insufficiency

In preliminary trials an attempt at producing valvular insufficiency via venous catherization was made. However, without the aid of a fluoroscope and because of the inconsistency of the damage produced, this technique was abandoned. A second method as described by Markowitz et al. (98) by sectioning the chordae tendinae was used, but after problems with fibrillation and failure to produce clinically obvious pathology, this method was also abandoned.

A technique described for the removal of heart worms (Dirofilaria immitis) (157) was modified by the author and Cholvin[1] and used to produce valvular insufficiency in five dogs. An incision was made over either the right or left fourth intercostal space depending on whether a left or a right A-V valvular insufficiency was being produced. Dogs were atropinized at the rate of .02 grain for average-sized dogs. Once inside the thoracic cavity a 15-inch piece of 1/8 inch wide umbilical tape was passed around the anterior vena cava. This was much easier from the right side approach than from the left. The free ends were passed through a

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[1]Ibid. page 56.

3-inch section of 3/16 inch rubber tubing. The posterior vena cava was similarly exposed and prepared for occlusion. The pericardium was then incised approximately 1 cm. ventral to the phrenic nerve and parallel with it and extending from the anterior to the posterior extremities of the sac. The sac was retracted dorsally and ventrally lifting the heart up and held in place with stay sutures. The placement of stay sutures varied depending upon which method presented the surface of the atrium best for the surgical procedure. Two, 12-inch pieces of vetafil suture were passed through the atrium 3 to 4 cm. apart with an atraumatic needle. These were used to apply tension to the atrium to attach the non-crushing clamp.[1] The clamp was then applied to the atrium while lifting it up with the vetafil sutures as far away from the auricle as possible. A 2 to 3 cm. incision was made in the atrium in the clamped tissue. The venous flow through the posterior vena cava was interrupted by applying tension to the ends of the umbilical tape and forcing the rubber tubing against the vessel. This tension was maintained by applying hemostatic forceps on the umbilical tape at the distal end of the rubber tubing. The anterior vena cava was similarly occluded. The blood supply from the brain was never occluded for more than 2 1/2 minutes. The clamp was

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[1]Satinsky Vena Cava Clamp. J. Sklar Mfg. Co. 38-04 Woodside Ave., Long Island City 4, New York.

removed by an assistant who at the same time applied mild tension to the vetafil suture in the atrium. The surgeon then used a spay hook to pick up the chordae tendinae and the amount of damage desired is made with a scalpel on the cusps of the valve. A hook with an inside cutting edge may also be used but it is more difficult to control the amount of damage.

The valve was then released and the clamp was applied under the tension sutures while the assistant applied tension to them. The hemostats on the umbilical tape were released thus restoring the patency of the vessels. The amount of regurgitation can be fairly well ascertained at this time. If more damage is desired, the process can be repeated after several minutes.

The incision was sutured with 5-0 black braided cardiovascular silk with a swaged atraumatic needle.[1] One row of simple continuous was made. The assistant applied tension to the stay sutures and the clamps were released to allow a slight amount of hemorrhage. Then a second row of simple continuous suture was continued back over the first, making sure to place the needle between sutures already present. The clamps were then removed and the area checked for signs of hemorrhage. If no hemorrhage occurred the stay sutures were removed and the pericardial sac and thorax were closed

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[1]K-870 A Cardiovascular silk - Ethicon, Inc., Somerville, New Jersey.

as described previously. If some hemorrhage occurred which did not cease shortly, several interrupted matress sutures were applied. This procedure allowed a very minimal amount of blood loss and provided a method where the amount of insufficiency produced could be well controlled and failure to produce damage was almost impossible.

One left A-V valvular insufficiency and four right A-V valvular insufficiencies were produced in this manner. Five dogs were sham-operated. All procedures were the same except for production of valvular damage. Two of these were approached from the left and the other three from the right.

## E. Postsurgical Care

Care of dogs following surgery was intentionally kept to a minimum except for routine examinations. Immediately following surgery the air was aspirated from the pleural cavity so that the lungs would assume their normal functioning rapidly and lack of oxygen would not be an additional stress factor. Antibiotics were not used routinely following surgery and bandaging was found not to be necessary. Each animal was examined daily for the first 7 to 10 days and then at 2 to 4 day intervals. Only two animals developed an infection in the surgical incision, both of them were cured shortly with antibiotic therapy. No cardiac drugs were given to any of the dogs. They were not exercised, but remained in a cage at all

times except for bleeding and taking ECG and phonocardiogram recordings.

One dog in the valvular insufficiency group was administered a diuretic and was treated for anasarca by surgical drainage several times. Other treatments were merely routine.

F. Necropsy and Histopathology

Dogs were sacrificed and necropsied at various times following the production of pathology. Necropsy procedure as outlined by Coffin was followed (28). Following gross post-mortem examination, a descriptive protocol was written and, where appropriate, photographs of the lesion were taken. Representative portions of tissues from lesionswere selected and fixed in 10 percent buffered formalin (4 percent formaldehyde solution) for histological examination. Tissue samples were taken routinely from the heart, liver, kidney and spleen. Other tissues were taken if a part appeared abnormal grossly, including tumorous-like growths.

Tissues were processed in the usual manner and the sections stained with hematoxyin and eosin. In a few cases a connective tissue (Mallory Triple Stain) stain was used to show a greater contrast between normal and abnormal tissues.

Samples for microbiological examination were not taken routinely. However, in three cases of epicarditis which were cultured there was no growth seen on blood agar.

# IV. RESULTS AND DISCUSSION

## A. Hematological Studies

Dogs were given a routine hematological examination as they were obtained for experimentation. Table 5 is a summary of the presurgical hematological data of 50 normal dogs used in this research project.

Table 5. Pretreatment hematologic data from 50 normal dogs

| | Average | Range |
|---|---|---|
| Hemoglobin | 16.5 | (12.3-19.4) gm/100 ml. |
| Hematocrit | 45.5 | (36-55) percent |
| RBC $10^6$ | 7.1 | (4.5-8.7) cu. mm. |
| WBC $10^3$ | 12.0 | (6.1-22.2) cu. mm. |
| Differential WBC (%) | | |
| Mature Neutrophils | 70.0 | (51-90) |
| Immature Neutrophils | 3.0 | (0-10) |
| Eosinophils | 3.5 | (0-13) |
| Basophils | .5 | (0-4) |
| Lymphocytes | 19.0 | (7-34) |
| Monocytes | 4.0 | (0-15) |

Hematological studies are of utmost importance in any physiological study as well as in any clinical study. Therefore, blood samples from each dog were studied at intervals to follow any possible changes occurring in the blood picture. Since dogs were sacrificed at various intervals throughout the research, some dogs were studied only once postsurgically while others were studied several times over periods of up

to 45 weeks. Generally, speaking, infarction dogs were sacrificed from 1 to 4 weeks following surgery whereas the epicarditis and valcular insufficiency dogs were kept for much longer periods.

Serial hematological data are shown in the appendix for all dogs arranged in groups as to type of operation performed. Very little can be said as to any constant changes which occurred in the hematology of the dogs undergoing surgery. All dogs stayed within normal limits in hemoglobin, hematocrit, and total erythrocyte count. Most dogs remained within normal ranges in total leukocyte and differential leukocyte counts; however, epicarditis dogs generally had elevated leukocyte counts early in the syndrome (not seen in shams) which returned to lower regions after several weeks. The only other general trend noted was in the valvular insufficiency dogs where a higher eosinophil count was present following surgery. It was thought that this might be due to a reaction to the silk foreign body in the atrium; however, this could not be confirmed on histological study of the area involved.

It can be said generally that hematological studies are of little aid in determining the presence or extent of lesions of the heart in the three conditions produced when strict asepsis was practiced as done in this research.

## B. Normal Serum Enzyme Activity Assay Methods and Factors Involved

As dogs were selected for surgery, serum samples were collected for GOT, GPT, and LDH determinations. These pre-surgical values are within the normal range and compare very well with those previously reported (31, 32, 116, 144). Table 6 is a summary of the presurgical serum enzyme activity. At least one other presurgical determination was made on each dog and occasionally as many as three different pre-surgical samples were analyzed. These varied as much as 10 units on SGOT and SGPT and up to 200 units on SLDH. However, all determinations were within the normal ranges set by original samples.

Table 6. Pretreatment serum enzyme values (Karmen Units)

| | Transaminase (50 dogs) | | | SLDH (22 dogs) |
|---|---|---|---|---|
| | SGOT | | SGPT | |
| | Method | | Method | Method |
| | Hergt | Sigma | Sigma | Sigma |
| Average | 24.6 | 24.2 | 21.2 | 220.7 |
| Range | 18-37 | 12-53 | 12-36 | 45-455 |

The Sigma method (145), which is a commonly used clinical method for SGOT, was compared for accuracy with the method of Hergt and Langin (64). It was found that there

was no significant difference between the two methods when SGOT was within normal range. In addition, the reproducibility of the two methods was studied. Both methods showed no significant difference in reproducibility; however, the Hergt method was more variable and approached the 5 percent significance level in variability. Tables 7 and 9 present the details of this study.

Table 7. Comparison of Hergt and Sigma methods on duplicate SGOT determinations

| | Method | |
|---|---|---|
| | Hergt | Sigma |
| Average difference | 1.0 | 1.0 |
| Range of difference | -8.0 to 5.0 | -6.0 to 4.0 |

No significant differences in either method on repeatability.

Hergt method is approaching 5 percent significance level on variability.

Since it had been reported that repeated freezing and thawing decrease serum enzyme activity, samples were frozen, thawed, assayed and refrozen, thawed and assayed again a few days to a few weeks later. Table 8 shows the results of this study. It was found that there was a significant loss of activity at the 1 percent level with the Sigma method and at the 5 percent level, approaching the 1 percent level, with the Hergt method.

Table 8. Comparison of samples analyzed after a second freezing and thawing on duplicate SGOT determinations

| | Method | |
|---|---|---|
| | Hergt | Sigma |
| Average difference | 2.4 | 2.3 |
| Range of differences | 0 to -8*** | 0 to -6*** |

**Significant at 5 percent level approaching the 1 percent level.
***Significant at 1 percent level that there is a loss of activity.

Table 9. Comparison of Hergt vs Sigma method at various SGOT concentrations

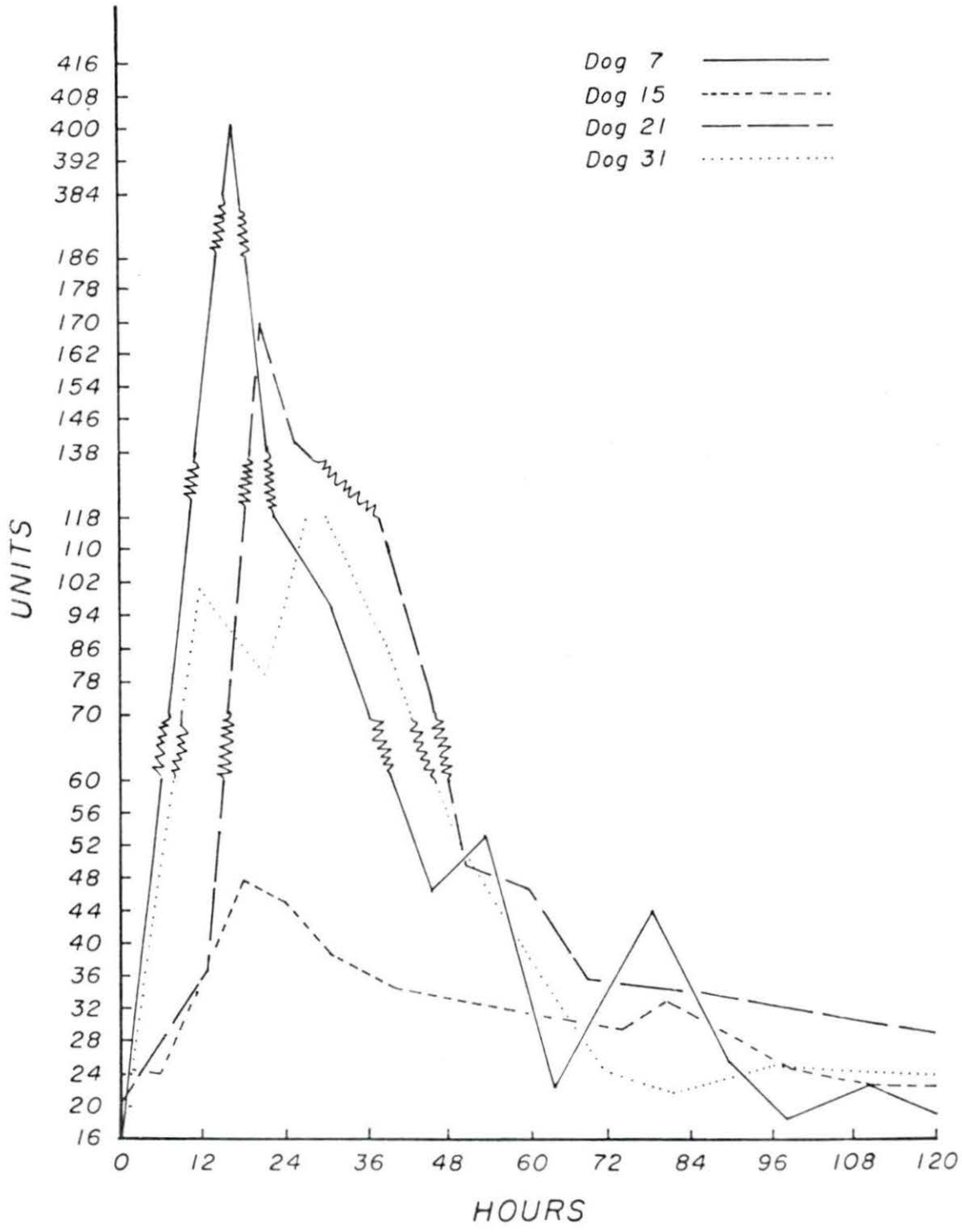
| | SGOT Concentration (units) | | | | | |
|---|---|---|---|---|---|---|
| | 15 - 39 | | 41 - 69* | | 70 - 410* | |
| | Hergt | Sigma | Hergt | Sigma | Hergt | Sigma |
| Average | 25.6 | 24.6 | 48.8 | 51.8 | 159.1 | 202.4 |
| Range | 18-38 | 17-38 | 40-62 | 41-69 | 70-320 | 75-410 |

*Significant at the .1 percent level.

In comparing the Hergt method with the Sigma method at increased serum enzyme concentrations, it was found that there was a significant difference between the two methods at the 0.1 percent level (See Table 9, and Figures 1 and 2). However, since neither method could be compared with a known concentration of enzyme, it is impossible to say which method

Figure 1. Graphic representation of SGOT levels of selected dogs following myocardial infarction (Sigma method).

UNITS


Dog 7
Dog 15
Dog 21
Dog 31
UNITS
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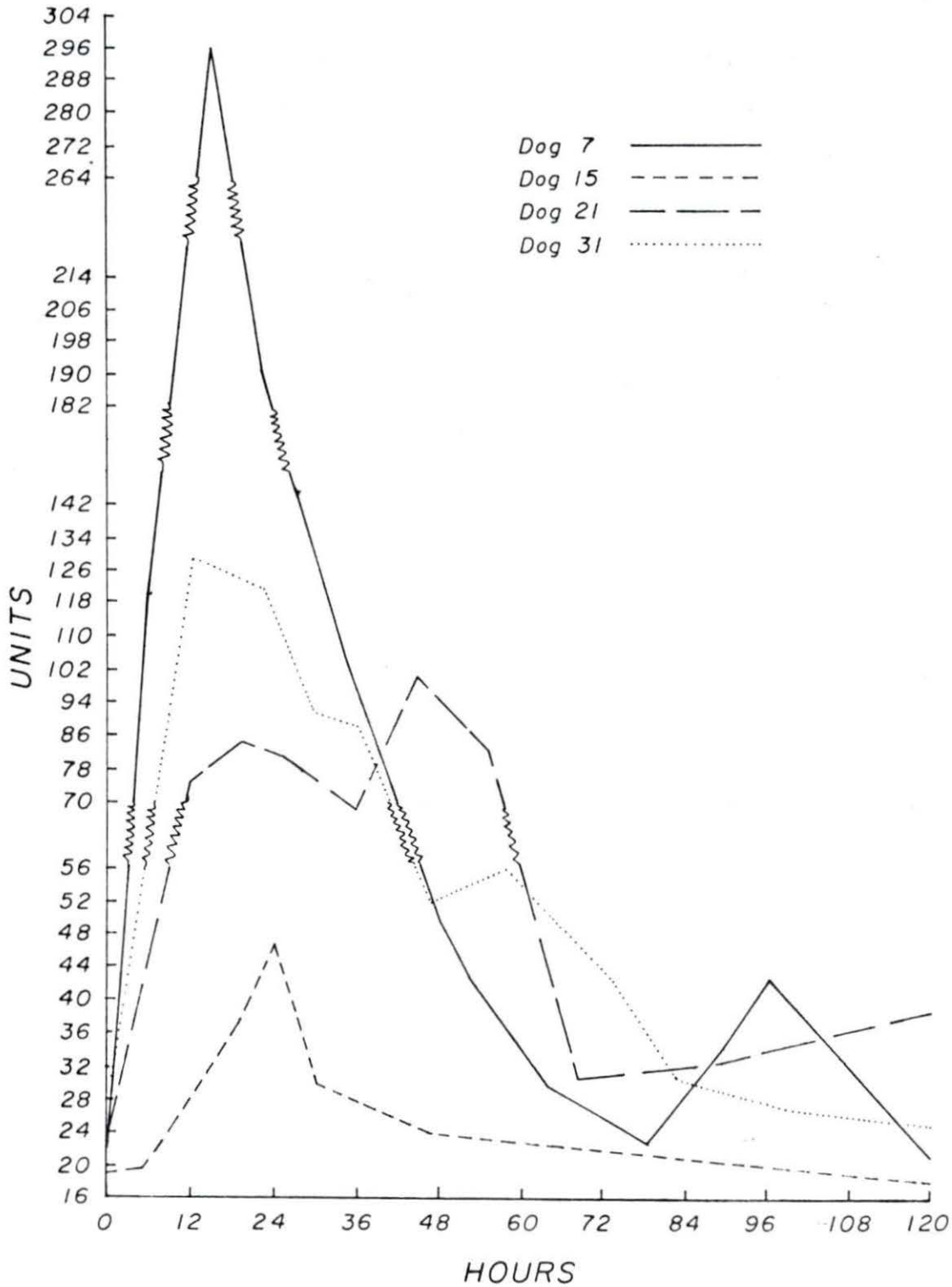
408
400
392
384
186
178
170
162
154
146
138
118
110
102
94
86
78
70
60
56
52
48
44
40
36
32
28
24
20
16
0
12
24
36
48
60
72
84
96
108
120
HOURS

Figure 2. Graphic representation of SGOT levels of selected dogs following myocardial infarction (Hergt method).

UNITS


304
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272
264
214
206
198
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182
142
134
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12
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108
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HOURS
Dog 7
Dog 15
Dog 21
Dog 31

is the most accurate at these increased concentrations. It is thought that the Sigma method is the more advantageous because it spreads out the range of activities, thus separating abnormally high activities from the normal low activities.

Since it was reported by Ranke et al. (126) that there may be a difference in serum enzyme concentration in human subjects with age, dogs which were divided into three age groups for ECG purposes were analyzed as to serum enzyme concentration from presurgical data. Table 10 is a compilation of the results. It was found there was no significant difference in serum enzyme activity due to age differences, and age was not considered as a factor in further studies.

Table 10. Normal serum enzyme levels arranged as to age[a] (Sigma method)

| Age | No. dogs | SGOT[b] | SGPT[b] | No. dogs | SLDH[b] |
|---|---|---|---|---|---|
| 1 | 11 | 22.1 (12-28) | 21.4 (13-30) | 8 | 160.6 (70-325) |
| 2 | 28 | 25.8 (13-53) | 21.2 (12-35) | 12 | 260.4 (140-455) |
| 3 | 11 | 21.6 (14-30) | 21.1 (16-36) | 4 | 136.2 (45-200) |

[a]Age differences are not significant statistically.

[b]Average and range.

## C. Surgery and Associated Factors

As described previously, three types of cardiac pathology were produced by surgical methods. Each surgical procedure was followed by serial enzyme and ECG studies. All dogs were necropsied and gross and histopathological studies were made of organs and tissues. Each of these studies will be discussed under separate headings. Table 11 is a summary of surgery performed.

Table 11. Summary of experimental surgery

| Procedure | No. Operated | No. Lived | No. Necropsied |
|---|---|---|---|
| Infarction | 17 | 14 | 17 |
| Sham | 8 | 8 | 8 |
| Vulvular insufficiency | 11 | 5 | 11 |
| Sham | 5 | 5 | 5 |
| Epicarditis | 6 | 6 | 6 |
| Sham | 3 | 3 | 3 |
| TOTALS | 50 | 41 | 50 |

## D. Serial Enzyme Studies Following Surgery

### 1. Myocardial infarction studies

Seventeen dogs underwent surgery to produce myocardial infarction. Two of these dogs did not survive surgery and one

died 10 hours after production of myocardial infarction. Fourteen dogs had various degrees of myocardial infarction produced. Table 12 is a summary of enzyme activity presurgically and peaks reached.

Eight dogs were sham-operated. All dogs lived and serial enzyme activity determinations were made for three to four weeks. Table 13 is a summary of enzyme activity presurgically and peaks reached.

One dog, No. 25, had two infarctions produced. While attempting to put a ligature around the anterior left descending coronary artery, it was accidentally punctured. The artery had to be ligated to stop the hemorrhage. Several weeks later the dog underwent surgery again and a second infarct was produced by ligating the left posterior descending coronary artery (See Figure 14, Appendix B). Upon necropsy one dog, No. 38, was found to have an accompanying epicarditis.

The serial serum enzyme studies indicate that activity starts to increase at 8 to 15 hours following infarction. Peaks are reached at 18 to 24 hours with SGOT and SGPT at 25 to 30 hours with SLDH. It has been reported (60, 85, 132), as described previously, that SGPT does not rise to any significant level in myocardial infarction of humans. However, it was reported (132) that SGPT may rise following myocardial infarction in the dogs. Cornelius (31) feels

Table 12. Enzyme levels of infarction dogs (Karmen units)

| | Method | | | |
|---|---|---|---|---|
| | Hergt | Sigma | | |
| | SGOT(14)[a] | SGOT(14) | SGPT(14) | LDH(9) |
| Presurgery I | | | | |
| average | 23.5 | 23.4 | 22.8 | 242 |
| range | 19-32 | 14-37 | 17-36 | 130-450 |
| Presurgery II | | | | |
| average | 25.2 | 22.4 | 21.0 | 283 (5) |
| range | 20-38 | 13-32 | 18-25 | 170-500 |
| Range of peaks[b] | 40-320 | 49-410 | 21-240 | 280-1225 |

[a]Number of dogs in parenthesis.

[b]Began to elevate from 8-18 hours post surgery. Transaminase reach peak in 12-31 hours and LDH, 5-10 hours later usually. Transaminase remain elevated for 25-60 hours, LDH, 10-20 hours longer.

Table 13. Enzyme levels of sham infarction dogs (Karmen units)

| | Method | | | |
|---|---|---|---|---|
| | Hergt | Sigma | | |
| | SGOT(8)[a] | SGOT(8) | SGPT(8) | LDH(3) |
| Presurgery I | | | | |
| average | 22.9 | 22.2 | 19.5 | 257 |
| range | 18-27 | 18-27 | 12-26 | 205-325 |
| Presurgery II | | | | |
| average | 22.9 | 26.1 | 22.1 | - |
| range | 20-26 | 21-30 | 15-40 | - |
| Range of peaks[b] | 26-41 | 25-43 | 18-39 | 130-280 |

[a]Number of dogs in parenthesis.

[b]Time to reach peaks varied from 14 to 61 hours -- all returned to normal shortly after peak was reached and continued within normal ranges.

that elevated SGPT in dogs is indicative of liver necrosis alone. The results from this research indicate that SGPT may rise to high levels but not as high a level as SGOT (Figures 1, 2 and 3). This fact must be kept in mind when suspecting liver damage because of the high SGPT values, the heart could be involved instead of the liver. From the reports of other workers it would seem that the time-concentration curve would be more prolonged in cases of liver pathology than in those involving the myocardium. In addition, the increased SGPT activity will be nearly as high as, or higher than SGOT in necrosis of the liver.

This work indicates that SLDH is not as reliable as SGOT as an index of myocardial necrosis (Table 12 and Figure 4). Results are erratic (See Table 27, Appendix A) and because of the wide range of normal values, it was frequently impossible to confirm a diagnosis of myocardial infarction with this enzyme assay, i.e., although the serum activity might be increased several times the normal value for the particular dog involved, it often falls within what must be considered the normal range. This presents a real problem in clinical work where the clinician seldom has a "normal" for the dog presented.

SGOT and SGPT levels remained elevated for 40 to 80 hours depending somewhat on the amount of necrosis present, i.e.,

Figure 3. Graphic representation of SGPT levels of selected dogs following myocardial infarction.

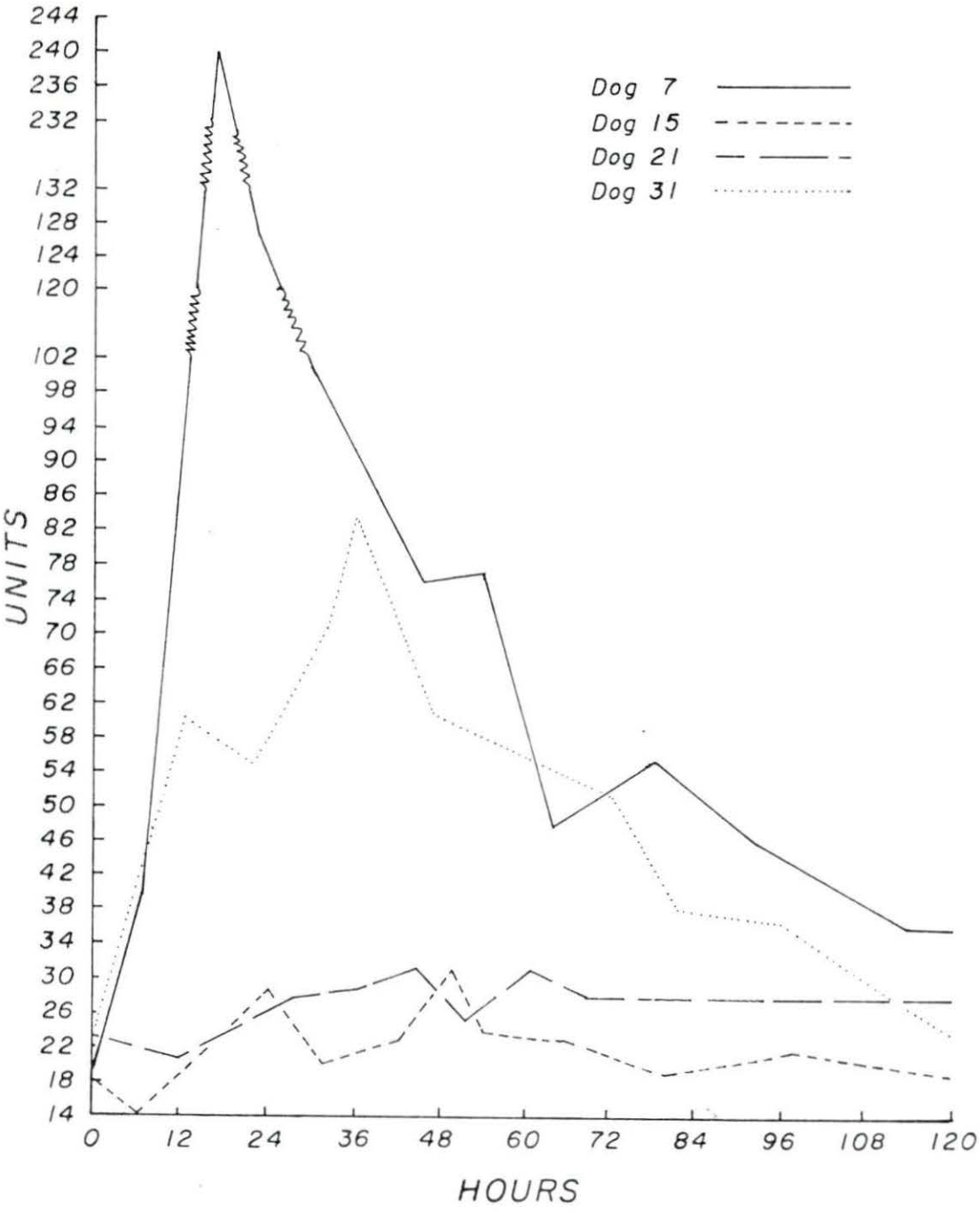
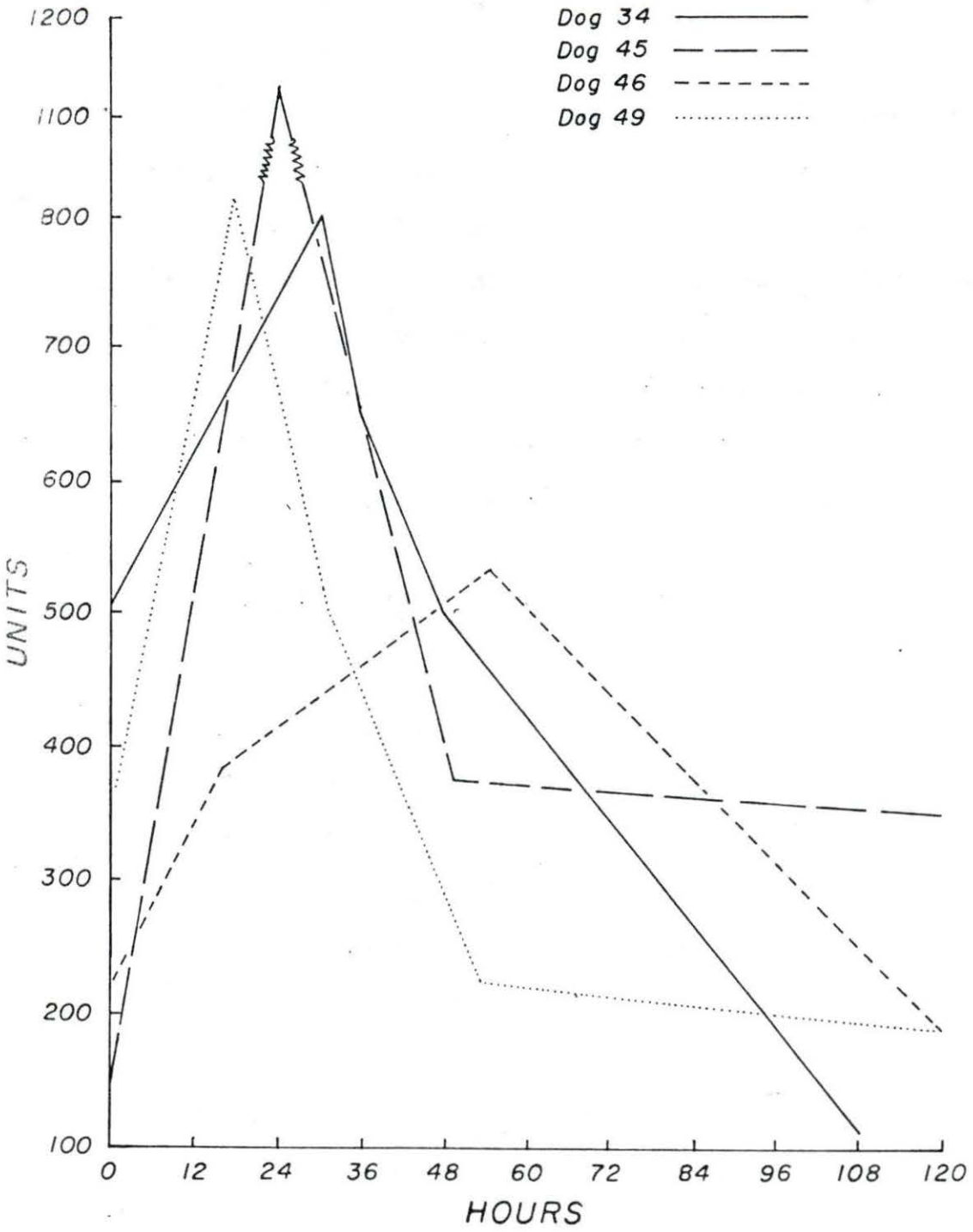

Dog 7
Dog 15
Dog 21
Dog 31
UNITS
244
240
236
232
132
128
124
120
102
98
94
90
86
82
78
74
70
66
62
58
54
50
46
42
38
34
30
26
22
18
14
0
12
24
36
48
60
72
84
96
108
120
HOURS

Figure 4. Graphic representation of SLDH levels of selected dogs following myocardial infarction.


Dog 34
Dog 45
Dog 46
Dog 49
1200
1100
800
700
600
500
400
300
200
100
UNITS
0
12
24
36
48
60
72
84
96
108
120
HOURS

as the amount of infarcted tissue is increased the peak concentrations are higher, and the length of time the enzyme remains elevated is prolonged (See Table 14). However, this is still a relatively short period since frequently the patient may not be presented to the clinic until three to four days after the onset of symptoms. It is reported (46, 72, 161) that SLDH levels remain elevated for longer periods (six days) in humans. It was found in this work that SLDH activity remained at abnormally high levels in dogs with myocardial infarction for 10 to 20 hours longer than the transaminases. Figures 1, 2, 3, and 4 depict the changes in enzyme concentrations in dogs suffering from various amounts of infarction. (See Tables 25 through 27, Appendix A for serial enzyme changes of each dog with myocardial infarction.)

Once the enzyme returns to the normal range there was seldom any marked elevation of the serum levels again in these studies. However, one dog, No. 11, did show a rise several weeks after production of an infarct. Upon necropsy, two small infarcted areas were found, one where the ligature had been placed and one more posteriorly on the endocardial surface of the left ventricle. It is thought that the second rise was due to this infarct; however, the dog did not show any outstanding symptoms at this time. This will be discussed in more detail later.

Infarcts were measured at the time of necropsy and these were correlated with the SGOT elevations. Table 14 gives the results. The results compare very favorably with the reports of other workers where tissue enzyme analyses were compared from necrotic areas and correlated with serum concentration. As a matter of interest SGPT was also correlated with amount of necrosis found at necropsy (Table 15). From this table it appears that only the more severe infarcts result in a great increase in SGPT and this may partially explain the controversy appearing in the literature regarding the elevation of SGPT in myocardial infarction. One can be misled by measuring only the diameter of the lesion since the depth of the infarcted area is not considered. In general the total amount of infarcted tissue correlated very well with the rise in SGOT. Individual dog serial SGOT and SGPT relating to amount of infarction are presented in Tables 25 and 26 (Appendix A).

As can be seen from Table 13 most of the sham-operated dogs remained well within normal serum enzyme ranges following surgery. However, a few animals did approach very high normal values or low abnormal values (40 units or over). Individual serial levels for the first 90 hours following surgery are shown in Table 28 (Appendix A) for sham-operated dogs. Serum levels continued within normal ranges until

Table 14. Comparison of presurgical and postsurgical SGOT levels of dogs with myocardial infarction (Sigma)

| No. dogs | Dia. inf. | Pre-surg. | Hours postsurgery 17 - 22 | 23 - 27 | 28 - 34 | 35 - 41 | 42 - 50 | 51 - 72 |
|---|---|---|---|---|---|---|---|---|
| 4 | < 1 cm. | 20.5 (18-22) | 63.5 (48-75) | 59.0 (45-64) | 45.5 (39-57) | 39.2 (35-51) | 34.0 (30-42) | 32.0 (30-35) |
| 3 | 1-2 cm. | 27.7 (18-37) | 150.3 (121-170) | 108.3 (88-140) | 105.7 (82-130) | 78.3 (50-118) | 51.0 (30-75) | 39.3 (29-50) |
| 7 | > 2 cm. | 23.1 (14-32) | 277.1 (80-400) | 236.6 (118-410) | 139.7 (72-280) | 69.9 (45-150) | 64.7 (40-130) | 38.6 (25-65) |

Table 15. Comparison of presurgical and postsurgical SGPT levels of dogs with myocardial infarction

| No. dogs | Dia. inf. | Pre-surg. | Hours postsurgery 17 - 22 | 23 - 27 | 28 - 34 | 35 - 43 | 44 - 50 | 51 - 72 |
|---|---|---|---|---|---|---|---|---|
| 4 | < 1 cm. | 24.5 (17-36) | 38.5 (24-48) | 39.2 (29-48) | 34.2 (20-42) | 36.2 (23-41) | 36.2 (31-46) | 29.5 (20-40) |
| 3 | 1-2 cm. | 26.0 (20-30) | 34.3 (25-44) | 33.7 (27-42) | 33.3 (28-39) | 34.7 (29-38) | 30.7 (25-36) | 23.3 (20-28) |
| 7 | > 2 cm. | 20.4 (17-24) | 125.9 (60-240) | 118.9 (55-220) | 110.4 (65-220) | 105.1 (69-171) | 83.1 (55-139) | 46.7 (29-64) |

sacrifice. Figure 5 depicts the levels of each of the enzymes at various times following surgery.

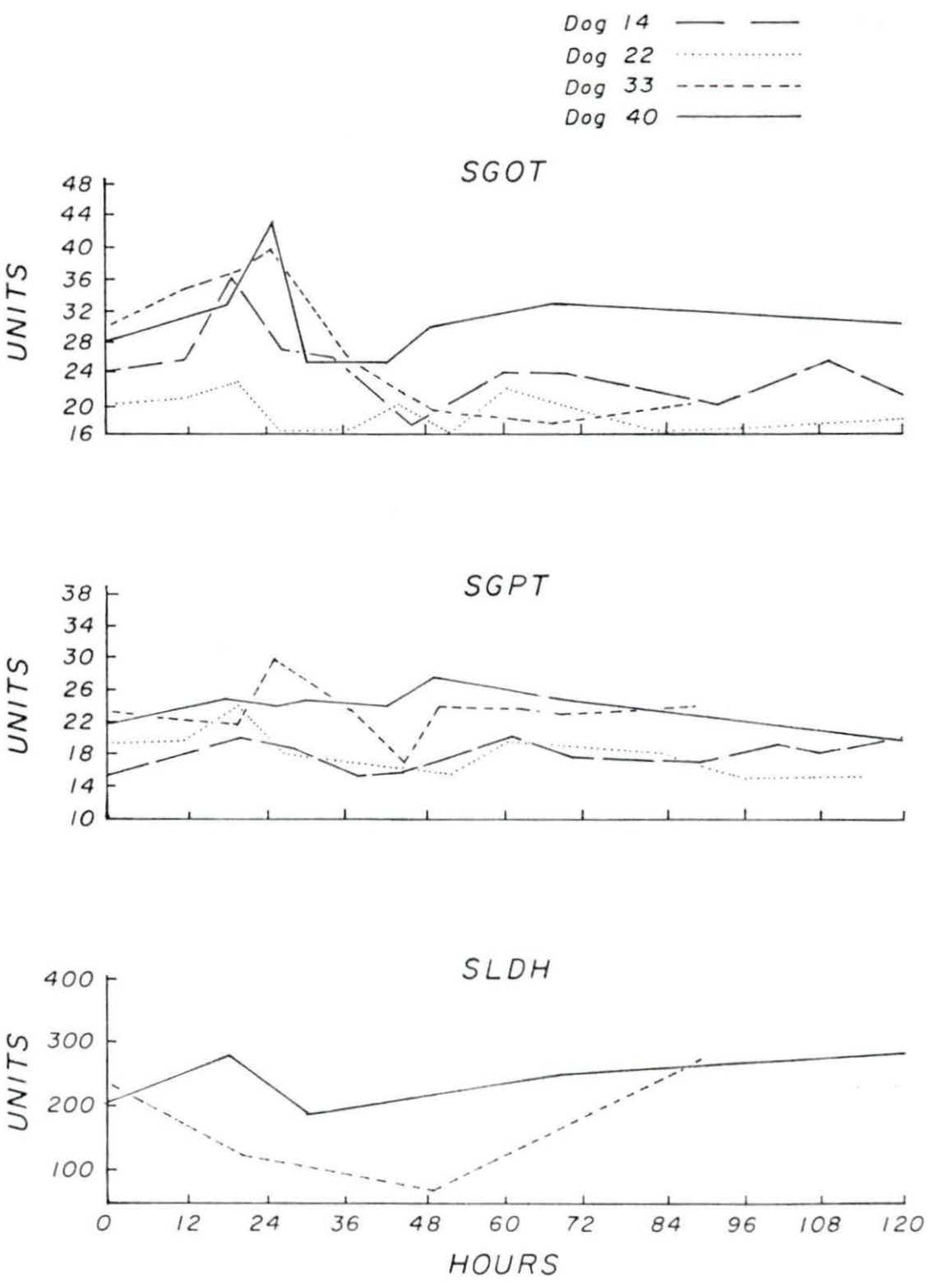
The exact reason for the small elevation occurring has not been determined. It may be just coincident with a normal physiological elevation, but more likely it is from damage to skeletal muscles which occurred on two or three occasions in attempting to isolate ligatures. However, this does not seem quite logical since transaminase activities following thoracic surgery were seldom over 100 units GOT and then much more skeletal muscle was damaged. A third possibility could be some liver damage following the repeated use of sodium pentobarbital, however, here again, it seems the liver could tolerate the anesthetic dose without causing any harm. Perhaps it is a combination of these and other factors. Since much of the physiology involved is not understood at this time, the answer will have to remain speculative for the present.

Serum GOT and GPT concentrations can be a definite aid to the diagnosis of myocardial infarction in dogs. More work needs to be done with SLDH before it is valuable in the diagnosis of myocardial infarctions in dogs.

2. Epicarditis studies

Nine dogs have been included in this group. Of these, three were sham-operated. Table 16 presents presurgical data as well as postsurgical averages on dogs with pathology

Figure 5. Graphic representation of enzyme levels of selected sham-operated myocardial infarction dogs (Sigma).


Dog 14
Dog 22
Dog 33
Dog 40
SGOT
UNITS
48
44
40
36
32
28
24
20
16
SGPT
UNITS
38
34
30
26
22
18
14
10
SLDH
UNITS
400
300
200
100
0
12
24
36
48
60
72
84
96
108
120
HOURS

produced. All but one dog remained well within normal range. This dog, No. 10, presented an increase in both SGOT and SGPT of 48 and 37 units, respectively, four months after surgery. This was apparently a transient rise which had disappeared when another serum sample was taken two weeks later. No pathology which could account for this rise was found upon necropsy four weeks later. Dog 43 showed a progressive rise in SLDH from a presurgical value of 200 units up to 555 units at 5 weeks. This level then declined gradually back to 255 units at 4 months. The reason for this rise is not known, but is believed to be a result of the natural variation of the normal levels and of the test. Individual serial enzyme data are shown in Table 29 (Appendix A).

Table 16. Enzyme levels of dogs with epicarditis (Karmen units)

| | Method | | | |
|---|---|---|---|---|
| | Hergt | Sigma | | |
| | SGOT(6)[a] | SGOT(6) | SGPT(6) | SLDH(2) |
| Presurgery I | | | | |
| average | 22.7 | 24.7 | 18.8 | - |
| range | 18-29 | 14-33 | 13-23 | 120-200 |
| Presurgery II | | | | |
| average | 28.0 | 25.0 | 20.5 | - |
| range | 22-31 | 16-30 | 16-27 | 125-130 |
| Postsurgery[b] | | | | |
| average | 26.6 | 26.0 | 22.9 | 246 (4) |
| range | 18-44 | 13-48 | 12-37 | 100-555 |

[a]Number of dogs in parenthesis.

[b]Average for samples taken at intervals for three months or more.

Data from sham-operated dogs appeared to be well within normal ranges for the three enzymes upon serial study. Table 17 shows presurgical and postsurgical averages and ranges. Individual serial enzyme data are shown in Table 30 (Appendix A).

From this study it can be concluded, concurring with the reports of other workers, that serum enzyme levels are of little value in diagnosing an epicarditis and pericarditis. However, they might be an important negative finding when considered in conjunction with clinical symptoms and ECG recordings. Figures 6 and 7 depict serial enzyme levels in epicarditis dogs and shams.

Table 17. Enzyme levels of sham-operated dogs for epicarditis (Karmen units)

| | Method | | | |
|---|---|---|---|---|
| | Hergt | Sigma | | |
| | SGOT(3)[a] | SGOT(3) | SGPT(3) | LDH(2) |
| Presurgery I | | | | |
| average | 23.0 | 19.3 | 19.7 | - |
| range | 20-27 | 18-21 | 16-23 | 75-150 |
| Presurgery II | | | | |
| average | 20.7 | 18.3 | 19.0 | - |
| range | 16-26 | 16-20 | 18-20 | 75-130 |
| Postsurgery[b] | | | | |
| average | 25.1 | 23.8 | 19.9 | 124 |
| range | 14-36 | 14-36 | 14-30 | 75-225 |

[a]Number of dogs in parenthesis.

[b]Average for samples taken at intervals for three months or longer.

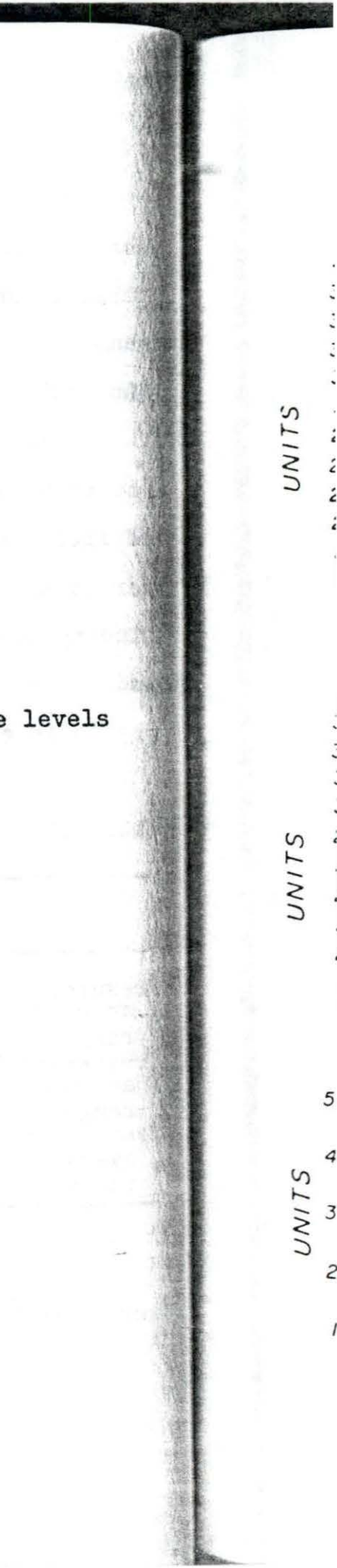



Figure 6. Graphic representation of serial enzyme levels of selected epicarditis dogs (Sigma).

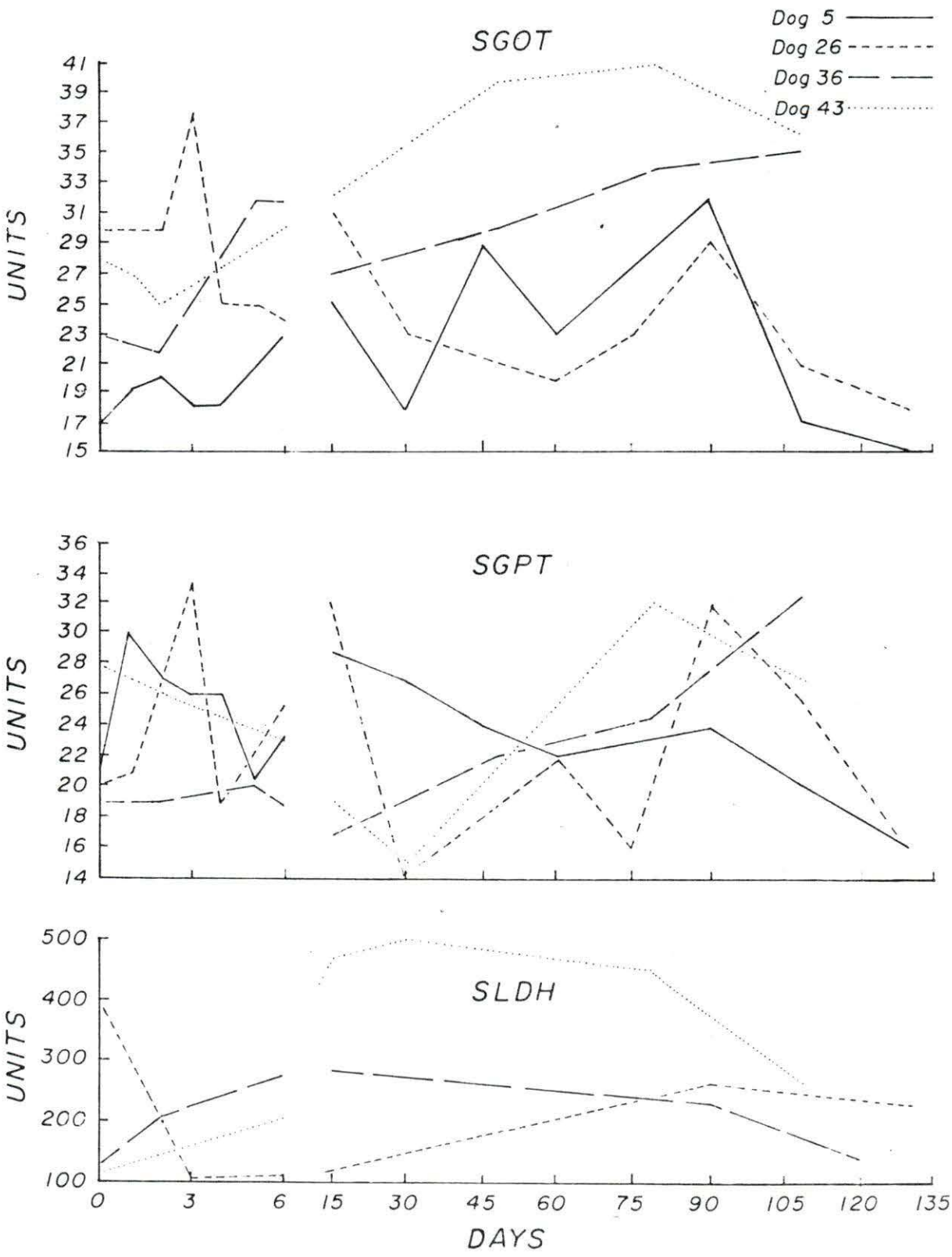
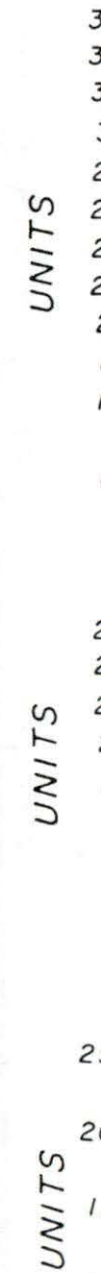
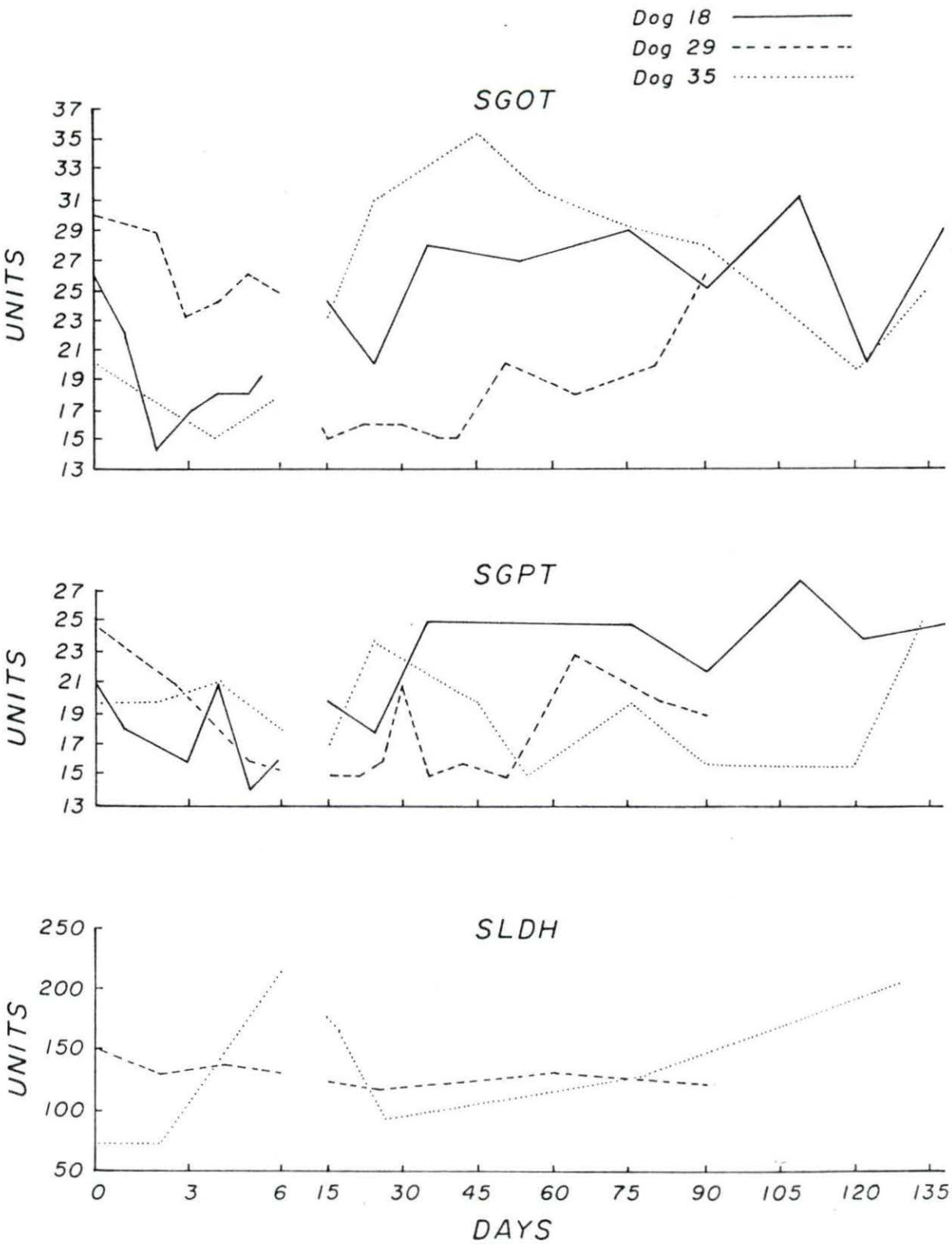

Dog 5
Dog 26
Dog 36
Dog 43
SGOT
SGPT
SLDH
UNITS
DAYS
41
39
37
35
33
31
29
27
25
23
21
19
17
15
36
34
32
30
28
26
24
22
20
18
16
14
500
400
300
200
100
0
3
6
15
30
45
60
75
90
105
120
135

Figure 7. Graphic representation of serial enzyme levels of selected sham-operated epicarditis dogs (Sigma).




Dog 18
Dog 29
Dog 35
SGOT
UNITS
37
35
33
31
29
27
25
23
21
19
17
15
13
SGPT
UNITS
27
25
23
21
19
17
15
13
SLDH
UNITS
250
200
150
100
50
0
3
6
15
30
45
60
75
90
105
120
135
DAYS

3. Valvular insufficiency studies

Sixteen dogs had surgery. Five of them were sham-operated. Four dogs had a right A-V valvular insufficiency produced and one a left A-V valvular insufficiency. The rest died during surgery or shortly after from a variety of causes. All dogs were necropsied and varying grades of pathology observed in the dogs with lesions produced. These lesions will be described in more detail later. Dog 42 showed the greatest amount of damage. This dog had shown severe symptoms from the pathology and succumbed 72 days following surgery from excessive fluids in the pleural cavity. Table 18 summarizes the results of the serial enzyme study. Figure 8 depicts the serial studies after the fourth day when enzyme levels returned to near normal following the surgical procedure. Table 31 (Appendix A) shows serial studies of individual dogs through 12 weeks.

All dogs returned to normal following surgery and remained at normal levels for several weeks. There was then a trend to a slight increase in enzyme levels; however, for the most part these stayed within normal ranges. Dogs 41 and 42 showed a rise in SGOT and SGPT over a period of about two weeks which were definitely elevated beyond normal levels. In both cases SGPT rose to higher levels than SGOT. The sampling at this time was at two-week intervals so no time-concentration curve was made. Still, it seems reasonable to

expect that some liver damage was occurring from the passive hyperemia. This theory was confirmed upon necropsy when histological and gross studies indicated necrosis of the liver. However, this damage apparently was not severe enough to cause clinical symptoms over and above those of the cardiac pathology.

Table 18. Enzyme levels of valvular insufficiency dogs (Karmen units)

| | METHOD | | | |
|---|---|---|---|---|
| | Hergt | Sigma | | |
| | SGOT(5)[a] | SGOT(5) | SGPT(5) | LDH(4) |
| Presurgery | | | | |
| average | 29.2 | 25.0 | 21.8 | 231.2 |
| range | 24-34 | 18-34 | 15-31 | 45-455 |
| Peak | | | | |
| average | 120.8 | 139.8 | 56.6 | 421.8 |
| range | 75-215 | 80-230 | 23-115 | 177-590 |
| Time to peak | | | | |
| hours | 17-21 | 17-25 | 17-21 | 25-50 |
| Time to normal[b] | | | | |
| hours | 55-100 | 55-100 | 50-90 | 60-100 |

[a]Number of dogs in parenthesis.

[b]Dogs returning to normal remained within normal range except dogs 41 and 42.

Table 19 is a summary of the sham-operated dogs. Results were very similar to those seen in the dogs with lesions produced. However, once dogs returned to normal serum levels they continued within normal ranges. The serum levels of all three enzymes remained quite stable throughout

the research. Dog 4 remained in the doubtful zone of 40 to 60 SGOT units throughout the rest of his life; however, this dog had a high presurgical level of 53 units. This points out the fact that certain animals may have normal levels in what could be considered indicative of pathology in the majority of cases. Hence, the clinician must consider the overall clinical picture very carefully in those dogs showing serum levels between 40 to 60 units. It is fairly safe to assume that anything below 35 is normal. Figure 9 indicates serial enzyme changes in representative sham-operated dogs. Individual serial serum enzyme activity up to eight weeks is given in Table 32 (Appendix A).

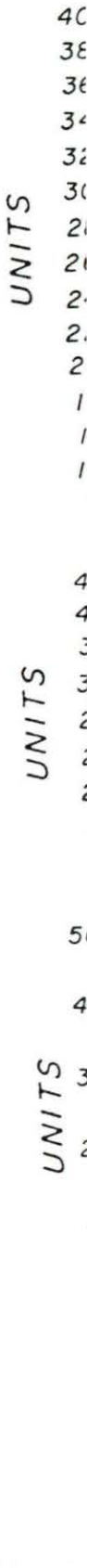
Table 19. Enzyme levels of dogs sham-operated for valvular insufficiency (Karmen units)

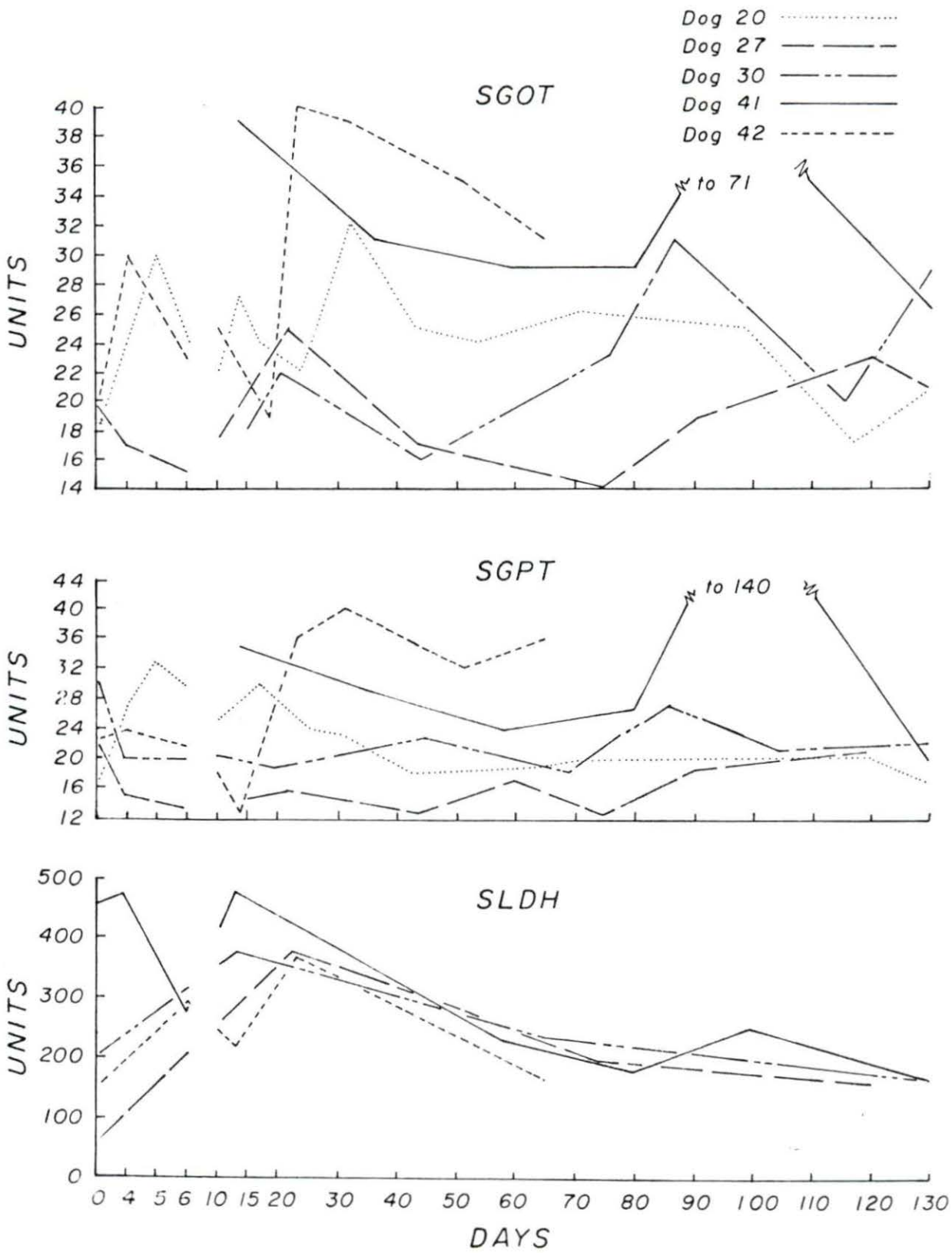
| | Method | | | |
|---|---|---|---|---|
| | Hergt | Sigma | | |
| | SGOT(5)[a] | SGOT(5) | SGPT(5) | LDH(2) |
| Presurgery | | | | |
| average | 24.6 | 31.2 | 22.4 | 185 |
| range | 18-30 | 20-53 | 17-32 | 160-210 |
| Peak[b] | | | | |
| average | 119.0 | 119.6 | 43.6 | 260 |
| range | 56-178 | 42-232 | 18-70 | 230-550 |

[a]Number of dogs in parenthesis.

[b]Peak at 17-27 hours. Peaks are usually a few hours later in GPT and 10-25 hours later in LDH. Shams return to normal in 50-100 hours and continue within normal range.

Figure 8. Graphic representation of serial enzyme levels of valvular insufficiency dogs (Sigma).




Dog 20
Dog 27
Dog 30
Dog 41
Dog 42
SGOT
UNITS
40
38
36
34
32
30
28
26
24
22
20
18
16
14
to 71
SGPT
UNITS
44
40
36
32
28
24
20
16
12
to 140
SLDH
UNITS
500
400
300
200
100
0
0 4 5 6 10 15 20 30 40 50 60 70 80 90 100 110 120 130
DAYS

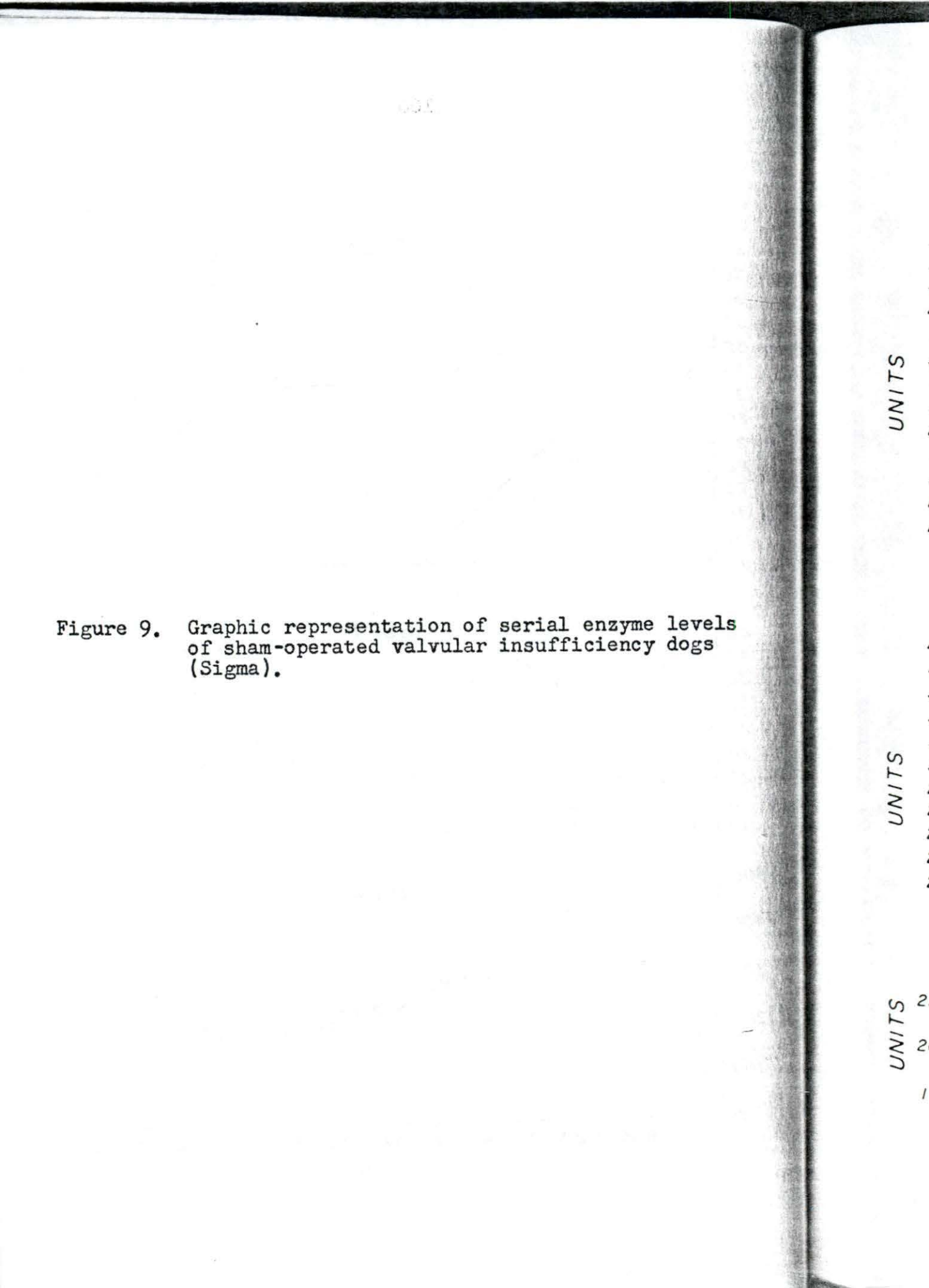
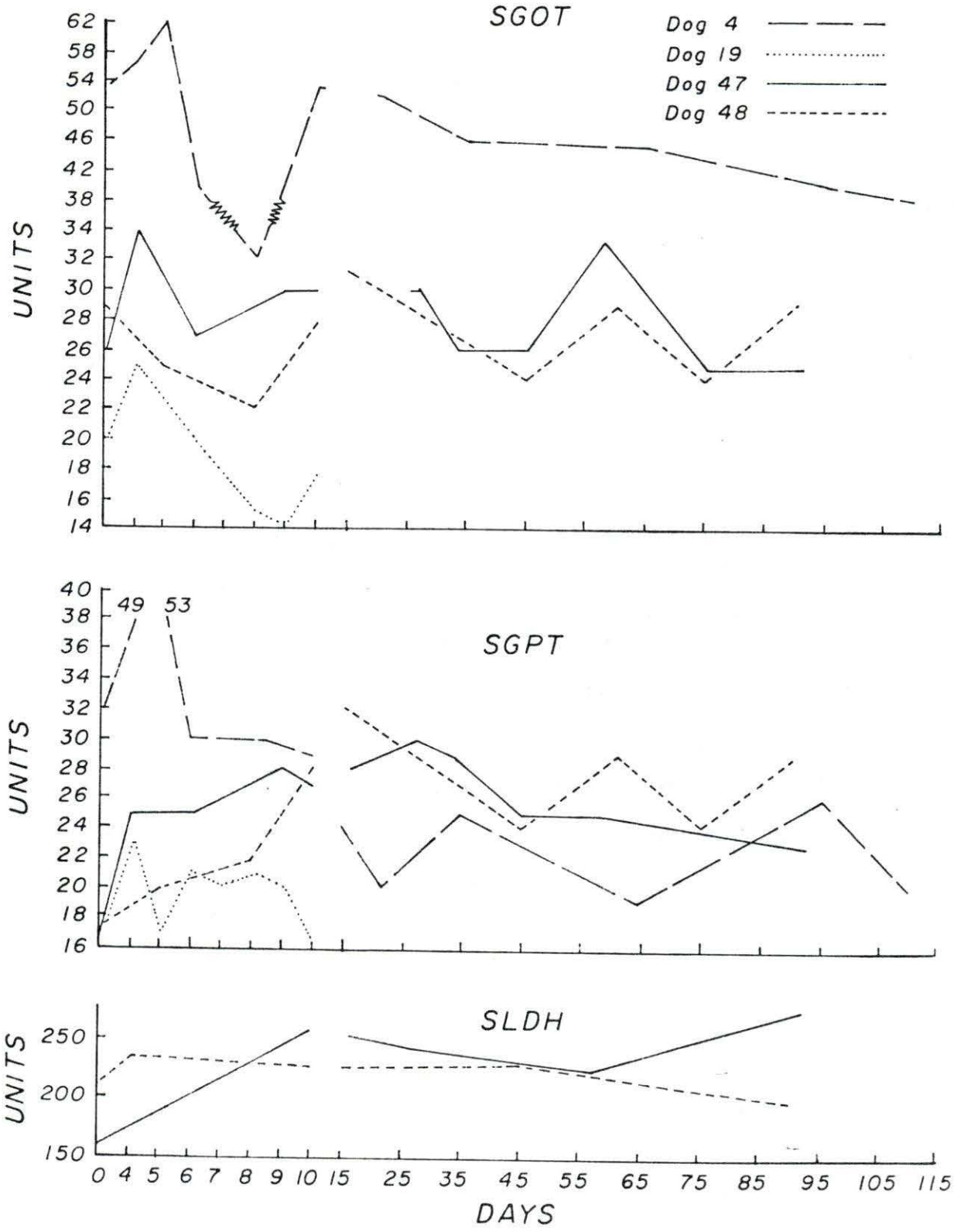



Figure 9. Graphic representation of serial enzyme levels of sham-operated valvular insufficiency dogs (Sigma).


SGOT
Dog 4
Dog 19
Dog 47
Dog 48
UNITS
62
58
54
50
46
42
38
34
32
30
28
26
24
22
20
18
16
14
SGPT
49
53
UNITS
40
38
36
34
32
30
28
26
24
22
20
18
16
SLDH
UNITS
250
200
150
0 4 5 6 7 8 9 10 15 25 35 45 55 65 75 85 95 105 115
DAYS

From this study it can be concluded that serum enzyme levels are of no aid in diagnosing valvular insufficiency. However, it must be pointed out that liver damage may occur secondarily to valvular insufficiency and increased SGOT and SGPT may follow. Therefore, the clinician must not lose sight of the cause and effect relation which occurs in many conditions.

E. Presurgical ECG Data and Factors Involved

It was found that ECG data could not be analyzed statistically with any degree of confidence because of the wide variability within any one recording with respect to voltage and time. However, measurements were made carefully and averages obtained from records of all individual leads and these means were used in further generalizations for comparison purposes and for discussion. Presurgical ECG's were taken on 46 dogs which were normal, healthy animals and in which the ECG appeared to be normal. Since the normal ECG in the dog has such a wide variability, certain parameters were examined as described previously in the hope of finding that factor or combination of factors which could be accepted as being normal in the greatest majority of recordings.

Because of the discrepancies reported in the literature on electrodardiographic values and the wide variability

found on preliminary studies on amplitudes of the various complexes from day to day, a ratio was made and this is reported instead of absolute measurements. This ratio in each case is compared to R with other values, Q, S, or T, taken as 1 mm. (0.1 mv.). In cases where Q, S, or T is 0, the value was omitted in calculating averages. However, these values are shown in Tables 33 through 38 (Appendix A). In cases where Q, R, or S was less than 1 mm., necessary computations were made to compare R to 1 mm. In these cases the relative error is quite large due to measuring difficulties and the fact that increasing such ratios magnifies error. Tables 20, 21, and 22 are a compilation of the normal values obtained from 46 mongrel dogs of both sexes and all ages. Since age had been suggested as being a factor affecting ECG values, these data were analyzed statistically for age differences. The only significant change was in heart rate which decreased with age.

Table 20. Age effect of dogs on heart rate

| Age[a] | Heart rate | |
|---|---|---|
| Group 1 | 151 | (100-175) |
| Group 2 | 141 | (100-185) |
| Group 3 | 120 | (65-175) |

[a]Group 1: 4 to 12 months; Group 2: 1 to 5 years; Group 3: over 5 years.

Presurgical electrocardiographic values are presented as standards which is the usual way of reporting such data, since it is unwise to break with tradition all together.

Table 21. Electrocardiographic standards of normal dogs (1 mm. = .1 mv.)

| | Lead | | | |
|---|---|---|---|---|
| | I | II | III | aVF |
| Q average | 4.15 | 2.51 | 1.68 | 1.46 |
| range | 0.5-12.0 | 0.5-7.75 | 0.5-5.25 | 0.5-5.0 |
| R average | 6.95 | 19.20 | 15.78 | 18.42 |
| range | 1.5-15.0 | 3.0-33.0 | 5.0-31.0 | 8.0-33.0 |
| S average | 1.84 | 4.42 | 4.91 | 5.10 |
| range | 0.25-12.0 | 0.5-17.0 | 0.5-16.0 | 0.5-20.0 |
| ST± average | .80 | 1.08 | 1.14 | .91 |
| range | 0.5-1.0 | -1.25-2.0 | -1.5-2.0 | -1.0-1.5 |

| | Durations (seconds) | | |
|---|---|---|---|
| | PR | QRS | QT |
| Average | .105 | .052 | .230 |
| Range | .06--.18 | .03-.08 | .18-.28 |

Other workers (71, 86) have reported high incidences of $Q_I$ followed by an inverted T-wave and of $S_{III}$ followed by an upright T. The results of this study agree with such reports. It was found that 49 percent of the recordings had a $Q_I$, 88 percent had an $S_{III}$ and 60 percent showed a negative

T in lead I. A negative T followed a Q-wave in lead I 95 percent of the recordings and an upright T-wave followed an S-wave in lead III in 89 percent of the records. (See Table 23). $S_{II}$ and $S_{aVF}$ occurred together in approximately 90 percent of the records. Upward T-waves occurred in 80 to 90 percent of the records in leads II, III and aVF. Q-waves and S-waves occurred most often in lead II and because of this it was used for all further R/Q, R/S, and R/T ratios. ST deviation occurred in only 20 percent of all recordings but in 46 percent of the dogs in one or more leads. However, as can be seen from Table 21, this deviation was quite small, being $\pm$ 1 mm. or less in 90 percent of the recordings showing deviation and never over 2.0 mm. (in two leads). ST deviation was always up in lead I and was seen in only 25 percent of the recordings showing deviation, or less than 11 percent of all dogs, and was always less than 1 mm.

Records taken on separate days presurgically followed similar patterns; however, there was a lot of difference in absolute amplitudes and some difference in duration of complexes. Ratios, although varying considerably, did not vary as much as the absolute amplitudes in R/Q and R/S. However, R/T varied about as much as absolute T amplitudes and T also varied back and forth from positive to negative in all leads; however, there was a trend to continue as positive in most instances in lead II, III and aVF. This same pattern was seen in sham-operated dogs.

Table 22. ECG data from normal dogs

| | Lead[a] | | | |
|---|---|---|---|---|
| | I | II | III | aVF |
| R/Q average | 3.4 | 11.2 | 14.9 | 16.8 |
| range | 0.5-22.0 | 3.0-42.0 | 2.5-44.0 | 4.5-46.0 |
| R/S average | 6.0 | 8.12 | 8.8 | 6.8 |
| range | 1.0-20.0 | 1.5-56.0 | 1.0-62.0 | 1.0-27.0 |
| R/+T average | 6.0 | 9.2 | 8.5 | 8.5 |
| range | 1.75-12.0 | 2.25-40.0 | 1.5-50.0 | 1.75-34.0 |
| R/-T average | 7.4 | 21.0 | 13.8 | 16.4 |
| range | 2.5-20.0 | 1.25-42.0 | 5.25-28.0 | 10.0-30.0 |
| ST± deviation[b] | 11 | 22 | 24 | 24 |

[a]Ratios expressed on basis of R:1 and 0 values omitted.

[b]Percent of total dogs showing deviation in lead.

Notching and slurring are very rare in occurrence in the presurgical records with the exception of the T-wave which takes on many odd forms. The only other notching which occurred was in the R-wave in lead I in one dog and in lead III in another.

P-waves were always upright in leads II, III, and aVF with one exception of an isoelectric P-wave in one lead III. P-waves are usually upright in lead I, but may occasionally be inverted or diphasic.

Sinus arrhythmia is a normal occurrence; other types of arrhythmias were not seen.

## F. Postsurgical ECG Data

Since no absolute ECG pattern could be found consistently in recordings taken on dogs following production of the various pathological conditions, only those values which showed a trend to be different from the presurgical controls will be discussed.

Table 23 presents as percentages some of the complexes which were thought to possibly have some importance in diagnosis of cardiac pathology as compared with normal presurgical percentages.

Table 23. Incidence of ECG values expressed in percent during presurgical and postsurgical periods

| Treatment | $Q_I$ | $Q_I$-T | $S_{III}$ | $S_{III}$+T | -$T_I$ |
|---|---|---|---|---|---|
| Presurgery | 49 | 95 | 88 | 89 | 60 |
| Infarct[a] | 75 | 14 | 100 | 71 | 11 |
| Shams[a] | 44 | 75 | 55 | 100 | 56 |
| Infarct[b] | 68 | 54 | 95 | 81 | 32 |
| Shams[b] | 13 | 100 | 75 | 100 | 12 |
| Epicarditis | 33 | 38 | 75 | 94 | 17 |
| Shams | 62 | 75 | 92 | 100 | 54 |
| Valvular Insuff. | 62 | 63 | 77 | 95 | 58 |
| Shams | 94 | 88 | 83 | 80 | 88 |

[a]Two to twelve days postsurgery.

[b]Two to seven weeks postsurgery.

Q-waves occur in approximately 50 percent of the cases in each lead and any two leads having a Q-wave from the same dog at any one time is a very random type phenomenon. Although $S_{II}$ and $S_{aVF}$ occurred at the same time in 90 percent of the presurgical records, the same pattern was found postsurgically. $S_{III}$ occurred in the greatest percentage of records in each of the six groups, and it is followed by an upright T in 90 percent or more of the records except in the infarcted and valvular insufficiency dogs. It is thought that the failure of the S in lead III to be followed by an upright T is an important indication of myocardial damage. In addition to the dogs with infarction and epicarditis, three records in which it appeared in the valvular insufficiency shams came from one dog in which the left ventricle had been punctured rather then using the atrial approach, and consequently had a small amount of myocardial damage. Another factor which appears to be important is the presence of a Q-wave in lead I being followed by an upright or absent T-wave. In all cases the incidence of this phenomenon was higher in dogs with lesions. This is especially true in the recently infarcted dogs and in the epicarditis dogs. Twenty-four of 30 recordings taken on 14 dogs, 2 to 12 days, following infarction showed: a) 16 with Q-waves with absent or upright T-waves in lead I, b) 6 with S-waves with inverted T-waves in lead III, and c) 2 showing both a and b. In

addition, 3 showed a Q-S complex in lead I and one showed it in lead III. Six showed ectopic beats at 2 to 3 days following surgery and 4 showed inverted P-waves in one or more leads.

In the six remaining records four had inverted P-waves in lead I and three of these had ST deviation in 2 or more leads. However, two looked normal. Figures 39 through 48 (Appendix B) show the appearance of the various complexes discussed.

It was thought that the decreased percentage of negative T-waves in lead I might be of some importance. However, its occurrence in only about 50 percent of normal records and infarct shams having a decreased percentage prevent this observation from being of any consequence. ST deviation did not turn out to be as good an index of cardiac pathology as had been anticipated from preliminary studies. ST deviation did occur following the production of pathology, however, a large percentage of normal ECG's showed ST deviation also. But, as was pointed out earlier, the deviation in presurgical normals were quite small. This same pattern was seen in sham-operated dogs (See Table 24) where a good share of the dogs showed ST deviation in 2 or more leads. Here again the values were usually 1 mm. or less. ST deviation did occur more often in dogs with pathology than in shams except the early infarction dogs. This in itself cannot be considered

to be especially important in the light of the normals. Here is one phase where absolute measurements are probably warrented for in the infarcted dogs 64 percent of the records in which deviation occurred were 2 mm. or more in the early stages and 82 percent in the later stages. The R/ST ratios did not show this difference as well as expected. Deviation up or down seemed to be a random occurrence.

R/Q ratios varied considerably. Q-waves were not present in many cases and when they were present they were often so small that measuring and calculation of ratios were subject to a large error. No particular pattern was noticed. Individual dog data on a serial basis are presented in Tables 33 through 38 (Appendix A) with respect to certain complexes and values (R/S, R/T, $Q_I$, $S_{III}$, T-waves, QT and PR).

An observation which was noted, but which is not evident from Table 24, was that the R/S and R/T ratios decreased in the recordings following production of lesions. Actually, the R did not decrease in size so much, in fact it may have even increased, but both S and T amplitudes were increased. The reason the table does not show this well is that some large ratios increased the over-all average. The figures for negative T are not very reliable because only a very few records showed negative $T_{II}$ (12.5 percent and 59 percent of these were in infarcted dogs). Since negative T occurs in lead II in a relatively high percentage of the records after

infarction it might be considered as having some importance. However, if one examines individual serial records (Table 33, Appendix A) one sees the pattern is very random.

Time durations of the various complexes varied so much that they are relatively unimportant as diagnostic aids for the three conditions present.

Notching of R did not occur as often as had been anticipated. Only 25 percent of infarction records from 2 to 12 days showed notching and less than 10 percent of the records after 12 days. The incidence of notching in recordings from the other groups was less than 3 percent.

Other than infarction dogs, ectopic beats were seen in only two recordings of a valvular insufficiency subject (dog 41).

In summary it may be said it is quite understandable as to why canine electrocardiography remains an empiric science. With the tremendous variations in the normal recording it is difficult to set up definite standards and deviations from these standards which can be considered definitely abnormal. From the above data the following points indicate pathology:

1. Presence of ventricular extrasystole (ectopic beats).
2. Presence and persistence of inverted P-waves.
3. ST deviation of 2.0 mm. or more.
4. Q-waves in lead I followed by positive T-waves.

Table 24. Comparison of complexes of dogs with lesions to shams

| | Lead II[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | I | IS | I | IS | E | ES | VI | VIS |
| | 2 - 12 days | | 2 - 7 weeks | | | | | |
| R/S mean | 6.0 | 7.6 | 6.1 | 6.8 | 12.6 | 17.5 | 13.8 | 15.2 |
| range | 0.3-28.0 | 1.0-21.0 | 1.25-22.0 | 1.0-16.0 | 3.0-29.0 | 1.5-44.0 | 1.25-66.0 | 1.75-64.0 |
| R/+T mean | 6.7 | 3.5 | 8.0 | 9.2 | 6.2 | 10.8 | 7.6 | 10.6 |
| range | 1.0-20.0 | 1.45-6.0 | 1.0-32.0 | 2.5-16.5 | 3.0-25.0 | 3.25-36.0 | 1.25-24.0 | 2.0-21.25 |
| R/-T mean | 7.1 | 17.0 | 20.9 | 0 | 7.5 | 20.0 | 32.0 | 3.75 |
| range | 1.0-28.0 | - | 6.75-39.0 | - | - | - | - | 3.0-4.5 |
| ST± %[b] | 33 | 45 | 73 | 28 | 62 | 31 | 42 | 14 |
| % record[c] | 50 | 75 | 55 | 33 | 62 | 56 | 66 | 25 |
| PR mean | .114 | .118 | .108 | .115 | .123 | .108 | .121 | .125 |
| range | .08-.14 | .10-.14 | .08-.16 | .10-.12 | .10-.16 | .10-.16 | .08-.18 | .08-.14 |
| QT mean | .221 | .229 | .212 | .234 | .208 | .228 | .215 | .208 |
| range | .16-.28 | .20-.26 | .16-.26 | .20-.26 | .16-.26 | .20-.28 | .18-.24 | .16-.24 |

[a]Amplitudes expressed as ratio of R to complex durations expressed in seconds.

[b]Percent of dogs showing deviation.

[c]Percent of records having deviation of dogs with deviation.

5. S-waves in lead III followed by inverted T-waves.

6. Decreased R/S and/or R/T ratios.

No correlation could be found between ECG recordings and the severity of cardiac lesions found upon necropsy or with the rise in serum enzymes. There are apparently no consistent changes in the ECG with respect to amount of pathology i.e., a small lesion will cause as much change as a large lesion if a change occurs.

## G. Necropsy Findings

### 1. Infarction dogs

Fourteen dogs in which infarction was produced were sacrificed at various intervals from 10 days to 20 weeks following production of pathology. Three others were necropsied immediately after death. One of these dogs died of massive myocardial infarction 10 hours after surgery. The other two died from hemorrhage when the coronary artery was punctured or burst.

The 14 dogs sacrificed showed various amounts of infarcted tissue in the left ventricle ranging from less than 1 cm. in diameter and involving only the endocardium to those involving nearly one-half the left ventricular musculature and varying in depth from involving only the endocardial portion of the myocardium to being transmural in nature. (See Figures 14 through 20, Appendix B.)

Generally speaking most dogs had a very slight amount of adhesions of the lung to the pleural wall and to the pericardial sac in the area of surgery. There were adhesions of the sac to the epicardium in every case. It seemed that the larger the infarcted area was the more adhesions of the sac to the epicardial surface occurred. Usually little other gross pathology was noted. Those cases in which other abnormalities occurred will be found in the individual dog necropsy synopsis in the appendix (Appendix C).

Sham-operated dogs showed very little evidence of any abnormalities. There was generally a small amount of adhesions of lungs to pleural wall and/or pericardial sac in the surgical area. Adhesions of the sac to the epicardium were minimal and often completely absent. The kidneys of dog 14 were very jaundiced in appearance but were of normal size and no signs of pathology could be seen. Dog 32 showed two small infarcted areas in the left kidney, one old depressed scar tissue area and one raised and red. The liver of dog 33 seemed to be enlarged and quite congested; however, no other signs of pathology were noticed. A small circumscribed tumor was found in the area of the bifurcation of the uterine horns in dog 40.

Other dogs appeared to be completely normal. It is believed that pathology found in the four dogs discussed above did not contribute large enough amounts of enzymes to the

serum to be of any great significance. The adhesions which occurred could possibly affect the ECG because of the effect of position. The exact effect, if any, could be very difficult to access.

Figures 12 and 13 (Appendix B) show examples of hearts of sham-operated dogs.

2. Epicarditis dogs

Six dogs were necropsied in which an epicarditis and pericarditis were produced. (See Figures 21, 22, and 23, Appendix B). The amount of pathology did not vary much among individuals. In most cases about three-fourths of the surface of the heart was involved. In one case the entire pericardial sac was adhered to the epicardium and was very difficult to remove (Figure 21). This dog died from pathology produced. The surface of the heart was generally granulated in appearance with some areas of small hemorrhage and hyperemia. A purulent exudate was never seen. There was no odor from any of the specimens. However, one had a small amount of dark viscous fluid in the pericardial sac. This dog had been dead several hours before it was necropsied and there was a slight odor which was attributed to post-mortem changes.

As in the infarction dogs there were a few adhesions of lungs to the pleural wall in the area of the incision and

also to the pericardial sac. In all cases the amount of adhesions of the sac to the epicardium was extensive.

Three of the six specimens were cultured aerobically and anaerobically on blood agar. No growth was seen. It was thought that all cases were sterile.

The three sham-operated epicarditis dogs were very near to normal. There were no adhesions of the pericardial sac to the epicardium. There was no indication of any type of lesion in any of the hearts. Dog 18 had a fairly severe pleuritis on the right side. This was cultured and found to be negative for bacterial growth. This same dog had a slight right A-V valvular proliferative endocarditis. In dog 35 there was a slight amount of irritation (about 5 mm. in diameter) on the left ventricle apparently from the silicone rubber tube.

All other organs and tissues appeared to be normal.

3. Valvular insufficiency dogs

Eleven dogs were operated on to produce a valvular insufficiency. Four of these were done using the Markowitz (98) technique. Two died of cardiac rupture and one died from too great of insufficiency. Seven were done by the method described previously. The first two of these died during surgery, one from ventricular fibrillation and one from rupture of the anterior vena cava. The other five survived surgery and upon necropsy showed various degrees of insufficiency. Dog 41 had a left A-V insufficiency which was quite severe. There was also a negatative endocarditis

found on the right A-V valves, apparently a result of the left A-V insufficiency. The liver was swollen and appeared to be mildly cirrhotic. Dog 42 had the greatest amount of insufficiency of the 5 dogs in this group. This dog showed necrosis and cirrhosis of the liver grossly, plus a passive congestion throughout. The other three dogs had milder A-V insufficiencies, and showed nothing spectacular other than some right ventricular dilation. See Appendix C for more details. (See Figures 24 through 27 and 36 through 38, Appendix B.)

Five dogs were sham-operated, one by the Markowitz Method (No. 3) and four by open heart surgery. Dog 3 showed a small area of scar tissue in the left ventricle where it had been punctured. The other four hearts appeared to be normal except for the area of silk in the atria where the incision was made. There was a slight amount of adhesion of lungs to the pleural wall and pericardial sac. There were also adhesions of the sac to the heart in the region of the sutures. These adhesions were easily broken down. There were no other gross indications of pathology.

## H. Histopathological Findings

### 1. Infarction dogs

The type of histopathology found in the infarcted area of the ventricle of each dog was very similar in appearance.

(See Figures 27 through 30, Appendix B.) These lesions were characterized by predominant replacement fibrosis with scar tissue formation, myocardial necrosis and infiltration of monocytes and some calcification of the necrotic myocardium. In some cases it appeared the area was enlarging as the cells adjacent to the fibrotic area showed swollen granular myocardial fibers with light, round, swollen or pyknotic nuclei. Congestion and hemorrhage were often seen in and adjacent to necrotic areas. In addition, adjacent areas of myocardium which were pale and grey, yet not frankly necrotic, showed considerable cloudy swelling with granular cytoplasm microscopically. Nuclear changes were not prominant in these cells.

Sections taken from the myocardium of sham-operated animals appeared to be normal.

In those dogs sacrificed earliest following myocardial infarction there was some cloudy swelling of the liver with some fatty infiltration. There was no evidence of actual necrosis, however. In a few cases there were small foci of swelling and some cells appeared granular, otherwise livers appeared normal. In the dogs sacrificed later the livers appeared to be normal.

Other organs, which were sectioned and studied microscopically, generally appeared normal. In one case an infarct was seen in a kidney. This was very small and the

rest of the section was normal. Sham-operated dogs were normal throughout.

The tumor found in dog 40 was a fibroma.

2. Epicarditis dogs

Pathology was confined almost entirely to the epicardial surface of the heart. In only one case was there any indication of the myocardium being involved. This dog showed some necrotic changes, pyknotic nuclei and some hyaline degeneration of the muscle fibers. In the other dogs the myocardium appeared to be entirely normal except for a hyperemia of the outer layers of muscle. The main changes seen were confined to a fibrosis with many spindle shaped nuclei of the connective tissue cells appearing. Polynuclear cells were minimal as was the appearance of other circulatory cells. In some areas there was hemorrhage on the epicardial surface. A few giant cells were noted throughout the area involved. There was also an increase in capillaries throughout the involved area. Figures 31 through 34 (Appendix B) are examples of histopathologic findings.

Studies of other organs and of the sham-operated dogs indicated no significant changes.

3. Valvular insufficiency dogs

Grossly cardiac dilatation in dogs was based upon the finding of enlarged cavernous ventricular chambers, thin

ventricular walls, and flattened papillary muscles. Microscopic changes were also present. These changes were thin and sometimes wrinkled or curled myocardial fibers.

In most cases thickening of the cusps of the A-V valve involved occurred. In dog 41, where left A-V valve was impaired, the opposite A-V valve also showed this pathology. The edge of the cusps consisted of proliferative fibroblasts and collagen fibers. Figure 35, Appendix B, shows an example of the valvular change.

No other changes were noted in the cardiac muscles. At the sight of entry in the atrium there was a thin line of scar tissue and some monocyte infiltration. However, cellular changes were minimal and there was a noticable lack of eosinophils which had been expected to appear in this area because of the increase in the peripheral circulation.

In dogs 41 and 42 there was distinct evidence of early cardiac cirrhosis of the liver. Central veins were very enlarged and cells surrounding these veins were in various stages of degeneration (Figures 37 and 38, Appendix B). Other tissues showed excess interstitial fluids and most organs showed cells with cloudy swelling. Both dogs were suffering from a passive congestion. The other three dogs in this group showed no significant changes upon histological examination of any organs other than the heart.

Histological examination of the five sham-operated dogs revealed only an area of fibrosis in the left ventricular

musculature of dog 3, and a thin line of scar formation in the atrium of the other four dogs. All other organs appeared to be normal.

## I. Symptoms

Dogs in which myocardial infarction was produced showed symptoms of severe depression for three to seven days following surgery. Heart rate was usually increased as was respiration. In the first few days there were indications of pain. The dogs then showed rapid improvement and appeared to be normal in two to four weeks. It must be pointed out that dogs were maintained under cage rest and were not permitted to exercise or were not bothered for any reason other than routine care, taking blood samples, and ECG recordings.

Epicarditis dogs showed severe depression for approximately one week following surgery. Upon auscultation of the heart, fluids could be heard for a few days. This sound disappeared and no abnormalties could be auscultated on most dogs. One dog did seem to have a sound which sounded gritty for one day on the fourth day following surgery. The dogs generally showed improvement and appeared normal for several weeks. This was followed by a mild depression which became very severe in dog 37. This dog died three months after production of the epicarditis. Several dogs had a fremitus or thrill upon palpation of the femoral arteries and a decreased

pulse. This continued throughout the remainder of the experimental period once it had appeared. Dog 5 showed no symptoms after the first week and got along very nicely. In all cases appetite seemed to be normal.

Dogs with valvular insufficiency generally showed increased respiration and heart rate. They were more depressed than the epicarditis dogs but not as much as the infarction dogs early in the experiment. Dogs 41 and 42 suffered from periods of anorexia. They both developed a cough and some dyspnea. In addition dog 41 definitely suffered from an anasarca. This dog was treated with a diuretic and had a peritoneal puncture several times to remove the ascites fluid.

All dogs indicated a valvular insufficiency upon auscultation by various degrees of systolic murmur. The pulse of dog 41 was barely palpable. Other dogs had periods of intermittent fremitus in the femoral artery, but pulses remained fairly strong except dog 42 which had a fairly strong pulse at first but which gradually became weaker and was impalpable shortly before death.

# V. SUMMARY

Various types of cardiac pathology (myocardial, infarction, epicarditis, and A-V valvular insufficiency) were produced in 50 dogs by surgical methods. These dogs were classified prior to surgery as normal based on physical examination, hematological studies, and electrocardiograms.

The upper ranges for normal serum enzyme levels were 40 units for glutamic oxalacetic transaminase (SGOT), 35 units for glutamic pyruvic transaminase (SGPT), and 500 units for lactic dehydrogenase (SLDH). The clinical Sigma method, which has been used in research also, for the assay of SGOT, was compared with an established research method of Hergt and Langin. It compared very favorably with the latter method and in some respects was more advantageous.

It was found that repeated freezing and thawing of serum significantly reduced the amount of enzyme activity.

It was further found that age is not a factor in normal serum enzyme levels.

Based upon serial enzyme studies following the production of myocardial infarctions it was found that SGOT was the most reliable, of the three enzymes studied, as a diagnostic aid. In addition the relative amount of infarction could be fairly well ascertained by the rise in SGOT activity. It was found that SGOT began to elevate 8 to 15 hours

following infarction, reached peak levels (up to 15 times normal values) from 18 to 24 hours and returned to normal ranges within 40 to 90 hours. Also, SGPT may rise considerably, up to 10 times normal values, following the more severe infarctions. Its pattern was similar to SGOT although SGPT never reached the elevations of SGOT following infarction. SLDH was much more erratic, both for normal serum levels and for those following infarction. This enzyme reached peak values at 25 to 30 hours post-infarction and did not return to normal until 80 to 100 hours in most cases. However, because of its wide range of normal activity among dogs and because of its radical behavior following infarction, it cannot be recommended as a diagnostic aid for myocardial infarction in dogs at this time. Both SGOT and SGPT should be assayed because of the high levels of SGPT and SGOT seen in liver necrosis as reported by other workers.

Serum enzyme assays are of little aid in diagnosing epicarditis or valvular insufficiency in dogs; however, they may be an important negative finding when coupled with symptoms and ECG findings.

Routine hematological studies are of little aid in diagnosing any of the conditions presented.

Electrocardiograms from normal dogs were studied and it was revealed 1) when a Q-wave occurred in lead I, it was followed by an inverted T-wave 95 percent of the cases;

2) when an S-wave occurred in lead III, it was followed by an upright T-wave in 89 percent of the records; 3) although an ST deviation occurred in one or more leads fairly frequently (20 percent of all recordings in one or more leads I, II, III, and aVF), it was never more than 2.0 mm. and in 95 percent was not over 1.5 mm.; 4) inverted P-waves in lead II, III and aVF and ventricular extrasystoles (ectopic beats) in any lead never occurred; and 5) that there is considerable variability from dog to dog and from day to day in voltages and time intervals of the ECG.

Following the production of certain pathological conditions of the heart it was found that certain changes in the ECG may be indicative of pathology. These changes included: the presence of ventricular extarsystoles; the presence and persistence of inverted P-waves in leads II, III, or aVF; the presence of ST deviation $\pm$ 2.0 mm. or more in one or more leads; the presence of Q-waves in lead I followed by an upright T-wave; the presence of S-waves in lead III followed by inverted T-waves; and the presence of decreased R/S and/or R/T ratios. No correlation could be made with changes in the ECG following surgery with the amount of pathology or with the rise of serum enzymes.

This research has presented many questions which need to be investigated. It has been only a beginning into a vast area of study in the realm of enzymatic chemistry in

veterinary medicine. Investigations should continue on all species of domestic animals as there are known species variabilities and there is always much danger in extrapolating results from one species to another. In addition, because of the many tissues containing relatively high concentrations of the various enzymes, it is imperative that all pathological processes which may contribute to the serum activity be investigated so there is less chance of error in diagnosis. Finally, more basic biochemistry and physiology involved in these processes must be studied further to understand the significance of observed changes.

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# VIII. APPENDIX A: TABLES

Table 25. Serial SGOT levels of dogs with myocardial infarction (Sigma method)

| Lesion size | Dog. no. | Pre-surgery | Hours post surgery 17-22 | 23-27 | 28-34 | 35-43 | 44-50 | 54-72 |
|---|---|---|---|---|---|---|---|---|
| < 1 cm. | 15 | 18 | 48 | 45 | 39 | 35 | 31 | 31 |
| | 11 | 22 | 75 | 64 | 41 | 35 | 33 | 30 |
| | 38 | 20 | 68 | 64 | 45 | 36 | 30 | 35 |
| | 46 | 22 | 63 | 63 | 57 | 51 | 42 | 32 |
| 1-2 cm. | 21 | 18 | 170 | 140 | 130 | 118 | 75 | 50 |
| | 25 | 37 | 160 | 97 | 105 | 50 | 30 | 29 |
| | 51 | 28 | 121 | 88 | 82 | 67 | 48 | 39 |
| > 2 cm. | 7 | 14 | 400 | 118 | 95 | 46 | 53 | 30 |
| | 24 | 18 | 140 | 193 | 72 | 45 | 49 | 25 |
| | 31 | 23 | 80 | 130 | 105 | 62 | 58 | 40 |
| | 34 | 27 | 320 | 260 | 110 | 66 | 76 | 47 |
| | 45 | 21 | 370 | 410 | 280 | 150 | 130 | 65 |
| | 49 | 32 | 400 | 305 | 235 | 70 | 47 | 33 |
| | 50 | 27 | 230 | 240 | 81 | 50 | 40 | 30 |

Table 26. Serial SGPT levels of dogs with myocardial infarction

| Lesion size | Dog no. | Pre-surgery | Hours post surgery | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | 17-22 | 23-27 | 28-34 | 35-43 | 44-50 | 51-72 | 73-90 |
| < 1 cm. | 15 | 25 | 24 | 29 | 20 | 23 | 31 | 24 | 20 |
| | 11 | 17 | 48 | 46 | 42 | 41 | 46 | 32 | 30 |
| | 38 | 20 | 46 | 48 | 40 | 38 | 32 | 31 | 28 |
| | 46 | 36 | 36 | 34 | 35 | 34 | 36 | 33 | 40 |
| 1-2 cm. | 21 | 20 | 25 | 27 | 28 | 29 | 31 | 25 | 28 |
| | 25 | 28 | 44 | 42 | 39 | 37 | 25 | 24 | 22 |
| | 51 | 30 | 34 | 32 | 33 | 38 | 36 | 35 | 20 |
| > 2 cm. | 7 | 19 | 240 | 125 | 101 | 87 | 76 | 77 | 56 |
| | 24 | 20 | 88 | 93 | 89 | 80 | 55 | 62 | 29 |
| | 31 | 17 | 60 | 55 | 65 | 83 | 61 | 56 | 38 |
| | 34 | 24 | 100 | 100 | 92 | 86 | 64 | 53 | 44 |
| | 45 | 18 | 160 | 220 | 220 | 171 | 139 | 100 | 49 |
| | 49 | 24 | 150 | 140 | 130 | 160 | 125 | 78 | 64 |
| | 50 | 21 | 83 | 99 | 76 | 69 | 62 | 50 | 47 |

Table 27. Serial SLDH levels of dogs with myocardial infarction

| Dog no. | Pre-surgery | Post surgery | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Hours | | | Days | | | |
| | | 18-30 | 31-48 | 49-70 | 4-8 | 9-13 | 14-18 | < 19 |
| 46 | 130 | 385 | 495 | 535 | 180 | - | - | 140 |
| 25 | 165 | 1225 | 1075 | 1150 | 360 | - | 175 | |
| 51 | 100 | 580 | 265 | 240 | - | 550 | | |
| 31 | 170 | 280 | 230 | - | 100 | | | |
| 34 | 450 | 800 | 650 | 500 | 110 | - | - | 280 |
| 45 | 140 | 1125 | 375 | 370 | 350 | - | 190 | |
| 49 | 350 | 820 | 500 | 225 | 190 | 210 | | |
| 50 | 365 | 375 | 290 | 180 | | 225 | | |

Table 28. Serial enzyme levels of dogs sham-operated for myocardial infarction (Sigma)

| Dog no. | Pre-surgery | Hours post surgery 17-22 | 23-27 | 28-34 | 35-43 | 44-50 | 51-72 | 73-90 |
|---|---|---|---|---|---|---|---|---|
| | | | | SGOT | | | | |
| 1 | 23 | 25 | 23 | 23 | 25 | 23 | 21 | 16 |
| 13 | 25 | 35 | 32 | 24 | 32 | 33 | 27 | 30 |
| 14 | 24 | 36 | 26 | 25 | 22 | 17 | 24 | 20 |
| 22 | 20 | 23 | 16 | 16 | 18 | 20 | 20 | 16 |
| 32 | 20 | 41 | 41 | 39 | 29 | 25 | 27 | 25 |
| 33 | 18 | 37 | 40 | 25 | 25 | 19 | 18 | 20 |
| 39 | 27 | 27 | 20 | 25 | 19 | 24 | 28 | 24 |
| 40 | 21 | 37 | 43 | 25 | 25 | 30 | 33 | 32 |
| | | | | SGPT | | | | |
| 1 | 12 | 15 | 14 | 12 | 15 | 15 | 19 | 18 |
| 13 | 23 | 38 | 39 | 38 | 36 | 35 | 32 | 38 |
| 14 | 18 | 20 | 19 | 17 | 15 | 16 | 20 | 17 |
| 22 | 20 | 24 | 18 | 17 | 17 | 16 | 20 | 18 |
| 32 | 15 | 32 | 29 | 29 | 34 | 20 | 20 | 20 |
| 33 | 18 | 22 | 30 | 26 | 24 | 24 | 24 | 24 |
| 39 | 26 | 26 | 21 | 20 | 29 | 18 | 28 | 25 |
| 40 | 24 | 25 | 24 | 25 | 24 | 28 | 25 | 22 |
| | | | | SLDH | | | | |
| 33 | 240 | 130 | 110 | 70 | 120 | | | |
| 39 | 325 | 85 | 165 | - | - | - | 150 | |
| 40 | 205 | 280 | 190 | 175 | - | 160 | | |

Table 29. Serial enzyme levels of epicarditis dogs (Sigma)

| Dog no. | Pre-surgery | Post surgery | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Days | | | | Weeks | | | |
| | | 2 | 5 | 8 | 12 | 3 | 4 | 5 | 6 |
| | | | | | SGOT | | | | |
| 5 | 17 | 20 | 21 | 24 | 13 | 22 | 18 | - | 29 |
| 10 | 27 | 16 | 22 | 17 | 14 | 23 | 25 | 22 | 22 |
| 26 | 29 | - | 25 | 37 | 31 | 25 | 23 | 22 | 21 |
| 36 | 14 | 22 | 32 | 20 | 27 | - | 28 | 29 | 30 |
| 37 | 28 | 28 | 30 | 23 | - | 27 | 19 | 26 | 27 |
| 43 | 33 | 25 | 32 | 30 | - | 23 | - | 21 | - |
| | | | | | SGPT | | | | |
| 5 | 18 | 27 | 20 | 23 | 27 | 18 | 24 | - | 27 |
| 10 | 17 | 13 | 21 | 12 | 14 | 15 | 25 | 19 | 15 |
| 26 | 22 | 21 | 36 | 37 | 25 | 25 | 16 | 20 | 22 |
| 36 | 13 | 19 | 30 | 19 | 17 | - | 22 | 25 | 23 |
| 37 | 23 | 28 | 25 | 24 | - | 21 | 26 | 23 | 21 |
| 43 | 20 | 26 | - | 23 | - | 19 | - | 15 | - |
| | | | | | SLDH | | | | |
| 26 | 400 | | 100 | - | - | - | 110 | - | 190 |
| 36 | 120 | 205 | - | - | - | 290 | - | - | 260 |
| 37 | 70 | - | - | 205 | - | - | - | - | 265 |
| 43 | 200 | - | 200 | - | 300 | 470 | - | 555 | - |

Table 30. Serial serum enzyme levels of sham-operated epicarditis dogs (Sigma)

| Dog no. | Pre-surgery | Post surgery: Days 2 | 5 | 8 | 12 | Weeks 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | SGOT | | | | |
| 18 | 18 | 14 | 18 | 17 | 24 | 20 | 28 | 27 | 27 |
| 29 | 19 | 29 | 26 | 25 | 15 | 16 | 16 | 15 | 15 |
| 35 | 21 | 17 | 17 | 19 | 23 | 31 | 33 | 35 | 34 |
| | | | | | SGPT | | | | |
| 18 | 16 | 17 | 14 | 18 | 20 | 18 | 21 | 25 | 25 |
| 29 | 23 | 22 | 16 | 19 | 15 | 15 | 21 | 15 | 16 |
| 35 | 20 | 20 | 20 | 18 | 17 | 24 | 22 | 21 | 20 |
| | | | | | SLDH | | | | |
| 29 | 150 | 130 | 175 | - | - | - | 140 | - | 150 |
| 35 | 75 | 75 | - | 225 | - | - | 90 | - | 120 |

Table 31. Serial enzyme levels of valvular insufficiency dogs (Sigma)

| Dog no. | Pre-surgery | Post surgery: Days 4 | 6 | 9 | 12 | Weeks 3 | 5 | 8 | 12 |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | SGOT | | | | |
| 20 | 18 | 24 | 24 | 23 | 27 | 22 | 32 | 24 | 25 |
| 27 | 20 | 17 | 15 | 16 | 19 | 25 | 20 | 15 | 19 |
| 30 | 34 | 26 | 18 | 26 | 18 | 22 | 19 | 18 | 28 |
| 41 | 29 | 34 | 29 | - | 39 | 36 | 31 | 29 | 71 |
| 42 | 24 | 30 | 23 | 25 | 24 | 19 | 39 | 35 | - |
| | | | | | SGPT | | | | |
| 20 | 16 | 27 | 33 | 25 | 26 | 30 | 23 | 18 | 20 |
| 27 | 23 | 15 | 13 | 14 | 15 | 16 | 14 | 17 | 18 |
| 30 | 31 | 20 | 20 | 20 | 20 | 19 | 22 | 21 | 24 |
| 41 | 24 | 38 | 24 | - | 35 | 32 | 29 | 24 | 140 |
| 42 | 15 | 24 | 22 | 21 | 16 | 36 | 40 | 32 | - |
| | | | | | SLDH | | | | |
| 27 | 45 | - | 175 | - | - | 380 | - | 250 | 180 |
| 30 | 200 | - | - | - | 380 | - | 325 | 250 | 200 |
| 41 | 455 | 475 | 280 | - | 480 | - | 355 | 230 | 250 |
| 42 | 225 | - | 290 | - | 225 | 375 | 300 | 170 | - |

Table 32. Serial enzyme levels of sham-operated valvular insufficiency dogs (Sigma)

| Dog no. | Pre-surgery | Post surgery: Days 4 | 6 | 9 | 12 | Weeks 3 | 4 | 6 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | SGOT | | | | |
| 3 | 26 | 24 | 23 | 15 | 22 | | | | |
| 4 | 53 | 56 | 40 | 41 | 51 | 50 | 46 | 46 | 45 |
| 19 | 20 | 25 | 20 | 14 | 22 | | | | |
| 47 | 28 | 35 | 27 | 30 | 30 | 30 | 26 | 26 | 33 |
| 48 | 29 | 27 | 24 | 25 | 28 | 29 | 26 | 24 | 29 |
| | | | | | SGPT | | | | |
| 3 | 20 | 22 | 21 | 15 | 21 | | | | |
| 4 | 32 | 49 | 30 | 29 | 31 | 30 | 28 | 24 | 29 |
| 19 | 17 | 23 | 21 | 20 | 18 | | | | |
| 47 | 26 | 25 | 25 | 28 | 26 | 30 | 29 | 25 | 25 |
| 48 | 17 | 19 | 21 | 25 | 28 | 30 | 28 | 24 | 29 |
| | | | | | SLDH | | | | |
| 47 | 160 | - | - | 255 | - | 240 | - | 230 | 225 |
| 48 | 210 | 230 | - | 230 | 225 | - | - | 230 | - |

Table 33. ECG data from myocardial infarction dogs - arranged by age and as to severity of lesion found at necropsy

| | | | Post surgery | | | | |
|---|---|---|---|---|---|---|---|
| | | | Days | | Weeks | | |
| Age | PS I | PS II | 2-5 | 6-12 | 2-4 | 5-7 | 9-17 |
| | | | R/S ratios | | | | |
| 1 | 5.5 | 5.5 | 7.25 | 8.0 | | | |
| 1 | 3.0 | 2.5 | 3 | 3.5 | 3.0 | 2.0 | |
| 1 | 9.5 | 8.0 | 13.25 | 7.0 | 10.5 | 9.75 | |
| 2 | 5.0 | 56.0[a] | 28.0 | 25.0 | 6.75 | - | 8.0 |
| 2[b] | 15.0 | 50.0[a] | 18.0 | - | 21/0 | | |
| 2 | 10.0 | 20.0 | - | 20.0 | - | 2.25 | |
| 2 | 2.5 | 3.5 | 3.0 | 16/0 | 3.5 | | |
| 2 | 14.0 | 17.0 | 10.0 | - | 22.0 | | |
| 2 | 11.5 | 24.0 | 8.0 | 16/0 | 12.0 | | |
| 2 | 4.0 | 6.0 | 1.5 | - | 5.75 | | |
| 2 | 2.0 | - | 0.3 | 0.5 | 1.75 | 1.25 | 2.5 |
| 3 | 3.0 | 2.5 | 2.75 | 1.75 | 2.0 | 3.0 | |
| 3 | 20.0 | 19/0 | 5.0 | | | | |
| 3 | 1.5 | 2.0 | 1.0 | 1.25 | 1.0 | | 2.75 |
| | | | R/T ratios | | | | |
| 1 | 11.0 | 10.0 | 6.0 | 8.0 | | | |
| 1 | 6.0 | 3.75 | 3.0 | 3.5 | 2.5 | 1.0 | |
| 1 | -9.5 | 1.25 | 11.25 | -28.0 | 32.0 | -39.0 | |
| 2 | 6.75 | 14.0 | 7.0 | 17.0 | -6.75 | - | 6.0 |
| 2[b] | -30.0[a] | -10.0 | -6.0 | - | 21.0 | | |
| 2 | 6.25 | -6.0 | - | 20.0 | - | 2.25 | |
| 2 | -40.0[a] | 8.5 | 3.0 | -4.0 | -17.0 | | |
| 2 | 5.5 | 5.5 | 4.0 | - | 7.25 | | |
| 2 | 6.5 | 8.0 | 3.0 | 4.0 | 12.5 | | |
| 2 | 13.25 | 12.5 | -1.0 | - | 3.25 | | |
| 2 | 4.75 | - | -2.0 | 1.0 | 3.5 | 2.0 | 6.0 |
| 3 | 7.5 | 3.25 | 8.5 | 5.5 | 3.5 | 5.5 | |
| 3 | 10.0 | 9.5 | 7.5 | | | | |
| 3 | 2.75 | 4.0 | -1.5 | 1.5 | 1.0 | - | -4.25 |

[a] < 1 mm.

[b] Second infarction.

Table 33. (Continued)

| Age | PS I | PSII | Post surgery: Days 2-5 | Days 6-12 | Weeks 2-4 | Weeks 5-7 | Weeks 9-17 |
|---|---|---|---|---|---|---|---|
| | | | P-R interval (sec.) | | | | |
| 1 | .08 | .08 | .10 | .08 | | | |
| 1 | .10 | .14 | .10 | .12 | .10 | .10 | |
| 1 | .12 | .12 | .12 | .10 | .12 | .10 | |
| 2 | .12 | .12 | .14 | .12 | .16 | - | .14 |
| 2[b] | .12 | .10 | .10 | - | .10 | | |
| 2 | .12 | .12 | - | .14 | - | .12 | |
| 2 | .12 | .08 | .08 | .12 | .10 | | |
| 2 | .10 | .10 | .10 | - | .08 | | |
| 2 | .10 | .12 | .14 | .12 | .12 | | |
| 2 | .08 | .10 | .12 | - | .10 | | |
| 2 | .10 | - | .10 | .10 | .08 | .10 | .08 |
| 3 | .12 | .12 | .12 | .12 | .12 | .12 | .12 |
| 3 | .10 | .10 | .10 | | | | |
| 3 | .10 | .14 | .14 | .14 | .12 | - | .10 |
| | | | Q-T interval (sec.) | | | | |
| 1 | .24 | .24 | .22 | .20 | | | |
| 1 | .22 | .22 | .24 | .24 | .22 | .24 | |
| 1 | .20 | .20 | .20 | .20 | .22 | .18 | |
| 2 | .24 | .26 | .24 | .24 | .26 | - | .26 |
| 2[b] | .20 | .18 | .16 | - | .22 | | |
| 2 | .24 | .20 | - | .24 | - | .24 | |
| 2 | .22 | .22 | .20 | .20 | .18 | | |
| 2 | .20 | .18 | .20 | - | .20 | | |
| 2 | .24 | .22 | .22 | .22 | .20 | | |
| 2 | .20 | .20 | .20 | - | .16 | | |
| 2 | .20 | - | .24 | .22 | .20 | .20 | .22 |
| 3 | .26 | .26 | .28 | .24 | .20 | .26 | |
| 3 | .24 | .24 | .24 | | | | |
| 3 | .22 | .20 | .24 | .20 | .20 | - | .22 |

Table 33. (Continued)

| Age | PS I | | PS II | | Post surgery: Days 2-5 | | Days 6-12 | | Weeks 2-4 | | Weeks 5-7 | | Weeks 9-17 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $Q_1$ | -T | $Q_1$ | -T | $Q_1$ | -T | $Q_1$ | -T | $Q_1$ | -T | $Q_1$ | -T | $Q_1$ | -T |
| 1 | - | | + | + | + | + | - | | | | | | | |
| 1 | + | + | + | + | + | - | - | | + | - | - | | | |
| 1 | - | | - | | - | | - | | - | | + | - | | |
| 2 | - | | + | - | + | + | + | - | + | + | | | - | |
| 2[b] | - | | + | + | + | + | | | + | + | | | | |
| 2 | - | | + | + | | | + | - | | | - | | | |
| 2 | - | | + | + | + | - | + | + | + | + | | | | |
| 2 | - | | - | | - | | | | + | + | | | | |
| 2 | - | | + | - | + | - | + | - | + | + | | | | |
| 2 | - | | + | - | + | - | | | + | + | | | | |
| 2 | + | + | | | - | | + | - | + | - | + | - | + | - |
| 3 | + | + | + | - | + | - | - | | - | | | | | |
| 3 | + | - | + | - | + | - | | | | | | | | |
| 3 | + | + | + | + | + | + | + | - | | | | | + | + |
| | $S_3$ | +T | $S_3$ | +T | $S_3$ | +T | $S_3$ | +T | $S_3$ | +T | $S_3$ | +T | $S_3$ | +T |
| 1 | + | + | + | + | + | + | + | + | | | | | | |
| 1 | + | + | + | + | + | + | + | + | + | + | + | + | | |
| 1 | + | - | + | + | + | - | + | - | - | | + | - | | |
| 2 | + | + | + | + | + | + | + | + | + | - | | | + | + |
| 2[b] | + | - | + | - | + | - | | | + | + | | | | |
| 2 | + | + | + | + | | | + | + | | | + | + | | |
| 2 | + | - | + | + | + | - | + | + | + | - | | | | |
| 2 | + | + | + | + | + | - | | | + | + | | | | |
| 2 | + | + | + | + | + | + | + | + | + | + | | | | |
| 2 | + | + | + | + | + | - | | | + | + | | | | |
| 2 | + | + | | | + | - | + | + | + | + | + | + | + | + |
| 3 | + | + | + | + | + | + | + | - | + | + | + | + | | |
| 3 | + | + | + | - | + | - | | | | | | | | |
| 3 | + | + | + | + | + | - | + | + | | | | | + | - |

Table 34. ECG data from sham-operated myocardial infarction dogs

| Age | PS I | PS II | Post surgery: Days 2-5 | Days 6-14 | Weeks 3-7 | Weeks 14-16 |
|---|---|---|---|---|---|---|
| | | | **R/S ratios** | | | |
| 1 | 1.5 | 1.75 | 2.75 | | | |
| 1 | 4.25 | 5 | 6.75 | - | 7.0 | |
| 2 | 10.5 | 4.25 | - | 5.0 | | |
| 2 | 1.5 | 1.5 | - | 2.0 | 1.0 | |
| 2 | 7.25 | 9.0 | - | 21.0 | 5.5 | |
| 2 | 13.0 | 12.0 | 8.5 | 13.0 | - | 16.0 |
| 2 | 2.25 | 2.5 | 4.5 | 4.5 | 2.5 | 2.25 |
| 3 | 12.5 | 13.5 | 25/0 | - | 12.5 | 8.0 |
| | | | **R/T ratios** | | | |
| 1 | 3.0 | 2.75 | 1.5 | | | |
| 1 | -17.0 | 4.5 | 3.25 | - | 8.75 | |
| 2 | 8.0 | 5.25 | - | 6.0 | | |
| 2 | 3.5 | 2.75 | - | 4.0 | 2.75 | |
| 2 | 11.0 | -44.0[a] | - | 4.25 | 11.0 | |
| 2 | 2.25 | 5.0 | -17.0 | 2.25 | - | 8.0 |
| 2 | 4.75 | 4.0 | 4.5 | 1.75 | 2.5 | 2.5 |
| 3 | 50.0[a] | 9.0 | 4.0 | - | 16.5 | 4.0 |
| | | | **PR interval** | | | |
| 1 | .10 | .08 | .12 | | | |
| 1 | .14 | .12 | .12 | - | .12 | |
| 2 | .12 | .12 | - | .12 | | |
| 2 | .08 | .12 | - | .10 | .12 | |
| 2 | .08 | .08 | | .12 | .10 | |
| 2 | .12 | .12 | .10 | - | - | .12 |
| 2 | .12 | .12 | .10 | .14 | .12 | .12 |
| 3 | .12 | .12 | .14 | - | .10 | .12 |

[a] T was on 0.5 mm.

Table 34. (Continued)

| Age | PS I | PS II | Post surgery: Days 2-5 | Days 6-14 | Weeks 3-7 | Weeks 14-16 |
|---|---|---|---|---|---|---|
| | | | QT interval | | | |
| 1 | .20 | .20 | .24 | | | |
| 1 | .24 | .22 | .24 | - | .24 | |
| 2 | .22 | .20 | - | .22 | | |
| 2 | .24 | .24 | - | .24 | .24 | |
| 2 | .24 | .24 | - | .26 | .26 | |
| 2 | .20 | .24 | .20 | | | |
| 2 | .24 | .26 | .22 | .20 | .20 | .24 |
| 3 | .24 | .24 | .24 | - | .20 | .26 |
| | $Q_T$ -T | $Q_T$ -T | $Q_T$ -T | $Q_T$ -T | $Q_T$ -T | $Q_T$ -T |
| 1 | + + | - | - | | | |
| 1 | + + | - | - | | - | |
| 2 | + + | + - | | + + | | |
| 2 | - | - | | - | - | |
| 2 | - | + + | | - | - | |
| 2 | - | + - | + + | - | | |
| 2 | + + | + + | + + | + - | | + + |
| 3 | - | + - | + - | | + + | |
| | $S_{III}$+T | $S_{III}$+T | $S_{III}$ +T | $S_{III}$+T | $Q_{III}$+T | $Q_{III}$+T |
| 1 | + + | + + | + + | + + | + + | + + |
| 1 | + + | + + | + + | | + + | |
| 2 | - | - | | + + | | |
| 2 | + + | + + | | + + | + + | |
| 2 | + + | + - | | + + | + + | |
| 2 | - | - | - | - | | |
| 2 | + + | + + | + + | + + | + + | + + |
| 3 | + + | + + | - | | - | + + |

Table 35. ECG data from epicarditis dogs

| Age | PS I | PS II | Weeks 1-3 | 4-6 | 7-12 | 15-17 | 18-24 |
|---|---|---|---|---|---|---|---|
| | | | | R/S ratios | | | |
| 1 | 23.0 | 7.25 | - | 15.0 | 12.5 | 11.0 | |
| 1 | 12.0 | 7.5 | 8.0 | 6.5 | 4.0 | | |
| 2 | 11.0 | 6.0 | 12.0 | 10.0 | 16.5 | 13.5 | 29.0 |
| 2 | 3.25 | 5.1 | 5.5 | 4.0 | 4.5 | 3.0 | 3.5 |
| 2 | 5.5 | 9.25 | 10.0 | 11.25 | 16.0 | 9.0 | 12.5 |
| 3 | 4.75 | - | 5.75 | - | - | 23.0 | 6.25 |
| | | | | R/T ratios | | | |
| 1 | 5.75 | 2.0 | - | 8.0 | 4.0 | 7.25 | |
| 1 | 8.0 | 10.0 | 2.25 | 4.25 | 3.0 | | |
| 2 | 16.0 | 8.0 | 3.0 | 4.0 | 3.75 | 6.75 | 10.0 |
| 2 | 8.0 | 12.0 | 4.5 | 3.0 | 7.0 | 4.0 | 3.0 |
| 2 | 14.0 | 14.0 | 1.5 | 7.0 | 5.0 | 6.25 | 25.0 |
| 3 | 4.75 | - | 1.75 | - | - | -7.5 | 17.0 |
| | | | | PR interval | | | |
| 1 | .12 | .12 | - | .14 | .14 | .12 | |
| 1 | .10 | .10 | .12 | .10 | .12 | | |
| 2 | .06 | .08 | .10 | .12 | .12 | .14 | .10 |
| 2 | .10 | .10 | .16 | .14 | .16 | .12 | .14 |
| 2 | .10 | .10 | .12 | .12 | .12 | .10 | .10 |
| 3 | .12 | - | .14 | - | - | .10 | .12 |
| | | | | QT interval | | | |
| 1 | .22 | .24 | - | .20 | .20 | .20 | |
| 1 | .22 | .20 | .20 | .20 | .26 | | |
| 2 | .24 | .18 | .20 | .18 | .20 | .20 | .20 |
| 2 | .22 | .24 | .24 | .22 | .24 | .24 | .22 |
| 2 | .20 | .20 | .18 | .20 | .22 | .20 | .16 |
| 3 | .24 | - | .24 | - | - | .18 | .22 |

Table 35. (Continued)

| Age | PS I | | PS II | | Weeks 1-3 | | 4-6 | | 7-12 | | 15-17 | | 18-24 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T |
| 1 | - | | - | | | | + | + | + | - | + | + | | |
| 1 | + | + | + | + | - | | - | | - | | | | | |
| 2 | - | | + | + | - | | - | | - | | - | | - | |
| 2 | - | | + | + | + | - | - | | - | | - | | + | - |
| 2 | - | | + | + | - | | + | - | - | | + | - | - | |
| 3 | - | | | | - | | | | | | + | + | - | |
| | $S_{III}$ | +T | $Q_{III}$ | +T | $S_{III}$ | +T | $S_{III}$ | +T | $S_{III}$ | +T | $S_{III}$ | +T | $S_{III}$ | +T |
| 1 | + | + | + | + | | | + | + | + | + | + | + | | |
| 1 | + | + | + | + | + | + | + | + | + | + | | | | |
| 2 | + | + | + | + | - | | - | | - | | + | + | - | |
| 2 | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 2 | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 3 | + | + | | | - | | | | | | + | - | + | + |

Table 36. ECG data from sham-operated epicarditis dogs

| Age | PS I | PS II | Weeks 1 | 6-8 | 10-14 | 20-23 | 28 | 30 |
|---|---|---|---|---|---|---|---|---|
| | | | | | R/S ratio | | | |
| 1 | 7.5 | 14.0 | 25.0 | 20.0 | 9.5 | 20.0 | | |
| 2 | 21/0 | 22.0 | 44.0[a] | 1.5 | 18/0 | 42.0[a] | 10.5 | 10.0 |
| 2 | 3.5 | 2.75 | - | 6.0 | 4.5 | | | |
| | | | | | R/T ratio | | | |
| 1 | 7.5 | -14.0 | 3.25 | 6.75 | 12.5 | 10.0 | | |
| 2 | -42.0 | 11.0 | 5.5 | 9.0 | 36.0 | 21.0 | 6.5 | -20.0[a] |
| 2 | -24.0 | 8.25 | - | 4.0 | 4.0 | | | |
| | | | | | PR interval | | | |
| 1 | .08 | .08 | .10 | .10 | .10 | .10 | | |
| 2 | .10 | .12 | .14 | .16 | .12 | .16 | .12 | .10 |
| 2 | .10 | .10 | - | .12 | .10 | | | |
| | | | | | QT interval | | | |
| 1 | .20 | .20 | .24 | .22 | .20 | .20 | | |
| 2 | .22 | .28 | .28 | .26 | .24 | .20 | .22 | .22 |
| 2 | .22 | .20 | - | .22 | .24 | | | |

| | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | + | + | + | + | + | + | + | + | + | + | + | + | | | | |
| 2 | + | + | + | + | - | | + | + | + | - | + | + | + | + | - | |
| 2 | + | + | + | + | | | - | | - | | | | | | | |

| | $S_{III}$ | +T | $S_{III}$ | +T | $S_{III}$ | +T | $S_{III}$ | +T | $S_{III}$ | +T | $S_{III}$ | +T | $S_{III}$ | +T | $S_{III}$ | +T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | + | + | + | + | + | + | + | + | + | + | + | + | | | | |
| 2 | - | | + | + | - | | + | + | | | + | + | + | + | + | + |
| 2 | + | - | + | + | | | + | + | + | + | | | | | | |

[a] < 1 mm.

Table 37. ECG data from valvular insufficiency dogs

| Age | Pre-surgery | Weeks 2 | 4-7 | 10-12 | 13-20 | 22-27 | 31-32 | 36-38 |
|---|---|---|---|---|---|---|---|---|
| | | | | | R/S ratio | | | |
| 2 | 3.0 | 7.5 | 6.0 | 6.5 | 8.5 | 12.0 | 14.0 | 15.25 |
| 2 | 5.75 | 1.75 | 3.0 | 3.0 | 32.0 | 3.0 | | |
| 2 | 5.0 | 3.0 | - | 1.25 | 10.0[a] | | | |
| 3 | 11.5 | - | 7.25 | 5.75 | 4.5 | 12.0 | | |
| 3 | 29/0 | - | - | 29/0 | 30/0 | 62.0[a] | 62.0[a] | 35/0 |
| | | | | | R/T ratio | | | |
| 2 | 5.0 | 8.5 | 7.5 | 6.5 | 2.5 | 8.75 | 4.5 | 7.5 |
| 2 | 4.25 | 1.75 | 2.0 | 1.75 | 9.0 | 3.5 | | |
| 2 | 6.25 | 1.25 | - | 2.0 | 10.0[a] | | | |
| 3 | 23/0 | - | 7.25 | 7.5 | 4.5 | 24.0 | | |
| 3 | 10.0 | - | - | 8.25 | 15.0 | -32.0 | 13.0 | 17.5 |
| | | | | | PR interval | | | |
| 2 | .10 | .12 | .12 | .12 | .16 | .14 | .16 | .10 |
| 2 | .10 | .10 | .12 | .12 | .10 | .12 | | |
| 2 | .12 | .12 | - | .08 | .18 | | | |
| 3 | .08 | - | .10 | .12 | .10 | .10 | | |
| 3 | .12 | - | - | .14 | .10 | .10 | .16 | .12 |
| | | | | | QT interval | | | |
| 2 | .24 | .26 | .24 | .24 | .24 | .24 | .22 | .22 |
| 2 | .24 | .20 | .24 | .22 | .24 | .20 | | |
| 2 | .22 | .20 | - | .22 | .18 | | | |
| 3 | .20 | - | .18 | .20 | .18 | .24 | | |
| 3 | .24 | - | - | .22 | .20 | .18 | .20 | .20 |

[a] < 1 mm.

Table 37. (Continued)

| Age | Pre-surgery | | Weeks 2 | | 4-7 | | 10-12 | | 13-20 | | 22-27 | | 31-32 | | 36-38 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T |
| 2 | + | + | + | + | + | + | - | | - | | + | - | + | - | + | + |
| 2 | + | + | + | - | + | - | - | | - | | + | + | | | | |
| 2 | - | | - | | | | + | + | - | | | | | | | |
| 3 | + | + | | | + | - | + | + | + | - | + | + | | | | |
| 3 | + | + | | | | | - | | - | | - | | + | + | + | + |
| | $S_{III}$ | +T | $S_{III}$ | +T | $S_{III}$ | +T | $S_{III}$ | +T | $S_{III}$ | +T | $S_{III}$ | +T | $S_{III}$ | +T | $S_{III}$ | +T |
| 2 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 2 | + | + | + | + | + | + | + | + | + | + | + | + | | | | |
| 2 | + | + | + | + | | | + | + | + | + | | | | | | |
| 3 | + | + | | | + | + | + | + | + | + | + | + | | | | |
| 3 | - | | | | | | - | | - | | - | | - | | - | |

Table 38. ECG data from sham-operated valvular insufficiency dogs

| Age | Pre-surgery | Days 2 | Days 10-12 | Weeks 4 | Weeks 6 | Weeks 10 | Weeks 13 |
|---|---|---|---|---|---|---|---|
| | | | | R/S ratio | | | |
| 1 | 4.0 | 6.0 | 22/0 | | | | |
| 2 | 3.0 | 19.0 | | | | | |
| 2 | 56.0[a] | | 30/0 | | 64.0[a] | 37.0 | 8.5 |
| 2 | 1.5 | | 9.0 | 4.5 | 6.0 | | 2.75 |
| 3 | 38/0 | 30/0 | 20/0 | | | | |
| | | | | R/T ratio | | | |
| 1 | - 8.0 | -4.5 | 7.25 | | | | |
| 2 | - 1.25 | -3.0 | | | | | |
| 2 | 28.0 | | 15.0 | | 21.25 | 18.5 | 17.25 |
| 2 | 3.0 | | 6.0 | 3.0 | 2.5 | | 2.0 |
| 3 | -19.0 | 15.0 | 9.25 | | | | |
| | | | | PR interval (sec.) | | | |
| 1 | .08 | .14 | .12 | | | | |
| 2 | .12 | .08 | | | | | |
| 2 | .10 | | .12 | | .12 | .12 | .12 |
| 2 | .10 | | .14 | .14 | .14 | | .14 |
| 3 | .14 | .12 | .12 | | | | |
| | | | | QT interval (sec.) | | | |
| 1 | .20 | .24 | .16 | | | | |
| 2 | .28 | .20 | | | | | |
| 2 | .18 | | .20 | | .18 | .20 | .20 |
| 2 | .22 | | .20 | .22 | .24 | | .22 |
| 3 | .24 | .20 | .24 | | | | |

| | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T | $Q_I$ | -T |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | + | + | + | + | + | - | | | | | | | | |
| 2 | + | + | + | + | | | | | | | | | | |
| 2 | + | + | | | + | + | | | + | + | + | + | - | |
| 2 | - | | | | + | + | + | + | + | + | | | + | + |
| 3 | + | + | + | + | + | + | | | | | | | | |

| | $S_{III}+T$ | | $S_{III}+T$ | | $S_{III}+T$ | | $S_{III}+T$ | | $S_{III}+T$ | | $S_{III}+T$ | | $S_{III}+T$ | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | + | - | + | - | + | + | | | | | | | | |
| 2 | + | - | + | - | | | | | | | | | | |
| 2 | + | + | | | + | + | | | + | + | + | + | + | + |
| 2 | + | + | | | + | + | + | + | + | + | | | + | + |
| 3 | - | | - | | - | | | | | | | | | |

[a] < 1 mm.

Table 39. Hematological studies of dogs during course of experimentation

| No. | Date | HT | Hb | WBC | RBC | Baso | Eos | Lymph | Stab | Ses | Mono |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Infarction | | | | | | |
| 14 | Pre-surgery | 45.0 (37-53) | 16.4 (12.9-19.4) | 13.04 ( 6.25-22.2) | 6.91 (5.22-7.9 ) | .71 (0-4) | 2.0 (0-7) | 19.0 ( 7-28) | 3.3 (0- 7) | 70.6 (61-73) | 4.14 (1-15) |
| 9 | 1 wk. | 44.4 (40-50) | 16.2 (13.9-17.8) | 16.18 ( 6.04-25.2) | 7.09 (5.93-7.74) | .67 (0-1) | 2.67 (1-4) | 16.67 (12-22) | 5.1 (2- 9) | 72.8 (67-76) | 3.1 (1- 5) |
| 3 | 2 wk. | 41 (37-43) | 4.1 (12.7-15.6) | 15.67 (12.9-18.52) | 6.14 (5.26-6.85) | .33 (0-1) | 3 (1-5) | 25.6 (18-35) | 4 (1- 6) | 63 (60-66) | 4 (3- 6) |
| 6 | 3 wk. | 45.5 (42-49) | 16.6 (14.3-18.8) | 14.09 (7.65-23.5 ) | 6.9 (5.74-8.5 ) | .75 (0-5) | 6.2 (1-20) | 14.2 ( 9-18) | 4.2 (1-10) | 69.3 (53-74) | 4.7 (2- 8) |
| 1 | 4 wk. | 48 | 16.0 | 10.85 | 7.35 | 0 | 2 | 33 | 3 | 59 | 3 |
| 1 | 15 wk. | 46 | 18.2 | 8.76 | 7.66 | 1 | 2 | 18 | 3 | 74 | 2 |
| | | | | | Infarct Sham | | | | | | |
| 8 | Pre-surgery | 46.4 (43-51) | 17.0 (16.0-19.1) | 12.28 ( 6.72-19.4) | 6.51 (6.69-8.68) | .625 (0-1) | 2.63 (1-9) | 20.0 (11-29) | 2.12 (0- 4) | 70.0 (61-80) | 4.1 (2- 7) |
| 4 | 1 wk. | 46 (43-49) | 16.5 (16 -17 ) | 10.77 ( 8.5-12.6) | 7.23 ( 6.87-8.07) | .75 (0-1) | 4 (2-7) | 18.5 (15-24) | 3 (2- 5) | 68.8 (65-72) | 5 (4- 7) |
| 2 | 2 wk. | 44.5 (44-45) | 17.3 (16.7-18.0) | 14.13 (12.36-15.9) | 7.08 (6.76-7.4) | 0 (0) | 5.5 (2-9) | 21.5 (13-30) | 4 (3- 5) | 66 (63-69) | 3 (2- 4) |
| 2 | 4 wk. | 46 (45-47) | 16.2 ( 16.2 ) | 9.8 ( 8.99-10.6) | 7.19 (7.1 -7.28) | .5 (0-1) | 2 (1-3) | 21 (16-26) | 1.5 (0- 3) | 71.5 (71-72) | 4 (2- 6) |

Table 39. (Continued)

| | HT | HB | WBC | RBC | Baso | Eos | Lymph | Stab | Seg | Mono |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Infarct sham (cont.) | | | | | |
| 1 6 wk. | 47 | 18.8 | 13.72 | 7.39 | 0 | 5 | 14 | 3 | 74 | 4 |
| 2 12 wk. | 50 (50) | 18.5 (18-19) | 10.93 (10.24-11.62) | 8.12 (7.32-8.92) | 0 (0) | 4 (2-6) | 19.5 (19-20) | 5.5 (4-7) | 64 (61-67) | 7 (7) |
| | | | | | Epicarditis | | | | | |
| 6 Pre-sur-gery | 48.5 (45-55) | 19.95 (16.4-19.0) | 9.298 (6.12-12.94) | 7.107 (5.38-8.59) | .5 (0-2) | 2.5 (1-7) | 17.0 (9-23) | 5.5 (1-10) | 70.5 (66-74) | 4.0 (2-6) |
| 5 1 wk. | 43.2 (37-51) | 16.2 (14.2-17.8) | 13.576 (8.86-18.4) | 7.12 (4.84-9.24) | .8 (0-3) | 3.6 (2-4) | 14.2 (10-19) | 3.8 (0-7) | 73.8 (70-79) | 3.8 (2-5) |
| 2 2 wk. | 45 (44-46) | 17.5 (17.4-17.6) | 15.56 (12.5-18.6) | 7.12 (6.52-7.72) | 1 (1) | 5 (4-6) | 10 (8-12) | 6 (6) | 76.5 (74-79) | 2 (1-3) |
| 4 4 wk. | 47 (43-50) | 17.5 (16.0-18.8) | 14.75 (7.6-25.4) | 7.568 (6.78-8.5) | 0 (0) | 3.25 (0-12) | 11.25 (10-13) | 4.5 (3-7) | 76.5 (67-84) | 4.5 (8-0) |
| 4 7 wk. | 46.25 (38-50) | 16.85 (15.1-18.0) | 14.91 (10.0-24.3) | 7.717 (6.0-8.48) | .8 (0-2) | 3.5 (2-8) | 14.75 (13-16) | 4 (1-9) | 72.25 (69-75) | 4.5 (3-6) |
| 6 11 wk. | 47.67 (43-55) | 16.86 (14.0-18.8) | 12.02 (7.55-19.35) | 7.672 (6.65-8.55) | 1.5 (0-4) | 7 (3-13) | 17 (13-21) | 2.33 (0-7) | 66.8 (59-72) | 5.33 (2-8) |

Table 39. (Continued)

| No. | Date | HT | Hb | WBC | RBC | Baso | Eos | Lymph | Stab | Seg | Mono |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Epicarditis (cont.) | | | | | | |
| 3 | 15 wk. | 47.67 (56-51) | 17.8 (17.5-18.5) | 15.26 (10.4-22.5) | 7.672 (7.33-8.26) | .33 ( 0-1) | 6.67 (1-12) | 19 (1-8) | 4.6 (1-8) | 67 (63-72) | 4.3 (2-7) |
| 5 | 19 wk. | 46.2 (43-51) | 17.3 (15.0-18.8) | 10.8 (5.56-15.88) | 7.31 (5.73-8.32) | .6 (0-2) | 7.4 (3-11) | 18.8 (13-23) | 3.4 (0-7) | 65.4 (57-72) | 4.4 (2-10) |
| 3 | 23 wk. | 49 (45-56) | 17.6 (12.5-18.8) | 12.29 (10.96-13,22) | 7.83 (7.15-9.39) | .6 (0-2) | 4.67 (4-6) | 20 (14-25) | 1.67 (0-3) | 68.3 (64-74) | 4.67 (4-5) |
| 1 | 27 wk. | 40 | 18.8 | 11.22 | 5.83 | | | | | | |
| 1 | 29 wk. | 43 | 17.0 | 14.78 | 7.29 | 0 | 3 | 18 | 4 | 68 | 7 |
| | | | | | Epicarditis sham | | | | | | |
| 3 | Pre-sur-gery | 44 (40-46) | 16.6 (15.2-18.4) | 14.293 (13.44-14.82) | 6.92 (6.23-7.27) | .33 (0-1) | 2 (0-3) | 18.67 (9-25) | 3 (0-7) | 71.3 (56-91) | 4.67 (0-11) |
| 3 | 1 wk. | 42.67 (41-44) | 16.67 (15.2-17.8) | 14.95 (13.7-16.64) | 6.76 (6.4-7.1) | .33 (0-1) | 1.67 (1-2) | 18.3 (13-21) | 6 (4-9) | 69.3 (63-77) | 4.33 (0-8) |
| 3 | 4 wk. | 42 (39-43) | 16 (15.1-16.8) | 14.62 (13.3-16.3) | 6.79 (6.46-7.43) | 0 (0) | 1.67 (1-3) | 18 (12-22) | 2.87 (2-3) | 73.67 (71-78) | 4.67 (4-6) |

Table 39. (Continued)

| No. | Date | HT | Hb | WBC | RBC | Baso | Eos | Lymph | Stab | Seg | Mono |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Epicarditis sham (Cont.) | | | | | | |
| 2 | 7 wk. | 49.5 (47-52) | 17.5 (17- 18) | 15.32 (13.9-16.74) | 8.28 (8.18-8.38) | (0) ( 0) | 8 (2-14) | 15.5 (10-21) | 9.5 (3-16) | 62 (54-70) | 5 (4-6) |
| 2 | 12 wk. | 45.5 (45-46) | 15.2 (15-15.23) | 8.81 (6.65-10.96) | 7.11 (5.94-8.28) | .5 (0-1) | 2.5 (2-3) | 28 (22-34) | 2 (0-4) | 56.5 (44-69) | 10.5 (1-20) |
| 2 | 16 wk. | 44.5 (44-45) | 16.3 (15.6-17) | 11.37 (10.2-12.54) | 6.86 (7.39-6.32) | 0 (0) | 10 (5-15) | 19.5 (11-28) | 1 (0-2) | 64 (51-77) | 5.5 (5-6) |
| 1 | 20 wk. | 46 | 17.4 | 5.40 | 6.20 | 0 | 2 | 21 | 6 | 62 | 10 |
| 1 | 24 wk. | 45 | 18.0 | 9.74 | 6.72 | 0 | 6 | 24 | 4 | 59 | 7 |
| 1 | 27 wk. | 46 | 18.4 | 12.96 | 8.28 | 0 | 2 | 25 | 3 | 67 | 3 |
| 1 | 30 wk. | 38 | 18.6 | 10.60 | 6.33 | 0 | 3 | 20 | 2 | 63 | 12 |
| 1 | 36 wk. | 45 | 16.0 | 8.24 | 7.50 | 0 | 6 | 21 | 4 | 63 | 6 |
| | | | | | Valvular Insufficiency | | | | | | |
| 5 | Pre-sur-gery | 44.6 (36-50) | 16.1 (14-17.2) | 12.86 (10.2-15.75) | 7.28 (6.18-8.71) | .6 ( 0-1) | 6.8 (1-13) | 13.8 (2-19) | 1.8 (0-4) | 57.6 ( 8-77) | 4.6 ( 0-9) |
| 5 | 1 wk. | 44.0 (34-48) | 16.2 (13.3-18.2) | 14.57 (9.57-21.74) | 6.95 (5.23-7.64) | .8 (0-2) | 4.6 (1-13) | 1.68 (14-21) | 2 (0-4) | 69.6 (64-77) | 6 (3-11) |

Table 39. (Continued)

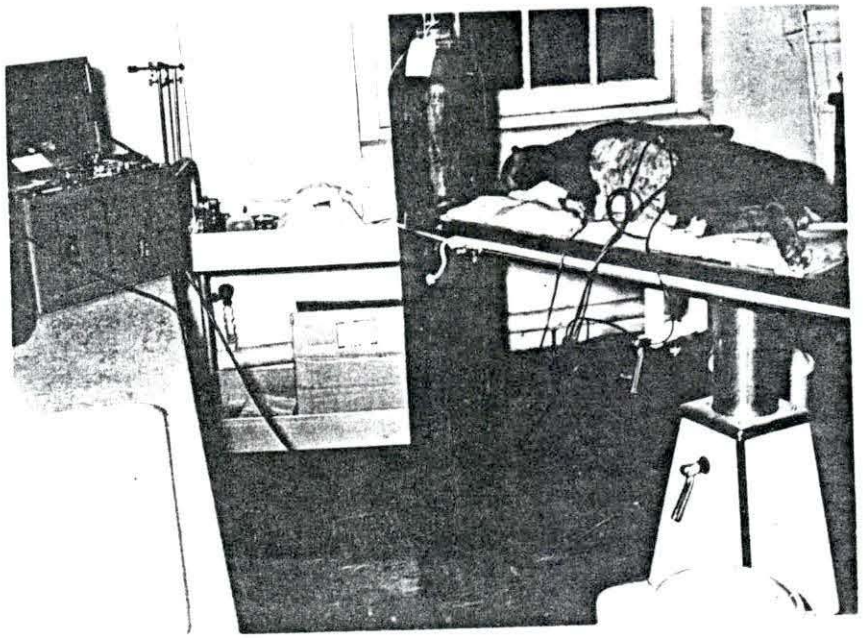
| No. | Date | HT | Hb | WBC | RBC | Baso | Eso | Lymph | Stab | Seg | Mono |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | VI (cont.) | | | | | | |
| 4 | 3 wk. | 46.0 (45-47) | 16.4 (15-17.5) | 12.08 (6.35-18.62) | 7.11 (6.04-8.14) | .75 (0-2) | 6 (1-15) | 18 (14-22) | 2 (1-3) | 66.5 (59-78) | 6.75 (3-10) |
| 5 | 7 wk. | 44.6 (30-52) | 16.08 (13-19) | 12.17 (7.25-16.74) | 7.18 ( 4.8-8.4) | .2 (0-1) | 6.6 (1-14) | 15.2 (13-19) | 5.6 (0-16) | 67.2 (54-78) | 5.2 (3-9) |
| 4 | 12 wk. | 50 (48-54) | 16.13 (15.2-16.8) | 13.5 (11.3-19.96) | 8.38 (8.15-8.77) | .5 (0-1) | 6.5 (2-11) | 18 (16-19) | 2.5 (1-5) | 68.5 (64-73) | 4 (3-5) |
| 3 | 16 wk. | 52 (48-58) | 16.67 ( 16-17 ) | 8.29 (7.98-8.65) | 8.34 (8.12-8.65) | 0 (0) | 3.33 (2-4) | 22 (14-34) | 2.67 (1-4) | 64.67 (56-70) | 7.33 (2-11) |
| 3 | 20 wk. | 49.3 (43-54) | 16.6 (15.8-17.2) | 9.93 (8.9-10.58) | 7.89 (6.56-8.58) | 2 (0-5) | 8.3 (5-14) | 22 (13-29) | 4.3 ( 1-8) | 60.3 (50-74) | 4 (3-5) |
| 2 | 24 wk. | 50 (49-51) | 17.4 (16.8-18) | (13.11) (9.65-16.56) | 8.09 (7.95-8.22) | .5 (0-1) | 1.5 (1-2) | 17 (15-19) | 4 ( 2-6 ) | 70.5 (69-72) | 6 (4-8) |
| 2 | 28 wk. | 52 (51-53) | 17.1 (16.2-18) | 11.44 (7.76-15.12) | 7.11 (66.8-7.53) | 1.5 (1-2) | 10 (6-14) | 14.5 (10-19) | 3 (2-4) | 64.5 (63-66) | 6.5 (6-7) |
| 2 | 32 wk. | 505 (48-53) | 17.2 (16.8-17.5) | 8.54 (8.18-8.9) | 7.76 (7.13-8.35) ( | 1 (1) | 11 (9-11) | 21 (14-29) | 4.5 (4-5) | 60.5 (51-70) | 2 (2) |
| 1 | 37 wk. | 50 | 17.3 | 7.91 | 7.91 | 0 | 15 | 16 | 1 | 64 | 4 |
| 1 | 42 wk. | 50 | 16.9 | 10.5 | 8.46 | 6 | 3 | 23 | 3 | 65 | 6 |
| 1 | 45 wk. | 46 | 16.0 | 10.52 | 5.64 | 4 | 9 | 11 | 5 | 68 | 3 |

Table 39. (Continued)

| No. | Date | HT | Hb | WBC | RBC | Baso | Eos | Lymph | Stab | Seg | Mono |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Valvular Insufficiency Sham | | | | | | |
| 5 | Pre-surgery | 45.8 (38-52) | 15.96 (12.3-19.4) | 11.23 (7.04-14.75) | 7.04 (6.0-8.34) | .8 (0-4) | 3.4 (1-9) | 26.2 (21-34) | 3 (0-6) | 62.2 (51-70) | 4.4 (1-10) |
| 4 | 1 wk. | 46.0 (44-49) | 16.5 (1.49-18.6) | 10.99 (8.7-12.9) | 7.21 (6.8-7.96) | .5 (0-2) | 4 (2-8) | 24.5 (21-27) | 3.5 (0-5) | 63 (57-68) | 4.5 (2-8) |
| 2 | 4 wk. | 42 (38-46) | 13.3 (12.6-14) | 13.0 (10.35-15.65) | 6.67 (5.57-7.77) | 5.5 (3-8) | 5 (4-6) | 12 (12) | 6.5 (3-10) | 60.5 (56-65) | 10 (5-15) |
| 2 | 8 wk. | 42 (42) | 15.05 (15-15.1) | 14.3 (13.8-14.8) | 6.43 (5.9-6.95) | 1 (0-2) | 10.5 (10-11) | 29.5 (26-33) | 6 (5-7) | 48.5 (45-52) | 4.5 (2-7) |
| 2 | 13 wk. | 46 (45-47) | 16 (15.3-16.8) | 16.6 (16.14-17.06) | 7.77 (7.69-7.86) | 0 (0) | 9.5 (3-16) | 25.5 (15-46) | 8 (2-14) | 505 (49-52) | 6.5 (3-10) |
| 1 | 16 wk. | 50 | 17.0 | 14.04 | 7.47 | 1 | 6 | 26 | 8 | 53 | 6 |
| 1 | 25 wk. | 49 | 18.8 | 11.2 | 7.44 | 1 | 4 | 20 | 7 | 62 | 6 |

Figure 10. Positioning of dog and placement of electrodes for serial ECG studies.

Figure 11. Placement of electrodes for serial ECG study.



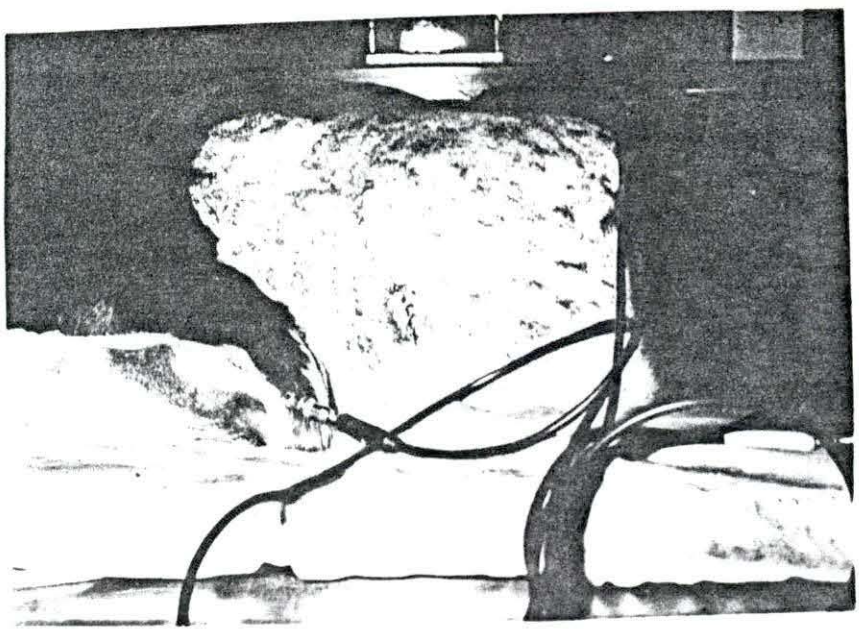
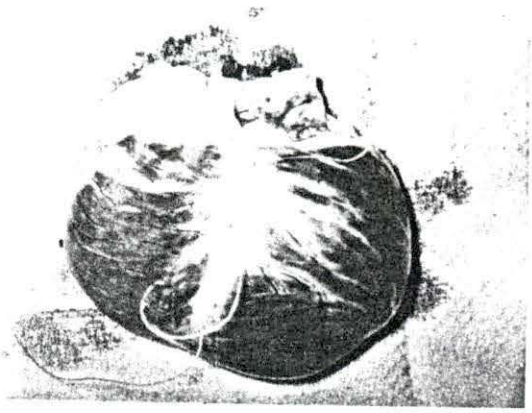
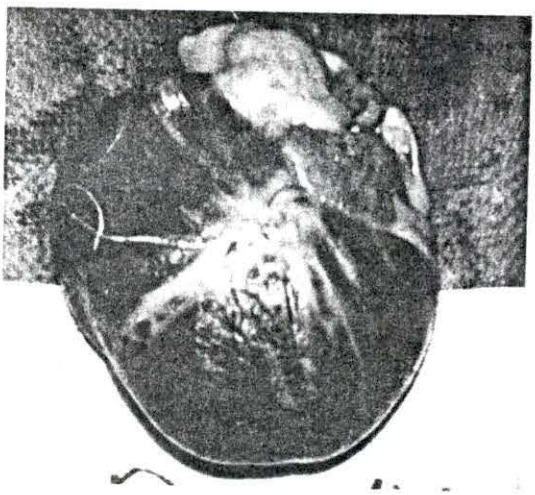


Figure 12. Heart from a dog sham-operated for myocardial infarction.

Figure 13. Closeup of a heart from a dog sham-operated for myocardial infarction.

Figure 14. Heart from dog 45 showing area of infarction at apex and a second smaller linear infarct between the papillary muscles.





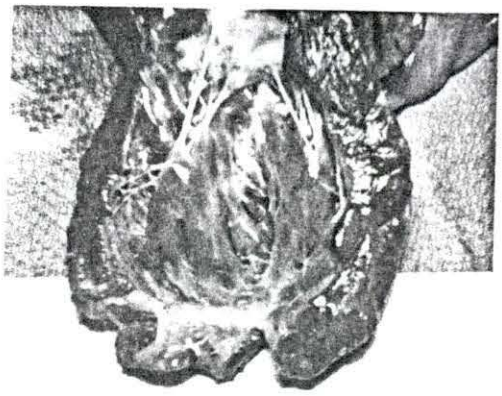
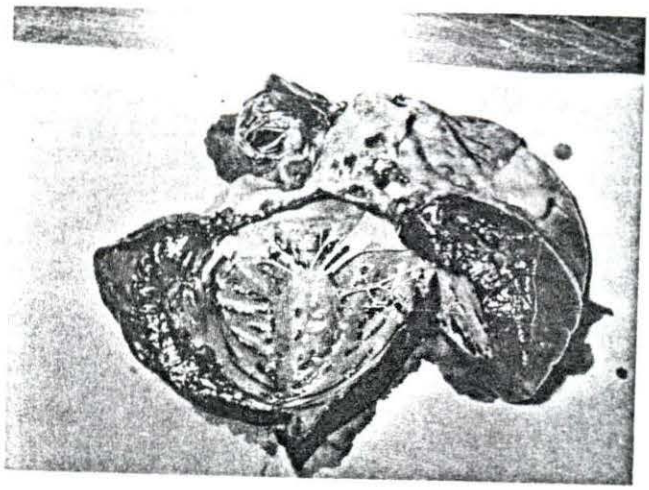
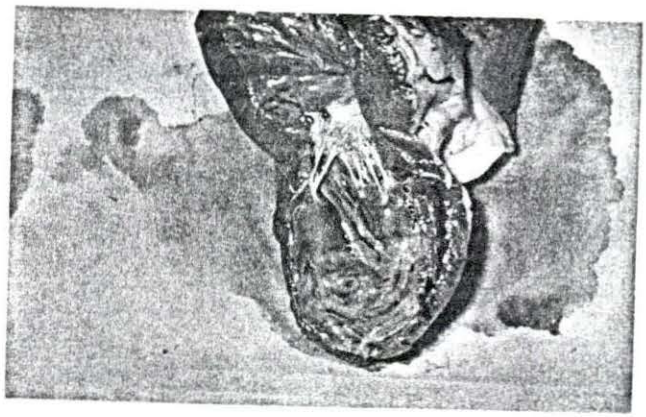


Figure 15. Heart from dog 46 showing small infarct on tip of papillary muscle.

Figure 16. Heart from dog 49 showing large area of fibrosis involving nearly one-half of the left ventricle musculature.

Figure 17. Heart from dog 31 showing classical staircase infarction in posterior laterial wall of the left ventricle.





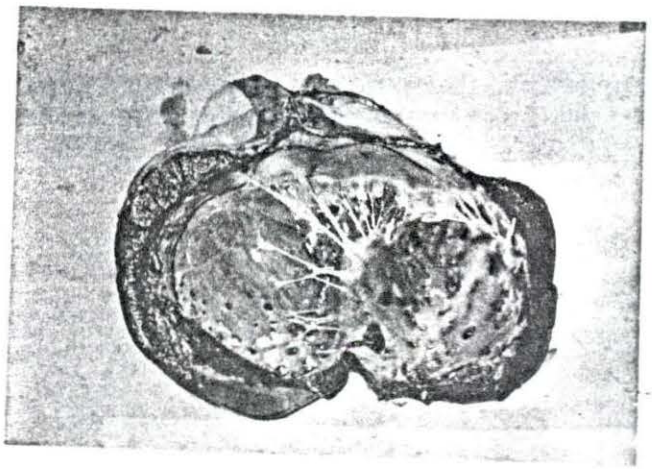
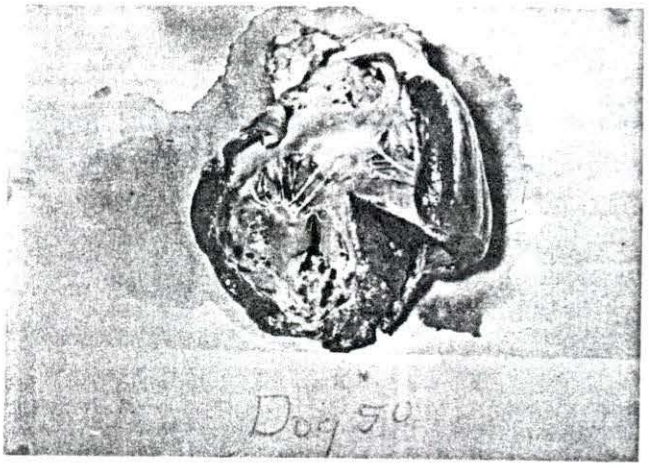
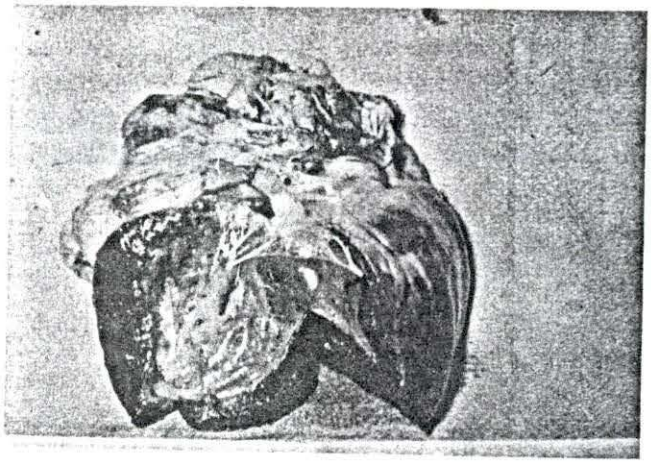


Figure 18. Heart from dog 50 showing fibrosis and the severe decrease in thickness of the left ventricle musculature.

Figure 19. Heart from dog 34 showing large infarction involving nearly all of the papillary muscle.

Figure 20. Heart from dog 7 showing infarction near apex of left ventricle and the decrease in the thickness of the muscle mass.


Dog 50



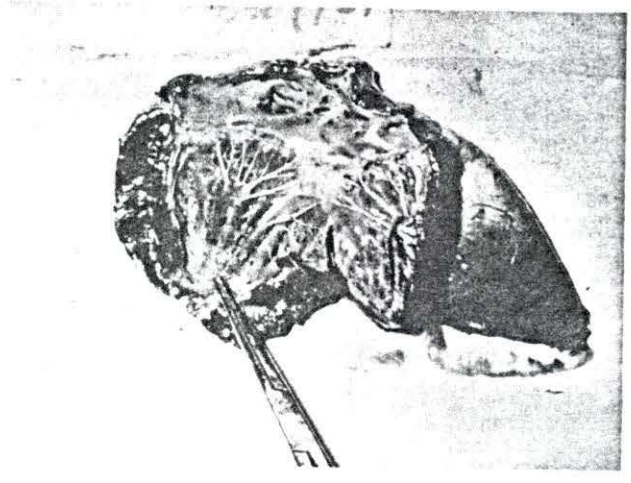
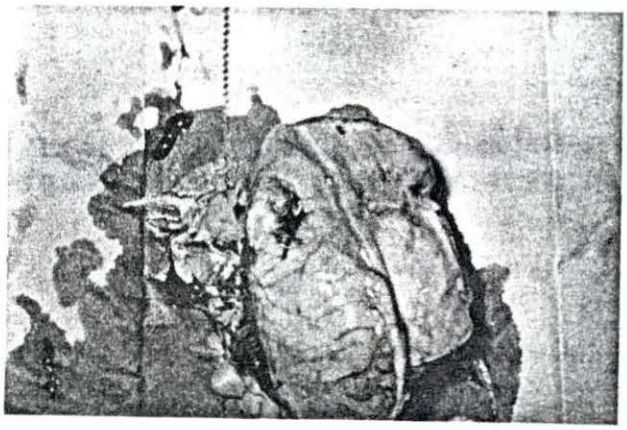
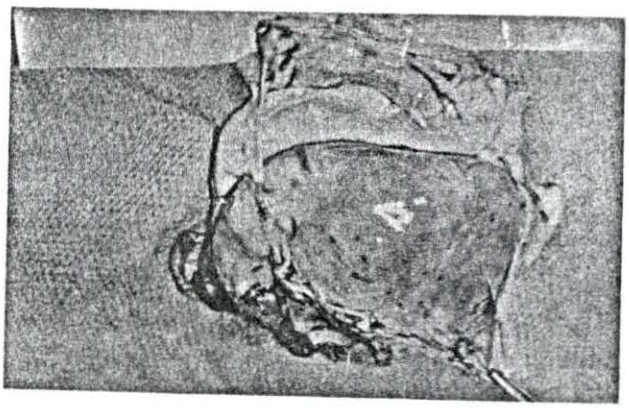


Figure 21. Heart and pericardium from dog 37. (The pericardium could not be removed from the heart.)

Figure 22. Heart and pericardium from dog 26 showing adhesions and epicarditis.

Figure 23. An epicarditis-pericarditis heart from dog 36.





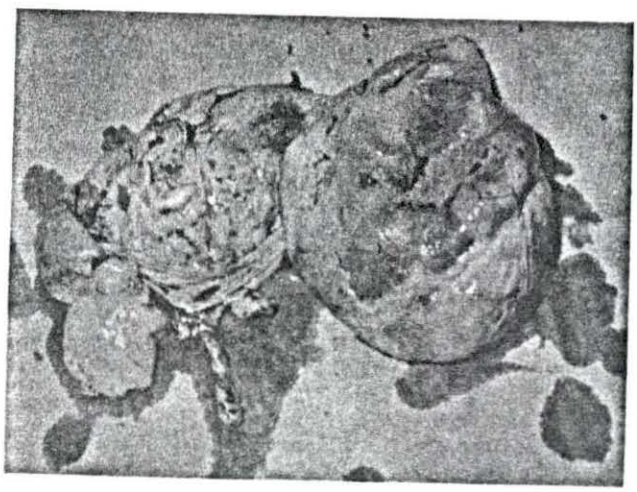
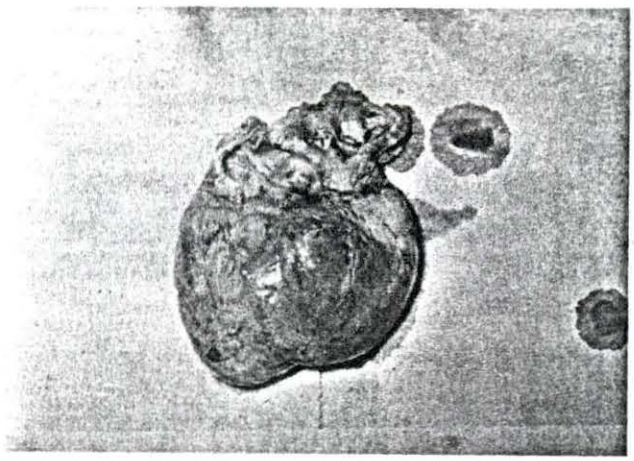
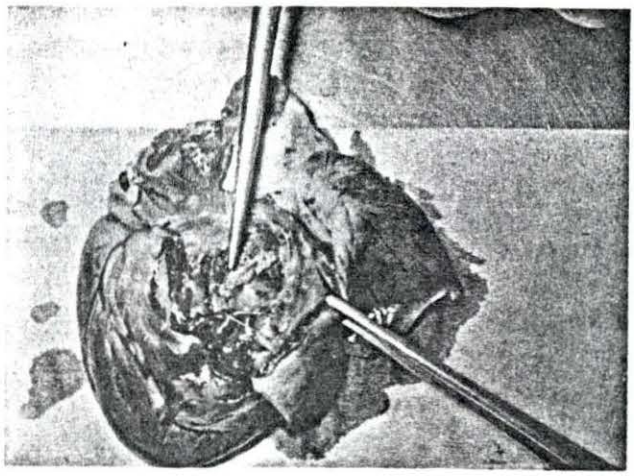


Figure 24. Heart from dog 42 (notice dilatation of right ventricle and "pumpkin-shape" of the heart).

Figure 25. Example of valvular insufficiency (Dog 27).

Figure 26. Example of valvular insufficiency (Dog 20).





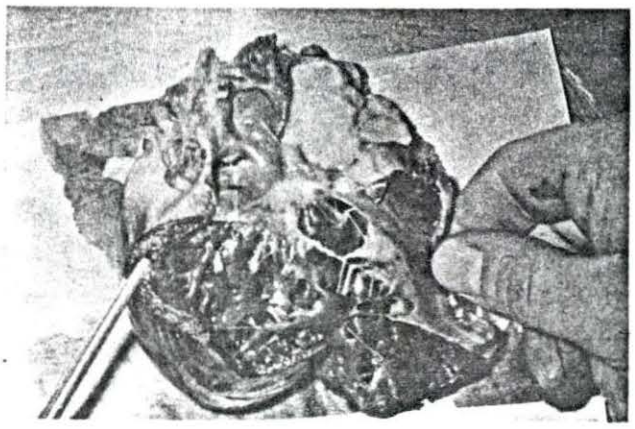


Figure 27. Section of fibrosis of an infarcted area. (Shows junction with normal cardiac muscle tissue fixed in buffered, neutral formalin and stained with the Mallory triple stain. 36X).

Figure 28. Section of fibrotic area of an infarction. (Shows junction with normal cardiac muscle. Fixed in buffered, neutral formalin and stained with hematoxylin and eosin. 36X.)

Figure 29. Increased magnification of Figure 28 (160X).

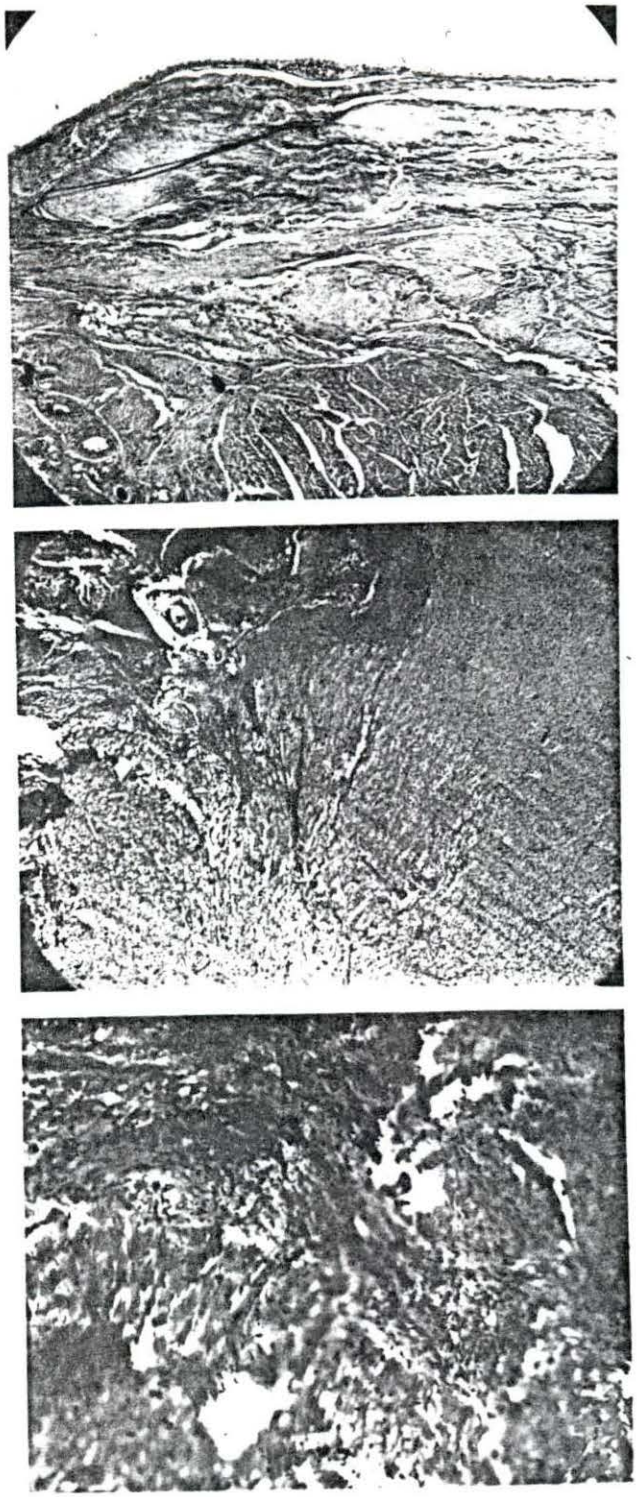
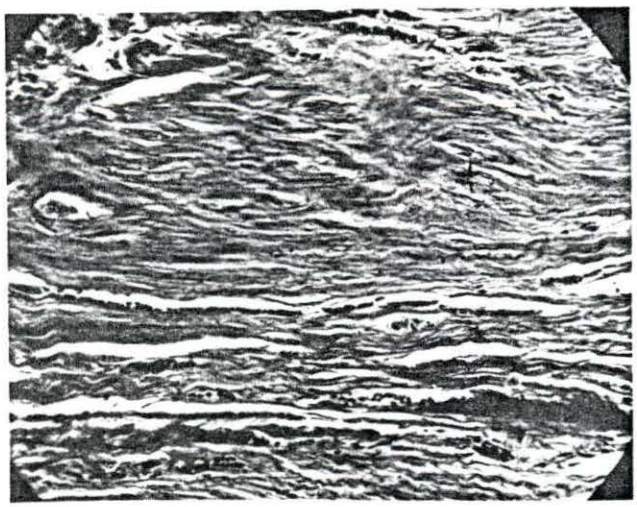
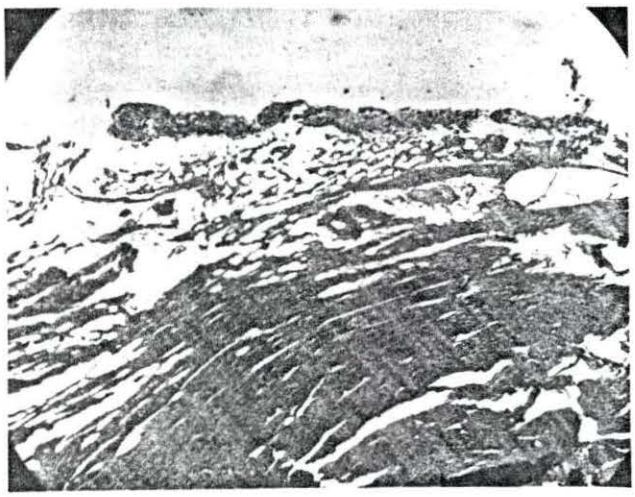


Figure 30. Section of fibrosis of an infarcted area. (Tissue fixed in buffered, neutral formalin and stained with the Mallory triple stain. 240X.)

Figure 31. Section of heart at junction of area of epicarditis with normal myocardium in dog 5. (Tissue fixed in buffered, neutral formalin and stained with the Mallory triple stain. 36X.)

Figure 32. Increased magnification of Figure 31. (Notice presence of giant cells (240X).)





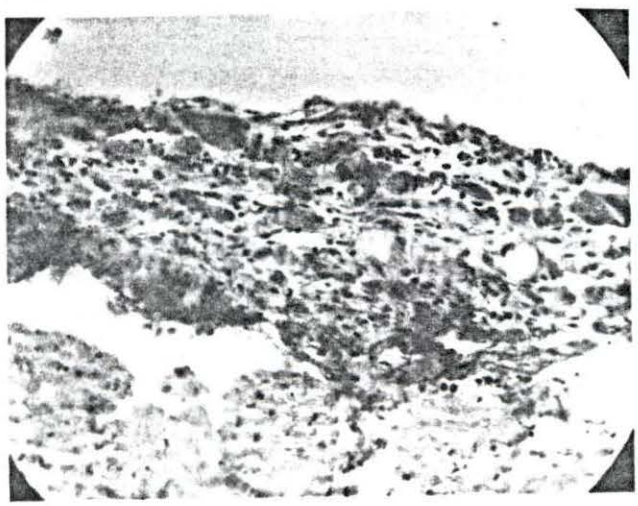
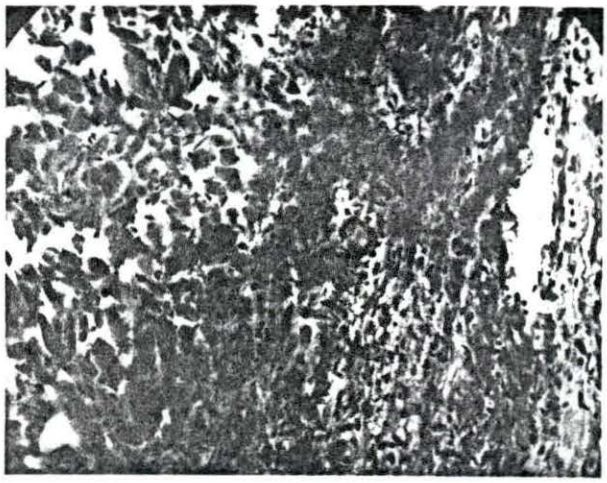


Figure 33. Section of heart at junction of area of epicarditis with normal myocardium in dog 37. (Notice involvement of the myocardium. Fixed in buffered, neutral formalin and stained with hematoxylin and eosin 36X.)

Figure 34. Increased magnification of Figure 33 (240X).

Figure 35. Section of a cusp from the right A-V valve showing increased thickness. (Tissue fixed in buffered, neutral formalin and stained with hematoxylin and eosin 36X.)



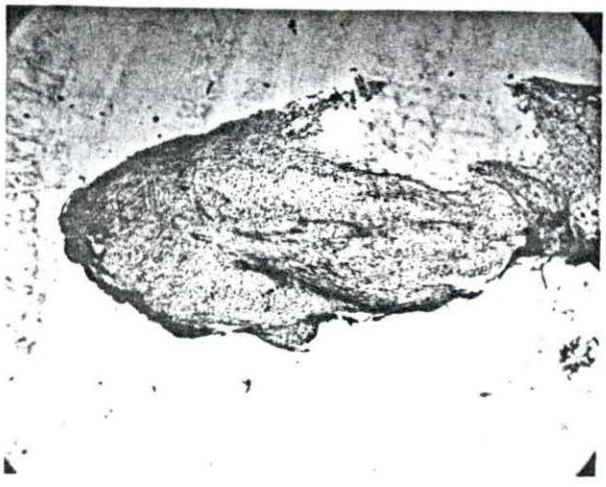
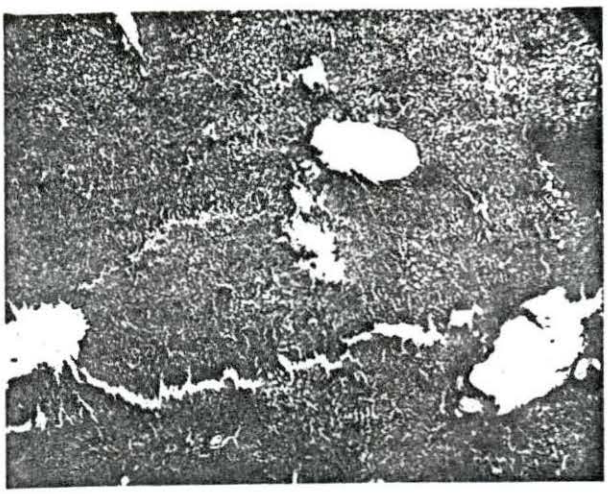


Figure 36. Liver from dog 42 showing early cardiac cirrhosis.

Figure 37. Section of liver from dog 42. (Notice greatly enlarged central veins. Fixed in buffered, neutral formalin and stained with hematoxylin and eosin. 36X.)

Figure 38. Increased magnification of Figure 37. (Notice cells around central vein in various stages of degeneration. 240X.)



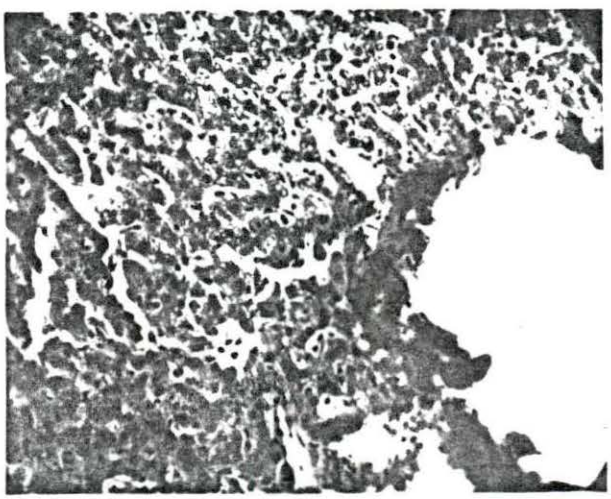
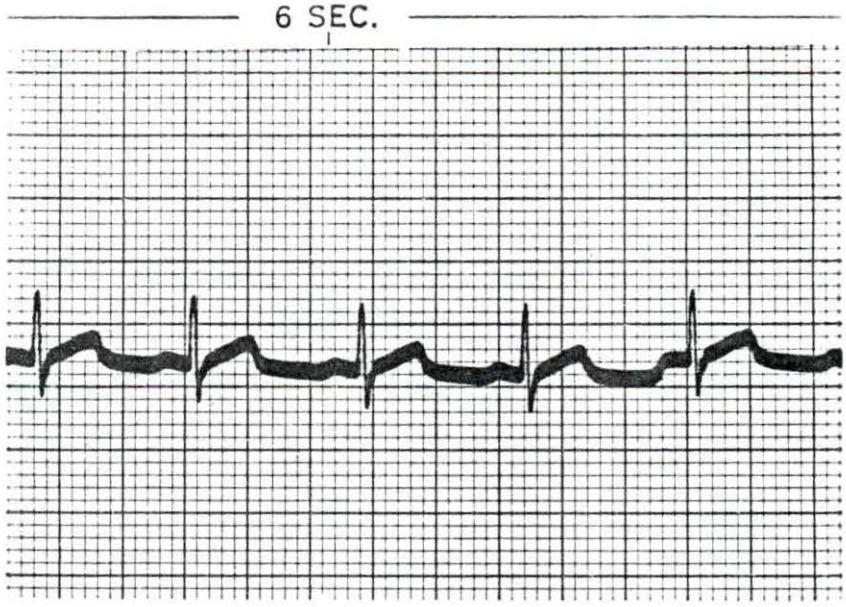


Figure 39. ECG recording of a normal lead I with no Q-wave and an upright T-wave.

Figure 40. ECG recording of a normal lead I with no Q-wave and an isoelectric T-wave.


6 SEC.

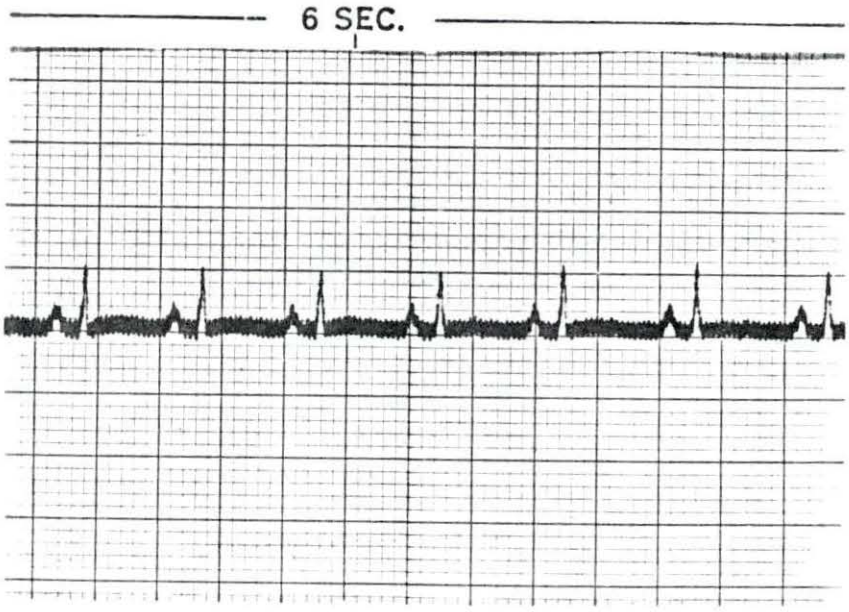
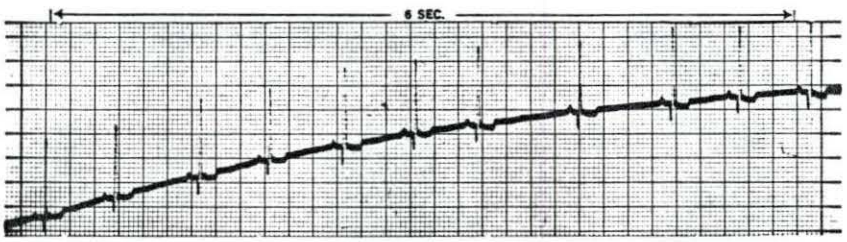

6 SEC.

Figure 41. ECG recording of a normal lead I with a Q-wave followed by an inverted T-wave.

Figure 42. ECG recording of an abnormal lead I with a Q-wave followed by an upright T-wave.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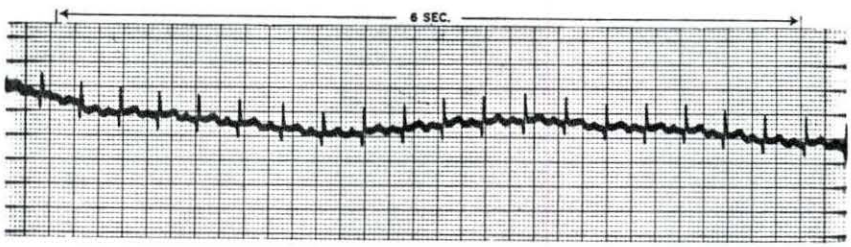
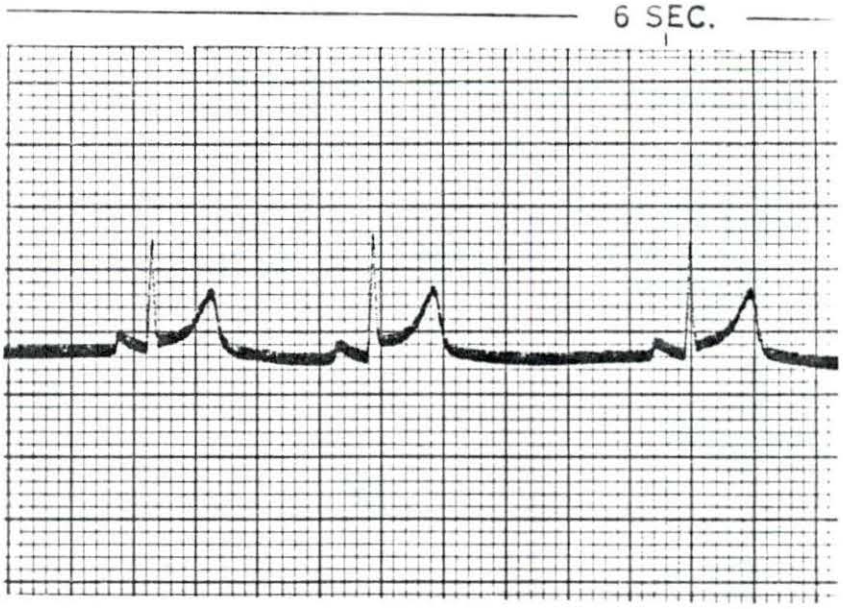

6 SEC.

Figure 43. ECG lead II recording from a dog taken 9 days following myocardial infarction showing a small S/T ratio and elevated ST segment.

Figure 44. ECG lead III recording from a dog following infarction showing an inverted P-wave.


6 SEC.

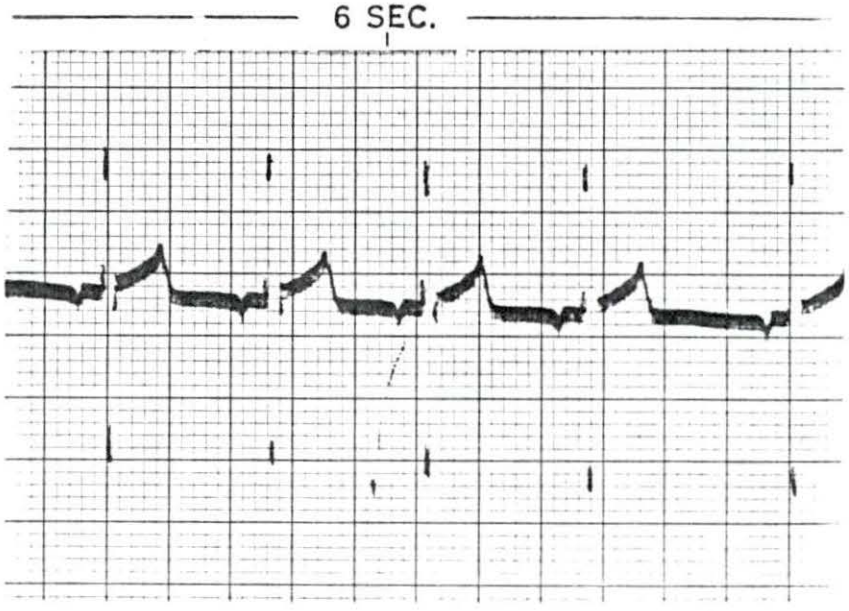
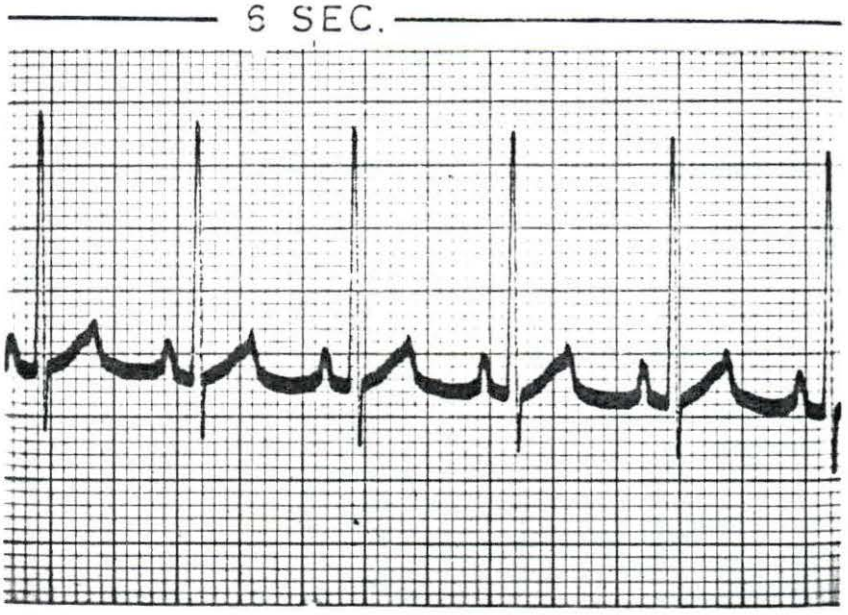

6 SEC.

Figure 45. ECG recording of a normal lead III with S-wave followed by an upright T-wave.

Figure 46. ECG of an abnormal lead III with an S-wave followed by an inverted T-wave.


6 SEC.

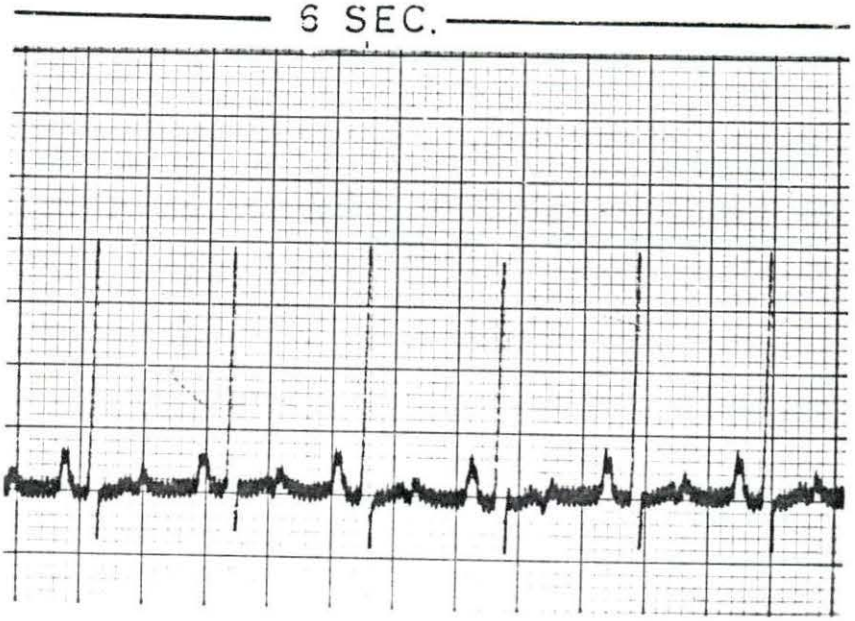
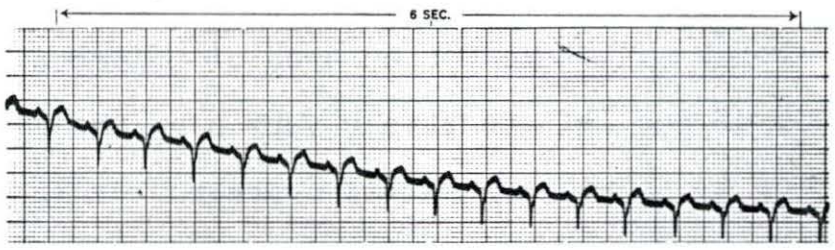
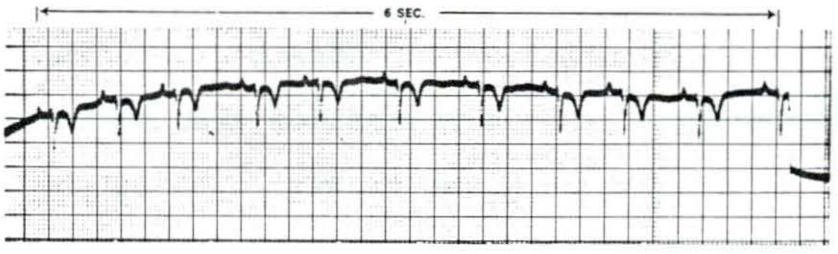

6 SEC.

Figure 47. Abnormal ECG recording showing a Q-S complex in lead I.

Figure 48. Abnormal ECG recording showing a Q-S complex in lead III.


6 SEC.


6 SEC.

# X. APPENDIX C: DATA

## A. Pathological Findings of Dogs with Cardiac Pathology

### 1. Myocardial infarction

Dog 7 There was an area of infarction at the right border of the left ventricle very near the apex. From the outside it showed as a small depressed area. Upon opening the heart there was an area of scar tissue about 2 cm. square and the thickness of the ventricular mass was about 1 cm. There were very few adhesions of lungs to thoracic wall and pericardial sac. Other organs and tissues appeared to be normal.

Dog 11 A very small infarct was found on the endocardial surface just ventral to the left anterior papillary muscle. This area was about 5 mm. in diameter. A second slightly larger infarction was seen more posteriorly on the endocardial surface of the left ventricle. There were no adhesions and other organs appeared to be normal.

Dog 15 A small, shallow area of infarction slightly less than 1 cm. in diameter was found in the left ventricle about midway between the apex and base of the heart. There was a small area of adhesions of lung to the pericardium. The kidneys were yellow and appeared to be friable from fatty infiltration. Other organs and tissues appeared normal.

Dog 21 An area of infarction approximately 1.5 cm. in diameter was seen on the anterior portion of the left endocardial surface about midway down the ventricular mass. Other organs and tissues were normal.

Dog 24 The heart of this dog showed a transmural infarction. It was slightly over 1 cm. in diameter on the epicardial surface; however, it spread in three fingers down to the apex on the endocardial surface. The involved area commenced over the interventricular septum about one-half of the way down the left ventricle. There were some adhesions of the pericardial sac to the epicardium. There were no adhesions of the lungs. The liver appeared to be swollen grossly and upon microscopic examination there was evidence of fatty infiltration. Other tissues seemed to be normal.

Dog 25 On the epicardial surface there was very little indication of pathology. Upon palpation a very thin area was found in the apex of the left ventricle. A hard mass was found near the region of the atrial-ventricular junction in the posterior portion of the left ventricle. Upon opening the heart a large fibrotic area was seen in the apex of the left ventricle about 3 cm. in diameter. There was a fibrotic streak 5 mm. wide and about 2.25 cm. in length between the two papillary muscles extending from the A-V junction to the base of the anterior papillary muscle.

There were some adhesions of the pericardial sac to the epicardium and to the lungs. The left diaphragmatic lobe showed areas of grey hepatization. There were no significant changes in the other tissues.

Dog 31 There was no indication of pathology from the external appearance of the heart except for a small area of adhesions of the pericardial sac to the epicardium in the area of pericardial suturing. Upon opening the left ventricle an abnormal area could be seen extending diagnally across the heart wall. It extended from below the papillary muscle almost to the attachment of the A-V valves on the posterior lateral wall. Its total length was nearly 7 cm. and it was 2 cm. wide at its greatest width very near the papillary muscle. Its width generally was between 1 and 1.5 cm. There were two fresh infarcts in the spleen. There was an old infarct on the left kidney. These were all small infarctions. The liver was hyperemic, but appeared normal otherwise. Other tissues were normal.

Dog 34 There were a few adhesions of the pericardial sac to the lungs and epicardium. There was a fibrotic area on the surface of the left ventricle. Upon opening the heart an extensive fibrotic area was evident. It extended for a distance of 7 cm. from the base of the papillary muscle to the apex of the heart in the area of the ventricular septum; however, the septum did not appear to be

involved. This area was approximately 3.5 cm. at its widest point and was more or less wedge-shaped with its point at the apex of the heart. The area around it was hyperemic and there were several rays of necrotic tissue extending from the mass of fibrosis. Liver appeared to be swollen and there was some cloudy swelling upon microscopic examination. Other tissues seemed to be normal.

Dog 38 This dog showed a fairly extensive area of epicarditis. The pericardial sac was adhered to approximately two-thirds of the epicardial surface. A small area of infarction was present on the endocardial surface in the base of the papillary muscle. This did not extend very deeply into the myocardium; however, there appeared to be 3 small projections of necrotic tissue extending towards the apex of the heart from the area of fibrosis. There seemed to be no significant changes in any of the other tissues.

Dog 45 This dog was allowed to exercise several weeks after production of the infarct. He did real well. However, he became tired in a relatively short time and would refuse to play or run for several minutes. After this short rest he would again resume normal activity. There were no other indications of any stress. Upon necropsy all tissues and organs were normal with the exception of the heart. An area of infarction about 3 cm. long and 2 cm. wide was seen in the left ventricular wall. This was not transmural, but

the entire muscle-fibrotic mass was reduced to a width of about 1 cm. In addition a slight valvular endocarditis was present on the left A-V valve.

Dog 46 There were no adhesions of the pericardial sac to the lungs or of the lungs to the pleura. There was a very small area of adhesions of the sac to the epicardium which was easily broken down. Heart appeared normal from the epicardial surface. On opening the heart an area of fibrotic tissue about 1 cm. in diameter was found on the papillary muscle where the chordae tendinae attach. Other tissues were normal.

Dog 49 This dog had the greatest amount of infarcted tissue. Upon palpation the left ventricle was nearly as thin as the atria and had much the same consistency. The fibrotic area covered the entire extent of the endocardial surface from the apex to about one-third of the way up the papillary muscles. The interventricular septum was also involved somewhat near the apex. The epicardial surface appeared to be normal.

The left lobe of the lung had some exudate in the bronchi and a few small areas of pneumonia were present. The left kidney was swollen and hyperemic; the other kidney was normal. Other organs seemed to be normal.

Dog 50 This dog showed a transmural infarct of the anterior left apex. It was about 3 cm. in diameter

encroaching on the lower part of the anterior papillary muscle. There was also some involvement of the septum near the apex. The center area was very thin, approximately 5 mm. in thickness. Other organs showed no significant changes.

Dog 51 There were a few adhesions of lungs to the pleura and pericardial sac and of the sac to the epicardium. There was no evidence of pathology from external appearance of the heart. Upon opening the heart, the involved area was not nearly so well defined as seen previously in other dogs. There was necrosis, but not nearly as much fibrosis had occurred. The infarct covered an area from the middle of the anterior papillary muscle to near the apex of the heart in a wedge-shape with its apex in the papillary muscle. This area was about 1.5 cm. wide at its widest point and nearly 3 cm. long. It appeared to be only about 1 cm. deep.

2. Epicarditis

Dog 5 There were no adhesions of the lungs. The pericardial sac of this animal was nearly normal. There was a diffuse epicarditis with the right side being more involved than the left. There was a slight valvular endocarditis of one cusp of the tricuspid valve. There was a slight right heart dilatation. The spleen was large and appeared nodular. Other organs looked normal.

Dog 10 This dog showed a severe pericarditis and much of the epicardium was involved. Some areas of petechiation and a lot of hyperemia were present. Fluids were minimal. The liver was slightly swollen and congested. The spleen was large and congested. The kidneys were very dark, nearly black, in color but of normal size and consistency. Other organs appeared to be normal.

Dog 26 The pericardium of this dog was severely involved. It was nearly 5 mm. thick. There was a mild epicarditis throughout with a minimum of hyperemia and almost no hemorrhage. There were two small cysts which appeared to be encased talcum powder. There were no adhesions of the lungs to the pericardium or the pleura. Other organs indicated no pathology.

Dog 36 The pericardial sac was adhered to the lungs and the pleural wall, and part of the cardiac lobe of the right lung was atelectatic. There was an extensive epicarditis on the right side which extended to the left. There was a large amount of petechial hemorrhage and hyperemia. Other tissues and organs were normal.

Dog 37 This dog died during the night about three months after pathology had been induced and had severe postmortem changes when necropsied. The thoracic cavity was full of fluids and there was little functional lung remaining. There were many large vessels extending into the pericardium.

The pericardial sac was entirely adhered to the epicardium and was impossible to remove. There was a small amount of dark, viscous fluid in the sac. It did have an odor. The heart muscle was hypertrophied and the chambers were smaller than normal. The auricles were almost entirely fibrotic. Other organs were badly decomposed and could not be studied effectively.

Dog 43 There were some adhesions of the right lung to the pleura and pericardium. Mediastinum was thickened with many large vessels in it. The pericardial sac was completely adhered to the epicardium. Epicarditis involved the entire surface of the heart. There was little or no exudate. The right ventricle was considerably dilated and the tissue had a very soft, flabby consistency. Other organs showed no significant changes.

3. Valvular insufficiency

Dog 20 This dog showed a minimal amount of right valvular insufficiency with one cusp having a cut about one-half of the way through, yet the right atrium was very dilated. The heart looked normal other than this. Other organs and tissues appeared to be normal.

Dog 27 The right ventricle was hypertrophied and the atrium was dilated. One cusp of the right A-V valve was completely destroyed. Liver was swollen slightly and somewhat congested, but there was no indication of necrosis upon

microscopic examination. The spleen was large and knobby. Other organs and tissues showed no significant changes.

Dog 30 The heart showed very little evidence of pathology from external appearance. The medial cusp of the right A-V valve was cut and the edges were granulated. Two tendinae were also cut, but the valve probably remained partially functional. There was a small lesion on the right kidney which may have been a cyst. Other tissues and organs looked normal.

Dog 41 This dog had a very dilated heart. The left A-V valve had one cusp which was adhered to the endocardium. The right A-V valve had a mild vegetative endocarditis on all cusps. The liver was swollen and very hyperemic. There was evidence of necrosis around the central veins upon microscopic examination. Other organs were hyperemic but showed no other pathology.

Dog 42 This dog had the most severe valvular insufficiency of the five dogs in this group. One cusp of the right A-V valve was completely destroyed and a small cut was present in the second cusp. There was also a piece of granulation tissue extending from the area of the cut into the atrium.

The pleural and peritonal cavities were partially filled with fluids. The left lung was collapsed and very little of

the right was still functional. Other organs and tissues were very edematous.

The liver was partially cirrhotic and swollen. A severe passive hyperemia was present throughout the body. Upon histological examination there was necrosis around the central veins; other liver cells were in various stages of degeneration.

Hergt and Langin Method (64) for SGOT Determination

(All materials listed below, except the buffer, are commercially obtainable from the California Foundation of Biochemical Research, 3408 Fowler Street, Los Angeles 63, California.)

Phosphate Buffer, 0.1 M., pH 7.5: 84 parts 0.1 M. dibasic sodium phosphate ($Na_2HPO_4$) are combined with 16 parts 0.1 M. monobasic potassium phosphate ($KH_2PO_4$). The solutions are stored separately in the refrigerator and discarded if growth of mold appears.

Reduced diphosphopridine nucleotide (DPNH): DPNH in solution is very unstable, but it can be stored dry indefinitely with no loss of activity.

Five mg. of powdered DPNH are weighed on a sheet of parafilm and divided into 12 parts of about equal size with a scalpel blade and each portion is placed into a small (12 x 75 mm.) test tube. The tubes are placed in a desicator

over night in the dark, then sealed with paraffinized corks, and stored in the refrigerator. Each tube contains enough DPNH for two determinations. The contents are dissolved in 1.4 ml. of buffer for use.

Aspartic Acid, 0.05 M: 333.0 mg. aspartic acid are dissolved in 50.0 ml. of buffer, and the pH adjusted to approximately 7.5 with 1 or 2 drops of saturated sodium hydroxide (NaOH). The solution is then stored in the refrigerator and prepared anew if growth of mold appears.

Alpha - ketoglutaric acid, 0.1 M: Because of the instability of this material in solution, a similar technique as in the preparation of DPNH is employed.

Seventy-six mg. of alpha -ketoglutaric acid are weighed on a sheet of parafilm and divided into 12 equal portions. The material is then distributed into 12 small test tubes, placed in a desicator over night, then stoppered with parafinized corks, and stored at room temperature. For use, the contents of a tube are dissolved in 0.5 ml. of buffer. Each tube contains enough material for two determinations.

Malic Dehydrogenase, stabilized: This is used as it is supplied; the enzyme concentration varies with each lot. It is indicated on the label and must be checked carefully before use. A total of 200 units is required for each determination and the quantity is transferred directly from the vial into the cells by means of a micropipette at the time

of actual determination. The material is stored in the refrigerator.

Procedure:

1. Set up the Beckman Model B spectrophotometer in the usual manner using a blue-sensitive phototube with a 500 megohm resistor and a blue filter. The wave length setting is 340 mμ. 10 mm. cells are used (pyrex quality).
2. Use nonhemolyzed serum fresh or after freezing or refrigeration.
3. Remove all test constituents from the refrigerator and allow to come to room temperature. Mix the phosphate solution proportionately as indicated above.
4. Add 0.5 ml. and 1.4 ml. of the buffer to the tubes containing alpha - ketoglutaric acid and DPNH, respectively.
5. Using two cells, pipette as follows:

| | Blank | Unknown |
|---|---|---|
| Buffer | 2.9 ml. | ____ |
| DPNH | ____ | 0.7 ml. |
| Aspartic Acid | ____ | 2.0 ml. |
| Malic Dehydrogenase | ____ | 200 units |
| Serum | 0.1 ml. | 0.1 ml. |

Mix well, let stand for 10 minutes at room temperature.

6. Add 0.2 ml. of alpha - ketoglutaric acid to the unknown. Mix well.
7. Set the instrument to 0 O.D. with the blank and then read O.D. of the unknown immediately. Repeat readings after timed intervals, usually 5 minutes. Two equally timed intervals are sufficient if the results agree within 10 percent of each other.

Calculations:

The change (decrease) in O.D. during timed intervals is averaged and divided by the average time per interval to obtain the average change in O.D. in one minute. Since one unit of GOT activity is defined as a decrease in O.D. of 0.001 per minute, using 1.0 ml. serum, the above quotient is multiplied by 10,000 to obtain "units of transaminase activity per ml." of the unknown material.

Example of Statistical Analysis

(Hergt vs. Sigma Methods for Measuring SGOT Concentrations)

| Sample No. | Unit SGOT : Hergt | Sigma | X= Diff. |
|---|---|---|---|
| 1 | 113 | 133 | 20 |
| 2 | 82 | 140 | 58 |
| 3 | 85 | 170 | 85 |
| 4 | 100 | 75 | -25 |
| 5 | 188 | 193 | 5 |
| 6 | 106 | 140 | 36 |
| 7 | 87 | 105 | 18 |
| 8 | 70 | 80 | 10 |
| 9 | 128 | 139 | 11 |
| 10 | 280 | 320 | 40 |
| 11 | 241 | 260 | 19 |
| 12 | 122 | 102 | -20 |
| 13 | 320 | 410 | 90 |
| 14 | 295 | 370 | 75 |
| 15 | 110 | 150 | 40 |
| 16 | 185 | 305 | 120 |
| 17 | 260 | 400 | 140 |
| 18 | 155 | 235 | 80 |
| 19 | 160 | 240 | 80 |
| 20 | 95 | 82 | -13 |
| Total | 3182 | 4049 | 909 |

$$S^2 = \frac{\Sigma(X-\bar{x})^2}{N-1} = \Sigma X^2 - \frac{(\Sigma X)^2}{N}$$

$$\Sigma X^2 = 78135 \quad (\Sigma X)^2 = 826281$$

$$\Sigma X^2 - \frac{(\Sigma X)^2}{N} = 78135 - \frac{826281}{20}$$

$$= 78,135 - 41,314.05$$

$$S^2 = \frac{36,820.95}{19}$$

$$S^2 = 1,937.94$$

$$S_{\bar{x}}^2 = \frac{S^2}{20} = 96.90$$

$$S_{\bar{x}} = \sqrt{\frac{S^2}{20}} = \sqrt{96.90} = 9.83$$

$$t = \frac{45.45}{9.83} = 4.62^*$$

*Student t-test significant at the 0.1 percent level that there is a difference in the two tests.

$$\bar{x} = 45.45$$