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LIVER FUNCTION IN ECK PISTOLA DOGS

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY

Emerson D. Colby

1959





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by

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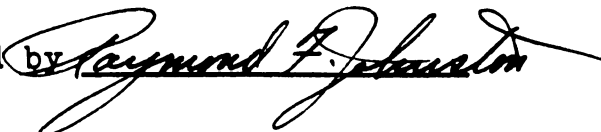
AN ABSTRACT

Submitted to the College of Veterinary Medicine
Michigan State University of Agriculture and
Applied Science in partial fulfillment of
the requirements for the degree of

MASTER OF SCIENCE

Department of Physiology and Pharmacology

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ABSTRACT

Liver function was studied in eight mongrel dogs surgically prepared with Eck fistulae. Clinical tests included bromsulfalein dye clearance, serum glutamic oxalacetic transaminase and serum glutamic pyruvic transaminase tests, total protein, alkaline phosphatase and serum bilirubin determinations. In addition, blood non-protein nitrogen was determined as an indication of kidney function. Hematological data included differential leucocyte counts and microhematocrits. A post-mortem examination was made on each dog at the end of the test period of seven months. Tissues from the heart, liver, and kidneys were prepared for pathological study with hematoxylin and eosin, and Sudan IV.

The data indicated weight loss in all the dogs as well as some fatty metamorphosis and passive congestion at the periphery of the liver lobes. Of the eight dogs, three showed extensive liver damage.

Since the results indicated that liver damage was not excessive during the test period, it is possible that under controlled conditions the liver may be by-passed by the portal circulation and yet remain functional over an extended period of time.

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TO MY WIFE

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INTRODUCTION

"A classic method of altering portal vein circulation is the creation of an Eck fistula." (Popper and Schaffner, 1957; p. 142.) In man, the principle of the Eck fistula is in the form of a porto-caval shunt and is recommended for reducing portal decompression. The best results from this surgical procedure are observed in young people (Popper and Schaffner, 1957).

The basis for this study was an attempt to determine liver function in dogs with Eck fistula by means of clinical tests. Since fat has been noted in the periphery of the lobes of the liver in such dogs (Markowitz et al., 1954), an investigation was also made to determine the extent of liver degeneration caused by the lack of portal blood flow to this organ.

It is hoped that this information might lead to a more adequate knowledge of the advantages and disadvantages of the Eck fistula in alleviating portal hypertension.

A number of liver function tests were used, including bromsulfalein dye clearance, serum glutamic oxalacetic transaminase, serum glutamic pyruvic transaminase, bilirubin, alkaline phosphatase, and total serum protein. Hematological studies included microhematocrits and differential leucocyte counts. Non-protein nitrogen determinations were used as a check on kidney function. The dogs

were kept on a meat-free diet and body weight recorded at each bleeding date.

Gross and microscopic examinations were made on the liver, heart, and kidneys at the termination of the experiment.

REVIEW OF LITERATURE

The Eck fistula has aroused much clinical interest in recent years as a technique for the treatment of portal hypertension in the final stage of hepatic cirrhosis. Since the time of Von Eck in 1882, there have been many modifications of this type of fistula. The present surgical method of Fischler and Schroeder (Markowitz et al., 1954) is the one most often used. This is a side-to-side anastomosis of the portal vein and the inferior vena cava, with subsequent ligation of the portal vein.

Such an anastomosis provides an alteration in the function of the liver and in its histological makeup.

After a period of much controversy concerning the use of dyes as an indication of liver function, bromsulphalein sodium, BSP, is the most often used and most widely accepted.

It has been determined that BSP is mainly metabolized by the liver. Klein and Levinson (1933), in attempting to prove that the reticulo-endothelial system offers the main source of BSP uptake, used India ink injections to block the Kupffer cells of the liver. They noted that subsequent BSP uptake by the liver was reduced.

Mills and Dragstedt (1938) reported that BSP disappears from the blood with great rapidity, 85 - 90% being removed in the first five minutes.

A rectilinear relationship has been found to exist between the time involved and the logarithms of the values of concentration (Inglefinger et al., 1948).

Many studies have been conducted in an attempt to measure hepatic blood flow and hepatic maximal transfer capacity using constantly infused BSP. Cohn et al. (1948) disproved this theory on the grounds that extrahepatic sites of BSP removal prevent such measurements.

Bauer and Pessotti (1949) reported that there are apparently three controlling factors in BSP removal from the blood stream. The substance of their work indicates that the liver favors BSP dye and has a method of excretion of the injected dye crystals into the bile. Thus the BSP is eliminated by way of the biliary system.

Bauer (1950) has also reported that when using S^{35} , tagged BSP under constant infusion techniques, a nearly quantitative recovery of BSP can be made from the liver, blood, and bile. This tends to indicate that the extrahepatic sites of BSP uptake are limited and must have a saturation point. This allows an almost maximal uptake by the liver. It has been shown by this method that a nearly quantitative recovery of BSP can be made from the bile. Bile BSP is known to be in four different fractions, none of which is colorless. Since the rate of BSP uptake by the bile is much slower than that of the liver, a longer period of time will be required for all BSP to be excreted

by the biliary system. The elimination of BSP by the bile may be accomplished in a period of several hours, and the apparent maximum concentration attainable in the dog is 292 mg./100 cc. of bile (Cantarow and Wirts, 1941).

Wirts and Cantarow (1942) studied the differing rates of BSP uptake by the liver and of BSP excretion by the bile. They attributed this to the functional ability of the reticulo-endothelial system to remove foreign substances from the blood and to Kupffer cell activity in the liver. These workers have formulated the hypothesis that the transfer of BSP from liver to bile may be an indication of hepatic polygonal cell activity. This same hypothesis has been made by Mendeloff et al. (1949). They also reported that extrahepatic sites of BSP removal play a minor role unless liver function is severely impaired.

The rate of BSP disappearance and hence percentage retention in dogs is independent of dosage between 5 and 20 mg. of BSP per kilogram of body weight (Moses et al., 1948; Gornall and Bardawell, 1952).

Cornelius (1957) reported that during infusion, BSP may be found in increasing amounts in the bile canaliculi and bile ducts of the liver prior to elimination to the gall bladder. The significance of dosage and time factors in the value of the BSP test for liver function have also been studied by Helm and Machella (1942). Formulas and

equations to be used in figuring hepatic clearance rates with BSP were studied by Lewis (1948).

Bromsulfalein is still not universally accepted as the best dye to test liver function, but it is by far the most widely used. The most common method of analysis is described by Klein and Levinson (1933).

Transaminase activity is used as an indication of disease or organ infarction in both man and animals. Wroblewski and Ladue (1956b) noted that serum glutamic oxalacetic transaminase (SGO-T) is more closely associated with heart muscle activity than with activity of other organs, in spite of the fact that it is found in many other tissues of the body, as is the serum glutamic pyruvic transaminase enzyme. These workers have also reported that another enzyme, glutamic pyruvic transaminase (SGP-T) is more specific for liver injury than SGO-T. These enzymes are liberated from cells of tissues at the point of infarct or injury.

Chinsky and Sherry (1957) showed that hepatic necrosis of various types produced significant changes in both humans and experimental animals. Careful diagnoses of transaminase levels have proved useful in determining different types of hepatic disorders. Cardiac and skeletal muscle defects have been noted in the same way.

Post-mortem examinations by Chinsky et al. (1957) have shown the comparison between SGO-T and SGP-T in specific

types of tissue necrosis. Because SGO-T is found in nearly all organs and tissues of the body, these workers have contrasted resulting levels of the enzyme with that of the pyruvic transaminase to establish various levels of activity in conjunction with specific diseases. SGO-T has been related to hepatic injury through histological examination and post-mortem examination by Donato (1957).

A number of clinical procedures for determining transaminase levels in plasma have been developed (Humoller et al., 1957; Umbreit et al., 1957; and Karmen, 1954). The method of Karmen (1954) was used in this work.

MATERIALS AND METHODS

This experiment was designed to study the effects of liver function in Eck fistula dogs. The function of the liver was determined through the use of various clinical blood tests.

The dogs were examined for visual symptoms of disease and vaccinated for distemper prior to the start of the experiment.

Three of the eleven dogs used in the experiment were control animals. The remaining eight dogs were surgically prepared with Eck fistulae.

A. SURGICAL PROCEDURES

Eight mongrel dogs of both sexes ranging in weight from 13.5 to 23 kilograms were surgically prepared with a straight Eck fistula, made by joining the portal vein to the inferior vena cava. An end-to-side anastomosis was performed in seven of the dogs and a side-to-side anastomosis in the eighth dog.

The anesthetic used was a 3% solution of pentobarbital sodium, or a 4% solution of surital. A 3% solution of mikedimid was used after surgery as a respiratory stimulant. All dogs were kept on a meat-free diet* (no fresh meat) following surgery.

B. BLOOD SAMPLES AND INJECTIONS

All blood samples were drawn under aseptic conditions.

*Borden's Mash - 20% protein.

The ventral surface of the neck and the cranial surface of the foreleg were clipped to make accessible the external jugular and cephalic veins respectively. All blood samples were drawn from the external jugular vein. All injections were made into the cephalic vein.

A 20 cc. sample of blood was drawn, microhematocrits were taken, and blood smears made and stained with Wright's stain. The remainder of the blood was centrifuged with the exception of 5 cc. used for the non-protein nitrogen determinations. All blood was drawn into syringes moistened with heparin. Plasma not used was immediately frozen.

A Bausch and Lomb Spectrophotometer Model 20 was used for all colorimetric determinations. A heavy-duty 6-volt storage battery with charger was utilized to provide a more constant light source.

Weights were taken on each dog before the drawing of blood samples and making each BSP clearance determination.

All clinical determinations were performed once a week for the first eight weeks, bi-weekly for the next five weeks, and once a month for the remaining three months.

C. CLINICAL PROCEDURES

1. Bromsulfalein Liver Function Test

The bromsulfalein used in this study was the commercial* 5% (50 mg./ml.) aqueous solution of sulfobromophthalein sodium,

*Supplied by Hynson, Wescott, and Dunning, Inc., Baltimore, Maryland.

U.S.P. (Phenoltetrabromphthalein-disodiumsulfonate). The commercial solution was injected into the cephalic vein, using 10 milligrams per kilogram of body weight. After the injection of BSP, blood samples were drawn at 2-, 5-, 30-, 45-, and 60-minute intervals. The blood was immediately centrifuged to obtain the plasma for colorimetric analysis.

2. Alkaline Phosphatase Test

This procedure was taken from the work of Gutman and Gutman (1940). The procedure was modified to make the volume to 50 ml. (1 - 100 dilution) by using 50 ml. volumetric flasks. The incubation times were extended to 30 minutes from Gutmans' recorded time of 15 minutes. This new time was used to compensate for the increase in dilution and volume.

3. Non-protein Nitrogen Determination

Lieboff digestion flasks of 25 ml. volumetric capacity were used in the Koch-McMeekin (1924) method.

4. Transaminase Activity

Tests were done utilizing both serum glutamic oxalacetic transaminase (SGO-T) and serum glutamic pyruvic transaminase (SGP-T). The method was taken from a study by Karmen (1954).

5. Total Proteins

This determination made use of the Hand Protein

Refractometer*. The procedure suggested by the manufacturer was followed.

6. Bilirubin

Total, direct, and indirect bilirubin determinations were done according to the method of Powell (1944).

D. POST-MORTEM

At necropsy each dog was thoroughly examined and specimens of the heart, liver, and kidneys were collected in 10% formalin for histological study.

Representative samples were cut and then dehydrated and infiltrated according to the methods of Johnson et al. (1943). Tissuemat** was used as the embedding media. Sections six micra in thickness were cut and stained with hematoxylin and eosin. Frozen sections were prepared on the liver and kidneys and stained with Sudan IV.

An analysis of variance was done on the BSP data and is represented by the vertical lines in Figure III, A-H. Sums of concentrations, sums of concentrations squared, and squares of the sums of concentrations were figured. The following formulae were then used in the analysis.

$$S^2_x = \frac{\sum x_i^2 - \frac{(\sum x_i)^2}{N_1} + \sum x_j^2 - \frac{(\sum x_j)^2}{N_2}}{N_1 + N_2 - 2}; \quad S_{\bar{x}_1 - \bar{x}_2} = \sqrt{S^2_x - \bar{x}_1 - \bar{x}_2};$$

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{S_{\bar{x}_1 - \bar{x}_2}}$$

*National Instrument Company; Baltimore, Maryland.

**Fisher Scientific Company; Pittsburgh, Pennsylvania.

RESULTS

Data obtained from liver function tests, hematological studies, and weights on the eight experimental dogs have been tabulated. Total, direct, and indirect bilirubins were not included since the results fell within normal limits. The three dogs that died in the fourth month of the study were autopsied immediately, and the other five were autopsied at the termination of the experiment. Specimens were taken for histological study. Normal values for BSP, alkaline phosphatase, SGO-T and SGP-T were taken from the work of Cornelius (1957).

A. BODY WEIGHT

The body weights of all the experimental dogs declined over a period of seven months. The decline was not uniform in any dog for a given period of time (see Table I).

B. HEMATOLOGICAL DATA

Microhematocrits for all the dogs (Table II) are within the normal range. The differential leucocyte counts are within normal limits as given by Albritton (1953).

C. BROMSULFALEIN

Experimental BSP data showed normal values for Dogs 2, 3, 4, 5, and 8. The livers of Dogs 1, 6, and 7 exhibited abnormal dye clearance (see Table III).

TABLE I

BODY WEIGHT IN KILOGRAMS

DATE	DOG NUMBER							
	1	2	3	4	5	6	7	8
June 18	14	14	23.5	13	14.5	14	13.5	14.5
June 23	12.5	13.3	22.5	12.5	--	--	--	--
June 25	--	--	--	--	14	12.8	13	13.5
June 30	11.5	13	22	12	--	--	--	--
July 2	--	--	--	--	13.5	11.5	12.7	11.7
July 7	10	12.5	21.5	10.5	--	--	--	--
July 9	--	--	--	--	14	11	13	12.5
July 14	9.5	13	21.5	11.5	--	--	--	--
July 16	--	--	--	--	14	10.5	13	13
July 21	9.5	13.5	21.5	11	--	--	--	--
July 23	--	--	--	--	13.7	10.5	13	13.5
August 4	9	13.5	22.3	11.5	13.3	10	12.8	13
August 18	8.5	13	22.5	11	13	9	11.7	13
September 2	8	12.5	22.5	11.5	12.8	8.5	11	12
September 15	8.5	12.5	23.5	12.5	12.2	7.5	--	12.5
October 18	--	12.5	22.8	12.5	12.3	--	--	13
November 15	--	12.5	20.0	12.3	12.3	--	--	13.3
December 17	--	12.3	20.8	10.8	12.3	--	--	12.3

TABLE II

MICROHEMATOCRIT IN PERCENT

DATE	DOG NUMBER							
	1	2	3	4	5	6	7	8
June 18	46.5	38.9	36.5	42.2	--	--	--	--
June 23	44.0	35.0	37.7	39.5	--	--	--	--
June 25	--	--	--	--	40.5	42.3	41.7	40.8
June 30	44.3	41.5	36.8	33.8	--	--	--	--
July 2	--	--	--	--	37.5	40.3	43.3	42.5
July 7	41.0	44.8	43.5	34.5	--	--	--	--
July 9	--	--	--	--	39.2	40.6	42.0	41.3
July 14	43.0	48.7	38.5	37.3	--	--	--	--
July 16	--	--	--	--	40.0	40.8	50.0	44.0
July 21	42.1	47.5	34.2	39.5	--	--	--	--
July 23	--	--	--	--	38.8	41.5	46.8	45.3
August 4	39.0	41.7	42.7	42.7	--	--	--	--
August 6	--	--	--	--	39.5	44.3	44.8	44.0
August 18	44.6	30.6	41.3	42.0	41.6	43.8	49.6	46.3
September 2	43.0	44.5	47.0	40.3	39.8	42.5	45.5	44.8
September 15	46.5	40.0	37.7	39.0	43.0	43.1	54.2	40.0
October 18	--	41.5	42.5	42.3	37.5	--	--	44.0
November 15	--	42.5	39.8	40.2	38.8	--	--	41.8
December 17	--	45.3	33.5	35.7	39.7	--	--	42.3

Normal: 42 - 52%

TABLE III

BROMSULFALEIN RETENTION BY THE BLOOD
MILLIGRAMS PERCENT

DATE	MINUTES AFTER DYE INJECTION	1	2	3	4	5	6	7	8
June 18	30	3.9	.82	.25	.87	.21	--	--	--
	45	2.5	.33	.05	.71	.05	--	--	--
June 23	30	3.9	.75	.63	1.6	--	--	--	--
	45	2.6	.18	.44	1.4	--	--	--	--
June 25	2	--	--	--	--	8.9	10.1	9.1	8.9
	30	--	--	--	--	.58	2.2	2.7	.55
	45	--	--	--	--	.03	.82	.82	.11
June 30	2	7.9	4.6	6.1	4.9	--	--	--	--
	30	4.4	.50	.95	.88	--	--	--	--
	45	--	.15	.45	.82	--	--	--	--
July 2	2	--	--	--	--	1.5	2.6	1.6	1.9
	30	--	--	--	--	.18	3.1	.15	.37
	45	--	--	--	--	.04	1.7	.57	.18
July 7	2	4.3	2.2	2.1	2.8	--	--	--	--
	30	4.3	.57	.67	.32	--	--	--	--
	45	3.3	.32	.32	.12	--	--	--	--
	60	2.2	.18	.10	.12	--	--	--	--
July 9	2	--	--	--	--	1.6	2.4	2.4	2.1
	30	--	--	--	--	.57	2.3	.67	.74
	45	--	--	--	--	.09	1.4	.18	.41
	60	--	--	--	--	.04	--	.04	.11
July 14	2	3.7	2.3	1.5	1.9	--	--	--	--
	30	5.1	.62	.80	.25	--	--	--	--
	45	--	.44	.33	.30	--	--	--	--
	60	3.1	.33	.30	.05	--	--	--	--
July 16	2	--	--	--	--	--	4.9	--	.55
	30	--	--	--	--	.05	4.4	--	.02
	45	--	--	--	--	.05	2.1	--	.02
	60	--	--	--	--	.02	1.8	--	.02
July 21	2	5.0	1.7	1.9	2.2	--	--	--	--
	30	4.0	.32	.62	.57	--	--	--	--
	45	2.9	.37	.70	.37	--	--	--	--
	60	1.9	.02	.75	.05	--	--	--	--

TABLE III (continued)

		1	2	3	4	5	6	7	8
July 23	2	--	--	--	--	.98	2.4	1.4	1.3
	30	--	--	--	--	1.1	3.8	1.6	.70
	45	--	--	--	--	.82	2.5	.82	.38
	60	--	--	--	--	.55	1.9	.83	--
August 4	2	5.9	2.0	1.9	2.2	2.1	--	--	--
	30	5.7	.50	.57	.37	1.1	--	--	--
	45	4.4	.05	.30	.30	.37	--	--	--
	60	3.6	.02	.05	.12	.25	--	--	--
August 6	2	--	--	--	--	--	4.0	3.0	2.2
	30	--	--	--	--	--	3.8	1.8	.67
	45	--	--	--	--	--	2.3	1.2	.32
	60	--	--	--	--	--	1.7	.68	.30
August 18	5	7.3	1.6	2.0	1.4	4.8	7.2	6.4	1.6
	30	5.9	.30	.65	.15	1.7	5.1	2.1	.55
	45	4.8	.18	.32	.12	.90	3.3	1.3	.03
	60	3.8	.05	.15	.02	.63	2.5	.75	.01
September 2	5	8.6	4.2	5.1	2.7	7.3	8.6	6.1	2.0
	30	5.0	.68	.63	.50	1.3	--	1.5	.38
	45	3.4	.62	.37	.37	.65	3.7	.95	.15
	60	2.4	.37	.18	.25	.41	2.9	.65	.05
September 15	5	9.2	4.8	5.6	4.5	6.2	6.3	--	6.1
	30	1.8	.67	.50	.62	.95	4.4	--	.80
	45	.67	.43	.10	.37	.70	2.9	--	.43
	60	.44	.25	.02	.30	.12	--	--	.32
October 18	5	--	3.9	4.7	3.8	1.1	--	--	4.0
	30	--	.95	.57	.45	.41	--	--	.10
	45	--	.15	.14	.32	--	--	--	.02
	60	--	.05	.10	.05	.30	--	--	.01
November 15	5	--	5.6	1.5	6.4	8.2	--	--	3.1
	30	--	.67	.50	1.4	1.9	--	--	.05
	45	--	.37	.30	.98	.57	--	--	.03
	60	--	.32	.10	.41	.33	--	--	.02
December 17	5	--	2.5*	2.3*	4.0	2.5*	--	--	3.4
	30	--	0.5	.63	.57	.68	--	--	.55
	45	--	.03	.15	.37	.15	--	--	.25
	60	--	.02	.02	.33	.05	--	--	.10

*11-minute samples

D. ALKALINE PHOSPHATASE

All of the experimental dogs exhibited alkaline phosphatase values within the normal range of 4 to 6 Bodansky units. Dogs 1 and 6 were in the upper limits of the normal range (Table IV).

E. NON-PROTEIN NITROGEN

Non-protein nitrogen values were normal (Table V).

F. TRANSAMINASE ACTIVITY

1. Serum glutamic oxalacetic transaminase

Of the eleven animals, the three control dogs as well as experimental dogs 1, 2, 3, 4, 5, and 8 showed normal values. Dogs 6 and 7 showed a significant increase over the normal range of 10 to 40 Sigma-Frankel units (see Table VI). There was no apparent peak of SGO-T activity. Since there were differences in values between the months of June and December, the data indicate that SGO-T activity was increasing with time.

2. Serum glutamic pyruvic transaminase

SGP-T values for normal dogs range from 20 to 40 Sigma-Frankel units. The data showed increases of SGP-T values in Dogs 1, 5, 6, and 7 (see Table VII).

G. TOTAL PROTEIN

Table VIII shows the results of total protein determination. With the exception of Dogs 1 and 4, which showed levels

TABLE IV

ALKALINE PHOSPHATASE IN BODANSKY UNITS

DATE	DOG NUMBER							
	1	2	3	4	5	6	7	8
June 18	6.25	7.81	4.68	3.12	3.13	--	--	--
June 23	4.68	3.13	3.13	3.12	--	--	--	--
June 25	--	--	--	--	10.9	9.37	6.26	7.82
June 30	3.13	3.13	3.12	3.12	--	--	--	--
July 2	--	--	--	--	3.13	6.25	3.18	3.12
July 7	4.68	3.12	3.12	4.68	--	--	--	--
July 9	--	--	--	--	4.68	9.38	4.72	3.12
July 14	7.81	4.68	3.13	3.12	--	--	--	--
July 16	--	--	--	--	3.13	3.13	4.68	3.12
July 21	7.81	4.68	3.12	3.13	--	--	--	--
July 23	--	--	--	--	6.25	3.12	3.13	3.12
August 4	6.25	6.25	3.13	4.68	4.68	3.13	6.25	4.68
August 18	7.81	3.13	1.00	3.12	3.12	4.70	3.12	4.70
September 2	6.25	3.13	3.12	3.12	3.12	4.68	3.12	3.12
September 15	4.68	3.13	3.12	3.13	3.12	3.12	3.12	4.68
October 18	--	3.12	--	3.13	3.13	--	--	3.12
November 15	--	6.25	4.68	4.50	3.13	--	--	3.12
December 17	--	4.68	3.12	3.13	3.13	--	--	3.12

Normal: 3 - 6 Bodansky Units

TABLE V

NON-PROTEIN NITROGEN IN MILLIGRAMS PERCENT

DATE	DOG NUMBER							
	1	2	3	4	5	6	7	8
June 18	57.7	34.7	75.2	63.5	51.5	--	--	--
June 23	53.2	36.8	71.0	19.8	--	--	--	--
June 25	--	--	--	--	39.8	52.2	47.6	50.2
June 30	36.5	42.5	38.0	41.0	--	--	--	--
July 2	--	--	--	--	59.0	49.5	38.0	48.7
July 7	26.5	35.0	35.5	36.2	--	--	--	--
July 9	--	--	--	--	27.5	23.0	24.8	28.0
July 14	31.5	42.2	35.0	39.5	--	--	--	--
July 16	--	--	--	--	42.7	39.7	27.5	39.2
July 21	25.5	56.0	28.7	33.0	--	--	--	--
July 23	--	--	--	--	45.2	36.7	37.0	50.5
August 4	37.5	49.2	38.0	37.7	--	--	--	--
August 6	--	--	--	--	32.5	20.5	20.5	18.0
August 18	23.5	36.8	30.2	35.5	40.2	27.2	40.2	28.5
September 2	30.5	18.4	25.0	20.0	11.5	14.0	9.7	10.0
September 15	29.4	27.8	32.8	31.5	35.2	21.3	43.2	31.6
October 18	--	12.7	13.0	16.8	12.0	--	--	16.7
November 15	--	21.7	22.7	12.0	20.3	--	--	18.3
December 17	--	37.8	52.7	24.5	28.5	--	--	25.8

Normal: 20 - 40 mg./100 cc.

TABLE VI

SERUM GLUTAMIC OXALACETIC TRANSAMINASE IN SIGMA-FRANKEL UNITS

DATE	DOG NUMBER							
	1	2	3	4	5	6	7	8
June 18	5	5	8	7	25	--	--	--
June 23	20	25	10	24	--	--	--	--
June 25	--	--	--	--	20	24	28	22
June 30	16	2	2	1	--	--	--	--
July 2	--	--	--	--	56	52	7	2
July 7	36	26	16	11	--	--	--	--
July 9	--	--	--	--	6	68	7	26
July 14	46	16	21	6	--	--	--	--
July 16	--	--	--	--	20	37	28	26
July 21	36	22	12	28	--	--	--	--
July 23	--	--	--	--	16	62	16	20
August 4	2	4	9	12	16	40.8	28	24
August 18	16	11	10	10	12	48	31	5
September 2	22	16	22	10	18	68	68	10
September 15	68	24	22	20	20	54	232	28
October 18	--	32	46	42	48	--	--	60
November 15	--	35	26	37	32	--	--	35
December 17	--	14	42	47	54	--	--	46

Normal: 10 - 40 Sigma-Frankel Units

TABLE VII

SERUM GLUTAMIC PYRUVIC TRANSAMINASE IN SIGMA-FRANKEL UNITS

DATE	DOG NUMBER							
	1	2	3	4	5	6	7	8
June 18	80	40	60	60	51	--	--	--
June 23	240	90	90	70	--	--	--	--
June 25	--	--	--	--	60	132	22	12
June 30	184	14	17	22	--	--	--	--
July 2	--	--	--	--	22	252	24	22
July 7	198	11	12	10	--	--	--	--
July 9	--	--	--	--	54	222	51	10
July 14	176	11	10	4	--	--	--	--
July 16	--	--	--	--	52	184	107	22
July 21	176	16	22	28	--	--	--	--
July 23	--	--	--	--	48	202	24	17
August 4	25	6	20	3	64	214	112	20
August 18	112	2	14	2	107	210	132	16
September 2	110	20	10	12	51	210	180	14
October 18	--	17	52	25	112	--	--	44
November 15	--	17	9.5	7.5	34	--	--	10
December 17	--	46	34	22	23	--	--	32

Normal: 20 - 40 Sigma-Frankel Units

TABLE VIII

TOTAL PROTEIN IN GRAMS PERCENT

DATE	DOG NUMBER							
	1	2	3	4	5	6	7	8
June 18	4.2	5.4	5.4	3.7	5.1	--	--	--
June 23	4.8	6.1	5.9	4.5	--	--	--	--
June 25	--	--	--	--	6.4	6.2	6.0	6.4
June 30	3.2	5.0	5.6	4.3	--	--	--	--
July 2	--	--	--	--	5.8	5.2	5.6	6.4
July 7	4.4	5.6	5.8	5.0	--	--	--	--
July 9	--	--	--	--	5.8	5.2	6.5	6.7
July 14	5.0	6.0	6.0	4.4	--	--	--	--
July 16	--	--	--	--	6.0	6.5	6.6	5.6
July 21	4.4	5.8	6.0	4.4	--	--	--	--
July 23	--	--	--	--	5.8	5.2	6.0	6.6
August 4	4.1	6.3	5.4	4.0	5.0	5.7	5.5	6.2
August 18	4.9	6.5	6.0	4.1	5.4	5.4	5.0	6.0
September 2	5.0	6.1	6.2	4.3	5.2	4.8	5.2	6.5
September 15	6.7	6.2	6.0	5.4	4.4	5.0	7.0	6.2
October 18	--	6.3	6.1	4.4	5.3	--	--	6.6
November 15	--	6.2	5.8	4.2	5.7	--	--	6.4
December 17	--	6.1	6.1	4.5	5.9	--	--	6.3

Normal: 5 - 7 g.%

of 4.67 and 4.40 grams percent, respectively, all values were within the normal range.

H. POST-MORTEM EXAMINATIONS

1. On autopsy the right side of the heart in every dog showed dilatation. The livers were friable with 0.3 to 1.5 mm. serrations along the edges of each lobe. In Dogs 1, 6, and 7, there were occasional small grayish areas at the periphery of the lobes.

The kidneys in all the dogs appeared normal; the capsules peeled with ease and the cut surfaces exhibited no abnormal coloration. The surgical areas were also examined for signs of collateral circulation. Dogs 3 and 8 exhibited some signs of apparent accessory circulation.

2. Histological studies on the liver, kidneys, and heart showed the following:

Livers in all the dogs showed some evidence of fatty metamorphosis and passive hyperemia. The livers in Dogs 1, 6, and 7 were more severely affected than the others (Plate I). Plates II and III show portal trinities with peripheral congestion.

The kidneys of Dogs 1, 6, and 7 showed a small amount of fat deposited in a few of the collecting tubules.

Sections of the right ventricle of the heart showed no abnormalities.

DISCUSSION

An analysis of the data shows an increase in the SGO-T and SGP-T values, a decrease in BSP clearance rates in some cases, and a gradual decline in body weight. However, this surgical procedure did not seriously alter the liver function in five of the eight dogs.

A. BODY WEIGHT

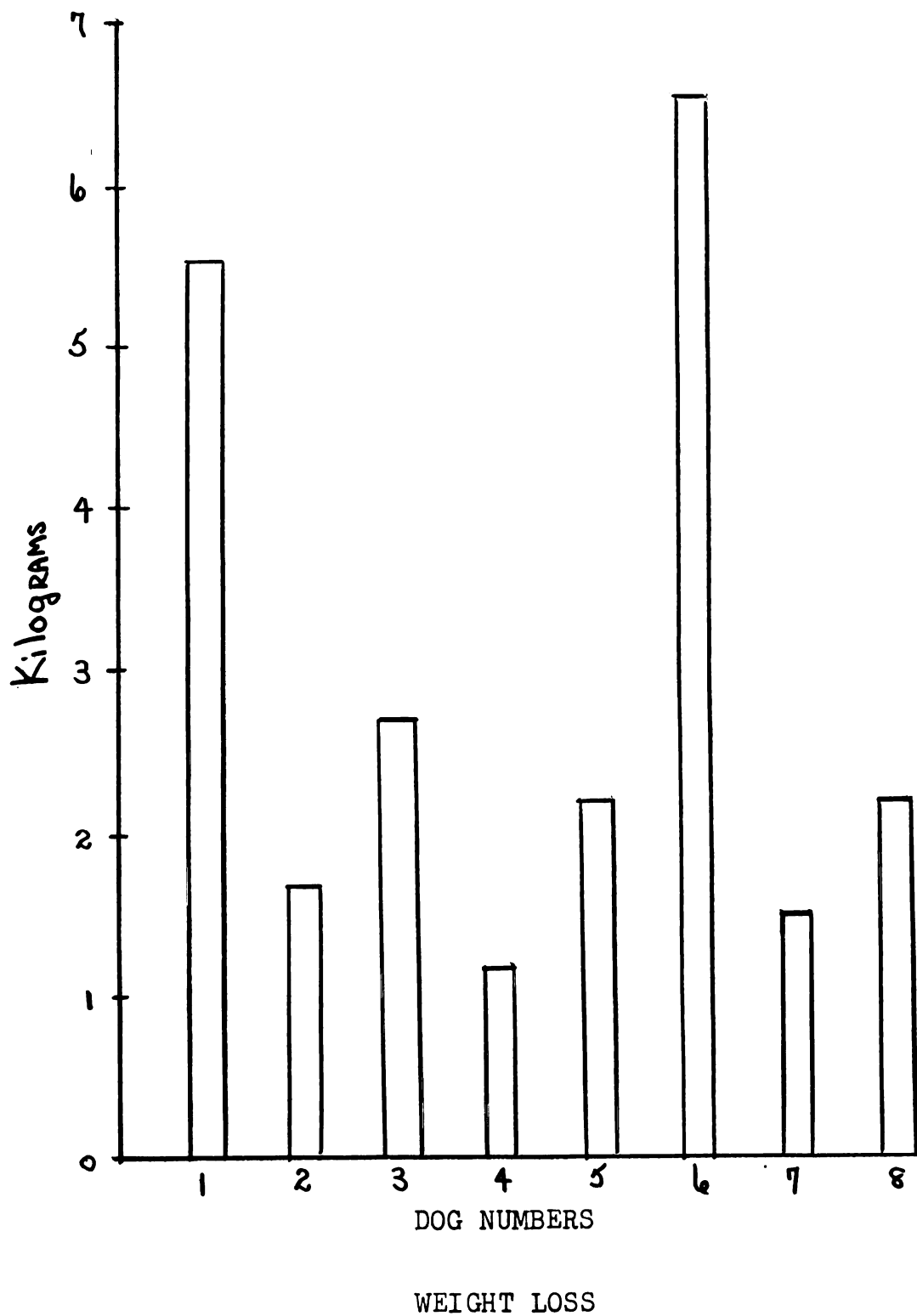
The fact that the body weight declined (Figure I) in all the dogs over the test period of seven months may not be attributed solely to the formation of the Eck fistula. As this study was conducted from June through December with the dogs being allowed a minimum of exercise, it is thought that environment and temperature also played a role in this weight loss. Control dogs were noted to exhibit no appreciable gain or loss in weight during this time. The eight dogs on this experiment were kenneled with other experimental dogs and a mild infection of any kind could have resulted in a loss of weight.

The depletion of body fat depots found at autopsy offers another explanation for the loss of weight in the experimental dogs. This depletion may be more closely associated with the loss of portal blood flow to the liver than with environmental conditions.

B. HEMATOLOGICAL STUDIES

Microhematocrits done on all the dogs were normal

FIGURE I



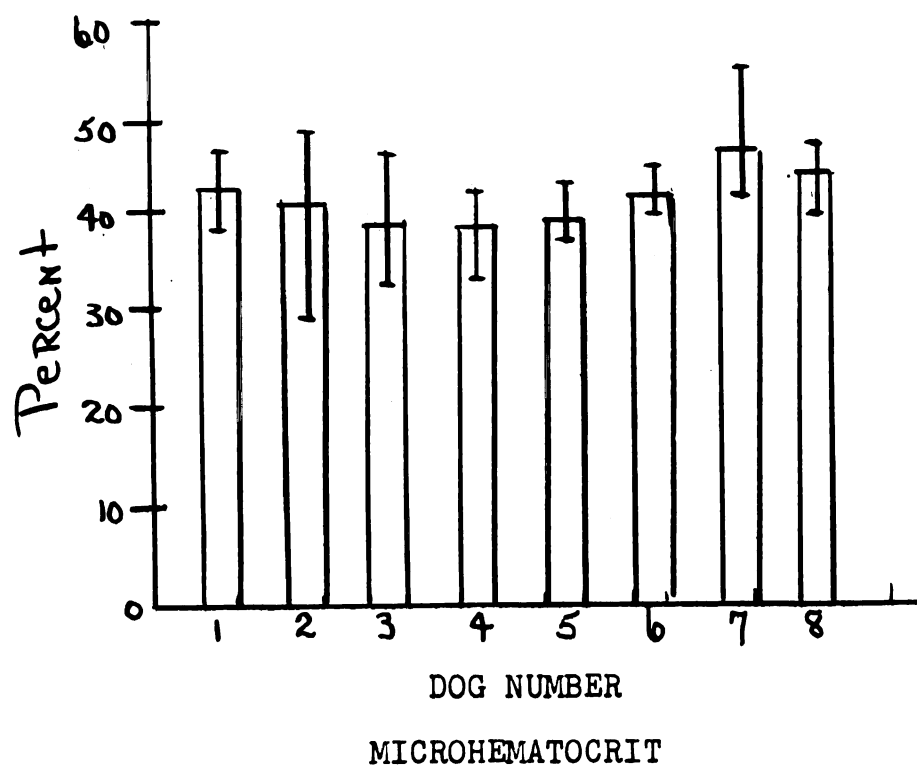
(Figure II) (Albritton, 1953). This indicates that the number of circulating red blood cells was not affected by the Eck fistula. The normal distribution of white blood cells seen in the differential leucocyte counts shows no rise or shift to the left in the blood picture as would be caused by disease or infection (Todd et al., 1955).

C. BROMSULFALEIN

An analysis of variance (refer to p. 30 for procedure) done on the BSP data shows that three of the eight experimental dogs exhibited abnormal BSP clearance rates (Figure III-A through H). This is not substantial evidence to indicate that Eck fistula formation will always elicit a decrease in dye clearance rates by the liver. The hepatic polygonal cells, in conjunction with the reticulo-endothelial system, are the main sites of BSP removal (Wirts and Cantarow, 1942). Polygonal cells located at the periphery of the liver lobes were seen to be congested with lipoid droplets (see Plate I). As a result, BSP removal is blocked here, and the dye must be removed by the more centrally located lobules.

It is suggested by the 2- and 5-minute values (Figure III-A through H) that the BSP had not reached an equilibrium in the blood at these times. It is possible that the dye was not being removed by the liver at a normal rate due to some passive congestion in this organ. The passive congestion in this case may have been caused by the dilatation of the right ventricle, which was evident in all of the eight dogs at autopsy.

FIGURE II



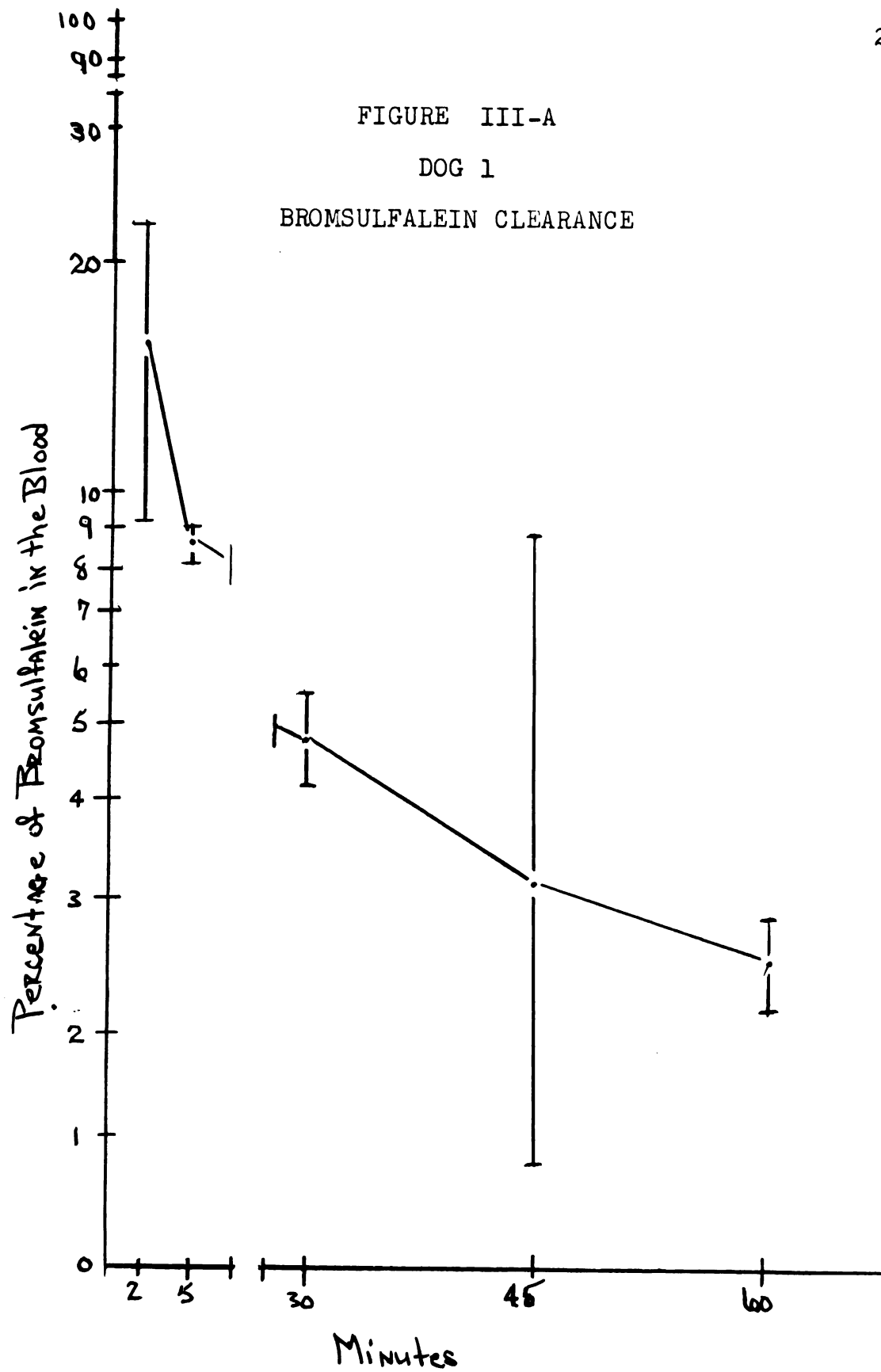


FIGURE III-B

DOG 2

BROMSULFALEIN CLEARANCE

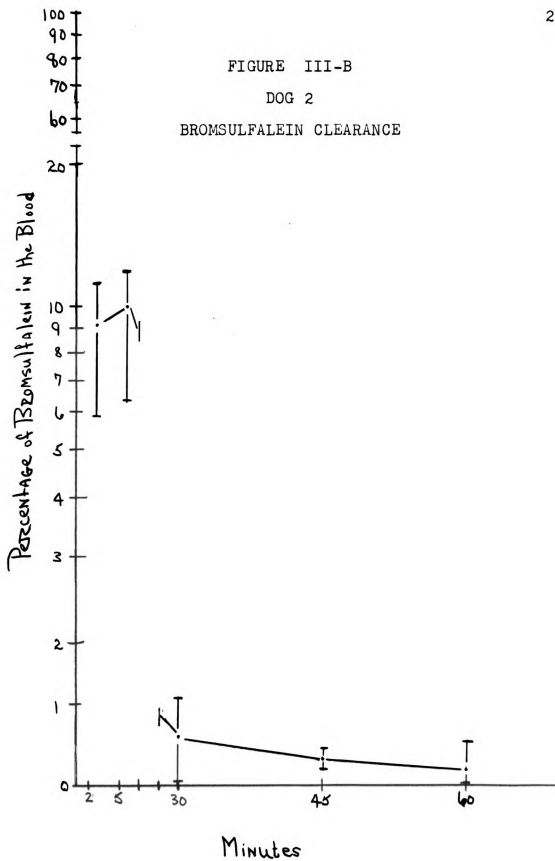
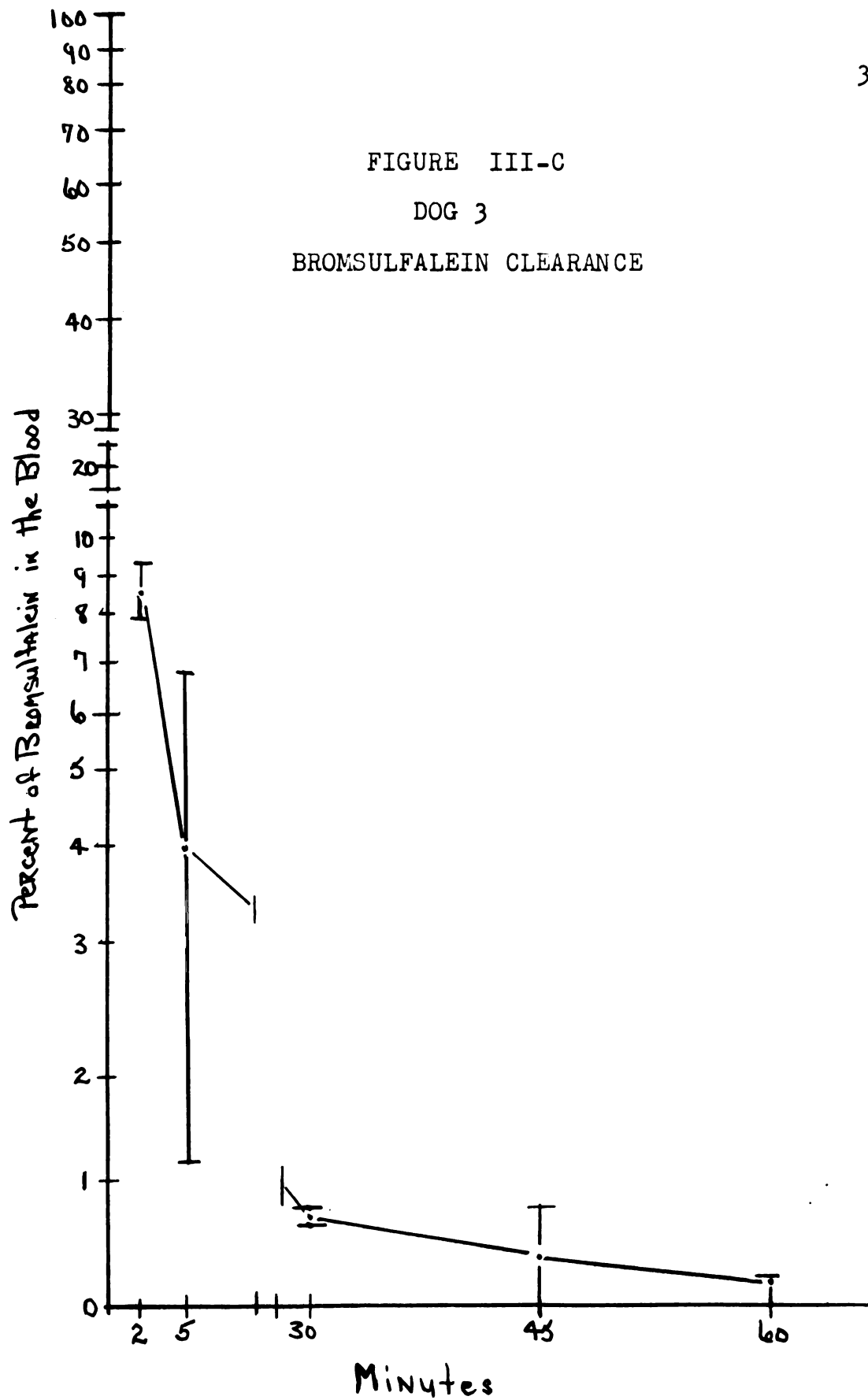


FIGURE III-C
DOG 3
BROMSULFALEIN CLEARANCE



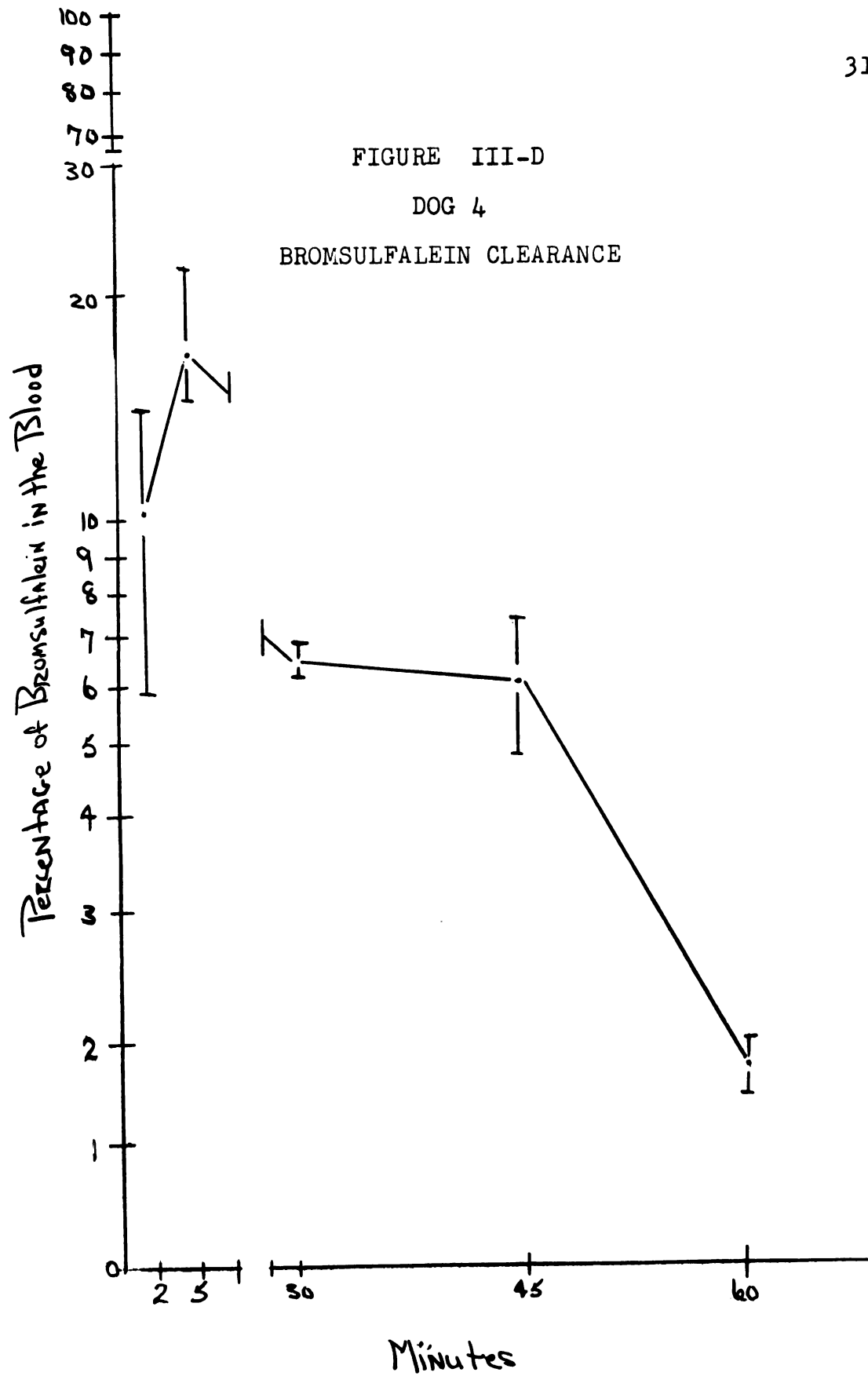


FIGURE III-E
DOG 5
BROMSULFALEIN CLEARANCE

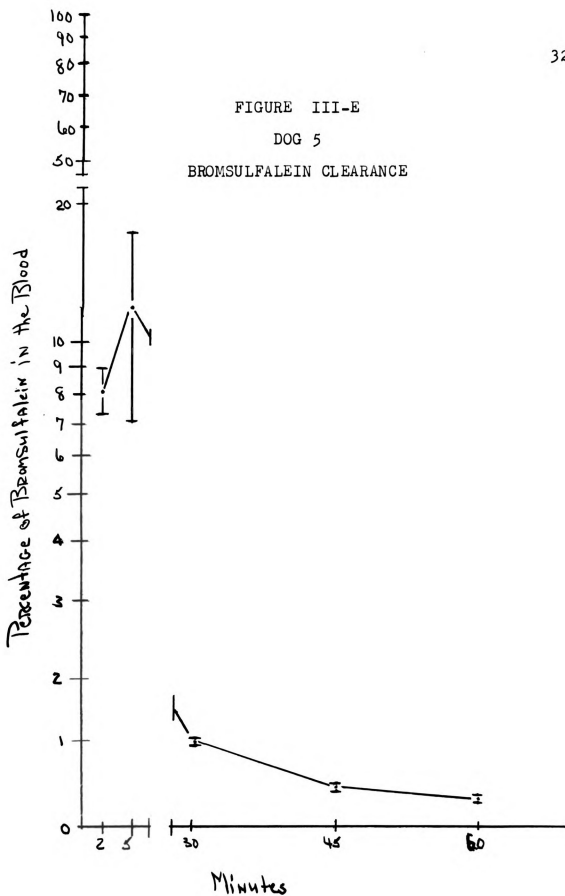


FIGURE III-F
DOG 6
BROMSULFALEIN CLEARANCE

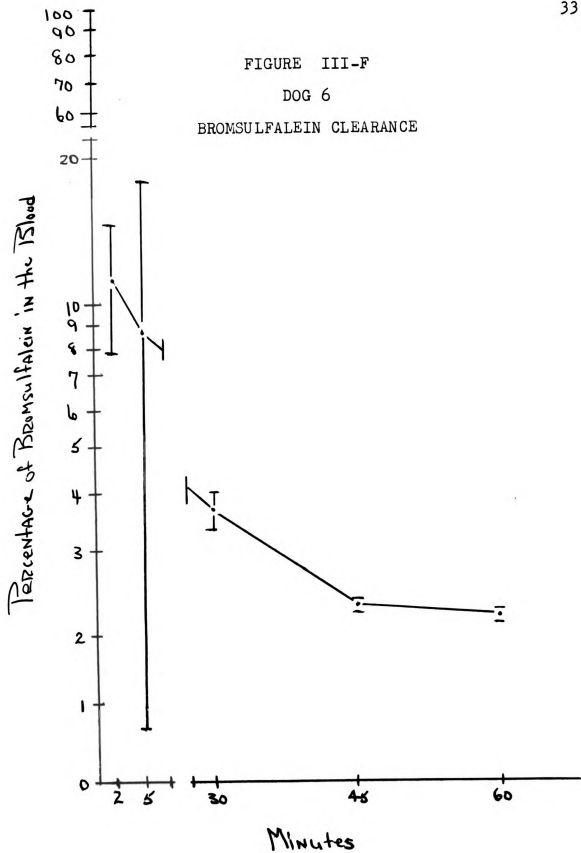


FIGURE III-G
DOG 7
BROMSULFALEIN CLEARANCE

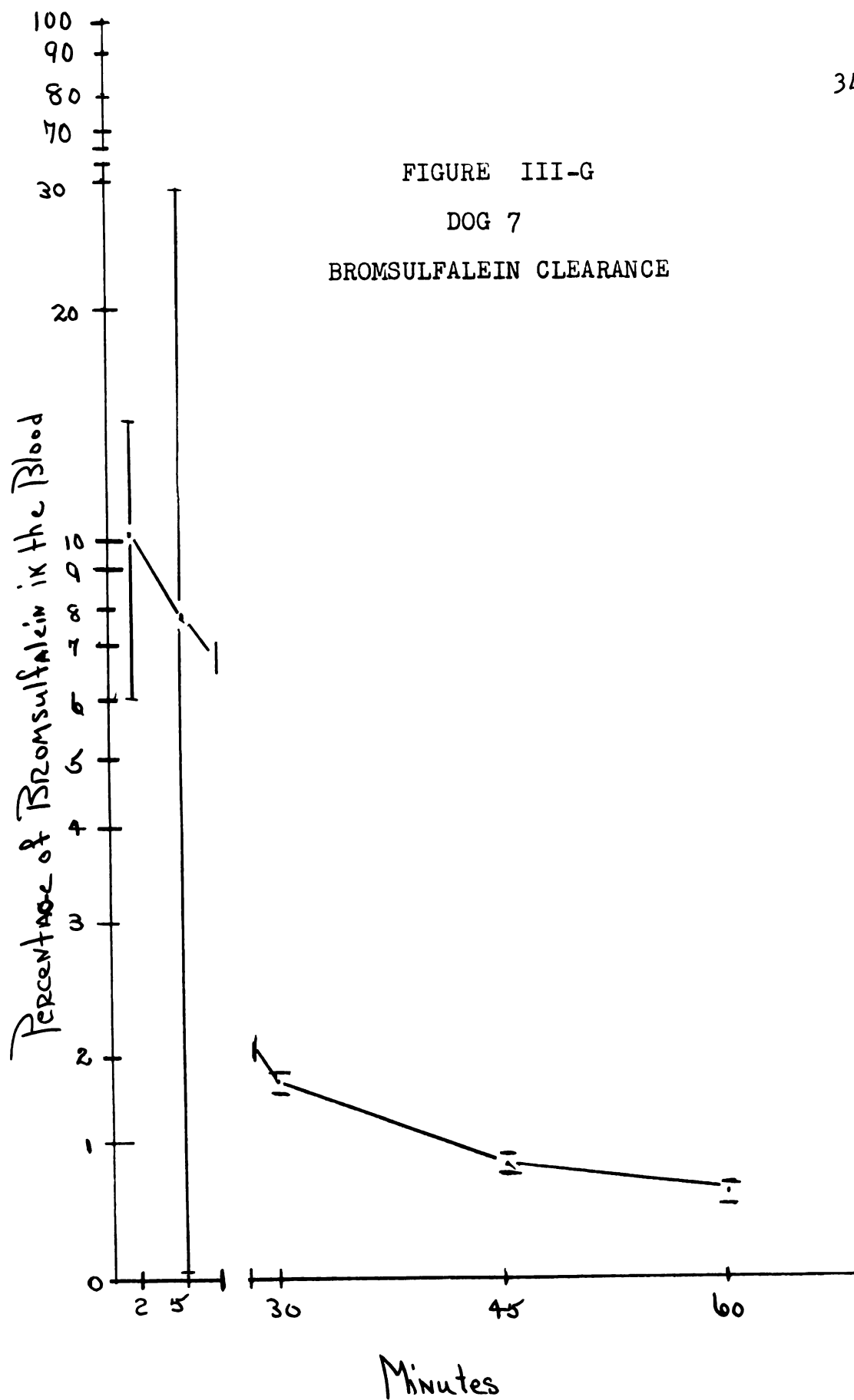
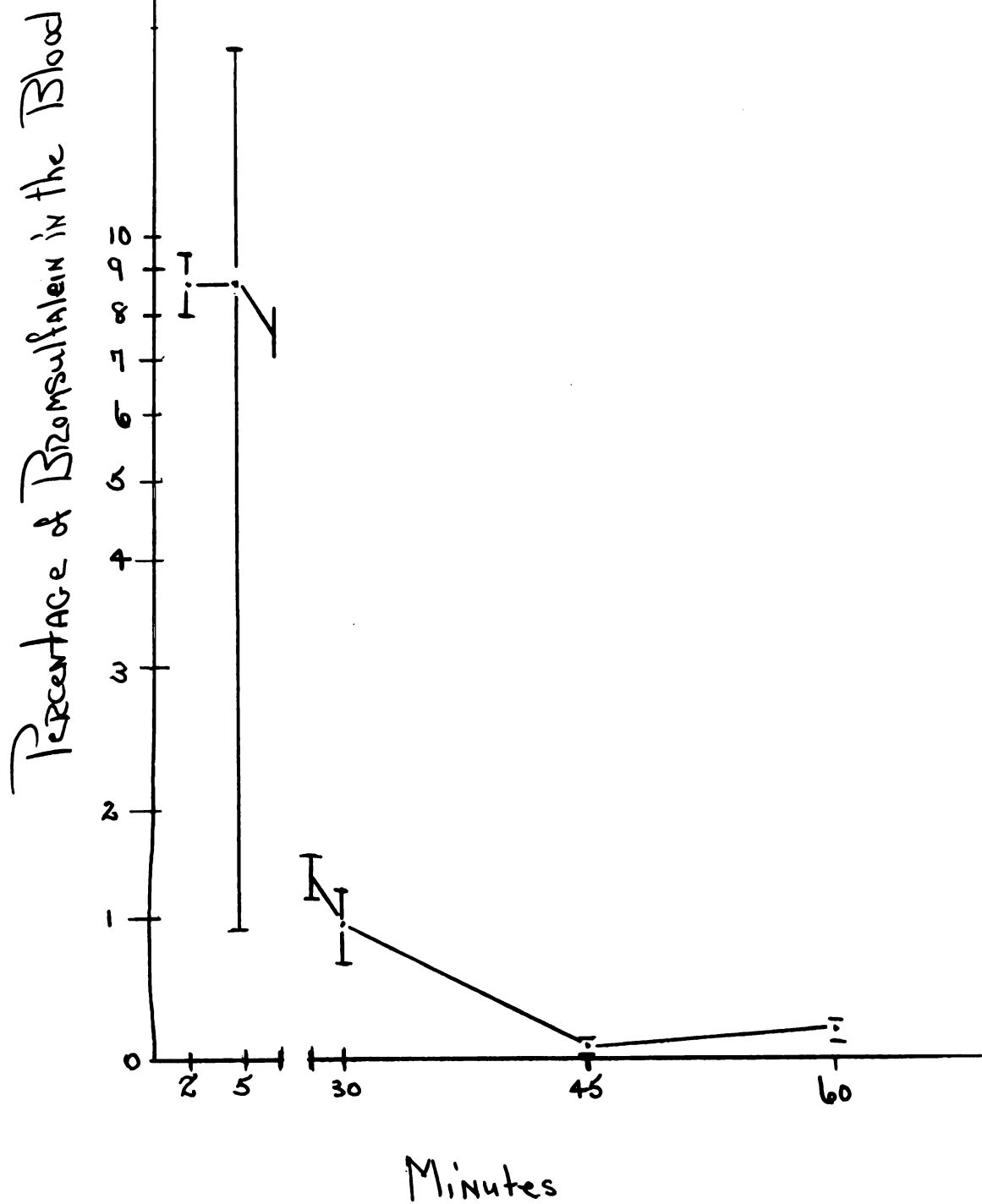


FIGURE III-H

DOG 8

BROMSULFALEIN CLEARANCE



In these instances blood is thrown back into the vena cava and its tributaries, the first appreciably expansible vascular channels being those of the liver (Smith and Jones, 1957).

The more extensive fatty metamorphosis observed in Dogs 1, 6, and 7 may be partly responsible for the decrease in BSP clearance rates in these dogs.

Extrahepatic uptake of BSP accounted, to a small extent, for the dye removal from the blood (Cohn et al., 1948; Mendeloff et al., 1949; Mason et al., 1948).

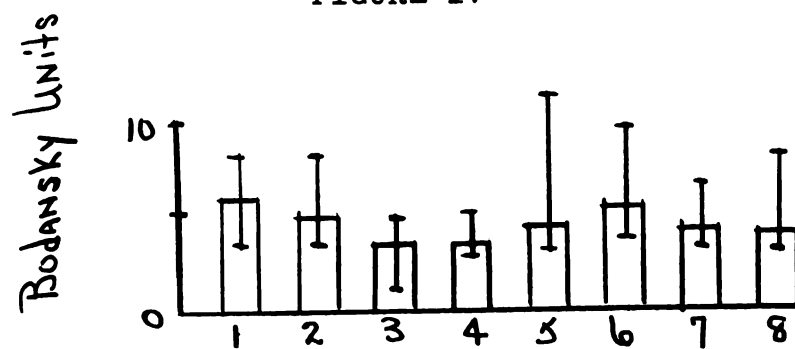
The dilatation of the right ventricle noted in all the dogs at autopsy would indicate a myocardial weakness and, according to Smith and Jones (1957), a resulting passive congestion in the liver. BSP data on Dogs 2, 3, 4, 5, and 8 show normal liver clearance rates. This indicates sufficient hepatic arterial flow and apparent functional ability of the liver cells to remove the dye within the normal length of time.

D. ALKALINE PHOSPHATASE

Normal alkaline phosphatase values (Cornelius, 1957) in the experimental dogs (Figure IV), when correlated with normal values for serum bilirubin (Cornelius, 1957), indicate that there was no biliary obstruction caused by the formation of the Eck fistula.

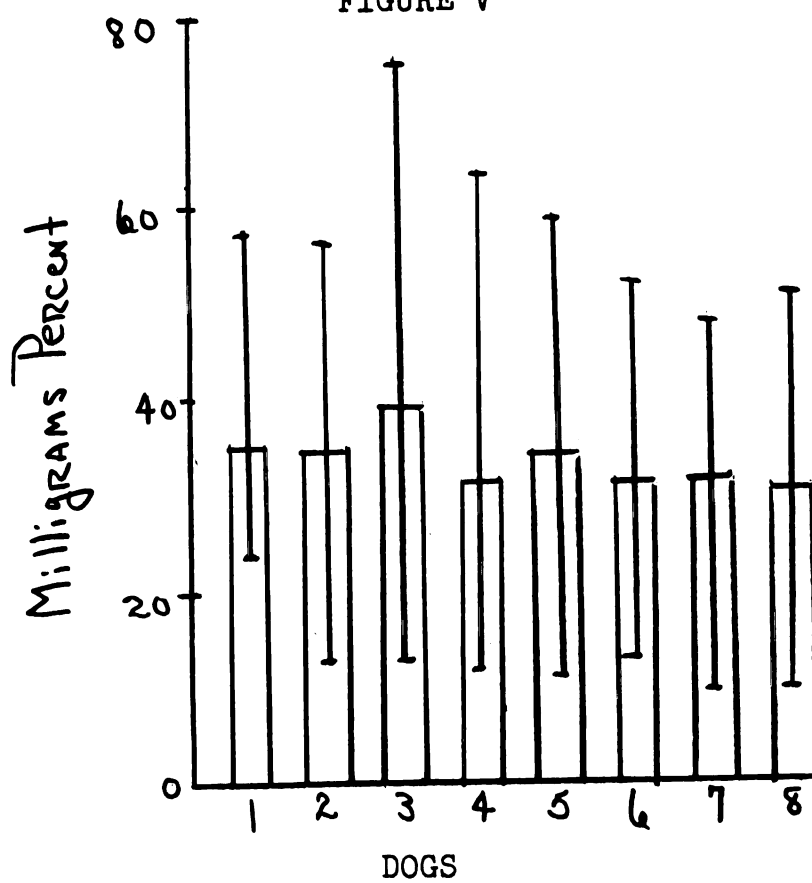
ALKALINE PHOSPHATASE

FIGURE IV



NON-PROTEIN NITROGEN

FIGURE V



E. NON-PROTEIN NITROGEN

Some evidence as to the functional state of the kidney is indicated by the non-protein nitrogen determinations (Figure V). The results of this data are within the normal range as established by Dukes (1955). This test also shows an absence of acute hepatic insufficiency (Todd et al., 1955).

F. TRANSAMINASE ACTIVITY

1. Serum Glutamic Oxalacetic Transaminase

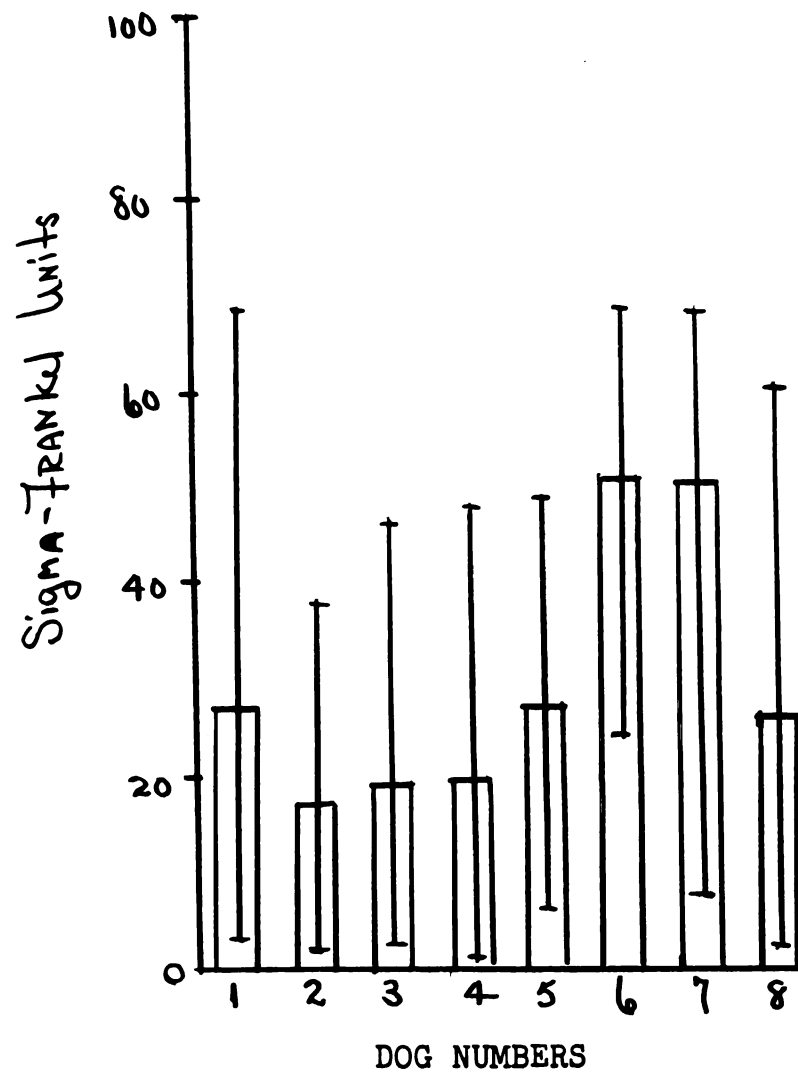
This enzyme is found in all the tissues of the body with the greatest concentration in cardiac muscle (Wroblewski and Ladue, 1956b). Ladue et al. (1956) have shown a correlation between a rise in SGO-T levels and myocardial damage. Wroblewski and Ladue (1956a) have also shown that a rise in SGP-T is followed by a rise in SGO-T, although the latter does not equal SGP-T in Sigma-Frankel units.

Hepatic cell injury also causes an increase in the amount of glutamic oxalacetic transaminase liberated into the blood stream (Nickell and Allbritten, 1957). The fatty metamorphosis, as seen in Plate I, and passive congestion (Plates II and III) would account for the rise in SGO-T, as shown by dogs 6 and 7 in Figure VI. Apparently these two factors caused excessive release of the enzyme into the blood stream.

2. Serum Glutamic Pyruvic Transaminase

Glutamic pyruvic transaminase is liberated by the hepatic cells to catalyze the metabolic process which breaks down pyruvic acid in the blood (Popper and Schaffner, 1957).

FIGURE VI



SERUM GLUTAMIC OXALACETIC-TRANSAMINASE

PLATE I

Intracellular fat at the periphery of a liver lobe.

H & E; x 195.

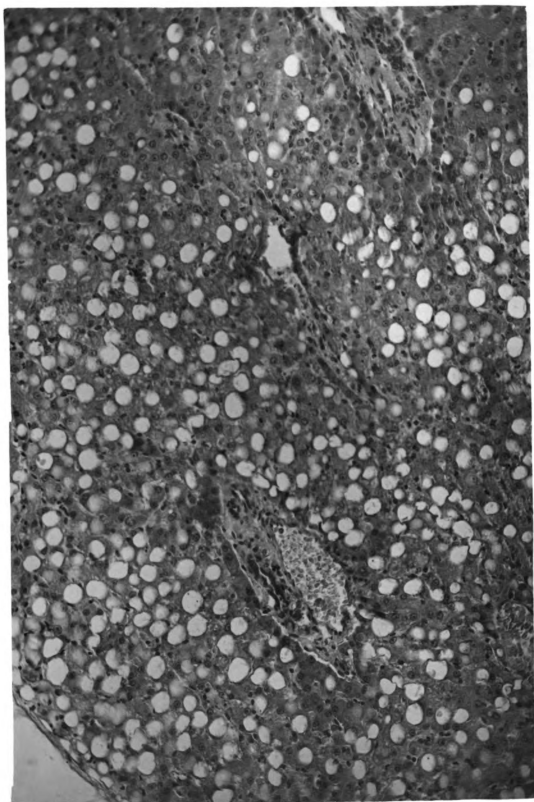


PLATE II

Portal trinity showing congestion and enlargement
of the interlobular vein. H & E; x 190.

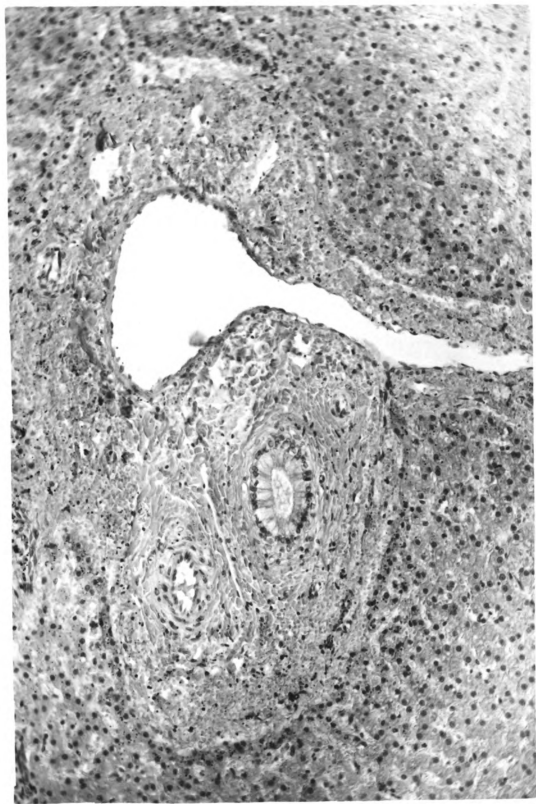
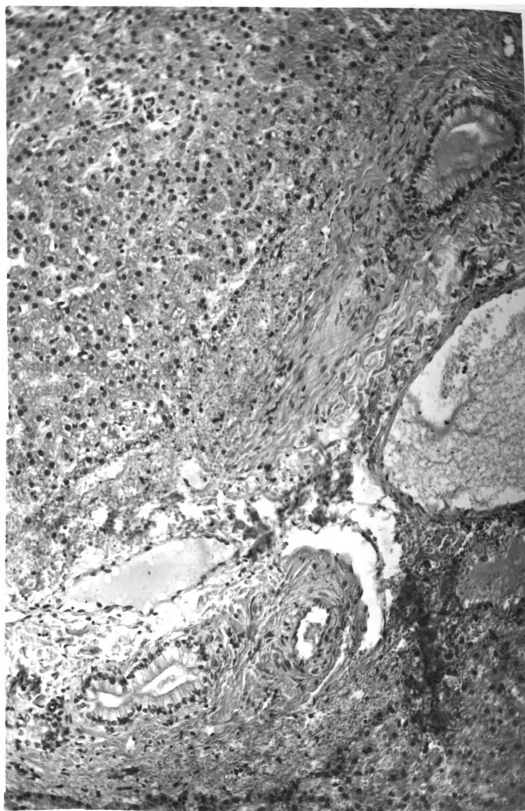


PLATE III

Portal trinity showing congestion and enlargement
of the interlobular vein. H & E; x 180.



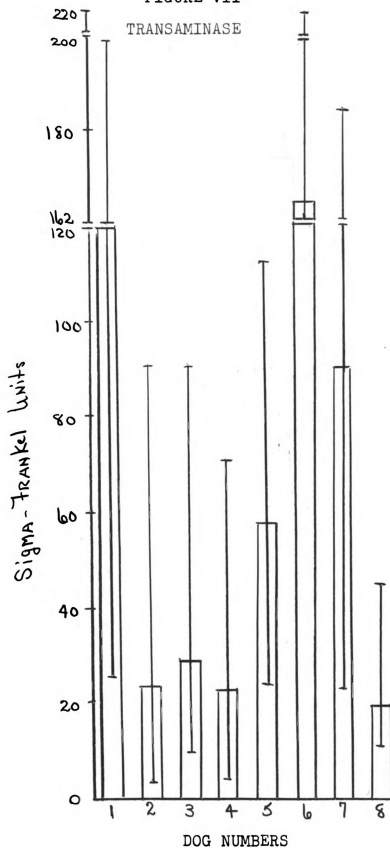
When the hepatic cells are damaged in any way the secretion of this enzyme becomes more rapid. The pyruvic acid is a metabolic end product of the Krebs cycle. By-products of these reactions are the keto acids which are concerned with carbohydrate metabolism (Hawk et al., 1954). Wroblewski and Ladue (1956a) have shown that an increase in the amount of SGO-T in the blood is an indication of hepatic cell malfunction. They have also reported that although glutamic pyruvic transaminase is known to be liberated by cells other than hepatic cells, its primary source is the liver.

The data in Figure VII suggest that there was damage to the hepatic cells in Dogs 1, 6, and 7. This rise in transaminase correlates with the results of the bromsulfalein clearance data. As the amount of neutral fat from the storage depots is absorbed and added to the phospholipid already present, the functional metabolism of the cell diminishes (Runnells, 1954). This results in transaminase being liberated into the blood stream.

G. TOTAL PROTEIN

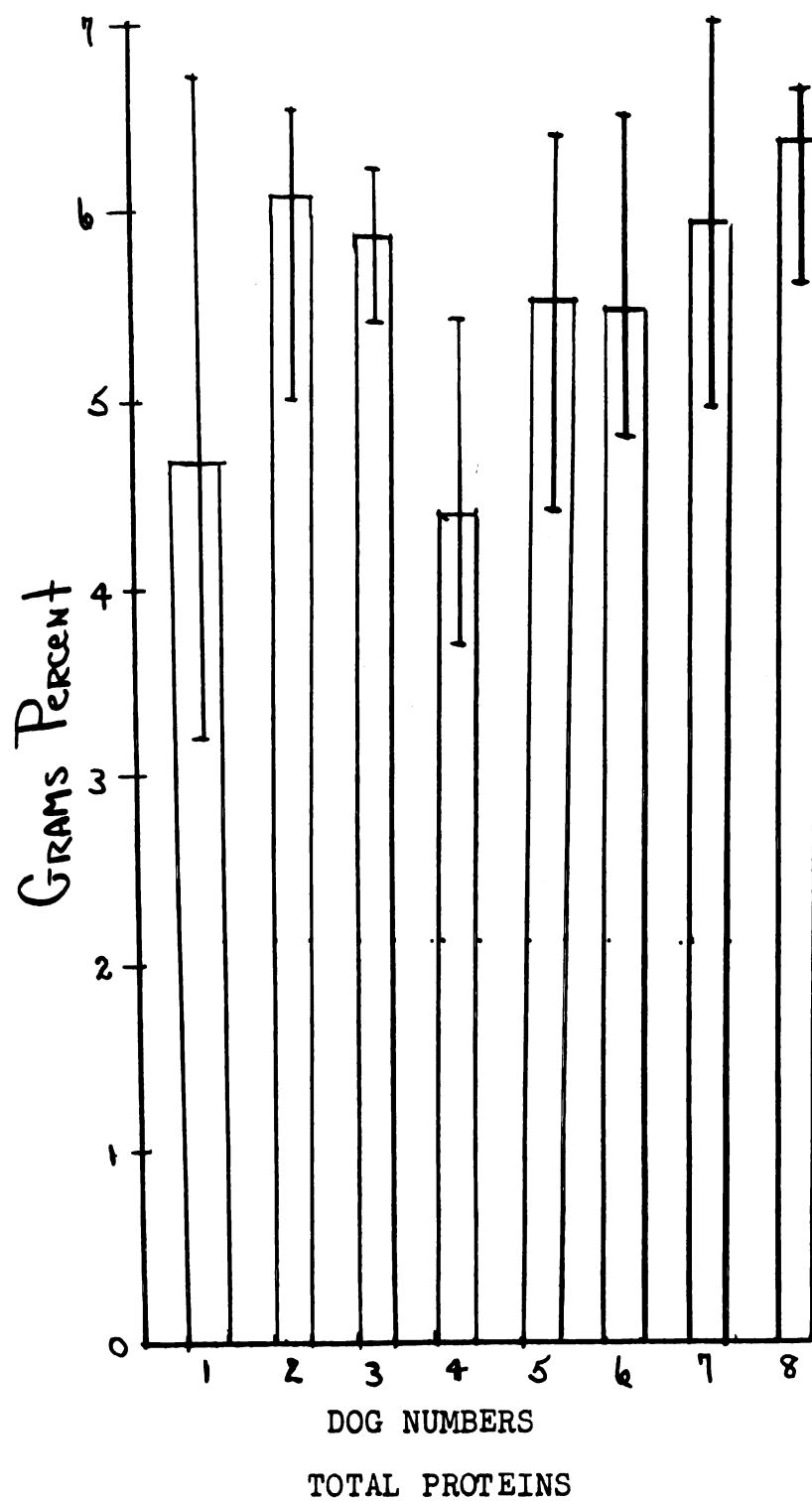
The data in Figure VIII indicate that the meat-free diet in conjunction with the Eck fistula did not result in lowered serum protein. Popper and Schaffner (1957) note that the liver is not only the main site of storage of serum protein, but is also concerned with the greater part of their production. This indicates that the hepatic artery supplied the liver with sufficient absorbed nutrients to maintain normal

FIGURE VII
TRANSAMINASE



SERUM GLUTAMIC PYRUVIC-TRANSAMINASE

FIGURE VIII



protein metabolism.

H. POST-MORTEM EXAMINATIONS

The distension of the right ventricle observed in all the dogs at autopsy could have been caused by an increased venous pressure (Katz and Rodbard, 1939) due to the formation of the Eck fistula, or by the absence of the liver's detoxifying action on the portal blood prior to entering the heart (Best and Taylor, 1955). The grayish foci seen in the liver proved to be those areas most involved with fatty infiltration. Sections taken through the serrated edges of the liver (Miller, 1955) evidenced no abnormal changes and no discontinuity of the serosa.

Histological examination of the liver showed evidence of fatty metamorphosis at the periphery of each lobe (Plate I). This degeneration was not extensive and did not include the main portion of any lobe. Runnells (1954) states that two causes of fatty metamorphosis are toxic agents and diminished oxygen supply. BSP is less toxic than most inert dyes and if infused slowly will cause no reaction at low dosage levels (Popper and Schaffner, 1957). One study on cats proved that two-thirds of the oxygen supply to the liver is via the portal blood (Mann and Bollman, 1939). Assuming that this is also the case in dogs, the lack of portal blood alone may have caused the fatty degeneration noted in Dogs 1, 6, and 7.

The passive congestion (Plates II and III) may be explained by dilatation of the right side of the heart causing the blood to back up in the vena cava and subsequently into the liver via the hepatic vein. Smith and Jones (1957) indicate that enlargement of the interlobular veins in the portal trinitities may be due to congestion. Some passive congestion was noted in all the experimental dogs.

SUMMARY AND CONCLUSIONS

The data supplied by this experiment indicate that the Eck fistula did not seriously alter the liver function in five of the eight dogs.

In all of the dogs the general loss of body weight is thought to be due more to temperature, environment, and the possibility of a mild infection than to the Eck fistula. Lack of body fat at autopsy is suggestive of a decrease in fat synthesis of digested material by the liver, due to the loss of portal blood flow in all of the experimental dogs. This fat loss was not noted in the control animals.

The microhematocrits on all the dogs were normal, and differential leucocyte data indicated no abnormal shift to the left which would result from disease or infection.

Low dye clearance rate and SGP-T data in three of the eight dogs showed some form of hepatic malfunction. This may be caused by passive congestion. The 2- and 5-minute values in the BSP data suggest that an even distribution of BSP throughout the blood stream had not occurred at these times. This may have been caused by the technique used in bleeding procedures. These values also show that the liver was not removing BSP at the usual rate, due to the loss of the supply of portal blood to the liver. Normal values were obtained on five of the eight dogs at the end of 30 minutes. The abnormally slow dye clearance in Dogs 1, 6, and 7 may be correlated with a greater

degree of fatty metamorphosis and congestion seen in these animals.

The normal values shown by the alkaline phosphatase and blood bilirubin determinations denote a normal function of the biliary system.

Some evidence as to the normal functional state of the kidney and the absence of acute hepatic insufficiency is furnished by normal non-protein nitrogen values of 20 - 40 mg./100 cc. of blood. The kidneys were grossly normal at autopsy and showed only slight fatty infiltration and no congestion upon histological examination. No harmful effects were noted as a result of this infiltration.

The rise in SGO-T in Dogs 6 and 7 is thought to be due to decreased liver function resulting from the passive congestion and fatty metamorphosis in this organ. An increase in SGO-T always accompanies, but does not equal, in Sigma-Frankel units, an increase in SGP-T.

The rise in SGP-T noted in Dogs 1, 6, and 7 was probably caused by the fatty metamorphosis and passive congestion. This enzyme is specific to the hepatic cell and is liberated into the blood stream when the liver cells are under stress from either pathological or physical conditions. (Wroblewski and Ladue, 1956a).

The dilatation of the right ventricle observed in all the dogs at autopsy could have been caused by an increase in vena cava pressure due to the formation of the Eck fistula.

Apparent toxicity as a result of the portal blood carrying absorbed nutrients from the intestine directly to the heart via the venous return may also have caused the dilatation.

The incomplete fatty metamorphosis at the periphery of the lobes of the liver (not in serrated edges) may be due to a diminished oxygenation of this organ through lack of portal flow. Congestion of the interlobular veins undoubtedly resulted from a backing up of blood into the liver from the dilated right ventricle.

In spite of the fact that three of the eight dogs with Eck fistula succumbed during the seven months of the experiment, it is believed that this study provides some positive evidence that the Eck fistula is capable of relieving portal hypertension. The normal values, obtained by clinical testing, and the absence of severe pathological changes, indicated by post-mortem and histological examinations, support the feasibility of using Eck fistulae in severe cases of portal decompression in humans.

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