STUDIES ON SERUM TRANSAMINASE IN CALVES EVALUATED BY THE USE OF LIVER BIOPSY

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Edward J. Hinsman

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by

Edward J. Hinsman

AN ABSTRACT

Submitted to the College of Veterinary Medicine of
Michigan State University of Agriculture and
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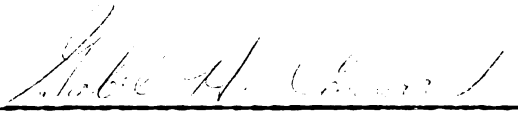
Approved 

EDWARD J. HINSMAN ABSTRACT

The enzymatic liver function tests are presently being evaluated in human medicine. The value of such tests as applied to veterinary medicine is yet to be established. This paper describes the evaluation of the serum transaminase level as a test of liver destruction in the bovine.

Carbon tetrachloride was administered in varying dosages to two groups of calves. This is a known way of producing liver destruction. The first group of calves was two months of age and the second group was six months of age.

Liver biopsy specimens for histopathological study were taken at intervals to evaluate the amount of damage occurring in the liver.

The serum glutamic pyruvic and serum glutamic oxalacetic transaminase tests were run at the same time in an attempt to correlate the amount of liver damage and transaminase levels.

There appeared to be some correlation between the transaminase levels and the damage observed on liver biopsy specimens in the two-month old calves. The serum glutamic oxalacetic transaminase was more sensitive as an indicator of liver destruction than the serum glutamic pyruvic transaminase.

There appeared to be no correlation between the amount of damage seen on histopathological examination of liver biopsy specimens in the six-month old calves and the transaminase levels.

Normal values for two- and six-month old calves were established and included in this paper.

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INTRODUCTION

The diagnostic problem of hepatic malfunction has long been one of the enigmas of medicine. The functions of the liver are numerous. There are almost as many liver function tests as there are functions. No one function test can be expected to completely reflect the biological state of the liver. A battery of function tests are usually run in anticipation of obtaining a more accurate picture of the liver.

The more recent tests being evaluated in hepatic conditions are enzymatic. The problem arises as to the value of such tests as applied to veterinary medicine.

Liver biopsy is one method of following the course of hepatic disease with live animals. The major problem remaining in evaluation of function tests is the production of known liver damage.

This paper describes the experimental procedure for causing known liver damage in calves, evaluating the amount of liver damage by liver biopsy, and evaluating two enzymatic liver function tests in the bovine.

REVIEW OF LITERATURE

The use of enzymatic tests in clinical medicine is rapidly becoming widespread. Two of the more recent enzymatic tests being evaluated in human medicine are the serum glutamic oxalacetic transaminase*, and the serum glutamic pyruvic transaminase**.

Transamination is the chemical reaction in which there is an exchange of the alpha-amino group of one amino acid for the keto groups of an alpha-keto acid, with the resulting synthesis of a second alpha-amino acid and a new alpha-keto acid (21).

$$\text{Glutamic acid} + \text{Oxalacetic acid} \xrightleftharpoons{\text{SGO-T}} \text{Alpha-keto glutaric acid} + \text{Aspartic acid}$$

$$\text{Glutamic acid} + \text{Pyruvic acid} \xrightleftharpoons{\text{SGP-T}} \text{Alpha-keto glutaric acid} + \text{Alanine}$$

This type of chemical reaction was first described in 1937, and thought to be a reaction which occurred with any amino acid and alpha-keto glutarate or oxalacetate using pigeon breast muscle as a source of transaminase (3). In 1940 pig heart was shown to transaminate other substrate systems, but was still more limited in scope than originally postulated (11). By the use of a coenzyme, pyridoxal phosphate, muscle homogenates were capable of

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* Hereafter refered to in this paper as SGO-T
** Hereafter refered to in this paper as SGP-T

catalyzing the transfer of alpha-amino groups of 25 different amino acids (6). This enzyme was present in pig heart, liver and kidney tissue (6). By 1952 it was reported that transaminase activity was not limited to pigeon breast, pig heart, liver and kidney, but present as well in varying activities in eight organs of the rat. Glutamic oxalacetic transaminase* was present in heart homogenates to the greatest extent. Smaller amounts were demonstrated in skeletal muscle, lung, brain, liver, spleen, prostate and testes in decreasing order (1). Subsequent observations indicated that the organ distribution of GO-T was species specific, and in man was present in heart, liver, skeletal muscle, and kidney in decreasing order (42).

The transaminases have been measured in a multitude of ways. SGO-T has been measured chromatographically (29), spectrophotometrically (2) (28) (29) (41) and colorimetrically (4) (45) (52) (56).

Spectrophotometrically, SGO-T is assayed by employing a double enzyme system. GO-T is coupled to the enzymatic oxidation of reduced diphosphopyridine nucleotide, using either malic dehydrogenase or lactic dehydrogenase (2) (22) (29) (41). All of the spectrophotometric techniques measure SGO-T by estimating the rate of the enzymatic reaction rather than any of the end products of the transamination. Spectrophotometrically, SGO-T activity is

* Hereafter refered to in this paper as GO-T

expressed as units per milliliter of serum per minute. One unit equals a decrease in optical density of 0.001 mμ under the conditions specified in the literature. Although simple, rapid and accurate, these techniques require malic or lactic dehydrogenase and these reagents are expensive, unstable and not generally available.

The colorimetric method estimates SGO-T activity by measuring the amount of pyruvic acid formed under standard incubation conditions (4) (33) (45). The oxalacetic acid formed by transamination is converted by aniline citrate to pyruvic acid. This method is simple and required no enzymes as reagents, but it is somewhat less accurate than other methods. Colorimetrically, SGO-T is expressed as units per milliliter of serum. One unit equals the formation of 1γ of pyruvate under the conditions specified (45).

When SGP-T is determined colorimetrically the substrate is changed to L-alanine and pyruvate determined without the use of aniline citrate (45).

The maximal activity of GO-T in cardiac musculature suggested that alterations in metabolic behavior of this tissue might reflect itself in changes in enzymatic levels in the blood. In 1953 the presence of GO-T was sought for and demonstrated in whole blood, serum and plasma (29). The normal range for activity was established (29), and elevations in some patients with clinical diagnosis of myocardial infarction were observed (33).

Early in the use of SGO-T as a diagnostic aid many investigators noted that necrosis in other tissues resulted in an elevated level of the SGO-T. The possibility of the SGO-T determination as a diagnostic aid to liver conditions was postulated.

Virus hepatitis in mice has been associated with rises in SGO-T activity. The rise in SGO-T activity appears to be associated with the size of the virus inoculum, the blood virus titer and the degree of liver necrosis. A rise in the SGO-T of mice has also been noted in partial hepatectomy. This was probably due to trauma of hepatic tissue (18).

Rats and dogs have been used by investigators as laboratory animals in experiments with toxic hepatitis due to carbon tetrachloride. Levels of activity have been reported that are 50 to 1,000 times the normal SGO-T levels (39) (64) (65) (66) (60). There appeared to be a correlation between the extent of centrilobular zonal necrosis and the height of the SGO-T activity attained. Whether judged by the gross or microscopic picture the highest levels were seen in the animals with the most severely damaged livers (39). Neither the myocardium nor kidneys of the rats with carbon tetrachloride poisoning showed any gross or microscopic damage (39).

Two days after exposure to carbon tetrachloride elevations in SGO-T were regularly observed in the canine. On the sixth day following administration of the toxic

agent practically normal levels of SGO-T were found in surviving dogs (17).

Common-duct occlusion experimentally produced in dogs has resulted in temporary elevations in SGO-T activity. Elevated levels returned to normal within a week following the relief of the biliary tract obstruction (36).

Experimental ligation of the common duct in rats was rapidly followed by a rise in the SGO-T activity. Sham operated controls showed no significant changes in SGO-T activity. Six hours following duct ligation a peak level was reached after which a recession took place despite continuation of the obstruction. Upon release of the common duct obstruction the SGO-T rapidly returned to normal (9).

Cirrhosis and hepatic tumors experimentally produced in rats with butter yellow 18 have been shown to be accompanied by elevation of SGO-T activity (39).

From the experimental data the following theory was postulated, "Any process causing death of enough liver cells should theoretically increase the serum transaminase activity" (38).

Investigators have reported many compounds which gave rise to elevated SGO-T levels due to toxic hepatitis; chlorpromazine (7) (63), salicylate (35), cinchophen (36), azasirine (36), pyrazinamide (63). SGO-T increased proportionally with the continued administration of these drugs when they proved to be hepatotoxic. However,

elevations of toxic hepatitis were greater when caused by carbon tetrachloride than when caused by any of the above mentioned drugs (39) (60) (64) (65) (66). Discontinuance of the heptotoxic drug resulted in a rapid fall of SGO-T toward normal (36).

Acute hepatic diseases have been shown to be associated with rises in SGO-T activity. In many instances the serial changes and/or levels of SGO-T activity were sufficiently characteristic to assist in differential diagnosis of liver disease (7) (8) (19) (38) (39) (58) (60) (63) (66).

The highest levels of SGO-T activity were observed in acute toxic hepatitis due to carbon tetrachloride, and in patients with acute infectious or homologous serum hepatitis (7) (66). The elevations following carbon tetrachloride exposure occurred within 24 hours and have been reported to reach levels of 27,000 units (66). After cessation of exposure to the toxin the SGO-T rapidly returned to normal values.

Acute infectious and homologous serum hepatitis resulted in alterations of SGO-T to levels of 10 to 40 times greater than normal (8) (14) (37) (43) (58) (60) (63) (65) (66) (33). The SGO-T rise in virus hepatitis began during the prodromal phase of the disease (8) (60) (63) (66), and reached its peak elevation when the patients were the sickest as judged clinically (7) (66). With clinical evidence of improvement the hepatitis was accom-

panied by a fall of SGO-T toward normal (7) (38) (66). Relapse and/or development of chronic hepatitis was reflected in continued abnormal SGO-T levels (60) (63) (66).

The theory has been suggested that serial determinations of SGO-T during the course of hepatitis reflected the liver damage better than conventional liver function tests (66). Because it does reflect liver cell injury rather than function it does not necessarily correlate with the routinely employed liver function tests (7) (38) (39) (66).

There was a correlation between the extent of damage to hepatic cells and the degree of elevation of SGO-T (15) (63). SGO-T may be useful as a guide in following the progress of restoration of hepatocellular integrity following acute injury (39). The determination of the SGO-T activity will become a useful tool in studying the natural course of hepatic diseases (15).

Epidemiological data has shown that SGO-T levels in persons exposed to infectious hepatitis rose one to four weeks before other clinical laboratory evidence of liver injury became evident (60). In homologous serum hepatitis the SGO-T may be elevated 8 to 14 days following exposure to contaminated serum (49). This permits diagnosis of hepatitis in the preicteric stage and aids in controlling epidemics (60).

Infectious mononucleosis unless associated with hepatitis was accompanied by normal SGO-T levels. Increased

levels of SGO-T accompanying infectious mononucleosis appeared to be related quantitatively to the severity of the hepatitis (7) (60) (63) (65).

Elevations of SGO-T in the range of 50 to 250 units have been reported in active or decompensated Laennec's cirrhosis (7) (8) (65). Cirrhosis complicated by acute hepatitis has been reported to present SGO-T alterations characteristic of acute hepatitis but of prolonged elevations (58) (65). Active cirrhosis presents much the same picture (58) (65).

Biliary tract obstruction, either extrahepatic or intrahepatic, has been accompanied by elevations in SGO-T as high as 300 units (7) (8) (14) (19) (38) (58) (65). Upon relief of biliary obstruction the SGO-T level fell to normal within 7 to 14 days (65).

"Acute and chronic hepatic disease is associated with quantitative and serial elevations of SGO-T which are sufficiently characteristic to permit diagnostic differentiation" (60).

Both primary and secondary carcinomas of the liver were associated with elevations in SGO-T. Neoplastic involvement of the liver appeared to be very sensitively indicated by the SGO-T levels (7) (8) (58) (65), except in the lymphomas and leukemias (65) where normal values were found. The absence of elevated SGO-T levels did not exclude the possibility of hepatic metastases (8).

In bone disease, either malignant or nonmalignant,

the SGO-T values were normal unless there was also hepatic involvement (65).

Although kidney tissue contains a high level of GO-T, elevations of SGO-T in renal disease were found in only a few cases (47) (48) (58) (59). Most of the patients showing elevated SGO-T levels with renal disease had accompanying hepatic damage (59).

Acute pancreatitis has been associated with elevation in SGO-T (8) (14) (43) (58). The reason for this elevation was not clear at the time but was thought to be due to hepatic factors involved with the acute pancreatitis (8).

Patients with prolonged shock for periods greater than 24 hours showed elevated SGO-T levels (8) (58). In those patients observed at post-mortem following prolonged shock, well developed centrilobular liver cell necrosis was present in each case. This necrosis was thought to have caused the elevations in SGO-T. With shorter periods of shock the SGO-T levels appeared to be unrelated to the vascular collapse and primarily related to the underlying disease (8).

Skeletal muscle necrosis due to severe trauma, infectious processes or following surgery was associated with elevated SGO-T levels (8) (37) (42) (43).

Elevations seen with very rapid arrhythmias were probably due to the centrilobular liver cell necrosis secondary to diminished cardiac output (8).

Because there was such a lack of specificity in the SGO-T activity in tissue necrosis, other serum enzyme systems were studied in an attempt to locate ones more specific for individual tissue. SGP-T was one such enzyme studied. SGP-T was claimed to be more sensitive than the SGO-T in depicting acute hepatocellular necrosis (62) (63). The relative differences in enzyme concentration of the tissues involved was reported to reflect itself in the relative differences found in the serum concentrations. The following table of GO-T and Glutamic Pyruvic Transaminase* values for human tissue was taken from the literature (61).

Comparison of Glutamic Oxalacetic Transaminase and Glutamic Pyruvic Transaminase in Normal Adult Tissue Homogenates (61)

TISSUE	GO-T	GP-T
	Units/gm. wet tissue	
Heart	156,000	7,100
Liver	142,000	44,000
Skeletal Muscle	99,000	4,800
Kidney	91,000	19,000
Pancreas	28,000	2,000
Spleen	14,000	1,200
Lung	10,000	700
Serum	20	16

* Hereafter refered to in this paper as GP-T

Experimental animals were used to test the effect of liver damage on SGP-T levels. Common duct ligation in rats resulted in an increased SGP-T level. This level reached its peak six hours following the operation and continued to recede toward normal despite the continuation of the obstruction (9). The use of carbon tetrachloride poisoning in dogs to cause liver damage resulted in greatly elevated SGP-T levels (17).

Since elevation of SGP-T was usually accompanied by elevations in SGO-T it was suggested by many investigators that the two be run simultaneously.

Many disease states have been reported in the literature evaluating SGO-T and SGP-T measurements as diagnostic aids. Whereas SGO-T was greatly elevated in myocardial infarction SGP-T was not unless the SGO-T rise was greater than 150 to 200 units. This was explained by the lower concentration of GP-T in heart muscle as compared to GO-T (61) (62).

In very rapid arrhythmias SGP-T was elevated along with the SGO-T. Necropsy of one such case showed evidence of extensive liver cell necrosis (9).

Viral hepatitis has been associated with elevations of SGP-T greater than SGO-T (9) (13) (61) (62). By the simultaneous measurement of SGO-T and SGP-T a diagnosis of viral hepatitis was reported to be possible. The normal ratio of SGO-T to SGP-T was usually greater than 1.0 (9) (13). If the ratio was less than 1.0 the diagnosis of viral hepatitis was proposed (13).

In infectious mononucleosis elevations in SGP-T were associated only with involvement of the liver. The rise in SGP-T was less that that associated with viral hepatitis or homologous serum hepatitis (61) (62).

In hepatic cirrhosis the elevation in SGP-T was variable. In patients with active cirrhosis the SGP-T and SGO-T levels were both elevated. In patients with inactive cirrhosis the SGP-T and SGO-T were both within the normal range (61) (62).

In obstructive jaundice, in a limited number of observations, the elevations of SGP-T were so variable that conclusions could not be drawn (9) (61) (62).

SGP-T level elevations have been noted in metastatic cancer of the liver. The levels attained were usually close to the normal levels, and of little diagnostic value (61).

Although the SGP-T has been reported to be elevated in hepatic disease its specificity as an indicator of hepatic disease when used alone was questioned (9). The procedure of using both the SGO-T and SGP-T simultaneously to help in differentiating acute from chronic liver disease has been suggested (61). The SGP-T should have its greatest use as an adjunct in the interpretation of an elevated SGO-T level (9).

The use of the transaminase levels to aid in the differential diagnosis of liver disease in no way relieved the physician of the necessity of interpreting the test in the light of the total clinical setting (58).

MATERIALS AND METHODS

Nine calves were obtained from local sales barns at about one week of age. The calves were of mixed breeding mainly from dairy animals. Both males and females were represented. No experimental work was started until the calves were judged to be clinically normal. Due to various origins all calves were treated prophylactically for calf scours immediately upon arrival. One-quarter gram of oral tetracycline hydrochloride* was given per day for one week. When experimental work was begun the calves ranged in age from two to six months, and in weight from 50 to 172 kilograms.

Fecal examinations for parasitic infestation were run on arrival and immediately preceeding the experimental work. The sugar flotation method of fecal examination was used (10).

At least two urine examinations were made before proceeding with experimental work. The urine examination included the following tests: specific gravity by urinometer**, pH by paper strip**, urine bilirubin***, urine albumin***, urine occult blood***, and urine acetone***. Urine was collected without catherization by stimulation of external genitalia.

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* Charles Pfizer & Co. Inc., Brooklyn, N.Y.
25 Gm. Oxytetracycline HCl
** Squibb, Chicago Apparatus Co., Chicago, Ill.
*** Ames Co. Inc., Elkhart, Ind.

Blood was drawn from the external jugular vein using a 16 gauge needle. Thirty milliliters of blood for serum was collected in two tubes to eliminate the possibility of hemolysis occurring in any one complete sample. Upon clotting, this blood was incubated for one hour at 37° Centigrade and then centrifuged. Serum was then removed and transferred to clean tubes. All samples were refrigerated until examination, and no samples were stored longer than 24 hours before determination of any tests requiring serum other than electrophoresis (7) (67).

Blood for cell counts was collected in citrated vials. Both red and white cell counts were run by standard dilution techniques and counted in a bright line hemacytometer (10). Differential white blood cell counts were made on a thin blood smear, stained with Wright's stain and counted as described in the literature (23).

Hematocrit values were determined by allowing freshly drawn blood to flow into heparinized capillary tubes, sealing one end with a gas flame, centrifuging for five minutes at 11,000 r.p.m., and then evaluating as percent packed cell volume.

Biopsy specimens were taken using a Vim-Silverman punch biopsy needle, and a technique modified from the literature (55). (Figure I and II) A liberal area around the eleventh and twelfth rib on the right side was clipped. The area was washed with soap and water and then disinfected with alcohol. A local infiltrative anesthetic of ten

milliliters of four percent procaine was used in the subcutaneous and muscular tissues. Most of this anesthetic was placed between the eleventh and the twelfth rib, about nine to ten centimeters below the vertebral-rib junction. A one centimeter incision was made through the skin with a scalpel blade. With a sharp thrust the biopsy stylet and outer needle were inserted in an anteroventral direction toward the left olecranon process. When hepatic tissue was entered and movement of the needle corresponded to diaphragmatic movements the stylet was removed. The inner biopsy needle was inserted with a rotating motion. The outer needle was forced over the inner needle for a distance of two centimeters and the entire unit was then removed. A topical antibiotic powder was applied to the incision. Animals were observed for an hour for any signs of internal hemorrhage or unusual distress.

Biopsy specimens were taken immediately preceeding carbon tetrachloride administration and during the subsequent 24, 72 and 144 hour periods. Specimens were placed in isotonic formalin for fixing. They were later embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination.

Daily clinical examination included observations on appetite, temperature and stethoscopic auscultation of thoracic and abdominal cavities.

Total serum protein was determined using the Hitachi hand protein refractometer*. Electrophoresis was run on

* National Instrument Company, Baltimore, Md.

serum samples on a paper electrophoreses system*. Six ten-thousandths milliliter of serum was run for 16 hours at 2.5 milliampere constant current using a veronal buffer of 8.6 pH and 0.075 ionic strength. Paper strips were analyzed on an analytrol following the procedure for human serum* (25).

SGO-T and SGP-T were determined colorimetrically (50) on a Bausch and Lomb Spectronic 20 Colorimeter. Only serum samples which had been refrigerated no longer than 24 hours and which showed no hemolysis were used (7) (45) (50). An incubation temperature of 37° Centigrade was used.

Serum bilirubin was determined by the colorimetric method as described in the literature (23). Both the one minute and the thirty minute bilirubin were determined. When possible, samples were run on the day collected, but never were they stored longer than overnight before processing (67).

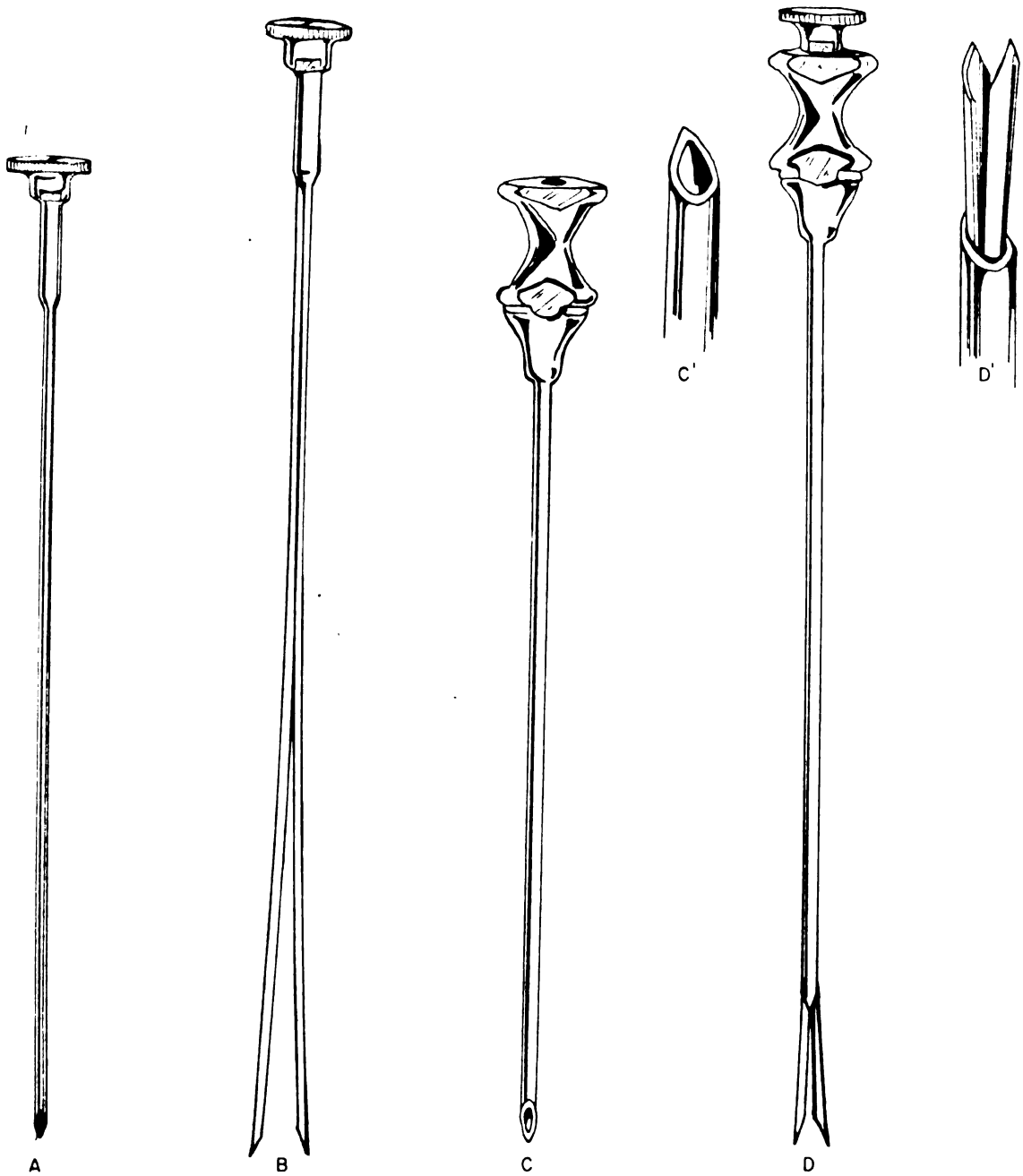
Prothrombin clotting times were determined on the six month old calves before any biopsy specimens were taken (23).

Liver poisoning was accomplished by varying doses of carbon tetrachloride given by stomach tube. Twenty-five milliliters of mineral oil were given following the carbon tetrachloride to carry the total dose of toxin to the stomach. Control animals were given twenty-five milliliters of mineral oil by stomach tube. Dosages were arbitrarily selected due to the lack of literature on this subject on the bovine.

* Spinco model R Paper Electrophoresis System, Beckman Spinco Division.

Because of the absence of reports in the literature concerning normal values in the bovine for either SGC-T or SGP-T, it was necessary to establish normal values. Random samples on the experimental calves were used to establish these normals. During the course of the experimental work two reports of normal values in the bovine were published (12) (32).

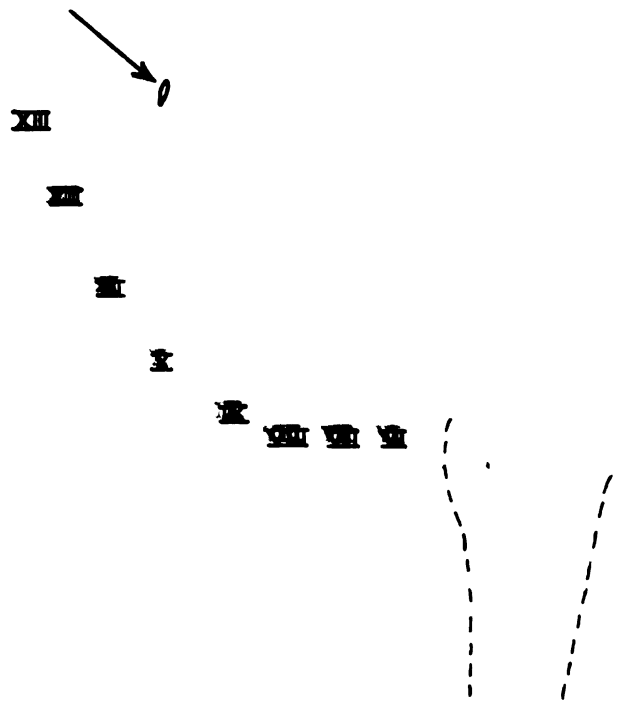
FIGURE I



A. Stylet
B. Inner needle
C. Outer needle C'. Magnification to show detail of outer needle
D. Inner needle in place within outer needle D'. Magnification of needle tips in "D".

OVERLAY FOR

Needle is angled toward the left olecranon process



OVERLAY FOR

Needle is angled toward the left electron process

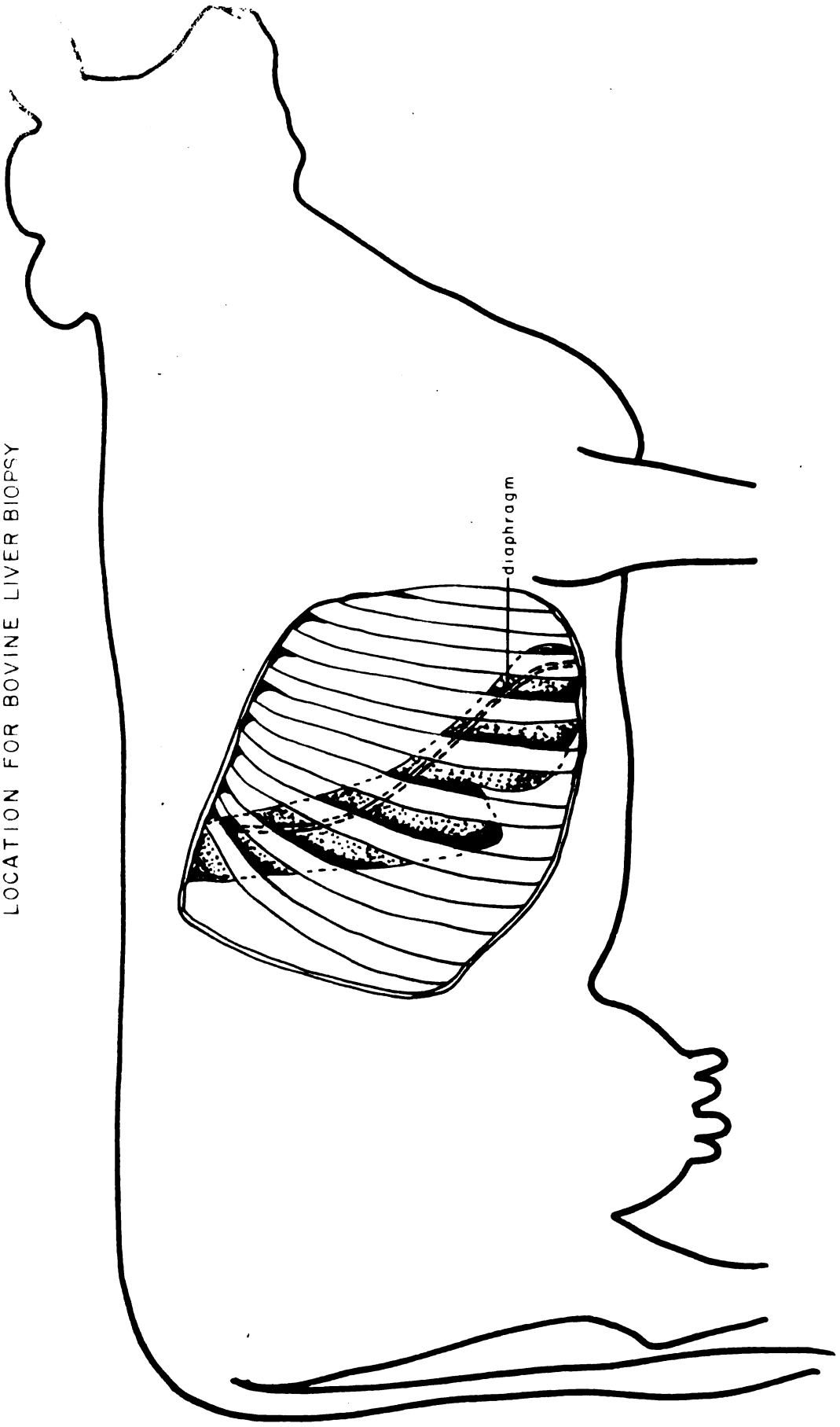


FIGURE - II
LOCATION FOR BOVINE LIVER BIOPSY

Daily rectal temperatures never rose over 103° Fahrenheit. At no time did the calves appear depressed or give any indications of being clinically ill. At the beginning of the experimental work all temperatures were 102° Fahrenheit. At no time were there any indications of increased pneumonic sounds of abnormal character.

All fecal examinations were negative for parasitic ova on initial examinations and remained negative throughout the duration of the experiment.

The results of urine examinations for control and experimental animals are placed in Chart I and II. The only abnormalities were three transient elevations in the urine albumin. These elevations were a trace as determined by the information provided by the supply company. The rest of the urine samples were all within the ranges of normal as reported in the literature (10). The prothrombin clotting times ranged from 19.2 to 22.6 seconds. The results from the six month old calves were placed in Chart III.

A total of 26 biopsy specimens were taken during the course of the experiment. The technique as described in the materials and methods adequately locates the liver for needle punch biopsy. On five different occasions it was necessary to re-enter the animal due to the absence of the liver tissue when the needle was withdrawn from the animal.

Discomfort was not noted in animals once the intercostal muscles were entered, although animal 46 showed a mild discomfort and dyspnea following biopsy on the first day. This subsided in less than one-half hour and no recurrences of a similar nature were noted. The biopsy specimen obtained was usually five millimeters in length and one-half millimeter in diameter.

Opinions formed upon histological examination of the biopsy specimens were confirmed by the Michigan State University Pathology Department*.

Two calves two months of age received ten millliliters of carbon tetrachloride orally by stomach tube. The biopsy specimens taken 24 hours following showed generalized changes. The normal cord cell arrangement was no longer present, and there was cloudy swelling, fatty changes, pyknotic nuclei and hydropic changes throughout the lobule.

The biopsy specimens taken before and 144 hours following carbon tetrachloride administration were normal. Biopsy specimens from control calves were normal.

The biopsy specimens taken before experimentation with the five calves six months of age were judged to be normal. Specimens taken during experimentation never showed generalized changes as seen in the two-month old calves.

Twenty-four hours following the administration of 17 millliliters of carbon tetrachloride orally the specimens from calf 45 showed extensive areas of zonal coagulation

* Dr. Ralph D. Barner, MSU Pathology Department, E. Lansing, Michigan.

necrosis with hemorrhage. Surrounding the zonal necrosis was a rim of cloudy swelling, vacuolation and both fatty and hydropic changes.

Both calves 44 and 46 showed cloudy swelling and small areas of focal necrosis, lymphatic infiltration and small amounts of hemorrhage in the 24 hour specimen. By the end of 144 hours no changes from normal were evident.

In calves receiving 35 milliliters of carbon tetrachloride the 24 hour specimen showed zonal necrosis, some cloudy swelling and in calf 47 some hydropic changes.

Calf 45 after receiving 100 milliliters of carbon tetrachloride showed necrosis, pyknotic nuclei, and cloudy swelling in 24 hours. The normal hepatic architecture was lost in the 48 hour specimen. All pathological changes were gone in the 144 hour specimen.

The SGO-T normals were run on two groups of calves. The first group consisted of four calves, all about two months old. There were three males; two Holstein and one Holstein-Angus cross, and one Holstein female. The weight range was from 50 kilograms for the Angus cross to 75 kilograms for one Holstein male. A total of 25 SGO-T samples were run on these calves to establish normal values. The results showed a range of 10 to 76 units, the average is 54 units with a standard deviation of 14.6 units (Chart IV).

The SGP-T normals were determined on the same calves by 30 samples. The range in units was from 3 to 22; the average was 14.1 units with a standard deviation of 4.0 units (Chart IV).

A second series of normal values was determined for six month old calves. This consisted of five calves; two Holstein females, two Holstein males and one Guernsey male. The weight range at the time experimental work was begun was 145 to 172 kilograms.

SGO-T for the second group was determined on 36 samples with the following results. The range was 12 to 95 units, the average value being 59.3 units and the standard deviation 24.2 units (Chart V).

The same group of six month old calves was used to establish normals for SGP-T. The results were a range of 4 to 40 units, an average value of 11.68 units and a standard deviation of 8.82 units (Chart V).

Graph I shows the results following the oral administration of ten milliliters of carbon tetrachloride to a 70 kilogram male Holstein calf, 163, and its effect on the SGO-T levels. One day following the administration of the toxin the SGO-T level rose from the normal range to 1,480 units. An abnormal level was noted until the tenth day after exposure to the toxin.

The SGP-T levels for the same animal are shown in Graph II. The elevation in SGP-T level was noted two days after the toxin was given by stomach tube. The peak level reached was 80 units four days following the administration of the toxin; three days later the level had again descended to the normal range.

A second animal, 164, demonstrated a reaction similar to 163. Following the administration of ten milliliters

of carbon tetrachloride, both SGO-T and SGP-T were elevated. Outstanding SGO-T elevation was noted in the 24 hour sample following toxin administration. The peak level, occurring in 24 hours, was 1,960 units. Again ten days was required for SGO-T to decline to normal values. The values declined rapidly 24 hours following the peak elevation and then receded to normal ranges more slowly (Graph III).

The SGP-T elevations as shown in Graph IV for animal 164 were somewhat different than those observed on animal 163. The peak elevation again was not noted until the fourth day following toxin administration. Two days after the peak level was reached the values were at a normal level. The total duration of elevated levels was shorter than observed in animal 163.

The six-month old calves used in the experimental work were tha same as those described in the establishment of normals for SGO-T and SGP-T.

Seventeen millliliters of carbon tetrachloride were administered to a 150 kilogram Holstein female calf, 44. This oral dosage of carbon tetrachloride was followed by an elevation to 300 units 48 hours later. The SGO-T level was elevated for a period of 48 hours (Graph VI). The SGP-T was never significantly elevated.

Calf 45 also received 17 millliliters of carbon tetrachloride orally. This was a 162 kilogram Guernsey male. Twenty-four hours following the toxin a level of 1,480 units of SGO-T was reached. The SGP-T level was elevated for

four days (Graph VII). No elevation was noted in the SGP-T level of this calf.

Thirty-five milliliters of carbon tetrachloride were administered orally to a six month old Holstein female, 43, weighing 150 kilograms. The toxin was administered on the ninth day as shown in Graph V.

Two days later an elevation in SGO-T was noted to a peak of 96 units. The following day the level fell to 38 SGO-T units. On the third day post-administration a second rise to 148 units was noted that lasted 24 hours and then fell to normal levels.

The SGP-T levels on the same calf, 43, showed a pattern similar to that of the SGO-T in its lack of any great elevations. The third day following the toxin a SGP-T level of 36 units was seen which declined to normal in 24 hours.

A similar protocol was followed on a male Holstein calf of six months of age weighing 172 kilograms. Thirty-five milliliters of carbon tetrachloride were administered on the eighth day as shown in Graph IX. The SGO-T levels on calf 47 rose sharply to 176 units on the day following the carbon tetrachloride, but the rise was only transitory and fell to normal levels in 24 hours. A second rise to 124 units was seen on the third day which also proved to be transitory and rapidly returned to normal. The SGP-T levels on calf 47 were never significantly elevated.

A Holstein male, six months old, and weighing 170 kilograms, 46, was given a total dose of 35 milliliters of

carbon tetrachloride, but administered in divided doses on the fourth and eighth day as shown in Graph VIII.

A response in the SGO-T level was noted in 24 hours after the first 17 milliliters of carbon tetrachloride. A peak level of 611 units was reached and elevated levels were seen for three and one-half days.

The second dose of carbon tetrachloride resulted in levels that were elevated but still within the normal range. The SGP-T levels were not changed following either the first or second dosage of carbon tetrachloride.

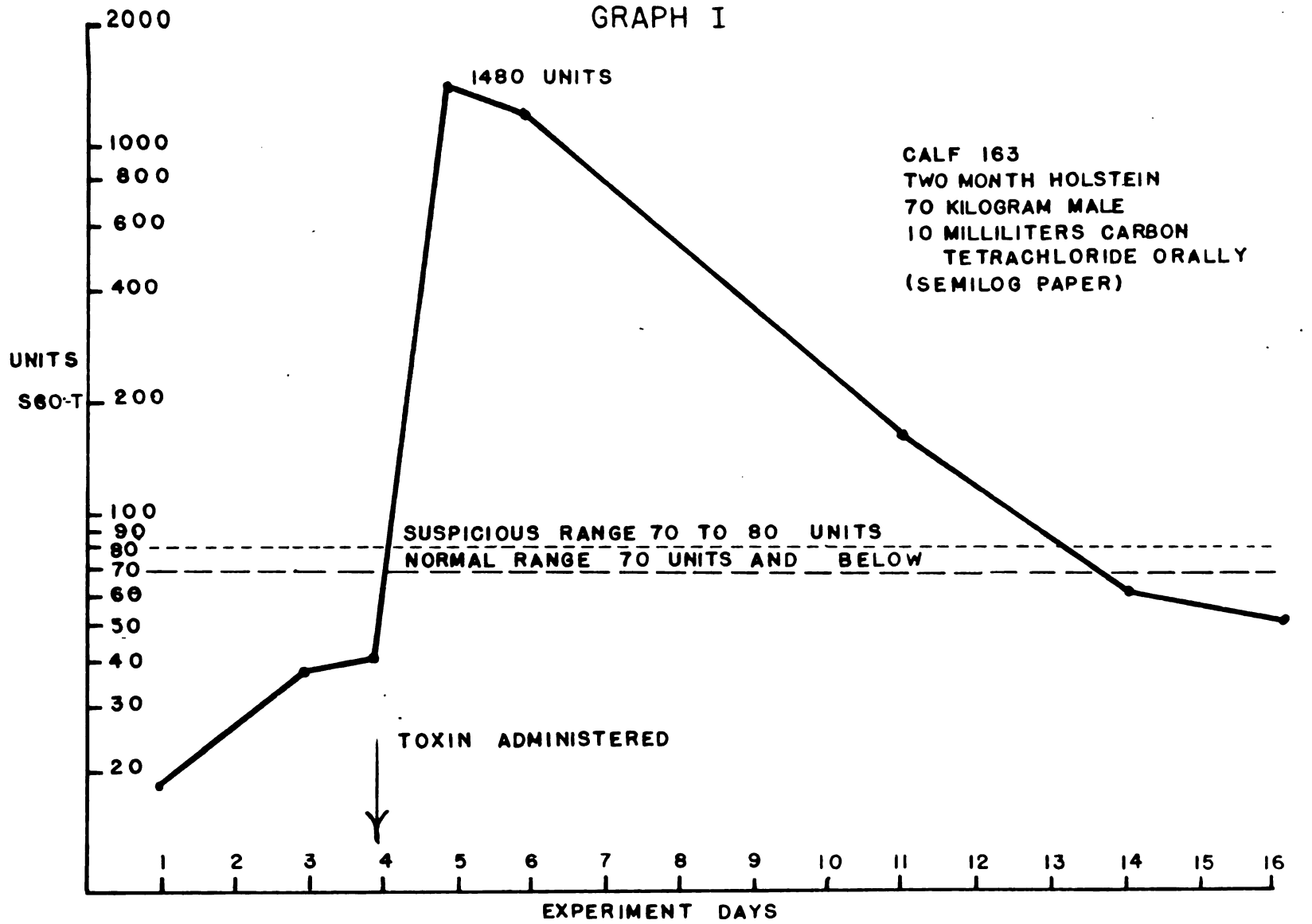
Eleven days after normal SGO-T levels were reached animal 45 received 100 milliliters of carbon tetrachloride orally (Graph VII). The response to this dosage of carbon tetrachloride was an elevation of SGO-T on the second day of 280 units. The SGP-T level was elevated for 24 hours to 68 units. Both SGO-T and SGP-T levels were within the normal range for the remainder of the experiment.

Erythrocyte, leucocyte and differential leucocyte counts are reported in Chart VI for the two-month old calves and in Chart VII for the six-month old calves.

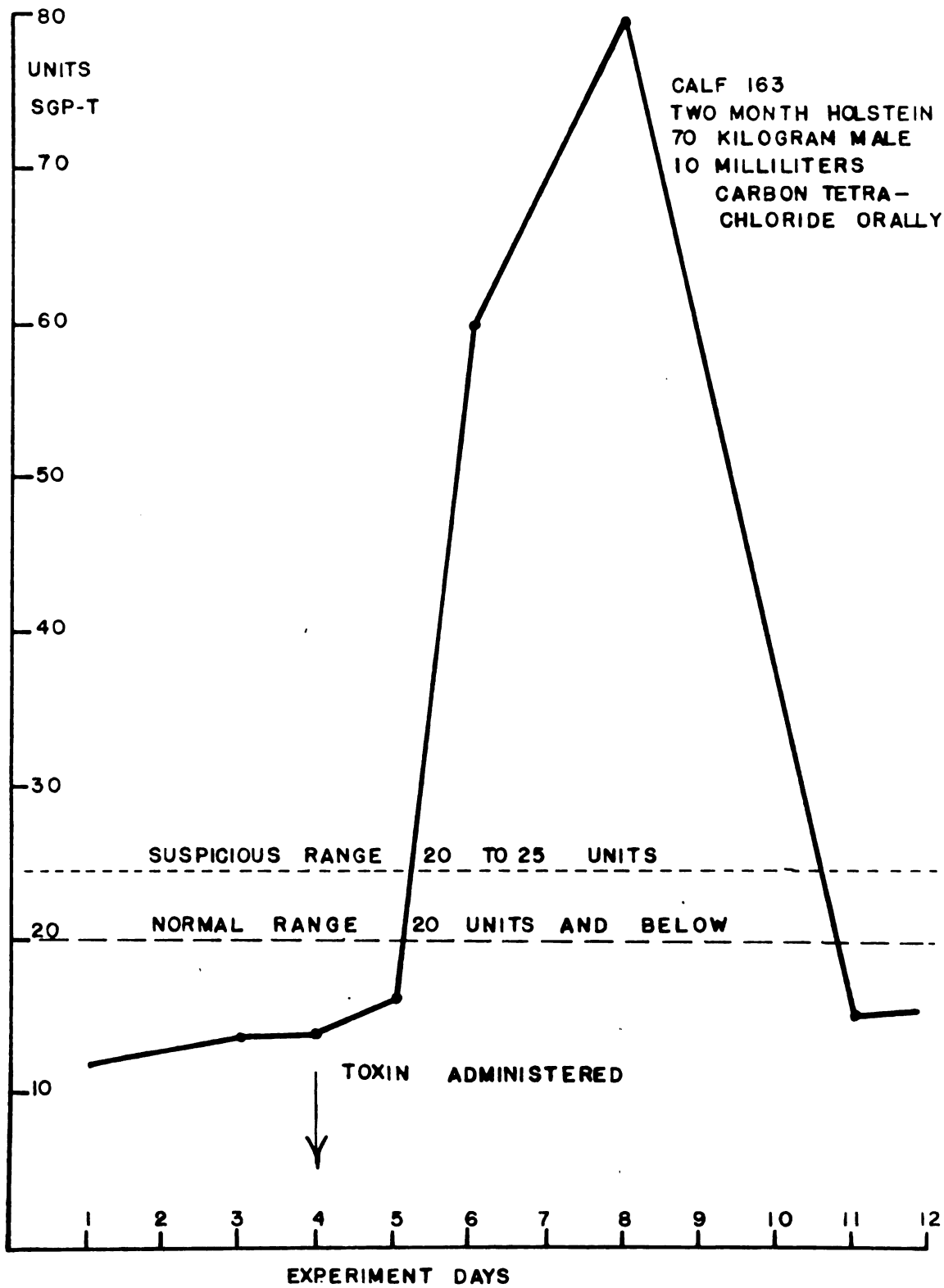
Paper electrophoresis usually resulted in separation of the following bovine serum protein fractions: albumin, alpha globulin, beta globulin, and gamma globulin. A peak at the point of application on the paper strip was identified as fibrinogen and foreign protein. In those samples in which gamma globulin was identifiable separately from the fibrinogen it was reported separately. Otherwise,

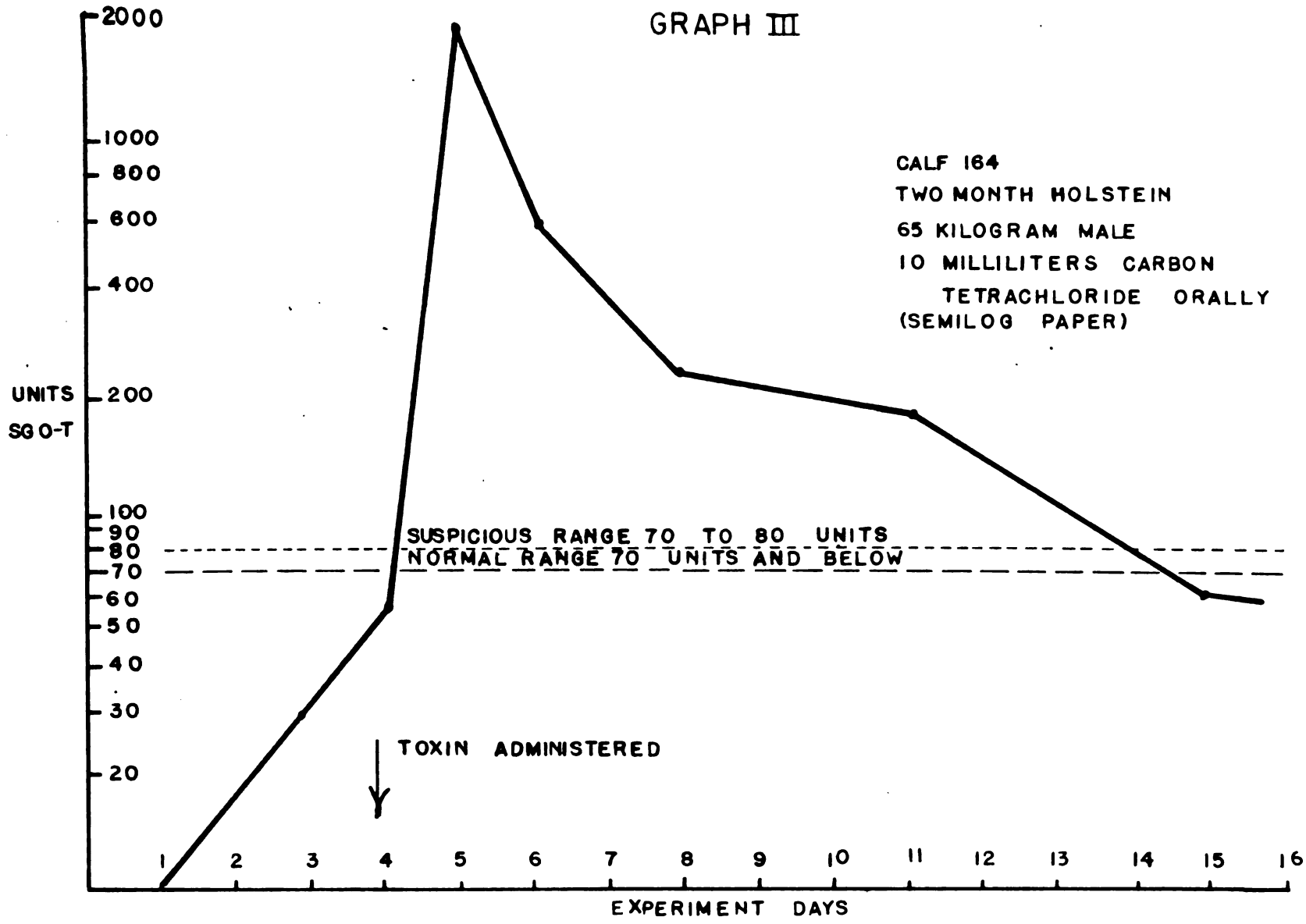
the two fractions were considered as one called fibrinogen and gamma globulin. The range of values are shown in Charts VIII, IX and X. A total of 45 samples were run on the six-month old calves spaced from the beginning to the end of the experimental work.

The serum bilirubin test gave very variable results. The daily variations were so great that normals for individual calves could not be established. The results are shown in Chart X. The serum bilirubin test was discontinued after completion of the experimental work on the two-month old calves and not utilized on the six-month old calves.

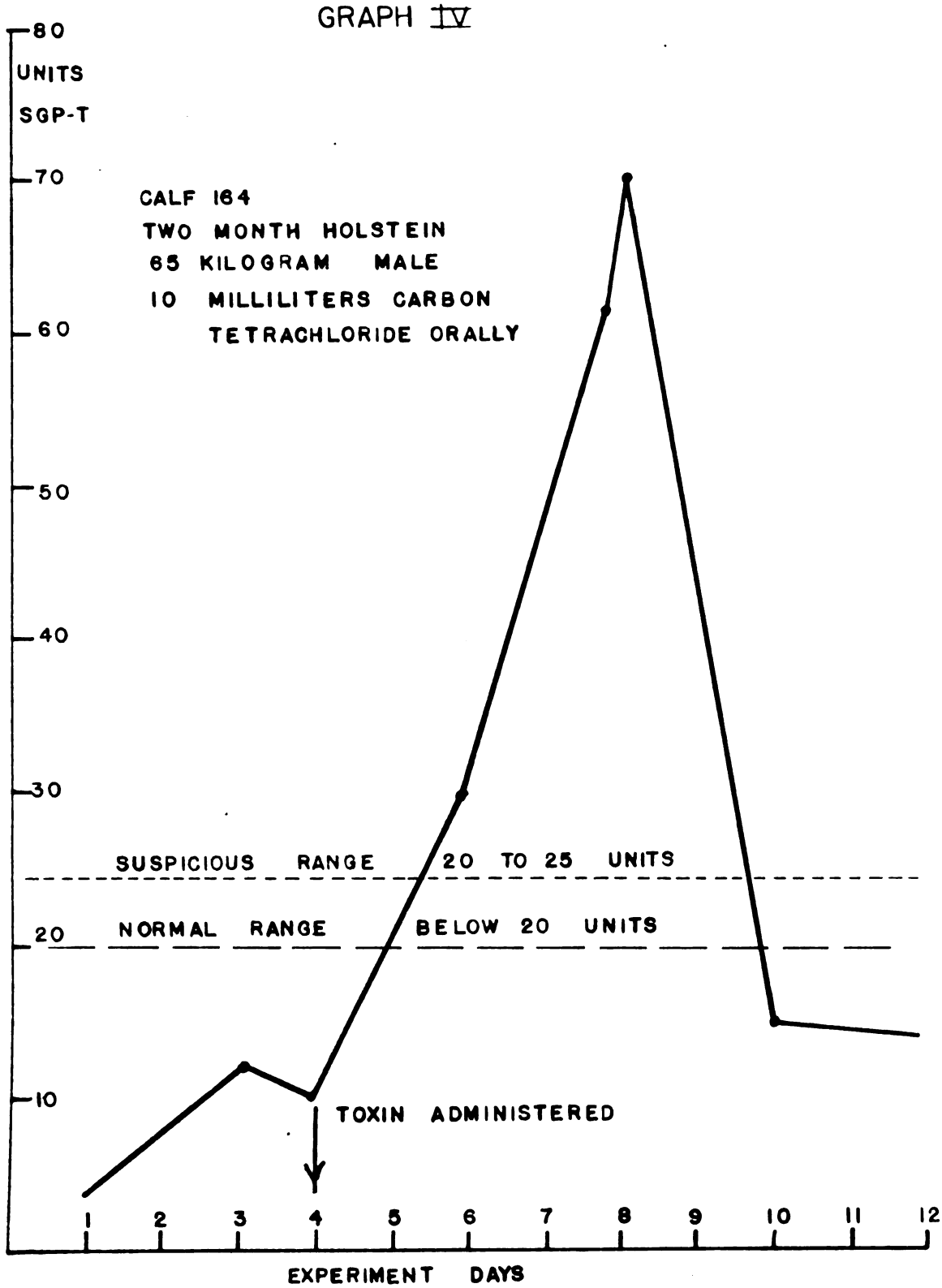

GRAPH I
2000
1000
800
600
400
200
100
90
80
70
60
50
40
30
20
UNITS
SGO-T
1480 UNITS
CALF 163
TWO MONTH HOLSTEIN
70 KILOGRAM MALE
10 MILLILITERS CARBON
TETRACHLORIDE ORALLY
(SEMILOG PAPER)
SUSPICIOUS RANGE 70 TO 80 UNITS
NORMAL RANGE 70 UNITS AND BELOW
TOXIN ADMINISTERED
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
EXPERIMENT DAYS

GRAPH III

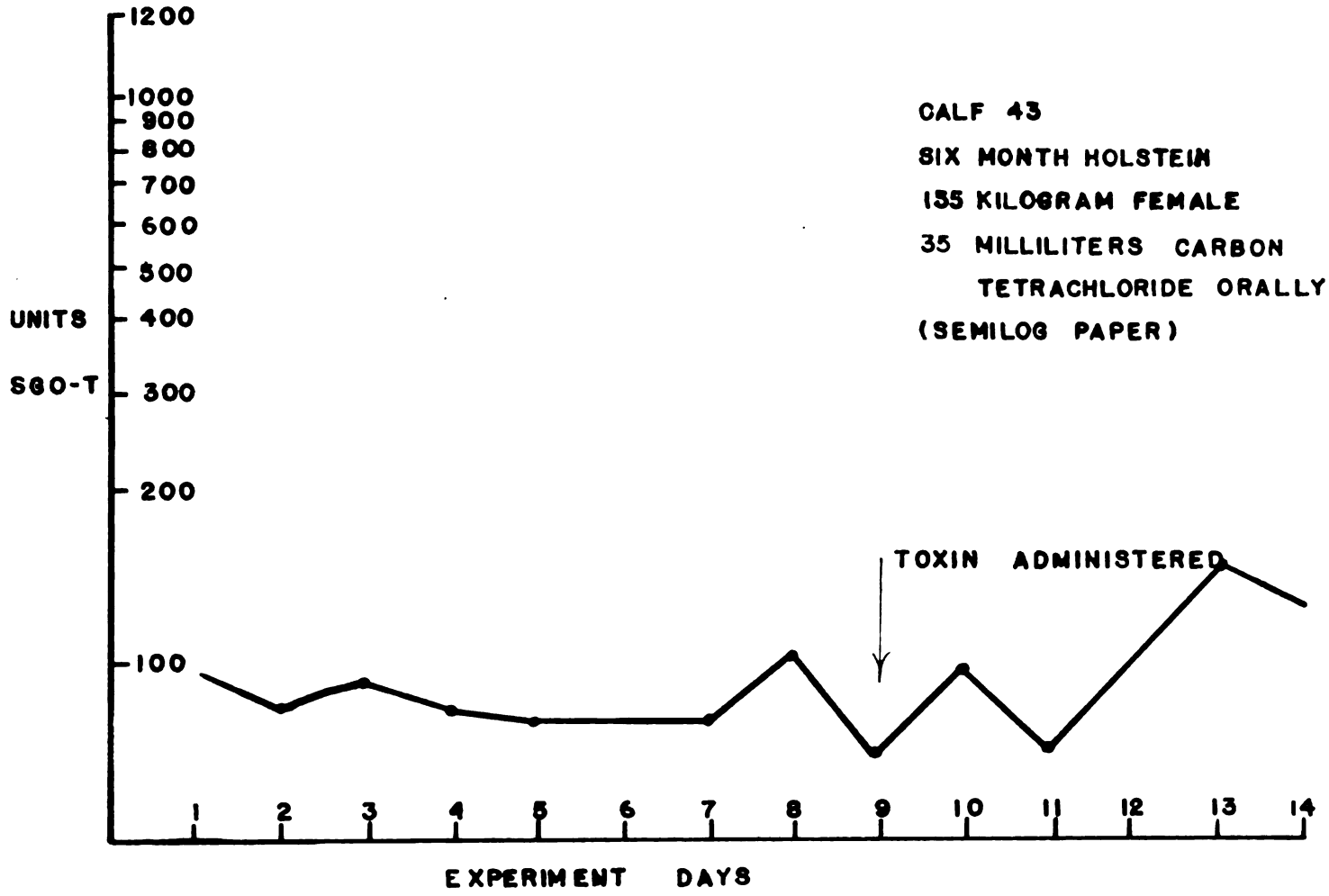

80
UNITS
SGP-T
70
60
50
40
30
SUSPICIOUS RANGE 20 TO 25 UNITS
20
NORMAL RANGE 20 UNITS AND BELOW
10
CALF 163
TWO MONTH HOLSTEIN
70 KILOGRAM MALE
10 MILLILITERS
CARBON TETRA-
CHLORIDE ORALLY
TOXIN ADMINISTERED
1 2 3 4 5 6 7 8 9 10 11 12
EXPERIMENT DAYS


GRAPH III
CALF 164
TWO MONTH HOLSTEIN
65 KILOGRAM MALE
10 MILLILITERS CARBON
TETRACHLORIDE ORALLY
(SEMILOG PAPER)
UNITS
SGO-T
2000
1000
800
600
400
200
100
90
80
70
60
50
40
30
20
SUSPICIOUS RANGE 70 TO 80 UNITS
NORMAL RANGE 70 UNITS AND BELOW
TOXIN ADMINISTERED
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
EXPERIMENT DAYS

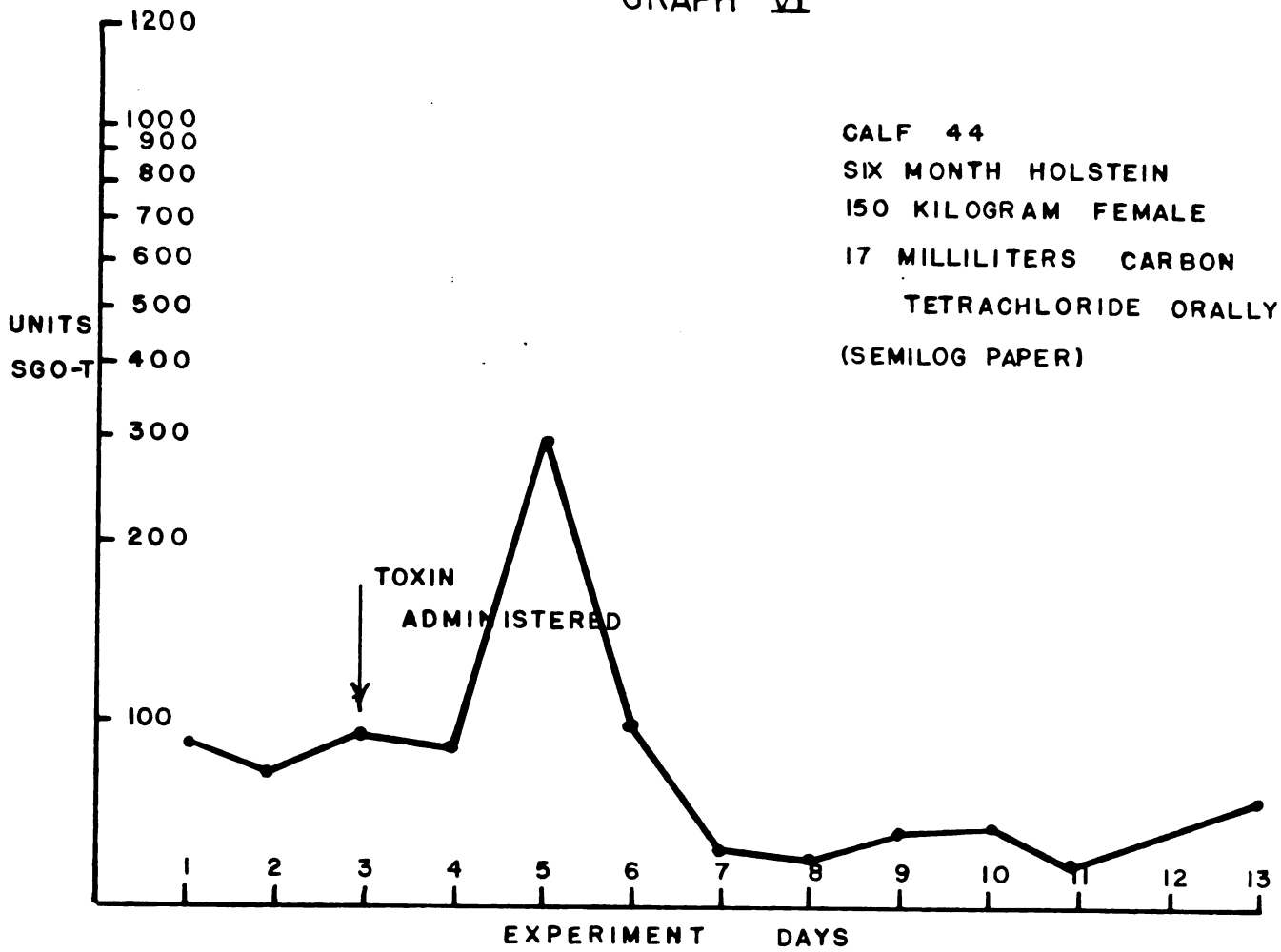
GRAPH IV

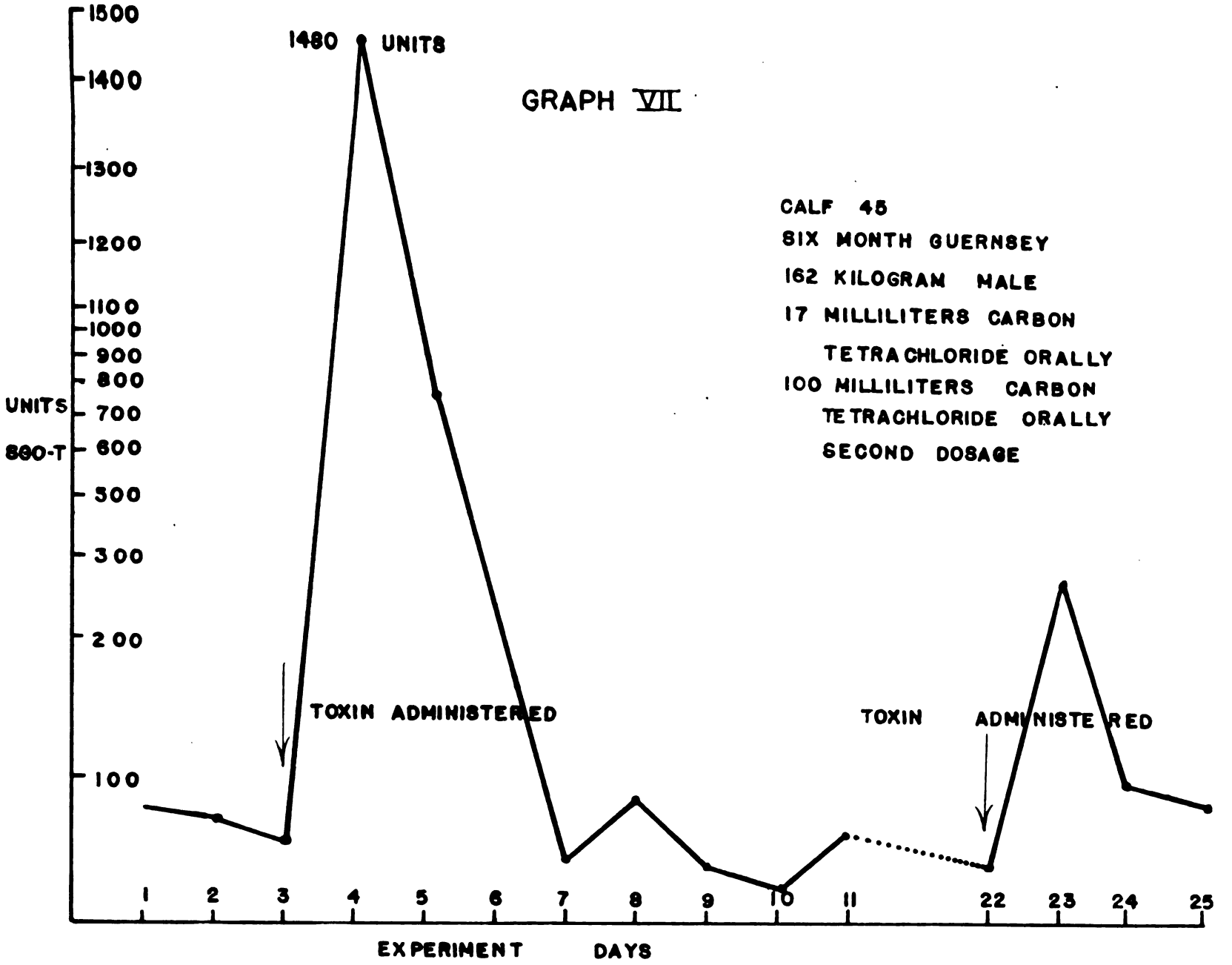

80
UNITS
SGP-T
70
60
50
40
30
20
10
CALF 164
TWO MONTH HOLSTEIN
65 KILOGRAM MALE
10 MILLILITERS CARBON
TETRACHLORIDE ORALLY
SUSPICIOUS RANGE 20 TO 25 UNITS
NORMAL RANGE BELOW 20 UNITS
TOXIN ADMINISTERED
1
2
3
4
5
6
7
8
9
10
11
12
EXPERIMENT DAYS

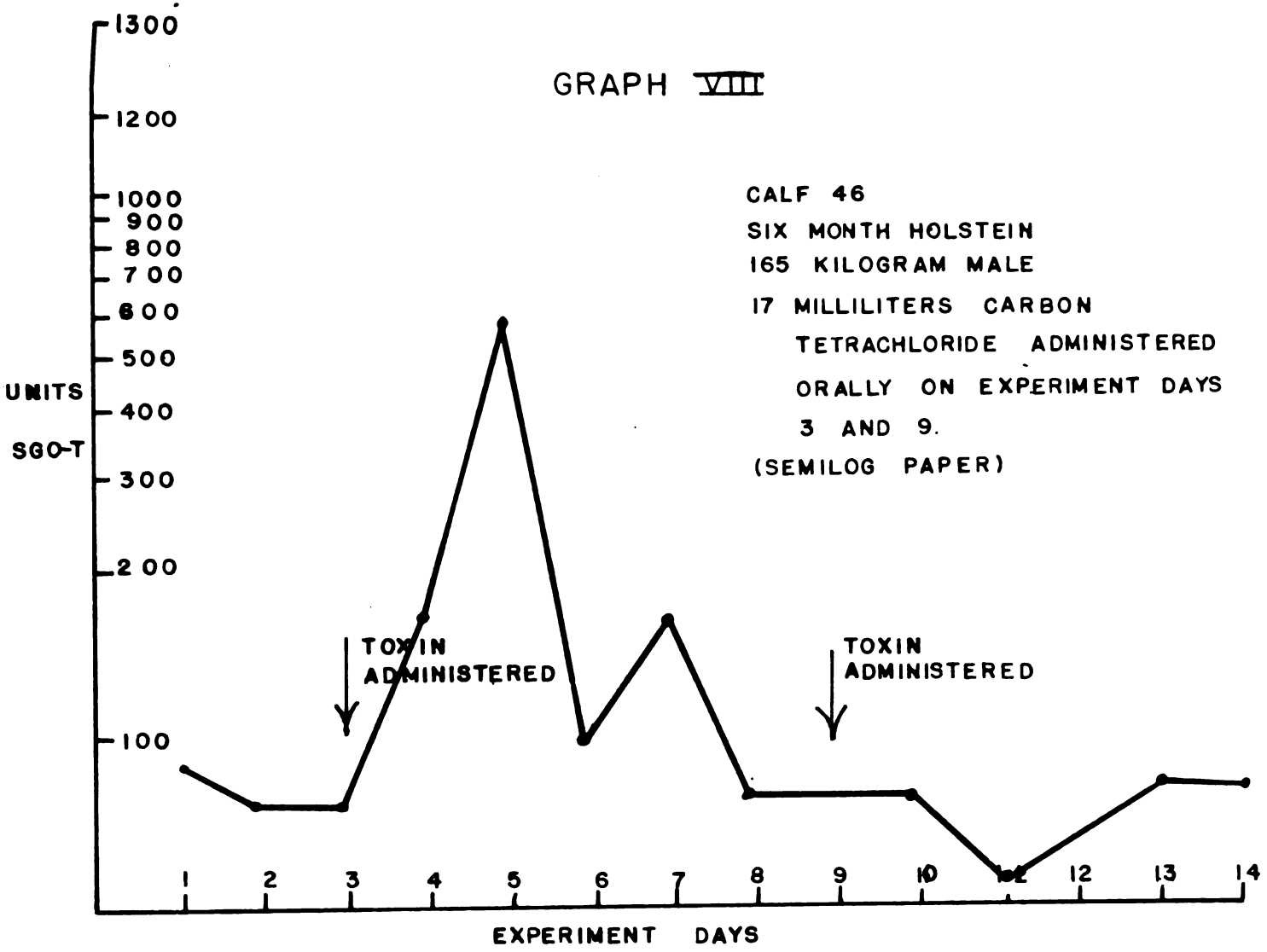
GRAPH V


1200
1000
900
800
700
600
500
UNITS
400
SGO-T
300
200
100
CALF 43
SIX MONTH HOLSTEIN
135 KILOGRAM FEMALE
35 MILLILITERS CARBON
TETRACHLORIDE ORALLY
(SEMILOG PAPER)
TOXIN ADMINISTERED
1
2
3
4
5
6
7
8
9
10
11
12
13
14
EXPERIMENT DAYS

GRAPH VI

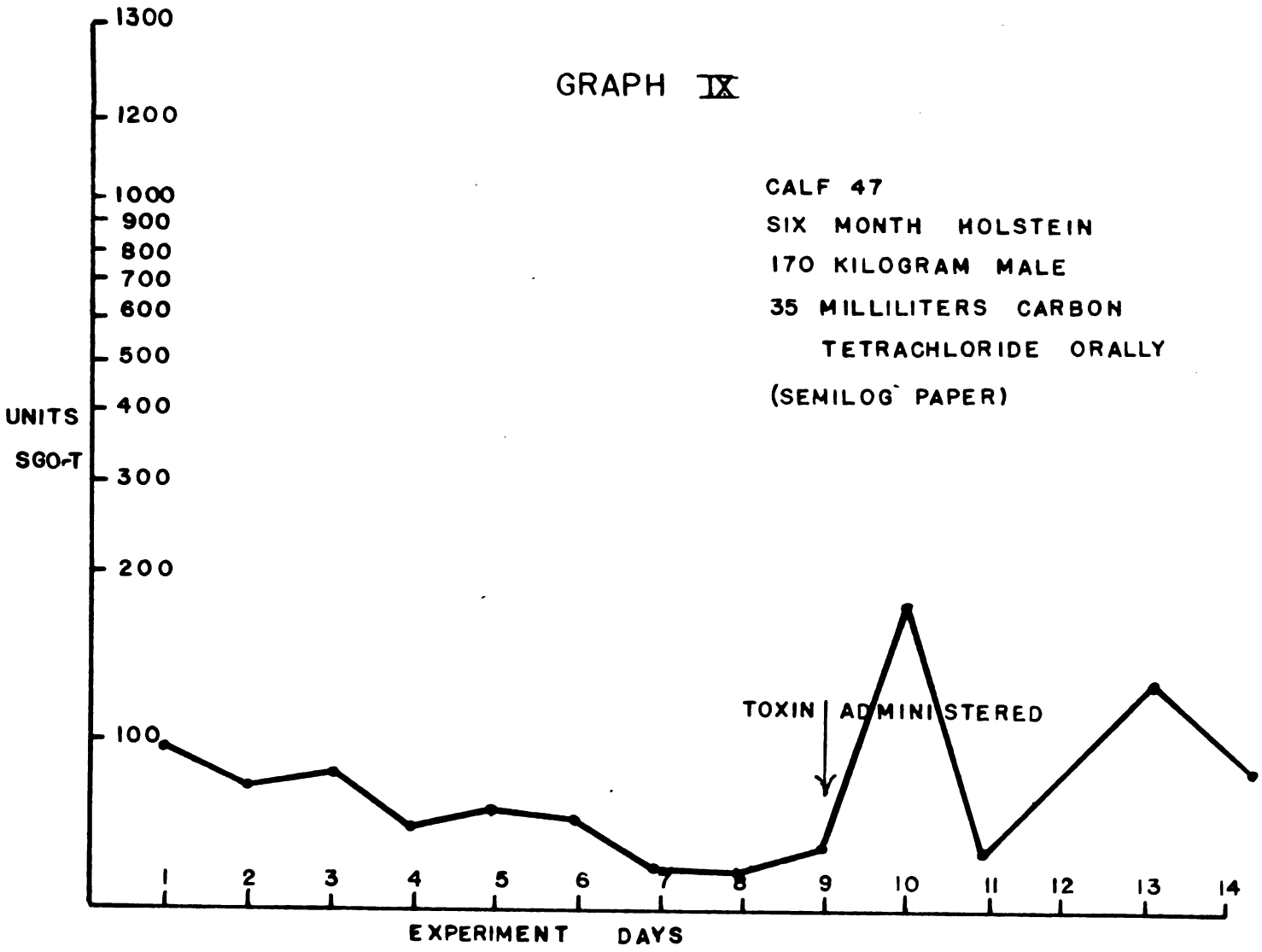

1200
1000
900
800
700
600
500
UNITS
SGO-T
400
300
200
100
TOXIN
ADMINISTERED
1
2
3
4
5
6
7
8
9
10
11
12
13
EXPERIMENT DAYS
CALF 44
SIX MONTH HOLSTEIN
150 KILOGRAM FEMALE
17 MILLILITERS CARBON
TETRACHLORIDE ORALLY
(SEMILOG PAPER)


GRAPH VII
1480 UNITS
CALF 45
SIX MONTH GUERNSEY
162 KILOGRAM MALE
17 MILLILITERS CARBON TETRACHLORIDE ORALLY
100 MILLILITERS CARBON TETRACHLORIDE ORALLY
SECOND DOSAGE
UNITS
1500
1400
1300
1200
1100
1000
900
800
700
600
500
300
200
100
TOXIN ADMINISTERED
TOXIN ADMINISTERED
1 2 3 4 5 6 7 8 9 10 11 22 23 24 25
EXPERIMENT DAYS


GRAPH VIII
CALF 46
SIX MONTH HOLSTEIN
165 KILOGRAM MALE
17 MILLILITERS CARBON TETRACHLORIDE ADMINISTERED ORALLY ON EXPERIMENT DAYS 3 AND 9.
(SEMILOG PAPER)
UNITS
SGO-T
1300
1200
1000
900
800
700
600
500
400
300
200
100
TOXIN ADMINISTERED
TOXIN ADMINISTERED
1
2
3
4
5
6
7
8
9
10
12
13
14
EXPERIMENT DAYS

GRAPH IX

CALF 47
SIX MONTH HOLSTEIN
170 KILOGRAM MALE
35 MILLILITERS CARBON
TETRACHLORIDE ORALLY
(SEMILOG PAPER)



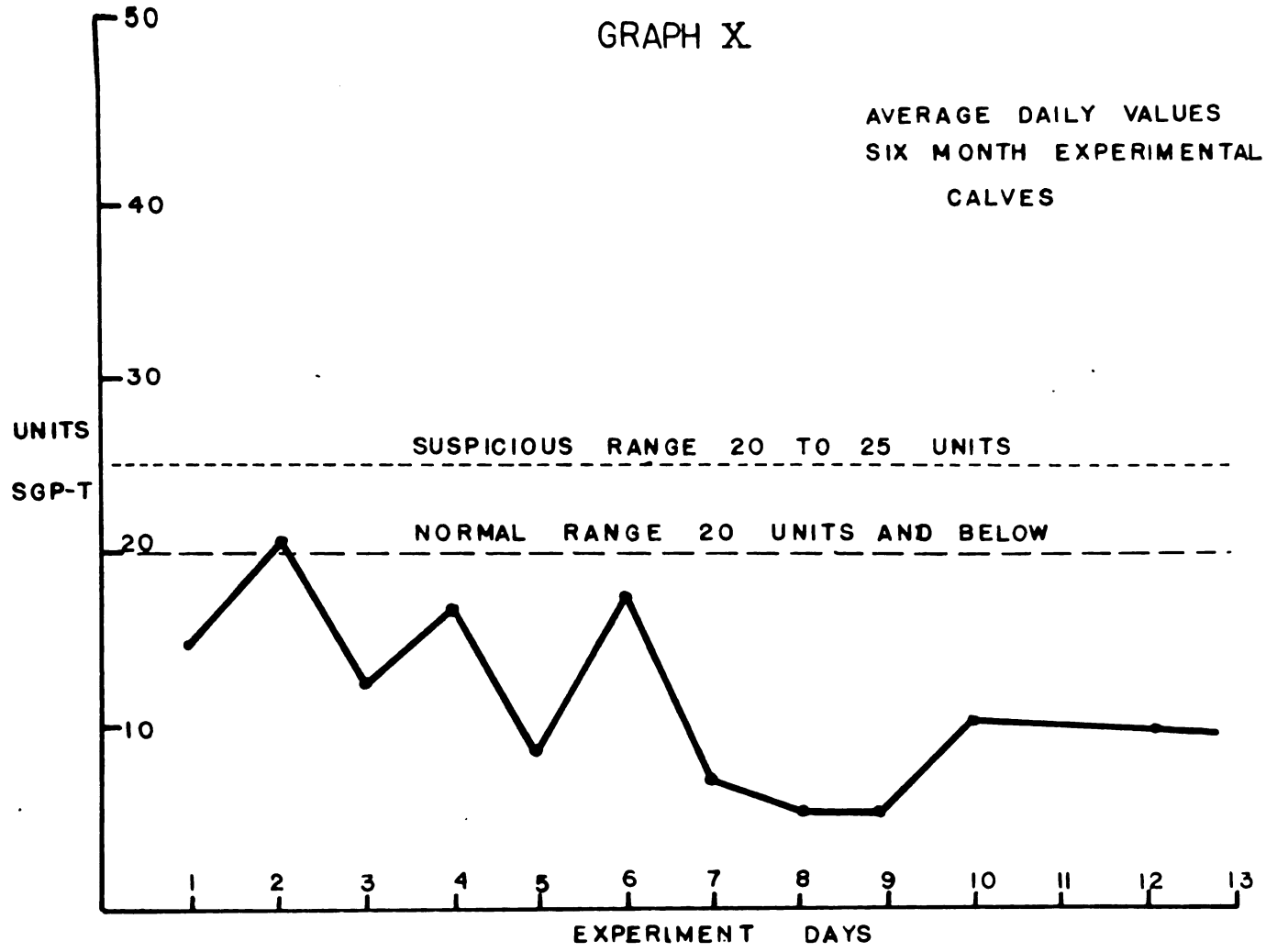

GRAPH X
AVERAGE DAILY VALUES
SIX MONTH EXPERIMENTAL
CALVES
50
40
30
UNITS
SGP-T
SUSPICIOUS RANGE 20 TO 25 UNITS
NORMAL RANGE 20 UNITS AND BELOW
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
EXPERIMENT DAYS

CHART I

Urine Examination Results

Two-month old calves

Experiment day	1				3				5			
Calf number	63	64	65	66	63	64	65	66	63	64	65	66
Specific gravity	1.030	1.037	1.040	1.037					1.035	1.035	1.042	1.032
pH	7.0	7.2	7.0	7.0					7.2	7.0	7.2	7.0
Bilirubin	N*	N	N	N	N	N	N	N	N	N	N	N
Occult blood	N	N	N	N	N	N	N	N	N	N	N	N
Acetone	N	N	N	N	N	N	N	N	N	N	N	N
Albumin	N	N	N	N	N	N	N	N	N	N	N	N

Experiment day	8				11				14			
Calf number	63	64	65	66	63	64	65	66	63	64	65	66
Specific gravity	1.035	1.039	1.040	1.035					1.035	1.037	1.040	1.038
pH	7.0	7.0	7.0	7.0					7.2	7.0	7.0	7.0
Bilirubin	N	N	N	N	N	N	N	N	N	N	N	N
Occult blood	N	N	N	N	N	N	N	N	N	N	N	N
Acetone	N	N	N	N	N	N	N	N	N	N	N	N
Albumin	N	N	N	N	N	N	N	N	N	N	N	N

*N = Negative

CHART II

Urine Examination Results

Six-month old calves

Experiment day			1		
Calf number	143	144	145	146	147
Specific gravity	1.035	1.039	1.039	1.037	1.039
pH	7.5	7.5	7.5	7.5	7.5
Bilirubin	N*	N	N	N	N
Occult blood	N	N	N	N	N
Acetone	N	N	N	N	N
Albumin	N	N	N	N	N
Experiment day			3		
Calf number	143	144	145	146	147
Specific gravity					
pH					
Bilirubin	N	N	N	N	N
Occult blood	N	N	N	N	N
Acetone	N	N	N	N	N
Albumin	tr.	N	N	tr.	N
Experiment day			6		
Calf number	143	144	145	146	147
Specific gravity	1.030	1.037	1.042	1.035	1.030
pH	7.5	7.5	7.5	7.5	7.5
Bilirubin	N	N	N	N	N
Occult blood	N	N	N	N	N
Acetone	N	N	N	N	N
Albumin	N	N	N	N	N
Experiment day			9		
Calf number	143	144	145	146	147
Specific gravity					
pH					
Bilirubin	N	N	N	N	N
Occult blood	N	N	N	N	N
Acetone	N	N	N	N	N
Albumin	N	N	N	N	N
Experiment day			11		
Calf number	143	144	145	146	147
Specific gravity	1.035	1.036	1.042	1.030	1.035
pH	7.5	7.5	7.5	7.5	7.5
Bilirubin	N	N	N	N	N
Occult blood	N	N	N	N	N
Acetone	N	N	N	N	N
Albumin	N	N	N	tr.	N

*N = Negative

CHART III

Prothrombin Clotting Times

Results on six-month old calves

Calf number		43	44	45	46	47
Determination	1	20.0	21.0	19.2	21.6	20.1
	2	20.6	22.2	19.4	22.6	19.4
	3	19.6	21.6	20.0	22.0	21.8

(all times reported in seconds)

CHART IV

Normal Transaminase Values

Two-month old calves

	Number of Samples	Range in Units	Average plus Standard Deviation
SGO-T	25	10-76	54.0 ± 14.6
SGP-T	30	3-22	14.1 ± 4.0

CHART V

Normal Transaminase Values

Six-month old calves

	Number of Samples	Range in Units	Average plus Standard Deviation
SGO-T	36	12-104	59.3 ± 24.2
SGP-T	34	4-40	11.6 ± 8.2

CHART VI

Blood Examination Results

Two-month old calves

Experiment Day	Calf Number	163	164	165	166
	RBC*	7,960	6,750	6,650	6,460
	WBC**	7,680	10,500	7,650	8,750
1	Lymphocytes	60	49	56	53
	Neutrophils	35	45	43	45
	Eosinophils	3	4	1	2
	Monocytes	2	2	0	0
	RBC*	7,780	6,500	6,970	5,980
	WBC**	8,550	9,800	7,900	7,950
6	Lymphocytes	58	52	57	51
	Neutrophils	38	43	41	44
	Eosinophils	4	3	2	4
	Monocytes	0	2	1	1
	RBC*	7,800	6,350	6,780	6,270
	WBC**	8,430	8,750	7,850	8,250
12	Lymphocytes	61	50	54	49
	Neutrophils	37	46	43	45
	Eosinophils	2	3	2	4
	Monocytes	0	1	1	2

* Red blood cells reported in thousands
** White blood cells

CHART VII

Blood Examination Results

Six-month old calves

Experiment Day	Calf Number	43	44	45	46	47
	RBC*	5,360	5,560	6,750	5,780	5,270
	WBC**	9,650	7,350	9,880	7,650	8,750
1	Lymphocytes	47	48	45	48	49
	Neutrophils	50	49	53	49	50
	Eosinophils	2	3	0	3	1
	Monocytes	1	0	2	0	0
	RBC*	6,100	5,860	6,270	5,980	5,780
	WBC**	10,500	8,750	11,600	8,050	9,350
5	Lymphocytes	45	51	48	42	39
	Neutrophils	51	48	49	55	58
	Eosinophils	3	1	2	3	1
	Monocytes	1	0	1	0	2
	RBC*	5,800	6,270	6,050	5,890	6,050
	WBC**	8,650	7,680	10,050	7,850	8,950
11	Lymphocytes	50	45	52	48	49
	Neutrophils	49	50	47	49	49
	Eosinophils	1	3	1	2	0
	Monocytes	0	2	0	1	2

* Red blood cells reported in thousands
** White blood cells

CHART VIII

Results of Serum Protein and Paper Electrophoresis Determinations

Six-month old calves

Calf 43

Experiment Day	1	2	3	5	7	9	11	13
Total serum protein	6.3	6.5	5.8	6.5	6.8	6.8	6.4	6.3
Percent albumin	46.8	48.5	48.3	47.5	45.7	46.9	47.5	47.8
Percent alpha globulin	14.6	14.2	12.8	14.0	13.9	14.1	14.2	14.0
Percent beta globulin	13.6	13.5	12.1	13.5	13.6	13.4	13.2	13.9
Percent gamma globulin and fibrinogen	24.9	23.8	26.8	25.5	25.8	24.9	25.8	24.2

Calf 44

Experiment Day	1	2	3	5	7	9	11	13
Total serum protein	5.7	6.3	6.2	6.8	6.2	6.8	6.5	6.0
Percent albumin	45.7	46.9	46.0	46.2	47.2	45.9	46.1	47.1
Percent alpha globulin	17.6	13.9	14.7	16.1	14.1	15.2	15.7	14.9
Percent beta globulin	14.6	12.6	12.7	12.7	12.7	15.1	13.8	13.9
Percent gamma globulin and fibrinogen	21.5	26.9	26.9	24.5	26.0	22.3	24.4	23.5

CHART IX

Results of Serum Protein and Paper Electrophoresis Determinations

Six-month old calves

Calf 45

Experiment Day	1	2	3	5	7	9	11	13
Total protein	6.5	6.1	6.5	6.8	6.7	6.7	7.0	6.8
Percent albumin	51.3	50.2	51.5	50.1	49.8	49.7	50.1	51.7
Percent alpha globulin	15.6	13.7	13.5	14.9	15.2	15.1	15.7	15.9
Percent beta globulin	15.1	15.0	13.5	14.8	15.3	13.9	14.8	15.1
Percent gamma globulin and fibrinogen	17.9	20.0	21.2	20.2	19.7	20.1	19.4	17.3

Calf 45

Experiment Day	22	23	24	25	26
Total protein	6.7	7.0	6.8	7.0	6.5
Percent albumin	51.2	50.4	51.9	50.1	50.7
Percent alpha globulin	15.1	14.8	14.9	16.0	15.2
Percent beta globulin	15.0	15.3	15.4	13.9	14.8
Percent gamma globulin and fibrinogen	15.6	19.5	17.4	19.9	18.9

CHART X

Results of Serum Protein and Paper Electrophoresis Determinations

Six-month old calves

Calf 46

Experiment Day	1	2	3	5	7	9	11	13
Total protein	6.7	6.4	6.3	6.5	6.2	6.5	6.7	6.3
Percent albumin	45.5	45.9	44.9	45.2	46.0	44.5	44.8	45.4
Percent alpha globulin	12.9	12.6	13.0	12.8	12.1	13.2	12.7	12.6
Percent beta globulin	13.6	13.2	13.6	13.7	13.8	14.1	13.1	14.0
Percent gamma globulin and fibrinogen	28.0	29.5	28.3	27.2	27.7	28.1	28.3	27.8

Calf 47

Experiment Day	1	2	3	5	7	9	11	13
Total protein	6.5	7.0	6.8	6.3	6.5	6.7	6.5	6.8
Percent albumin	45.7	43.9	44.7	45.3	46.0	47.6	45.5	44.9
Percent alpha globulin	11.5	10.9	11.9	11.2	11.7	11.2	12.6	12.6
Percent beta globulin	12.8	12.9	12.7	12.6	12.2	13.0	12.6	12.6
Percent gamma globulin and fibrinogen	29.2	32.2	31.1	29.9	29.3	28.1	30.9	28.6

CHART XI

Serum Bilirubin Determination Results

Two-month old calves

Experiment Day	1		3		5		8	
	1 min.	30 min.	1 min.	30 min.	1 min.	30 min.	1 min.	30 min.
Calf 163	.10	.84	.05	.55	.00	.24	.10	.63
Calf 164	.16	.88	.20	.59	.20	.59	.20	.35
Calf 165	.15	.38	.24	1.02	.47	.97	.10	.88
Calf 166	.00	.93	.43	.67	.84	.97	.05	.43

DISCUSSION

As described in the results the calves were judged to be normal clinically before any experimental work was begun. The temperature range, blood and urine examinations plus the use of clinical observation and auscultation would substantiate this view.

The urine examinations, although showing a transient albuminuria on three occasions, were not found to correlate with renal toxicity of carbon tetrachloride. Since kidney tissue contains a large amount of GO-T the possibility of kidney damage had to be ruled out. The results as shown in Chart I and II would lead one to believe that any kidney damage was of a minor nature, if present at all. These tests do not rule out the possibility of cellular changes that could impair renal function undetected by these tests. They do show that any damage was slight enough to be overlooked with routine urine examinations.

The use of a urine test for bilirubin is a form of liver function tests. Both obstructive jaundice and hepatitis with intrahepatic obstruction result in increased urine bilirubin (40). The urine bilirubin was never elevated in this experiment.

Prothrombin clotting time has been used as a liver function test and also to check the clotting time of blood. In this study the purpose of prothrombin clotting times was to anticipate any untoward reaction due to hemorrhage

following liver biopsy. All levels found during the experiment were within the lowest ranges as reported in the literature (27). Prothrombin times were not determined on the two-month old calves.

The high degree of stability of both SGO-T and SGP-T facilitates its clinical usefulness. Freezing and lyophilization of serum fail to influence either SGO-T or SGP-T. Serum and plasma have equivalent activities, and storing at room temperature for 24 hours, at refrigerator temperature for up to three weeks (29) (53), or at four degrees Centigrade for five days does not significantly influence SGP-T levels. The daily variations in the normal adult are insignificant. Reports vary as to the effect of the fasting state on SGO-T and SGP-T levels (7) (29) (57). Normal pregnancy does not influence SGO-T levels (31).

The mechanism of release of SGO-T and SGP-T from the damaged cells is thought to be release of GO-T upon damage to the cells. The mechanism of excretion from the body is unknown at this time. The concentration in the bile has been shown to be much greater than the serum levels in the same animal (9). The possibility of biliary excretion has been postulated by this fact, and also because SGO-T levels are increased following the use of the bromsulphalein (BSP) liver function test. One explanation of the increased level is that the BSP may be in competition for a common excretory mechanism (44). For this reason BSP was not used in this study.

GO-T is cleared from the blood at a considerably faster rate than is GP-T. This may be due to the smaller molecular size of GO-T (60,000) as compared to GP-T (180,000) (17) (20). The absence of GO-T in the urine is probably due to impermeability of the glomeruli and the large size of the GO-T molecule (16).

The normal values for SGO-T in the two-month old calves determined in this experiment fell well within the range described in the literature (32). They are much lower than those values in adult cattle (26).

A normal range of activity for SGO-T was arbitrarily established to include 93 percent of all normal samples. This is the average value with the addition of one standard deviation. Since there is the possibility that some normal values would be above this range, a suspicious level was fixed to include 100 percent of all normals. There is the possibility that some animals having slight liver pathology would have SGO-T levels in the suspicious level. The determination of serial samples taken in 12 to 24 hours would be indicated in such cases to check for changes in SGO-T levels.

The normal range of SGO-T in two-month old calves is up to 70 units, and the suspicious level is from 70 to 80 units.

At this time there are no reports in the literature for normal SGP-T levels for two-month old calves. Therefore normal values were established for this experiment

in a similar manner to that used for SGO-T. The normal values average 14.1 units and up to 20 units was considered normal for two-month old calves. Again a suspicious level was fixed to include 100 percent of the normal values. The suspicious level for SGP-T in two-month old calves is from 20 to 25 units.

Since the values determined for the two-month old calves were lower than those for SGO-T reported for adults in the literature, a second set of normal values for six-month old calves was devised.

Again a normal range and a suspicious level were fixed for both SGO-T and SGP-T in six-month old calves. The normal SGO-T range for six-month old calves is 0 to 85 units, and the suspicious level is from 85 to 95 units. The normal range of SGP-T levels for six-month old calves is 0 to 20 units. The suspicious level is from 20 to 25 units.

Erythrocyte, leucocyte, and differential leucocyte counts all fell within the normal ranges reported. The values for the blood examinations are shown in Charts VI and VII. The values being normal substantiate the view that the calves were normal throughout this study.

Serum paper electrophoresis was used to determine the effect of acute liver injury on serum proteins. Since this was an acute experiment the results were not conclusive. The variations noted on daily serum samples could be within the range of human error. The results obtained were within the ranges reported for the bovine (5) (46).

A peak was often noticed at the point of application of the serum to the paper strip. This fraction appears in association with the gamma globulin fraction of the blood. The 16 hour, 2.5 milliampere, constant current system used does not give the best resolution of bovine gamma globulin. It does give good resolution of albumin and alpha globulin. It was felt the albumin-globulin ratio could be determined adequately by the results obtained. It has been reported possible to reduce the amount of foreign material at the site of serum application to the paper strip by centrifuging or filtering (25). Because all samples received similar treatment, and the peak did not effect the albumin-globulin ratio, neither special centrifuging or filtering were attempted.

TWO-MONTH OLD EXPERIMENTAL CALVES

The elevations of both SGO-T and SGP-T seen in the two-month old calves due to carbon tetrachloride can be explained by microscopic examination of hepatic biopsy specimens. In all instances while the transaminase levels were within normal range, and throughout the experiment on control calves, the livers appeared histologically normal.

Two days following toxin administration both SGO-T and SGP-T were elevated and the liver specimens showed generalized changes throughout the lobule. The normal cord cell arrangement was no longer present. The cells showed cloudy swelling, hydropic changes, fatty changes,

and pyknosis of numerous nuclei. The total dose of carbon tetrachloride administered to the two experimental calves was ten milliliters per calf. This is a low dosage on a milliliter per kilogram basis, but the lack of literature on this subject prompted the use of small dosages. These calves were not ruminating to any great extent and it was assumed they would react to carbon tetrachloride similarly to the simple stomached animal.

The biopsy specimens never showed extensive centrilobular zonal necrosis as reported in the literature (34) (54). The biopsy specimens taken 144 hours after toxin administration were judged to be normal. This agrees with the literature in that the cytoarchitecture of the liver was restored in five to six days following carbon tetrachloride administration in the rat (34).

The possibility remains that a representative sample of the liver was not obtained at biopsy. According to the literature the punch biopsy is the best type for obtaining a representative sample of the liver because artifacts are avoided and a deeper, more representative sample is obtained (30) (51).

If there was a representative sample of the liver obtained, then it is possible to say that both SGO-T and SGP-T were elevated in two-month old calves following carbon tetrachloride administration when the livers showed no necrosis. The literature states that elevations were seen with necrosis of tissue. Elevations were seen in this

experiment with early cellular degenerations. These cellular degenerations may lead to necrosis, but this was never seen on subsequent biopsy specimens (52).

There was no correlation between serum transaminase levels and other tests of liver function used in this experiment. There does appear to be a correlation in the amount of tissue damage as judged microscopically and by the height of the SGO-T and SGP-T rise. The normal appearing livers were associated with normal levels of SGO-T and SGP-T, while the pathological appearing livers were associated with increased SGP-T and SGO-T levels.

The initial rise of SGO-T occurred 24 hours before the rise in SGP-T. This can probably be explained by the greater concentration per gram of liver tissue of SGO-T as compared to SGP-T. With greater amounts of tissue destruction the elevations of SGP-T should increase to greater levels. Since neither the SGO-T or SGP-T rose to levels as high as reported in the literature for carbon tetrachloride poisoning, it was assumed the liver damage was most likely less in this experiment.

The SGO-T and SGP-T ratio was always greater than one, even at the peak elevations of both enzymes. The SGP-T levels are elevated enough to be of diagnostic value, but with generalized necrosis would probably be elevated more.

The elevations in SGO-T for three days following the return to normal of SGP-T values are difficult to explain. According to the literature, SGO-T is cleared from the

blood stream at a faster rate than SGP-T (17). This is probably due to the smaller molecular size of GO-T as compared to GP-T (20). The elevations seen for ten days in the SGO-T, and only seven days in SGP-T, may be caused by the quantitative differences at the peak levels. Since SGO-T and SGP-T reach equilibrium with the interstitial fluid at six to twelve hours following peak blood levels (16) (17), it follows that clearance from the body fluid would require more time for return to the vascular system.

SIX-MONTH OLD EXPERIMENTAL CALVES

In this group of calves elevations were seen only in SGO-T following administration of carbon tetrachloride. Varying dosages of carbon tetrachloride were tried in an attempt to cause a rise in the SGP-T levels. The SGO-T levels attained were lower at their peak than the peak levels reached in the two-month old calves.

Two calves received 17 millliliters of carbon tetrachloride orally followed by 25 milliliters of mineral oil. Elevations in the SGO-T levels were noted in twelve hour serum samples. Peak elevations were seen in the 24 hour serum samples. These levels of SGO-T were elevated for 84 hours. The peak elevation in one calf, 45, reached 1,480 units, while the peak level for the other calf, 44, was only 300 units. The SGP-T levels were not elevated.

Microscopic examination of biopsy specimens from these calves helps explain the small and transient elevations in the SGO-T levels. The liver appeared to be

involved with focal areas of necrosis. The liver biopsy from the calf, 45, with the higher peak elevation in SGO-T showed extensive coagulation necrosis with hemorrhage, surrounded by vacuolation from fatty changes, cloudy swelling and hydropic degeneration. The other 24 hour biopsy specimen (calf 44) showed only very small areas of focal necrosis and some lymphocytic infiltration upon microscopic examination. These findings agree with the reports in the literature correlating amount of liver cell damage and elevations of SGO-T (15).

The failure of the SGP-T to rise can be explained in calf 44 by the relatively minor amount of liver tissue necrosis. The amount of SGP-T would not be great enough to elevate the serum level. An explanation for the lack of elevation in the SGP-T in calf 45 will be discussed later in this paper.

In an attempt to produce greater elevations in the SGO-T, larger volumes of carbon tetrachloride were administered to two calves. Thirty-five millliliters of carbon tetrachloride followed by 25 millliliters of mineral oil were administered to calves 43 and 47.

Elevations in SGO-T were very slight. The peak elevation was 176 units (calf 47) and SGO-T was elevated only three days. SGP-T was not elevated in either calf.

Biopsy specimens 24 hours after administration of the toxin showed focal areas of necrosis. Cloudy swelling and vacuolation due to fatty changes were evident surround-

ing the areas of necrosis. An explanation for the lack of elevation in the SGO-T or SGP-T, in spite of liver necrosis, cannot be made at this time.

Calf 46 received two 17 milliliter doses of carbon tetrachloride six days apart. The first dosing resulted in an elevated SGO-T level for 84 hours with a peak elevation of 600 units. No elevation of SGP-T was noted. The 24 hour biopsy specimen showed focal areas of necrosis and hemorrhage. The cells showed evidence of cloudy swelling, while the nuclei in the area showed pyknosis.

The second dosage of 17 milliliters of carbon tetrachloride was not accompanied by an elevation in the SGO-T level. The only change noted in the 24 hour biopsy specimen was albuminous degeneration which is an early stage of cloudy swelling. The next biopsy specimen taken at 96 hours appeared normal.

In an attempt to produce massive necrosis 100 milliliters of carbon tetrachloride were administered by stomach tube to calf 45. A mild elevation in SGO-T was seen with a peak of 280 units, and persisted for three days. In this case the SGP-T was elevated, in the 24 hour sample only, with a peak of 68 units.

The 24 hour biopsy specimen demonstrated a loss of normal hepatic architecture and focal areas of necrosis. The SGP-T level was at normal levels when the 48 hour biopsy specimen was taken. The 48 hour biopsy specimen showed focal areas of necrosis, pyknosis of nuclei, some cloudy swelling and restoration of the hepatic architecture.

In an attempt to explain the failure of carbon tetrachloride to raise the SGO-T and SGP-T in six-month old calves the factor of rumination was considered. There appears to be some mechanism in ruminating calves that increases their resistance to oral carbon tetrachloride. Factors which might influence this such as ruminal dilution, biochemistry of the rumen and biochemistry of bovine hepatic tissue were looked for in the literature, but to no avail. The problem still remains unanswered.

The damage to the livers from carbon tetrachloride in the six-month old calves could not be correlated with the quantity administered. The damage produced to the livers was variable and could not be correlated with the SGO-T or SGP-T levels in these calves. Biopsy specimens did show the liver damage, but the reasons for the lack of elevations of SGO-T and SGP-T cannot be offered on the basis of this study.

The liver biopsy punctures described in this paper resulted in no elevations in either SGO-T or SGP-T. At no time did a level of either SGO-T or SGP-T rise in a control calf following biopsy.

SUMMARY

Normal values for SGO-T and SGP-T in both two- and six-month old calves are presented. SGO-T is a more sensitive index of liver cell destruction than SGP-T in the bovine calf when judged by liver biopsy. There possibly is a correlation between the amount of damage as seen on biopsy and the rise in SGO-T levels in two-month old calves. No such correlation could be determined in the six-month old calves.

Neither SGO-T or SGP-T could be correlated with the total serum protein alterations, albumin-globulin alterations, clinical observations or urine bilirubin determination.

There appears to be some mechanism of detoxification of carbon tetrachloride that is associated with ruminating bovines. Carbon tetrachloride is still capable of causing necrosis in ruminating calves when administered orally, but not in proportion to that seen in nonruminating calves.

Hepatic biopsy as performed in this study was associated with little if any undesirable side effects and could be a valuable diagnostic tool in bovine medicine. A biopsy needle of slightly larger diameter to obtain more tissue for microscopic examination would be advantageous.

CONCLUSIONS

1. Serum glutamic oxalacetic transaminase is a sensitive index of liver cell damage caused by carbon tetrachloride in the two-month old bovine.

2. Serum glutamic pyruvic transaminase is not as sensitive as serum glutamic oxalacetic transaminase as an indicator of liver cell damage due to carbon tetrachloride in the young bovine.

3. There is no correlation between the serum glutamic oxalacetic transaminase, or serum glutamic pyruvic transaminase and any of the liver function tests used in this study.

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