OBSERVATIONS ON ANAPLASMOSIS IN THE CALF

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CHAPTER I

INTRODUCTION

Anaplasmosis is an infectious disease of animals of the genera, Bos, Ovis, Capra and some species of Bison and Cervidae. The disease is characterized by a rapid progressive normocytic normochromic anemia. In the convalescent animal the anemia becomes a macrocytic hypochromic anemia. As the animal recovers, the erythrocytes subsequently return to normal numbers, size and hemoglobin content (1).

Anaplasma marginale (Theileri) is the name given to small round bodies, 0.5_u to 1.0_u in diameter, which are consistently found in the erythrocytes of animals experiencing the progressive phase of the anemia.

The infective form has an average diameter of 0.30_u (2), and eight or more subunits in a more or less spherical arrangement with or without variously shaped delicate projections. The organism's oxygen requirements lie somewhere between those of the virus and the malarial protozoan parasite (3). The causative agent is transmitted mechanically by transfer of intact erythrocytes by fomites and by insects of the genera, Tabanus and Culicoides. It is biologically transmitted by the genus Ixoididae (1).

Although the question of distinctly different isolates is still undecided, Murphy (4) reports an apparently successful cross-challenge

of a splenectomized calf infected initially with the Florida-isolate (or strain). He cross-challenged with a California-isolate (or strain). (See Appendix, Figure 46). Other workers have reported differences in isolates from various parts of the United States (5).

The clinical signs and symptoms of anaplasmosis in cattle are those characteristic of a severe anemia. These signs and symptoms are usually not evident until there has been a 40 to 60 percent reduction in the erythrocyte packed cell volume (PCV). The onset of the disease is signaled by depression, weakness, anorexia and fever followed in two to four days by weight loss, dyspnea, diarrhea or constipation, rumen atony, pale mucus membranes, tachycardia, irregular heart sound and, in some cases, icterus (6).

It has been suggested that upon entering the new host, the infective agent has a short development period in the bone marrow, after which it infects an increasing number of certain stages of the maturing erythroid cell (1). There is a 10-20 percent reduction in bone marrow erythroblasts that starts about ten days prior to the appearance of 1 percent infected erythrocytes.* This apparent suppression continues for about 6 days after the appearance of 1 percent infected erythrocytes (7). The infected circulating erythrocytes are rapidly destroyed by hemolysis, causing the typical anemic syndrome (8). The bilirubinglobin from the hemolized erythrocytes is converted to bilirubin and excreted. Icterus, when present, indicates anoxic liver damage and is caused by bilirubin retention. Icterus usually is seen

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*In these cases the average incubation period was 21 days.

after the peak anemia has passed. The recovered animal, in most cases, remains a clinically inapparent carrier of the infectious agent for life (1).

The syndrome in cattle less than one year old is usually mild, often clinically undetectable. The disease is of moderate intensity in yearlings and two-year-olds. In older cattle the disease is usually severe and is frequently fatal (9).

Although anaplasmosis appears to be an acute hemolytic anemia, hemoglobinemia and hemoglobinuria are not apparent (6, 8, 10). An autoimmune mechanism has been proposed as the mechanism of erythrocyte destruction (11, 12). The writer has certain reservations about the statistical validity of work done in this area. There are conflicting laboratory reports (11, 13). Although an autoimmune mechanism has some merit, such a mechanism would not appear to be compatible with the increased susceptibility resulting from splenectomy. The exact mechanism of erythrocyte destruction remains undefined.

The Spleen-(Mysterii Plenum Organon-Galen)

As previously mentioned, various writers have cited the increased susceptibility of the bovine animal to anaplasmosis after splenectomy (1, 9). It has been shown that the removal of the spleen has a marked influence on the course of anaplasmosis, the course of the disease being more severe in the splenectomized calf (4). Splenectomy of the carrier calf cause a severe relapse to the anemic phase of the disease (4, 14).

The spleen is a large gland-like, but ductless, organ situated

in the upper part of the abdominal cavity on the left side. It has a flattened oblong shape and a pliable consistency (15). Sisson (16) describes the bovine spleen as being 50 cm. by 15 cm. in size, weighing approximately 900 Gms. and being equal to 1/6 of one percent of the animal's body weight. With the possible exception of the hemal lymph nodes, all the lymphatic tissue of the body that is specialized to filter blood is concentrated in the spleen (17). Recent evidence indicates rather conclusively that the spleen has a closed circulation (18). Krumbhaar (19) states that the spleen is directly concerned with fetal erythropoiesis and that, upon physiological demand during later periods of life, it may revert to an erythropoietic status.

Many functions have been ascribed to the spleen. It removes "wornout" erythrocytes from the circulation and manufactures bilirubin from the hemoglobin in these cells. It liberates this bilirubin for processing by the liver. Iron is also extracted from the hemoglobin in these cells for future use by the bone marrow. Many of the circulating lymphocytes and probably most of the circulating monocytes are produced in the spleen (17).

The sequestration of erythrocytes appears to be dependent upon the degree of erythrocyte injury rather than the type of injury. Slightly injured erythrocytes are removed from the circulation almost exclusively by the spleen. Moderate injury causes removel by both the spleen and the liver. Cells severely damaged are removed at a very high rate by the liver. It is suggested that the spleen has the capability of detecting smaller changes in the erythrocytes undergoing various types of damage (20).

Bennett and co-workers have reported the active removal of micro-organisms from the circulation by the spleen (21); weight for weight, it is more effective than the liver (22).

Hypersplenism has been used to describe "hypernormal" splenic function, i.e., a larger-than-normal spleen associated with anemia, leukopenia, granulopenia, and thrombopenia usually accompanied by concomitant bone marrow hyperplasia. Following splenectomy, the blood picture usually becomes normal (23).

Many writers list the spleen as a specific reservoir of blood (24, 25, 26, 27). Schalm (27) states that the spleen is a significant reservoir of erythrocytes in the dog and the horse. Zetterstrom (28) has shown that this function in the dog is greatly impaired by denervation.

Turner has reported highly variable jugular hematocrits in sheep due to splenic pooling (up to 1/4 the erythrocyte mass) as related to neurogenic factors, i.e., handling, excitement, etc. (29). The writer has not observed this variability in the calf.

Following splenectomy, appearance of increases of the following elements have been reported in various animals: leptocytes (target cells), reticulocytes, Howell-Jolly bodies, nucleated erythrocytes, siderocytes, and Heinz bodies (30, 31). According to Craddock (32), there is evidence that the erythrocyte becomes less fragile, i.e., more resistant to destructive agents and hypotonic salt solutions.

In the dog a marked, but sometimes inconsistant, postsplenectomy anemia occurs due to inhibition, or lack of stimulation, of erythropoiesis (33). The writer has not observed this postsplenectomy in the

calf. Crosby has suggested that postsplenectomy anemia is due to lost splenic reservoir function and subsequent reduction of the erythrocyte mass (31).

Mezhlumyan's work in the rabbit indicates that significant regeneration of the spleen is possible after extensive subtotal splenectomy. Regeneration can be stimulated by the use of "antireticular cytotoxic serum" and ethanolamine (34). There is evidence for some sort of humoral control to splenic size. Regardless of the number of splenic implants after subtotal or total splenectomy, the final regenerated mass very closely approximates the original splenic weight (31).

Experimental atrophy of the spleen has been induced in animals by removal of the pituitary gland. Conversely, splenectomy has been shown to cause pituitary enlargement (35).

The spleens of the dog and guinea pig have been shown to be capable of removing endotoxin, in vivo (36, 37). However, presumably because of its location in the circulation, it was not found to be significantly important in this role. Splenectomized guinea pigs were no more susceptible to endotoxin than intact animals (37).

The spleen is thought to be a complicating factor in the study of Factor VIII Hemophilia (A.H.F.-Anti-Hemophilic Factor). While it does not produce A.H.F., it has been shown to store significant quantities of A.H.F. (38). Henn (39) reports a greatly shortened coagulation time in the splenectomized dog, but little change under similar conditions in the rat and rabbit.

It is well known that the spleen produces antibodies in response to an antigenic stimulus (17, 39, 40, 41). Although detectable anti-

body production may or may not indicate immune protection (42), several writers report positive correlations between increases in the incidence of fulminating infections and splenectomy of the less than one year old child (43, 44). Rogers, et al. (45) have shown a positive relationship between the concentration of circulating globulin components and Anaplasma complement-fixing (CF) titer.

Schenberg (46) has demonstrated that as a primary response to an antigenic stimulus there is a rapid appearance of 19S macroglobulin antibodies. He presents strong evidence for the production of 19S globulin by large basophilic cells in the spleen (which resemble, and may be, large lymphocytes) and production of 7S globulin by plasma cells. This is consistent with Andersen's (47) report that splenectomy significantly decreases circulating gamma-M-macroglobulins (19S) in the guinea pig. A significant decrease in circulating immunoglobulins has been shown in the rat after 80 percent subtotal splenectomy (49). Borel (41) quotes Davidson as saying that the rabbit spleen is responsible for the formation of 7S antibody in the anamnestic response. He also states that the 19S antibody "immunologic memory" is short in contrast to that of the 7S antibody.

The study of the pre and postsplenectomy responses is somewhat complicated by the observation of various writers that the antibody response is dependent upon antigen, dose and route of administration. (40, 46, 47, 48, 49). Saslaw (40) has shown that there is no difference in antibody response of the intact splenectomized monkey when the antigen was given via the subcutaneous route. However, when the

antigenic stimulus was given intravenously, there was significantly less antibody response in the splenectomized animals than in the control intact group.

Borel (41) states that in the neonatal rabbit large antigen doses, given intraperitoneally, produce a persistant anamnestic response in the 19S antibody. The same response is seen with a small intradermal dose. Small intravenous antigen doses produce only a 7S anamnestic response capability.

In intraperitoneal injections of mice with sheep erythrocytes, the sequence of antibody production was found to be spleen, pancreatic lymph nodes and mesenteric lymph nodes (50). In the mouse and rabbit the lymph nodes developed immunologic competance 48 hours later than splenic cells (50). Murphy (4) has shown that the bovine animal responds with 19S - 7S antibody production during anaplasmosis. (See Appendix - Calf 84E and Calf 1049, Figures 45, 46, 47, 48).

The observations cited above seem to verify the words of Krumbhaar (19):

"The mammalian spleen, while not necessary for the maintenance of normal existence and sharing many of its functions with other members of the hemolyto-poietic system, is useful in several ways and an organ whose presence under certain stresses may even be the deciding factor between life and death. Its functions are largely indicated by its structure, its reticuloendothelial cell content and by the changes produced in other organs by its absence."

CHAPTER II

EXPERIMENTAL DESIGN AND METHODS

This study was undertaken in an attempt to better describe the relationship of the spleen to the anemia of anaplasmosis in the calf. There are five treatment groups.

Classification of Treatment Groups

Treatment Group I. The primary disease in the intact calf: six normal uninfected* calves were infected with anaplasmosis and the subsequent disease course recorded.

Treatment Group II. The primary postsplenectomy anemia relapse in the calf: six anaplasmosis carrier calves were splenectomized and the subsequent disease course recorded. These are different animals from those used in Group I.

Treatment Group III. The primary disease response in the splenectomized calf: six normal uninfected calves were surgically splenectomized. Six weeks after splenectomy the calves were infected and the subsequent disease course recorded.

Treatment Group IV. The effect of laparotomy on the anaplasmosis

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*Uninfected, unless otherwise defined, means free of anaplasmosis as determined by two or more negative compliment fixation tests for anaplasmosis (51).

carrier calf: laparotomies and splenic manipulations (without removal) were performed on five carrier calves. The postlaparotomy means were established from data taken during the period of expected maximum anemia. This period was days "+8" through "+12". Day "0" was designated as the twelfth day after laparotomy since the Group II calves showed 1 percent parasitized erythrocytes twelve days (on the average) after splenectomy. The period of maximum anemia in the Group II calves also occurred on days +8 through +12.

Treatment Group V. A comparison of pre and postsplenectomy hematological values in the same calf: presplenectomy "normal" values were established on six normal uninfected calves using five observations per calf to calculate the means. Six weeks after splenectomy new means were calculated using the previously described regimen.

Experimental Procedure

The data presented in this study were obtained using recognized procedures (52). Where it was found necessary to modify existing procedures, the modification of the procedure is described.

Five or more pretreatment determinations were made on each test for each animal to establish normal values. In general, each test was performed 2 or 3 times weekly during the prepatent period, daily during the acute anemic phase of the patent period and 2 or 3 times weekly during the convalescent period of the patent period. The onset of patent disease was defined as the first day that 1 percent of the erythrocytes contained anaplasma bodies.*

*These were counted on Wright's stained blood films with a light microscope.

Experimental Animals

Calves of mixed breed and sex weighing less than 250 lbs. were obtained from institutional and local herds. They were maintained on a standard commercial calf feed, supplemented with steamed bone meal* and a commercial vitamin-trace mineral preparation**. Prairie and alfalfa hay were fed free choice. The animals were given free access to water from the municipal water supply. They were maintained under standard conditions of husbandry throughout the study period. During the three to six week pre-experiment acclimatization period, the animals were vaccinated against Clostridium chauvoei and septica, Leptospira pomona and treated with phenothiazine for internal parasites. Periodic fecal flotation examination, using saturated sodium sulphate, indicated that internal parasites were not a problem during the experimental period. Animals were top sprayed twice a month with a topical chlorinated hydro-carbon insecticide for external parasite control.

Exposure to Anaplasmosis

Prior to experimental infection, a negative reaction to the complement fixation test for anaplasmosis was used as the basis for selecting a calf free from the disease. Each animal was inoculated subcutaneously with 5 ml. of carrier blood from a known infected cow.

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*Superior Feed Mills, Oklahoma City, Oklahoma.

**Clovite, Fort Dodge Laboratories, Fort Dodge, Iowa.

The carrier blood was treated with neoarsphenamine (25 mg. per 10 ml. of whole blood) at 40 degrees Fahrenheit for 24 hours to eliminate possible eperythrozoon organisms in the carrier blood (53). All animals in each treatment group received treated blood from the same container at the same time with the exception of Treatment Group I, in which each half of the group was infected from separately prepared containers.* All cattle subsequently became infected. This technique provides, as nearly as possible, the same infective dose for each animal.

Hematology

Blood samples were drawn between 6:30 and 8:30 a.m. from the jugular vein into tubes containing 10 mg. of dry dipotassium ethylenediaminetetraacete (EDTA) for hematological studies the same day.** At the same time 10 ml. of whole blood was collected and set aside at room temperature (approximately 72 degrees Fahrenheit) for serum separation. The coagulated blood sample was centrifuged each afternoon, the serum decanted, frozen at -10 degrees Fahrenheit, and stored for later examination.

Erythrocyte counts were done by standard laboratory procedures using an electronic grid partical counter+ with a 100_u aperture tube.

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*This was done because of its relation to another experiment to be reported separately.

**Animals were either bled in their stable or moved to an adjacent chute handling facility for bleeding.

+Coulter Counter, Model B, Coulter Electronics Co., Hialeah, Florida.

The counter control settings were amplitude -4; current - 0.177; lower threshold - 7 (erythrocytes); upper threshold - open (full counter-clockwise); threshold control switch - "separate" position. To reduce the coincidence factor, the standard erythrocyte dilutions were doubled.

The raw count was corrected for coincidence using the company recommended chart; the background count of the saline diluent was subtracted out, and the resultant number doubled to give the final true count. The reproducibility, accuracy, and simplicity of the electronic counting technique is well documented and will not be dealt with here (54). Theoretically, this technique can increase the statistical accuracy of individual cell counts by a factor of 10 over standard hemocytometer hand counting methods; this is in accordance with our laboratory findings and those of others (55). Repetitive counts on the same sample using the hemocytometer were far more variable than similar counts using the electronic grid technique.

Packed cell volumes (P.C.V.) were determined using the micro-capillary tube method and a microhematocrit centrifuge (56). Each sample was measured on an International* rotary reading unit.

Hemoglobin determinations were made colorimetrically using the acid hematin technique (57). A 60 minute color developing time and a reading wavelength of 525 mm_u were used.

Blood films of the erythrocytes were made with the coverslip technique and counterstained with Wright's stain. A quantity of

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*International Electric Co., Evanston, Illinois.

distilled water buffered to pH 6.8 with phosphate buffer, equal to the amount of stain, was used to dilute the Wright's stain. The stained smears were washed in approximately neutral distilled water (58).

The percentage of erythrocytes (PPE) containing anaplasma bodies among 500 red blood cells was estimated from the stained blood smears. A Whipple's disk was placed in the microscope ocular to facilitate counting. The total number of infected erythrocytes (PRBC) was determined by multiplying the total erythrocyte count by the percent of infected erythrocytes.

The estimates of average erythrocyte size (Mean Corpuscular Volume-MCV) and cell hemoglobin contents (Mean Corpuscular Hemoglobin Concentration-MCHC) were made using the standard erythrocyte indices calculating formulae (59).

The compliment fixation (CF) test for anaplasmosis was used to detect antibodies produced against anaplasma and their by-products (51). A modification of the photoelectric nephelometric method was used to interpret the CF reaction. (See Appendix Figure 44). A positive reaction was reported as the log to the base 10 of the denominator of the highest dilution giving a 4+ reaction. This method was chosen to facilitate electronic data processing of the CF titers.

CHAPTER III

TABULATION OF DATA

The tabular and graphic system used in reporting the results is a slight modification of that used by Brock (1). It has been shown that data from various animals can best be combined by adjusting them to some common phase point in the disease (60). In this case, the data of each animal was adjusted to the day in which 1 percent of the erythrocytes was first observed to contain anaplasma bodies. Daily means within treatment groups were calculated on this basis. This day was designated as Day Zero ("0"), the start of the patent period. The prepatent days were designated with a minus sign, and the days of the patent period were designated with a plus sign.

Values for the following tests were reported in tabular form as actual values and in graphic form as percentage deviations from pre-treatment mean ($\bar{X}$) values. The following abreviations were used:

HB - Hemoglobin, Gms. per 100 ml. whole blood.

PCV - Packed Cell Volume, Volumes percent.

RBC - Erythrocyte count, x 10^6 cells per $mm.^2$

MCV - Mean Corpuscular Volume, cubic microns.

MCHC - Mean Corpuscular Hemoglobin Concentration, percent.

PPE - Percent Parasitized Erythrocytes, percent.

PRBC - Parasitized erythrocytes, x 10^6 cells per $mm.^2$

CF - Complement Fixation Titer, $\log_{10}$ of the reciprocal of the highest titer giving a 4 + reaction. (See Appendix Figure 44).

$\bar{X}$ - Pretreatment "normal" mean value.

N - Number of animals in the group.

TABLE I

PRIMARY DISEASE RESPONSE IN THE INTACT CALF. THE DAILY MEAN VALUES OF TESTS PERFORMED ON GROUP I: N = 6.

Days	HB	PCV	RBC	MCV	MCHC	PPE	PRBC	CF
$\bar{X}$	8.9	31.1	8.39	37.5	28.4	0.0	0.000	0.00
- 48	8.9	34.5	7.75	44.5	25.7	0.0	0.000	0.00
- 43	8.0	30.4	8.24	37.0	26.2	0.0	0.000	0.00
- 38	8.7	31.2	8.33	37.4	28.1	0.0	0.000	0.00
- 35	8.6	29.2	8.12	35.9	29.6	0.0	0.000	0.00
- 30	8.8	29.5	8.15	36.3	29.8	0.0	0.000	0.00
- 28	9.1	31.3	8.49	36.9	29.2	0.0	0.000	0.00
- 24	8.7	2.88	8.07	35.7	30.2	0.0	0.000	0.00
- 21	8.8	30.7	8.16	37.7	28.8	0.0	0.000	0.00
- 18	9.6	32.6	8.93	36.5	29.3	0.0	0.000	0.00
- 15	9.6	32.3	9.65	33.5	29.8	0.0	0.000	0.00
- 13	9.8	32.6	9.89	33.0	29.9	0.0	0.000	0.00
- 10	9.5	26.1	10.13	25.7	36.5	0.0	0.000	0.00
- 9								0.00
- 8	9.4	33.2	10.08	33.2	28.4	0.0	0.000	0.95
- 7								2.91
- 6								2.91
- 5	9.6	30.1	10.19	29.8	31.9	0.0	0.000	2.96
- 4								2.51
- 3								2.81
- 2	9.8	31.0	9.99	31.0	31.5	0.0	0.000	2.58
- 1								3.33
0	9.6	29.9	9.19	32.6	31.9	1.0	0.092	3.11
+ 1	9.3	30.3	9.19	33.0	30.6	2.5	0.230	3.23
+ 2	8.9	29.3	8.22	35.9	30.1	3.7	0.307	3.17
+ 3	8.4	28.7	8.00	35.8	29.3	4.7	0.376	3.26
+ 4	7.8	26.0	8.44	30.8	30.0	7.0	0.591	2.74
+ 5	7.2	23.3	7.37	31.6	31.0	8.7	0.641	3.31
+ 6	6.4	21.2	6.10	34.9	30.2	10.0	0.610	3.16
+ 7	6.2	19.7	5.93	33.2	31.2	11.8	0.700	3.36
+ 8	5.6	17.8	4.99	35.8	31.2	10.2	0.509	3.42
+ 9	4.8	15.8	4.38	36.0	30.8	9.1	0.400	3.23
+ 10	5.1	16.8	4.31	39.0	30.2	9.2	0.400	3.31
+ 11	5.1	16.8	4.28	39.3	30.1	8.3	0.355	3.06
+ 12	5.5	18.3	3.73	49.3	30.1	6.8	0.254	3.11
+ 13	5.7	19.3	3.90	49.6	29.7	4.6	0.179	3.18
+ 14	6.4	20.9	4.20	49.8	30.6	2.7	0.113	3.16
+ 15	6.4	21.3	4.56	44.8	30.1	1.1	0.050	3.11
+ 16	6.4	22.0	4.55	48.5	28.9	2.2	0.100	3.11
+ 17	6.9	23.0	4.34	53.0	29.8	1.8	0.078	3.26
+ 18	7.1	24.3	4.09	59.5	29.1	1.4	0.057	3.11

Table I (Continued)

Days	HB	PCV	RBC	MCV	MCHC	PPE	PRBC	CF
+ 19								3.11
+ 20	7.5	24.5	4.61	53.3	30.1	1.2	0.055	3.11
+ 21								3.11
+ 22	7.5	25.5	4.40	58.0	29.3	1.6	0.070	3.11
+ 23								3.18
+ 25	8.3	26.9	5.25	51.3	30.8	0.5	0.026	2.99
+ 28	8.6	28.9	5.54	52.2	29.9	0.5	0.028	2.86
+ 32	9.2	30.1	5.87	51.3	30.6	0.0	0.000	2.69
+ 35	10.1	31.3	6.77	46.3	32.3	0.0	0.000	2.66
+ 38	10.0	32.8	7.20	45.6	30.5	0.0	0.000	2.71
+ 44	10.1	34.0	7.82	43.5	29.6	0.0	0.000	2.57
+ 46	9.9	33.9	8.63	41.7	29.3	0.0	0.000	2.35

TABLE II

PRIMARY RELAPSE IN THE SPLENECTOMIZED CARRIER CALF. THE DAILY MEAN VALUES OF THE TESTS PERFORMED ON GROUP II: N = 6.

Days	HB	PCV	RBC	MCV	MCHC	PPE	PRBC	CF
$\bar{X}$	9.7	29.4	7.61	38.6	32.9	0.03	0.002	1.88
- 31	9.4	28.2	6.94	40.6	33.4	0.03	0.002	*
- 27	9.6	29.0	7.43	39.0	31.8	0.03	0.002	*
- 24	9.4	29.4	7.33	40.1	31.9	0.03	0.002	*
- 21	9.5	28.8	7.77	36.3	33.1	0.00	0.000	2.51
- 18	9.2	27.8	7.77	35.8	32.9	0.03	0.002	2.21
- 13	10.7	30.7	7.69	39.9	34.7	0.03	0.002	1.80
- 10	9.0	29.8	7.95	37.5	30.1	0.01	0.001	1.78
- 7	10.4	31.5	7.96	39.6	33.0	0.03	0.002	1.42
- 3	10.6	29.7	7.71	38.5	35.6	0.03	0.002	1.58
0	10.4	30.9	8.03	38.5	33.6	0.96	0.077	0.75
+ 2	9.3	28.2	7.25	38.9	32.9	4.00	0.290	
+ 3	8.9	26.7	7.20	37.1	33.3	6.90	0.497	0.70
+ 4	8.7	25.6	6.87	37.3	33.8	10.10	0.694	
+ 5	8.2	24.6	6.48	37.9	33.1	13.50	0.875	1.02
+ 6	7.3	22.2	5.28	42.0	32.8	20.60	1.088	
+ 7	6.0	19.2	4.85	39.6	31.1	26.80	1.300	1.24
+ 8	4.9	15.5	4.07	38.1	31.6	25.60	1.042	0.87
+ 9	4.0	12.2	2.87	42.5	33.0	21.70	0.623	0.75
+ 10	3.2	11.5	2.70	42.3	27.5	15.50	0.419	1.07
+ 11	3.4	12.4	2.47	50.2	27.1	8.90	0.220	0.92
+ 12	3.6	13.9	2.29	60.7	25.5	5.00	0.115	1.08
+ 13	4.2	15.1	2.56	58.9	27.5	2.60	0.067	1.28
+ 14	4.6	16.5	2.61	63.2	27.9	1.20	0.031	1.23
+ 18	5.8	20.9	3.38	61.8	27.8	0.22	0.007	1.72
+ 21	6.5	22.8	3.38	67.5	28.3	0.31	0.010	1.48
+ 25	5.9	20.4	3.16	64.6	28.7	0.43	0.014	1.46
+ 28	6.2	20.3	3.96	51.3	30.4	0.27	0.011	2.20
+ 31	5.6	19.8	3.84	51.5	28.4	0.49	0.019	
+ 35	5.8	19.3	3.79	50.9	30.2	0.72	0.027	2.51
+ 42	6.3	21.1	4.04	52.2	29.6	0.03	0.001	2.20
+ 47	7.2	23.0	5.00	46.0	31.3	0.03	0.002	1.90
+ 49	6.7	22.6	4.85	46.6	29.6	0.05	0.002	1.60

*Titer not available: 4 + @ 1/10.

TABLE III

PRIMARY DISEASE RESPONSE IN THE SPLENECTOMIZED CALF. THE DAILY MEAN VALUES OF THE TESTS PERFORMED ON GROUP III: N = 6.

Days	HB	PCV	RBC	MCV	MCHC	PPE	PRBC	CF
$\bar{X}$	10.4	30.5	7.68	38.7	34.0	0.0	0.000	0.00
- 46	9.6	29.9	7.73	*	32.2	0.0	0.000	0.00
- 44	10.0	30.0	7.75	*	33.2	0.0	0.000	0.00
- 42	10.3	30.0	7.75	*	34.3	0.0	0.000	0.00
- 39	10.4	30.5	7.88	*	34.1	0.0	0.000	0.00
- 37	10.7	30.7	7.92	*	34.8	0.0	0.000	0.00
- 35	10.6	30.4	7.86	*	34.9	0.0	0.000	0.00
- 32	10.5	30.9	7.99	*	34.1	0.0	0.000	0.00
- 30	10.5	30.5	7.88	*	34.3	0.0	0.000	0.00
- 28	10.1	29.8	8.36	35.6	35.3	0.0	0.000	0.00
- 25	10.7	31.0	6.08	51.0	34.6	0.0	0.000	0.00
- 23	10.5	30.9	7.67	40.3	34.0	0.0	0.000	0.00
- 21	10.0	29.6	7.15	41.5	33.7	0.0	0.000	0.00
- 19	10.5	29.7	7.26	40.1	35.3	0.0	0.000	0.00
- 18	10.2	29.5	7.98	37.0	34.5	0.0	0.000	0.00
- 17	10.3	29.8	8.30	35.9	34.5	0.0	0.000	0.00
- 16	10.2	28.8	8.05	35.7	35.5	0.0	0.000	0.00
- 15	10.7	29.4	76.7	38.3	36.2	0.0	0.000	0.00
- 14	10.5	28.7	7.56	37.9	36.7	0.0	0.000	0.00
- 13	9.8	29.3	7.31	34.6	33.3	0.0	0.000	0.00
- 12	10.5	28.6	7.42	38.7	36.7	0.0	0.000	0.00
- 11	10.9	28.8	7.48	38.6	37.7	0.0	0.000	0.00
- 10	10.1	29.1	8.83	32.6	34.6	0.0	0.000	0.00
- 9	9.9	29.3	8.93	32.8	33.7	0.0	0.000	0.00
- 8	9.8	28.2	8.83	31.9	34.7	0.0	0.000	0.00
- 7	9.3	28.8	9.14	31.5	32.2	0.0	0.000	0.00
- 6	9.6	29.6	9.06	32.6	32.5	0.0	0.000	0.00
- 5	9.4	29.8	9.47	31.4	31.6	0.0	0.000	0.00
- 4	9.6	29.6	8.95	33.1	32.3	0.0	0.000	0.00
- 3	9.9	30.0	9.52	31.5	33.1	0.0	0.000	0.00
- 2	9.9	30.0	9.52	31.5	33.1	0.0	0.000	0.00
- 1	9.6	29.3	9.46	30.9	32.7	0.3	0.003	0.88
0	9.1	27.9	10.09	27.7	32.6	1.1	0.114	1.16
+ 1	9.4	28.6	8.95	32.0	32.7	3.3	0.292	2.10
+ 2	9.2	28.0	9.28	30.2	32.8	3.6	0.335	2.25
+ 3	9.2	28.1	8.12	34.6	32.6	6.6	0.532	2.40
+ 4	9.3	27.8	8.05	34.6	33.2	9.9	0.794	2.45
+ 5	8.3	26.2	7.48	34.9	31.5	14.8	1.108	2.50
+ 6	7.5	22.0	6.35	34.6	33.9	21.1	1.337	2.75
+ 7	5.6	16.8	5.30	31.6	33.1	27.2	1.442	2.90
+ 8	3.8	11.8	3.80	30.9	32.2	35.4	1.343	3.05

Table III (Continued)

Days	HB	PCV	RBC	MCV	MCHC	PPE	PRBC	CF
+ 9	2.9	9.5	2.80	34.1	30.1	35.3	0.985	3.04
+ 10	2.5	8.3	2.30	36.2	30.0	19.7	0.452	3.02
+ 11	3.4	10.8	1.82	59.1	31.6	14.9	0.271	2.87
+ 12	3.7	13.1	2.10	62.8	27.8	10.1	0.212	3.25
+ 13	4.3	14.3	2.23	43.1	30.1	6.6	0.148	3.25
+ 14	4.4	16.1	2.36	68.0	27.5	3.9	0.092	3.25
+ 16	5.2	18.4	2.96	62.0	28.3	2.2	0.065	2.80
+ 17	5.9	19.3	3.10	62.3	30.6	1.5	0.046	3.02
+ 18	6.6	20.4	3.31	61.5	32.3	1.3	0.042	3.17
+ 19	6.9	21.6	3.74	58.0	31.9	0.7	0.026	3.25
+ 20	7.1	22.2	3.28	67.1	32.0	0.5	0.015	3.17
+ 21	7.1	22.7	3.66	62.2	31.4	0.8	0.030	3.02
+ 23	7.7	23.9	3.74	64.0	32.1	1.0	0.037	2.95
+ 24	7.6	24.1	4.09	59.1	31.3	4.8	0.197	2.95
+ 25	7.7	23.9	5.36	44.5	32.1	5.5	0.292	3.02
+ 26	7.6	23.5	4.95	47.5	32.1	4.5	0.220	2.72
+ 27	7.3	23.6	3.69	64.1	30.7	3.0	0.111	2.65
+ 28	6.7	22.3	4.17	53.4	30.0	3.0	0.125	2.65
+ 30	6.4	21.9	4.27	49.8	30.2	5.2	0.220	2.42
+ 31	6.8	21.3	4.38	48.8	31.7	5.2	0.229	2.57
+ 32	6.8	21.4	4.17	51.2	31.7	4.5	0.118	2.50
+ 33	7.1	21.4	4.54	49.0	31.9	4.6	0.252	2.42
+ 34	7.6	22.3	3.45	64.8	34.0	5.4	0.185	2.50
+ 35	7.1	22.4	5.55	40.7	31.5	7.1	0.391	2.42
+ 36	7.0	22.6	6.37	34.1	32.3	9.6	0.608	2.50
+ 37	6.9	21.7	4.38	47.6	33.1	8.1	0.353	2.42
+ 38	6.4	20.9	3.92	50.1	32.5	7.0	0.274	2.12
+ 39	5.7	19.6	3.76	49.0	31.1	7.4	0.279	2.27
+ 40	5.3	18.4	3.33	51.4	30.9	9.8	0.325	2.42
+ 41	5.4	17.1	3.98	41.1	32.8	8.8	0.351	2.35
+ 42	5.1	16.4	3.70	43.2	31.7	10.6	0.390	2.57
+ 44	5.2	15.3	3.59	42.5	34.1	5.1	0.182	2.50
+ 45	4.9	14.9	3.40	43.9	32.8	7.8	0.264	2.27
+ 46	5.0	14.6	3.75	39.0	34.3	6.2	0.231	2.27
+ 47	5.1	16.1	3.40	47.4	31.8	4.0	0.136	1.97
+ 48	5.4	15.0	3.19	47.1	36.0	5.6	0.179	1.97
+ 49	5.8	16.6	3.47	48.0	35.0	5.3	0.184	1.90
+ 50	6.2	17.4	3.61	48.1	35.8	6.0	0.214	1.90
+ 51	5.9	17.8	3.53	50.3	33.2	5.9	0.207	1.90
+ 52	6.1	18.6	3.54	52.7	32.5	5.9	0.209	1.82
+ 53	6.5	19.6	3.95	49.7	33.2	5.0	0.198	
+ 54	6.6	19.4	4.06	47.8	33.8	5.1	0.208	
+ 55	6.9	19.6	4.22	46.5	35.2	5.4	0.228	
+ 57	7.0	19.9	3.87	51.3	35.2	4.6	0.179	

* Due to counting machine malfunction, no RBC values were available for these days. Since pretreatment data appeared homogeneous, RBC counts were estimated for these days using the formula (daily PCV times MCV pretreatment mean = RBC estimated count. These estimated data do not enter in any statistical calculations.

TABLE IV

CONTROL LAPAROTOMY AND SPLENIC MANIPULATION - CARRIER CALF. THE DAILY MEAN VALUES OF THE TEST PERFORMED ON GROUP IV: N = 5.

Days	HB	PCV	RBC	MCV	MCHC	PPE	PRBC	CF
$\bar{X}$	10.0	30.3	7.79	38.4	32.8	0.10	0.009	2.50
- 30	9.3	28.0	6.60	42.5	33.0	0.03	0.002	*
- 27	9.5	30.3	7.38	41.0	31.4	0.20	0.016	*
- 16	10.1	33.5	8.42	39.8	30.1	0.30	0.029	*
- 12	10.8	32.2	8.62	31.3	33.5	0.10	0.010	2.51
- 10	9.6	31.0	7.66	40.5	31.0	0.01	0.008	2.38
- 6	10.2	29.2	7.62	38.3	35.1	0.01	0.005	2.57
- 3	9.8	29.8	7.83	38.2	32.6	0.00	0.000	2.51
0	10.4	28.9	8.17	35.4	35.9	0.01	0.010	3.33
+ 2	8.1	28.9	7.98	36.1	28.2	0.20	0.018	2.28
+ 4	10.6	31.7	7.96	39.8	33.4	0.06	0.005	2.08
+ 7	9.6	29.2	7.89	37.0	33.9	0.03	0.024	2.20
+ 8	8.0	28.6	7.75	36.9	28.1	0.02	0.014	1.98
+ 9	8.4	29.3	7.81	38.0	28.8	0.02	0.012	2.20
+ 10	10.4	28.3	7.78	36.3	36.7	0.02	0.019	2.02
+ 11	9.6	28.0	7.74	36.1	34.2	0.02	0.019	1.84
+ 13	10.0	30.3	7.71	39.3	32.9	0.02	0.014	2.08
+ 15	9.3	28.4	7.61	37.4	32.7	0.01	0.005	1.96
+ 18	9.8	29.1	7.82	37.2	33.8	0.02	0.014	2.97
+ 22	9.3	29.0	7.84	37.0	32.0	0.00	0.000	2.14
+ 25	10.0	29.5	7.68	38.4	33.8	0.00	0.000	1.96
+ 32	9.4	28.5	7.52	37.9	32.8	0.01	0.005	1.90
+ 39	9.1	29.1	7.14	40.7	31.2	0.01	0.004	1.96
+ 44	10.6	29.9	7.87	38.0	35.3	0.01	0.005	1.66
+ 46	8.7	27.8	7.06	39.4	31.25	0.01	0.004	1.55

*Titer not available: 4 + @ 1/10.

TABLE V

PRE AND POST SPLENECTOMY TEST VALUES IN THE CALF WITH NEGATIVE REACTIONS TO THE ANAPLASMA CF TEST - GROUP V: N = 6.

HB	PCV	RBC	MCV	MCHC	*	HB	PCV	RBC	MCV	MCHC
Calf #06, 1 wk. presplenectomy						Calf #06, 6 wks. postsplenectomy				
10.2	33.0	9.73	33.9	30.9	*	10.7	30.0	12.70	23.6	35.7
9.4	32.0	8.45	37.8	29.4	*	9.9	29.0	11.76	24.6	34.1
8.3	27.5	7.07	38.9	30.2	*	9.9	29.0	9.88	29.3	34.1
9.0	29.5	7.99	36.9	30.5	*	10.7	30.0	11.06	27.1	35.7
11.4	33.0	8.03	41.0	34.5	*	9.9	29.0	11.66	24.9	34.1
Calf #07, 1 wk. presplenectomy						Calf #07, 6 wks. postsplenectomy				
10.2	33.0	9.73	33.9	30.9	*	11.4	32.0	12.36	25.9	35.6
10.2	30.0	9.04	33.1	34.0	*	11.4	31.0	11.98	25.9	36.8
10.0	34.0	8.97	37.9	29.4	*	11.0	32.0	12.72	25.1	34.4
11.0	33.5	8.72	38.4	32.8	*	11.0	32.0	12.90	24.8	34.4
10.7	33.0	9.44	34.9	32.4	*	11.6	32.0	11.30	28.3	36.2
Calf #08, 1 wk. presplenectomy						Calf #08, 6 wks. postsplenectomy				
9.9	33.5	8.40	39.9	29.6	*	8.3	29.0	10.58	27.4	28.6
9.9	33.5	8.69	38.5	29.6	*	9.9	29.0	8.98	32.2	34.1
9.0	28.5	8.04	35.4	31.6	*	9.9	28.5	7.30	39.0	34.6
10.5	33.0	8.39	39.3	31.8	*	10.7	30.0	11.26	26.6	35.7
11.4	35.0	7.38	47.4	32.6	*	9.9	29.0	10.94	26.5	34.1
Calf #09, 1 wk. presplenectomy						Calf #09, 6 wks. postsplenectomy				
9.0	30.0	11.26	26.6	30.0	*	9.9	31.0	10.94	28.3	32.0
7.8	28.0	7.40	37.8	27.8	*	10.2	31.0	11.10	27.9	33.0
8.8	29.5	7.02	42.0	29.8	*	10.7	31.0	9.80	31.6	34.5
7.8	26.0	6.38	40.7	30.0	*	11.0	29.5	10.10	29.2	37.2
9.4	27.5	6.32	43.5	34.1	*	11.0	30.5	11.94	25.5	36.0
Calf #10, 1 wk. presplenectomy						Calf #10, 6 wks. postsplenectomy				
10.5	32.0	12.82	24.9	32.8	*	9.6	28.5	10.32	27.6	33.7
9.9	31.0	7.43	41.7	31.9	*	10.7	30.0	9.72	30.9	35.7
10.5	33.5	8.03	41.7	31.3	*	11.4	31.0	9.62	32.2	36.8
11.0	33.0	8.38	39.4	33.3	*	11.0	31.0	11.78	26.3	35.4
10.5	31.0	7.18	43.1	33.9	*	10.5	30.0	9.06	33.1	35.0
Calf #11, 1 wk. presplenectomy						Calf #11, 6 wks. postsplenectomy				
9.0	30.0	8.00	37.5	30.0	*	10.7	30.0	12.12	24.8	35.7
9.6	30.0	7.63	39.3	32.0	*	10.5	31.0	8.84	35.1	33.9
8.8	28.0	6.36	44.0	31.4	*	11.0	32.0	13.10	24.4	34.4
9.4	30.0	8.16	36.8	31.3	*	10.7	32.0	9.98	32.0	33.4
10.2	34.0	8.51	40.0	30.0	*	11.0	32.0	10.36	30.9	34.3

CHAPTER IV

DATA TRANSFORMATION

Brock (1, 61) has suggested a percentage type of data transformation to facilitate the comparison of various groups with different pretreatment means. This was done by the construction of graphs which show the deviations, on a percentage basis, of the daily observations from the pre-treatment mean. In effect, this reduced the means of each treatment group to a common mean of zero, with treatment effects (plus experimental error) demonstrated as plus or minus percentage deviations from zero.

The following graphs (Figures 1 through 24) were constructed on a "mean zero" basis. A pre-treatment mean was calculated on five or more pre-treatment observations. The percentage deviation was calculated: observation divided by pre-treatment equals the deviation. The deviation was multiplied by one hundred to convert it to whole percentage points and recorded with its plus or minus sign.

The graphs showing the numbers of parasitized erythrocytes and the graphs showing the CF titers normally have zero means. (Figures 25 through 33). No transformation was necessary for these observations, and they are given as changes in test values and not as percentage deviations.

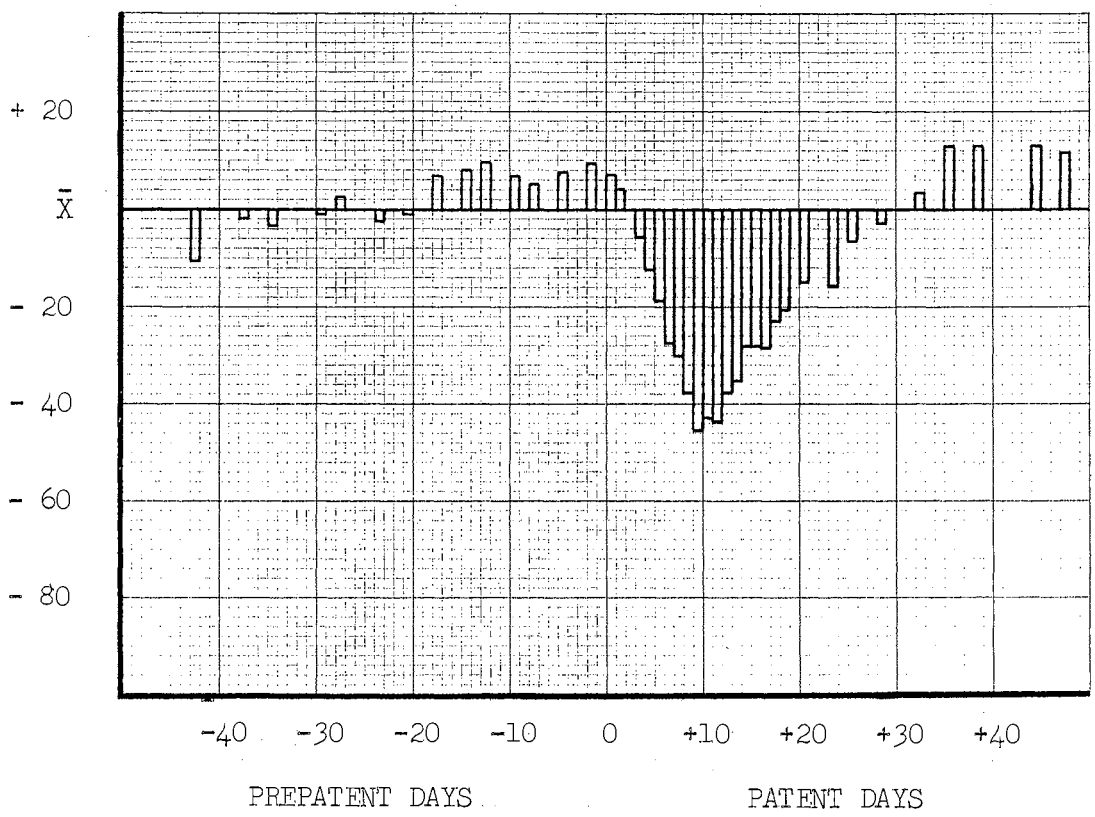

Figure 1. Deviations of the grams of hemoglobin per 100 ml. of blood from the pretreatment mean ($\bar{x}$ = 8.88 Gm. %) during the prepatent and patent periods of anaplasmosis in Group I - The primary disease in the intact calf: N = 6. (Y-axis is expressed as percent change.)

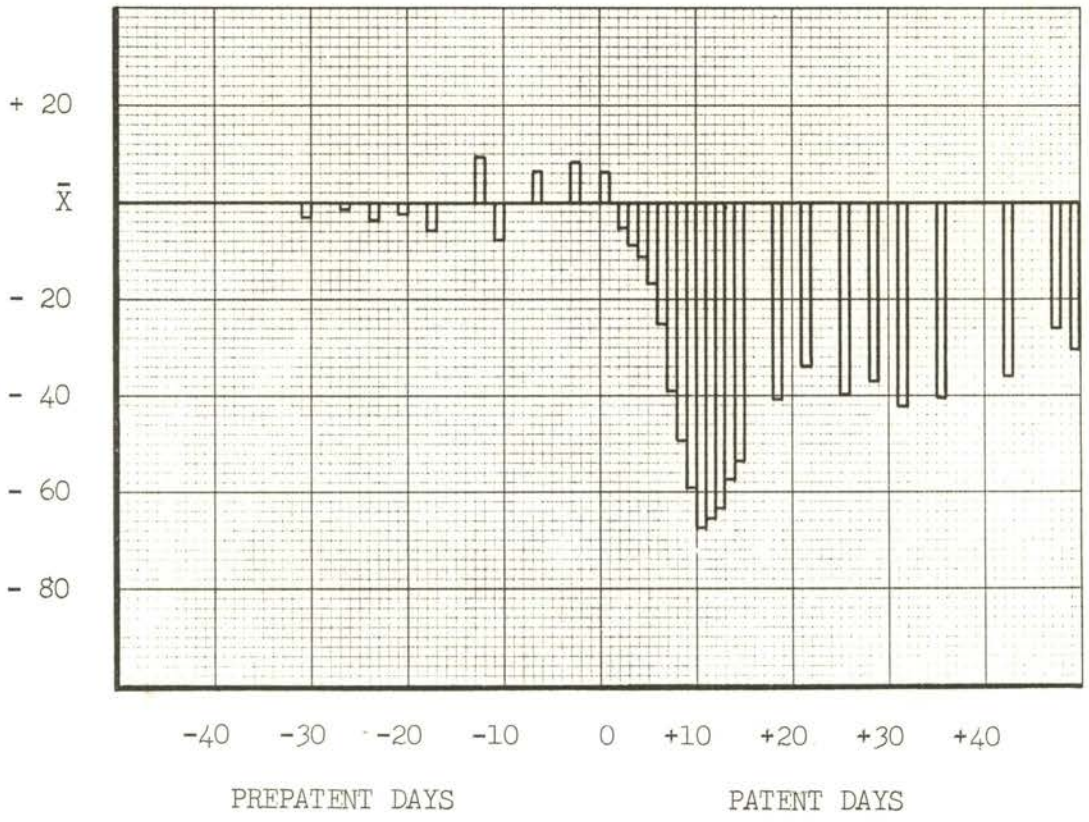

Figure 2. Deviations of the grams of hemoglobin per 100 ml. of blood from the pretreatment mean ($\bar{x}$ = 9.74 Gm. %) during the prepatent and patent periods of anaplasmosis in Group II - The primary relapse in the splenectomized carrier calf: N = 6. (Y-axis is expressed as percent change.)

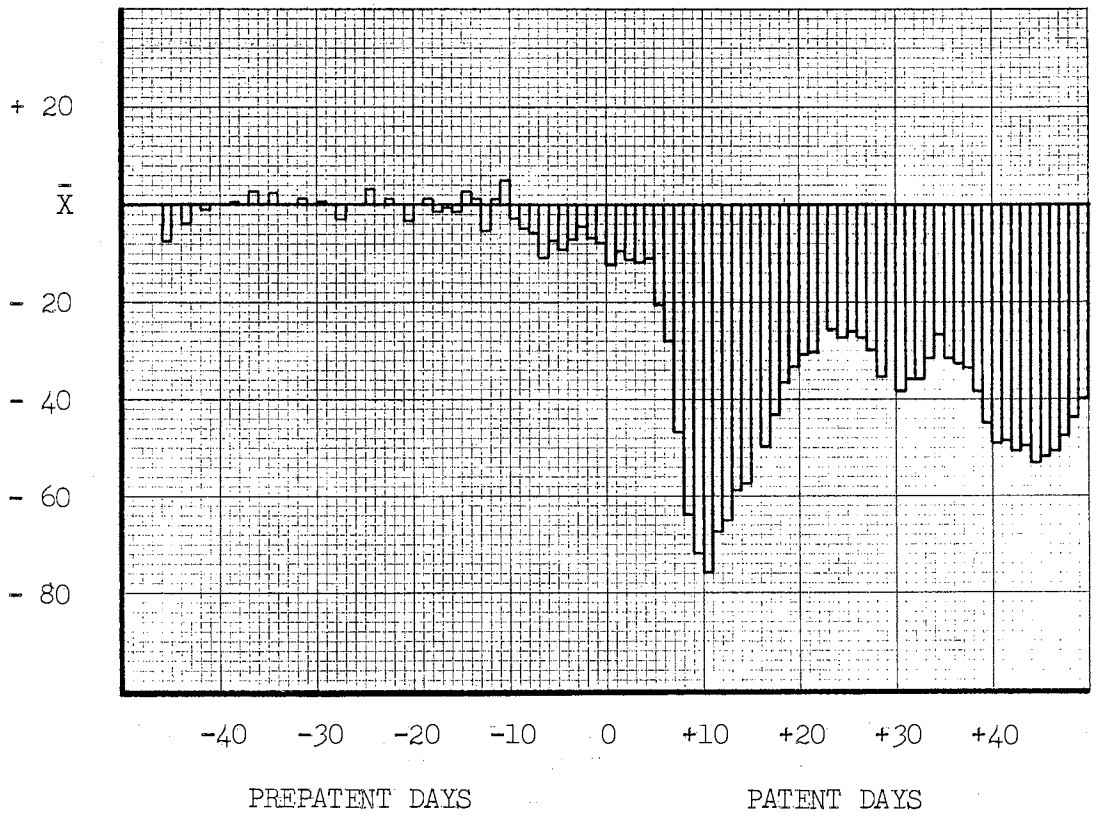

Figure 3. Deviations of the grams of hemoglobin per 100 ml. of blood from the pretreatment mean ($\bar{x}$ = 10.36 Gm. %) during the prepatent and patent periods of anaplasmosis in Group III - The primary disease response in the splenectomized calf: N = 6. (Y-axis is expressed as percent change.)

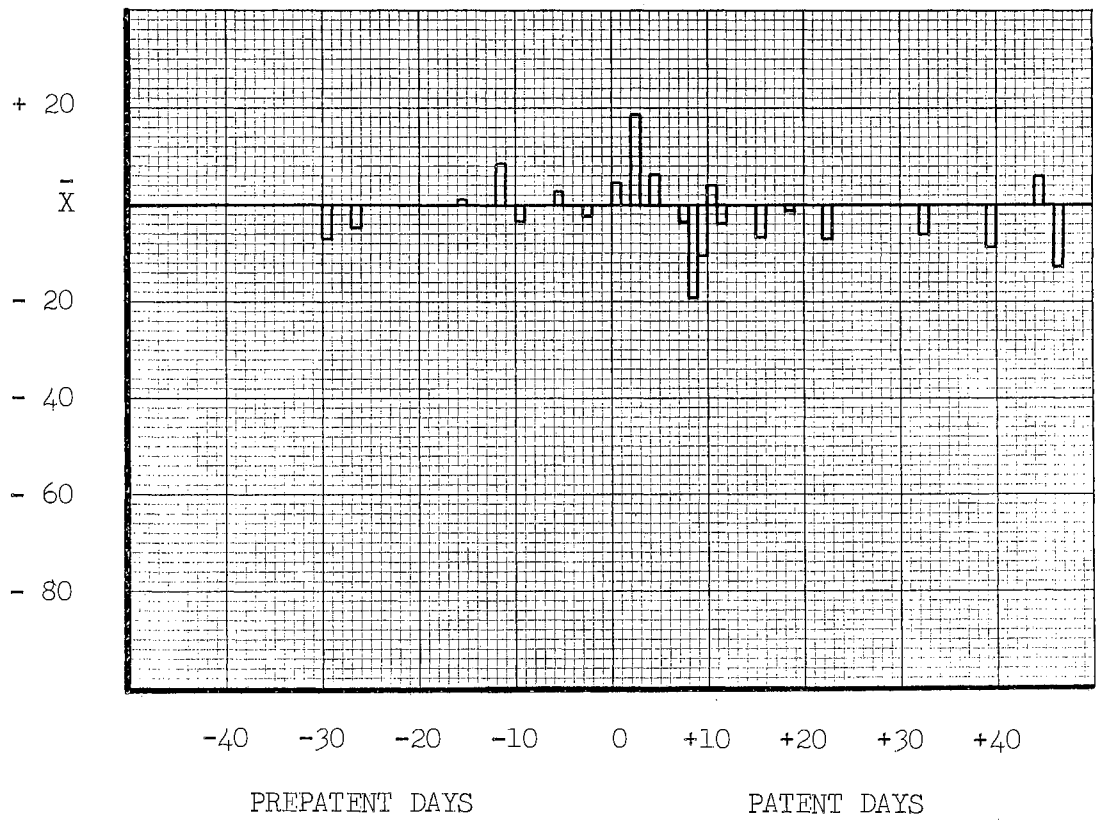

Figure 4. Deviations of the grams of hemoglobin per 100 ml. of blood from the pretreatment mean ($\bar{x}$ = 9.96 Gm. %) during the prepatent and patent periods of anaplasmosis in Group IV - Control laparotomy of the carrier calf: N = 5. (Y-axis is expressed as percent change.)

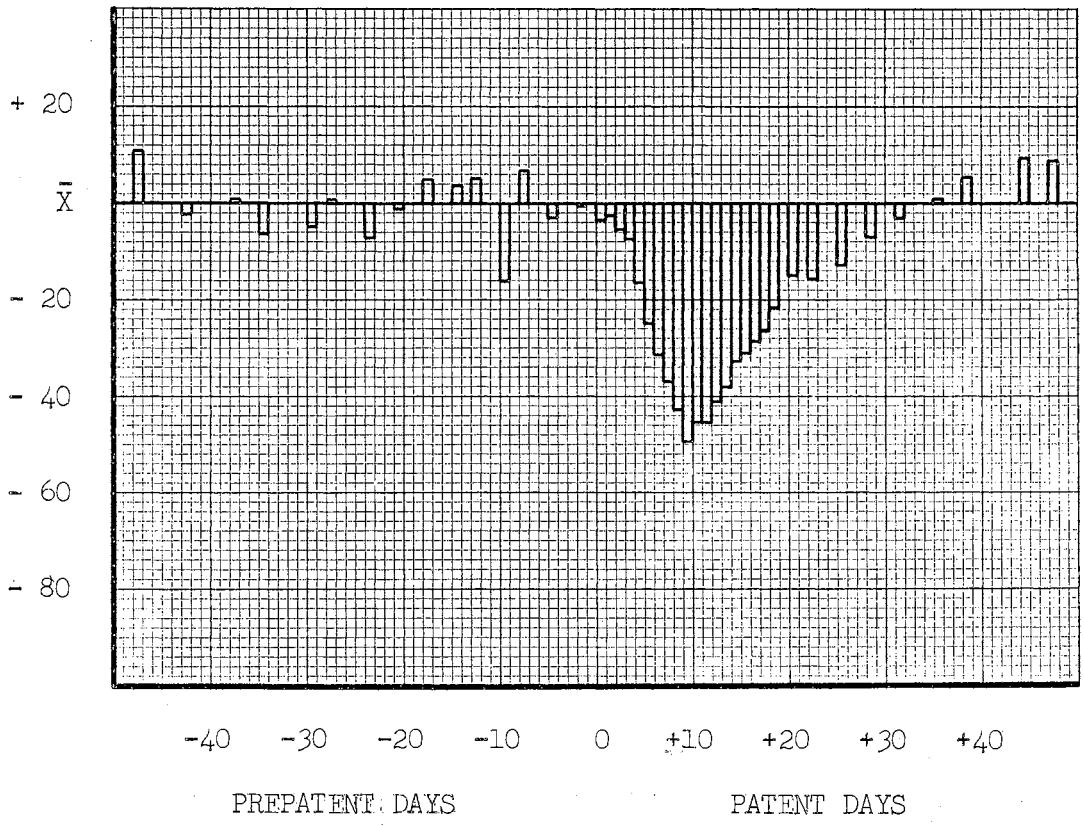

Figure 5. Deviations of the packed cell volume of blood from the pretreatment mean ($\bar{x}$ = 31.1 Vol. %) during the prepatent and patent periods of anaplasmosis in Group I - The primary disease response in the intact calf: N = 6. (Y-axis is expressed as percent change.)

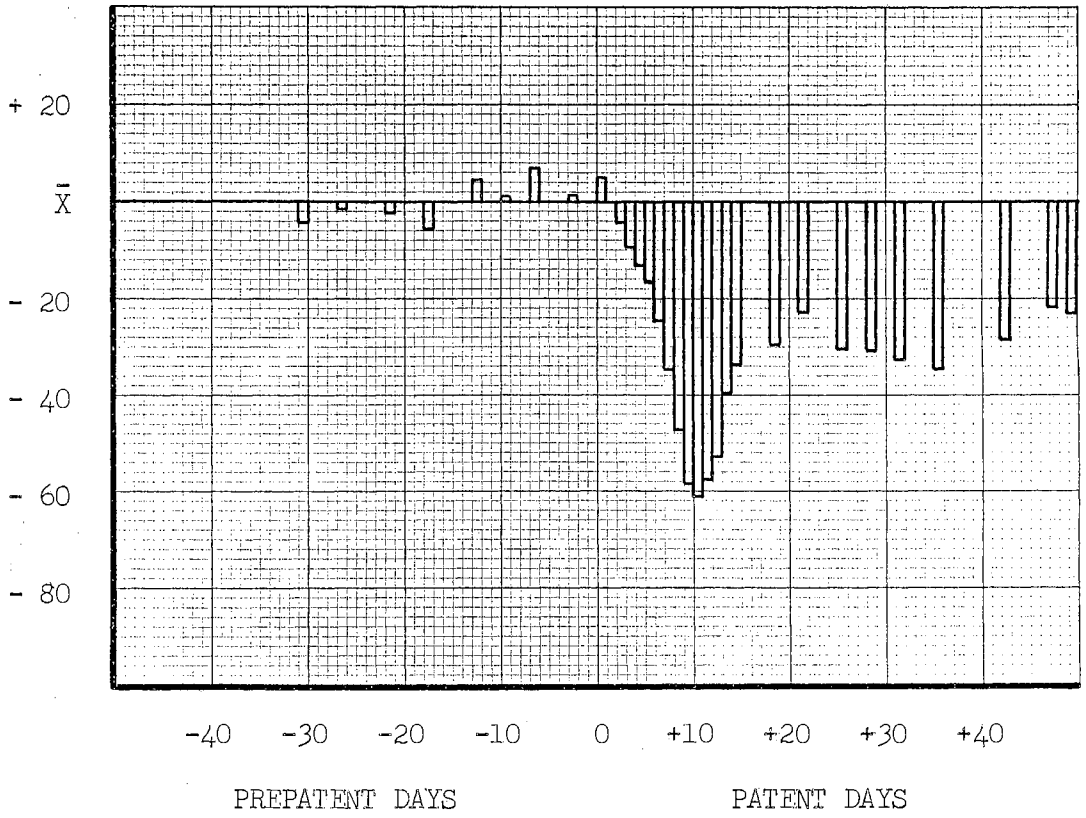

Figure 6. Deviations of the packed cell volume of blood from the pretreatment mean ($\bar{x}$ = 29.4 Vol. %) during the prepatent and patent periods of anaplasmosis in Group II - The primary relapse in the splenectomized carrier calf: N = 6. (Y-axis is expressed as percent change.)

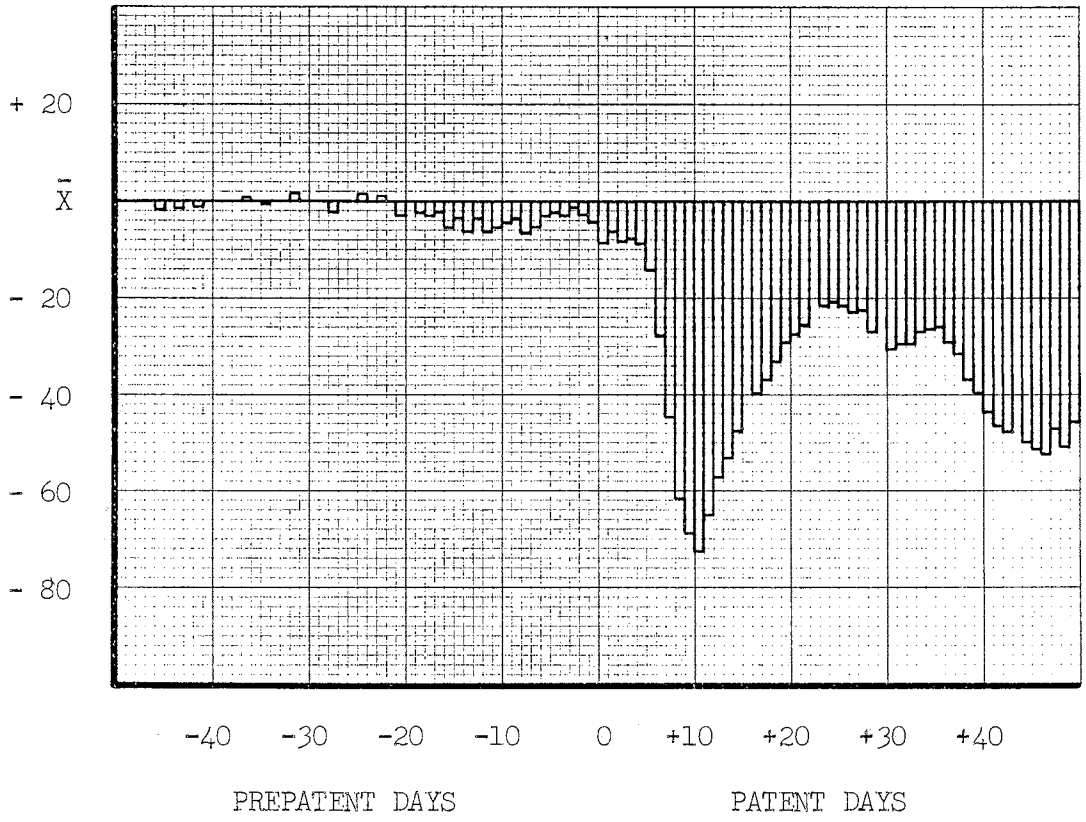

Figure 7. Deviations of the packed cell volume of blood from the pretreatment mean ($\bar{x}$ = 30.5 Vol. %) during the prepatent and patent periods of anaplasmosis in Group III - The primary disease response in the splenectomized calf: N = 6. (Y-axis is expressed as percent change.)

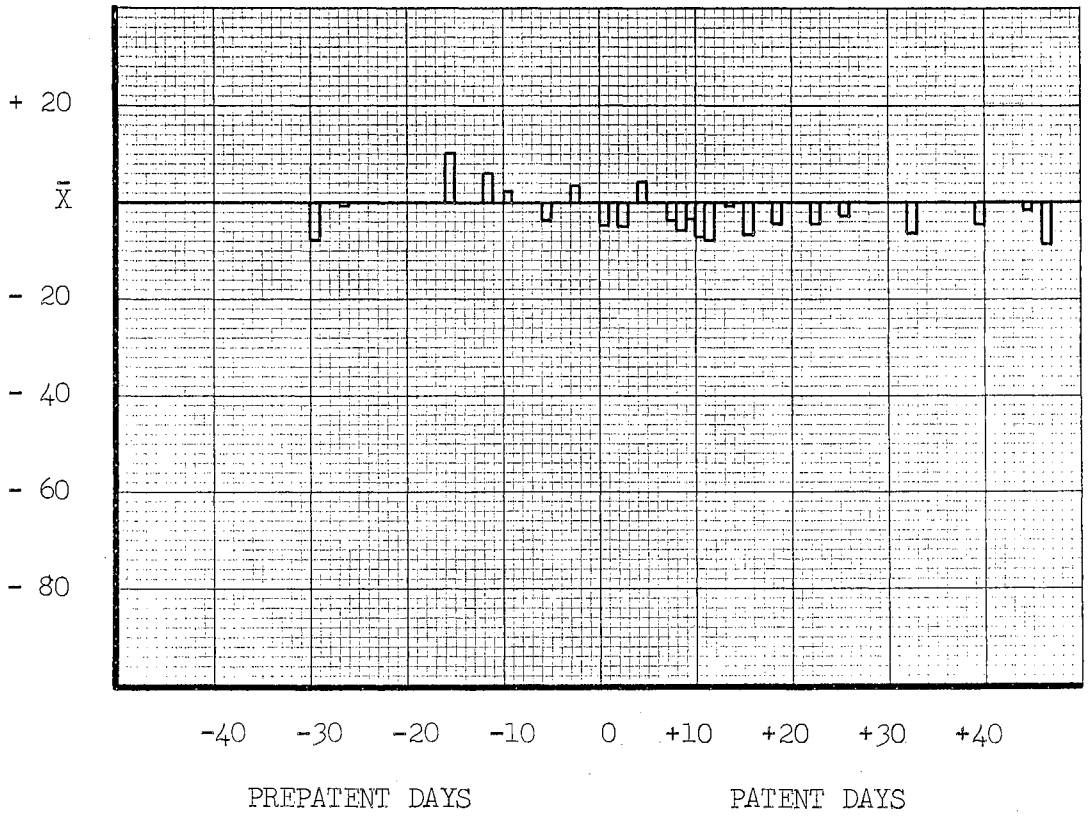

Figure 8. Deviations of the packed cell volume of blood from the pretreatment mean ($\bar{x}$ = 30.4 Vol. %) during the prepatent and patent periods of anaplasmosis in Group IV - Control laparotomy of the carrier calf: N = 5. (Y-axis is expressed as percent change.)

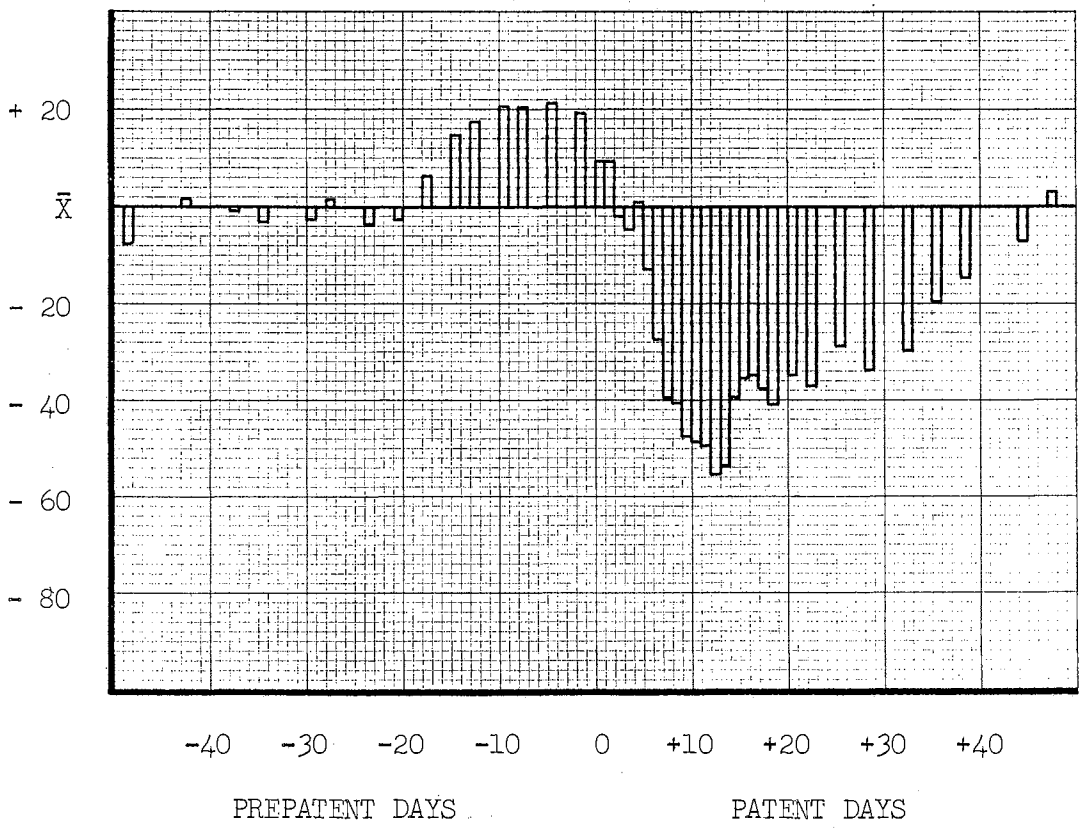

Figure 9. Deviations of the number of erythrocytes per cubic millimeter of blood from the pretreatment mean ($\bar{x}$ = 8.39 x 10^6/mm.^{2}) during the prepatent and patent periods of anaplasmosis in Group I - The primary disease response in the intact calf: N = 6. (Y-axis is expressed as percent change.)

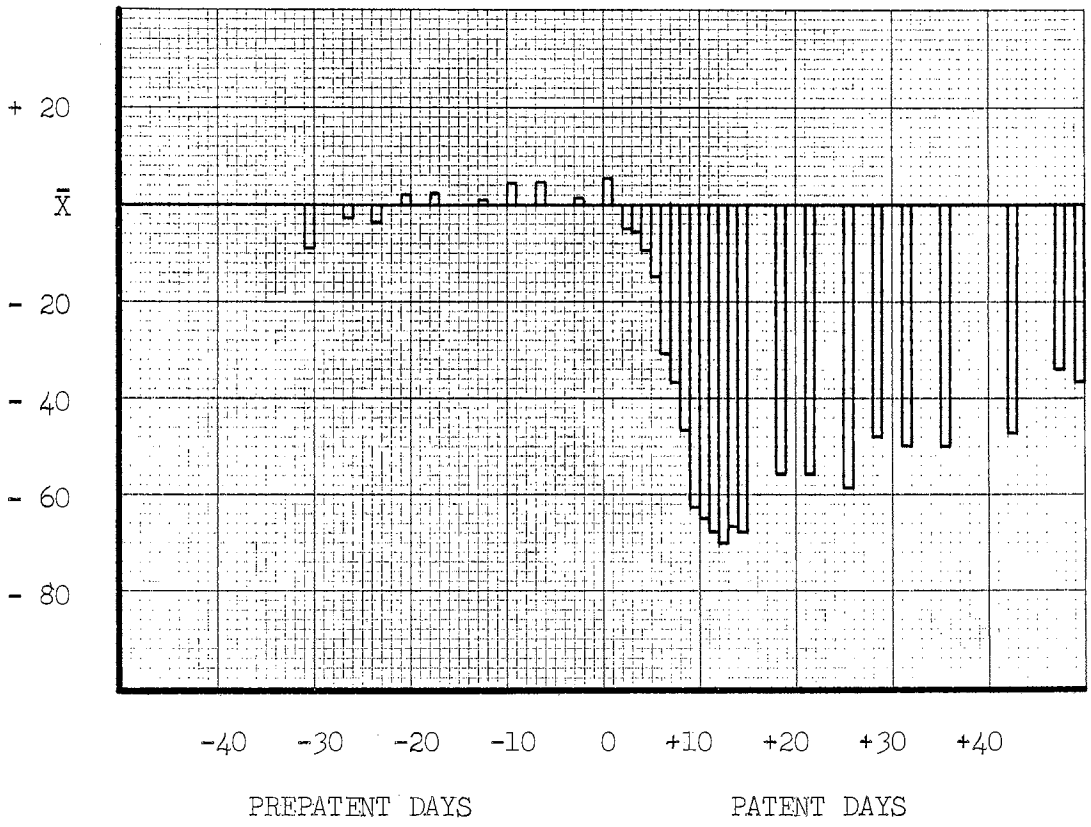

Figure 10. Deviations of the number of erythrocytes per cubic millimeter of blood from the pretreatment mean ($\bar{x}$ = 7.61 x 10^6/mm.^{2}) during the prepatent and patent periods of anaplasmosis in Group II - The primary relapse in the splenectomized carrier calf: N = 6. (Y-axis is expressed as percent change.)

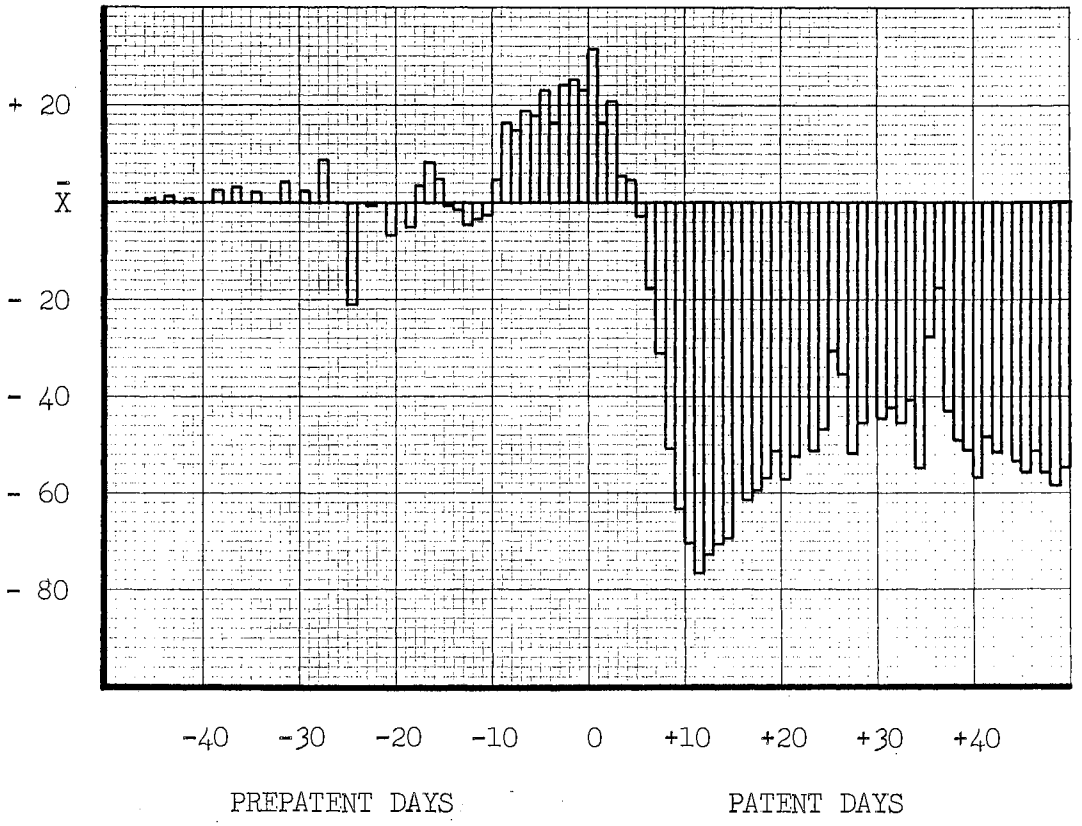

Figure 11. Deviations of the number of erythrocytes per cubic millimeter of blood from the pretreatment mean ($\bar{x}$ = 7.68 x 10^6/mm.^{2}) during the prepatent and patent periods of anaplasmosis in Group III - The primary disease response in the splenectomized calf; N = 6. (Y-axis is expressed as percent change.)

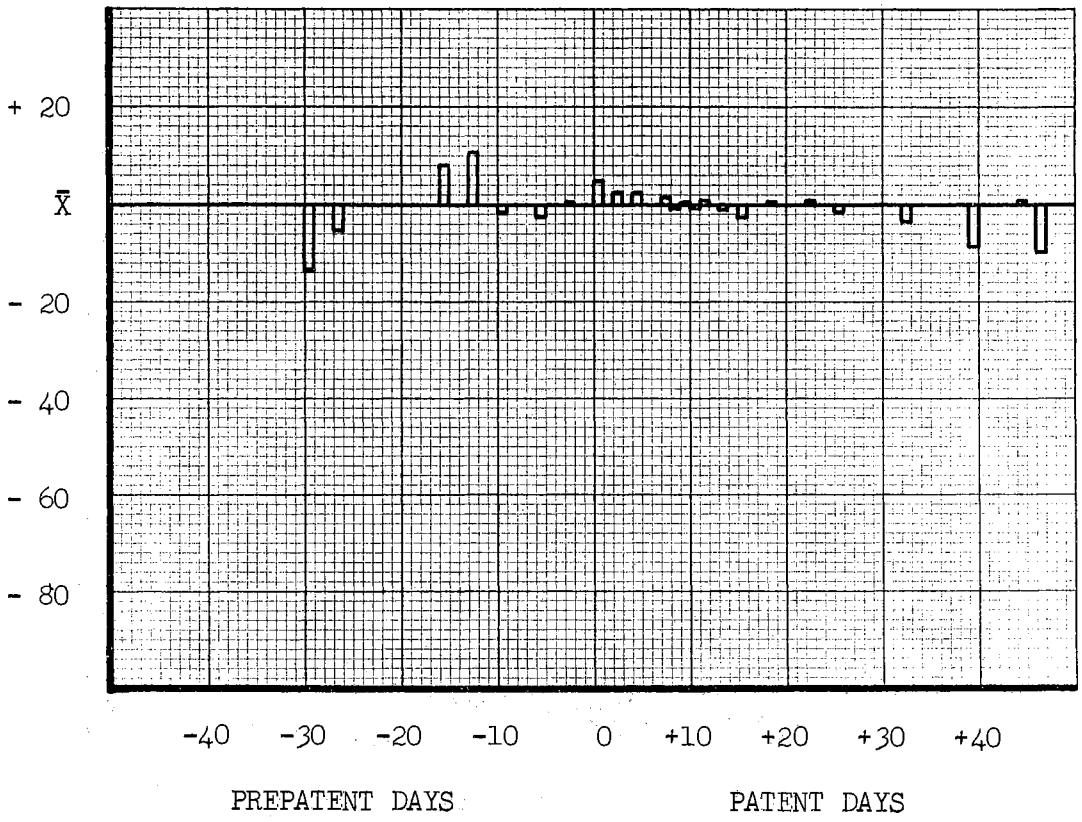

Figure 12. Deviations of the number of erythrycytes per cubic millimeter of blood from the pretreatment mean ($\bar{x} = 7.79 \times 10^6/mm.^2$) during the prepatent and patent periods of anaplasmosis in Group IV - Control laparotomy of the carrier calf: N = 5. (Y-axis is expressed as percent change.)

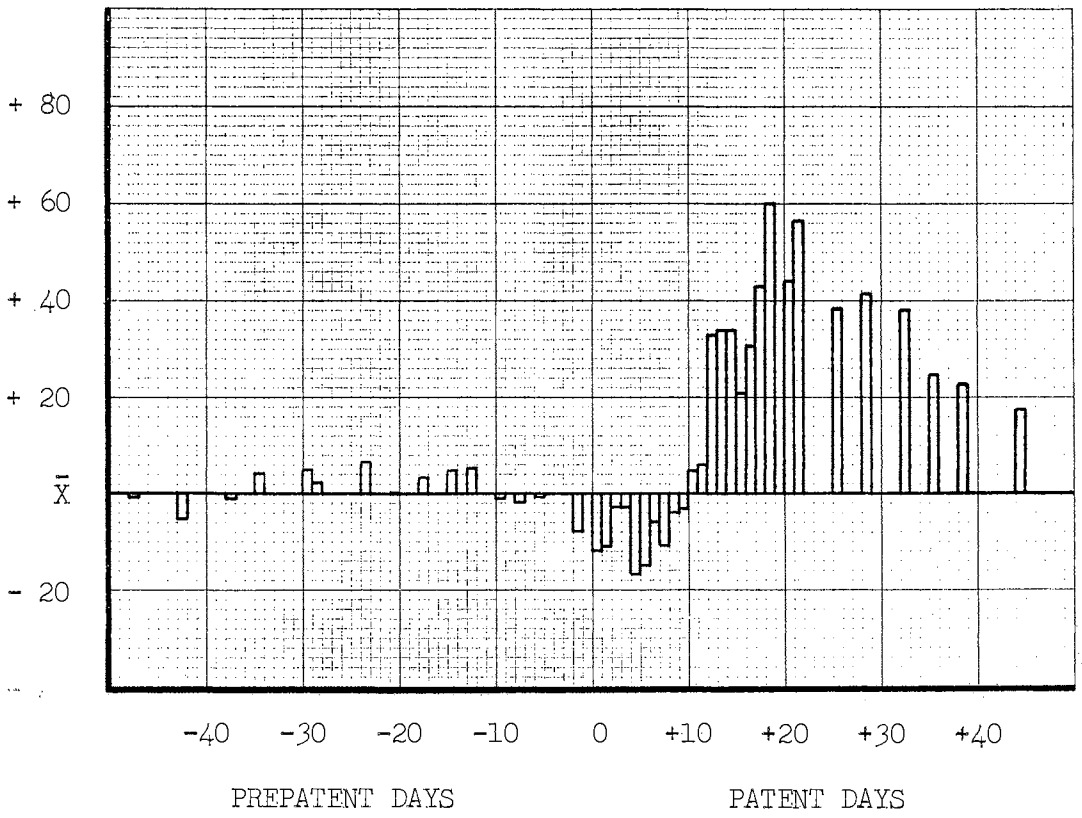

Figure 13. Deviations of the mean corpuscular volume of blood from the pretreatment mean ($\bar{x}$ = 37.5 c.u.) during the prepatent and patent periods of anaplasmosis in Group I - The primary disease response in the intact calf: N = 6. (Y-axis is expressed as percent change.)

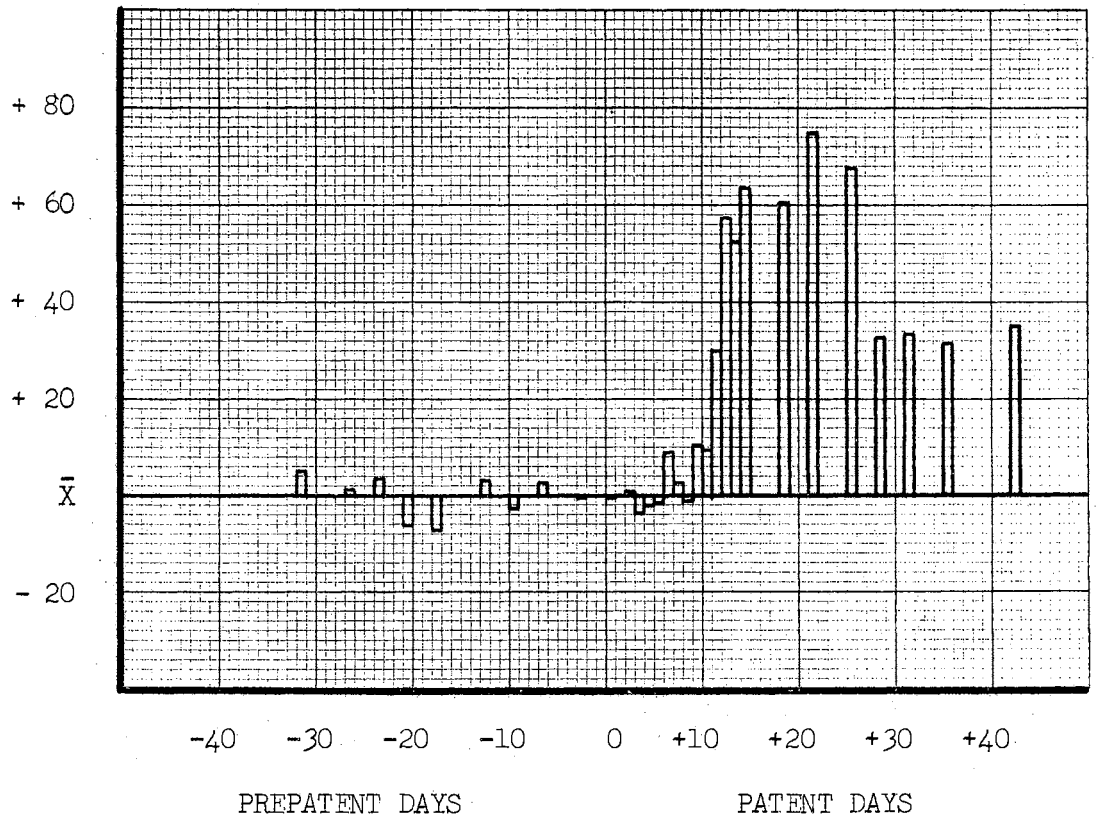

Figure 14. Deviations of the mean corpuscular volume of blood from the pretreatment mean ($\bar{x}$ = 38.6 c.u.) during the prepatent and patent periods of anaplasmosis in Group II - The primary relapse in the splenectomized carrier calf: N = 6. (Y-axis is expressed as percent change.)

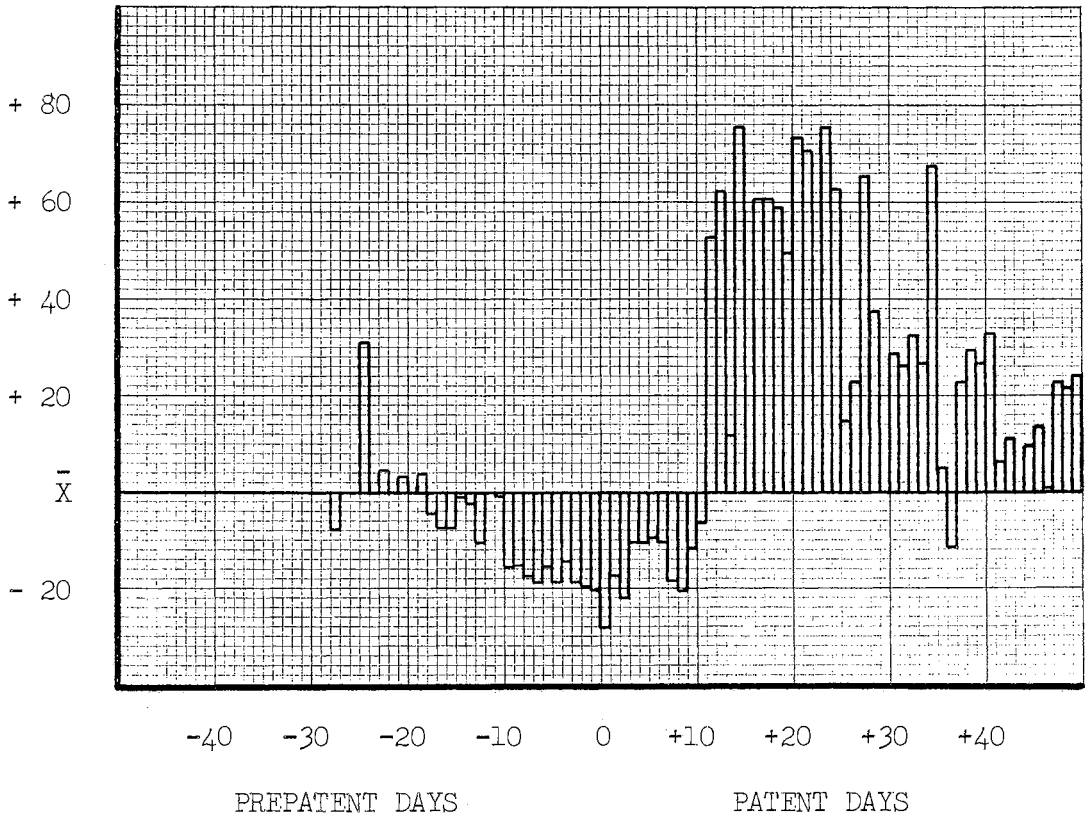

Figure 15. Deviations of the mean corpuscular volume of blood from the pretreatment mean ($\bar{x}$ = 38.7 c.u.) during the prepatent and patent periods of anaplasmosis in Group III - The primary disease response in the splenectomized calf; N = 6. (Y-axis is expressed as percent change.)

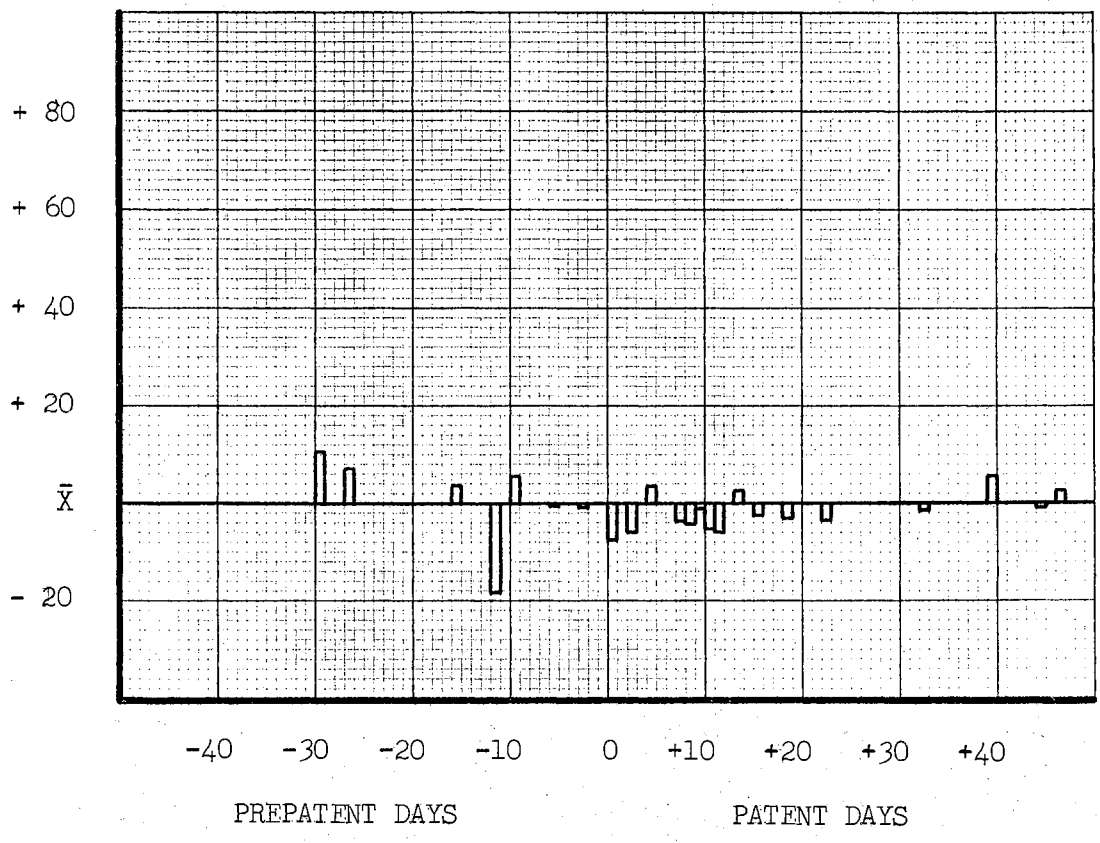

Figure 16. Deviations of the mean corpuscular volume of blood from the pretreatment mean ($\bar{x}$ = 38.4 c.u.) during the prepatent and patent periods of anaplasmosis in Group IV - Control laparotomy in the carrier calf: N = 5. (Y-axis is expressed as percent change.)

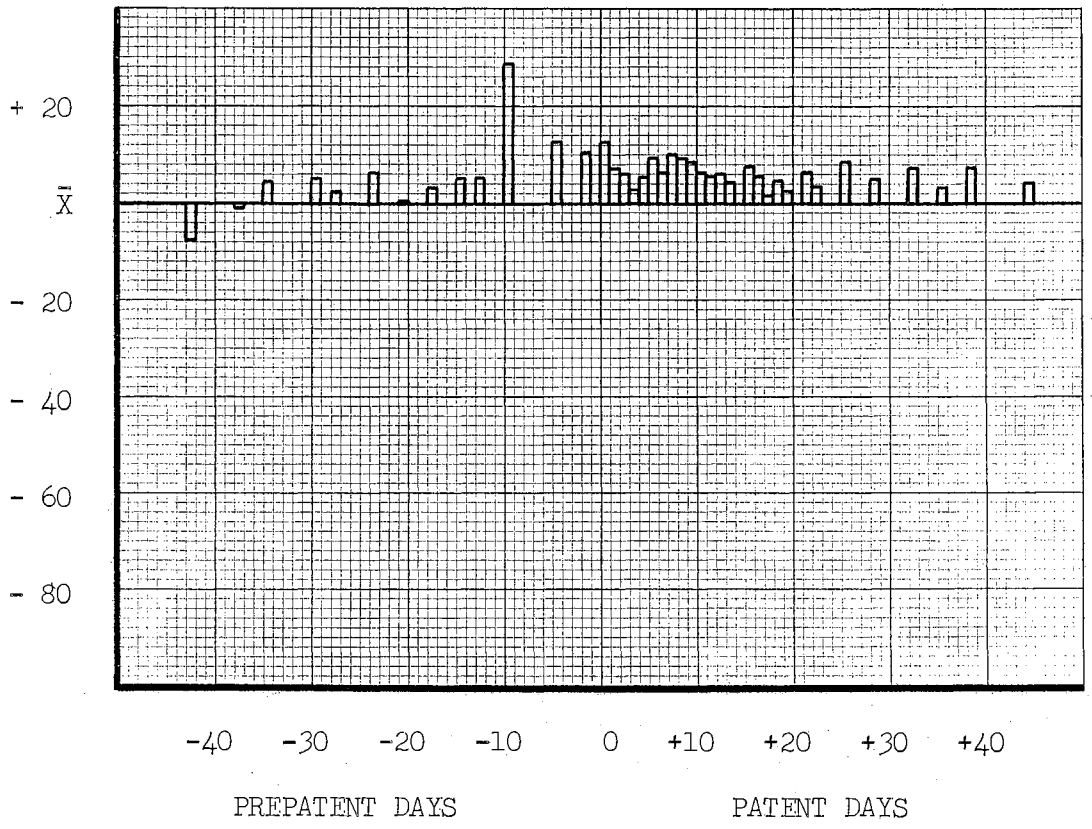

Figure 17. Deviations of the mean corpuscular hemoglobin concentration of blood from the pretreatment mean ($\bar{X}$ = 28.4 %) during the prepatent and patent periods of anaplasmosis in Group I - The primary disease response in the intact calf; N = 6. (Y-axis is expressed as percent change.)

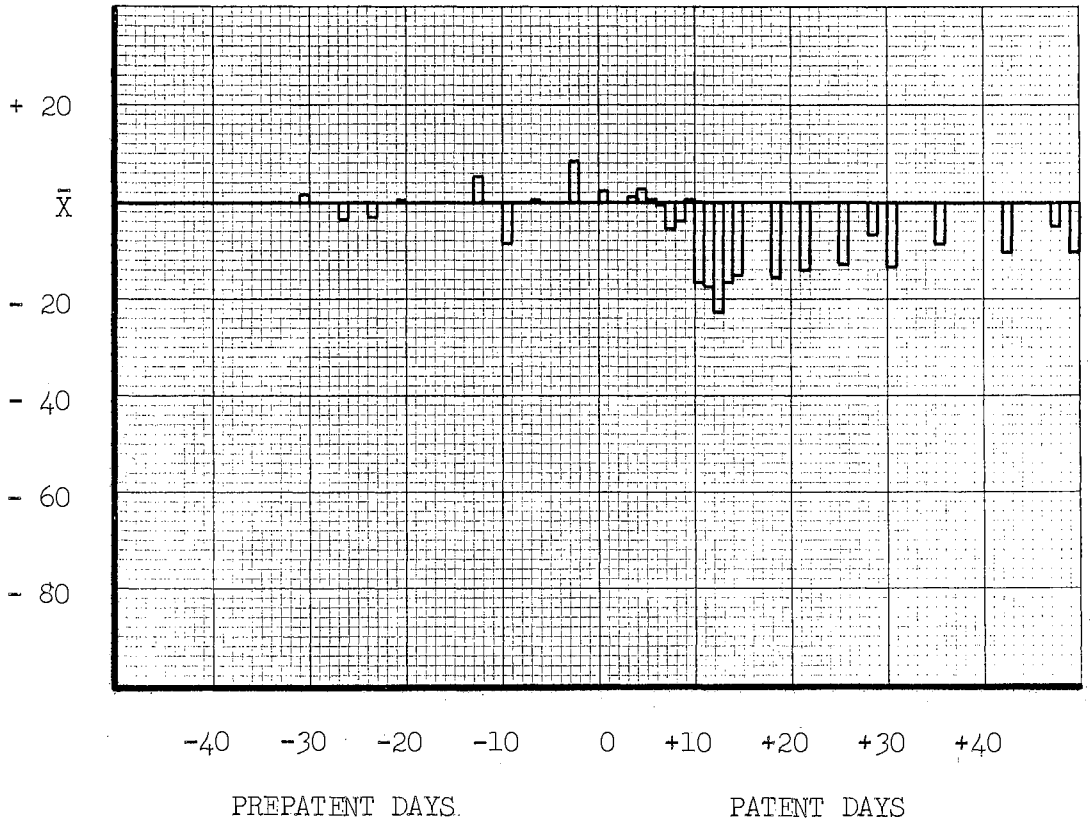

Figure 18. Deviations of the mean corpuscular hemoglobin concentration of blood from the pretreatment mean ($\bar{x}$ = 32.9 %) during the prepatent and patent periods of anaplasmosis in Group II - The primary relapse in the carrier calf: N = 6. (Y-axis is expressed as percent change.)

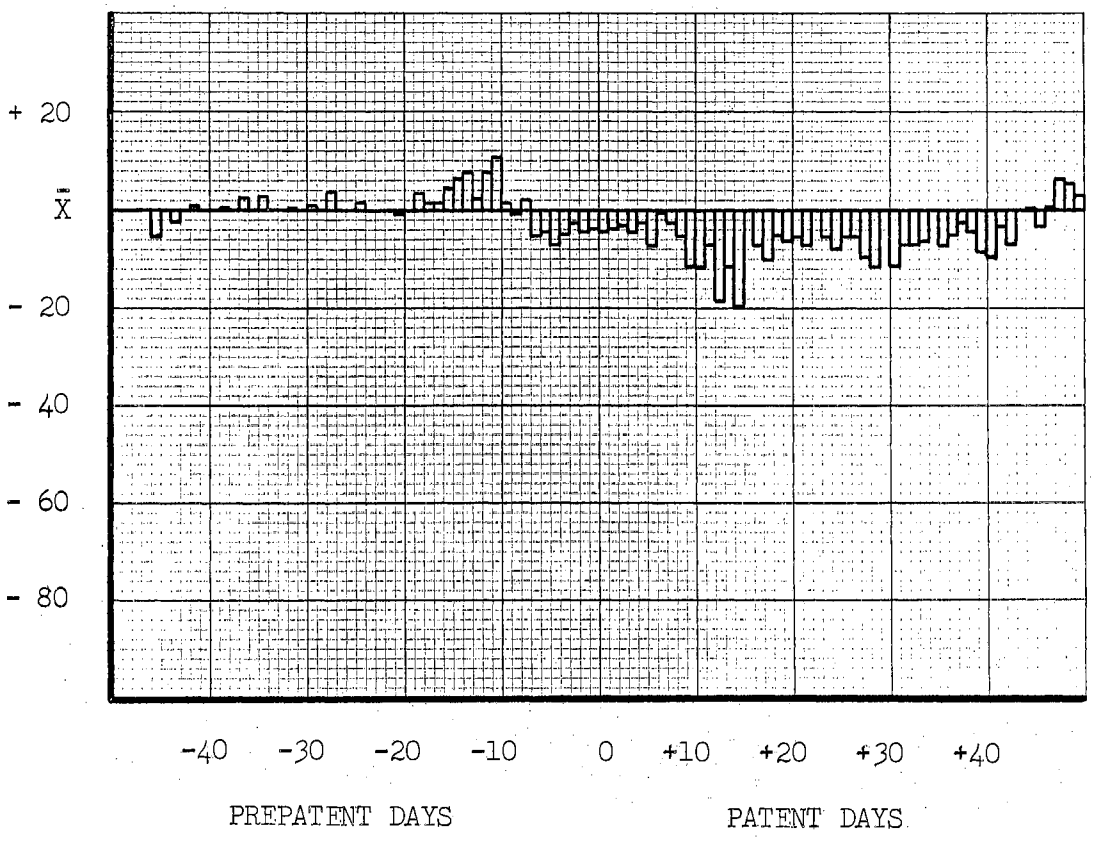

Figure 19. Deviations of the mean corpuscular hemoglobin concentration of blood from the pretreatment mean ($\bar{x}$ = 34.0 %) during the prepatent and patent periods of anaplasmosis in Group III - The primary disease response in the splenectomized calf: N = 6. (Y-axis is expressed as percent change.)

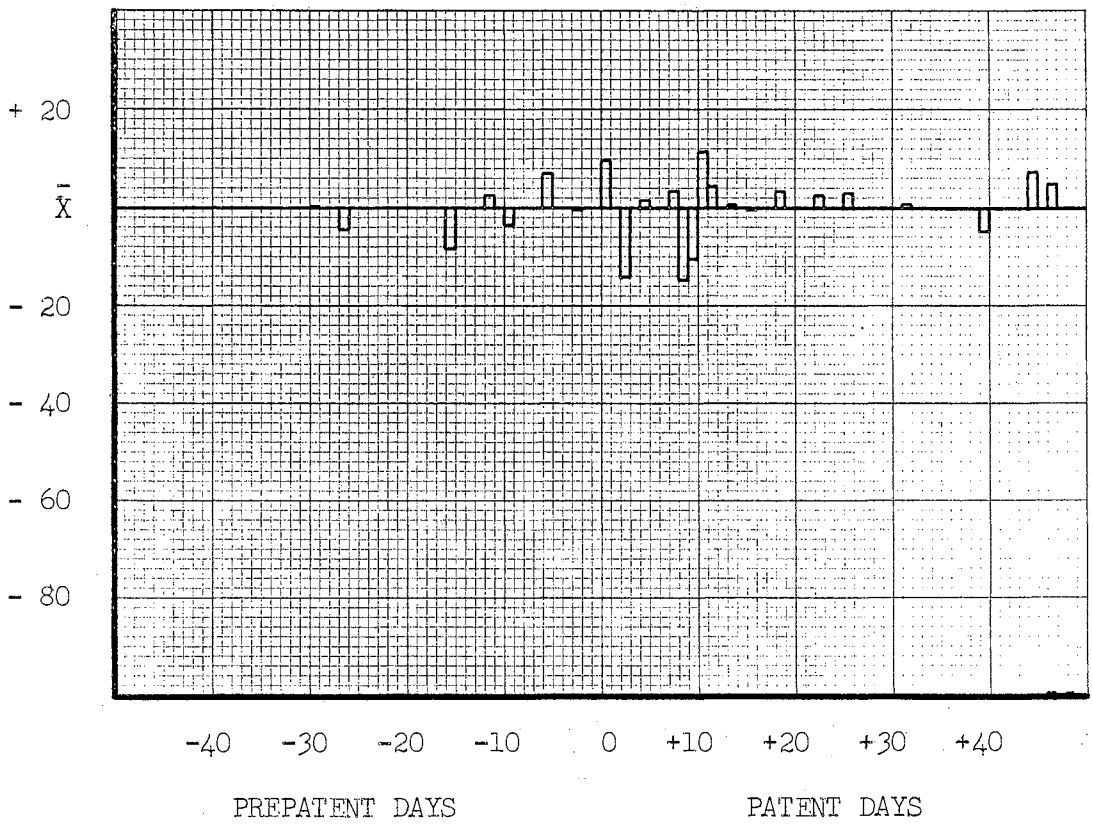

Figure 20. Deviations of the mean corpuscular hemoglobin concentration of blood from the pretreatment mean ($\bar{x}$ = 32.8 %) during the prepatent and patent periods of anaplasmosis in Group IV - Control laparotomy in the carrier calf: N = 5. (Y-axis is expressed as percent change.)

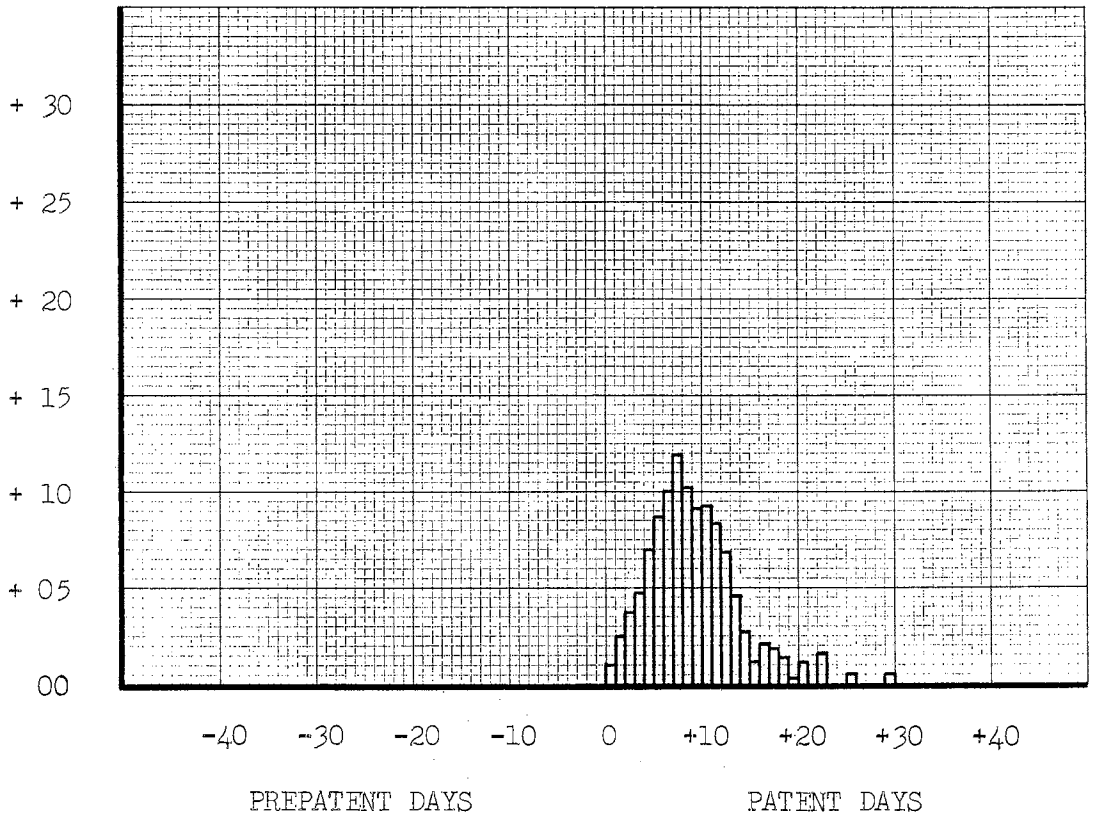

Figure 21. Deviations of the percentage of parasitized erythrocytes in the blood during the prepatent and patent periods of anaplasmosis in Group I - The primary disease response in the intact calf: N = 6. (Y-axis is expressed as percent change.)

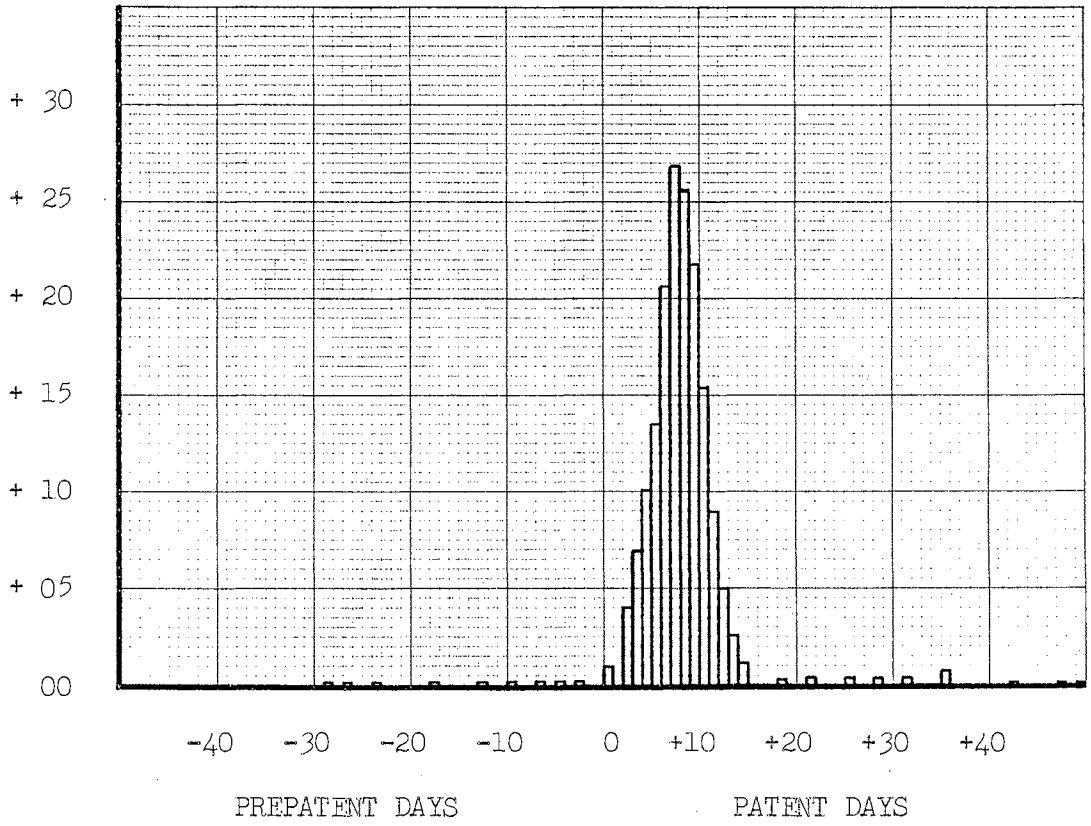

Figure 22. Deviations of the percentage of parasitized erythrocytes in the blood during the prepatent and patent periods of anaplasmosis in Group II - The primary relapse in the splenectomized carrier calf: N = 6. (Y-axis is expressed as percent change.)

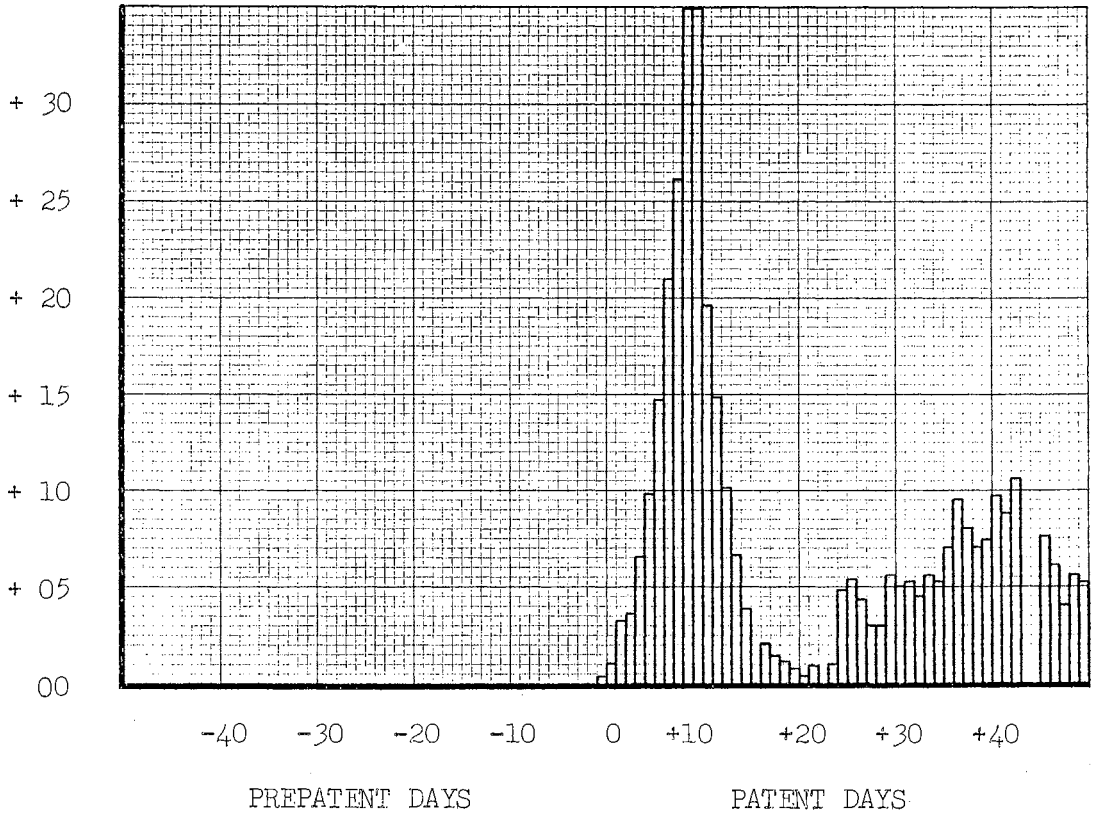

Figure 23. Deviations of the percentage of parasitized erythrocytes in the blood during the prepatent and patent periods of anaplasmosis in Group III - The primary disease response in the splenectomized calf: N = 6. (Y-axis is expressed as percent change.)

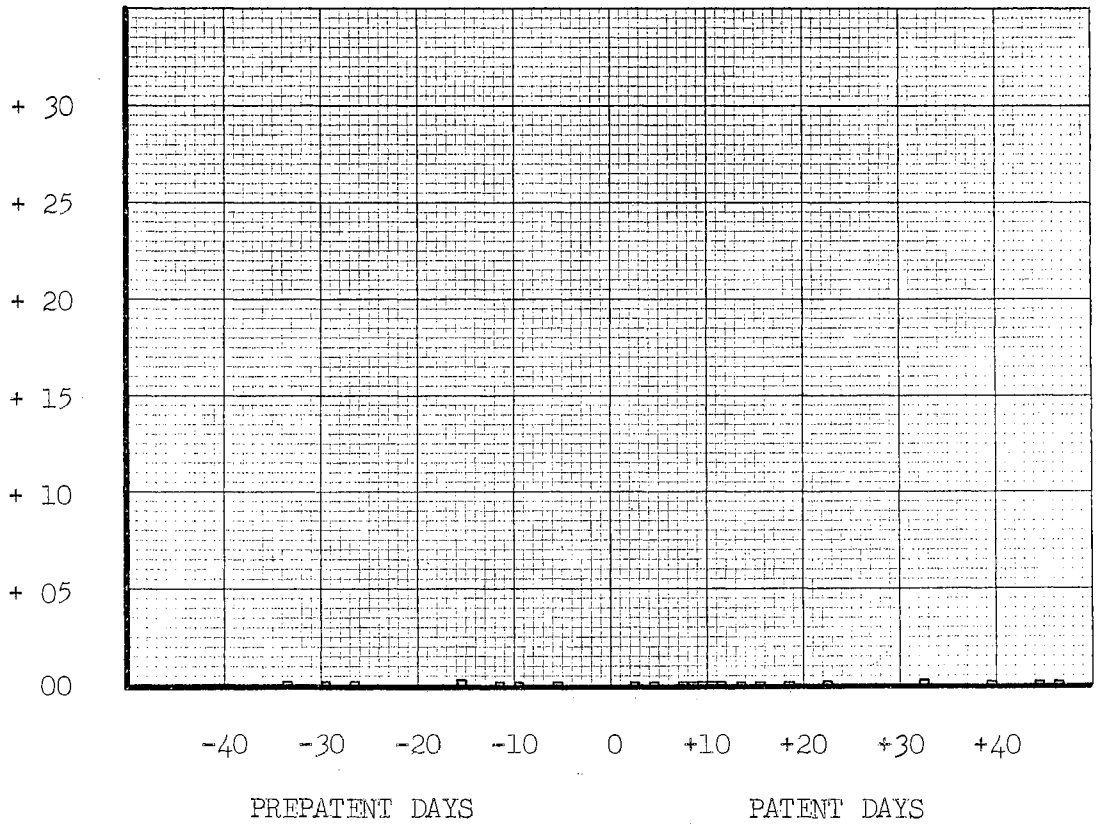

Figure 24. Deviations of the percentage of parasitized erythrocytes in the blood during the prepatent and patent periods of anaplasmosis in Group IV - Control laparotomy of the carrier calf: N = 5. (Y-axis is expressed as percent change.)

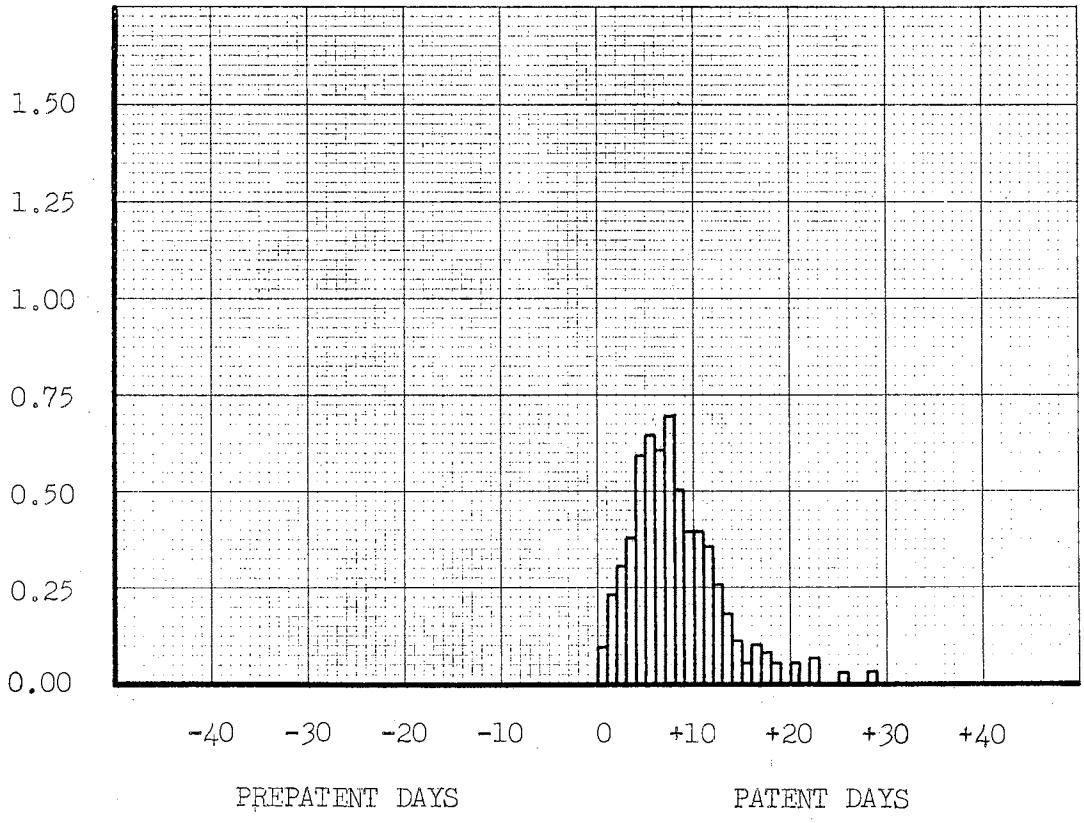

Figure 25. Deviations of the number of parasitized erythrocytes per cubic millimeter of blood during the prepatent and patent periods of anaplasmosis in Group I - The primary disease response in the intact calf: N = 6. (Y-axis is expressed as erythrocytes x 10^6 per $mm.^2$ of blood.)

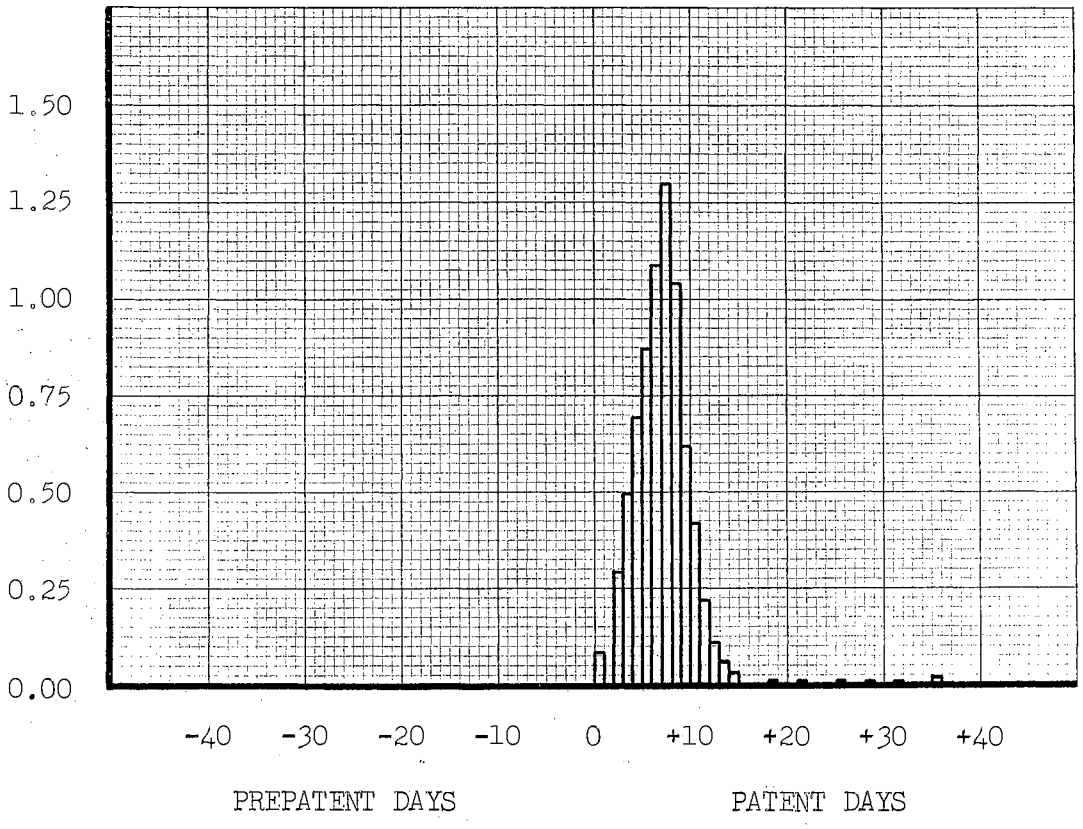

Figure 26. Deviations of the number of parasitized erythrocytes per cubic millimeter of blood during the prepatent and patent periods of anaplasmosis in Group II - The primary relapse in the splenectomized carrier calf: N = 6. (Y-axis is expressed as erythrocytes x 10^6 per $mm.^2$ of blood.)

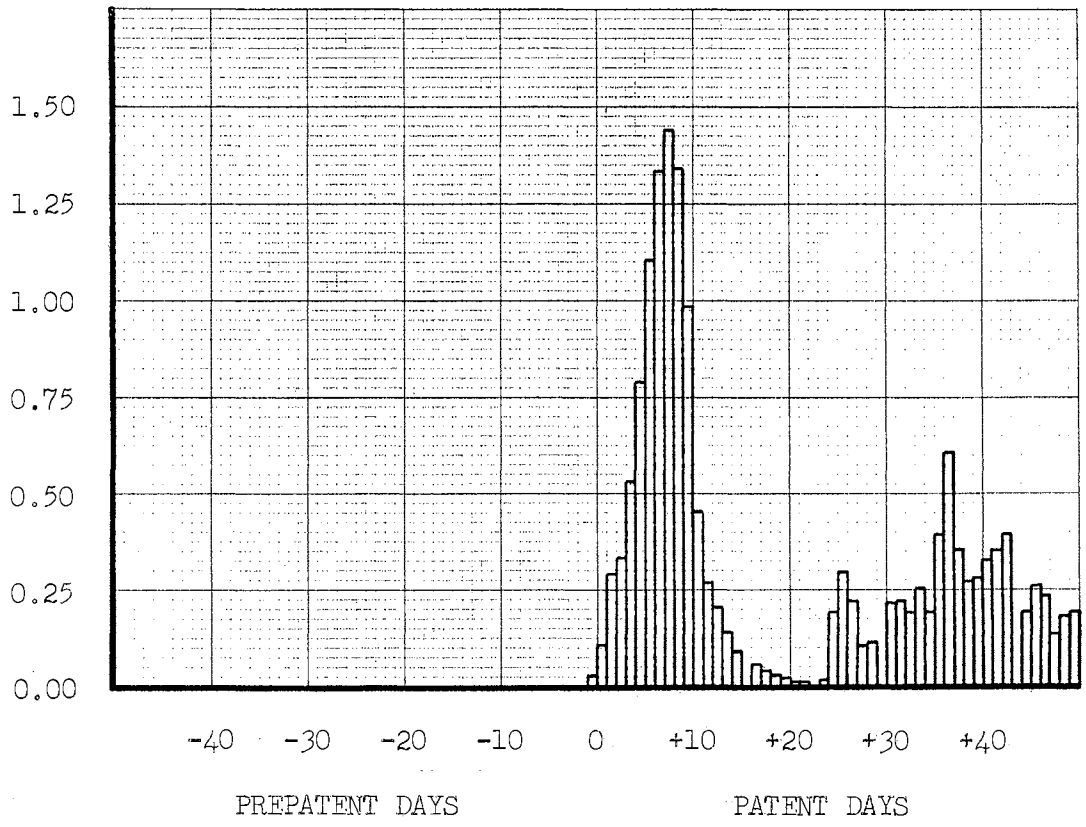

Figure 27. Deviations of the number of parasitized erythrocytes per cubic millimeter of blood during the prepatent and patent periods of anaplasmosis in Group III - The primary disease in the splenectomized calf: $N_2 = 6$. (Y-axis is expressed as erythrocytes x 10^6 per $mm.^2$ of blood.)

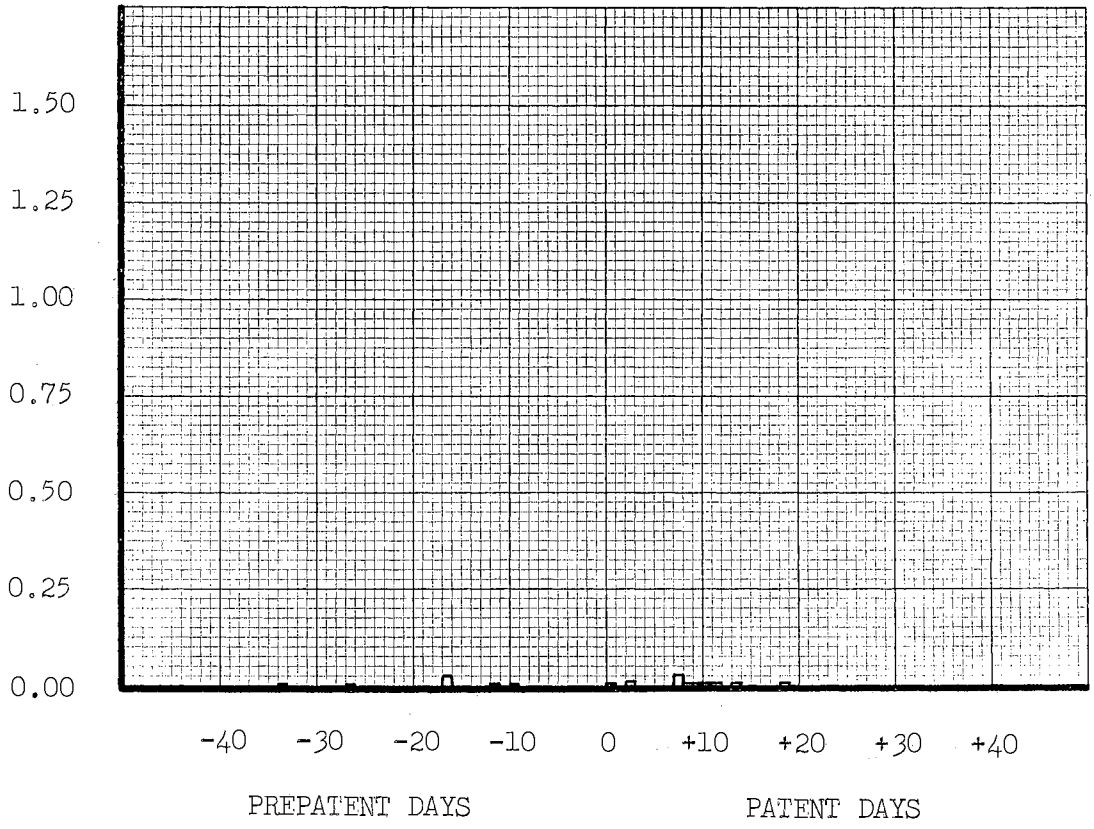

Figure 28. Deviations of the number of parasitized erythrocytes per cubic millimeter of blood during the prepatent and patent periods of anaplasmosis in Group IV - Control laparotomy of the carrier calf: $N_2 = 5$. (Y-axis is expressed as erythrocytes x 10^6 per $mm.^2$ of blood.)

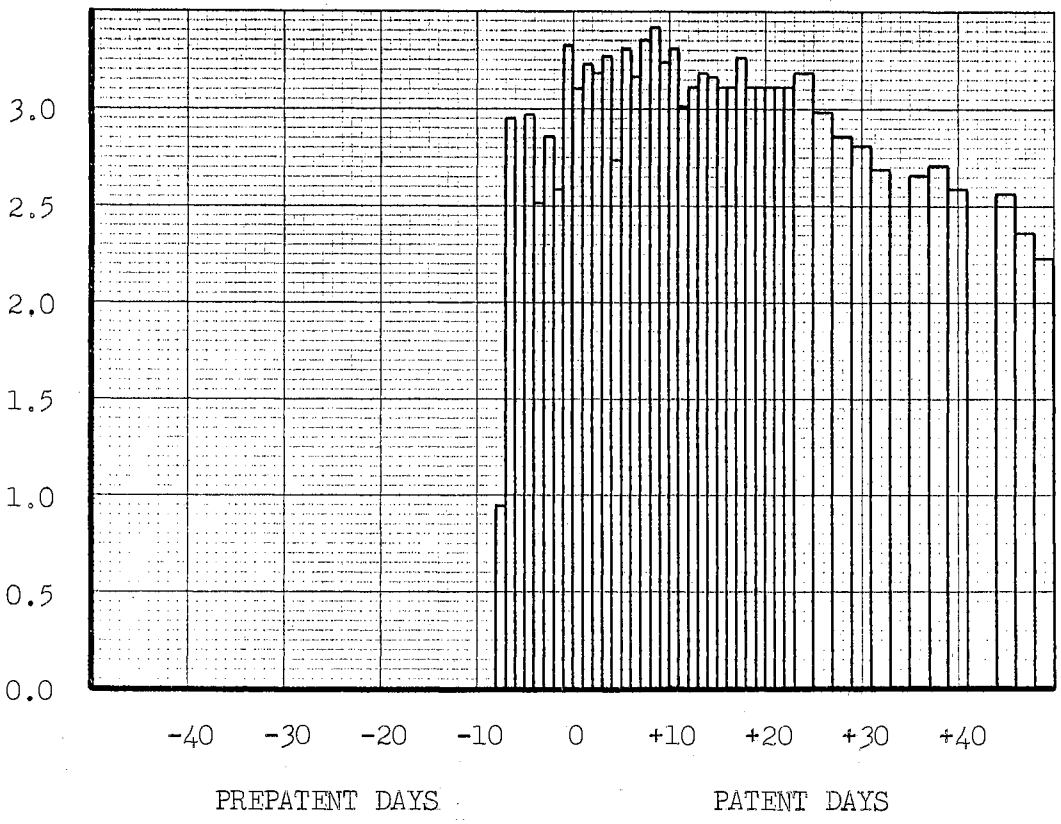

Figure 29. Deviations of the complement-fixation reaction curing the prepatent and patent periods of anaplasmosis in Group I - The primary disease response in the intact calf: N = 6. (Y-axis is expressed as the logarithm to the base 10 of the denominator of the highest dilution giving a 4 + reaction. See Figure 44.)

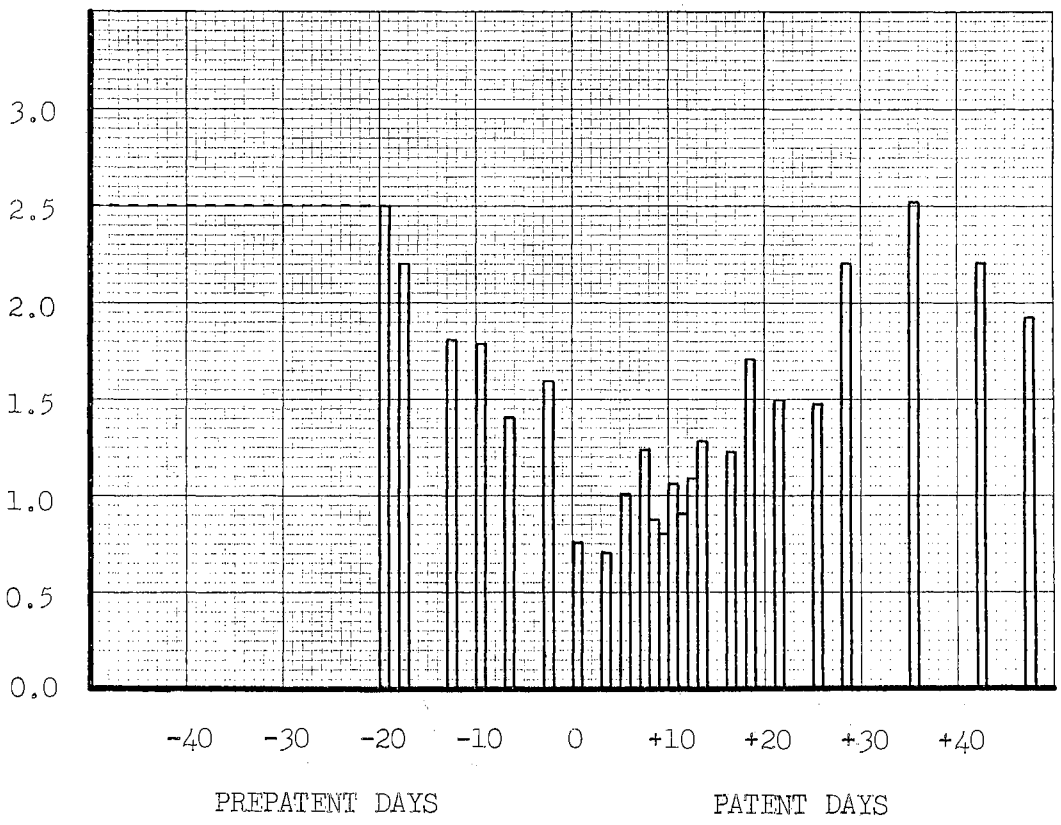

Figure 30. Deviations of the complement-fixation reaction during the prepatent and patent periods of anaplasmosis in Group II - The primary relapse in the splenectomized carrier calf: 6. The area under the dotted line indicates that although no high dilutions were made, the animals were CF positive at the standard titration of 4 + at 1/10. (Y-axis is expressed as the logarithm to the base 10 of the denominator of the highest dilution giving a 4 + reaction. See Figure 44.)

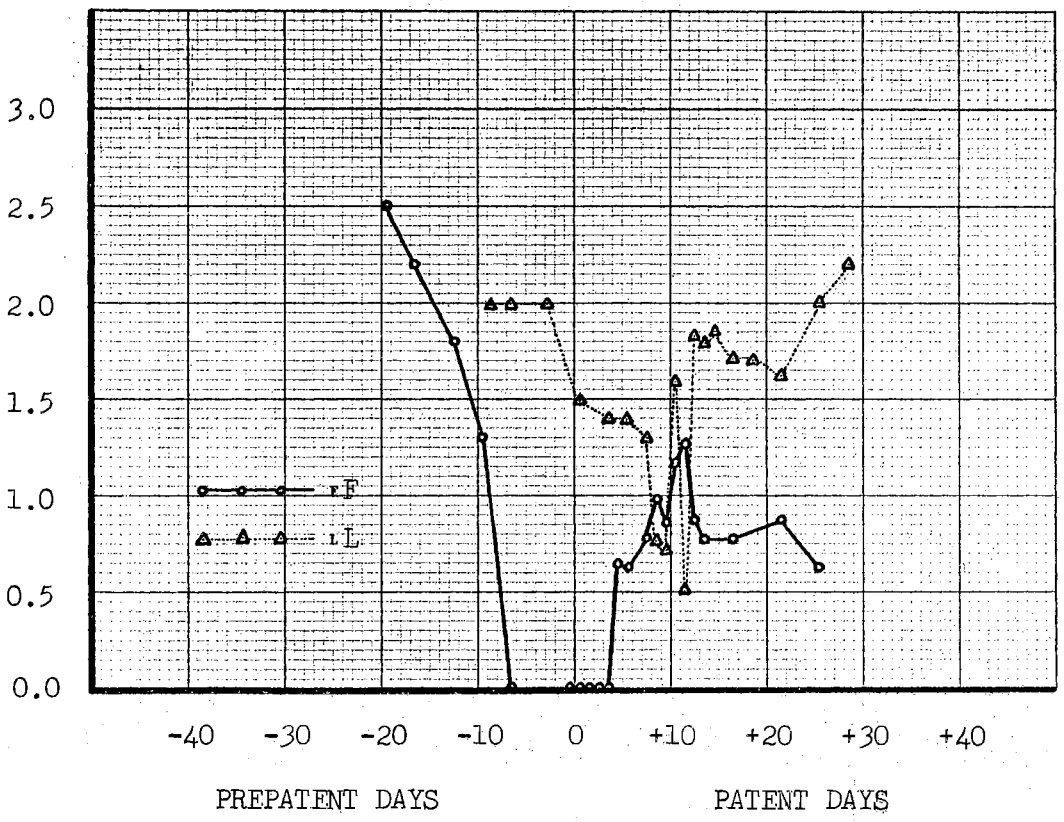

Figure 31. Comparison of the complement-fixation reactions during the prepatent and patent periods of anaplasmosis between subgroups of Group II - The primary relapse in the splenectomized carrier calf: N = 3 for each subgroup. F = the subgroup that had the earliest reduction in CF titer. L = the subgroup that had the later reduction. (Y-axis is expressed as the logarithm to the base 10 of the denominator of the highest dilution giving a 4 + reaction. See Figure 44.)

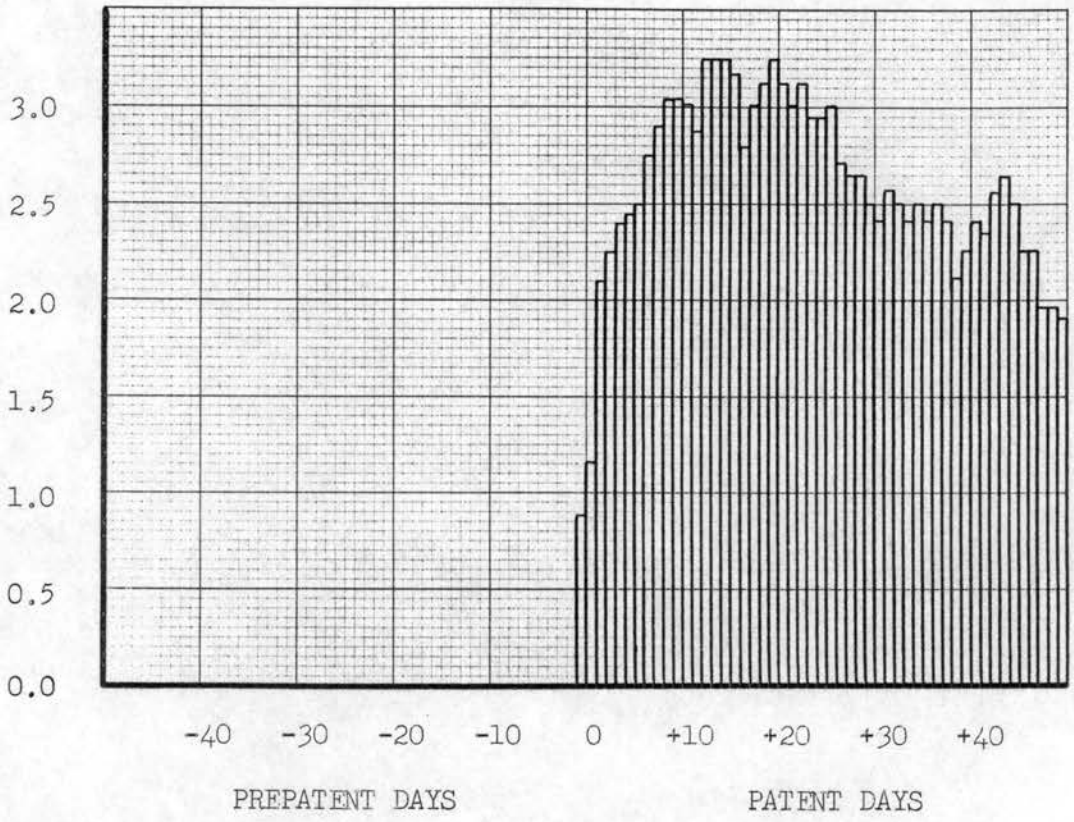

Figure 32. Deviations of the complement-fixation reactions during the prepatent and patent periods of anaplasmosis in Group III - The primary disease response in the splenectomized calf: N = 6. (Y-axis is expressed as the logarithm to the base 10 of the denominator of the highest dilution giving a 4 + reaction. See Figure 44.)

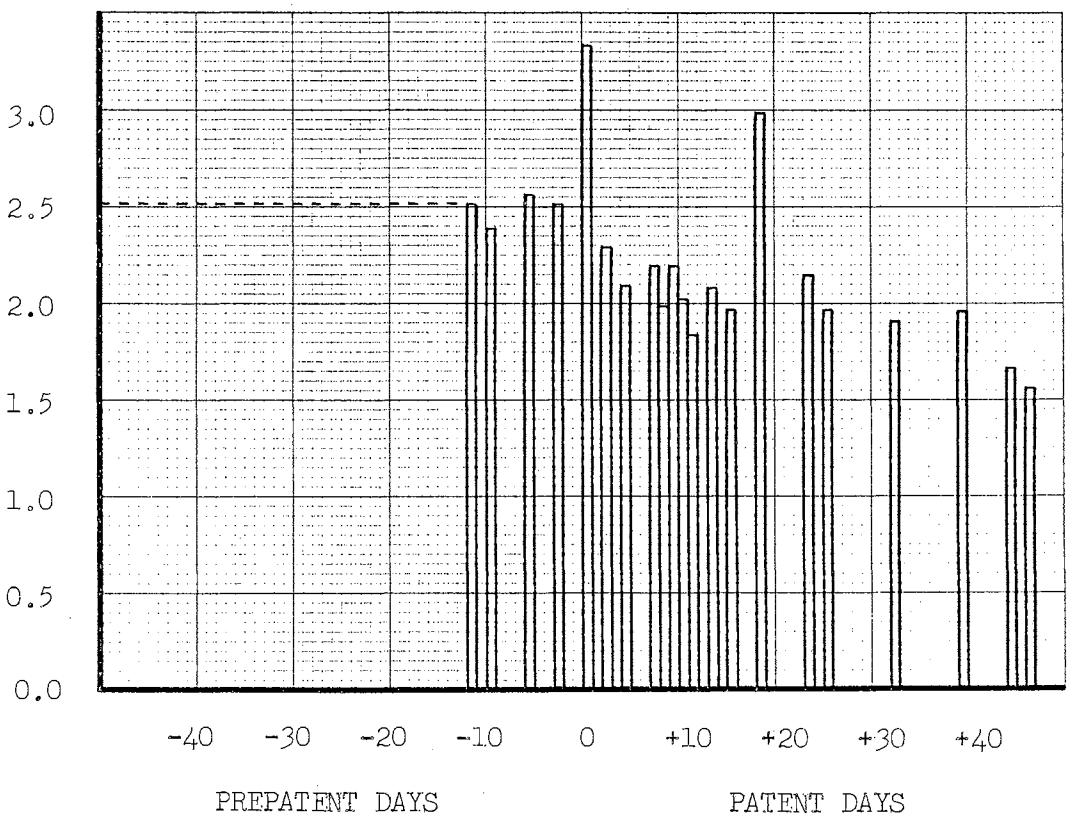

Figure 33. Deviations of the complement-fixation reaction during the prepatent and patent periods of anaplasmosis in Group IV - Control laparotomy in the carrier calf: N - 5. The area under the dotted line indicates that although no high dilutions were made, the animals were CF positive at the standard titration of 4 + at 1/10. (Y-axis is expressed as the logarithm to the base 10 of the denominator of the highest dilution giving a 4 + reaction. See Figure 44.)

CHAPTER V

RESULTS

Comparison of the Treatment Groups Using Fitted Curves

The following graphs are fitted curves taken from the individual group bar graphs. Although usual care was taken in fitting the curves, one should refer to the individual bar graphs for close analysis.

The writer has omitted the Group IV control values from certain fitted graph comparisons. These values are represented by the zero mean (horizontal continuous line) since their deviations approximate zero. This was done to avoid duplication of essentially identical lines. These control values may be inspected in their respective bar graphs.

Hemoglobin (Figure 34)

The deviations noted in the late prepatent period (days -15 through "0") appear to the writer to be definite changes. The progressive rate of fall in hemoglobin values appears to be the same for all three groups. The two groups of splenectomized animals give similar responses and maintain low values throughout the observed patent period. The group of intact animals does not reach such low values. The values of the intact animals return to the prepatent "normal" values on day +30 and appear to "over-compensate" during days +30 through +50.

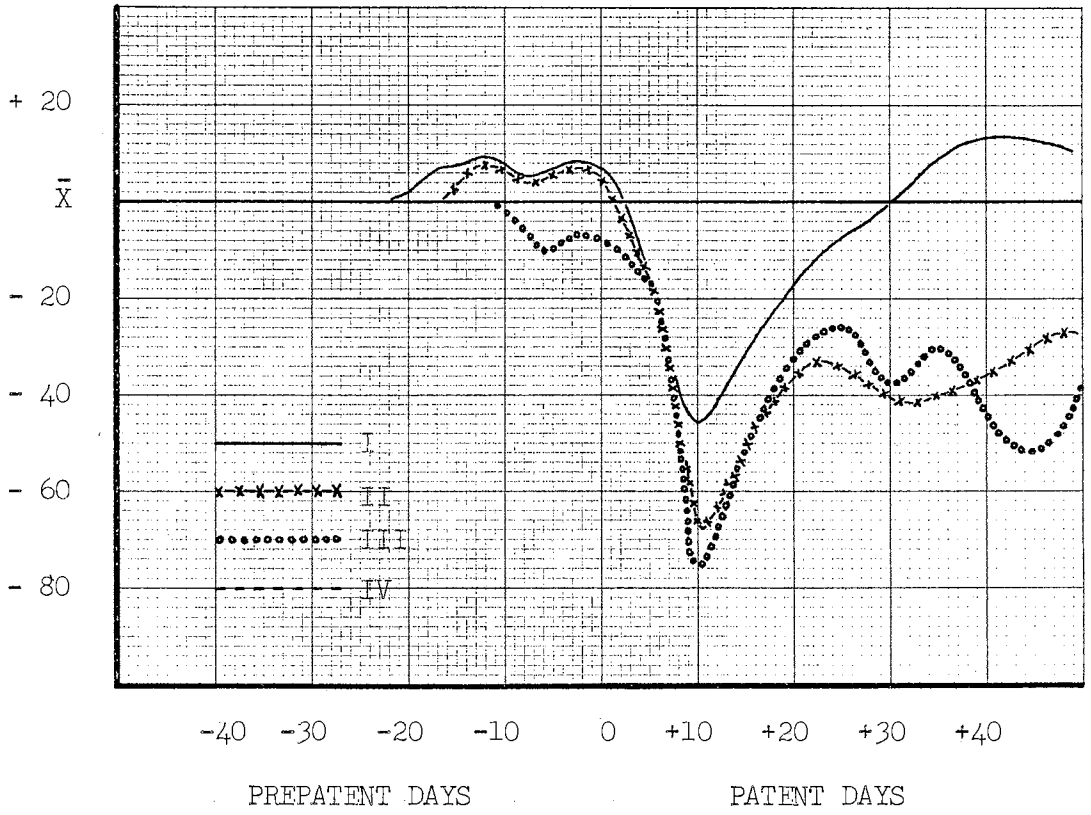

Figure 34. Comparison of the deviations of the hemoglobin values of blood during the prepatent and patent periods of anaplasmosis in: Group I - The primary disease response in the intact calf; Group II - The primary relapse in the splenectomized calf; Group III - The primary disease response in the splenectomized calf; Group IV - The control laparotomy in the carrier calf (this group omitted since it approximated zero). These fitted curves are taken from data in Figures 1, 2, 3, and 4. (Y-axis is expressed as percent change.)

Packed Cell Volume (Figure 35)

The fluctuations of these curves follow those observed in the graph on hemoglobin, with the exception of the late prepatent changes (days -15 through "0"). Only Group III appears to show significant changes in the late prepatent period. Again, the response of the two groups of splenectomized animals is similar and is more severe and of longer duration than the group of intact animals. The apparent over-compensation is noted in the intact animals beginning at day +30.

Numbers of Erythrocytes (Figure 36)

The late prepatent response is quite marked in this graph, with all groups showing some degree of late prepatent elevation. During the patent period the two groups of splenectomized animals maintain low values and appear similar until the last part (days +40 to +50) of the observed patent period. The similarity of shape of the curves of Groups I and II during the patent period should be noted, even though the difference of values is apparent, the long convalescence of the asplenic animals is dramatically different from that of the intact animals.

Mean Corpuscular Volumes (Figure 37)

The reduction of MCV is apparent in the late prepatent and early patent periods (days -10 through +10). On day +10, the start of a marked elevation in MCV values can be seen. Again, the asplenic groups appear similar (until day +28). The response of the intact animals was neither as marked nor as abrupt.

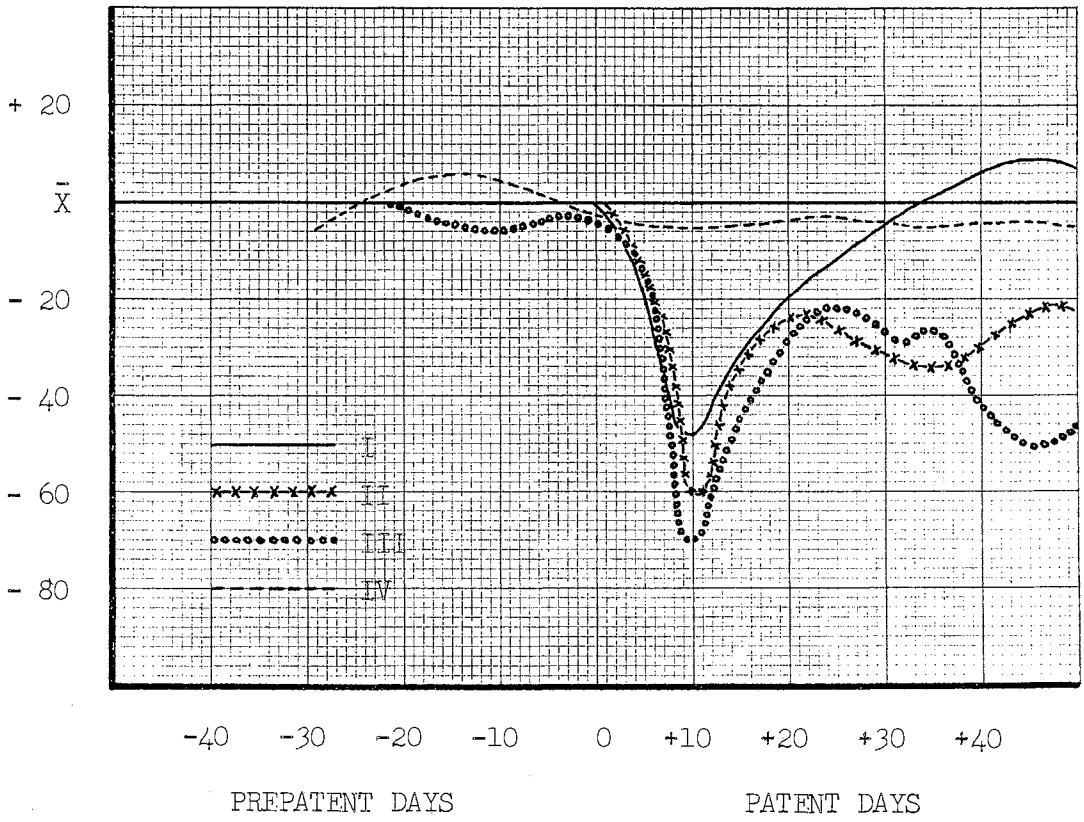

Figure 35. Comparison of the deviations of the packed cell volume values of blood during the prepatent and patent periods of anaplasmosis in: Group I - The primary disease response in the intact calf; Group II - The primary relapse in the carrier calf; Group III - The primary disease response in the splenectomized calf; Group IV - Control laparotomy in the carrier calf. These fitted curves are taken from data in Figures 5, 6, 7, and 8. (Y-axis is expressed as percent change.)

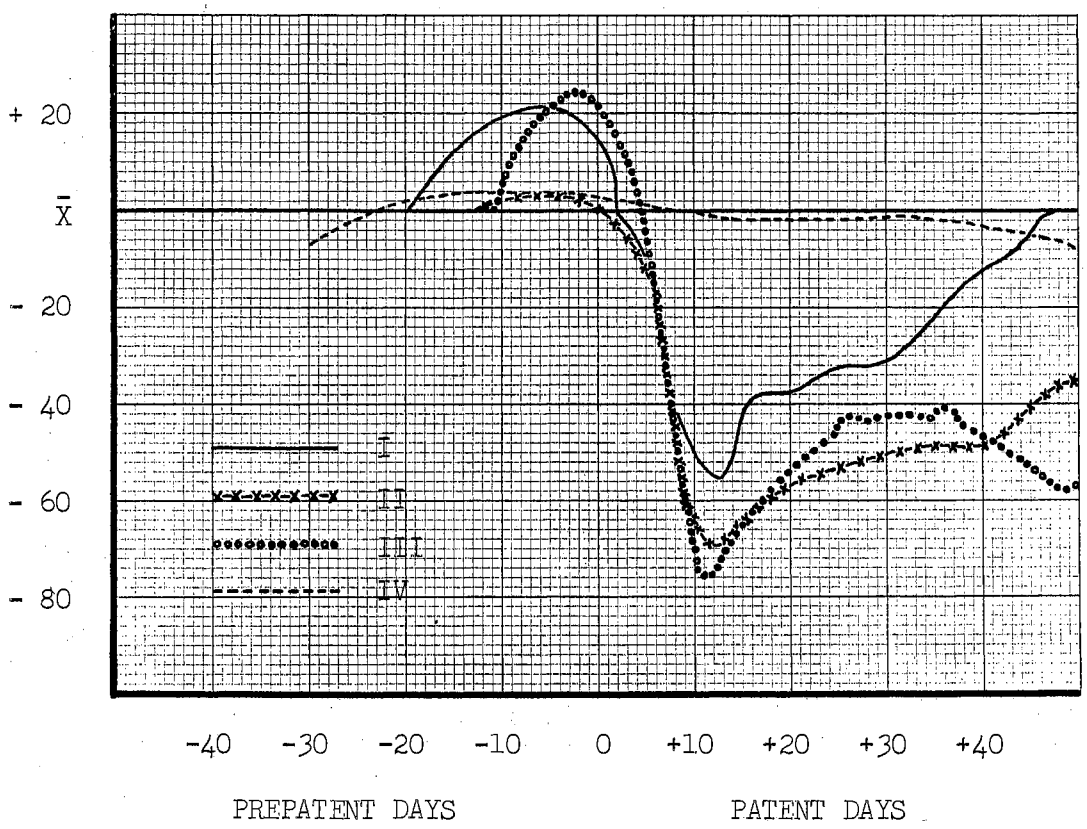

Figure 36. Comparison of the deviations of the erythrocyte counts of blood during the prepatent and patent periods of anaplasmosis in: Group I - The primary disease response in the intact calf; Group II - The primary relapse in the splenectomized carrier calf; Group III - The primary disease response in the splenectomized calf. These fitted curves are taken from data in Figures 9, 10, 11, and 12. (Y-axis is expressed as percent change.)

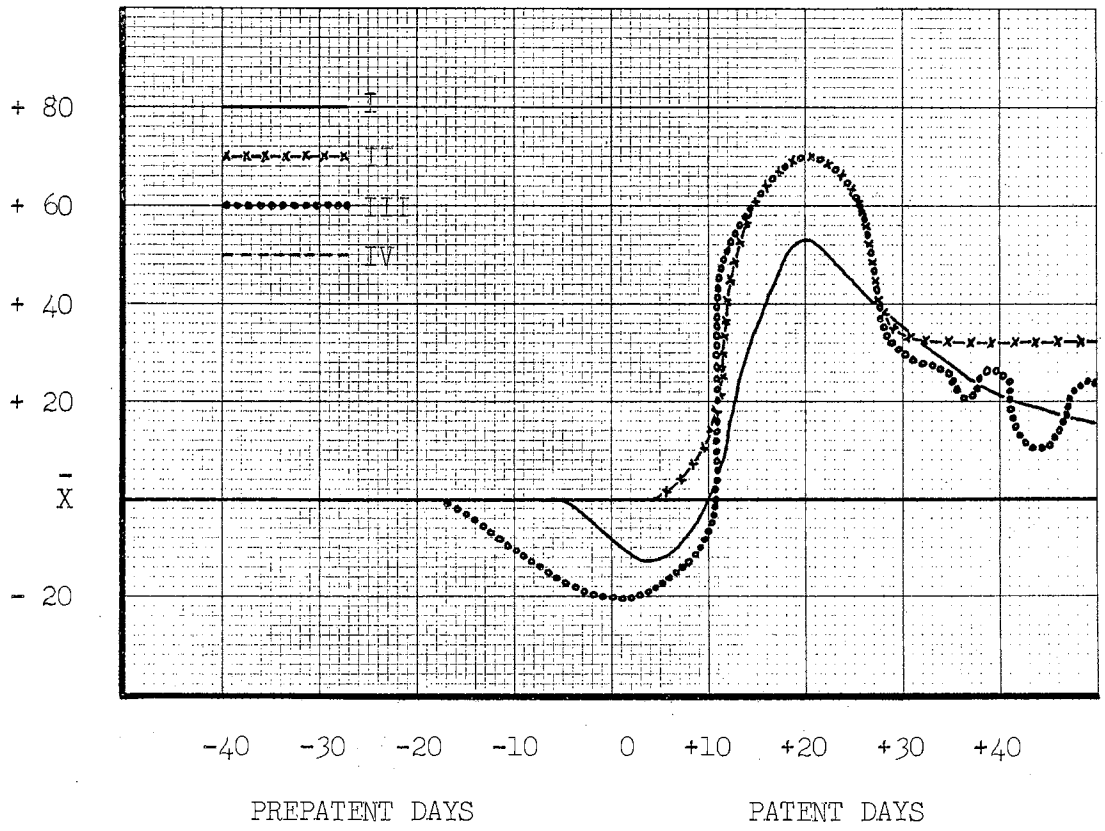

Figure 37. Comparison of the deviations of the mean corpuscular volumes of blood during the prepatent and patent periods of anaplasmosis in: Group I - The primary response in the intact calf; Group II - The primary relapse in the splenectomized carrier calf; Group IV - Control laparotomy of the carrier calf (this group is omitted because it approximated zero). These fitted curves are taken from data in Figures 17, 18, 19, and 20. (Y-axis is expressed as percent change.)

Mean Corpuscular Hemoglobin Concentration (Figure 38)

The late prepatent period (days -10 through "0") shows a difference in the response by Groups I and III. During the patent period there is an apparent long-term reduction in the MCHC values of the asplenic animals. There are two slight decreases in MCHC values (days +19 and +41) which appear to occur simultaneously in Groups II and III. The elevation of the MCHC in the intact group animals is related to the fact that this group had a lower MCHC pretreatment mean. (See Table I).

Percentage of Parasitized Erythrocytes (Figure 39)

The initial rate of rise is strikingly similar for all three groups. The asplenic animals maintain their high rate of change to their peak values. On day +2 the rate of change for the intact animals becomes reduced. The intact animals reach a considerably lower peak value. Group III maintains a marked parasitemia during the entire observed patent period. The fitting of the curve to data for Group III for days +29 through +49 is problematical. It is suggested that the reader review Figure 23 before making judgement on this section of the graph.

Numbers of Parasitized Erythrocytes (Figure 40)

This graph is similar in form to that for the percentage of parasitized erythrocytes. (Figure 39). The rise and fall of the curves in Figure 40 precede those in Figure 39 by one to two days. It appears that the rise by Group III presents itself one day earlier

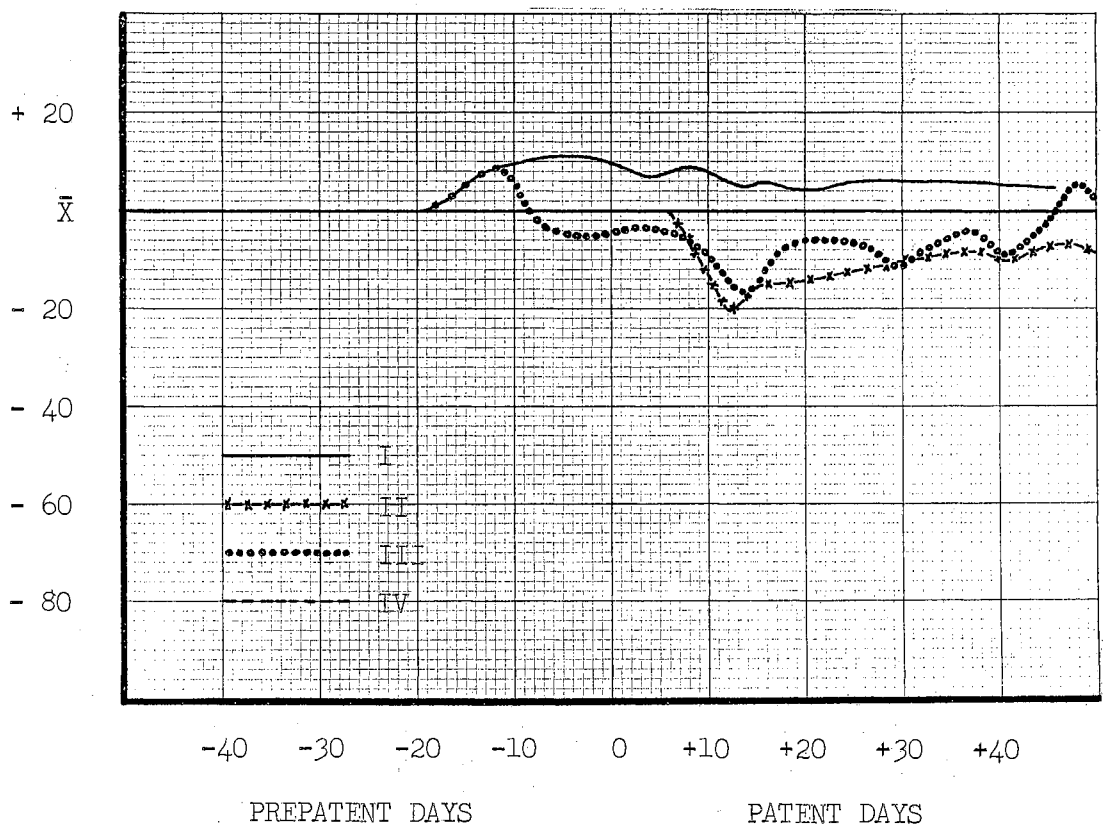

Figure 38. Comparison of the deviations of the mean corpuscular hemoglobin concentrations of blood during the prepatent and patent periods of anaplasmosis in: Group I - The primary disease response in the intact calf; Group II - The primary relapse in the splenectomized carrier calf; Group III - The primary disease response in the splenectomized calf; Group IV - Control laparotomy in the carrier calf (this group was omitted since it approximated zero). These fitted curves are taken from data in Figures 22, 23, 24, and 25. (Y-axis is expressed as percent change.)

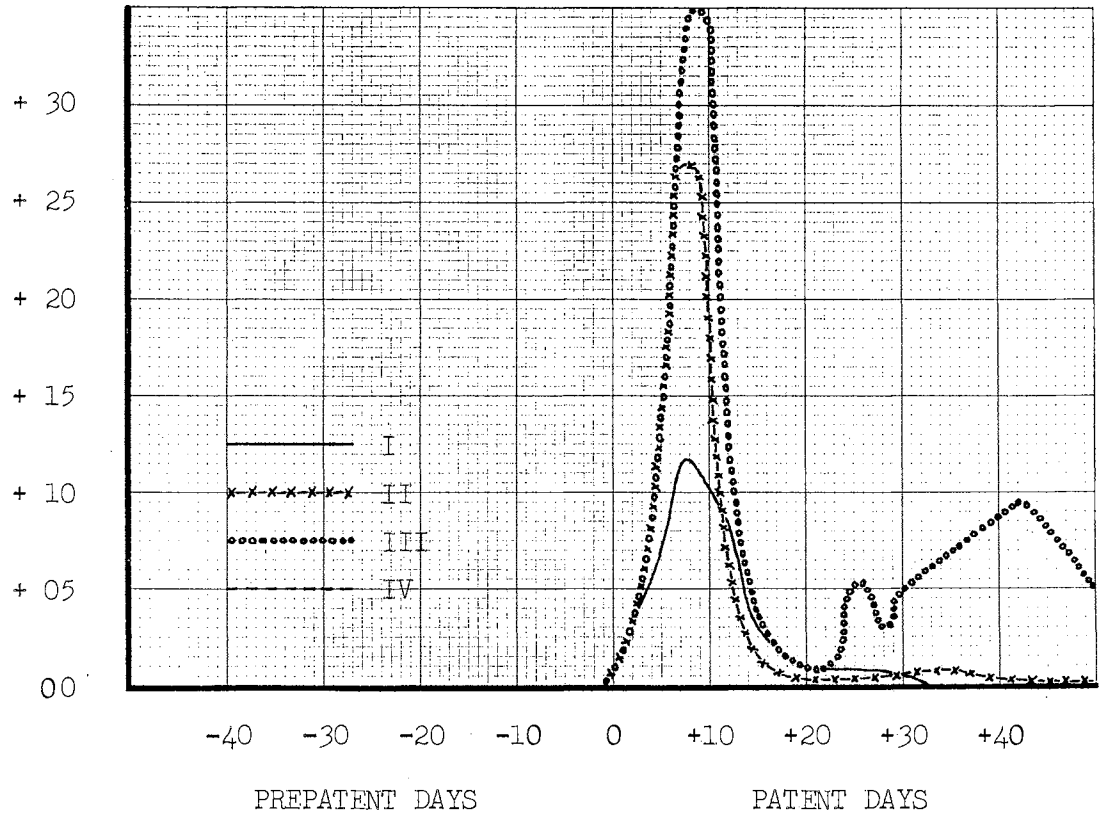

Figure 39. Comparison of the percentage of parasitized erythrocytes of blood during the prepatent and patent periods of anaplasmosis in: Group I - The primary disease response in the intact calf; Group II - The primary relapse in the splenectomized carrier calf; Group III - The primary disease response in the splenectomized calf; Group IV - Control laparotomy in the carrier calf (this group was omitted since it approximated zero). These fitted curves are taken from data in Figures 27, 28, 29, and 30. (Y-axis is expressed as percent change.)

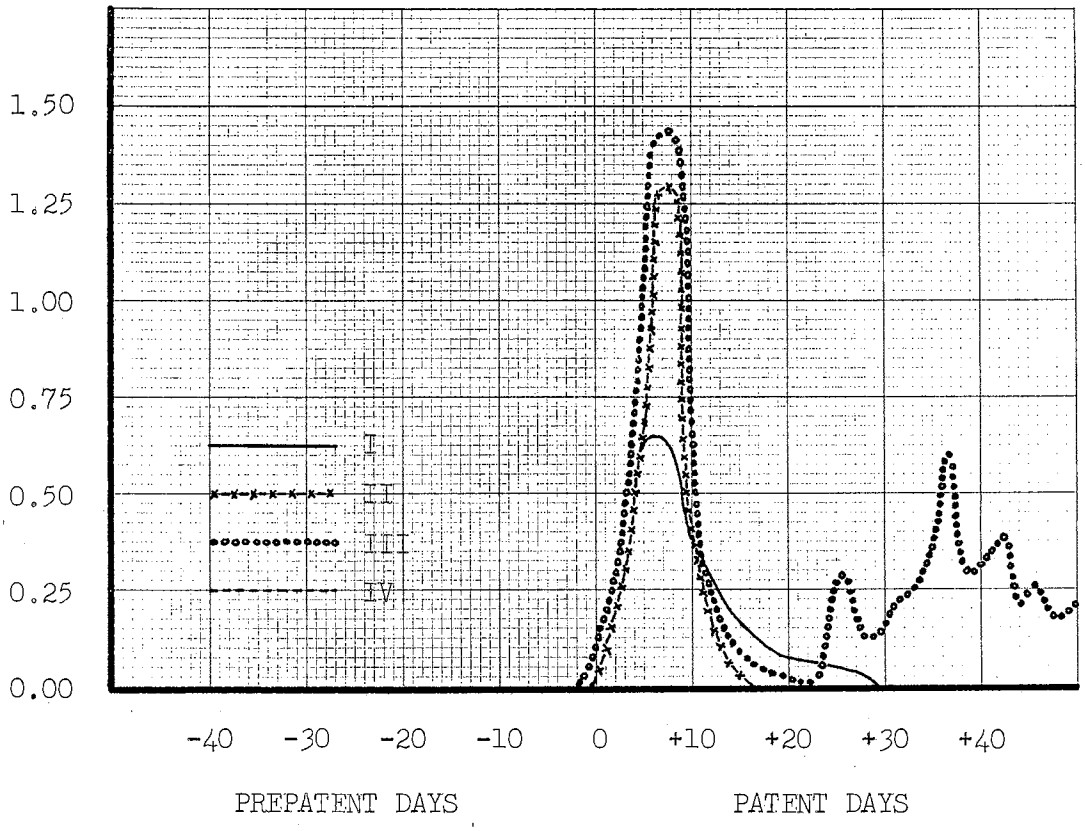

Figure 40. Comparison of the deviations of numbers of parasitized erythrocytes of blood during the prepatent and patent periods of anaplasmosis in: Group I - The primary disease response in the intact calf; Group II - The primary relapse in the splenectomized carrier calf; Group III - The primary disease response in the splenectomized calf; Group IV - Control laparotomy of the carrier calf (this group was omitted since it approximated zero). These fitted curves are taken from data in Figures 32, 33, 34, and 35. (Y-axis is expressed as cells x 10^6 per cubic millimeter of blood.)

than by the other groups. Attention is called to three points: (1) the rise and fall of Group III is different from the other groups. (2) the asplenic animals experience a higher parasitemia than do the intact animals. (3) a marked parasitemia persists only in Group III.

Complement-fixation Reactions (Figures 41 and 42)

There is a very abrupt rise in the CF titer for Group I. The rise is only less marked in Group III. However, the appearance of the CF titer for Group III is seven days later than for Group I. Note the "notches" in Groups I and III which follow the peak titers by four days. There is another titer peak at day +44 in Group III. There is a marked postsplenectomy titer fall in Group II. (Review Figure 31 since "mean" data tends to mask individual responses.) Figure 42 shows the splenic and asplenic response. (See explanation in the legend.)

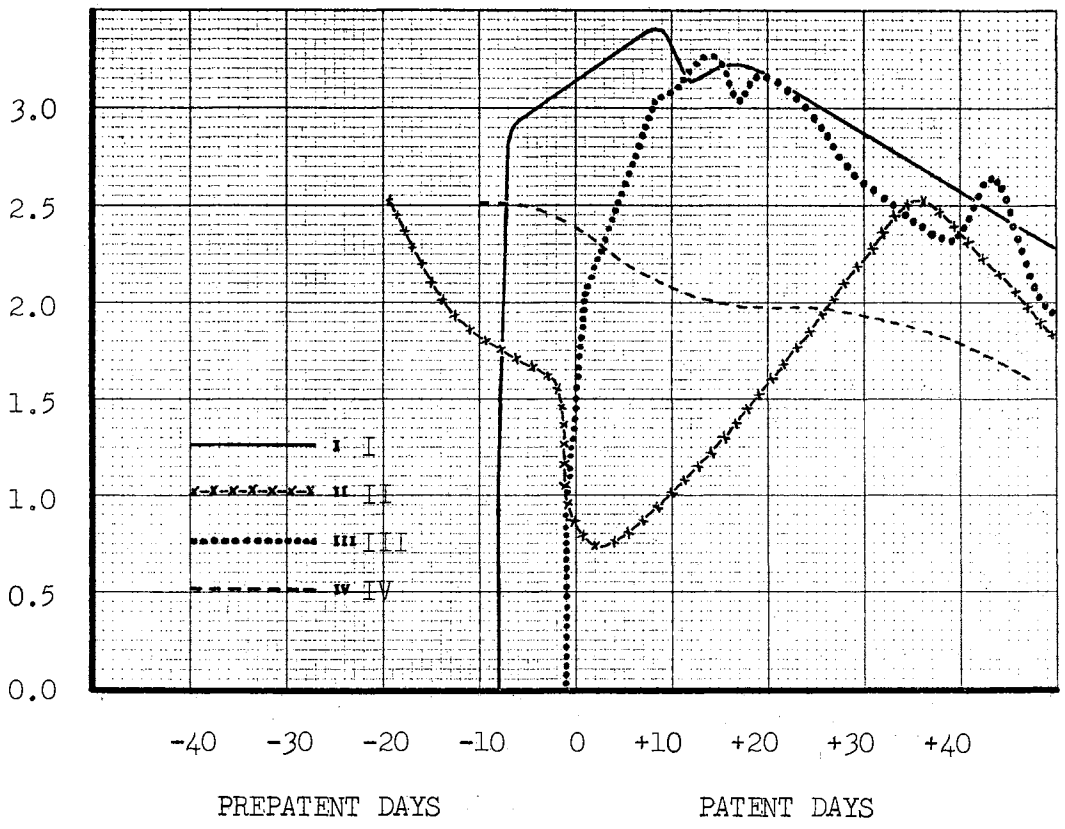

Figure 41. Comparison of the deviations of the complement-fixation reaction during the prepatent and patent periods of anaplasmosis in: Group I - The primary disease response in the intact calf; Group II - The primary relapse in the splenectomized carrier calf; Group III - The primary disease response in the splenectomized calf; Group IV - Control laparotomy in the carrier calf. These fitted curves are taken from data in Figures 29, 30, 32, and 33. (Y-axis is expressed as the logarithm to the base 10 of the denominator of the highest dilution giving a 4 + reaction.)

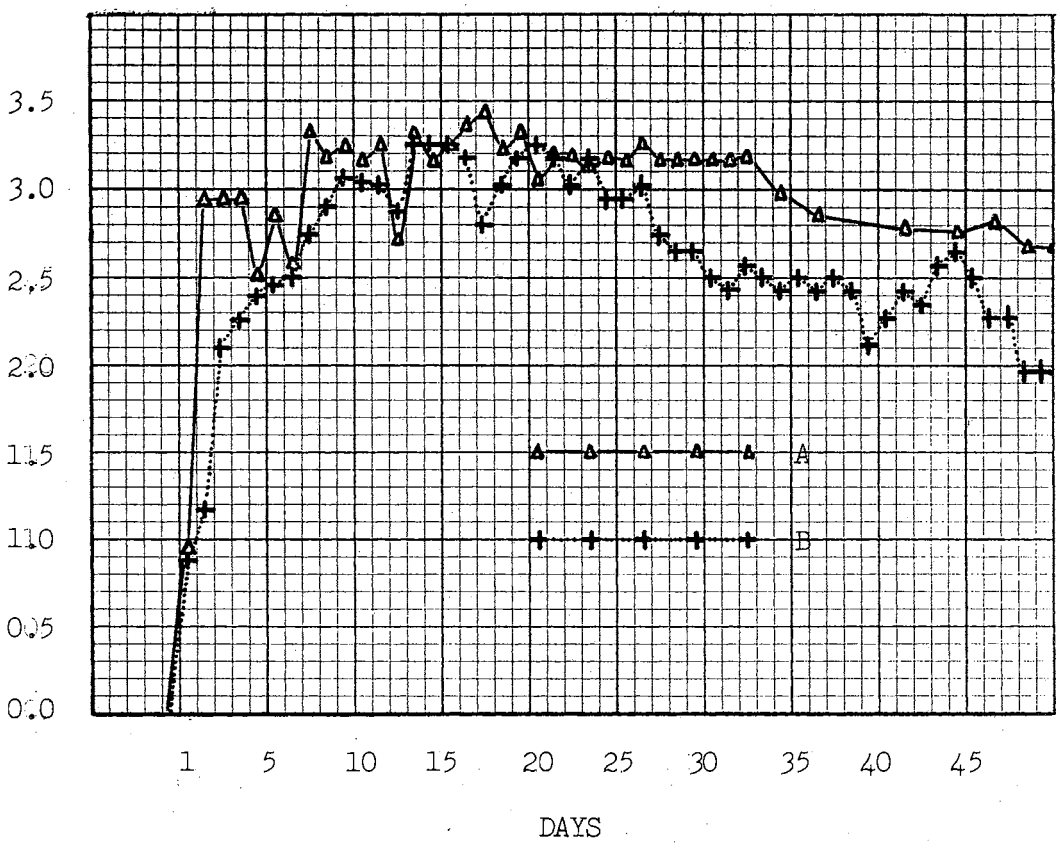

Figure 42. Comparison of the complement-fixing titer rise response in the intact calf (A) of Group I and the asplenic calf (B) of Group III. The data has been adjusted so the CF rises start together. (Y-axis is expressed as the logarithm to the base 10 of the denominator of the highest dilution giving a 4 + reaction.)

CHAPTER VI

DATA ANALYSIS*

A system of electronic data processing (EDP) using the I.B.M. 1620** was developed for analysis of hematological data generated in this set of experiments (62). Tukey's (63) "W" multirange test for unequal replicates was used to test for statistically significant differences between multiple treatment means. Student's "t" test was used on simple treatment pairs (64).

The statistical tables are comparisons of certain values during various phases of the disease of anaplasmosis. Values are reported as means, plus or minus their standard deviations.

In the comparison of multiple means, the value of "F" is calculated by standard analysis of variance (AOV) procedure (65). Those values of "F" followed by (*) are considered to be statistically different at the P.05 level of significance; those values followed by (**) are considered significant at the P.01 level. A "significant" value of "F" indicated that one or more of the means is different. Errors of "F" are considered to be decision-wise types of error.

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*The writer wishes to acknowledge the counsel of Leroy Folks, Ph.D., Oklahoma State University Statistical Laboratory, for certain statistical considerations in this analysis.

**International Business Machines, Inc., New York.

Those means which are underscored by a continuous line are not considered to be significantly different at the P.05 level, using Tukey's "W" test. The errors of Tukey's "W" are experiment-wise types of error.

When necessary to compare two treatment means, the Student's "t" method is used. Following a calculated value of "t", (*) or (**) indicate statistical significance at the P.05 and P.01 levels, respectively. The errors of "t" are decision-wise errors.

Mean values as encountered in the data tables and in the statistical analysis tables may vary slightly for the same group and the same test. They represent slightly different samples of the same original raw data. For example, pretreatment means in the data tables may be drawn from a slightly different period during the prepatent phase. Also, another example would be the lowest value reached by a certain test, i.e., the lowest value in the data tables is the lowest mean of six animals reached on the same day. In the statistical tables, the lowest value will represent the mean of the lowest value observed in each of the six animals, regardless of when that value occurred.

TABLE VI

HEMOGLOBIN COMPARISONS+

	I	II	III	IV	"F"
1. Prepatent "normal" values, HB - Gms. percent.	8.8 ± 0.9	9.6 ± 1.4	10.3 ± 0.4	9.9 ± 0.4	2.67
2. Lowest value at peak anemia, HB - Gms. percent.	4.5 ± 0.9	2.7 ± 0.5	2.5 ± 0.6	8.4 ± 0.5	119.03**
3. Patent day lowest value HB reached, days.	9.7 ± 1.6	10.8 ± 1.3	9.3 ± 0.5	28.0 ± 11.6	14.57**

TABLE VII

PACKED CELL VOLUME COMPARISONS+

	I	II	III	IV	"F"
1. Prepatent "normal" values, PCV - Volumes percent.	30.4 ± 2.0	29.8 ± 2.2	30.4 ± 1.1	30.8 ± 2.1	0.26
2. Lowest value at peak anemia, PCV - Volumes percent.	14.6 ± 2.2	10.0 ± 1.4	8.5 ± 0.5	26.0 ± 1.2	153.30**
3. Patent day lowest PCV reached, days.	9.6 ± 1.2	10.0 ± 1.6	9.8 ± 1.7	29.6 ± 8.9	28.00**

+ I - Primary disease response in the intact calf: n = 6.
II - Primary relapse in the splenectomized carrier calf: n = 6.
III - Primary disease in the splenectomized calf: n = 6.
IV - Control laparotomy of carrier calf: n = 5.

TABLE VIII

ERYTHROCYTE COUNT COMPARISONS+

1. Prepatent "normal" values, RBC - x 10^6 cells per $mm.^2$

I	II	III	IV	"F"
8.19 ± 0.36	7.77 ± 1.64	7.82 ± 0.33	7.87 ± 0.14	0.81

2. Lowest value at peak anemia, RBC - x 10^6 cells per $mm.^2$

I	II	III	IV	"F"
3.42 ± 0.56	2.06 ± 0.32	1.79 ± 0.51	6.39 ± 0.35	138.80**

3. Patent day lowest RBC count reached, days.

I	II	III	IV	"F"
10.2 ± 1.6	11.2 ± 1.3	10.8 ± 1.9	28.6 ± 14.5	9.00**

TABLE IX

MEAN CORPUSCULAR VOLUME COMPARISONS+

1. Prepatent "normal" values, MCV - cubic microns.

I	II	III	IV	"F"
37.0 ± 1.3	36.7 ± 0.9	40.6 ± 4.7	39.2 ± 2.1	2.82

2. Highest value reached during patent period, MVC - cubic microns.

I	II	III	IV	"F"
64.3 ± 5.7	70.3 ± 6.2	74.6 ± 7.8	41.6 ± 2.1	32.31**

3. Patent day that highest MCV value was reached, days.

I	II	III	IV	"F"
19.2 ± 3.3	14.7 ± 4.1	12.8 ± 1.0	18.8 ± 18.6	0.44

+ I - Primary disease response in the intact calf: n = 6.
II - Primary relapse in the splenectomized carrier calf: n = 6.
III - Primary disease in the splenectomized calf: n = 6.
IV - Control laparotomy of carrier calf: n = 5.

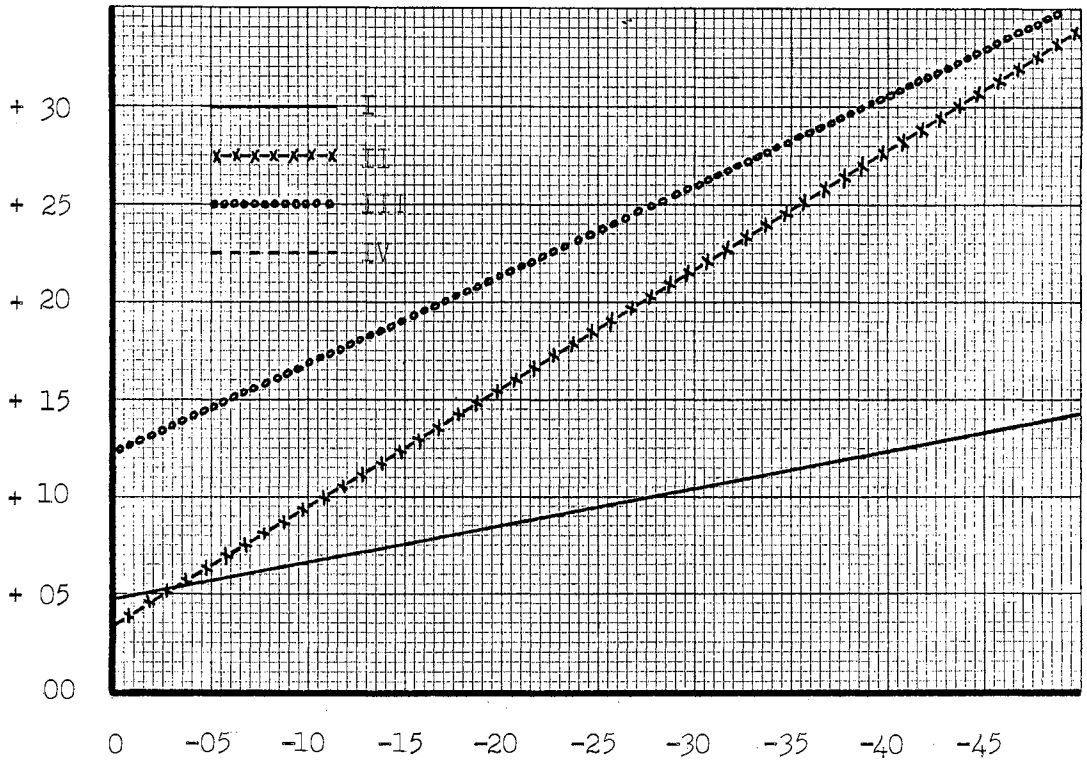

Figure 43. Linear regression of the percentage of parasitized erythrocytes on the percentage of erythrocytes lost during the anemic phase of the patent period of anaplasmosis in: Group I - The primary disease response in the intact calf, N = 6, beta = -0.186; Group II - The primary relapse in the splenectomized carrier calf, N = 6, beta = -0.612; Group III - The primary disease response in the splenectomized calf, N = 6, beta = -0.467. The calculated values of Student's "t" are: between $beta_I$ and $beta_{II}$ = 24.45^{**}; between $beta_I$ and $beta_{III}$ = 6.39^{**}; between $beta_{II}$ and $beta_{III}$ = 2.94^{*}. These regressions are calculated from data in Tables 1, 2, and 3. Group IV was omitted since it approximated zero. (Y-axis is expressed as the percentage of parasitized erythrocytes.)

TABLE X

MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION COMPARISONS+

1. Prepatent "normal" values, MCHC - percent.

I	II	III	IV	"F"
28.6 ± 1.2	32.5 ± 2.1	33.7 ± 0.6	32.0 ± 1.0	15.65**

2. Lowest MCHC value reached during the patent period, percent.

I	II	III	IV	"F"
27.2 ± 1.2	24.0 ± 5.4	25.2 ± 5.5	29.6 ± 5.6	1.46

3. Patent day when lowest MCHC occurred, days.

I	II	III	IV	"F"
17.8 ± 2.9	11.8 ± 1.9	10.3 ± 4.5	23.6 ± 12.4	4.91*

TABLE XI

PERCENT PARASITIZED ERYTHROCYTES (PPE) COMPARISONS+

1. Highest PPE reached during the patent period. (Note changed order)

IV	I	II	III	"F"
00.0	12.8 ± 3.8	30.7 ± 7.6	37.8 ± 21.8	11.26**

2. Patent day that highest PPE was reached.

I	II	III	IV	"F"
7.3 ± 1.0	8.3 ± 2.0	8.2 ± 0.8	00.0	57.95**

3. Highest PPE reached during patent period, excluding data from two animals in Group III which died of anaplasmosis. (Order changed)

IV	I	III	II	"F"
00.0	12.8 ± 3.8	24.3 ± 6.2	30.7 ± 7.6	34.41**

+ I - Primary disease response in the intact calf: n = 6.
II - Primary relapse in the splenectomized carrier calf: n = 6.
III - Primary disease in the splenectomized calf: n = 6.
IV - Control laparotomy of carrier calf: n = 5.

TABLE XII

NUMBER OF PARASITIZED ERTYHROCYTES (PRBC) COMPARISONS+

1. Highest PRBC count reached during the patent period, cells x $10^6/mm.^2$

IV	I	II	III	"F"
0.0	0.707 ± 0.224	1.028 ± 0.342	1.558 ± 0.904	9.86**

2. Patent day that highest PRBC count was reached.

IV	I	II	III	"F"
0.0	6.3 ± 1.5	6.8 ± 0.8	6.2 ± 1.2	45.77**

3. Highest PRBC count reached during the patent period, excluding data from two animals in Group III which died of anaplasmosis.

IV	I	II	III	"F"
0.0	0.707 ± 0.224	1.028 ± 0.342	0.698 ± 0.319	25.67**

TABLE XIII

COMPARISONS OF THE LENGTHS OF VARIOUS TIME PERIODS DURING THE DISEASE+

1. Length of the prepatent period; day of infection to day "0" in Groups I and III; day of splenectomy to day "0" in Group II. Group IV omitted since it showed no detected response. (Given in days)

II	I	III	"$t_{cal.}$" between I and III = 0.31
10.7 ± 1.9	30.0 ± 2.7	29.5 ± 2.8	

2. Length of time required to reach lowest PCV after day "0", Vol. %.

I	II	III	IV	"F"
9.6 ± 1.2	10.0 ± 1.6	9.8 ± 1.7	29.6 ± 8.9	28.00**

3. Time required to return to 75 % of the prepatent PCV after the anemic crisis. (Given in days)

I	II	III	IV	"F"
16.7 ± 3.4	19.0 ± 1.7	20.3 ± 1.6	0.0	89.67**

+ I - Primary disease response in the intact calf: n = 6.
II - Primary relapse in the splenectomized carrier calf: n = 6.
III - Primary disease response in the splenectomized calf: n = 6.
IV - Control laparotomy of the carrier calf: n = 5.

TABLE XIV

COMPLEMENT-FIXATION (CF) REACTIONS: THE COURSE OF THE EXPERIMENTS+

1. Day of first positive CF reaction in the primary disease response.

I	III	Calculated "t"
- 6.5 ± 1.4	- 0.8 ± 1.8	6.02**

2. Day that highest titer was reached, in the primary disease response.

I	III	Calculated "t"
+ 2.3 ± 2.9	+ 11.2 ± 2.6	17.70**

3. Highest CF titer reached in the primary disease response. Reported in the "standard" $\log_{10}$ mode. See Appendix - Fig. 44.

I	III	Calculated "t"
3.60 ± 0.05	3.36 ± 0.04	4.89*

4. Lowest CF titer reached in the primary relapse in the carrier calf.

II	IV	Calculated "t"
0.48 ± 0.75	1.36 ± 0.33	8.26**

5. Day that lowest CF titer was reached in the primary relapse.

II	IV	Calculated "t"
+ 1.8 ± 7.1	+ 42.1 ± 5.7	10.95**

+ I - Primary disease response in the intact calf: n = 6.
II - Primary relapse in the carrier calf: n = 6.
III - Primary disease response in the splenectomized calf: n = 6.
IV - Control laparotomy of the carrier calf: n = 5.

TABLE XV

COMPARISON OF HEMATOLOGICAL VALUES IN THE CALF ONE WEEK BEFORE AND SIX WEEKS AFTER SPLENECTOMY. GROUP V: N = 6.

	Presplenectomy Values	Postsplenectomy Values	Calculated "t" Value+
1. Grams of hemoglobin per 100 ml. of blood.	9.7 ± 1.0	10.5 ± 0.6	2.35
2. Packed cell volumes percent of blood.	31.1 ± 2.4	30.4 ± 1.2	0.79
3. Numbers of erythrocytes per cubic millimeter of blood.	8.29 ± 1.36	10.86 ± 1.71	7.34**
4. Mean corpuscular volume in cubic microns.	38.3 ± 4.6	28.3 ± 3.6	19.60**
5. Mean corpuscular hemoglobin concentration in percentage.	31.6 ± 2.2	34.6 ± 1.6	7.89**

+ "t" test drawn from paired observations, N = 6, with 5 samples per observation per calf.

CHAPTER VII

DISCUSSION

Miale (66) and Greatorex (67) list the measurements of erythrocyte count (RBC), hemoglobin content (HB), packed cell volume (PCV), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) as essential for the classification of the anemias. The writer has reported on these values, as well as those values indicating the degree of parasitemia; i.e., the percentage of parasitized erythrocytes (PPE) and the numbers of parasitized erythrocytes per cubic millimeter (PRBC). Complement-fixing titers (CF) were also measured because of their apparent relationship to the spleen and anaplasmosis (45, 47). Values for several other tests were also recorded and will be reported separately.

Pretreatment Normal Values

The pretreatment "normal" mean values for all the tests and all the groups were rather uniform. Only the MCHC pretreatment mean for the intact calves of Group I appeared to be significantly different ($P<.05$) from the other means. (See Table X, part 1). In the writer's experience with over 100 similar calves during a two year period in this section of Oklahoma, low MCHC values were not uncommon, even though the calves appeared to be on an adequate nutritional regime

and without apparent parasitic problems.

However, even including the low MCHC for Group I, the values for all pretreatment means appeared to be within the normal limits listed for the calf by Schalm (68) and Greatorex (67). The values observed by the writer more closely agreed with those described by Greatorex. Many of the writer's normal means were in the lower edge of the normal limits given by Schalm. These differences were even more pronounced when one considers that Schalm's values were drawn from a primarily female population, and the writer's values were drawn from a mixed population. Since Schalm described the male as normally having higher blood values than the female, the writer's values were indeed lower than Schalm's normal data might indicate.

Some of the greatest differences were observed in the RBC and MCV values and were probably due, in part, to technical differences and errors. Schalm (68) indicated that this type of error is common and difficult to control. When standard deviations were inferred from the erythrocyte data given by Schalm, there appeared to be little difference in the variability of the two sets of data (69). Those differences, though small, tended to favor a smaller variability in the writer's data.

Effects of Splenectomy on the Uninfected Calf

No changes were observed in HB or PCV values six weeks after splenectomy. There was an increase in MCHC and RBC values, and a marked decrease in MCV. (See Table XV). This could be interpreted as an increase of microcytic erythrocytes after splenectomy. Although

anemia, siderocytosis, leptocytosis, Howell-Jolly bodies, polycythemia, Heinz bodies, and other hematologic changes have been reported after splenectomy in other animals, few of these changes were observed in these calves (31). Only polycythemia and a reduced MCV were consistent features in the postsplenectomy state. Microcytosis is consistent with leptocytosis, even though typical "target cells" were not definitely identified in the observed hematologic changes.

Hematological Differences During the Prepatent Period

The only striking differences noted during this period were a marked rise in the RBC values by more than twenty percent during the late prepatent period (the last two weeks before day "0") and a concurrent decrease in MCV values by fifteen to twenty percent for Groups I and III. Since the calculation of MCV values are dependent upon RBC values, such a concurrent decrease might be expected.

Upon inspection of Figures 36 and 37, it will be noted that the shape and location (in time) of the RBC and MCV changes are distinctly different and appear to represent real changes. The fact that the PCV and HB values remained within normal limits suggests that this was a transient increase of microcytic erythrocytes. Transient prepatent increases in RBC values have been noted by other workers in anaplasmosis (70). Confirmation of this transient microcytosis awaits further cell-size distribution studies during the course of the disease using electronic sizing techniques.

Hematologic Differences During the Early Patent Period

Regardless of the prepatent response, as soon as the character-

istic parasitemia develops, there was a marked fall in the values for HB, PCV, and RBC (days +2 through +8). The rate of fall was very similar for the intact and asplenic animals. (See Figures 34, 35 and 36). Although the rate was similar, the peak anemia values were different for the various groups. The control laparotomy group showed no significant changes, and the intact animal became moderately anemic. The two asplenic groups experienced severe, statistically identical (P.05) anemias. (See part 2 of Tables VI, VII and VIII).

The anemia values were lowest for Group III, suggesting that Group II did show some immunologic benefit from its previous experience with the acute anemia. This was supported by the fact that only Group III experienced death loss during this experiment (two of six animals died with anaplasmosis). This was also suggested by the calculations presented in Figure 43. When the percentage of erythrocyte loss was compared with the degree of parasitemia, there were significant differences between all three groups.

There are two mechanisms which might explain these differences. One is an increased sensitivity of the erythrocyte sequestering mechanism (20). The other is an increase in the number of circulating cells susceptible to sequestration. Garnham (71) stated "....removal of the spleen renders animals susceptible to malarial parasites which otherwise would be confined to the tissue stages....".

Hematologic Differences During the Late Patent Period

Asplenic Group III exhibited a marked persistent parasitemia.

This is difficult to explain when one considers that the degree of anemia is similar in both asplenic groups. Again, it could be due to a persistent, though incomplete, acquired immunity (non-splenic in origin) in Group II.

In view of Wagner's (20) work, there is justification in assuming that there is some direct relationship between the circulating time of marginal-body carrying erythrocyte and the degree of damage experienced by that erythrocyte. This could explain, in part, the higher degree and persistence of the parasitemia in asplenic calves.

During the late patent period (up to day +50), only the RBC values for the intact animals returned to normal prepatent levels. Elevated HB and PCV values indicated an over compensation only in this group. The control group remained within normal limits. A macrocytosis persisted in all three treatment groups. Asplenic Group III experienced a marked recrudescence of the marginal bodies which peaked out at day +48. Asplenic Group II exhibited a very slight increase in circulating parasitized cells at day +35.

Classification of the Anemia

The anemia of anaplasmosis in two year old steers has been described as normocytic normochromic changing to macrocytic hypochromic after stimulation of erythropoiesis (1). The writer observed a similar type of anemia in both asplenic groups of calves. However, the intact animals, while exhibiting the macrocytic aspect, did not exhibit the hypochromic aspect of the anemia. Even though the fitted curve graph (Figure 38) shows a "hyperchromasia", this is an anomaly

due to data transformation (see MCHC values, Table I.) and the fact that the Group I pretreatment mean for the MCHC value was significantly lower than the other pretreatment means. If allowance is made for the low pretreatment mean, Group I still did not exhibit a significant hypochromic phase during the course of the anemia. This was consistent with other reports that the intact calf is the least susceptible of the bovine animals to anaplasmosis (6, 9, 72). Thus the anemia in the intact calf might be characterized as normocytic normochromic turning to macrocytic normochromic (or only slightly hypochromic). This illustrates the value and need for applying concurrent and integrated procedures to the study of the anemia of anaplasmosis (73).

Differences in the Complement-Fixing Reaction

By the inspection of Figure 41 and Table XIV, there is noted a marked difference of the onset of the primary CF titer rise between the intact calves in Group I and the splenectomized calves in Group III. The intact calves responded some eight days earlier than the asplenic group. This response was consistent with reports by Roby (72) and Murphy (4) on individual calves. (See Appendix Figure 45 and 46). After splenectomy of the carrier calves, there was a marked drop in CF titer. In both asplenic groups the initiation of the titer or the start of its return to presplenectomy values was concurrent with the initiation of the progressive parasitemia. (See Figures 40 and 41). This would suggest that demonstrable numbers of parasitized erythrocytes were necessary for the initiation of "non-splenic" antibody pro-

duction. If one equates a demonstrable parasitemia with a large dose of foreign protein given intravenously, then it is possible to draw a close analogy between the CF response in the asplenic calf and the circulating antibody formation capabilities of the splenectomized rabbit (49); i.e., both appear to require very strong antigenic stimuli when given intravenously in the asplenic state for the production of antibodies by non-splenic sources. Rowley's (49) work suggests that a significant amount of the non-splenic antibody production is in the liver and visceral lymph nodes.

Summary and Conclusions

The degree and severity of the anemia of anaplasmosis in the calf is related to splenic function and previous immunologic experience. The intact calf does not appear to experience the severe hypochromic phase of the anemia described for older animals. The persistant CF antibody in the intact calf is produced primarily in the spleen. The initiation of CF antibody production by non-splenic sources in the calf apparently requires a large antigenic dose. In the intact calf and the splenectomized calf, there is a transient microcytic erythrocytosis immediately preceding the primary parasitemia.

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A P P E N D I X

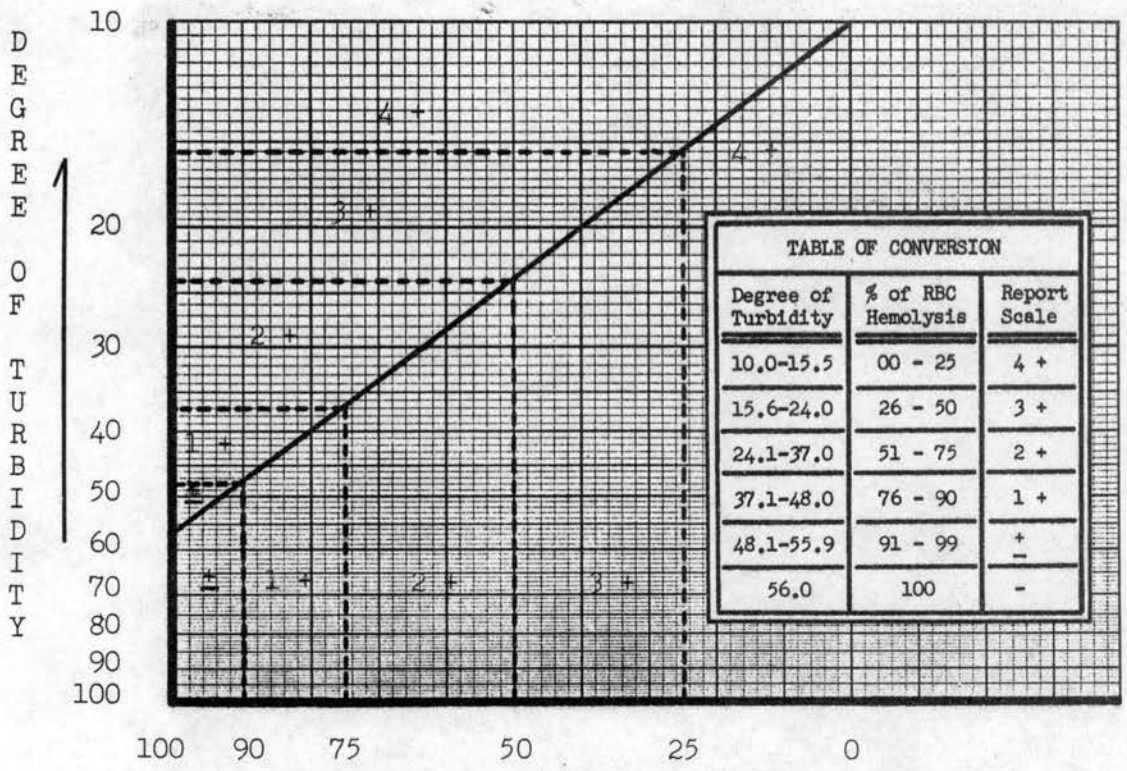

TABLE OF CONVERSION

Degree of Turbidity	% of RBC Hemolysis	Report Scale
10.0-15.5	00 - 25	4 +
15.6-24.0	26 - 50	3 +
24.1-37.0	51 - 75	2 +
37.1-48.0	76 - 90	1 +
48.1-55.9	91 - 99	±
56.0	100	-

Figure 44. Relationship of turbidity to the degree of hemolysis: An example of the conversion chart used in the photonephelometric determination of complement-fixation reactions used at the Veterinary Research Laboratories, Pawhuska Division, College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma. From this chart the reactions are then subjected to a $\log_{10}$ transformation on the following basis: 4 + = 1.00 at 1/10; 1.30 at 1/20; 1.6 at 1/40; 1.9 at 1/80; 2.2 at 1/160; 2.5 at 1/320; 2.8 at 1/640; 3.1 at 1/1,280; 3.4 at 1/2,560; 3.7 at 1/5,120; and 4.0 at 1/10,240. The log transformation facilitates data handling for statistical purposes.

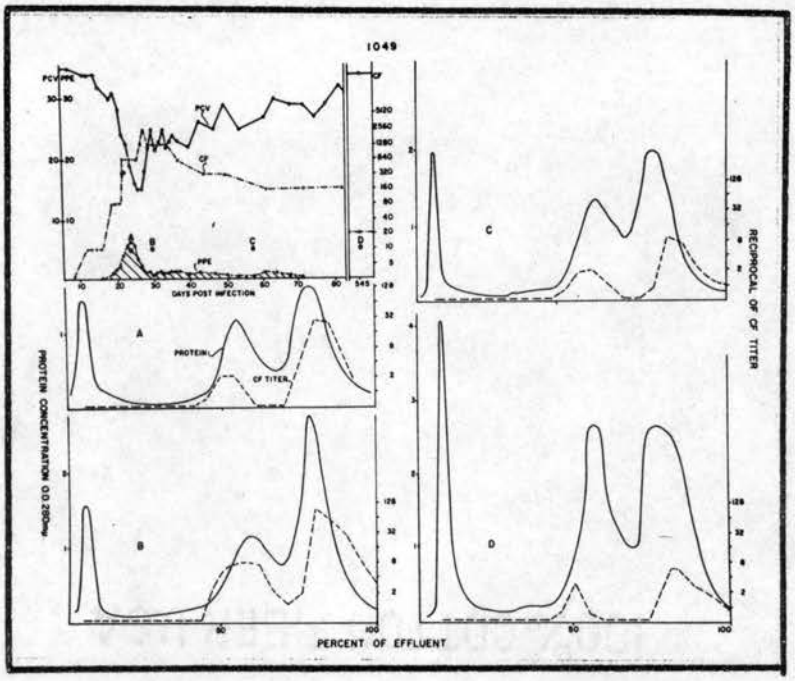

Figure 45. Calf 1049, 6 month old steer with intact spleen, infected with Anaplasma marginale (California isolate). Taken from Physical Heterogeneity of Bovine Gamma Globulins; Response to Experimental Anaplasma Marginale Infection by Frederick A. Murphy, Jr., Ph.D., Dissertation, University of California, 1964. Used by special permission of the author. (4).

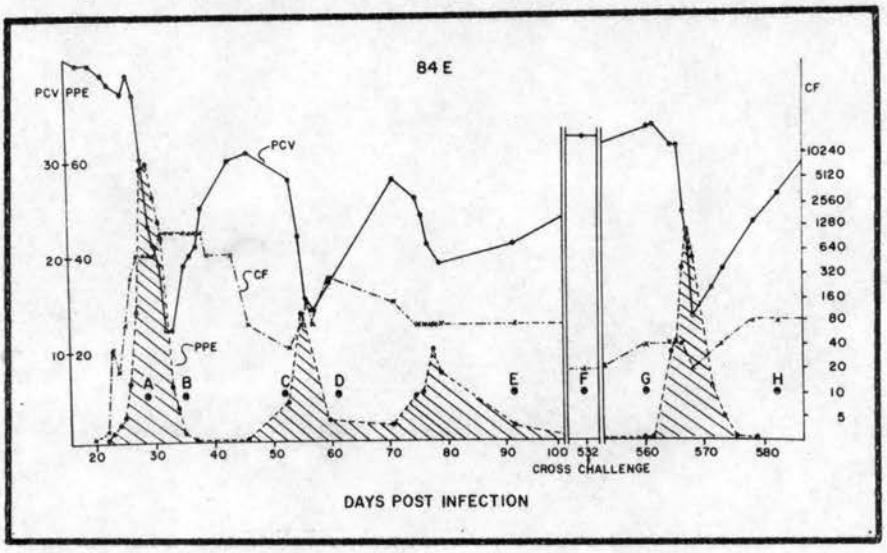

Figure 46. Calf 84 E, 6 month old Jersey steer splenectomized 3 days prior to infection with Anaplasma marginale (Florida isolate). Taken from Physical Heterogeneity of Bovine Gamma Globulins; Response to Experimental Anaplasma Marginale Infection by Frederick A. Murphy, Jr., Dissertation, University of California, 1964. Used by special permission of the author. (4).

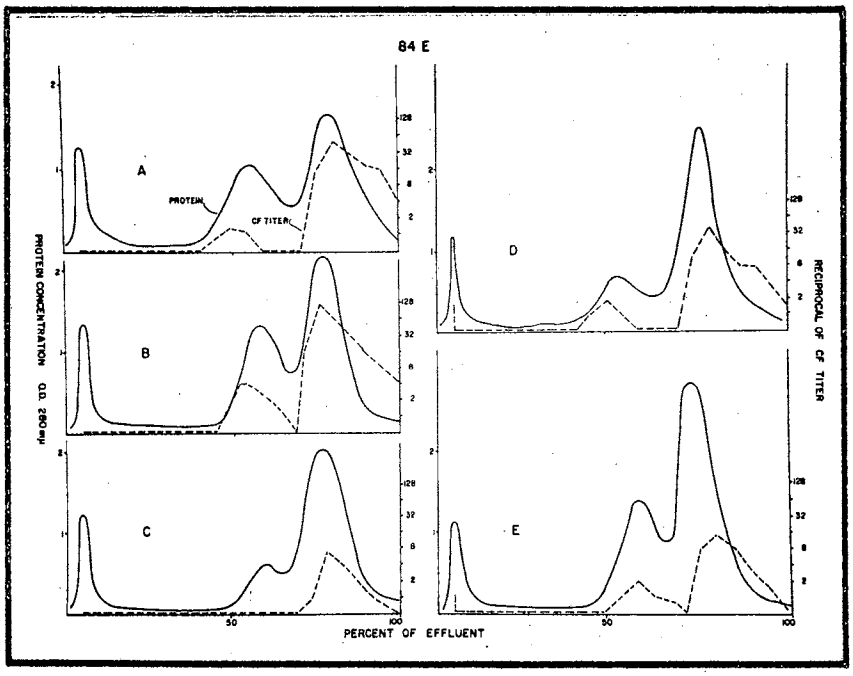

Figure 47. DEAE Sephadex chromatography of sera of sera A - E from calf 84 E. Taken from Murphy (4) with the author's permission.

Abbreviations used by Murphy (4):
PCV - Packed cell volume.
CF - Complement-fixation titer.
PPE - Percent parasitized erythrocytes.

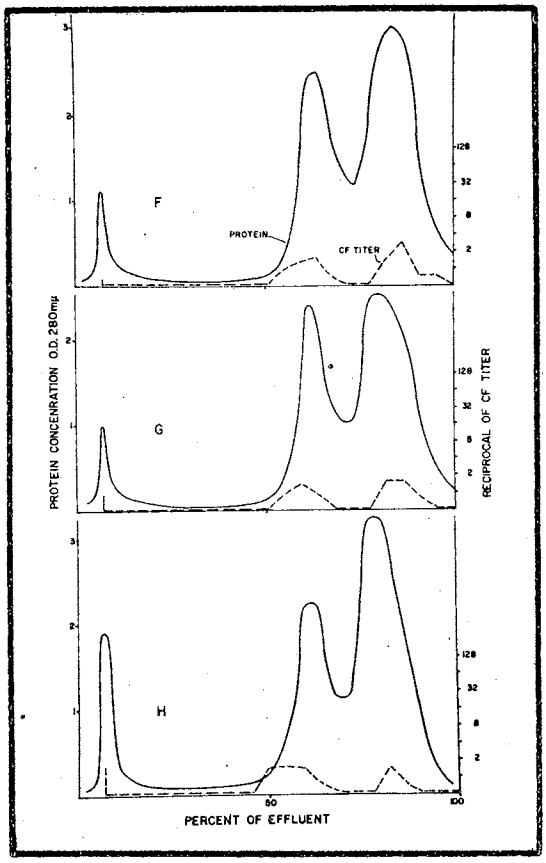

Figure 48. DEAE Sephadex chromatography of sera F - H from calf 84 E. Taken from Murphy (4) with the author's permission.

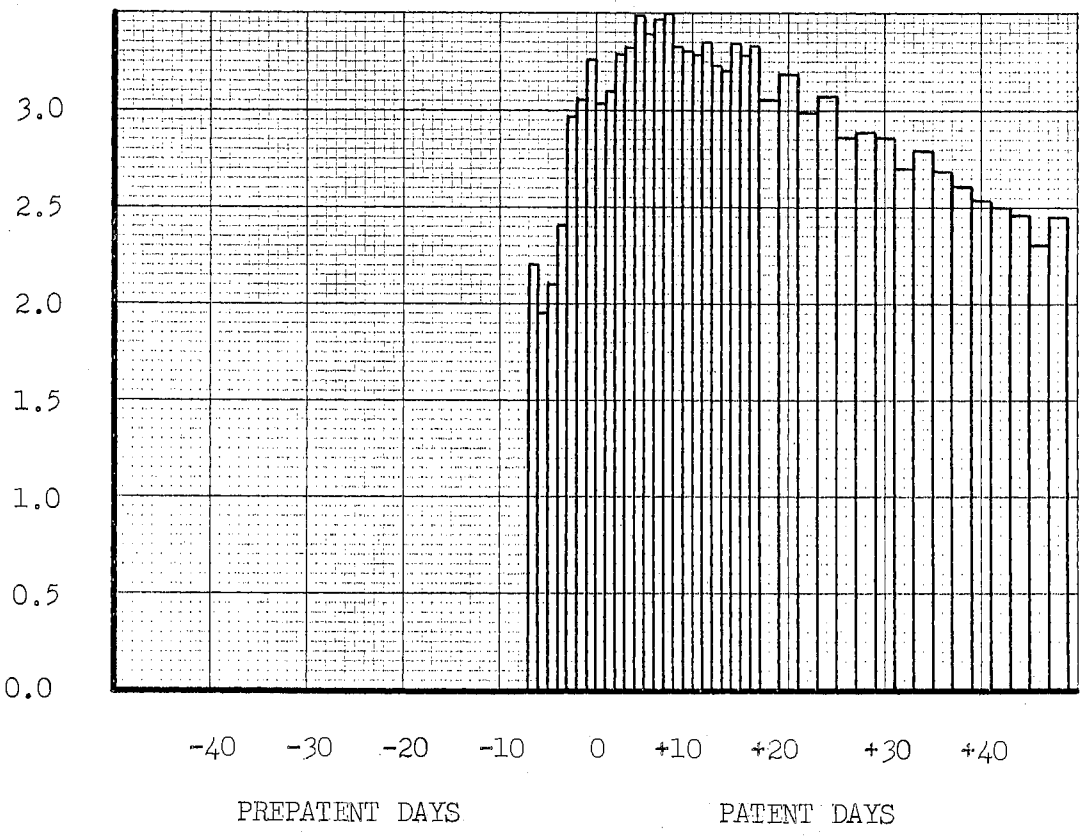

Figure 49. Deviations of the complement-fixation reactions during the prepatent and patent periods of anaplasmosis in Special Group I - The primary disease response in the intact calf; N = 12. (Y-axis is expressed as the logarythm to the base 10 of the denominator of the highest diluation giving a 4 + reaction.) The wide bars indicate that the reactions were summed for successive two day periods. Drawn from unpublished data of E. W. Jones - Used by permission.

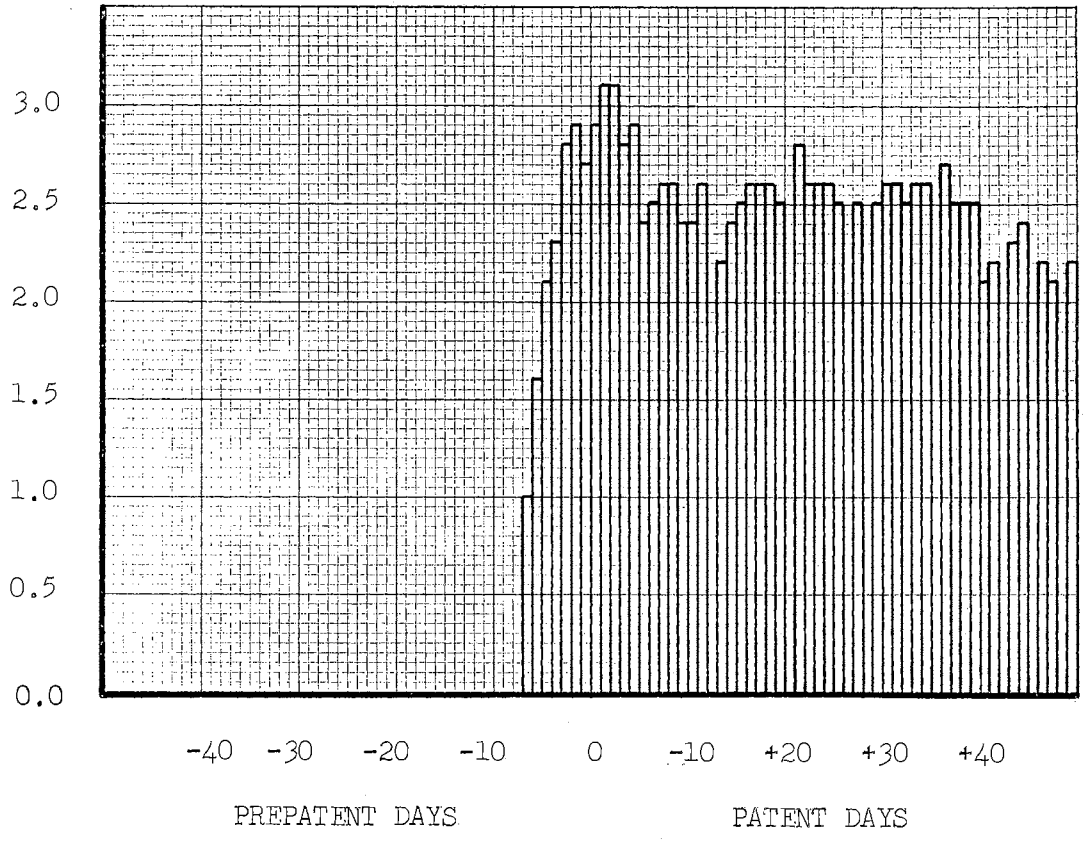

Figure 50. Deviations of the complement-fixation reaction during the prepatent and patent periods of anaplasmosis in Special Group 2 - The primary disease response in the calf when splenectomized during the initial days of the patent period: N = 3. (Y-axis is expressed as the logarythm to the base 10 of the denominator of the highest dilution giving a 4 + reaction.) Drawn from unpublished data of E. W. Jones - Used by permission.

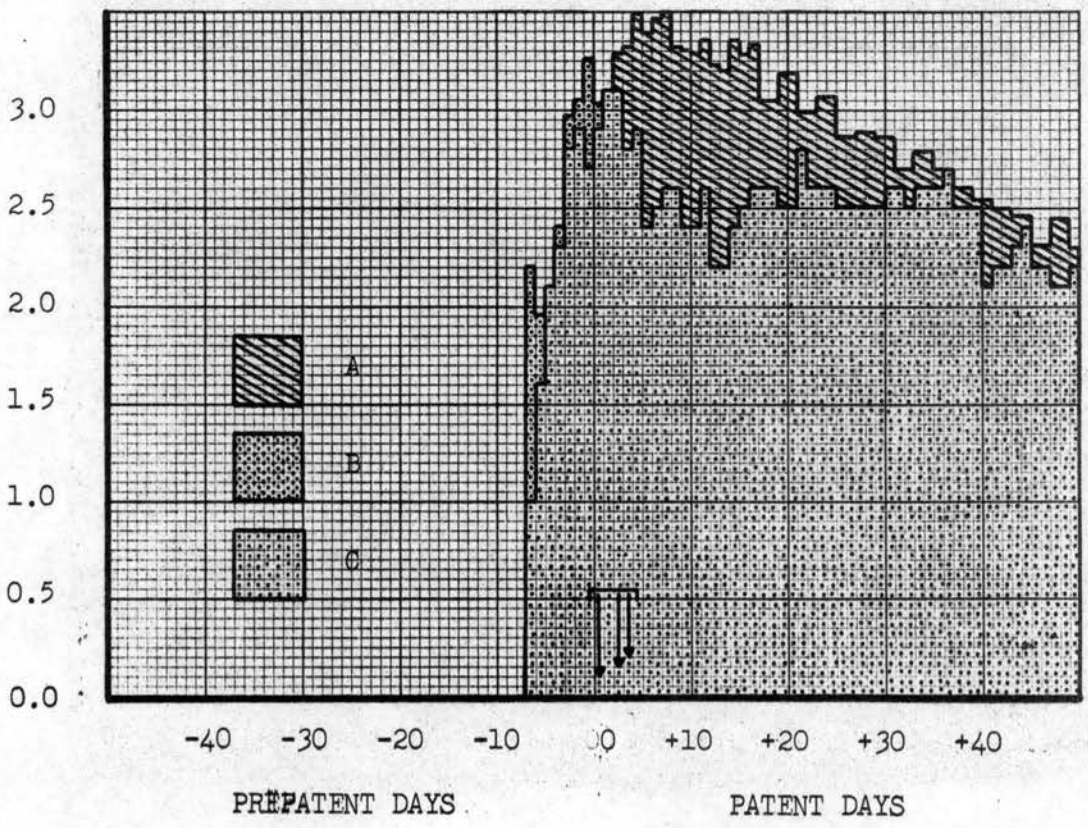

Figure 51. Comparison of the complement-fixation reactions during the prepatent and patent periods of anaplasmosis between Special Group I and Special Group II. A = Area representing the difference between the intact calf response and the response of the calf splenectomized in the initial part of the patent period. B = Area representing the difference of the two special groups prior to splenectomy. C = Area representing those calves which were splenectomized in the initial part of the patent period. Each arrow indicates the splenectomy of an individual calf. (Y-axis is expressed as the logarithm to the base 10 of the denominator of the highest dilution giving a 4 + reaction.) Drawn from unpublished data of E.W. Jones - Used by permission.

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