BLOOD TRANSFUSIONS IN ANAPLASMOSIS

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CHAPTER I

INTRODUCTION

Anaplasmosis is an infectious disease of cattle which is characterized by a progressive anemia with a significant absence of hemoglobinuria. The causative agent is Anaplasma marginale.

The disease has been recognized for over 50 years as a major economic loss to the cattle industry throughout the tropical and subtropical world (1). During recent years, it has spread into the temperate zones (2). Anaplasmosis is believed to have been brought into the United States by Spanish cattle. It has been reported from 39 of the 50 states. There are two main endemic zones: (a) Southeastern states, (b) Northwestern states and California (3). In Oklahoma, the disease may be found in any part of the state but is most prevalent in the eastern region. Hawaii eradicated anaplasmosis by means of a cattle blood testing program (4).

For many years the disease was confused with babesiosis. Smith and Kilborne (5) mistakenly believed that the intraerythrocytic bodies found in anaplasmosis were a stage in the life cycle of Babesia bigemina. Theiler (6) observed "marginal points" in blood smears from South African cattle. Later, he found similar objects in the erythrocytes of cattle which developed a marked anemia but which had been previously immunized against babesiosis. Theiler (7) obtained a pure infection of Anaplasma marginale and passed it through several cattle

free from babesiosis.

Examination by light microscopy reveals Anaplasma marginale as basophilic stained bodies, 0.2 u to 1.0 u in diameter, situated within and near the margins of erythrocytes. Electron microscopy and filtration indicate that the infective filterable form may be less than 0.3 u in diameter (8). The morphological description of the anaplasma body is still undecided but there appear to be two broad areas of agreement (9,10); viz: (a) the organism is composed of 8 or less sub-units in a more or less spherical arrangement without projections, (b) the organisms show variously shaped delicate projections or tails.

The oxygen requirements of the anaplasma body lie somewhere between those of a virus and the malarial parasite (29). None of the malarial drugs destroy or suppress the causative agent of anaplasmosis (11). The organism is susceptible to parenteral therapy with tetracycline drugs (11,12,13).

The conclusions of Dickmans (14) in 1933 still represent the position of research work today. He maintained that the anaplasma body is not (a) a stage in the life cycle of Babesia, (b) a degeneration product or Jolly body, (c) a reaction of the red cell to invasion by a filterable virus. He suggested it may be a protozoan parasite. Later studies have suggested that (a) the anaplasma body is not a reaction to any disease process, (b) it is probably a phase in the life of the etiological agent which may be viral or protozoan in nature (15).

Infection can be executed by the mechanical or biological transmission of blood from an infected animal (16). It has been shown that infection of a susceptible animal can be achieved with as little as

0.001 ml. blood from an anaplasmosis carrier animal (17,18). Infected cells can be transferred mechanically to a susceptible animal by the mouth parts of biting insects such as ticks, horse flies and mosquitoes (19,20). An important means of spread is through the use of unsterilized surgical instruments, following mass inoculations, or following dehorning and castration operations (21,22).

The readiness with which anaplasmosis can be transmitted mechanically is a pronounced difference from the transmittal modes of clinically similar diseases such as malaria, yellow fever, or theileriosis. These diseases require precise insect and arachnid vectors for their transmission (23).

Biological transmission of anaplasmosis by ticks may occur (20,24). Howell (20) stated that all stages in the development of the tick are infective, and transovarian passage of the etiological agent from the adult through the egg to the larva has been accomplished experimentally. The same author indicated that tick infested pastures where anaplasmosis has occurred cannot be considered free from the disease for at least six years.

Evidence has been presented to show that deer can be silent reservoirs of infection (25).

Cattle of all ages are susceptible to infection. Calves are generally more resistant to clinical infection than adults and usually develop a mild form of the disease (16,26).

The disease is most prevalent during the vector season.

The incubation period varies from about 15 to 45 days. It has been reported, however, that this period may be prolonged up to 3 months when the disease is transmitted by ticks (20,24). Experimen-

tally there appears to be a correlation between the quantity of the infective dose used as inoculum and the length of the incubation period (27).

The characteristic clinical feature of anaplasmosis has been described as being a severe anemia, accompanied by initial fever, depression, weakness, and normal urine (26); signs of anorexia, weight loss, dyspnea, constipation, more rarely diarrhea, tachycardia, and an accentuated jugular pulse develop in 2 to 4 days. Cases of anaplasmosis which are severely anemic often show irrational behavior, probably due to cerebral anoxia. The red blood cell (RBC) count may fall as low as 0.7 million cells per c.mm. In such animals, as many as 60 percent of the RBC may be infected with anaplasma bodies (28).

Lotze (27) noted that the hematocrit decreased most rapidly before the peak of infection, but increased after the appearance of macrocytes. Brock (15) found that reticulocytes appeared from the fifth to the tenth patent days* of the infection and reached a maximum of up to 14 percent from the fifth day onward.

Despite the massive RBC loss, hemoglobinemia and hemoglobinuria have not been observed in anaplasmosis (15,16,29). Icterus has been reported as a late symptom of the disease most frequently observed during early convalescence and is considered to be indicative of liver damage and bilirubin retention (15).

The mortality rate may reach 60 percent in adult cattle, especially in bulls and older animals. Pregnant cows may abort. Death

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*Patent days refer to the appearance of 1 percent or more of erythrocytes containing anaplasma bodies.

is rare in young animals (26).

Gross necropsy findings have been described as being typical of an acute anemia with pallor, icterus and splenomegaly as common features (1,30). Hepatomegaly, a distended gall bladder, petechial hemorrhages upon serosal surfaces, and a pale, flabby myocardium have been observed (16). Histologically there is evidence of renal, myocardial and centrilobular liver degeneration, hemosiderosis, and erythrophagocytosis (31). Christensen (16) referred to a hyperplastic bone marrow, with evidence of depletion in chronic cases.

Pathogenesis of Anaplasmosis

The pathogenesis of anaplasmosis poses a fascinating study. Factors requiring elucidation include, (a) the site of primary invasion, (b) the mechanism of erythrocyte destruction.

Site of Infection.

Foote *et al.* (32) showed evidence, by means of electron photomicrographs, that the anaplasma organism infects the erythroblasts in the bone marrow. He demonstrated the presence of abnormal bodies in normoblasts which resembled elementary bodies, and postulated that these bodies could form the commonly seen anaplasma bodies when the infected erythrocytes matured and entered the blood stream. This theory suggestes that the formation of the anaplasma body requires a few days after the red blood cell matures.

Brock (15) failed to observe the bodies in reticulocytes, basophilic cells or cells with basophilic stippling. He suggested that there is a developmental period of 3 to 7 days in the bone marrow when the anaplasma infectious agent enters a new host. Here it

infects increasing numbers of some stage in the maturation of the erythrocyte.

Ristic and Watrach (33) showed, by electronmicroscopy, that the initial bodies reproduce in mature erythrocytes by means of binary fission. They advanced a hypothesis that the initial anaplasma body is capable of growing, multiplying, and forming marginal anaplasma bodies in mature erythrocytes. There is no supportive evidence for these observations. The failure to grow the anaplasma organisms in RBC in vitro suggests that the actual site of anaplasma growth is in the bone marrow and that the appearance of the organism in the erythrocytes is merely a retarded manifestation of active growth.

Kreier and Ristic (34) demonstrated, by means of histochemical and immunofluorescent techniques, that Anaplasma marginale entered fully developed deer erythrocytes, after they had been transfused into anaplasma infected calves. Invasion of mature RBC by initial bodies from the plasma has been suggested (1,33).

Mechanism of Erythrocyte Destruction.

Brock (15) considered that the anemia found in anaplasmosis is principally due to hemolysis of the infected erythrocytes. There are features of the disease, however, which differ from those which might be expected, or those which are seen in other apparent hemolytic anemias of cattle, such as babesiosis and leptospirosis. These diseases are characterized by hemoglobinuria which does not occur in anaplasmosis (35). It appears, therefore, that the anemia in anaplasmosis results from the removal of parasitized erythrocytes by means other than intravascular hemolysis. It has been postulated that phagocytosis of infected RBC by the reticuloendothelial system

occurs in the disease (31,36,37,38). The observance of hemosiderin and even intact, infected erythrocytes in phagocytic cells adds weight to this theory. Summers (39) stated that phagocytic cells increase markedly during the acute phase of the disease. The same author maintained that it had not been made clear, however, whether such erythrocytes are phagocytized because they are physiologically abnormal, or because of some specific antigen-antibody reaction involving Anaplasma marginale per se as one would expect in opsonization.

Ristic and Sippell (40) suggested that the complement-fixing antibody might act as an opsonizing antibody to render the parasite more susceptible to phagocytosis. Coating of erythrocytes with antibodies supplied by the spleen is believed to occur in anaplasmosis (41). The existence of a positive reaction to the Coombs Test has been reported (41). Brock et al (42), however, were unable to confirm these findings. Jones (43), emphasized the fact that, if the formation and release of damaging immune bodies is chiefly localized in the spleen, splenectomy should not enhance the disease intensity.

Taliaferro and Mulligan (44) studied the immune mechanism of integrated cellular and hormonal activity in avian and simian malarias. The authors described the basic picture until parasitic crisis as one of scavenging phagocytosis of parasitic and cellular debris by cells of the lymphoid-macrophage (reticulo endothelial) system. After crisis, the picture changed to one of stimulated phagocytosis of debris, whole parasitized cells, and of erythrocytes which appeared in every way normal, thus leading to an "excessive" anemia.

Zuckerman (45) referred to an unexplained anemia common to a number of malarias in a wide variety of hosts, and stated that the

anemia may be induced by autoantibody or that the RBC, following crisis, may absorb soluble parasitic antigen from the plasma, and may be destroyed by the action of antiparasitic rather than anti-erythrocytic antibody, thus leading to anemia. The same author (45) suggested that RBC and parasite may have an antigen in common, and that antiparasitic antibody might then opsonize uninfected erythrocytes.

Brock (15) found that parasitized RBC are removed rapidly from the circulation in anaplasmosis. A ten fold increase in erythrocyte removal has been reported even in the splenectomized calf (36).

The presence of autoantibody (hemolysins) has been demonstrated in calves following the decline of percent packed cell volume (PPCV) (41). The low content of free complement in bovine serum and the absence of free hemoglobin in the plasma during the period of progressive anemia tend to disprove the intravascular hemolytic theory in anaplasmosis (46). It has been observed in man and other species, however, that certain plasma proteins called haptoglobins,* can combine with hemoglobin liberated into the plasma which transports it as a hemoglobin-haptoglobin complex. Evidence for this phenomenon was produced by the marked increase in plasma iron and the decrease in total hemoglobin and plasma iron turn over rate in anaplasmosis-induced calves observed 3 days before peak parasitized erythrocytes appeared (47,48).

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*Haptoglobins are alpha 2 globulins with a high affinity for circulating hemoglobin.

Immunity and Splenectomy

Cattle which have recovered from an initial attack of anaplasmosis remain carriers of the disease (26). This type of immunity is dependent on the causal organism remaining alive in the animal and is called premunition. A small quantity of blood from a carrier animal will reproduce the disease when inoculated into a susceptible animal.(17).

Splenectomy causes the temporary disappearance of a positive CF titer in a carrier animal, resulting in increased susceptibility to anaplasmosis, irrespective of age (49). This technique is widely used in anaplasmosis research to test for the presence of the carrier state (50). The influence of splenectomy has been reviewed by Norman (49).

The splenectomized carrier animal exhibits a persistent parasitemia which is characterized by cyclic recrudescences at 2 to 3 week intervals (50). During the intervening period, a basal parasitemia in which zero to 3 percent of the RBC are infected usually exists, especially in animals splenectomized preinfection.

This cyclic phenomenon and its relationship, if any, to a growth cycle of the organism, or to red blood cell maturation and clearance, remains to be elucidated. A recrudescing parasitemia, of reduced intensity, has been observed 3 to 4 weeks after the onset of patent disease in aged cattle (51). Brock (52) noted recrudescences in cattle receiving intermittent tetracycline therapy.

Horan and Colebatch (53) reported a characteristic pattern for postsplenectomy infections in children, in which a positive correlation exists between the incidence of infections and splenectomy of the

child less than one year old. The course is fulminative with a tendency to recurrent attacks. Wagner et al.(54) observed a pattern of sequestration of damaged human erythrocytes which depended on the degree of damage to the erythrocyte. Early signs of injury resulted primarily in splenic sequestration of the RBC while more severe injury to the cells resulted in both hepatic and splenic sequestration of the erythrocytes. Very severe damage, short of intravascular hemolysis, resulted in hepatic, rather than splenic sequestration, the rate of removal from the circulation exceeding that observed with complete hemolysis.

Attempts to evaluate the role of the reticuloendothelial system (RES) in acquired immunity in certain protozoal diseases by blockading its activity has been reviewed by various authors (55,56) and in anaplasmosis by Jiminez de Asua et al.(57). The latter authors used India ink, assuming that mechanical blockage of the RES would prevent further phagocytic activity, thus allowing uninhibited growth of the infectious agent. The severity of the disease was not greater than in unblocked animals which led the authors to conclude that blockage of the RES had little or no influence on this aspect of the disease. It is doubtful, however, if blockage was achieved in their experiments. Gingrich (58), studied the role of phagocytosis in natural and acquired immunity in avian malaria by attempting to blockade the macrophages. The blockading procedure consisted of daily intravenous injections of washed foreign avian red blood cells. This procedure was found to be superior to injections of India ink or Janus green. The author found that there was no effect on the daily rate of increase in the number of malarial parasites during the acute rise of the infection. The

crisis with subsequent recovery was prevented, recovery interrupted, and relapses were produced. It was inferred that phagocytosis is an active process and a major factor in acquired immunity to malarial infections in birds. Gingrich emphasized that successful blockage of the RES necessitated large volumes of the blockading agent.

Ristic et al.(59) gave cortisone to anaplasmosis infected calves prior to splenectomy. Anemia did not develop and the titer to the complement-fixation test remained high after the spleen was removed. Similar animals which received cortisone after splenectomy developed a severe anemia associated with a decline in the complement-fixation titer. The authors suggested a probable stimulation of extrasplenic reticuloendothelial tissue by cortisone during the prepatent period.

Klaus, Jones and Kliewer (60) found that large doses of estradiol cypionate caused a temporary disappearance of parasitemia. The mechanism of this temporary remission is unknown. It may represent a direct inhibitory or parasitical action upon the causal organism or it may be due to temporary hyperphagocytosis by extrasplenic reticuloendothelial tissue.

Allison (61) stated that many species of animals show genetically controlled differences in plasma proteins and suggested that some of these may affect the development of blood parasites, especially those which are extracorpuscular.

The relationship between resistance to malaria and hemoglobin types in certain human populations has been reported (62). Human fetal hemoglobin is insoluble in acid buffers and it has been suggested that the presence of this hemoglobin during the first 3 to 4 months of postnatal life plays a part in the resistance of the new-

born child to malaria (63). This factor may explain the relative resistance of young calves to anaplasmosis (51).

Blood Transfusions in Anaplasmosis

Blood transfusions have been recommended as supportive treatment for anaplasmosis (16,64). Franklin and Schmidt (65), however, failed to demonstrate any value from whole blood transfusions. Carricaburu (66) referred to the impractability of this type of therapy in wild or range cattle and stated that its use should be confined to gentle cattle. Return to pretransfusion PCV values has been observed within 18 hours after the administration of 8 ml./lb. of body weight of compatible whole blood (36). A study of the survival of transfused homologous erythrocytes in cattle revealed a rapid disappearance of radioactivity after the transfusion of labelled homologous cells, with an apparent half survival time of $2\frac{1}{2}$ days (67). This was contrasted with the apparent half survival time of 8 days after autotransfusion of labeled cells in the same cattle. The same survival time was found after homotransfusion of Fe^{59} labeled erythrocytes. These results indicate that benefits derived from blood transfusions are of short duration. They should be repeated within 24 hours. The latter authors emphasized that, if this is a general phenomenon in cattle, transfusion therapy may be dangerous unless compatibility is demonstrated, prior to transfusion, by serologic techniques.

CHAPTER II

EXPERIMENTAL DESIGN AND METHODS

This study was designed to evaluate the cyclic recrudescences in the parasitized erythrocytes (PE) which occur in splenectomized anaplasmosis-carrier animals and to ascertain whether a correlation exists between a recrudescence and the state of maturity of circulating RBC. Consequently, citrated whole blood was transfused into intact and asplenic carriers and acutely infected cows. Homologous, heterologous, infected and uninfected blood was used.

Experimental Animals

Male calves of mixed breeds and two Guernsey cows were obtained from local herds. They were maintained under standard conditions of husbandry throughout the study period. The calves were vaccinated against clostridial infections and leptospirosis and treated for internal parasites when indicated.

Exposure to Anaplasmosis

Prior to experimental infection, the complement-fixation (CF) test for anaplasmosis was performed on a serum sample taken from each animal (68). Five ml. of carrier blood from a known infected cow was inoculated subcutaneously into each animal. The carrier blood had been treated with neoarsphenamine (25 mg. per 10 ml. of whole blood)

at 40 degrees Fahrenheit for 24 hours to eliminate eperythrozoon organisms (69). All cattle subsequently became infected. The experimental calves were splenectomized a few weeks later.

Splenectomy Technique

The animal was restrained in a calf chute which was rotated into the horizontal position. The left flank from the last rib to the tuber coxae was prepared for surgical operation. This area was anesthetized by local infiltration of about 40 ml. of a 2 percent procaine hydrochloride solution. An incision was made in the skin, muscles, and peritoneum, commencing about 2 cm. below the left lumbar transverse processes and about 5 cm. caudad to the left costal arch and extended ventrally for about 15 cm.. The spleen was located on the lateral surface of the rumen and the splenic pedicle, containing the splenic blood vessels, was isolated by blunt dissection and ligated with No. 2 chromic catgut. The pedicle was transected, and the spleen was removed. The abdominal incision was closed in a conventional manner. Five ml. benzathine penicillin G and procaine penicillin G in aqueous solution* were administered intramuscularly.

Pilot Study

Pilot observations were made on the effects of transfusing blood obtained from (a) intact anaplasmosis-free, (b) intact carrier and (c) splenectomized carrier animals into splenectomized anaplasmosis-carrier animals.

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*Longicil Fortified: Fort Dodge Labs., Inc., Fort Dodge, Iowa.

Allocation of Treatment Groups

Experiment 1

Group A - Two 15 month old cross-bred splenectomized anaplasmosis-carrier male calves, which were in the convalescent period*, were transfused with a calculated volume of citrated whole blood obtained from a Holstein-Friesian cow which was known to be free from anaplasmosis.

Group B - Two 15 month old cross-bred splenectomized anaplasmosis-carrier male calves, in the convalescent period, were transfused with a calculated volume of citrated whole blood collected from an Angus cow which was known to be a carrier of anaplasmosis.

Group C - Two 15 month old cross-bred splenectomized anaplasmosis-carrier male calves, in the convalescent period, were transfused with a calculated volume of citrated whole blood, containing a considerable percentage of immature red blood cells, obtained from an anaplasmosis-free Guernsey cow.

Group D - Two 15 month old cross-bred splenectomized carrier male calves, which were in the convalescent period, were transfused with a calculated volume of citrated whole blood obtained from 4 to 5 day old Holstein-Friesian calves.

Experiment 2

Two 15 month old cross-bred nonsplenectomized anaplasmosis-carrier male calves were transfused with a calculated volume of blood obtained from an anaplasmosis free Holstein-Friesian cow.

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*Convalescent period means the period of minimal percentage of parasitized erythrocytes.

Experiment 3

Fifteen month old splenectomized carrier male calves, in the convalescent period, were transfused with a calculated volume of compatible citrated whole blood obtained from (a) Quarter horse, (b) deer.

Experiment 4

Two 6 year old Guernsey cows were inoculated subcutaneously with 5 ml. of carrier blood from a known infected cow. When these cows developed peak clinical signs of anaplasmosis, they were transfused with a calculated volume of citrated whole blood obtained from an anaplasmosis-free Guernsey cow.

Compatibility Test

A cross matching test was made on each occasion before a blood transfusion was administered, to establish compatibility between donor and recipient animal bloods. The method employed was similar to the technique described by Hepler (70), except that plasma was substituted for serum. The absence of agglutination or clumping, on examination under the lower power of the microscope, was used as an indicator of compatibility.

Collection of Whole Blood

Blood was collected from the donor animal into sterile 1 liter vacuum jars containing 100 ml. of 3 percent sodium citrate solution (71).

Dosage Rate

Citrated whole blood was transfused at the rate of 4 ml. of red

blood cells per pound body weight. It was considered that transfusion of donor blood at this calculated level would raise the PCV of the recipient animal significantly without causing undue hypervolemia.

The volume of whole blood required was calculated as follows:

Example: Weight of recipient animal = 300 lbs.

Volume of RBC required = 1200 ml.

Packed cell volume (PCV) of donor blood* = 40%

Volume of donor blood required = 3000 ml.

Blood Transfusion Technique

Blood transfusions were commenced within one hour after collection. The recipient animal was restrained in the standing position in a cattle chute. Transfusion was made into the jugular vein, using a standard technique. The rate of transfusion was approximately 1 liter per 30 minutes. The animal was kept under close observation for signs of cardiac embarrassment and transfusion reactions. In such events, the rate of transfusion was reduced, temporarily suspended, or discontinued.

Hematology

The data presented in this study were obtained by recognized procedures (72). Blood samples were collected from the jugular vein into tubes containing 10 mg. of dry dipotassium ethylene diamine tetra acetate (EDTA). Samples were taken 2 or 3 times weekly during

*The packed cell volume of the donor blood was made prior to transfusion.

the prepatent period*, and more frequently during the patent period. On the day of transfusion, 4 samples were collected at 2 hour intervals before and after treatment, and every 12 hours for the succeeding 3 days.

Blood films were made with the coverslip technique and counterstained with Wright's stain (73).

Packed cell volume (PCV) was determined by the microcapillary tube method and a microhematocrit centrifuge (74). The samples were measured on an International rotary reading unit**.

Erythrocyte counts were made by standard laboratory procedures using an electronic grid partical counter+ with a 100 u aperture tube. To reduce the coincidence factor, the standard erythrocyte dilutions were doubled.

Hemoglobin determinations were made colorimetrically using the acid hematin technique (75). A 60 minute color developing time and a reading wave length of 525 mmu were used.

The percentage of erythrocytes containing anaplasma bodies among 1000 red blood cells was estimated from the stained blood smears. Counting was facilitated by placing a Whipple's disk in the microscope ocular.

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*The prepatent period is the period before one percent parasitized erythrocytes are observed in the peripheral circulation.

**International Electric Co., Evanston, Ill.

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

CHAPTER III

RESULTS

The data in this study are reported in tabular and graphic forms, using the following abbreviations:

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

PCV - Packed cell volume, volumes percent

RBC - Erythrocyte count, x 10^6 cells per mm^3

PPE - Percent parasitized erythrocytes, percent

PRBC - Parasitized erythrocytes, x 10^6 cells per mm^3

In the pilot study, only values for PCV and PPE were obtained. The graphs present these data in relation to time. (Figure 1). In experiments 1 through 4, PRBC were calculated, and they are depicted graphically. Insert graphs are presented in Figures 2 through 7 to demonstrate the relationship of treatment time to the pattern of cyclic recrudescences.

Results of Pilot Study

There was an increase in the percentage parasitemia in experiments 1 and 2 following transfusion with non-infected and infected blood from intact animals respectively. There was only a slight increase in experiment 3, using infected blood from a splenectomized carrier animal. (Figure 1).

Results of Experiment One

The transfusion of homologous, non-infected blood into splenectomized, anaplasmosis-carrier calves produced an increase in parasitized erythrocytes from 83 thousand/mm^3 to 363 thousand/mm^3 of blood in calf no. 1 within 24 hours post-transfusion. There was an increase from 121 thousand/mm^3 to 545 thousand/mm^3 within 12 hours in calf no. 2. (Figure 2). The transfusion of infected blood, under the same conditions, produced an increased parasitized erythrocytes from 35 thousand/mm^3 of blood to 188 thousand/mm^3 within 6 hours post-transfusion in calf no. 1. A return to pre-transfusion values occurred within 24 hours. There was an increase from 50 thousand parasitized erythrocytes/mm^3 to 253 thousand/mm^3 in calf no. 2 within 24 hours post-transfusion, which was maintained for several days. (Figure 3).

The responses following transfusion of blood containing 16 percent reticulocytes (Figure 4) and blood from newborn calves (Figure 5) were insignificant.

Results of Experiment Two

There was no response following transfusion of homologous, non-infected blood into non-splenectomized, carrier calves. (Figure 6).

Results of Experiment Three

Transfusion of horse blood into a splenectomized, carrier animal did not result in an increased number of parasitized erythrocytes. There was a slight response following transfusion of deer blood. (Figure 7). Stained blood smears collected from the recipient animal, at various intervals post-transfusion, were examined under

light microscopy for evidence of parasitized deer erythrocytes. These cells, which normally appear sickle-shaped, could not be distinguished from recipient erythrocytes.

Results of Experiment Four

The transfusion of a cow during acute anaplasmosis produced an increased parasitized erythrocytes from 416 thousand/mm^3 of blood to 719 thousand/mm^3 within 2 hours post-transfusion. There was a decline to 181 thousand/mm^3 shortly before death which occurred 3 days post-transfusion. (Figure 8). The data from another cow in this experiment were discarded because the animal died from acute peritonitis due to a perforated abomasal ulcer, two days post-transfusion.

TABLE I

PILOT STUDY - EXPERIMENT ONE

Transfusion of splenectomized anaplasmosis-carrier calves with citrated whole blood from an intact anaplasmosis-free animal.

(A) Weight of recipient animal = 310 lbs.
PCV of donor blood = 28%
Volume of blood transfused = 4450 ml.

Date	PCV	PPE	PCV x PPE
2-28-65	20.0	2.8	56.00
2-22-65	21.0	2.2	46.20
2-23-65*	23.0	2.0	46.00
2-24-65	35.0	5.5	192.50
2-25-65	36.0	7.2	259.20
2-26-65	33.0	7.8	257.40
2-27-65	25.0	6.1	152.50
2-28-65	24.0	5.8	139.20

(B) Weight of recipient animal = 305 lbs.
PCV of donor blood = 30%
Volume of donor blood transfused = 4150 ml.

Date	PCV	PPE	PCV x PPE
3-4-65	23.5	3.5	82.25
3-5-65	22.5	3.4	76.50
3-6-65*	22.5	3.3	74.25
3-7-65	30.5	4.1	125.05
3-8-65	32.5	4.2	136.50
3-9-65	27.5	7.9	217.25
3-10-65	20.5	6.0	123.00
3-11-65	19.5	5.8	113.10

*Date of transfusion

TABLE II

PILOT STUDY - EXPERIMENT TWO

Transfusion of splenectomized anaplasmosis-carrier calves with citrated whole blood from an intact anaplasmosis-carrier animal.

(A) Weight of recipient animal = 320 lbs.
PCV of donor blood = 32%
Volume of blood transfused = 4000 ml.

Date	PCV	PPE	PCV x PPE
4-24-65	21.0	4.8	100.80
4-25-65	20.5	3.4	69.70
4-26-65	19.5	3.8	74.10
4-27-65*	19.5	3.8	74.10
4-28-65	30.5	5.4	164.70
4-29-65	30.5	8.4	256.20
4-30-65	24.5	8.7	213.15
5-1-65	22.0	7.0	154.00
5-2-65	19.5	6.2	120.90

(B) Weight of recipient animal = 375 lbs.
PCV of donor blood = 31%
Volume of donor blood transfused = 4300 ml.

Date	PCV	PPE	PCV x PPE
4-24-65	28.0	4.9	137.20
4-25-65	29.0	4.6	133.40
4-26-65	29.5	4.7	138.65
4-27-65*	29.0	4.7	136.30
4-28-65	35.0	8.1	283.50
4-29-65	31.5	9.1	286.65
4-30-65	27.5	8.9	244.75
5-1-65	27.0	8.8	237.60
5-2-65	27.0	8.0	216.00

*Date of transfusion

TABLE III

PILOT STUDY - EXPERIMENT THREE

Transfusion of splenectomized anaplasmosis-carrier calves with citrated whole blood from a splenectomized anaplasmosis-carrier animal.

(A) Weight of recipient animal = 125 lbs.
PCV of donor blood = 25%
Volume of blood transfused = 2000 ml.

Date	PCV	PPE	PCV x PPE
5-5-65	30.0	2.9	87.00
5-6-65	31.0	3.0	93.00
5-7-65	32.0	3.0	96.00
5-8-65	32.0	3.1	99.20
5-9-65	32.5	3.2	104.00
5-10-65	32.0	3.6	115.20
5-11-65*	33.0	3.5	115.50
5-12-65	37.5	2.4	90.00
5-13-65	37.5	2.9	108.75
5-14-65	37.5	2.2	82.50
5-15-65	37.0	1.9	70.30

(B) Weight of recipient animal = 280 lbs.
PCV of donor blood = 28%
Volume of donor blood transfused = 4000 ml.

Date	PCV	PPE	PCV x PPE
5-21-65	28.0	5.5	154.00
5-24-65	34.0	3.6	122.40
5-25-65*	34.5	2.8	96.00
5-26-65	33.0	2.8	92.40
5-27-65	35.5	2.7	95.85
5-28-65	37.5	2.9	108.75
5-29-65	38.0	2.6	98.80
5-30-65	38.0	2.5	95.00
5-31-65	33.0	3.1	102.30
6-1-65	32.0	6.6	211.20

*Date of transfusion

TABLE IV

EXPERIMENT ONE, GROUP A (1)

Transfusion with citrated whole blood from a Holstein-Friesian cow known to be free from anaplasmosis.

Recipient Animal: 15 month old cross-bred male calf (splenectomized anaplasmosis carrier).

Weight of recipient animal = 252 lbs.
PCV of donor blood = 34%
Volume of blood transfused = 2950 ml.

Date	Time	PCV	RBC	PPE	PRBC	PCV x PPE
1-1-66	8:00 AM	17.0	4.01	11.4	0.457	193.80
1-2-66	8:00 AM	19.0	4.12	8.9	0.366	169.10
1-3-66	8:00 AM	20.0	4.14	6.8	0.281	136.00
1-4-66	8:00 AM	20.0	4.01	5.6	0.224	112.00
1-6-66	8:00 AM	25.0	4.67	4.2	0.196	105.00
1-8-66	8:00 AM	27.0	5.22	1.6	0.083	43.20
1-9-66	8:00 AM	29.5	5.30	1.8	0.095	53.10
1-10-66	4:00 AM	28.0	5.22	1.8	0.093	50.40
	6:00 AM	27.5	5.21	1.5	0.078	41.25
	8:00 AM	27.5	5.20	1.6	0.083	44.00
	*10:00 AM	27.0	5.20	1.6	0.083	43.20
	+11:30 AM	32.0	6.34	1.5	0.095	48.00
	1:30 PM	35.0	6.64	2.3	0.152	80.50
	3:30 PM	37.0	6.95	3.1	0.215	114.70
	5:00 PM	36.0	6.90	3.0	0.207	108.00
	7:30 PM	35.5	6.85	4.5	0.308	159.75
1-11-66	8:00 AM	35.0	6.61	5.5	0.363	192.50
	8:00 PM	32.0	6.44	4.9	0.315	156.80
1-12-66	8:00 AM	32.0	6.42	4.5	0.288	144.00
	8:00 PM	29.0	5.95	4.9	0.291	142.10
1-13-66	8:00 AM	28.5	5.41	4.7	0.254	133.90
	8:00 PM	29.0	5.92	4.0	0.239	116.00
1-14-66	8:00 AM	29.0	5.51	3.9	0.215	113.10
1-15-66	8:00 AM	26.5	3.96	3.5	0.138	92.70
1-16-66	8:00 AM	23.0	4.20	3.3	0.138	75.90
1-17-66	8:00 AM	25.0	4.84	3.0	0.145	75.00
1-18-66	8:00 AM	27.5	5.84	3.5	0.204	96.20

*Transfusion commenced
+Transfusion completed

TABLE V

EXPERIMENT ONE, GROUP A (2)

Transfusion with citrated whole blood from a Holstein-Friesian cow known to be free from anaplasmosis.

Recipient animal: 15 month old cross-bred male calf (splenectomized anaplasmosis carrier).

Weight of recipient animal = 225 lbs.
PCV of donor blood = 35.5%
Volume of blood transfused = 2820 ml.

Date	Time	PCV	RBC	PPE	PRBC	PPE x PCV
1-1-66	8:00 AM	30.5	5.22	5.1	0.266	155.50
1-2-66	7:00 AM	35.0	5.42	2.8	0.152	98.00
1-3-66	8:00 AM	38.0	6.15	2.5	0.154	95.00
1-5-66	8:00 AM	35.0	6.86	1.6	0.109	56.00
1-7-66	8:00 AM	37.0	7.25	1.4	0.101	51.80
1-9-66	8:00 AM	34.5	7.20	1.6	0.115	55.20
1-10-66	8:00 AM	32.0	7.15	2.0	0.143	64.00
1-11-66	8:00 AM	31.0	6.95	1.9	0.132	58.90
1-12-66	6:00 AM	30.0	6.84	1.9	0.130	57.00
	8:00 AM	30.5	6.73	1.8	0.121	54.90
	10:00 AM	29.0	6.37	1.9	0.121	55.10
	*12:00 AM	30.0	6.25	2.0	0.125	60.00
	+1:30 PM	36.0	7.05	2.7	0.190	97.20
	3:30 PM	39.0	7.66	4.3	0.329	167.70
	5:30 PM	41.0	8.45	4.0	0.338	164.00
	7:30 PM	42.0	8.65	6.3	0.545	264.60
1-13-66	8:00 AM	41.5	8.42	5.2	0.438	215.80
	8:00 PM	39.0	7.89	5.0	0.394	195.00
1-14-66	8:00 AM	38.5	7.75	5.3	0.411	204.00
	8:00 PM	36.5	7.02	4.8	0.337	175.20
1-15-66	8:00 AM	32.0	5.52	3.9	0.215	124.00
	8:00 PM	23.0	5.01	4.2	0.210	96.00
1-16-66	8:00 AM	22.0	4.22	4.5	0.190	99.00
1-17-66	8:00 AM	20.5	3.74	4.1	0.153	84.00
1-18-66	8:00 AM	19.5	4.26	3.6	0.153	70.20
1-19-66	8:00 AM	17.5	3.90	2.9	0.113	50.70
1-20-66	8:00 AM	19.0	3.84	2.1	0.086	39.90

*Transfusion commenced
+Transfusion completed

TABLE VI

EXPERIMENT ONE, GROUP B (1)

Transfusion with citrated whole blood from an Angus cow known to be a carrier of anaplasmosis.

Recipient Animal: 15 month old cross-bred male calf (splenectomized, anaplasmosis carrier)

Weight of recipient animal = 375 lbs.
PCV of donor blood = 40%
Volume of blood transfused = 3750 ml.

Date	Time	PCV	RBC	PPE	PRBC	PPE x PCV
4-7-66	8:00 AM	25.5	5.56	1.1	0.061	28.00
4-8-66	8:00 AM	26.0	5.40	1.2	0.065	31.20
4-9-66	8:00 AM	26.5	5.50	1.0	0.055	26.50
4-10-66	8:00 AM	26.0	5.55	1.0	0.055	26.00
4-11-66	8:00 AM	27.0	5.74	0.9	0.051	24.30
4-12-66	8:00 AM	26.0	4.98	1.1	0.055	28.60
4-13-66	4:00 AM	26.0	4.95	0.7	0.035	18.20
	6:00 AM	26.0	4.98	0.6	0.029	15.90
	8:00 AM	26.0	5.06	0.7	0.035	18.20
	*10:00 AM	24.5	5.00	0.7	0.035	17.15
	+11:30 AM	29.5	6.08	0.7	0.042	20.65
	1:30 PM	34.5	7.10	1.8	0.127	62.10
	3:30 PM	34.0	7.24	2.6	0.188	88.40
	5:30 PM	37.0	7.32	2.0	0.146	74.00
	7:30 PM	37.0	7.35	1.7	0.125	62.90
4-14-66	8:00 AM	35.0	7.58	0.2	0.015	7.00
	8:00 PM	32.5	7.24	0.1	0.072	3.25
4-15-66	8:00 AM	31.5	6.04	0.2	0.012	6.30
	8:00 PM	32.0	6.20	0.1	0.006	3.20
4-16-66	8:00 AM	31.0	6.42	0.4	0.025	12.40
	8:00 PM	30.0	6.30	0.5	0.031	15.00
4-17-66	8:00 AM	28.5	5.82	0.4	0.023	11.40
4-18-66	8:00 AM	26.0	5.04	0.5	0.025	13.00
4-19-66	8:00 AM	24.0	5.34	0.2	0.011	4.80
4-20-66	8:00 AM	22.5	5.34	0.0	0.000	0.00

*Transfusion commenced
+Transfusion completed

TABLE VII

EXPERIMENT ONE, GROUP B (2)

Transfusion with citrated whole blood from an Angus cow known to be a carrier of anaplasmosis.

Recipient Animal: 15 month old cross-bred male calf (splenectomized, anaplasmosis carrier).

Weight of recipient animal = 380 lbs.
PCV of donor blood = 40%
Volume of donor blood transfused = 3800 ml.

Date	Time	PCV	RBC	PPE	PRBC	PPE x PCV
4-7-66	8:00 AM	28.5	5.50	1.2	0.066	34.2
4-8-66	8:00 AM	29.5	5.60	1.0	0.056	29.5
4-9-66	8:00 AM	29.0	5.55	1.3	0.072	37.7
4-10-66	8:00 AM	29.5	5.62	1.2	0.067	35.4
4-11-66	8:00 AM	30.0	5.36	1.6	0.085	48.0
4-12-66	8:00 AM	26.0	4.48	2.0	0.089	52.0
4-13-66	4:00 AM	27.0	4.56	1.4	0.063	37.8
	6:00 AM	27.5	4.82	1.0	0.048	27.5
	8:00 AM	28.5	5.32	0.9	0.047	25.6
	*10:00 AM	25.0	5.04	1.2	0.050	30.0
	+11:30 AM	31.0	6.02	1.0	0.060	31.0
	1:30 PM	30.5	5.92	1.4	0.082	42.7
	3:30 PM	35.0	6.38	1.3	0.082	45.5
	5:30 PM	36.5	6.30	2.0	0.126	73.0
	7:30 PM	37.0	6.32	2.9	0.183	107.3
4-14-66	8:00 AM	32.0	6.34	4.0	0.253	128.0
	8:00 PM	35.0	6.31	4.3	0.271	150.5
4-15-66	8:00 AM	35.0	6.32	4.5	0.284	157.5
	8:00 PM	34.0	6.51	4.6	0.299	156.4
4-16-66	8:00 AM	34.0	6.74	4.2	0.283	142.8
	8:00 PM	33.5	6.65	4.0	0.266	134.0
4-17-66	8:00 AM	33.0	6.50	4.2	0.273	138.6
4-18-66	8:00 AM	32.0	6.74	4.5	0.303	144.0
4-19-66	8:00 AM	26.5	5.20	4.3	0.223	113.9
4-20-66	8:00 AM	20.0	4.24	3.8	0.161	76.0

*Transfusion commenced
+Transfusion completed

TABLE VIII

EXPERIMENT ONE, GROUP C (1)

Transfusion with citrated whole blood containing a considerable percentage of immature RBC obtained from an anaplasmosis-free Guernsey cow.

Twenty two liters of blood were withdrawn from this animal (825 lbs. body weight) reducing the hemoglobin level to 7.0 Gms. per 100 ml. Ten days later blood from this animal was collected for transfusion.

Recipient Animal: 15 month old cross-bred male calf.

Weight of recipient animal = 305 lbs.
PCV of donor blood = 28% (Reticulocyte count = 16%)
Volume of blood transfused = 4350 ml. approximately

Date	Time	PCV	RBC	PPE	PRBC	PPE x PCV
6-9-66	8:00 AM	17.5	3.94	1.6	0.063	28.0
6-10-66	8:00 AM	19.5	4.04	1.0	0.040	19.5
6-11-66	8:00 AM	19.5	3.62	1.2	0.043	23.4
6-12-66	8:00 AM	18.5	3.82	1.9	0.072	35.1
6-13-66	8:00 AM	17.5	4.08	1.9	0.077	33.2
6-14-66	4:00 AM	19.5	4.00	2.0	0.080	39.0
	6:00 AM	19.0	3.78	1.7	0.064	32.3
	8:00 AM	16.5	3.58	1.5	0.053	24.7
	*10:00 AM	17.5	3.42	1.4	0.047	24.5
	+12:00 AM	23.0	4.01	1.2	0.048	27.6
	2:00 PM	22.0	4.32	1.3	0.056	28.6
	4:00 PM	22.5	4.25	1.1	0.046	24.7
	6:00 PM	22.5	4.20	1.2	0.050	27.0
	8:00 PM	21.5	4.18	1.0	0.041	21.5
6-15-66	8:00 AM	20.0	3.92	1.3	0.050	26.0
	8:00 PM	20.0	3.72	1.2	0.044	24.0
6-16-66	8:00 AM	20.5	4.16	1.4	0.058	28.7
	8:00 PM	20.5	4.20	1.0	0.042	20.5
6-17-66	8:00 AM	18.0	3.52	1.2	0.042	21.6
	8:00 PM	18.0	3.40	1.0	0.034	18.0
6-18-66	8:00 AM	17.5	3.39	1.1	0.037	19.5
6-19-66	8:00 AM	16.5	3.38	1.2	0.040	19.8
6-20-66	8:00 AM	16.5	3.38	1.3	0.043	21.5

*Transfusion commenced
+Transfusion completed

TABLE IX

EXPERIMENT ONE, GROUP C (2)

Transfusion with citrated whole blood containing a considerable percentage of immature RBC obtained from an anaplasmosis-free Guernsey cow.

Twenty two liters of blood were withdrawn from the jugular vein of this animal (825 lbs. body weight) reducing the hemoglobin level to 7.0 Gms. per 100 ml.. Ten days later blood from this animal was collected for transfusion. (Reticulocyte count = 16%)

Recipient Animal: 15 month old cross-bred male calf (splenectomized, anaplasmosis-free).

Weight of recipient animal = 286 lbs.
PCV of donor blood = 28%
Volume of blood transfused = 2650 ml. approximately

Date	Time	PCV	RBC	PPE	PRBC	PPE x PCV
6-9-66	8:00 AM	28.5	5.40	1.0	0.054	28.5
6-10-66	8:00 AM	30.0	5.50	1.0	0.055	30.0
6-11-66	8:00 AM	28.0	5.58	1.3	0.072	36.4
6-12-66	8:00 AM	29.0	5.59	1.2	0.067	34.8
6-13-66	8:00 AM	31.0	5.66	1.2	0.067	37.2
6-14-66	4:00 AM	31.5	5.56	1.4	0.077	44.1
	6:00 AM	31.0	5.52	1.2	0.066	37.2
	8:00 AM	26.0	5.36	1.0	0.053	26.0
	*10:00 AM	28.0	5.54	0.9	0.049	25.2
	+11:30 AM	34.0	7.38	1.3	0.095	44.2
	1:30 PM	30.5	5.34	1.4	0.074	42.7
	3:30 PM	31.0	5.55	1.2	0.066	37.2
	5:30 PM	29.0	5.40	1.3	0.070	37.7
	7:30 PM	28.0	5.32	1.1	0.058	30.8
6-15-66	8:00 AM	26.0	4.75	1.0	0.047	26.0
	8:00 PM	29.0	4.82	0.9	0.043	26.1
6-16-66	8:00 AM	28.0	5.34	1.2	0.064	33.6
	8:00 PM	27.5	5.21	1.0	0.052	27.5
6-17-66	8:00 AM	24.0	5.02	1.1	0.055	26.4
	8:00 PM	29.0	5.32	1.1	0.058	31.9
6-18-66	8:00 AM	29.0	5.35	1.1	0.058	31.9
6-19-66	8:00 AM	28.5	5.42	1.5	0.081	42.7
6-20-66	8:00 AM	28.5	5.56	2.3	0.127	65.5

*Transfusion commenced
+Transfusion completed

TABLE X

EXPERIMENT ONE, GROUP D (1)

Transfusion with citrated whole blood from two 5 day old Holstein-Friesian calves.

Recipient Animal: 15 month old cross-bred male calf (splenectomized, anaplasmosis carrier).

Weight of recipient animal = 310 lbs.
PCV donor blood = 30%
Volume of blood transfused = 4130 ml.

Date	Time	PCV	RBC	PPE	PRBC	PPE x PCV
5-17-66	8:00 AM	17.0	2.62	1.8	0.040	30.6
5-18-66	8:00 AM	16.0	1.88	0.7	0.013	11.2
5-21-66	8:00 AM	18.0	2.74	0.0	0.000	00.0
5-23-66	8:00 AM	19.0	2.98	0.0	0.000	00.0
5-24-66	8:00 AM	18.0	3.22	0.0	0.000	00.0
5-25-66	8:00 AM	19.0	3.21	0.0	0.000	00.0
5-26-66	4:00 AM	21.0	3.21	0.0	0.000	00.0
	6:00 AM	21.5	3.22	0.0	0.000	00.0
	8:00 AM	21.0	3.26	0.0	0.000	00.0
	*10:00 AM	21.5	3.30	0.0	0.000	00.0
	+12:00 AM	26.5	3.60	0.0	0.000	00.0
	2:00 PM	31.0	3.62	0.0	0.000	00.0
	4:00 PM	30.5	3.70	0.0	0.000	00.0
	6:00 PM	31.0	3.80	0.0	0.000	00.0
	8:00 PM	30.5	4.00	0.0	0.000	00.0
5-27-66	8:00 AM	30.0	5.74	0.0	0.000	00.0
	8:00 PM	32.0	5.80	0.0	0.000	00.0
5-28-66	8:00 AM	31.5	6.00	0.0	0.000	00.0
	8:00 PM	31.5	6.00	0.0	0.000	00.0
5-29-66	8:00 AM	32.0	5.88	0.0	0.000	00.0
	8:00 PM	32.5	5.88	0.0	0.000	00.0
5-30-66	8:00 AM	33.5	5.66	0.1	0.005	03.5
5-31-66	8:00 AM	27.5	4.64	0.2	0.009	05.0

*Transfusion commenced
+Transfusion completed

TABLE XI

EXPERIMENT ONE, GROUP D (2)

Transfusion with citrated whole blood from two 4 day old Holstein-Friesian calves.

Recipient Animal: 15 month old cross-bred male calf (splenectomized, anaplasmosis carrier).

Weight of recipient animal = 340 lbs.
PCV of donor blood = 31%
Volume of blood transfused = 4450 ml. approximately

Date	Time	PCV	RBC	PPE	PRBC	PPE x PCV
5-26-66	8:00 AM	18.5	2.54	3.3	0.083	61.05
5-27-66	8:00 AM	20.0	3.00	3.0	0.090	60.00
5-28-66	8:00 AM	21.5	2.92	2.8	0.081	60.20
5-29-66	8:00 AM	22.0	3.01	2.6	0.078	57.20
5-30-66	4:00 AM	23.0	3.20	2.0	0.060	46.00
	6:00 AM	22.0	3.20	1.9	0.060	41.80
	8:00 AM	24.5	3.38	2.5	0.084	61.25
	*10:00 AM	21.5	3.30	2.2	0.072	47.30
	+12:00 AM	27.5	3.40	2.0	0.060	55.00
	2:00 PM	31.5	3.70	1.9	0.070	59.85
	4:00 PM	32.0	3.75	2.3	0.086	73.60
	6:00 PM	33.0	4.20	2.1	0.088	69.30
	8:00 PM	34.0	4.30	1.5	0.064	51.00
5-31-66	8:00 AM	33.0	5.54	0.9	0.049	29.70
	8:00 PM	34.0	5.40	1.3	0.070	44.20
6-1-66	8:00 AM	33.5	5.30	1.2	0.063	40.20
	8:00 PM	33.5	5.25	1.4	0.073	46.90
6-2-66	8:00 AM	34.5	5.64	1.5	0.084	51.00
	8:00 PM	34.5	5.67	1.8	0.102	62.10
6-3-66	8:00 AM	34.0	5.68	1.5	0.085	51.00
6-4-66	8:00 AM	34.5	4.92	1.3	0.063	44.85
6-5-66	8:00 AM	34.0	4.80	1.3	0.062	44.20
6-6-66	8:00 AM	33.0	4.75	1.2	0.057	39.60

*Transfusion commenced
+Transfusion completed

TABLE XII

EXPERIMENT TWO (1)

Transfusion with citrated whole blood from a Holstein-Friesian cow known to be free from anaplasmosis.

Recipient Animal: 15 month old cross-bred male calf (nonsplenectomized anaplasmosis carrier).

Weight of recipient animal = 300 lbs.
PCV of donor blood = 35.0%
Volume of blood transfused = 3439 ml.

Date	Time	PCV	RBC	PPE	PRBC	PCV x PPE
2-8-66	8:00 AM	26.0	5.74	00.0	0.000	00.00
2-9-66	8:00 AM	27.0	5.80	00.0	0.000	00.00
2-11-66	8:00 AM	26.5	5.96	00.0	0.000	00.00
2-14-66	8:00 AM	27.0	6.56	00.0	0.000	00.00
2-15-66	8:00 AM	25.0	5.62	00.0	0.000	00.00
2-16-66	4:00 AM	28.5	6.80	00.0	0.000	00.00
	6:00 AM	29.5	7.22	00.0	0.000	00.00
	8:00 AM	25.0	6.68	00.0	0.000	00.00
	*10:00 AM	32.5	7.64	00.0	0.000	00.00
	+12:30 PM	32.5	7.64	00.0	0.000	00.00
	2:30 PM	34.0	7.52	00.0	0.000	00.00
	4:30 PM	35.5	7.64	00.0	0.000	00.00
	6:30 PM	38.0	7.92	00.0	0.000	00.00
	8:30 PM	37.5	8.26	00.0	0.000	00.00
2-17-66	8:00 AM	36.5	7.30	00.0	0.000	00.00
	8:00 PM	40.0	8.06	00.0	0.000	00.00
2-18-66	8:00 AM	38.0	7.88	00.0	0.000	00.00
	8:00 PM	39.5	7.55	00.0	0.000	00.00
2-19-66	8:00 AM	40.0	7.46	00.0	0.000	00.00
	8:00 PM	39.0	7.50	00.0	0.000	00.00
2-20-66	8:00 AM	38.5	7.36	00.0	0.000	00.00
2-21-66	8:00 AM	33.0	7.26	00.0	0.000	00.00
2-22-66	8:00 AM	32.5	7.21	00.0	0.000	00.00
2-23-66	8:00 AM	32.0	7.20	00.0	0.000	00.00

*Transfusion commenced
+Transfusion completed

TABLE XIII

EXPERIMENT TWO (2)

Transfusion with citrated whole blood from a Holstein-Friesian cow known to be free from anaplasmosis.

Recipient Animal: 15 month old cross-bred male calf (nonsplenectomized anaplasmosis carrier).

Weight of recipient animal = 280 lbs.
PCV of donor blood = 35.0%
Volume of blood transfused = 3200 ml.

Date	Time	PCV	RBC	PPE	PRBC	PPE x PCV
2-8-66	8:00 AM	29.5	6.16	0.0	0.000	00.00
2-9-66	8:00 AM	30.5	6.40	0.0	0.000	00.00
2-11-66	8:00 AM	30.5	6.24	0.0	0.000	00.00
2-14-66	8:00 AM	30.5	7.32	0.0	0.000	00.00
2-15-66	8:00 AM	31.0	6.90	0.0	0.000	00.00
2-16-66	4:00 AM	30.5	6.66	0.0	0.000	00.00
	6:00 AM	29.5	6.32	0.0	0.000	00.00
	8:00 AM	30.0	6.34	0.0	0.000	00.00
	*10:00 AM	33.5	7.32	0.0	0.000	00.00
	+12:30 PM	33.0	7.36	0.0	0.000	00.00
	2:30 PM	34.0	7.38	0.0	0.000	00.00
	4:30 PM	34.5	7.52	0.0	0.000	00.00
	6:30 PM	36.0	7.62	0.0	0.000	00.00
	8:30 PM	37.0	8.24	0.0	0.000	00.00
2-17-66	8:00 AM	35.0	7.36	0.0	0.000	00.00
	8:00 PM	41.0	8.16	0.0	0.000	00.00
2-18-66	8:00 AM	36.0	7.36	0.0	0.000	00.00
	8:00 PM	37.5	7.21	0.0	0.000	00.00
2-19-66	8:00 AM	40.0	7.10	0.0	0.000	00.00
	8:00 PM	38.0	7.00	0.0	0.000	00.00
2-20-66	8:00 AM	36.5	6.80	0.0	0.000	00.00
2-21-66	8:00 AM	29.0	6.18	0.0	0.000	00.00
2-22-66	8:00 AM	28.5	6.00	0.0	0.000	00.00
2-23-66	8:00 AM	28.5	6.01	0.0	0.000	00.00

*Transfusion commenced
+Transfusion completed

TABLE XIV

EXPERIMENT THREE (1)

Transfusion with citrated whole blood from a Quarter horse.

Recipient Animal: 15 month old cross-bred male calf (splenectomized anaplasmosis carrier).

Weight of recipient animal = 335 lbs.
PCV of donor blood = 45.0%
Volume of blood transfused = 3200 ml.

Date	Time	PCV	RBC	PPE	PRBC	PPE x PCV
3-30-66	8:00 AM	13.0	2.98	3.6	0.107	46.80
3-31-66	8:00 AM	17.0	3.68	3.8	0.139	64.60
4-1-66	8:00 AM	16.0	3.66	3.2	0.117	51.20
4-2-66	8:00 AM	17.5	3.88	2.1	0.081	36.75
4-4-66	8:00 AM	18.5	2.98	2.7	0.080	49.95
4-5-66	8:00 AM	16.5	2.66	0.9	0.023	14.85
4-6-66	4:00 AM	16.5	2.48	0.2	0.004	03.30
	6:00 AM	16.0	2.48	0.0	0.000	00.00
	8:00 AM	16.0	2.20	0.0	0.000	00.00
	*10:00 AM	15.5	2.10	0.2	0.004	04.20
	+12.30 PM	13.5	2.26	0.0	0.000	00.00
	2:30 PM	15.5	2.60	0.0	0.000	00.00
	4:30 PM	16.0	2.70	0.0	0.000	00.00
	6:30 PM	15.5	2.82	0.0	0.000	00.00
	8:30 PM	15.5	2.83	0.0	0.000	00.00
4-7-66	8:00 AM	20.0	2.96	0.0	0.000	00.00
	8:00 PM	20.0	3.10	0.0	0.000	00.00
4-8-66	8:00 AM	19.5	3.12	0.0	0.000	00.00
	8:00 PM	18.5	3.32	0.0	0.000	00.00
4-9-66	8:00 AM	19.0	3.68	0.0	0.000	00.00
	8:00 PM	19.5	3.69	0.0	0.000	00.00
4-10-66	8:00 AM	19.0	3.82	0.0	0.000	00.00
4-11-66	8:00 AM	18.5	3.74	0.0	0.000	00.00

*Transfusion commenced
+Transfusion completed

TABLE XV

EXPERIMENT THREE (2)

Transfusion with citrated whole blood from a deer.

Recipient Animal: 15 month old cross-bred male calf (spenectomized, anaplasmosis carrier).

Weight of recipient animal = 290 lbs.
PCV of donor blood = 35%
Volume of blood transfused = 1250 ml.

Date	Time	PCV	RBC	PPE	PRBC	PPE x PCV
3-1-66	8:00 AM	18.5	3.32	12.4	0.411	229.40
3-5-66	8:00 AM	18.0	3.38	2.9	0.098	52.20
3-6-66	8:00 AM	21.5	3.74	1.8	0.067	38.70
3-7-66	8:00 AM	22.5	4.20	2.0	0.084	45.00
3-8-66	4:00 AM	23.5	4.56	0.0	0.000	00.00
	6:00 AM	23.0	4.45	0.0	0.000	00.00
	8:00 AM	23.5	4.28	0.0	0.000	00.00
	*10:00 AM	23.0	4.30	0.0	0.000	00.00
	+11:00 AM	25.0	4.45	0.0	0.000	00.00
	1:00 PM	29.5	5.12	0.0	0.000	00.00
	3:00 PM	30.5	5.22	0.0	0.000	00.00
	5:00 PM	31.5	5.54	0.0	0.000	00.00
	7:00 PM	32.0	5.72	0.0	0.000	00.00
3-9-66	8:00 AM	30.5	6.54	0.8	0.052	24.40
3-10-66	8:00 AM	29.0	5.14	1.2	0.061	34.80
3-11-66	8:00 AM	30.5	5.40	2.6	0.140	79.30
3-12-66	8:00 AM	28.0	4.92	1.1	0.054	30.80

*Transfusion commenced
+Transfusion completed

TABLE XVI

EXPERIMENT FOUR (1)

Transfusion of a nonsplenectomized anaplasmosis-infected Guernsey cow showing a "peak" percentage parasitized erythrocytes with citrated whole blood from an anaplasmosis-free Guernsey cow.

Weight of recipient animal - 850 lbs.
PCV donor blood = 30.5%
Volume of blood transfused = 9.5 liters

Date	Time	PCV	RBC	PPE	PRBC	PPE x PCV
5-19-66	8:00 AM	38.0	5.32	00.0	0.000	000.00
5-20-66	8:00 AM	37.5	6.04	00.0	0.000	000.00
5-21-66	8:00 AM	35.5	5.90	00.0	0.000	000.00
5-23-66	8:00 AM	34.0	5.52	00.0	0.000	000.00
5-24-66	8:00 AM	37.0	6.38	00.0	0.000	000.00
5-25-66	8:00 AM	34.0	5.40	01.2	0.064	040.80
5-26-66	8:00 AM	33.0	5.04	02.6	0.131	085.80
5-27-66	8:00 AM	37.5	6.16	02.9	0.178	108.75
5-28-66	8:00 AM	33.5	5.84	05.1	0.297	170.85
5-29-66	8:00 AM	28.0	5.20	10.8	0.561	302.40
5-30-66	8:00 AM	24.5	4.04	16.6	0.670	406.70
5-31-66	8:00 AM	24.5	3.80	18.8	0.714	460.60
6-1-66	8:00 AM	21.0	3.80	24.6	0.934	516.60
6-2-66	8:00 AM	13.5	1.90	40.2	0.763	542.70
6-3-66	6:00 AM	10.0	1.16	36.4	0.422	364.00
	8:00 AM	09.5	1.16	35.7	0.414	339.15
	*10:00 AM	09.0	1.14	36.5	0.416	328.50
	12:00 AM	11.0	1.02	25.8	0.263	283.80
	+2:00 PM	12.5	1.94	23.1	0.448	288.75
	4:00 PM	14.5	2.58	27.9	0.719	404.55
	6:00 PM	14.5	2.32	25.6	0.593	371.20
	8:00 PM	15.5	2.15	20.1	0.432	311.55
	10:00 PM	16.0	1.75	16.5	0.288	264.00
6-4-66	8:00 AM	11.5	1.48	25.6	0.378	294.40
	8:00 PM	09.2	1.28	20.6	0.263	189.52
6-5-66	8:00 AM	09.2	1.20	24.1	0.289	221.72
	8:00 PM	08.5	1.10	24.8	0.272	210.80
6-6-66	8:00 AM	06.0	0.72	25.2	0.181	151.20
	2:00 PM	Died				

*Transfusion commenced
+Transfusion completed

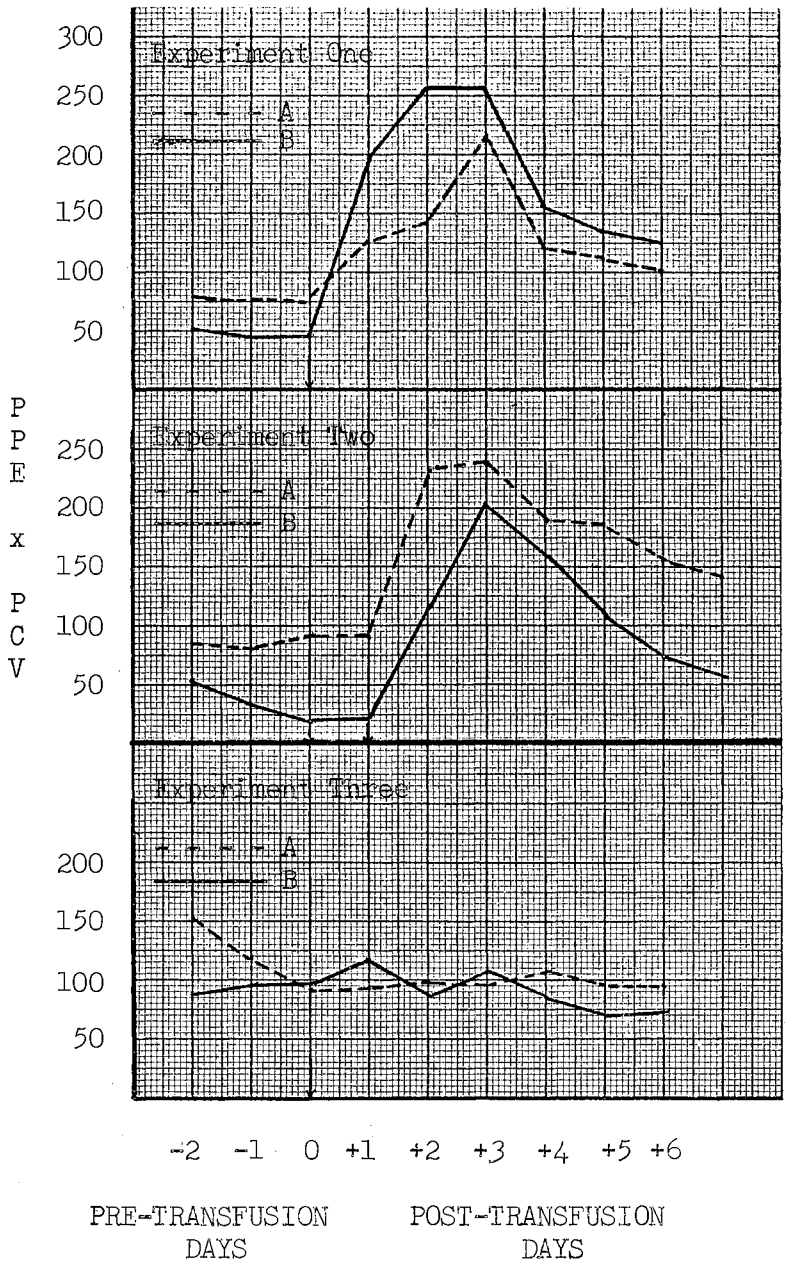

Figure 1. Pilot study on splenectomized anaplasmosis-carrier calves (A and B). Experiment One - the effect of blood transfusion from an intact, anaplasmosis-free animal; Experiment Two - the effect of a blood transfusion from an intact, carrier animal; Experiment Three - the effect of a blood transfusion from a splenectomized, carrier animal.

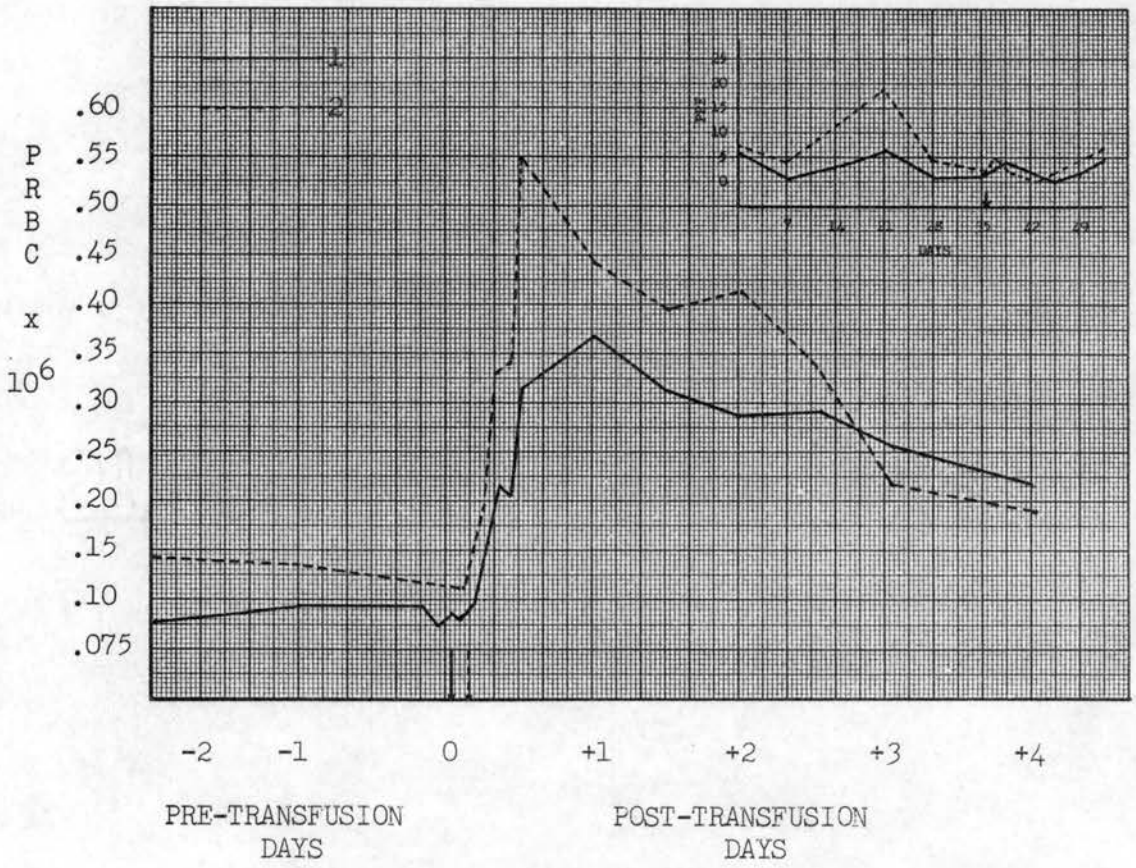

Figure 2. Experiment one, group A - the effect of transfusion of homologous, non-infected blood into splenectomized, carrier calves.

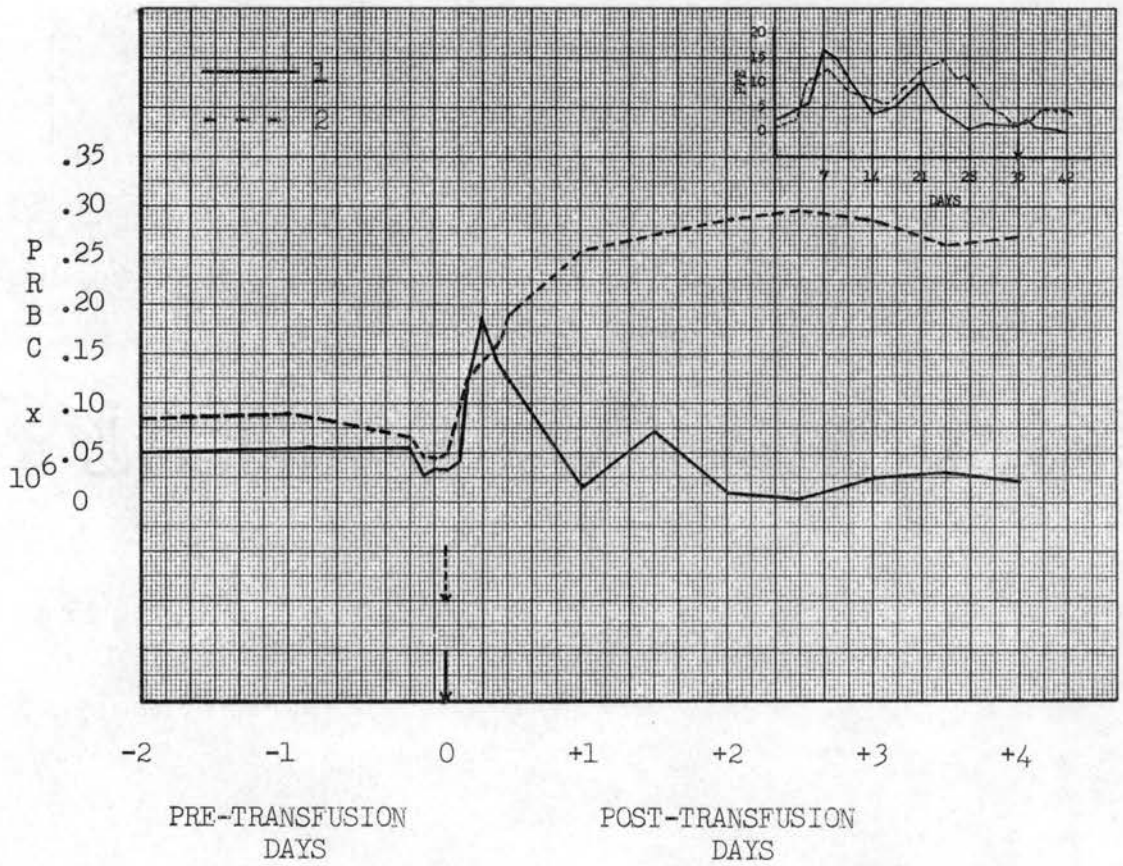

Figure 3. Experiment one, group B - the effect of transfusion of homologous, carrier blood into splenectomized, carrier calves.

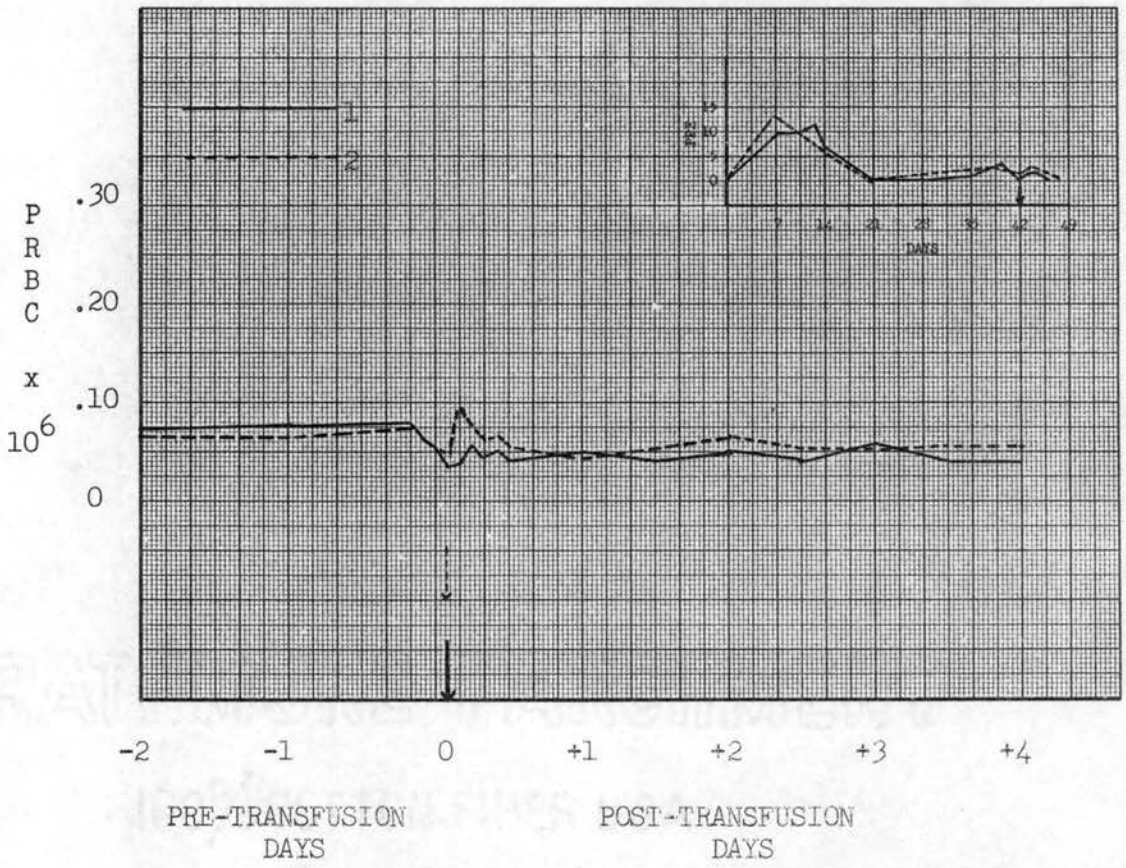

Figure 4. Experiment one, group C - the effect of transfusion of homologous, immature, non-infected blood containing 16% reticulocytes into splenectomized, carrier calves.

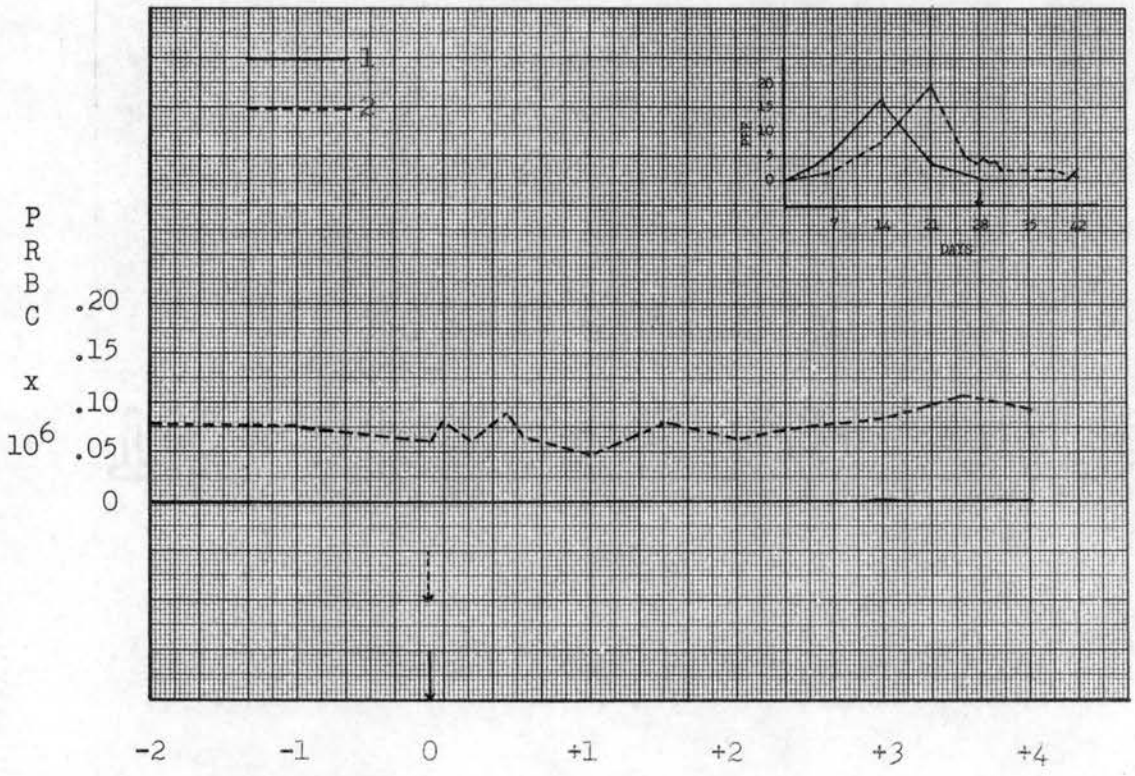

Figure 5. Experiment one, group D - the effect of transfusion of homologous, non-infected blood obtained from 4-5 day old calves into splenectomized, carrier calves.

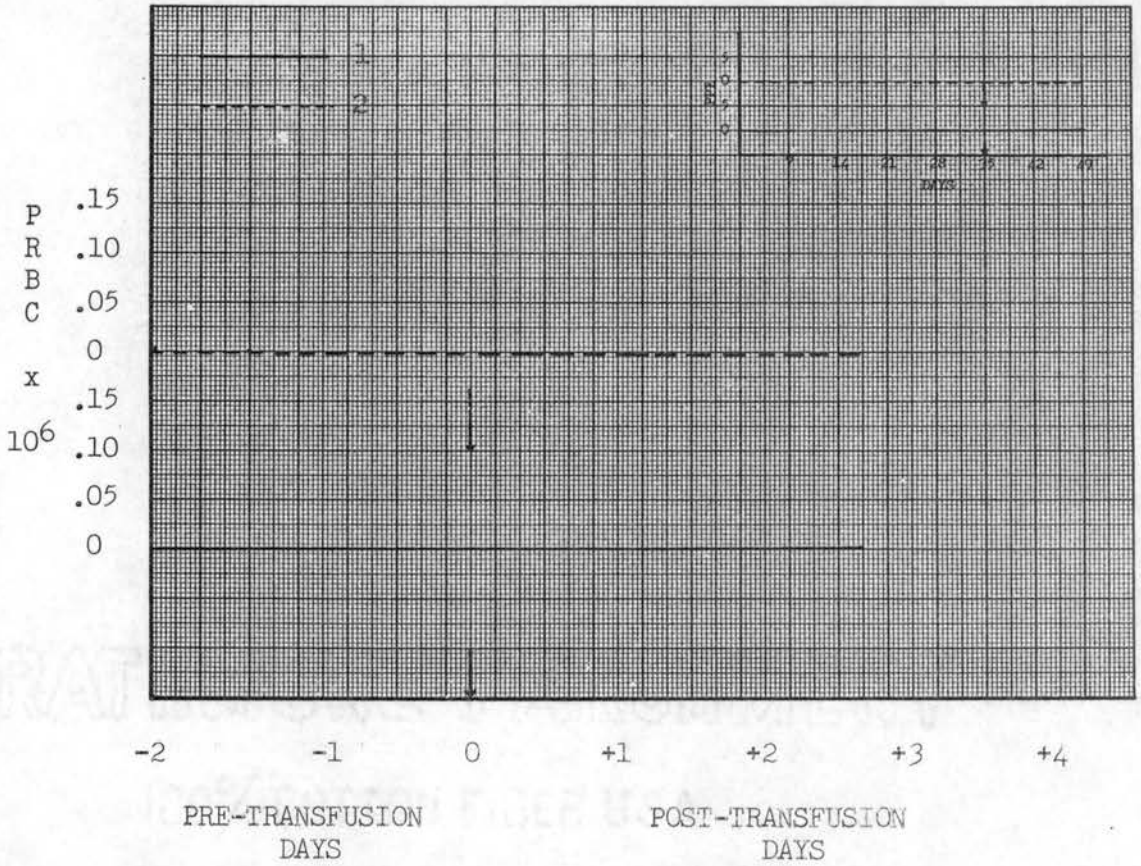

Figure 6. Experiment two - the effect of transfusion of homologous, non-infected blood into nonsplenectomized, carrier calves.

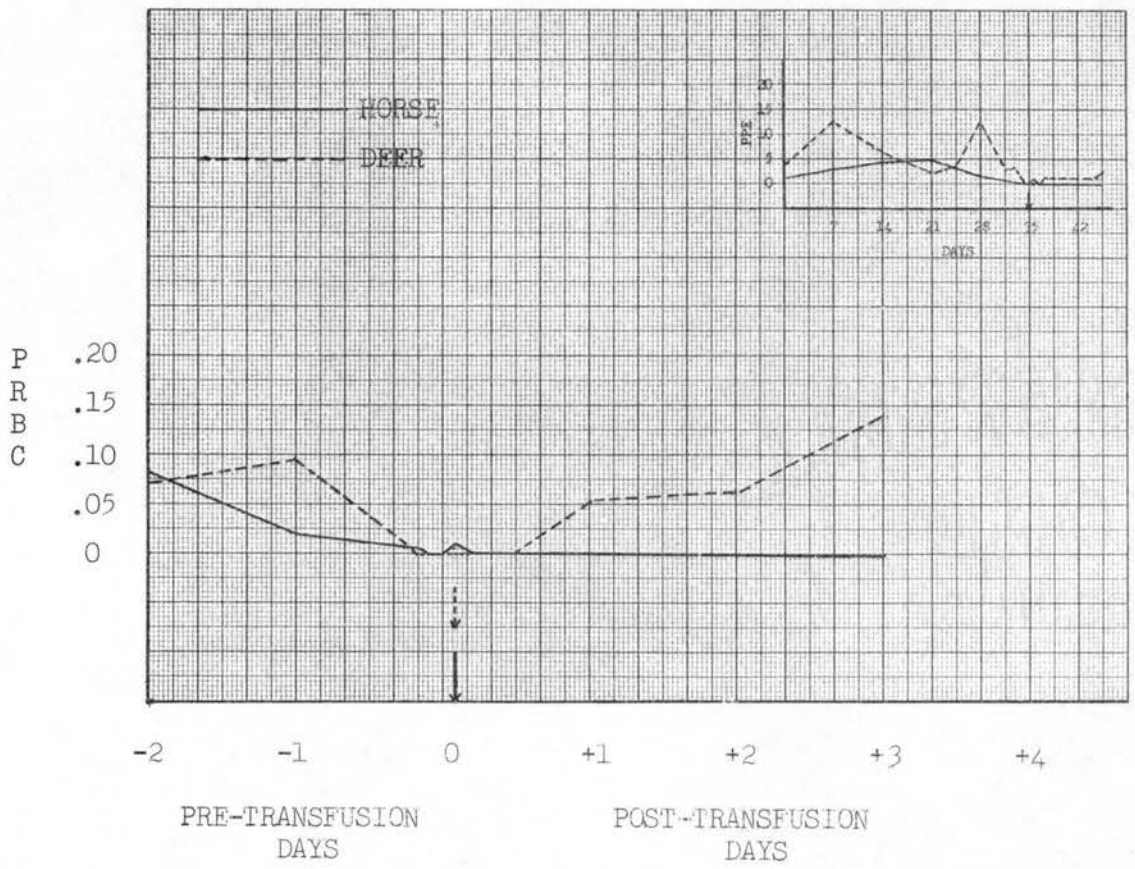

Figure 7. Experiment three - the effect of transfusion of heterologous, non-infected blood into splenectomized carrier calves.

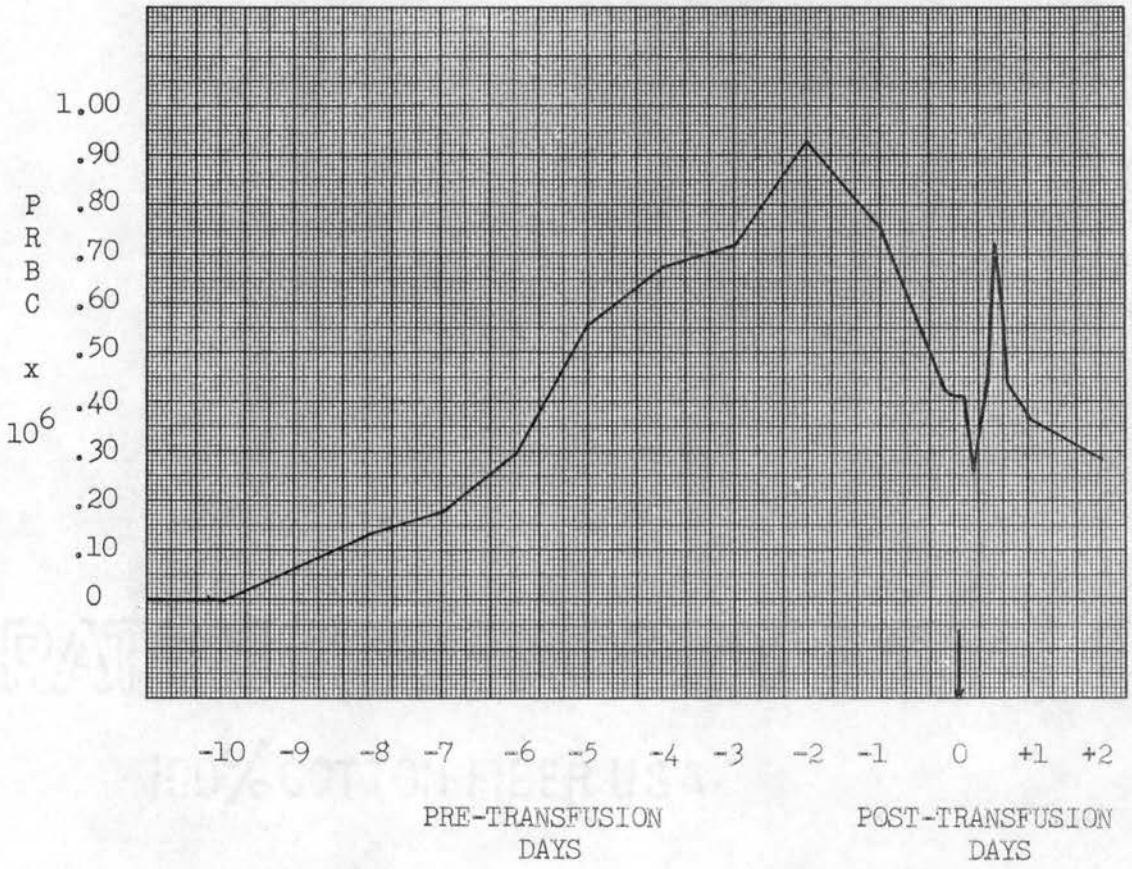

Figure 8. Experiment four - the effect of transfusion of homologous, non-infected blood into a cow during acute anaplasmosis.

CHAPTER IV

DISCUSSION

It has been postulated that cyclic recrudescences in the parasitemia, which are characteristic of anaplasmosis in the splenectomized calf, result from (a) defective clearance of the infective agent from the plasma and of infected red blood cells (RBC) following splenectomy (b) the insusceptibility of immature RBC to A. marginale infection.

Ristic (33) considered that invasion of circulating RBC by initial bodies from the plasma occurs in anaplasmosis. The same author (34) demonstrated A. marginale in deer RBC within 8 hrs. following transfusion into anaplasmosis-infected calves. The inoculation of a susceptible host with plasma from an infected animal will lead to infection. It has been shown that plasma may contain the anaplasma agent; the infective filterable form being less than 0.3 u in diameter (8). There is no evidence, however, to support the theory that the plasma stage of the infective agent is removed by the spleen and the remaining reticulcendothelial system (RES). The present study has not explored this aspect of anaplasmosis.

With reference to RBC clearance, there are indications that this mechanism is more efficient and more active in resistant animals as reflected by the ratio of RBC lost to RBC infected (52). Infected RBC clearance by the RES has been demonstrated (36).

Foote et al. (32) suggested that RBC are invaded during their formation in the bone marrow. Brock (15) failed to observe marginal bodies in immature RBC.

Cyclic recrudescences in asplenic calves could result from (a) cyclic activity of the organism (b) a transient immunity or (c) a suitable RBC population in a certain stage of maturity.

The typical pattern of recrudescence indicates that a development cycle of the organism would require 2 to 3 weeks. A short incubation period, less than one week, subsequent to massive challenge, and the direct invasion of deer RBC within a few hours, refute this possibility.

Transient immunity characterized by highly efficient clearance of A. marginale from the blood or by inhibition of the growth of the organism, for only a 2 to 3 week period, could be responsible for such a pattern of recrudescence. The CF antibody does not reflect such a possibility, although it is doubtful whether this antibody provides a high degree of protection. Brock et al. (42) were unable to detect any beneficial effect from the administration of bovine globulins.

Assuming that the majority of RBC are destroyed by the primary attack of the disease, a new population of RBC would be formed. The cells in this population would be of similar age. Brock (15) reported recrudescence following tetracycline treatment in the intact animal. The drug probably inhibited the growth of the organism which persisted for about 3 weeks thus preventing the invasion of all susceptible RBC. When the effect of the tetracycline had disappeared, anaplasma bodies invaded the remaining susceptible RBC and new popula-

tions of RBC which had reached a susceptible stage of maturity.

The findings by Foote et al. (76), that simultaneous infection by eperythrozoonosis interferes with the development of anaplasmosis, may also be explained on the basis of competition for susceptible RBC, although this could result from stimulation of the RES.

In most asplenic animals, a basal parasitemia, usually below 3 percent, exists between cyclic recrudescences. In the present study, relatively large volumes of blood were transfused at this point, namely the commencement of a convalescent period.

The results of the pilot study showed that the transfusion of infected and non-infected blood from intact animals produced an increase in the percentage parasitemia. (Figure 1). Since the increase occurred more rapidly than anticipated, the experiments were repeated and observations made more frequently. Increased parasitemia were likewise induced when infected and non-infected bloods were used. (Figure 2 and 3). It is surprising that transfusion of intact, carrier blood produced the same effect as non-infected blood, which suggests that circulating antibody does not appear to prevent invasion of the RBC. It is noteworthy that a return to pre-transfusion levels occurred within 24 hours, except in Experiment One, Group B2. A rapid clearance of transfused RBC has also been observed in normal animals (67). The delayed clearance in one animal could be due to a relatively poor clearance mechanism or to an impending recrudescence at the time of transfusion.

The rapid rise in the parasitemia agrees with Ristic's (34) findings with deer RBC. The short duration of the induced recrudescences refutes the possibility that they were caused by impending, naturally

occurring recrudescences.

The insignificant response following transfusion of blood containing immature RBC (Figure 4) supports the hypothesis that anaplasma organisms only invade mature RBC. The results of transfusion of blood from newborn calves (Figure 6) suggest that neonatal RBC contain some factor which resists invasion by A. marginale, such as fetal hemoglobin. The resistance of the newborn child to malaria has been attributed to a factor in hemoglobin (63). Jones (51) observed a prolonged incubation period following the inoculation of newborn calves with anaplasma organisms.

The failure to reproduce a response when non-infected blood was transfused into intact, carrier calves (Figure 7) was probably due to (a) an efficient splenic clearance mechanism, (b) insufficient submicroscopic anaplasma units in the plasma to invade the new population or (c) the removal of damaged RBC by the spleen before obvious marginal bodies could develop in the RBC.

The transfusion of heterologous blood, (horse and deer) into splenectomized carrier calves, was designed to eliminate the possibility of an induced recrudescence being due to blockading the RES. No response was produced with horse blood and only a slight response occurred with deer blood. (Figure 7). Gingrich (58) found that successful blockade of the RES in birds required large doses of the blockading agent which he repeated daily for 3 days. In the present study, it was not possible to ascertain whether deer RBC became parasitized, because they were indistinguishable from the host RBC by light microscopy examination.

The failure to produce a response with heterologous blood

provides supportive evidence that the increased parasitemia in Experiment One, Groups A and B was due to the invasion of mature RBC and not to RES blockade. The possibility that some humoral factor in the transfused blood stimulated this response cannot be ruled out.

The rapid response and the early return to pre-transfusion RBC levels, following transfusion of susceptible RBC during acute anaplasmosis in one cow, have practical significance. (Figure 8). Additional study is required on these lines. The present findings suggest that blood transfusions for therapeutic purposes should be repeated and combined with tetracyclines.

Summary and Conclusions

A significant increase in the number of parasitized erythrocytes was observed following transfusion of splenectomized anaplasmosis-carrier calves with infected and non-infected blood from mature animals. Blood containing a predominant population of immature red blood cells, newborn calf blood and equine blood failed to induce a parasitemic response. A slight response was observed when deer blood was transfused. An increased parasitemia was also induced in one cow during acute infection when transfused with normal non-infected blood. These results suggest that Anaplasma marginale invades the mature erythrocyte within the circulation. Erythrocytes from the newborn calf and immature erythrocytes appear resistant to this infection.

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APPENDIX

TABLE XVII

PRODUCTION OF IMMATURE ERYTHROCYTES

Subject: 6 year old Holstein-Friesian cow

Body Weight: 940 lbs.

Date	Volume of Blood Removed	PCV	Hb	% Reticulocytes
May 4	nil	39.0	13.5	nil
May 5	12 liters	39.0	13.5	nil
May 6	10 liters	34.0	11.5	nil
May 7	nil	27.0	10.0	nil
May 8	nil	22.0	9.0	nil
May 9	nil	19.0	8.5	2.5
May 10	nil	19.5	8.0	7.0
May 11	nil	23.0	8.0	9.0
May 12	nil	26.0	7.0	12.0
May 13	nil	27.0	7.0	14.0
May 14	nil	28.0	7.0	16.0

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