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WILLIAM ELIHU BROCK

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CHAPTER I

INTRODUCTION

The control of any disease, whether by prevention or treatment, is based on a thorough understanding of the etiology and pathogenesis of the disease in question. Neither the etiology nor the pathogenesis of anaplasmosis has been fully defined. Anaplasmosis is an infectious disease. The infectious agent, however, has not been isolated. Its development in the body has not been demonstrated, nor has its exact nature been sufficiently well determined to permit classification with certainty.

The characteristic sign of anaplasmosis is a severe anemia. While much is known about the pathology of the disease, no detailed studies have been made concerning the mechanism of this anemia. It is usually reported to be a hemolytic anemia and many of the clinical and laboratory findings support this classification. However, certain findings fail to fit those expected of a simple hemolytic anemia. These findings need to be studied in order to clarify the mechanism by which the anemia is produced. This study was undertaken to obtain further knowledge concerning the pathogenesis of the anemia found in anaplasmosis.

To provide a basis for understanding the problem studied in this experiment, the generally accepted information concerning anaplasmosis is briefly summarized. This information is reviewed in greater detail in Chapter II.

Anaplasmosis is an infectious disease of the domesticated species of the genera Bos, Capra, and Ovis, and certain wild species of Bison and Cevidae. It is characterized by a rapidly progressive anemia and is transmitted mechanically by the transfer of intact erythrocytes by Tabanus and Culicidae and biologically through genera of Ixodidae. A small round body, 0.5 u to 1.0 u in diameter, is consistently found in the erythrocytes of animals undergoing the typical acute anemia and is named Anaplasma marginale.

The signs and symptoms of anaplasmosis in adult cattle are those characteristic of a severe anemia. The onset of the disease is characterized by depression, weakness, anorexia, and fever; followed in 2 to 4 days by weight loss, dyspnea, constipation, paleness of mucous membranes, and tachycardia. Frequently slight to marked icterus is present late in the anemia. Hemoglobin in the urine or feces has not been detected. Death occurs 4 to 6 days after the appearance of clinical signs of the disease in 30 to 70% of the cases.

Hematological examinations in cases of anaplasmosis reveal erythrocytes infected with A. marginale in the peripheral circulation for 3 to 6 weeks following inoculation. The erythrocyte count in the peripheral blood falls from approximately 8.0 millions per c.mm. to approximately 1.5 millions per c.mm. within 8 to 10 days at the first appearance of infected erythrocytes in the peripheral circulation. A

decrease in hematocrit and hemoglobin values coincides with the decline in the erythrocyte count.

During convalescence there is an increase in hematopoiesis. This is indicated by the appearance of reticulocytes and macrocytes in the peripheral venous circulation, and by an increased mean corpuscular volume 5 to 8 days after patent infection. These indications of accelerated hematopoiesis are followed 2 to 4 days later by the appearance of basophilic stippled, polychromatophilic and poikilocytic erythrocytes, and by the appearance of normoblasts. Only slight irregular increases of total plasma bilirubin from 0.0 mg. to 2.9 mg. per 100 ml. of plasma have been detected during the most marked decline in the erythrocyte count.

Necropsy of cases that die of anaplasmosis show a marked decrease in blood viscosity. The spleen is enlarged, dark red, and friable. The liver is mottled, yellow, swollen and friable; while the gallbladder is enlarged and contains thick, viscous, brownish-green bile. The heart is pale and flabby. Other organs present no significant changes. Histopathological examination reveals a marked splenic congestion, fatty infiltration and centrolobular necrosis of the hepatic parenchyma with slight to marked biliary stasis.

As stated earlier in this chapter, many of the signs described above are those of a hemolytic anemia. These include a rapid progressive loss of erythrocytes, frequent icterus and, at necropsy, splenomegaly and enlarged liver. However, the magnitude and rapidity of the blood loss would lead one to expect hemoglobinuria, earlier and more marked bilirubinemia, earlier reticulocytosis, and consistent icterus. The

fact that these latter signs are mild, late, or absent, poses the problem whether this disease is a simple hemolytic anemia or is complicated by early bone marrow inhibition.

CHAPTER II

REVIEW OF ANAPLASMOSIS

History

For many years anaplasmosis was confused with babesiosis, or Texas fever. The two diseases have such similar signs, modes of transmission, and pathology that early separation of the two entities presented many difficulties. Both are contagious diseases of cattle caused by blood parasites and characterized by high fever, acute anemia, enlarged congested spleen, liver degeneration, distention of the gallbladder, and frequent icterus. Excluding identification of the respective organisms, or the use of the complement-fixation test, hemoglobinuria is the only sign distinguishing babesiosis from anaplasmosis.

Smith and Kilborne (1893), in their classical report demonstrating transmission of babesiosis by ticks, showed pictures of a blood parasite indistinguishable from Anaplasma marginale. They concluded that this body in the erythrocyte was a stage in the life cycle of Babesia bigemina. They remarked, however, on the fact that this stage was found more frequently in late Summer or Fall and followed a typical attack of "redwater."

Drawings, apparently of A. marginale, appeared in a paper by Kolle (1898), describing a disease of cattle in South Africa. Theiler

(1908) likewise noted "marginal points" in South African cattle. He did not realize their significance until some cattle previously immunized against babesiosis developed a severe anemia. Only the "marginal points" could be found in the erythrocytes of these cattle (Theiler, 1910). Theiler then concluded that these bodies were a pathogen, independent of B. bigemina, and not a stage in the life cycle of the latter organism. Subsequently Theiler (1911) obtained a pure infection of A. marginale and passed it through several cattle free from Babesia infection.

Geographical Distribution

Within a few years after Theiler had established that anaplasmosis was a separate entity, the disease was reported from many parts of the world. Giltner, reviewing anaplasmosis in 1928, attributed the early identification of the disease in various countries to the following workers: Balfour, 1911, in the Sudan; Lignieres, 1911, in Argentina; Carini, 1911, in Brazil; Carpano, 1912, in Eritrea and Italy; Koidzuma, 1912, in Formosa; deBlieck, 1912, in Java; Sergent and Beguet, 1913, in Algeria; Carrougeau, 1913, in Madagascar; Boynton, 1917, in the Philippines; Velu, 1920, in Morocco; Yakimoff and Belawine, 1927, in Russia. Descazeaux (1924) reported anaplasmosis in Chile. The disease was also recognized in sheep and goats by Schellhase (1913) in German East Africa.

Since these early reports, the disease has been found in most areas of the world. Leclainche (1930) found a focus of infection in France from an accidental source. Later a native source of the disease was reported by Cuille et al. (1935).

Other countries in Europe have reported the disease: Leitao

(1943) in Portugal, Garcia (1934) in Spain, Iriminoiu (1948) for A. marginale and Metiamu (1950) for A. centrale in Romania, Mlinac and Sterk (1937) in Serbia, and Mikacic (1952) in Yugoslavia.

After the discovery of anaplasmosis in Algeria in 1913, other foci of the disease were found in North Africa and Asia Minor. Stuart reported the disease in Palestine in 1924. Ducloux and Cordier (1930) worked on treatments for anaplasmosis in Tunisia. Carpano (1934) found the organism in buffalo in Egypt. Camels were reported to be infected with anaplasmosis in Libya by Monteverde (1937). The disease was observed in Iran by Delpy in 1946.

The disease was known in many parts of Asia, as shown by the reports of Crawford in 1935 from Ceylon, Jacotot and Evanno from Indochina in 1931, the reports from the Imperial Veterinary Research Institute in India, and Jauffret in 1934 from Cambodia.

Following Lignieres' discovery of the disease in 1911, anaplasmosis was reported from many places in South America. Morgan wrote of anaplasmosis in Venezuela in 1934. The disease as it occurred in Uruguay was discussed by Rubino (1938). Anaplasmosis was mentioned in an article on piroplasmosis by Velasquez (1938) from Columbia.

The earliest available reference to anaplasmosis in Australia was the discovery of the disease by Legg (1933) in Queensland in 1932. It was identified in New South Wales in 1949 (Edgar, 1953).

Van Volkenberg observed the disease in Puerto Rico in 1939. Hutson also found anaplasmosis in the West Indies on Antigua in 1940 and the disease was mentioned in the Annual Report of the Department of Agriculture in Jamaica in 1945.

In the United States the tick eradication program was in progress for babesiosis control even before Theiler's work on anaplasmosis. The belief was prevalent in this country during the years between 1910 and 1926 that, while anaplasmosis was known to be present (Meyer, 1913, as reported by Giltner, 1928), the disease could be controlled by the same measures used against babesiosis. That this was a mistaken hope was demonstrated when Darlington (1926) described the disease in an area of Kansas free from Boophilus annulatus, the Texas fever tick. It was then realized that vectors other than the tick might transmit the disease (section on transmission). This fact also forced the realization that anaplasmosis, as separate from Texas fever, was endemic throughout much of the southern United States.

It was not realized, however, the extent to which the disease could penetrate into the northern sections of the country. Reports from the more northern areas over the next 30 years demonstrated that anaplasmosis may be found in any part of the country and was just as prevalent in certain northern regions as it was in the South. The spread of the disease was greatly facilitated by modern methods of transportation. Determination of the incidence of the disease has been greatly aided by recent perfection of the complement-fixation test for identification of carrier cattle (section on serology).

Reports of the gradual spread of anaplasmosis northward were found between 1926 and 1951. Brill reported the disease in Iowa in 1929. In 1933 it was reported in Colorado by Stiles and in Pennsylvania by Marshall. Stiles also observed the death of 25 cattle from a herd of 2000 due to anaplasmosis in Wyoming in 1935. The disease was diagnosed in

Oregon in 1936 (Ore. Agri. Dept. Bul. No. 58). Of 82 cattle in a herd in Idaho, Stiles (1937) reported that 6 cattle died of anaplasmosis. Goss recognized the disease in Ohio in 1941. One case of anaplasmosis was diagnosed by Moore et al. in Michigan in 1950. An outbreak of the disease was observed by Griffiths and Hadlow (1951) in 400 cattle in Minnesota.

After the complement-fixation test became available in 1951, areas where the disease was prevalent could be more easily identified and outlined. While surveys of the incidence of anaplasmosis are only in their early stages, sufficient information is available to indicate the major boundaries of infection in the United States. Saulmon (1957) has summarized the latest reports on the incidence of anaplasmosis. He showed that there are 2 principal endemic areas: (1) Predominantly the southeastern United States and (2) the Northwest and California.

The southeastern area extends from Maryland along the South Atlantic states and around the Gulf of Mexico through Texas. The area extends north to Nebraska, then southeast through southern Illinois, Kentucky and back to the southeastern seaboard. The area of greatest incidence of the disease is along the low areas of the coast and the large rivers extending inland.

The northwestern endemic region covers most of Wyoming, Montana, Idaho, Oregon and California, extending south into northern Colorado, Utah and Nevada and extending northward into southeastern Washington. The Oregon and Washington coastal areas have almost no reported cases, while the California coast shows a high incidence of the disease. The 2 other areas of high incidence in this region are found in eastern Oregon and in a diagonal area running northeastward through Wyoming.

Saulmon (1957) stated that anaplasmosis has been diagnosed in 38 of the 48 states. He mentioned, however, that many of the cases from the north-central region have been traced to cattle imported from endemic areas. The New England states have not reported any cases of anaplasmosis.

Nature of the Etiologic Agent

There is general agreement among workers concerning the morphology of the body seen in the erythrocytes during the acute phase of anaplasmosis. It is round and stains reddish purple to almost black with Romanowsky-type stains. In unpublished work by the writer, it has been seen in unstained smears by phase contrast.

The average size of the body is approximately 0.5 u in diameter. Dikmans (1933f) gave a minimum size of 0.1 u while Carpano (1930) thought the disease should not be diagnosed without seeing bodies 0.6 u in size, distinctly rounded and on the margin of the erythrocyte. Sergent et al. (1945) gave the range of size as 0.3 u to 0.8 u. The writer has measured organisms up to 1.0 u in diameter.

The work in this laboratory has shown a maximum of 5 bodies in a single erythrocyte. Sergent et al. (1945) stated that there may be 4 to 5 bodies on the same margin of an erythrocyte. Henning (1956) reported that the bodies usually occur singly but 2, 3, or 4 of the parasites may be in the same erythrocyte.

Some workers have reported seeing forms differing from the usual coccoid shape. They were interpreted to be a stage in the division or multiplication of the organism. Organisms in the form of rods, commas, and triads with light centers associated with the typical anaplasma were

reported by de Faria (1928). Dikmans (1933f) showed similar forms in a calf sick with anaplasmosis. Quevado (1914) and Descazeaux (1924) also reported forms differing from the typical anaplasma body that may be similar to those of de Faria and Dikmans. Franklin and Redmond (1957) described bodies in the erythrocytes of splenectomized calves in the acute phase of anaplasmosis which have projections or tails 0.6 u to 0.9 u in length, extending beyond the red blood cell.

Studies of the organism by electron microscopy of infected erythrocytes are in general agreement although they differ in detail, due possibly to different techniques. Organisms containing either a central undifferentiated mass with elementary bodies on the periphery, or organisms composed entirely of elementary bodies, are described (de Robertis and Epstein, 1951). These elementary bodies are reported to be 170 mu. to 400 mu. in diameter and may be found dispersed throughout the cell. Espana (1957) described the organism as a central undivided mass surrounded by elementary bodies. Foote et al. (1958), using sectioned erythrocytes, showed that the anaplasma body contained a number of distinct round forms. Elementary bodies were also observed dispersed in the cytoplasm of normoblasts, both in the bone marrow and in the circulating blood.

In the years since Theiler's interpretation of the body as an intracellular parasite, many workers have disagreed with this hypothesis. The exact nature of the body is still in doubt. Henning (1956) mentioned that Schilling-Torgan (1912) and Dias and Aragao (1914) were opposed to the "protozoan theory." They thought the bodies were degeneration products of erythrocytes. Such products could be produced by injection

of trypan blue. However, du Toit (1928) showed that these bodies were produced by trypan blue only in the case of animals already infected with anaplasmosis, and not in uninfected cattle. Schilling and Meyer (1930) were of the opinion that the bodies appeared as a symptom of the disease but were not the agent. Meyer, in 1927, thought that the body might be similar to the Jolly body. Evidence was presented by Penha (1930) that anaplasma bodies were negative to the Feulgen reaction, while Jolly bodies were positive to the reaction.

Support for those workers who associated the anaplasma body with the etiologic agent was found in the work of Ristic et al. (1957). They were able to identify fluorescent anaplasma bodies after infected blood was incubated with fluorescein labeled antibodies. Likewise the high specificity of the antigen in the complement-fixation test, as prepared by Price et al. (1952), was evidence for associating the body with the cause of the disease.

Many attempts, published and unpublished, have been made to pass the etiologic agent through bacteriologic filters. Theiler (1911) and du Toit (1928) were unsuccessful in passing the agent through filters. Dikmans (1933c) was unable to produce the disease with filtrates either from serum or from washed laked erythrocytes. The unfiltered laked red blood cells did not produce anaplasmosis. While working with ovine anaplasmosis, de Kock and Quinlan (1926a) were able to show that the disease could be transmitted by serum, whole blood, washed erythrocytes, and hemolysed washed erythrocytes; but not by filtrates of any of those preparations. Transmission with filtrates of erythrocytes crushed by sterile sand were unsuccessful (de Asua et al., 1927). Of the many attempts by

the writer and his associates to filter the causative organism, none have produced the disease.

Another as yet unproductive inquiry into the nature of the organism has been the culture of the etiologic agent *in vitro*. Veglia, in 1915, discovered an increase in the number of anaplasma bodies when infected blood was incubated in several media. Lestoquard (1926) was able to show an increase in the bodies 72 hours after blood from recovered ovine cases was incubated. A media using cerebral tissue, horse blood, and vitamins was used by de Faria (1928). The anaplasma infected blood was then added to this media. A few days later bodies similar to anaplasma, but outside the cells, were observed. Cultures kept for 30 days or longer were non-infectious. He did not think that multiplication of the organism occurred. Dikmans (1933f) observed changes in the morphology of the bodies during the incubation of infected blood which contained dextrose. He attributed these to division of *A. marginale*. Rossi and Triozon (1953) found an increase in the number of bodies during the first 2 days of culture. A decrease occurred during the next 7 days. They did not find the changes described by Dikmans, nor did they feel that there was actual culture of the organism. The writer and associates have found numerous bodies similar to those of anaplasma, both in cultures of mixed carrier blood and in uninfected blood in tissue media after 14 days incubation in roller tubes. Multiplication of the organism was not considered to have occurred.

The etiologic agent of anaplasmosis is quite resistant to cold, but relatively sensitive to heat. Rees (1937) observed that the organism was not destroyed by -78° C. for 18 hours. Survival in heat varied from

48° C. to 60° C. for 20 minutes. Some samples were destroyed at 48° C., while a few samples survived 60° C. One sample removed from the donor animal during the incubation period of the disease was destroyed at 45° C. in 15 minutes. The incubation period was lengthened in the animals used for subsequent transmission when the sample had been treated but not killed. Australian workers (1950) have preserved the viability of A. centrale for 739 days at -80° C.

In a study of the histochemical reactions of the anaplasma body, Moulton and Christensen (1955) have identified desoxyribonucleic acid, ribonucleic acid, protein, and organic iron in the body. They were unable to find evidence that arginine, tyrosine, polysaccharide complexes, inorganic iron, calcium, lipids, fat peroxidases, dehydrogenase, or alkaline phosphatase were present in the body. They concluded that the anaplasma bodies were animate particles containing nuclear chromatin and were not degeneration products, Howell-Jolly bodies, or staining artifacts.

Although work has continued on the nature of the anaplasma body since the time Dikmans reported his conclusions in 1933, they still generally represent the position of the work today. His conclusions were: (1) the anaplasma body is not a stage in the life cycle of Babesia; (2) it is not a degeneration product or Jolly body; (3) it is not a reaction of the erythrocyte to invasion by a filterable virus; (4) it may be a reaction to an unfilterable virus; (5) it may be a protozoan parasite. These conclusions may be modified in the light of recent information gained by electron microscopy, histochemical, and fluorescein studies. Such modifications should include the following: (1) the

anaplasma body is not a reaction to any disease process; (2) it is probably a phase in the life of the etiologic agent; (3) the etiological agent may be viral or protozoal in nature.

Transmission

The transmission of anaplasmosis may be either biological or mechanical. Biological transmission by ticks was described by Smith and Kilborne in 1893. Ticks become infected by feeding on anaplasmosis infected cattle and later, in the same or subsequent stages, feed on susceptible cattle, thereby transmitting the disease. Mechanical transmission is accomplished by the transfer of infected erythrocytes from infected to susceptible animals. The infected cells may be transferred to a susceptible animal on surgical instruments or on the mouth parts of biting insects.

In the years since 1893, thirty species of ticks have been shown to transmit anaplasmosis. All stages in the development of the tick were infective. Adult ticks (Table 1) that had engorged on infected cattle transmitted the infectious agent via the eggs to their larvae, nymphs, and subsequent adult stages. Each stage was capable of producing the disease when feeding on a susceptible animal. The extensive literature, written since 1893 on the transmission of anaplasmosis, has been well reviewed by Dikmans (1950). Neitz (1956) has more recently reviewed the information concerning tick transmission of disease, including anaplasmosis. The references to work of various authors included in Table 1 have been entered in the present bibliography, but have not necessarily been reviewed by this writer.

TABLE 1*

RESULTS OBTAINED BY VARIOUS INVESTIGATORS
WITH TICKS TESTED EXPERIMENTALLY
AS VECTORS OF ANAPLASMOSIS
(POSITIVE TRANSMISSIONS)

Name of Tick	Stages of Ticks in Experiments: A	B	L	N	A	Authors
Boophilus decoloratus	X———				Y	Theiler, 1912
B. microplus	X———				Y	Quevedo, 1916
B. microplus	X———				Y	Rosenbusch et al., 1927
B. annulatus	X———				Y	Rees, 1934
Hyalomma lusitanicum	X———			Y—Y—		Sergent et al., 1945
Rhipicephalus simus	X———				Y	Theiler, 1912
R. bursa			X—X—		Y	Brumpt, 1931
R. sanguineus			X—		Y	Rees, 1930
R. sanguineus			X—		Y	Rees, 1934
R. sanguineus				X—	Y	Rees, 1934
Dermacentor andersoni			X—		Y	Rees, 1933
D. andersoni			X—		Y	Rees, 1934
D. andersoni				X—	Y	Rees, 1933
D. andersoni				X—	Y	Boynton et al., 1936
D. andersoni	X———				Y	Howell et al., 1941
D. variabilis			X—		Y	Rees, 1932
D. variabilis				X—	Y	Rees, 1932
D. variabilis				X—	Y	Rees, 1934
D. variabilis				X—	Y	Sanders, 1933
D. occidentalis	X———				Y	Boynton et al., 1936
D. albipictus				X—	Y	Boynton et al., 1936
Ixodes ricinus	X———				Y	Zeller et al., 1923
I. ricinus	X———				Y	Helm, 1924
I. scapularis			X—		Y	Rees, 1934

A-Adult, E-Egg, L-Larva, N-Nymph; X-Stage of tick engorging on infected animal, Y-Stage of tick engorging on susceptible animal.

*Adapted from Dikmans, 1950.

Dikmans (1950) in his review discussed the possible role of the various ticks found in the United States as natural vectors. On the basis of ecology, life cycle, and transmission experiments, he felt that Dermacentor occidentalis and D. albipictus were potential natural vectors of anaplasmosis. He felt that it was less probable that D. andersoni, D. veriabilis, Ixodes scapularis, and Rhipicephalus sanguineus were important as natural vectors. He stated, however, that the information available was inadequate to fully determine the importance of various ticks as vectors under natural conditions.

Many instruments contaminated with blood from an anaplasmosis infected animal have been incriminated in transmission of anaplasmosis. The following instruments have been reported: a prod for driving cattle (Descazeaux, 1924); dehorning instruments (Hilts, 1928; Sanborn, 1931; Stiles, 1936; Goss, 1941); needle (Sergent et al., 1924); scalpel (Rees, 1930); and pitchfork (Stiles, 1936). From these reports it is obvious that any object used around cattle that can carry blood from one animal to another may transmit anaplasmosis.

Stiles (1936) made the observation that the explosive outbreaks of the disease in which many cattle in a herd become ill with anaplasmosis at almost the same time can usually be traced to unclean surgery such as dehorning. It is interesting to note that the serious outbreak of anaplasmosis reported by Griffiths and Hadlow (1951) in Minnesota followed bleeding for brucellosis tests. When the same herd was bled again 5 months later, anaplasmosis was observed a second time.

In addition to mechanical transfer on instruments, the mouth parts of biting insects were shown to transmit the disease. The

following biting insects have been shown to transmit anaplasmosis mechanically under experimental conditions: Tabanus fumipennis (Sanders, 1933); T. americanus, T. oklahomensis, T. abactor, T. equalis, T. erythracus, T. fuscicostatus, T. venuatus, Chrysops sequox (Howell et al., 1941); T. sulcifrons (Howell et al., 1941; Lotze, 1944); Stomoxys calcitrans (Sanders, 1933); Psorophora columbiae, P. ciliata, Aedes aegypti (Howell et al., 1941). Many other unsuccessful attempts were made to transmit anaplasmosis both with other biting insects and with those mentioned above.

Transmission of anaplasmosis by biting insects apparently depends upon a number of factors. Howell (1957) has stated that a rough correlation exists between the size of horse flies and their ability to transmit the disease. He stated that no case of transmission was recorded in which more than 5 minutes elapsed between feeding on the infected animal and feeding on the susceptible animal. Dikmans (1950) has shown a correlation between the ease of mechanical transmission and the number of infected erythrocytes in the peripheral circulation. He mentioned that within one group of carrier animals it was only possible to effect insect transmission with one certain animal. Dikmans (1950) reported that the other two cases of recorded experimental insect transmission in carrier cattle happened during a period of recrudescence of the parasites in the blood (Lotze, 1944). Transmission from clinical cases, however, is accomplished quite readily.

In view of the fact that transmission from infected nonpatent animals by insects appears to happen infrequently, it has been difficult to explain the naturally occurring outbreaks in the absence of ticks or

human incited transmission. Sergent et al. (1946) and Lotze (1941 and 1944) demonstrated that there was, in certain carrier animals, an increase of infected erythrocytes some time following recovery from the initial infection. They saw this recrudescence of infected erythrocytes principally in the Spring (from February to May). At this time 0.4% to 2.0% of the red blood cells were infected. This parasitic relapse (Dikmans, 1950), provided sufficient infected erythrocytes to permit natural transmission by biting insect vectors. Once a newly infected animal became clinically sick, a highly infected source of blood was available for relatively easy dissemination.

Serological Diagnosis

It is generally accepted that cattle which have recovered from an initial attack of anaplasmosis remain carriers of the disease for life. These carrier cattle show no clinical evidence of the infection. Only by frequent hematological examinations over months is diagnosis possible (Sergent, 1946).

The presence of undiagnosed carrier cattle in herds has prevented the control of anaplasmosis. Many unsuccessful and therefore unpublished attempts to develop a test to identify the carrier cattle have been made. The first test to show promise in the diagnosis of carriers was reported by Boynton in 1935. The test depended upon the precipitation of a protein from carrier serum when placed in distilled water. It did not gain general acceptance because it lacked specificity and because it remained positive for only a short time following the initial attack of anaplasmosis.

The Report of the United States Bureau of Animal Industry for 1931-32 mentioned that an antigen prepared from water-lysed, infected blood showed good antigenicity in the complement-fixation test. Due to difficulties in producing quantities large enough to be useful in a control program, work on this antigen ceased.

Later another antigen prepared at the Bureau of Animal Industry from triturated and water extracted viscera of anaplasmosis infected ticks (Rhipicephalus sanguineus) was reported by Rees and Mohler (1934). This antigen showed good antigenicity and low anticomplementary action. Mohler and Gates (1953), however, reported that the antigen could not be produced in quantity.

Mott and Gates (1949) described the production of an antigen prepared by precipitating a substance from water-lysed infected red blood cells with carbon dioxide. The precipitate was washed and lyophilized for storage at -70° C. This antigen was used in the complement-fixation test by Mohler et al. (1949). They obtained a 90% accuracy in diagnosing known anaplasmosis carrier cattle. Additional batches of antigen prepared in this manner were reported by Mohler and Gates (1953) to be variable in antigenicity. This caused considerable difficulty in standardization of the complement-fixation test.

In 1952 Price et al. produced an antigen based on the water lysis of infected erythrocytes. The lysed red cells were purified by repeated fractional centrifugation. This antigen showed high antigenicity, low anticomplementary action and had little variability between batches. Large quantities of the antigen could be prepared by using a high speed continuous flow centrifuge (Price et al., 1954). In a cooperative project

between Maryland, Oklahoma, and Louisiana, 293 cattle, known to be free from or definitely infected with anaplasmosis, were tested with this antigen. Ninety-six percent accuracy was reported (Price et al., 1954).

The production of the last two mentioned antigens permitted the survey of large areas for the incidence of anaplasmosis. Preliminary surveys are now being made in the following states: Wyoming (Thomas, 1958), Florida (Sanders, 1957), Montana (Tunnicliff, 1957), Virginia and Texas (Roby, 1957), Oklahoma (Pearson, 1955), and Hawaii (Willers, 1957).

A few pilot experiments for the control of anaplasmosis are using the results of the complement-fixation test as a basis for segregation or removal of carrier cattle from herds. A large scale trial is being conducted in Hawaii to attempt eradication of the disease from island cattle (Willers, 1957). Small trials for control of the disease in single herds of cattle are being conducted in Montana (Tunnicliff, 1957), Virginia and Texas (Roby, 1957), and Oklahoma (Pearson, 1957).

Immunity

Cattle which have recovered from the initial attack of anaplasmosis do not clinically relapse or show clinical signs of the disease upon reinfection. This type of immunity is dependent upon the organism remaining alive in the animal and is called premunition. In unpublished work by the writer, it has been shown that cattle whose carrier state has been destroyed by antibiotics will be as severely affected upon reinfection as during the initial attack.

On rare occasions animals have lost the carrier state and thus their premunition to the disease. Mohler et al. (1949) reported a case

of anaplasmosis in which a carrier animal lost its titre to the complement-fixation test. Blood from this animal was then inoculated into a susceptible animal without producing the disease. Later the former carrier was splenectomized without showing the presence of anaplasmosis infection. Henning (1956) mentioned a personal communication from Neitz (1955) in which he found "autosterilization" of animals which were susceptible to reinfection.

A less virulent strain of Anaplasma (A. centrale) was found by Theiler in 1912 which would generally produce a mild reaction in susceptible cattle, but at the same time render them immune to the more serious reaction of A. marginale. This organism has been used since its discovery to premunize cattle against anaplasmosis.

Sergent et al. (1945) showed that there was a reaction to reinfection in premunized cattle which they called the "acces de premuni." They stated that this attack was always non-febrile, was not accompanied by morphological blood changes or death, regardless of the virulence or quantity of the virus used for reinoculation. There was, however, a mild reappearance of anaplasma bodies 40 to 47 days following reinfection, as shown in 2 cases that were presented.

Other methods have been used to attempt immunization of cattle against anaplasmosis. Pearson et al. (1953) reported that tissue vaccines prepared from various viscera of infected cattle and infected blood treated with chemicals and antibiotics showed no protection against anaplasmosis. Sergent et al. (1945) used blood taken during the first week of the incubation period as a premunizing agent during the years from 1926 to 1934. This method was discontinued because of the violent and

unequal reaction to the vaccine. Attenuation of A. marginale by serial passage through several sheep was claimed by Lignieres (1928). This work was not confirmed by Donatien and Lestoquard (1930), or Neitz and du Toit (1932).

Henning (1956) stated the present position of immunization in anaplasmosis by the following statement: "It would appear, therefore, that the only safe and reliable method of immunization against anaplasmosis is .....by means of blood containing a pure strain of A. centrale." Infection with A. centrale, or any less virulent strain of Anaplasma, has not been reported in the United States. Since the policy of animal disease control officials has been opposed to the introduction of A. centrale for immunization against A. marginale, there is no recognized method of vaccination employed in the United States.

It has been recognized that the spleen played an important role in the resistance of cattle to clinical signs of anaplasmosis since the work of de Kock and Quinlan (1926b). They discovered that splenectomy of carrier cattle resulted in clinical relapse. Rees (1931, 1933b) concluded that the virulence of A. marginale was increased in splenectomized cattle, although the attack resulting from the splenectomy was mild. He observed the period between splenectomy and relapse to be 3 to 28 days. The technique of splenectomy is widely used in anaplasmosis research to test for the presence of the carrier state.

The relation of the spleen to immunity was studied by Ristic et al. (1958). When cortisone was given to anaplasmosis infected calves prior to splenectomy, or prior to and following splenectomy, anemia did not develop and the titre to the complement-fixation test remained high

after the spleen was removed. The infected and splenectomized control calves, and the calves which received cortisone after splenectomy, developed severe anemia and a marked decline in the complement-fixation titre.

Pathology

The pathology of anaplasmosis was thoroughly described by Smith and Kilborne in 1893. Although they did not recognize anaplasmosis as a disease separate from babesiosis, their description of the "mild type" of Texas fever included most of the pathology of anaplasmosis known today.

The occurrence of anaplasmosis principally in the late Summer and Fall was noted by Smith and Kilborne (1893). Seasonal occurrence of anaplasmosis coinciding with the seasonal activity of natural vectors was mentioned by many later writers on the subject, including Sergent *et al.* (1945), Giltner (1928), and Farley (1948).

Descriptions of the signs and symptoms of acute anaplasmosis were in general agreement. In the report of the first recognized case of anaplasmosis in the United States, Darlington (1926) stated that there was temperature of 104° F. to 105.6° F., decreased milk production, weakness, gauntness, loss of appetite, constipation, more rarely diarrhea, labored breathing, tachycardia, anemia, icterus and jugular pulse. In addition to the signs and symptoms described by Darlington (1926), Giltner (1928) reported that infected animals showed a stiff gait, a tendency to lie down, cessation of rumination, tachypnea (60 per minute), tachycardia (120 per minute), and lachrymation. Henning (1956) mentioned a temperature rise as the first clinical symptom. The febrile

disturbance, however, followed the first appearance of infected erythrocytes in the blood by 3 to 6 days.

The lack of, or infrequent occurrence of hemoglobinuria in anaplasmosis was noted by many workers. Smith and Kilborne (1893) thought that hemoglobinuria was probably never present in the "mild type" of Texas fever. Dikmans (1933c) did not observe hemoglobinuria in his experimental cases of anaplasmosis. Henning (1956) stated that hemoglobinuria was not present in spite of the extensive destruction of red cells. According to Henning (1956), du Toit (1934) explained the presence of hemoglobinuria in babesiosis and its absence in anaplasmosis by the fact that the destruction of erythrocytes in babesiosis is due to erythrocytorrhexis, while in anaplasmosis it is the result of erythrocytolysis. Hemoglobinuria was observed in a milk cow during the course of an acute attack of anaplasmosis by Sergent et al. (1945). These writers stated, however, that the urine usually remained normal in anaplasmosis.

Smith and Kilborne (1893) mentioned that jaundice is rare. Icterus was observed to vary in different cases (Dikmans, 1933c). Crispell (1930) noted icterus in the cases he reported.

Changes in the blood during anaplasmosis has received much attention. Smith and Kilborne (1893) noted that the blood was thin and watery. In one of their cases of the "mild type," the low erythrocyte count was 1.18 million red cells. They observed that "the peripheral coccus-like bodies" were not seen in the true Texas fever, but these bodies infected 5 to 50% of the red cells for 1 to 5 weeks in the "mild type." Giltner (1928) demonstrated a minimal red blood cell count of 3.26 millions with 30% of the red cells infected with A. marginale. He

stated that anisocytosis, polychromasia, basophilic stippling and normoblasts were observed in the blood.

Sergent et al. (1945) reported red blood cell counts as low as 0.7 million and 60% infected erythrocytes. These workers, after studying the leukocyte picture, concluded that the acute attack was usually accompanied by leukocytosis; the increase in white blood cell count was 1,000 to 10,000 cells. Differential white cell counts showed slight neutrophilia in the incubation period and slight lymphocytosis in the acute attack of anaplasmosis.

Hemoglobin was demonstrated by Netto and Ribeiro (1955) to decrease from 14.2 gm. to 9.4 gm. per 100 ml. of blood during the development of acute anemia. In the same period the hematocrit percentage declined from 39.6% to 27.9%. These writers were unable to find any change in the plasma protein. More marked changes in hemoglobin and hematocrit were reported by Miller (1953) and Brock (1955). Depression of hemoglobin below 3.5 gm. per 100 ml. of blood and hematocrit values of 7% were observed by Brock (1955). Machado (1950) reported minimal values of hemoglobin and hematocrit of 3.4 gm. per 100 ml. of blood and 16% respectively on the forty-fifth day following infection. Lotze (1947) noted that the hematocrit decreased most rapidly before the peak of infection, but increased after the appearance of macrocytes.

Few workers had examined blood in anaplasmosis for the presence of reticulocytes. Machado (1950) mentioned that Jimenez de Sua and colleagues noted the presence of reticulocytes during anaplasmosis in 1928. Reticulocytes were observed by Machado (1950) on the forty-first day after inoculation of cattle with Anaplasma infected blood. The percentage of

reticulocytes increased to attain a maximum of 7% on the forty-ninth day. Reticulocytes were reported by Brock (1955) to appear in the peripheral circulation from the fifth to tenth patent days and to reach a maximum of 14% in 1 case on the fifth day thereafter. He observed the appearance of reticulocytes to be associated with an increase in the mean corpuscular volume.

Consistent changes in the blood, spleen, liver, and gallbladder were observed at necropsy on animals dead from anaplasmosis. Smith and Kilborne (1893) noted the following changes: Heart--epicardial petechia, paleness; spleen--enlarged diffluent pulp of "blackberry jam" consistency, disappearance of Malpighian corpuscles and trabeculae; liver--enlarged with rounded edges, pale, finely mottled and grayish-yellow; gallbladder--enlarged, containing 0.5 pt. to 1.0 qt. of a semisolid mass similar to "chewed grass;" blood--thin and watery. Microscopic examination of tissues by these workers revealed bile retention in the canaliculi, fatty change and centrolobular necrosis in the liver; congestion and clumps of yellowish pigment in the spleen. The other organs were normal.

Reports by other writers differed only in details. Darlington (1926) reported that the gallbladder is 3 to 5 times larger than normal and filled with viscid stringy, yellow to dark green or black bile. This writer also mentioned moderate gastrointestinal catarrh and swollen edematous lymph glands. Giltner (1928) observed marked petechiation and ecchymosis of the pericardium, epicardium and pleura. The myocardium contained grayish areas that appeared to be muscle degeneration when examined by microscope.

A liver showing an "anemic spot" in the center and otherwise

extremely yellow was reported by Derflinger (1936). All tissues were described as a deep lemon yellow. Crispell (1930) mentioned fluid in the pericardial sac. Ristic and Sipple (1958) observed thick and inflamed gallbladders, purple bone marrow, petechia on the pericardium, pleura and diaphragm at necropsy. Microscopic examination revealed cellular red pulp and exhaustion of the germinal centers in the spleen and lymph nodes, and exhaustion of the adrenals.

The loci of A. marginale in the body tissues were studied by Rees and Underwood (1939). They removed samples of bone marrow, liver, spleen, and lungs during the incubation, clinical, and carrier stages of anaplasmosis. No anaplasma bodies were observed during the incubation period. During the clinical period the concentration of bodies was similar in all tissues sampled. Anaplasma bodies were seen in erythrocytes in the lungs during the carrier state.

CHAPTER III

EXPERIMENTAL PROCEDURE

The methods used for obtaining the data presented in this study are recognized procedures. In certain instances, where it was necessary to modify existing procedures, the procedure or its modification is described.

In order to establish normal values, 5 pre-exposure determinations were made for each test on each animal used in this experiment. During the prepatent period each test was performed twice weekly for the first 2 weeks, 3 times during the third week, and daily during the patent period.

Experimental Animals

Ten 2-year-old Hereford steers in good condition and of approximately the same weight were used for this study. They were placed in a dry lot. They were fed 20 lbs. of good quality bluestem hay and 4 lbs. of cottonseed meal per animal per day. Free access to clean water was provided. At the times material was obtained from the cattle for laboratory examination, they were moved approximately 500 ft. to a working chute where they could be restrained for the necessary procedures.

The complement-fixation test for anaplasmosis (Price *et al.*, 1954) was performed on serum from each animal immediately prior to

placing them on the experiment. While the test has been shown to be only 96% accurate in identifying carrier cattle, the chance of failing to identify a carrier in this group was felt to be small. All experimental cattle became infected with anaplasmosis when subsequently exposed.

Exposure to Anaplasmosis

After adjustment to the conditions of husbandry and the determination of the normal values for the tests used, each animal was inoculated subcutaneously with 10 ml. of fresh carrier blood drawn at one time from an infected donor. This procedure of exposure provided, as nearly as possible, the same inoculation dose for each animal. Variations in the results were held to a minimum and were primarily the result of individual host-parasite relationships.

Hematology

Blood samples were drawn at approximately 8:30 a.m. from the right jugular vein into 10 ml. heparinized tubes. Blood films were made and stained, and the other determinations were performed during the morning of the same day the blood samples were obtained. Bone marrow aspirations from the dorsal vertebral spines were taken at the same time and slides were smeared immediately, to be stained later in the morning.

Erythrocyte counts were made by the standard clinical procedure. The cells were diluted in Hayem's solution and counted in a bright line hemocytometer.

Hematocrit values were determined by the microcapillary tube method. Capillary tubes were filled to approximately two-thirds capacity

and centrifuged for 4 minutes on an International microhematocrit centrifuge. The percentage of packed erythrocytes was then read on a microcapillary reader (Jones, 1956).

Hemoglobin determinations were made colorimetrically by placing 20 c.mm. of whole blood into 5.0 ml. of 1% hydrochloric acid solution. After 1 hour the percentage transmission of the acid hematin solution was read in the colorimeter at a wave length of 525 mmu.

The percentage of reticulocytes among 1000 erythrocytes was determined by placing 2 drops of the heparinized blood with 1 drop of a 1% saline solution of brilliant cresyl blue for 5 minutes. A film was then made of the stained red blood cells and counterstained with Wright's stain. A quantity of distilled water, equal to the amount of stain used, and buffered to pH 6.8 with phosphate buffer was used to dilute the Wright's stain. The slides were washed in approximately neutral distilled water.

The percentage of anaplasma body-containing erythrocytes among 500 red blood cells was estimated from the stained blood smears. A Whipple disk placed in the ocular of the microscope greatly facilitated the counting of both reticulocytes and infected erythrocytes.

Bone marrow aspiration from the sternum of adult cattle was found to be extremely difficult due to the inaccessibility of the region. The dorsal spines of the vertebrae were chosen because of their accessibility and freedom from contamination; and because the red marrow appears to be found there in large amounts throughout life, as determined by examination of dressed beef carcasses. Custer and Ahlfeldt (1932) have shown that the greatest amount of red marrow in the human being is present

throughout life in the vertebrae, and that the response to hemopoietic stimuli in those bones is relatively greater. It was hoped that a similar response would be present in the vertebrae of cattle.

Bone marrow was obtained by aspiration from the twelfth and thirteenth thoracic spines and from all of the lumbar vertebral spines. The vertebrae were aspirated in succession from anterior to posterior on successive biopsy dates, repeating that order when the last lumbar vertebra had been biopsied.

The technique of bone marrow aspiration (Jones, 1957) used in this study is described in detail because this is apparently the first application of the method to obtain bone marrow from vertebrae in cattle. The surgical area was clipped and scrubbed, followed by the application of a quaterinary ammonia antiseptic solution. The area over the dorsal spine of the vertebra was infiltrated with a 2% procaine solution. After sufficient time for the local anesthetic to take effect, a 16 guage Rosenthal bone marrow biopsy needle was inserted by slight rotating movement through the skin and cartilage into the shaft of the spine. Care must be taken to enter and remain in the relatively narrow marrow cavity. A solid feeling of being in bone is imparted by the needle when it is properly in place. After proper placement of the needle, a 10 ml. heparinized glass syringe was attached and 0.5 ml. to 1.0 ml. of marrow was aspirated. Slight withdrawal or rotation of the needle facilitated collection when a sample was not immediately obtained. Unless the needle is in the cavity, marrow can not be drawn into the syringe.

A small amount of the aspirated marrow was ejected from the syringe onto a clean glass slide. By using a white cell diluting pipette,

a few of the small grayish white beads of bone marrow scattered through the blood on the slide were picked up and transferred to a clean glass cover slip. A second cover slip was placed over the first and the blood and bone marrow allowed to spread by capillary action between the two slips which were then drawn apart and the film dried as rapidly as possible. The remainder of the specimen in the syringe was placed in a tube and saved to make additional smears if necessary. The smears of bone marrow were stained immediately with Wright's stain, as described for peripheral blood smears.

The percentage of erythroblastic cells among the nucleated cells of the bone marrow was estimated from the slides prepared by this method. One hundred nucleated cells were counted in each of 5 randomly selected areas on each bone marrow slide and the percentage of erythroblastic cells on that slide determined.

Estimations of the size and hemoglobin content of the erythrocytes were made by using the erythrocyte count and the hemoglobin and hematocrit values in the following formulae (Wintrobe, 1946):

$$\text{Mean corpuscular volume in c.u. (M.C.V.)} = \frac{\text{hematocrit} \times 10}{\text{RBC millions/c.mm.}}$$

$$\text{Mean corpuscular hemoglobin in uug. (M.C.H.)} = \frac{\text{hemoglobin, gm./1000 ml.}}{\text{RBC millions/c.mm.}}$$

$$\text{Mean corpuscular hemoglobin concentration in \% (M.C.H.C.)} = \frac{\text{hemoglobin gm./100 ml.} \times 100}{\text{hematocrit}}$$

Fecal Urobilinogen

Fecal samples were collected from each animal at approximately 8:00 a.m. on the days of collection. The urobilinogen content of each sample was determined by the method of Watson et al. (1946) on the same day the samples were obtained. Because of the low urobilinogen content found in normal bovine feces, it was necessary to use a 20 gm. aliquot of the feces in order to develop sufficient color to read in the Bausch and Lomb Spectronic 20 colorimeter. Adjustment for the increased amount of feces was made later when calculating the amount of urobilinogen.

Liver Biopsy

Liver biopsies were made on each animal 4 days following exposure to anaplasmosis; twice during the development of the anemia, at the peak of the anemia, and twice during the convalescent period. A 4 in. 16 ga. Vim Silverman biopsy needle was used. The skin over the eleventh and twelfth ribs on the right side, approximately 5 in. below the dorsal midline, was shaved and scrubbed. A small $\frac{1}{4}$ in. incision through the skin between the eleventh and twelfth ribs was made to admit the needle. The needle was passed between the ribs and then directed postero-medially through the diaphragm into the liver. With approximately 1 in. of the canula shaft remaining outside of the body, the trocar was removed and the split needle inserted through the canula. The canula was then pushed in to the hub and the split needle removed. The liver biopsy was transferred to 10% formalin immediately. A hematoxylin-eosin stained preparation was made from the liver tissue.

CHAPTER IV

RESULTS

Organization of the Results

In order to minimize individual variation in the measurements used to estimate the results of the infection, the data on the 10 cattle were combined and the means of the measurements used for analysis. Preliminary examination of the results, however, showed that 5 of the cattle had undergone a severe attack of anaplasmosis while the other 5 cattle had been less severely affected. Although each animal was approximately the same age and weight and received the same exposure dose of carrier blood, the individual natural resistance of each steer produced varying response to the infection.

Due to this varied response to the infection, means derived from a combination of data from the 10 cattle showed extremely large standard deviations. Such a combination of data also tended to mask the peaks of the various measurements. Since dividing the data into 2 groups would tend to correct these conditions, it was decided to place the data from the more severely affected cattle (numbered 643, 644, 646, 648, 651) into Group I, and those from the less severely affected cattle (numbered 645, 647, 653, 654, 655) into Group II. This does not affect the validity of the data since the 2 groups are not compared with each other.

Sergent et al. (1945) had shown that data from various animals infected with anaplasmosis can best be combined by adjusting them to the day the disease becomes patent, rather than the day the animals were exposed to the disease. Unpublished results by the writer and co-workers verify this observation. In view of this fact, the data in this experiment were adjusted to the day the infected erythrocytes reached 1% of the total red blood cells. In the following tables and figures this day was marked "0." The days of the prepatent period preceding the "0" day were given a negative designation, while the days of the patent period were marked with plus signs. The prepatent period began on the day of exposure to anaplasmosis.

The preinoculation mean for each test in the tables and figures was derived from the results of the 5 preinoculation tests performed on the cattle, since there was assumed to be no difference in the 2 groups of cattle prior to infection.

Table 2 was composed of the means of each test taken throughout the prepatent and patent periods of infection of Group I. Table 3 presents the same information for the less severely affected Group II.

The preinoculation mean for each test was used as a base line for the figure representing that test, except in cases where there was no preinfection value; i.e. in percentage of infected erythrocytes and reticulocytes. The mean for each day of the prepatent and patent periods was shown as bars representing the deviations from the base line. The "y" axis of each figure was divided into positive units of deviation above the mean base line and negative units of deviation below the base line. For the actual value of the mean for a given day, refer to the appropriate table.

Results Showing Infection with Anaplasmosis

Since the diagnosis of anaplasmosis depends upon demonstration of the typical bodies in the erythrocytes, blood films were examined daily for their presence. The incubation (prepatent) period of the disease was considered to terminate when 1% of the erythrocytes contained anaplasma bodies. There was a definite risk of mistaking other erythrocytic inclusions and artifacts for anaplasma when the percentage of infected erythrocytes was less than 1%.

The animals in Group I had incubation periods of 20 days, except animal number 644 which had an incubation period of 18 days. The incubation periods of the animals in Group II were as follows: numbers 645 and 647, twenty days; numbers 651 and 655, twenty-two days; and number 654, twenty-three days.

Since all of the data of this experiment were adjusted to the appearance of 1% infected erythrocytes, the rise in the percentage of erythrocytes containing anaplasma bodies began on day "0." The peak infections are shown in Figures 1 and 2 to be the fifth and sixth days of the patent period. As shown in Table 2, the greatest percentage of infected erythrocytes in Group I was 38.8% on the sixth patent day. Group II reached a peak infection of 13.7% on the fifth patent day (Table 3).

The percentage of infected erythrocytes declined following the peak infection at a rate approximately equal to the rise in infection, as shown in Figures 1 and 2.

TABLE 2

THE MEAN DAILY VALUES OF THE TESTS PERFORMED ON GROUP I; THE CATTLE MOST SERIOUSLY AFFECTED BY ANAPLASMOSIS

Days	RBC $x10^6$/c.mm.	Inf. RBC %	Ht %	Hb gm./100 ml.	MCV cu.
Normal $\bar{x}$	8.35		35.5	11.7	42.8
-16	8.75		39.1	12.4	44.8
-14	7.62		40.0	11.6	52.5
-12	8.80		35.5	11.5	40.4
-10	8.84		36.5	10.5	41.3
- 9	8.68		37.4	12.0	43.2
- 7	8.75		33.5	10.2	37.7
- 5	8.51		36.8	11.3	43.3
- 3	7.44		31.0	9.9	41.7
- 2	7.94		35.6	11.6	44.9
0	7.55	1.3	32.4	11.2	42.8
+ 1	5.96	3.3	26.8	8.6	45.2
+ 2	6.47	6.0	29.2	9.6	45.3
+ 3	5.32	18.0	24.9	7.8	46.8
+ 4	4.72	20.2	21.5	6.4	46.1
+ 5	3.55	38.4	16.4	5.1	46.4
+ 6	2.75	38.8	13.2	3.8	47.7
+ 7	1.97	28.9	10.5	2.8	53.8
+ 8	1.77	25.9	9.6	2.4	55.1
+ 9	1.37	12.0	10.3	2.0	77.2
+10	1.47	4.5	10.4	2.4	72.7
+11	1.29	2.3	12.5	2.4	99.3
+12	1.33	2.3	14.1	3.0	107.5
+13	1.84	2.3	17.3	3.5	96.4
+14	1.99	1.0	19.1	4.1	98.4
+15	1.72		17.0	3.7	99.5
+16	2.40		21.5	5.1	91.8
+19	3.14		23.4	6.1	74.8
+26	4.21		28.9	9.1	69.3

TABLE 2--Continued

MCH uug.	MCHC %	Urobilinogen mg./100 g.	Reticulocytes %	Erythroblasts %
13.9	32.9	0.66		43.3
14.1	31.6	0.96		47.1
15.2	28.7	0.99		52.8
13.1	32.4	0.75		28.0
11.9	28.7	0.77		21.2
14.0	32.2	0.73		18.3
11.7	30.9	0.77		18.0
13.4	32.4	0.96		28.5
13.3	31.9	1.53		30.0
14.6	32.6	1.16		35.5
15.0	35.0	2.01		29.4
14.6	32.2	4.62		20.7
15.0	33.0	4.49		26.3
14.3	31.3	7.34		43.0
13.9	29.9	12.09	0.1	36.2
13.1	31.0	14.68	1.0	25.4
15.5	28.8	48.58	3.3	28.0
13.8	25.9	78.79	7.5	48.8
13.3	24.4	96.09	6.6	49.0
14.7	19.3	67.43	18.7	51.0
16.6	22.9	47.57	28.5	58.4
19.1	19.2	38.91	34.0	70.1
22.4	20.9	7.26	33.3	71.6
19.5	20.2	14.63	32.0	64.9
21.0	21.5	7.42	16.0	68.8
21.4	21.4	14.10	17.8	72.4
21.3	23.5	3.68	11.1	74.3
19.5	26.1	3.76	0.5	71.9
21.7	31.2	2.63		

TABLE 3

THE MEAN DAILY VALUES OF THE TESTS PERFORMED ON GROUP II; THE CATTLE LESS SERIOUSLY AFFECTED BY ANAPLASMOSIS

Days	RBC $x10^6$/c.mm.	Inf. RBC %	Ht %	Hb gm./100 ml.	MCV cu.
Normal $\bar{x}$	8.35		35.5	11.7	42.8
-19	9.22		37.0	12.6	40.1
-18	8.61		34.8	12.2	40.3
-16	8.93		39.5	13.6	44.6
-15	9.15		35.0	12.3	38.3
-14	7.96		33.8	11.8	42.5
-12	9.15		37.0	12.3	40.1
-11	7.68		32.8	10.3	42.9
- 9	9.04		37.5	11.5	41.5
- 8	9.34		38.0	12.0	40.7
- 7	7.76		33.3	10.4	43.1
- 5	9.11		38.0	11.8	41.7
- 4	7.90		34.5	10.4	43.6
- 3	8.80		35.0	11.6	39.8
- 2	8.00		31.0	10.1	42.0
- 1	8.22		33.5	11.2	40.8
0	8.07	1.0	33.6	10.6	41.6
+ 1	7.12	1.9	29.6	9.9	41.6
+ 2	6.64	4.1	27.6	9.0	42.0
+ 3	6.37	6.5	25.7	8.4	40.3
+ 4	5.68	8.1	24.3	7.2	43.4
+ 5	5.20	13.7	21.3	6.6	41.7
+ 6	4.83	12.3	20.3	6.1	42.6
+ 7	4.21	12.8	17.4	5.2	41.7
+ 8	3.52	13.4	16.5	4.7	47.5
+ 9	3.53	8.3	17.2	4.8	49.4
+10	3.33	5.4	18.0	5.0	54.0
+11	3.35	5.0	19.0	5.3	58.3
+12	3.08	2.3	19.4	5.4	62.3
+13	3.90	4.0	22.2	6.3	59.0
+14	3.69	1.0	22.1	6.4	61.3
+16	4.60		25.5	7.0	58.5
+17	4.40		26.0	7.4	59.2
+19	4.49		26.3	7.9	58.6
+23	4.84		26.5	9.2	54.8
+24	4.90		27.0	9.1	55.7
+26	4.73		30.0	9.8	63.5

TABLE 3--Continued

MCH uug	MCHC %	Urobilinogen mg./100 g.	Reticulocytes %	Erythroblasts %
13.9	32.9	0.66		43.3
13.7	34.0	0.73		35.8
14.2	34.9	0.95		42.1
15.3	34.2	1.03		43.6
13.4	35.1	0.76		17.8
14.9	35.1	0.74		25.5
13.4	33.1	0.78		34.9
14.2	33.1	0.75		15.9
12.8	30.9	0.72		16.4
12.8	31.5	1.75		
14.1	32.9	1.10		24.9
19.9	31.0	0.72		19.2
13.8	31.7	1.01		23.0
13.2	33.1	1.65		34.8
12.6	30.2	1.64		22.1
13.6	33.4	2.64		15.0
13.2	31.8	2.85		33.2
13.9	33.4	3.47		23.2
13.8	32.7	5.61		22.8
13.3	32.9	7.82		15.7
13.5	31.1	8.18		30.6
12.8	30.8	35.10		31.1
12.9	30.2	16.80		25.3
12.4	29.6	22.57	2.0	42.6
12.8	28.1	33.62	4.8	51.4
13.6	27.4	17.25	8.5	42.7
14.9	27.5	39.22	15.5	63.5
15.8	27.2	22.01	9.0	58.4
17.4	27.7	9.92	10.7	69.1
16.3	27.8	3.78	7.6	69.5
17.5	28.6	2.80	7.5	63.1
15.8	27.4	3.68	5.2	75.9
16.7	28.1	3.37	4.0	79.4
17.6	30.1	3.22	0.7	73.5
19.0	34.7	11.28		
18.8	33.6	3.12		
12.0	33.0	1.18		

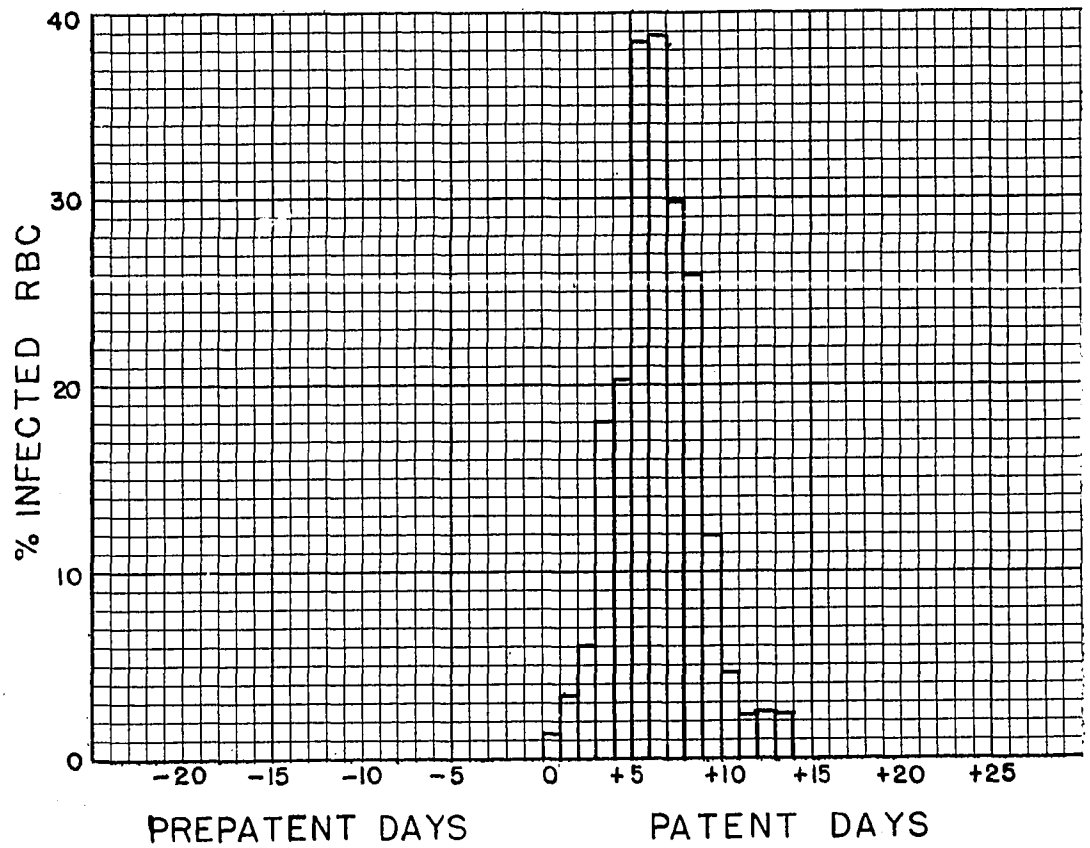

Fig. 1.--The percentages of erythrocytes infected with A. marginale during the prepatent and patent periods of anaplasmosis in Group I.

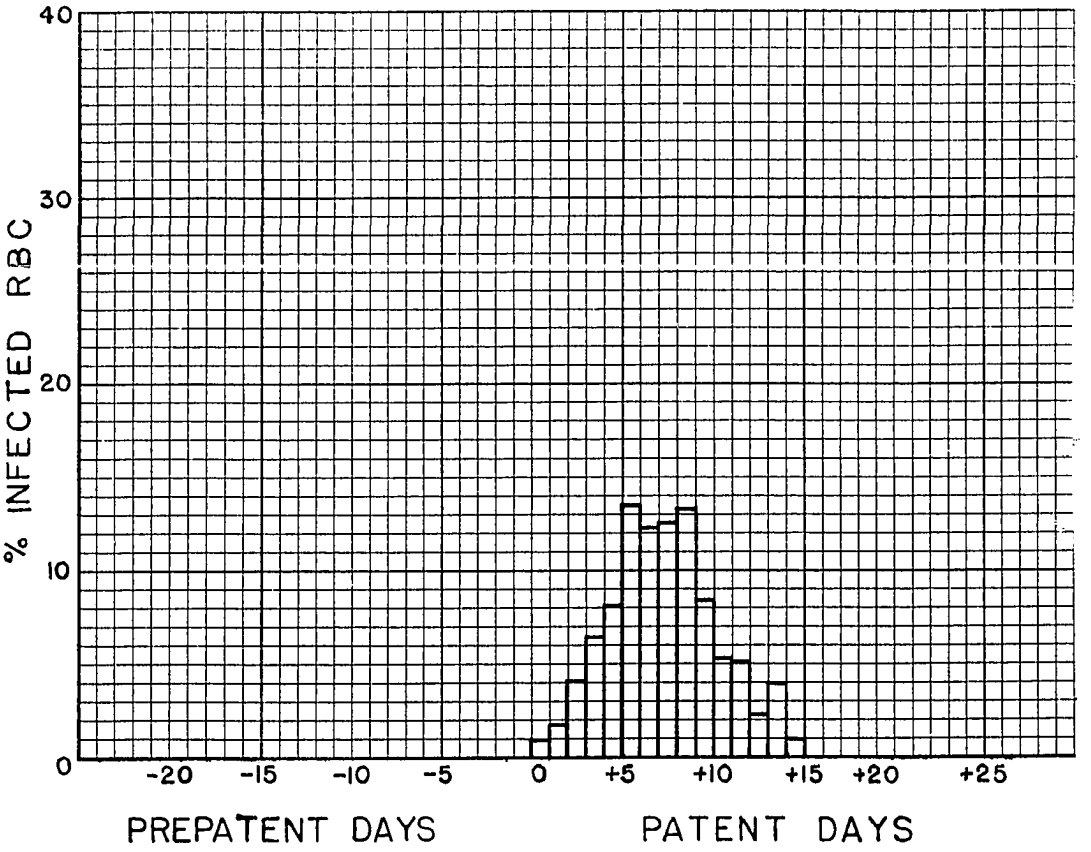

Fig. 2.--The percentages of erythrocytes infected with A. marginale during the prepatent and patent periods of anaplasmosis in Group II.

Results Showing the Degree of Anemia

Three tests were performed that indicate the severity of the anemia produced: (1) The number of erythrocytes per cubic millimeter of blood; (2) the volume of packed erythrocytes expressed as percentage of blood (hematocrit); (3) the hemoglobin expressed as grams per 100 ml. of blood.

The Erythrocyte Counts

During the prepatent period of the disease the number of erythrocytes per cubic millimeter remained within normal limits. There was variation on either side of the mean during this period (Figures 3 and 4).

As indicated in Figures 3 and 4, the erythrocyte count declined with the appearance of infected erythrocytes to a minimal number on the eleventh patent day in Group I and on the twelfth patent day in Group II. The minimal number of erythrocytes was 1,290,000/c.mm. of blood in Group I and 3,080,000/c.mm. of blood in Group II (Tables 2 and 3).

After the greatest erythrocytic depression, there was steady increase in the red blood cell count until the experiment was terminated. While the numbers did not reach prepatent levels, they had increased approximately 3 million in Group I and 1.5 million in Group II by the twenty-sixth patent day.

The Hematocrit Values

The hematocrit values (volume of packed erythrocytes expressed as percentage of blood) varied on either side of the mean during the

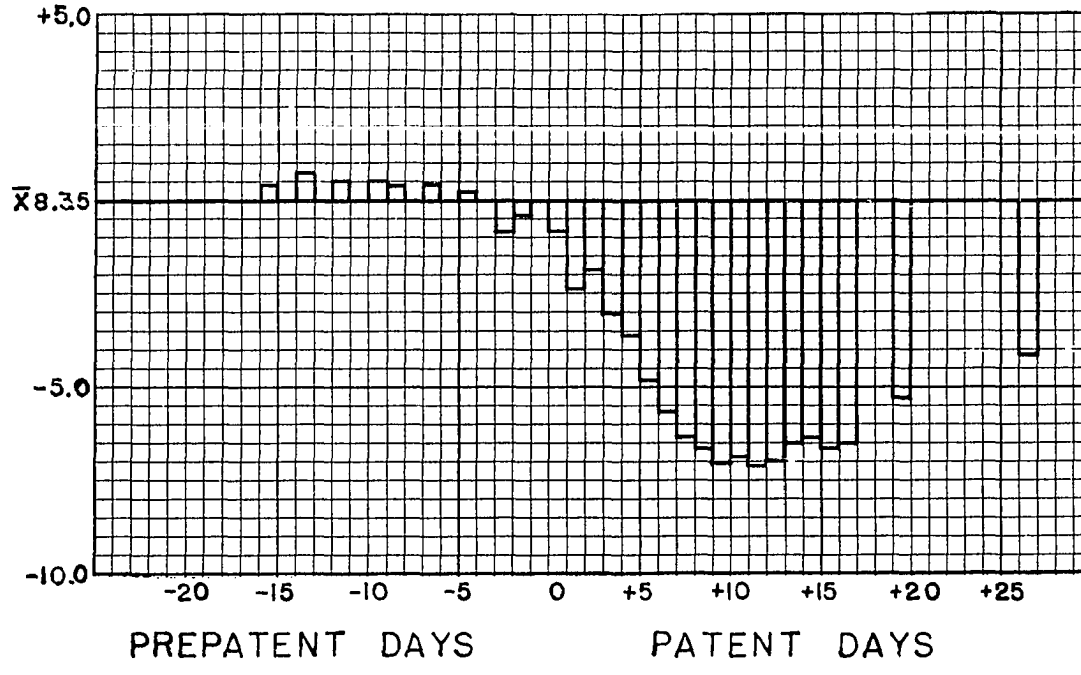

Fig. 3.--Deviations of the number of erythrocytes per cubic millimeter of blood from the preinoculation mean during the prepatent and patent periods of anaplasmosis in Group I.

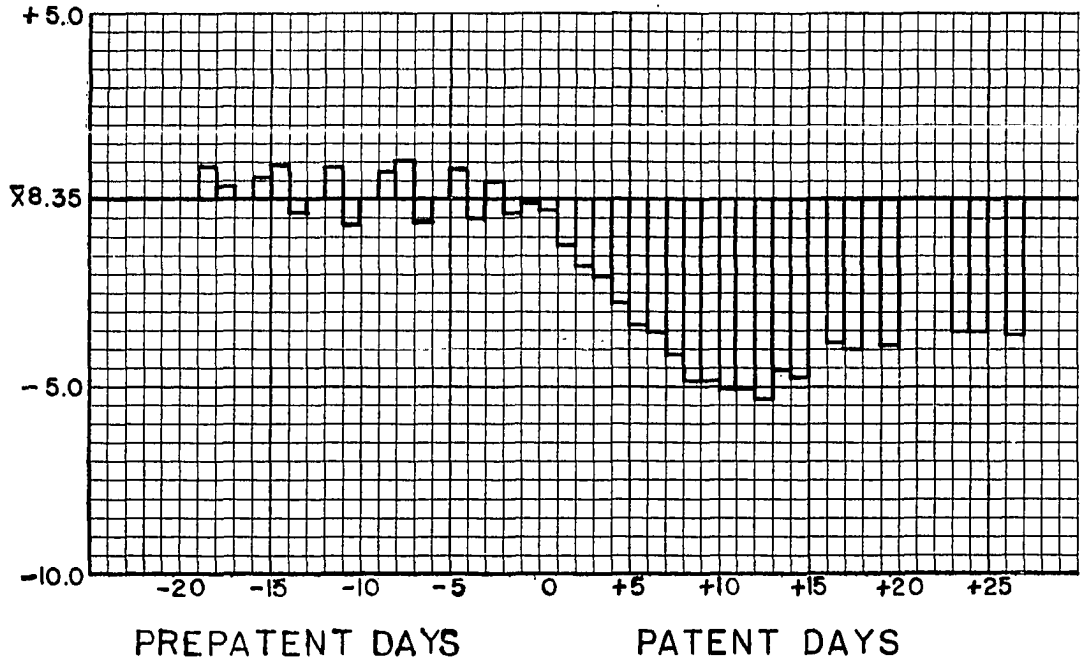

Fig. 4.--Deviations of the number of erythrocytes per cubic millimeter of blood from the preinoculation mean during the prepatent and patent periods of anaplasmosis in Group II.

prepatent period but were not significantly different from the preinoculation mean (Figures 5 and 6). A decline in the hematocrit values started on the "+1" day. This decline followed that of the erythrocyte counts to reach a minimal value of 9.6% in Group I and 16.5% in Group II (Tables 2 and 3). The minimal values were reached on the eighth patent day in both groups.

A steady increase in hematocrit values was seen following the eighth patent day, attaining 28.9% in Group I and 30.0% in Group II by the termination of the experiment.

Hemoglobin Determinations

The grams of hemoglobin per 100 ml. of blood followed the erythrocyte and hemoglobin values. There was no significant change during the prepatent period. The patent period showed a constant drop in the grams of hemoglobin to 2.0 gm. in Group I on the ninth patent day, and to 4.7 gm. in Group II on the eighth patent day of the infection. These values for hemoglobin paralleled the increase in erythrocyte counts and hematocrit determinations during the latter part of the patent period to attain values of 9.1 gm. and 9.8 gm. for Groups I and II respectively, at the end of the experiment (Tables 2 and 3, and Figures 7 and 8).

The results of the tests presented in this section indicate the development of a rapidly progressive, acute anemia starting with the appearance of anaplasma infected erythrocytes and becoming more marked for the following 8 to 10 days. The tests show that in the most severely affected group the values approach the minimum compatible with life.

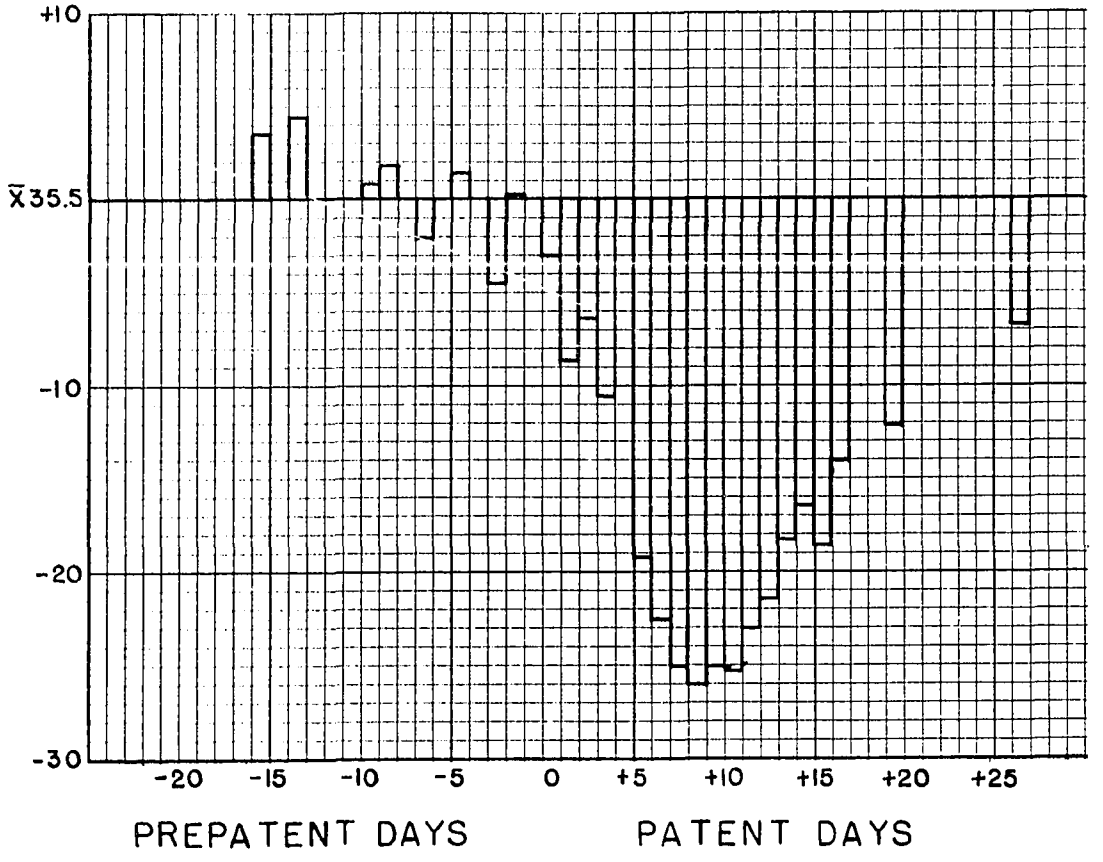

Fig. 5.--Deviations of the hematocrit values (expressed as percentage of blood) from the preinoculation mean during the prepatent and patent periods of anaplasmosis in Group I.

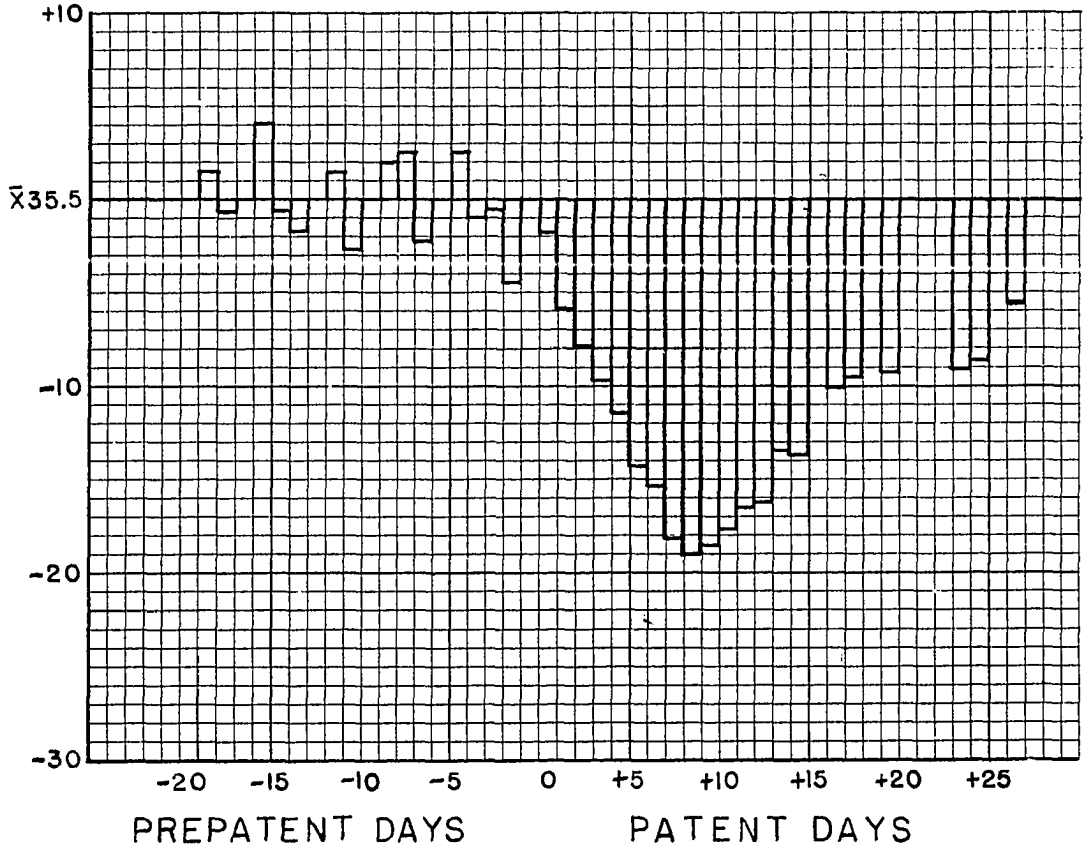

Fig. 6.--Deviations of the hematocrit values (expressed as percentage of blood) from the preinoculation mean during the prepatent and patent periods of anaplasmosis in Group II.

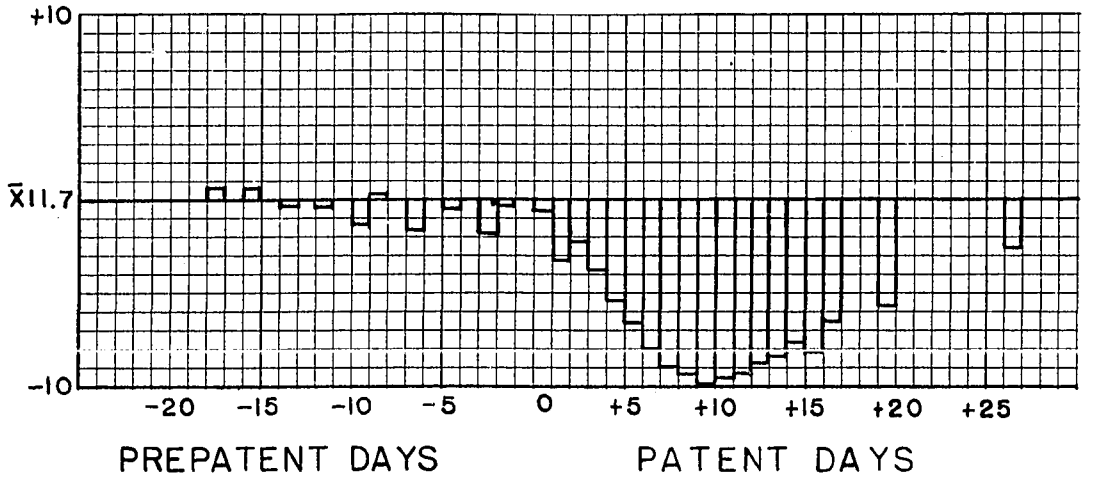

Fig. 7.--Deviations of the grams of hemoglobin per 100 ml. of blood from the preinoculation mean during the prepatent and patent periods of anaplasmosis in Group I.

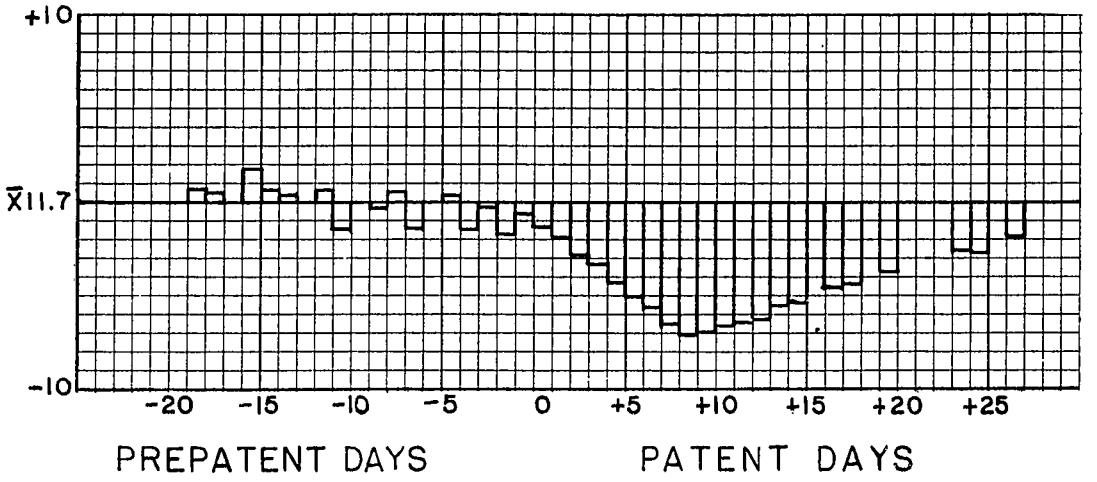

Fig. 8.--Deviations of the grams of hemoglobin per 100 ml. of blood from the preinoculation mean during the prepatent and patent periods of anaplasmosis in Group II.

Results Indicating the Types of Anemia

One of the most useful classifications of anemia is that based on the size and hemoglobin content of the erythrocyte. In this classification, anemias are designated as macrocytic, normocytic, microcytic, and hypochromic microcytic (Wintrobe, 1946). This classification depends on the mean corpuscular volume and the mean corpuscular hemoglobin concentration. In this experiment the mean corpuscular hemoglobin, while not as important, was also used.

Mean Corpuscular Volume

The mean corpuscular volume varied little from the preinoculation mean during both the prepatent and early patent periods (Figures 9 and 10). It is only on the seventh patent day in Group I and on the eighth patent day in Group II that the mean size of the erythrocytes showed a definite increase. The mean size of the erythrocytes then increased rapidly through the twelfth patent day to reach a maximal mean size of 107.5 cu. in Group I and 62.3 cu. in Group II (Tables 2 and 3). The size then decreased slightly from the peak values but remained high in comparison with the mean preinoculation level throughout the remainder of the period studied.

Mean Corpuscular Hemoglobin Concentration

No significant changes in the mean corpuscular hemoglobin concentration values were seen throughout the prepatent period of the infection or early patent period (Figures 11 and 12). On the fourth day of the patent period a decrease in the mean corpuscular hemoglobin concentration

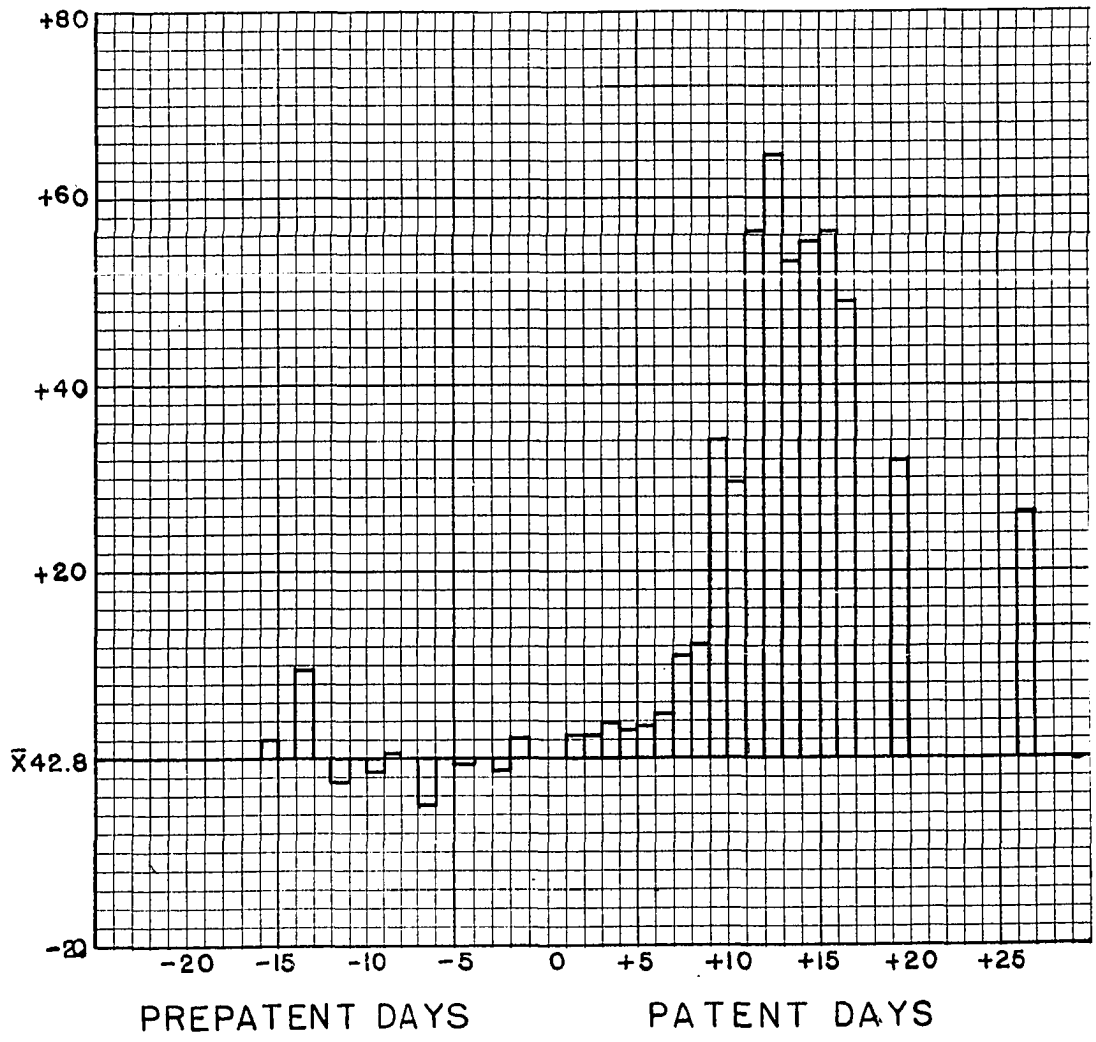

Fig. 9.--Deviations of the mean corpuscular volumes from the preinoculation mean during the prepatent and patent periods of anaplasmosis in Group I.

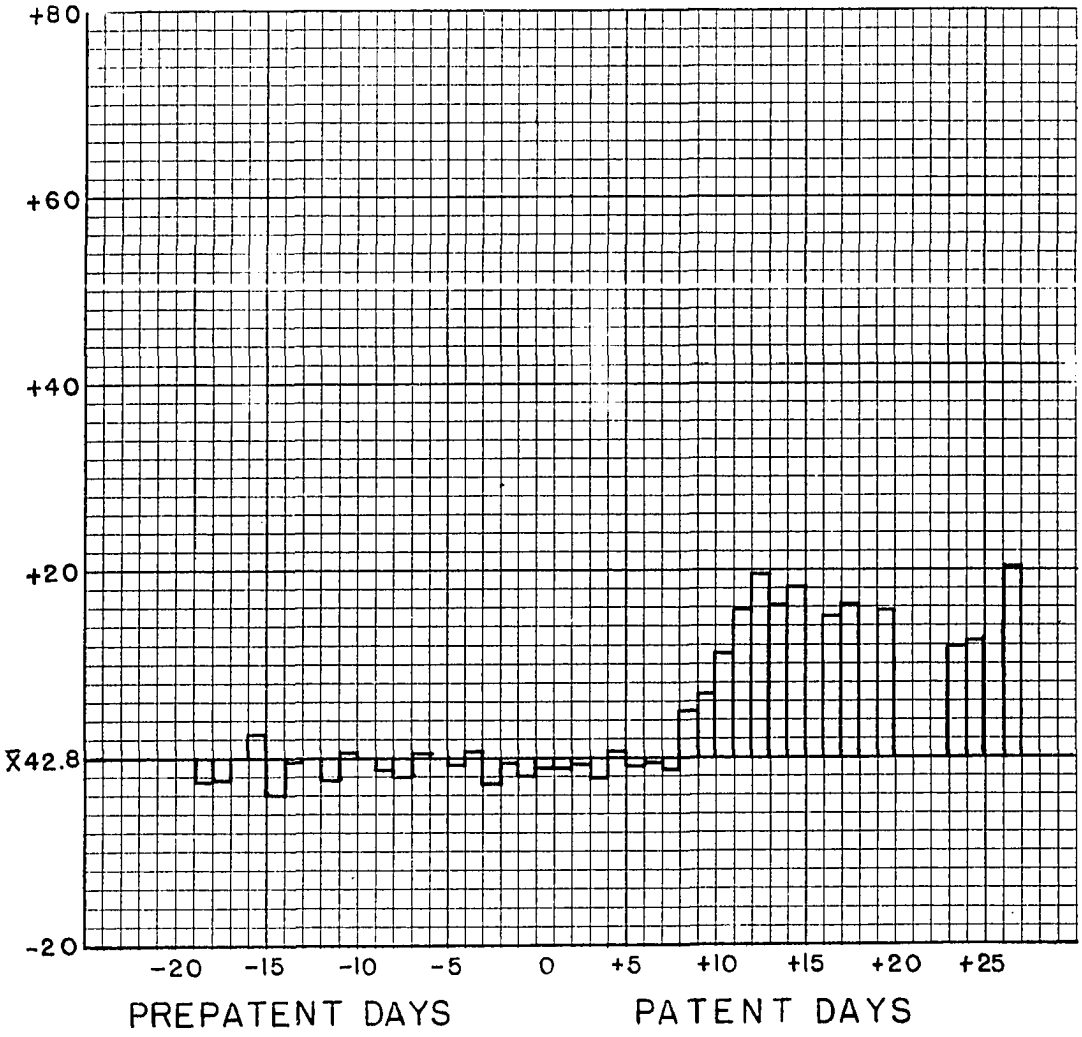

Fig. 10.--Deviations of the mean corpuscular volumes from the preinoculation mean during the prepatent and patent periods of anaplasmosis in Group II.

occurred. The percentage of hemoglobin continued to decrease until the eleventh patent day at which time the minimal percentage attained 19.2% in Group I and 27.2% in Group II (Tables 2 and 3). The mean corpuscular hemoglobin concentration increased during the late patent period and returned to normal at the termination of the experiment.

Mean Corpuscular Hemoglobin

The values of the mean corpuscular hemoglobin do not vary appreciably from the base mean until the tenth patent day in Group I and the eleventh patent day in Group II (Figures 13 and 14). During the next 3 days there was an abrupt increase to 22.4 uug. and 17.5 uug. in Groups I and II respectively (Tables 2 and 3). The mean hemoglobin per red blood cell then remained significantly above the preinoculation mean throughout the remainder of the experiment.

Calculations of the size and hemoglobin content of the erythrocytes during the disease course indicate that the early developing anemia in anaplasmosis was a normocytic normochromic type. In the later stages of the anemia, following increased hematopoiesis, a macrocytic hypochromic anemia developed. This is readily seen by comparing Figures 9 and 11, which show increasing mean cell size accompanied by a decrease in the percentage of hemoglobin present in the cell after the fifth patent day of the disease.

Results Indicating Bone Marrow Influence

Two tests were conducted primarily to estimate the influence of bone marrow activity on the anemia of anaplasmosis. These were firstly, the percentage of reticulocytes in the circulating blood as an estimate

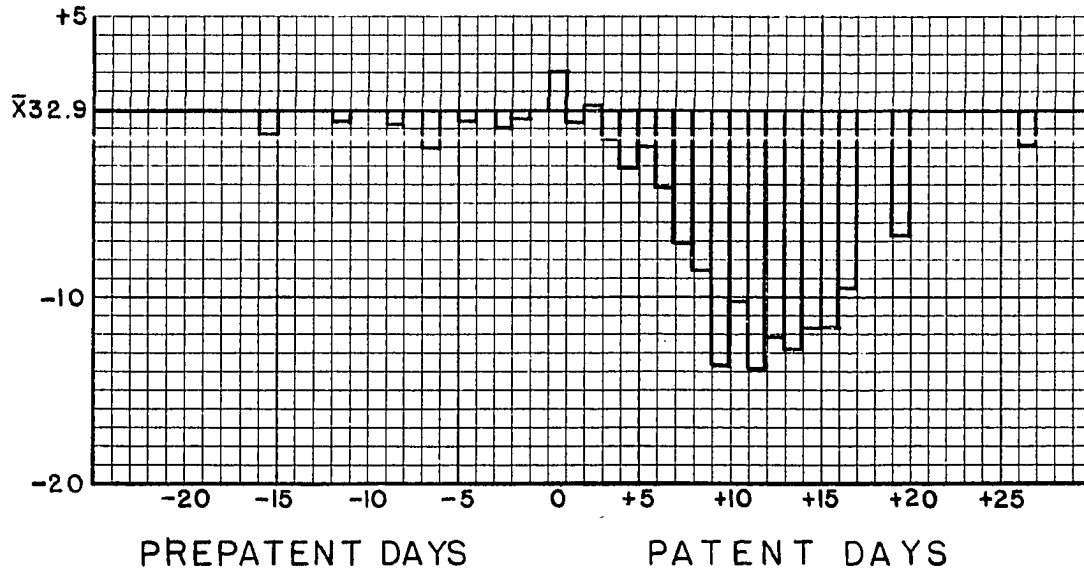

Fig. 11.--Deviations of the mean corpuscular hemoglobin concentration percentages from the preinoculation mean during the prepatent and patent periods of anaplasmosis in Group I.

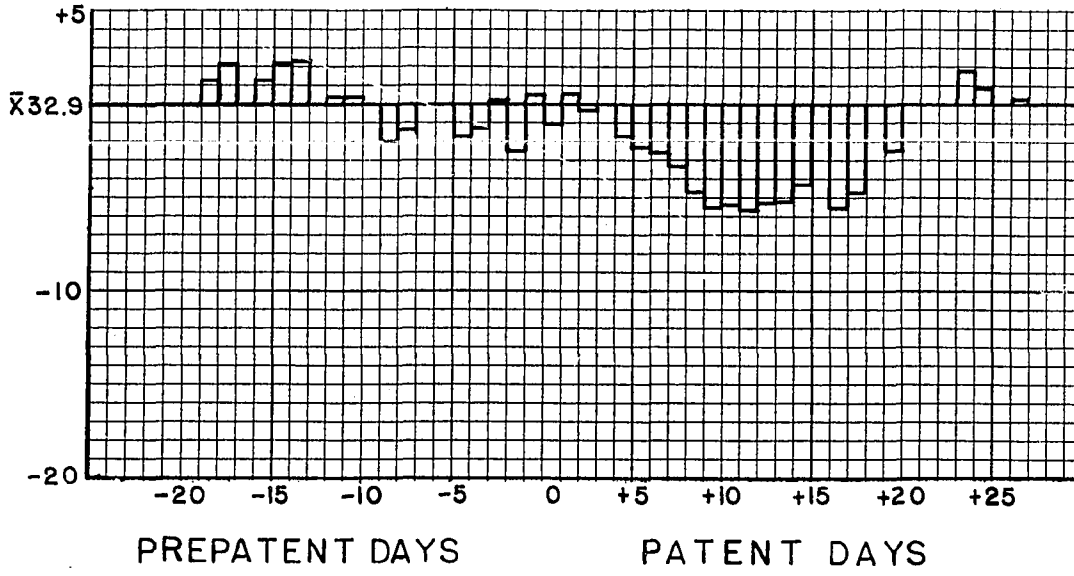

Fig. 12.—Deviations of the mean corpuscular hemoglobin concentration percentages from the preinoculation mean during the prepatent and patent periods of anaplasmosis in Group II.

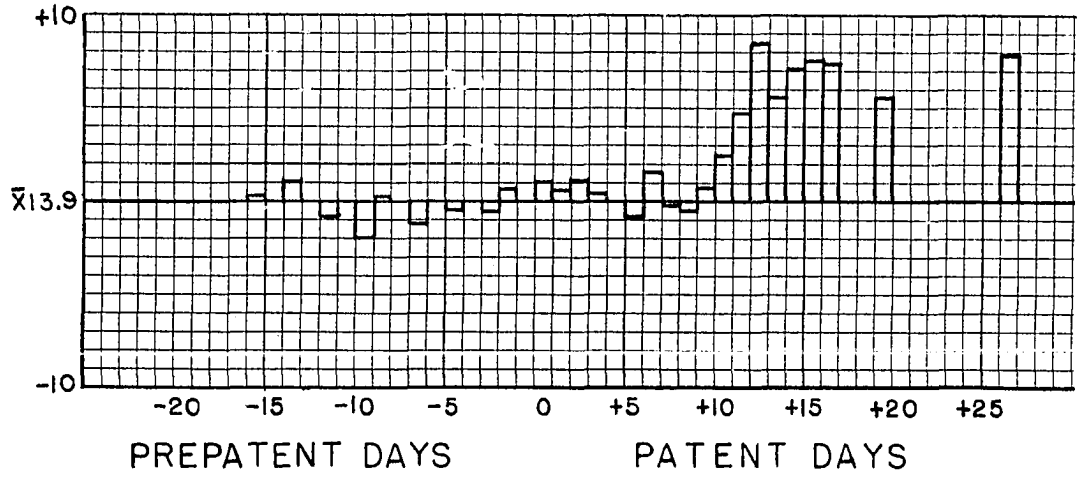

Fig. 13.--Deviations of the mean corpuscular hemoglobin from the preinoculation mean during the prepatent and patent periods of anaplasmosis in Group I.

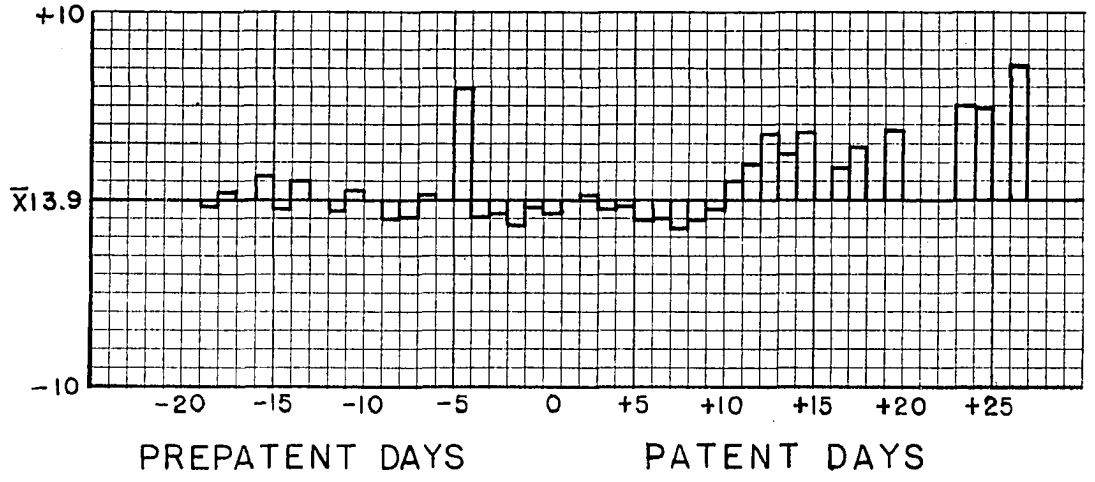

Fig. 14.--Deviations of the mean corpuscular hemoglobin from the preinoculation mean during the prepatent and patent periods of anaplasmosis in Group II.

of the number of newly formed erythrocytes reaching the blood stream; and secondly, the percentage of nucleated marrow cells composed of erythroblasts.

Reticulocytes

Coffin (1947) has stated that at the most only occasional reticulocytes are found in the normal blood of cattle. No reticulocytes have been observed in any of the normal or prepatent blood samples taken during this or previous experiments on anaplasmosis. The deduction naturally follows that the appearance of reticulocytes in the circulating blood of cattle is indicative of an increase in the number of young erythrocytes entering the blood stream.

Reticulocytes appeared in the circulating blood of the cattle in Group I on the fourth patent day (Figure 15). From the fourth to the eleventh patent days they increased rapidly to reach a maximum of 34.0% (Table 2), and then decreased at approximately the same rate to the nineteenth patent day. After this time they were no longer observed in the blood.

Reticulocytes appeared on the seventh patent day in Group II (Figure 16), and followed closely the pattern seen in Group I. The peak percentage of reticulocytes in Group II was 15.5% (Table 3). As in Group I, this peak occurred on the eleventh day.

Bone Marrow

Estimating the erythropoietic activity of the bone marrow by determination of the percentage of erythroblastic cells is relatively inaccurate when compared with other measurements used in this study.

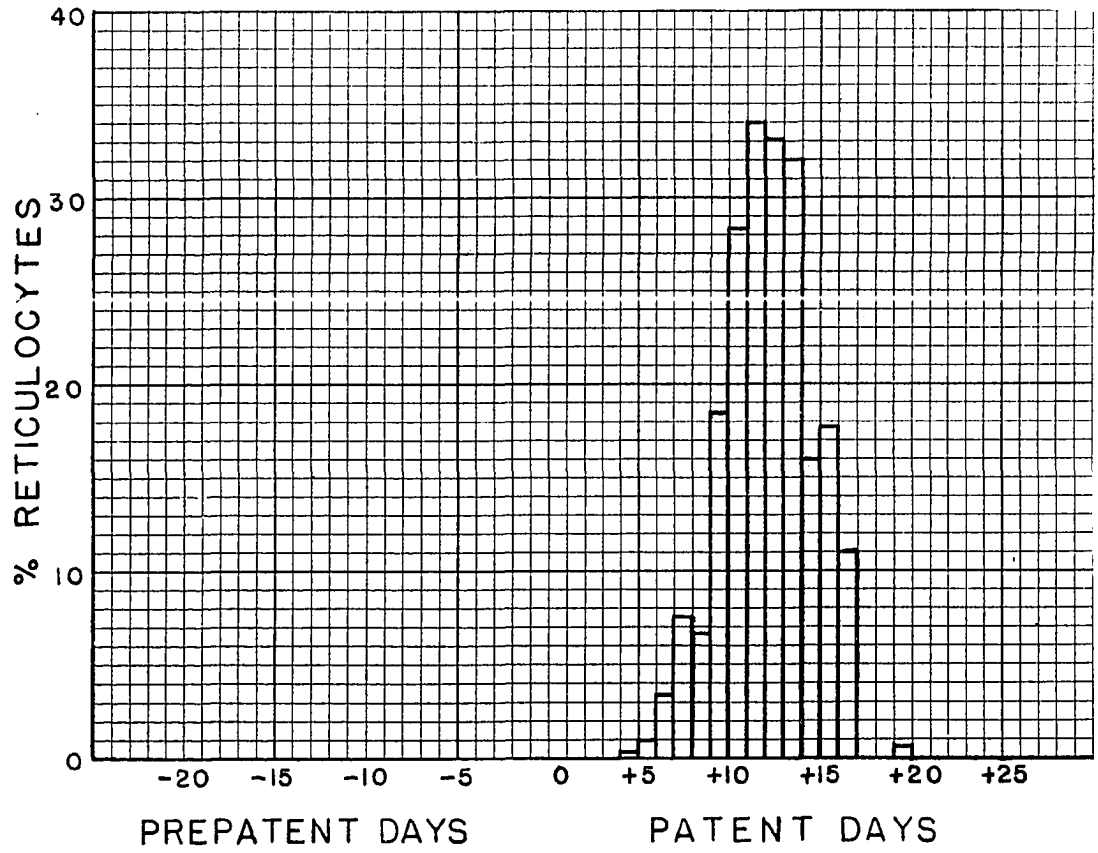

Fig. 15.--The percentages of reticulocytes during the prepatent and patent periods of anaplasmosis in Group I.

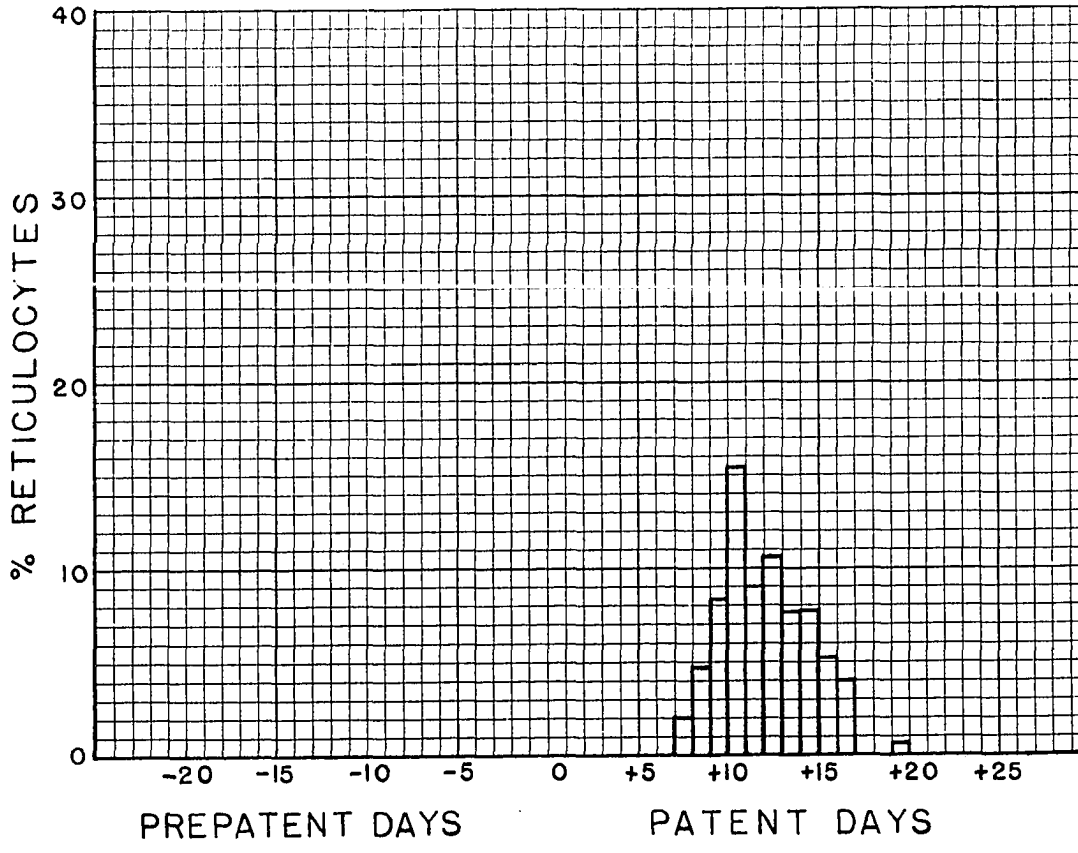

Fig. 16.--The percentages of reticulocytes during the prepatent and patent periods of anaplasmosis in Group II.

The difficulty of accurate sampling of both the bone marrow and the cells on the slide result in wide variation of the means (Figures 17 and 18).

Broad trends in the data can be followed however, if sufficient samples are taken. In this study 280 samples of bone marrow were taken; 50 of the samples were used to establish the normal mean, while the other 230 samples were used to establish the means for the days during the disease. Since the trends in the erythroblastic percentages in both groups of cattle followed the same pattern, the data from both groups may be considered together. This provided adequate sampling to indicate overall trends in erythropoietic activity in the bone marrow.

The trends of erythropoietic activity (Figures 17 and 18) indicated depression of the percentage of erythroid cells beginning on the twelfth to fifteenth day before the appearance of 1% infected erythrocytes in the circulating blood. This depression persisted through the sixth patent day when there was a reversal of the trend, indicating stimulation of erythropoietic activity. This increased activity persisted throughout the remainder of the experiment.

The results of the determinations of the percentage of reticulocytes in the circulating blood and of the percentage of erythroblastic cells in the bone marrow suggest that depression of erythropoiesis during the latter one-half to two-thirds of the prepatent period and first six days of the patent period may be a factor in the early anemia of anaplasmosis. The increases in both reticulocyte percentages and erythroblast percentages indicate a hematopoietic response of the bone marrow to the anemia after the fifth or sixth patent day.

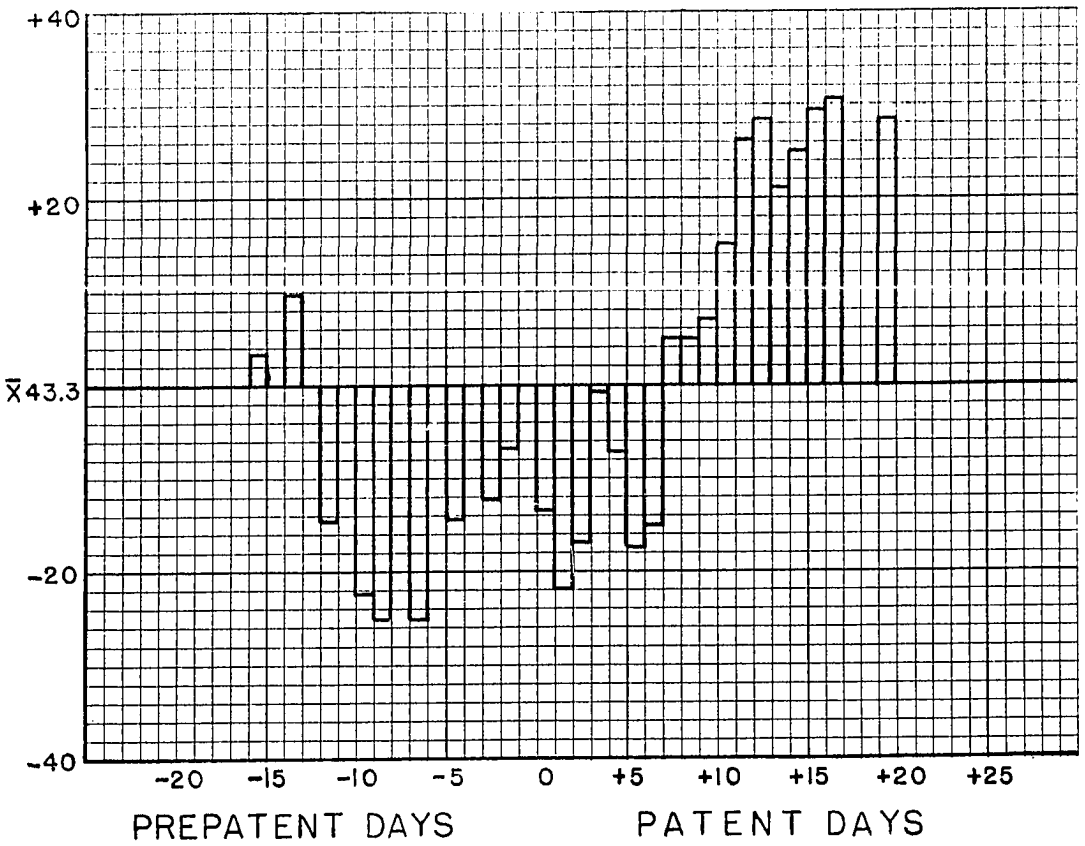

Fig. 17.--Deviations in the percentages of erythroblasts in the bone marrow from the preinoculation means during the prepatent and patent periods of anaplasmosis in Group I.

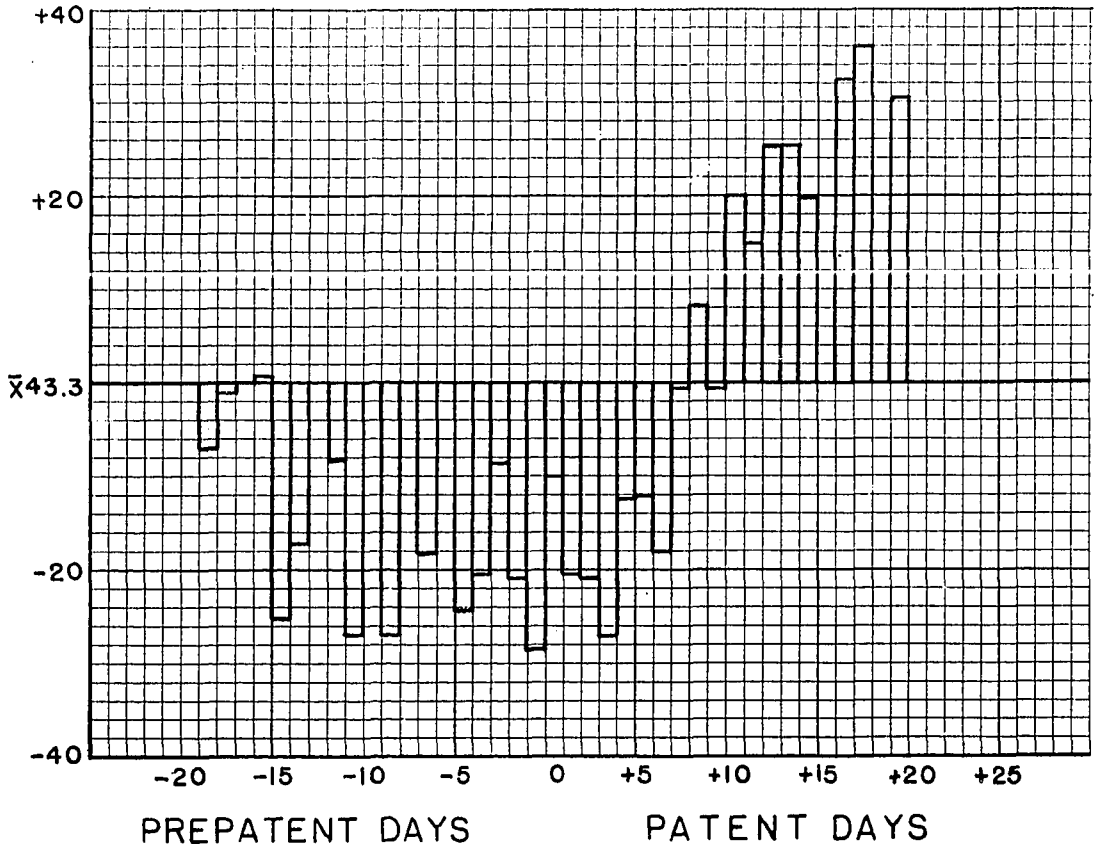

Fig. 18.--Deviations in the percentages of erythroblasts in the bone marrow from the preinoculation means during the prepatent and patent periods of anaplasmosis in Group II.

Results Indicating Hemolysis

It was not possible to gather 24 hour fecal samples from the cattle used in this study. Therefore total daily fecal urobilinogen production could not be determined. For the purposes of this investigation, in which interest was directed primarily toward the time and relative change in urobilinogen production, daily samples of feces were considered adequate.

Sources of error in the sampling method used should be pointed out. Little information was available on the time of bile excretion into the intestine in cattle. In case the excretion of bile was not uniform throughout the day, samples were taken at approximately the same time each day in an attempt to minimize this possible variable.

The concentration of urobilinogen in the feces was no doubt increased by the constipation the cattle developed during the anemia. While this contributed to the 90 fold rise in fecal urobilinogen observed in Group I (Figure 19) and the 40 fold increase in Group II (Figure 20), it did not fully account for this increase. The amount of feces produced during the constipation was not reduced in proportion to the rise in urobilinogen, and a marked rise in urobilinogen was noted before the animals became constipated.

A slight increase in fecal urobilinogen was detected on the day the disease became patent in Group I (Figure 19). There was a gradual rise in the amount of urobilinogen over the following 5 days. The urobilinogen content of the feces then abruptly increased for the next 3 days to reach a maximal value of 95.4 mg./100 gm. of feces on the eighth

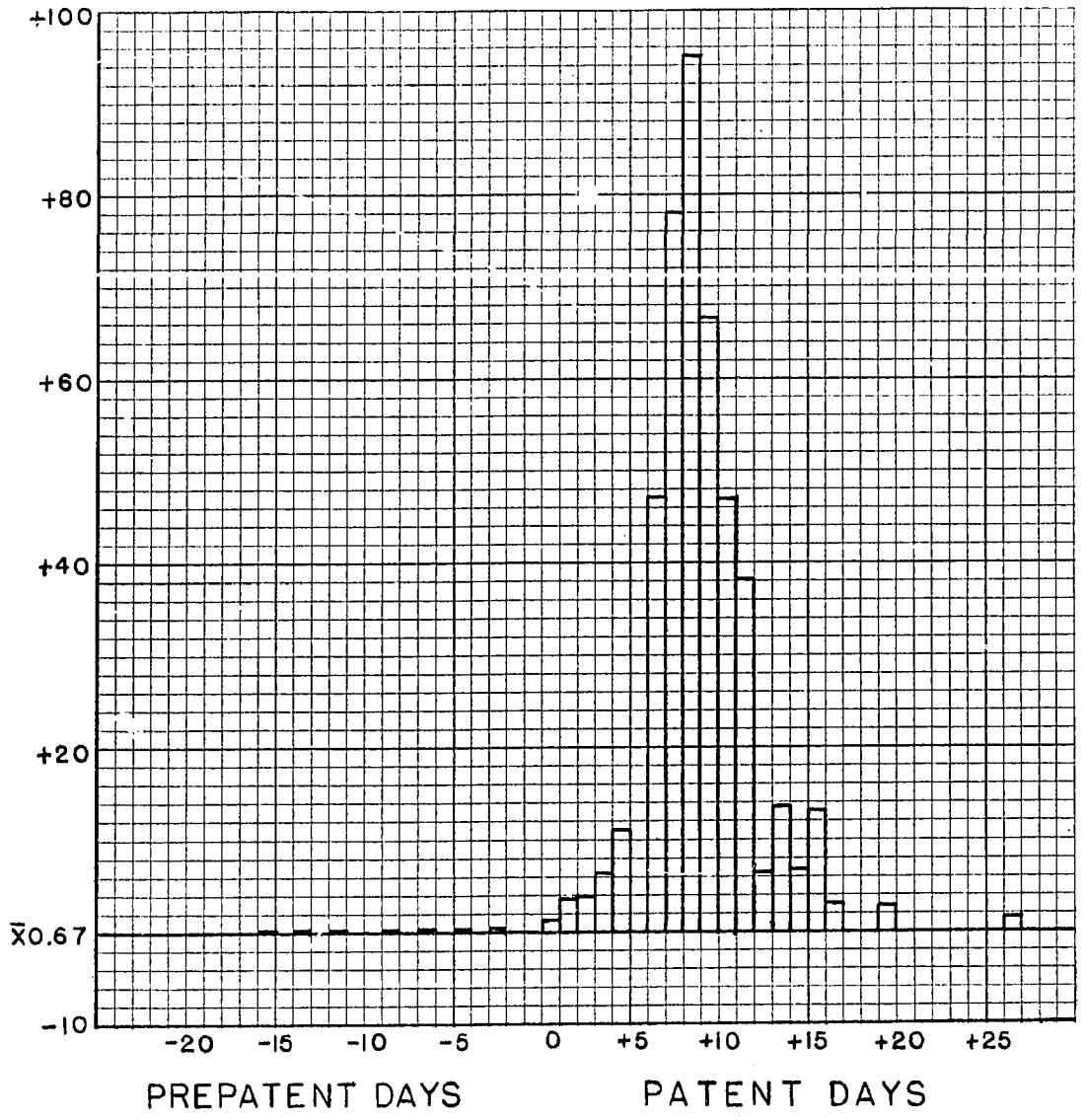

Fig. 19.--Deviations in the amount of fecal urobilinogen from the preinoculation mean during the prepatent and patent periods of anaplasmosis in Group I.

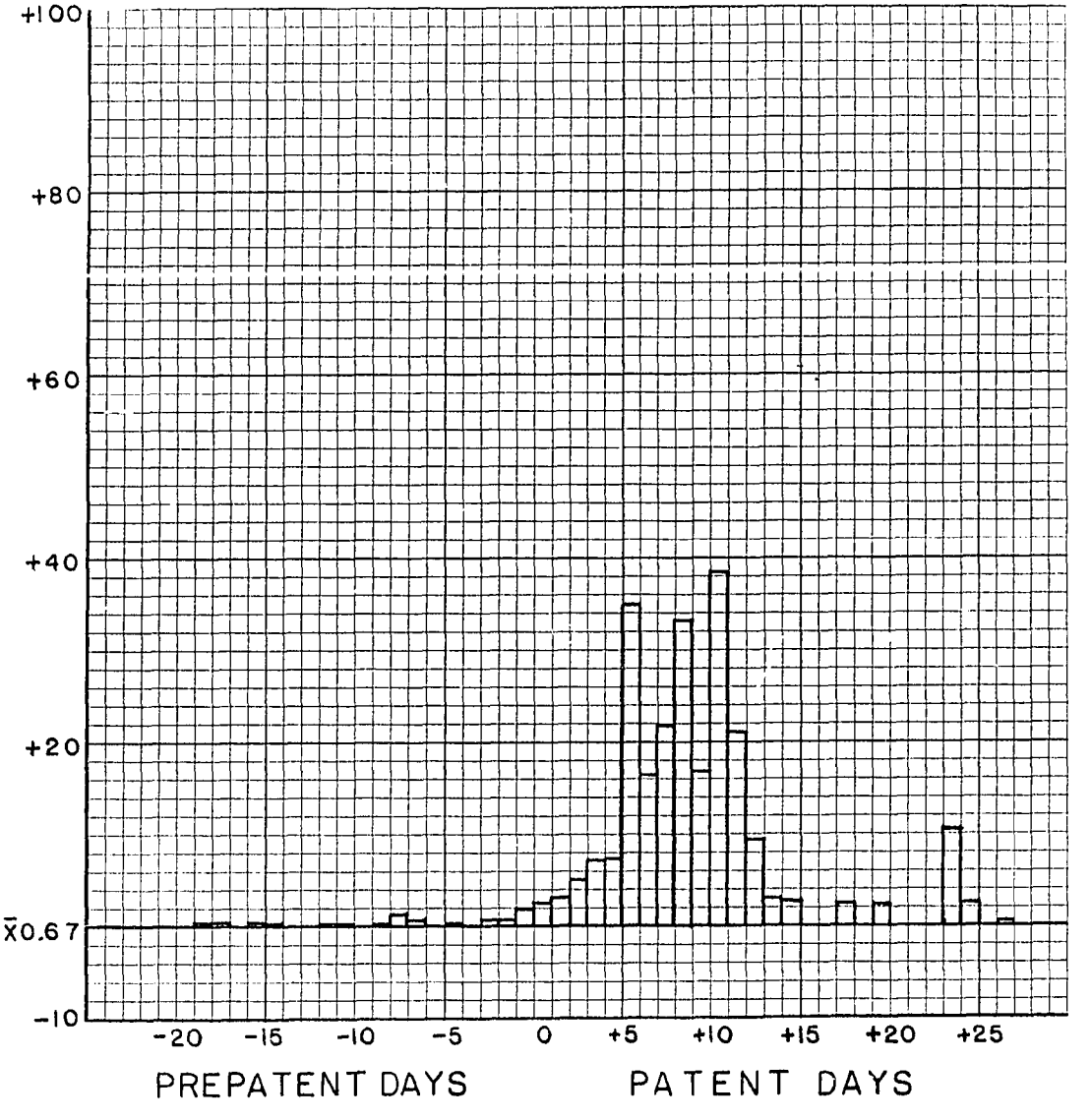

Fig. 20.--Deviations in the amount of fecal urobilinogen from the preinoculation mean during the prepatent and patent periods of anaplasmosis in Group II.

patent day (Table 2). This was followed by an almost equally rapid drop to near normal levels at the end of the experiment.

The pattern of increases in fecal urobilinogen for Group II seen in Figure 20 shows that the rise in urobilinogen began on the last prepatent day and gradually increased to the fifth patent day. On the fifth patent day the urobilinogen increased abruptly to near maximal level. In the following 6 days the means for each day varied widely between a maximum of 38.5 mg./100 gm. of feces to a minimum of 16.8 mg. (Table 3). The urobilinogen content then declined gradually to almost the preinoculation level on the twenty-sixth patent day.

The results of the fecal urobilinogen determinations indicate a hemolytic process present during the developmental period of the anemia in anaplasmosis. Hemolysis gradually increased as the anemia became more severe. The period of maximal hemolysis corresponded to the period of maximal anemia. Thereafter the hemolytic process declined rapidly, while recovery from anemia was gradual (compare Figures 3 and 4 with Figures 19 and 20).

Results of Liver Biopsy

Liver damage, as revealed by the liver biopsy sections, agreed closely with the other indications of the severity of the attack of anaplasmosis. In those animals that comprise Group I liver damage varied from slight to moderate, while only 1 animal in Group II showed even slight damage (Table 4).

The early biopsies, through the second patent day on all the cattle, were essentially normal. The biopsies from the cattle in Group II

were normal throughout the experiment with the exception of steer number 655. This animal developed slight bile duct proliferation and bile retention, seen first in the biopsy taken on the tenth patent day and in the subsequent biopsies.

In Group I biopsies from the two most severely affected cattle (644 and 648) showed slight fatty change and centrolobular necrosis on the eighth and sixth patent days respectively. Animal 644 died the following day. On the twelfth patent day, animal 648 had moderate fatty change, slight centrolobular necrosis, moderate bile duct hypertrophy and proliferation, and moderate bile retention. The principal changes found in biopsies taken later from this animal were some bile and cellular casts in the bile ducts and marked bile retention evidenced by cyst-like dilatations of many hepatic cells. The other 3 animals in Group I showed similar but less marked hepatic damage, which was first seen in the biopsies made on the twelfth patent day.

TABLE 4

THE PATHOLOGY FOUND IN THE LIVER BIOPSIES
ERYTHROCYTE COUNTS AND AMOUNT
OF UROBILINOGEN INCLUDED
FOR COMPARISON

				Pathology				
Animal	Day	RBC	Urob.	Fatty Change	Necrosis	Bile Duct Hypertrophy	Bile Retention	Bile Duct Casts
Group I								
643	2	6.76	7.20	N	N	N	N	N
	6	3.08	31.50	N	N	N	N	N
	12	1.73	3.24	Sl	Sl	N	Sl	N
	19	3.82	3.85	Sl	N	Sl	Sl	N
	26	4.10	3.20	Sl	N	Sl	Sl	N
644*	4	4.30	28.80	N	N	N	N	N
	8	1.38	68.25	Sl	Sl	N	N	N
	2	5.72	2.68	N	N	N	N	N
	6	3.21	31.50	N	N	N	N	N
	16	1.74	4.50	Mk	Sl	Sl	Sl	N
	19	2.88	3.85	Md	N	Sl	Sl	N
	26	3.42	1.05	Sl	N	Sl	Sl	N
648	2	5.50	5.01	N	N	N	N	N
	6	2.09	35.04	Sl	Sl	N	N	N
	12	1.38	2.04	Md	Sl	Md	Md	N
	19	2.31	6.05	Md	N	Md	Mk	Md
	26	4.20	5.10	Md	N	Md	Mk	Mk
651	2	8.10	2.16	N	N	N	N	N
	6	2.89	32.40	N	N	N	N	N
	12	1.14	16.50	Sl	Sl	Sl	Sl	N
	19	3.57	1.28	Sl	N	Sl	Sl	N
	26	5.12	1.16	N	N	Sl	Sl	N

N-Essentially Normal; Sl-Slight; Md-Moderate; Mk-Marked
* Died on +9 day.

TABLE 4—Continued

Animal	Day	RBC	Urob.	Pathology				
				Fatty Change	Necrosis	Bile Duct Hypertrophy	Bile Retention	Bile Duct Casts
Group II								
645	2	6.00	5.66	N	N	N	N	N
	6	4.21	20.70	N	N	N	N	N
	12	2.46	15.14	N	N	N	N	N
	19	4.62	3.45	N	N	N	N	N
	26	5.00	1.02	N	N	N	N	N
647	2	6.15	8.22	N	N	N	N	N
	12	3.22	5.88	N	N	N	N	N
	19	4.36	3.00	N	N	N	N	N
	26	4.47	1.34	N	N	N	N	N
653	0	6.98	2.73	N	N	N	N	N
	10	4.20	7.08	N	N	N	N	N
	14	5.10	1.12	N	N	N	N	N
	17	5.28	3.65	N	N	N	N	N
	24	5.50	5.11	N	N	N	N	N
654	-1	11.20	2.64	N	N	N	N	N
	9	4.75	5.88	N	N	N	N	N
	13	5.44	1.96	N	N	N	N	N
	16	6.31	4.70	N	N	N	N	N
	23	4.84	11.28	N	N	N	N	N
655	0	8.60	1.15	N	N	N	N	N
	10	3.46	61.40	Sl	N	Sl	N	N
	14	3.27	2.40	Sl	N	Sl	Sl	N
	17	3.52	3.10	N	N	Sl	Sl	N
	24	4.30	1.13	N	N	Sl	Sl	N

N-Essentially Normal; Sl-Slight

CHAPTER V

DISCUSSION

Any attempt to explain the pathogenesis of anemia in anaplasmosis must, of course, be consistent with the clinical and laboratory findings during the disease. The signs of the disease have been described in Chapters I and II. Many additional laboratory findings have been shown in Chapter IV. These observations require correlation to form a consistent and logical theory to account for the development of the anemia.

In review, the pertinent observations concerning the signs of the anemia in anaplasmosis are: a rapid progressive loss of erythrocytes from the circulating blood without internal or external signs of hemorrhage; the absence of hemoglobinuria; an apparent absence of marked bilirubinemia; the inconsistent appearance of clinical icterus; an enlarged and yellow liver; splenic enlargement and congestion. The lack of hemorrhage in this disease limits the cause of blood loss to hemolysis, depression of erythropoiesis, or perhaps, in part, to both hemolysis and depression. Since the observations presented in Chapter IV indicate that both hemolysis (Figures 19 and 20) and depression of the percentage of erythroblasts (Figures 17 and 18) are present, the influence of each process, both separately and combined, on the anemia needs to be assessed.

The Influence of Erythropoietic Depression

Estimation of the influence of the apparent depression of the bone marrow on anemia in anaplasmosis presents certain difficulties. Such an estimation, to be accurate, requires information that is not available. For this reason any deductions that are made must be guesses rather than accurate estimates.

The life span of the normal bovine erythrocyte has not been thoroughly investigated. Hansard and Kincaid (1956) place the life span of bovine erythrocytes at approximately 50 days. In human beings the results of studies to determine the longevity of red blood cells have varied widely from a few days to over 100 days. Investigations using isotope labelled erythrocytes indicate that the life span is approximately 125 days (Shemin and Rittenberg, 1945). Using a technique of reticulocyte showers following hemorrhage, Harne et al. (1945) estimated the life span of the red blood cell in the monkey to be approximately 100 days. Hawkins and Whipple (1938) determined the erythrocyte life span in dogs to be approximately 124 days.

The length of time necessary for the erythrocyte to mature from the erythroblast to the reticulocyte should be known before a good estimate can be made of the effect of the erythropoietic depression. Some deductions have been made from studies on pernicious anemia in the human being. Minot and Castle, cited by Isaacs (1937), have determined that it requires an average of 7 days for the reticulocyte to develop from the megaloblastic stage following therapy for pernicious anemia. In malaria the maximum reticulocytic response follows effective treatment

by 6 to 10 days (Sturgis, 1948).

Another factor to be considered in the effect of bone marrow depression on the production of anemia is the rate of normal erythrocytic destruction. Upon this factor and the degree of depression would depend the time of appearance and the degree of the anemia following the reduction of erythropoiesis. It has been estimated that 0.85% of the blood, or 50 ml., is replaced daily in human beings (Wintrobe, 1946). Hawkins and Whipple (1938) have demonstrated by the quantitative determination of bile pigments produced, that 1.92 gm. of hemoglobin is destroyed daily in the dog. They have calculated from these data that 0.75% of the total red cell mass is destroyed daily.

From the data obtained in this study, an extremely speculative value for the milliliters of bovine blood destroyed per day can be obtained. Previous information indicates that these cattle probably produced approximately 18,160 gm. of feces per day. The total milligrams of urobilinogen per day may be estimated by the following formula:

$$\frac{\text{Total gm. feces per day} \times \text{mg. of urobilinogen per 100 gm. of feces}}{100} =$$

total mg. urobilinogen per day

$$\frac{18160 \times 0.67}{100} = 121.7 \text{ mg. of urobilinogen per day}$$

The destruction of 23.9 mg. of hemoglobin is required to produce 1.0 mg. of urobilinogen (Wintrobe, 1946). The grams of hemoglobin destroyed per day may be estimated as follows:

$$\frac{\text{Total mg. urobilinogen per day} \times 23.9}{1000} =$$

total gm. of hemoglobin destroyed per day

$$\frac{121.7 \times 23.9}{1000} = 2.91 \text{ gm. hemoglobin per day}$$

The normal mean hemoglobin in these cattle was 11.7 gm. per 100 ml. of blood. The milliliters of blood destroyed per day may be estimated by the formula:

$$\frac{\text{gm. hemoglobin per day} \times 100}{\text{gm. hemoglobin per 100 ml. of blood}} =$$

total ml. of blood destroyed per day

$$\frac{2.91 \times 100}{11.7} = 24.9 \text{ ml. blood destroyed per day}$$

The value, 24.9 ml. of blood destroyed per day, is disproportionately low when compared with the estimated human value of 50 ml. of blood destroyed per day. It is probable that when less speculation can be used in estimating the value for cattle, the figure will be revised upward.

In the present study the depression in the percentage of erythroblasts in the bone marrow started approximately 15 days before there were signs of anemia in the peripheral blood. There was an average of about 17% fewer erythroblasts in the bone marrow than normal, or expressed more usefully, there was approximately 60% of the normal percentage of erythroblasts present. If the percentage of erythroblasts directly reflects the erythropoietic function of the bone marrow, then the bone marrows in these cattle were producing only 60% of the normal number of erythrocytes during the last 15 days of the prepatent period.

It must be assumed that the cattle were in a state of equilibrium insofar as erythrocyte destruction and production were concerned prior to exposure to anaplasmosis. A sudden decrease of 40% in the production of erythrocytes would be expected to produce a corresponding decrease in the number of circulating erythrocytes within a few days. As can be seen by comparing Figures 2 and 3 with Figures 17 and 18, there was no significant variation of the erythrocyte count from the normal mean during the 15 prepatent days in question.

Urobilinogen was being produced in the feces during these last 15 prepatent days at nearly normal levels. This suggests that there was no abnormal loss of erythrocytes by increased hemolysis. The normal loss, by the speculation above, is about 25 ml. of blood. Cattle the size of those used in this experiment (800 lbs.), as compared with the human, should normally destroy about 350 ml. of blood daily if the rates of destruction were proportional to their weights.

Even if the smaller value calculated from the data presented in this study proves to be correct, the normal daily destruction of erythrocytes should be sufficient to produce signs of anemia following a 40% decrease in erythropoiesis in less than 15 days.

It would appear from this discussion that there is an apparent paradox in the decreased percentage of erythroblastic cells in the bone marrow that is not, within a reasonable time, reflected in the peripheral blood picture. Ristic and Sipple (1958) show extramedullary hematopoiesis in splenectomized calves infected with anaplasmosis during anemia, but it seems unlikely to occur in the absence of anemia. While little is known concerning red blood cell storage in the spleen and

other tissues in the bovine, it appears improbable that such reservoirs would suddenly become exhausted at the appearance of infected erythrocytes in the blood. It is possible that areas of the bone marrow other than those sampled in this study showed a compensatory increase in activity. Custer and Ahlfeldt (1932), however, have shown the vertebral bone marrow to be most responsive to the need for additional cells. There is no known reason for anaplasmosis to affect certain sites of the marrow more than other sites.

At least a partial explanation for the paradox may be found to lie in the manner of measuring erythropoietic activity used in this experiment. The 40% decline in erythroblasts may be relative rather than absolute. The count was made as a percentage of the nucleated cells present in the bone marrow. The marrow was not aplastic in the sense that no myeloid cells were present, nor is an aplastic picture present in the circulating blood. Sergent et al. (1945), in extensive studies of the peripheral blood in anaplasmosis, have shown hyperleukosis with an increase in the percentage of neutrophiles during the incubation period of the disease. This condition, therefore, may not be entirely aplasia, but partially an increase of granulocytic elements without a corresponding relative increase of erythroblastic cells.

The leukocytosis observed during the incubation period possibly results from invasion of the bone marrow by the anaplasma organism, this invasion being reflected in the marrow as an increase in the myeloid cells. The absolute number of erythroblastic cells, however, remains approximately the same with the result that no signs of anemia develop during the prepatent period.

Evidence that the anaplasma organism infects the erythroblasts in the bone marrow has recently been presented by Foote (1958). By means of electron photomicrographs he has demonstrated the presence of abnormal bodies, possibly the organism, in normoblasts. In this cell, however, the bodies have the appearance of elementary bodies. Since anemia does not develop during the prepatent period, this organism does not appear to influence the maturation of erythrocytes. When the infected erythroblasts become mature and enter the blood stream the elementary bodies may form the commonly seen anaplasma body. If this theory is correct, formation of the anaplasma body requires a few days after the cell becomes mature. The writer has never observed the bodies in reticulocytes, basophilic cells or cells with basophilic stippling.

The influence, therefore, of the bone marrow on the production of anemia in anaplasmosis seems to be negligible. The bone marrow appears, rather, to be the primary site of infection where the early stages of the development of the etiologic agent takes place. It is there that the erythroblasts become infected, later appearing as infected erythrocytes in the circulation.

In recovery from the anemia, the bone marrow has a major role. Increased erythropoietic activity, as indicated by the appearance of reticulocytes in the blood, begins on the fourth or fifth patent day when the erythrocyte counts are approximately 4.50 million. This activity continues to increase, as indicated by such phenomena as an increase in the mean corpuscular volume, an increase in the percentage of erythroblasts in the bone marrow, and an increase in the percentage of reticulocytes.

The appearance of reticulocytes in the blood stream within 5 days of the start of the anemia indicates prompt bone marrow response. Harne et al. (1945) noted a delay of 2 to 4 days in reticulocyte increase in monkeys following hemorrhage. The promptness of the appearance of reticulocytes in these cattle is additional evidence that there was little actual depression of erythropoiesis.

The Influence of Hemolysis

The results of this experiment indicate that the major portion of the anemia in anaplasmosis, if not all of the anemia, is due to hemolysis. Examination of Tables 2 and 3 and Figures 19 and 20 reveals that hemolysis started with the earliest signs of anemia. By comparison of Figures 1 and 2 with Figures 19 and 20 it can be seen that the increase and decrease of urobilinogen accompanies, day by day, the increase and decrease of infected erythrocytes. It is also apparent, by comparing Figures 19 and 20 with Figures 3 and 4, that when the production of urobilinogen decreases sharply the erythrocyte count begins a gradual increase.

The high positive correlation between the rise and fall of infected erythrocytes and that of the production of urobilinogen seems to indicate a relation between the two factors. The most probable relationship is that the infection in some manner injures the erythrocyte, making it susceptible to hemolysis when it enters the circulation.

The role of hemolysis in the mechanism of the anemia in anaplasmosis appears to be the removal of the infected erythrocytes as they appear in the circulation.

Evidence from Liver Biopsy

From the results of the series of liver biopsy studies reported in Chapter IV, it appears that the degree of liver damage is a function of the severity of the anemia (Table 4). The animals whose erythrocyte counts remained above 2 million showed little or no damage (Group II), while the animals whose erythrocyte counts went lower than 2 million showed varying degrees of liver damage from slight to moderate (Group I). In none of the cases, including animal 648 whose liver showed the most pathology, was the damage more than moderate.

Slight liver damage is first seen on the sixth patent day in animal 648. In the other cases it appears in the biopsies made on the twelfth or sixteenth patent days. By comparison with Figures 19 and 20, it can be seen that the peak of urobilinogen production is passed at the time liver damage becomes evident. A portion of the decrease in urobilinogen is probably due to retention of bile in the liver, but it is evident from the increasing erythrocyte counts at this time that hemolysis is decreasing along with the urobilinogen production. The absence of high plasma bilirubin and hemoglobinuria in anaplasmosis, therefore, appears to be due to the ability of the liver to process the products of hemolysis almost as rapidly as they are formed.

Pathogenesis of the Anemia

From the results presented in Chapter IV and the discussion in this chapter, it is possible to form a theory of the pathogenesis of the anemia in anaplasmosis which corresponds with the known facts of the

disease. It is recognized that this preliminary attempt to explain the anemia is based on incomplete information and upon certain assumptions for which there is little factual support. A working hypothesis that may have to be revised or discarded, if it agrees with the known facts, is more to be desired, however, than illogical chaos.

When the anaplasma infectious agent enters the new host, there is a developmental period of 3 to 7 days in the bone marrow. There it infects increasing numbers of some stage in the maturation of the erythrocyte.

Due to damage from the infection, the infected erythrocytes in the circulation are rapidly destroyed by hemolysis, resulting in a rapidly progressive anemia.

The peak of infected erythrocytes and hemolysis is usually reached prior to appreciable anoxic damage to the liver. In such cases, the bilirubinglobin resulting from the hemolysis can adequately be processed by the liver to bilirubin and excreted. Those cases in which there is early liver damage or prolonged anemia and hemolysis with resulting liver damage, the liver is no longer able to handle even reduced amounts of bilirubinglobin. The result is an increased icteric index and clinical icterus from both retention and regurgitation of bilirubin.

If the animal survives the period of maximal anemia, increased erythropoietic activity in the bone marrow gradually leads to complete recovery. There are at least two factors that contribute to the infected animal's ability to survive the maximal anemia. One factor is prompt bone marrow response to the anemia. In the writer's experience, animals seldom die after the appearance of reticulocytes and increased

mean corpuscular volume. The other factor is the animal's freedom from exertion during the critical period of anemia. In the writer's experiments those animals which have died following the appearance of reticulocytes have done so while excited or while being moved. Other factors are no doubt also important, such as the role of the spleen and hormones in maintaining antibody titre, and bone marrow activity as shown by Ristic et al. (1958), but these factors are still little understood and await further evaluation.

CHAPTER VI

SUMMARY

Anaplasmosis is characterized by a normocytic normochromic anemia. If the developing anemia provokes erythropoietic stimulation of the bone marrow, the anemia becomes macrocytic hypochromic in character. As the anemic condition is gradually corrected the erythrocytes return to normal size and hemoglobin content.

The etiological agent of anaplasmosis probably enters erythroblastic cells in the bone marrow where it undergoes multiplication. Depression of the percentage of erythroblasts in the bone marrow may, in part, be a reflection of damage to the erythroblasts by the organism. Maturation of the infected red blood cells appears to continue. Mature infected erythrocytes enter the peripheral circulation and typical anaplasma bodies may develop in 2 or 3 days.

The anemia in anaplasmosis is principally due to hemolysis of the infected erythrocytes. Erythrocytes containing anaplasma bodies are quickly destroyed by the body. The liver remains essentially undamaged during the period of greatest hemolysis. It is able, therefore, to process the bilirubin produced by the hemolysis. This may explain the failure to find hemoglobinuria, infrequent icterus, and relatively low bilirubinemia in acute anaplasmosis. Only after the anemia becomes

severe is the liver damaged, with consequent bile retention and icterus. Hemolysis at this time, however, is greatly reduced from peak levels. The icterus, as a result, is due more to liver damage than to hemolysis.

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