

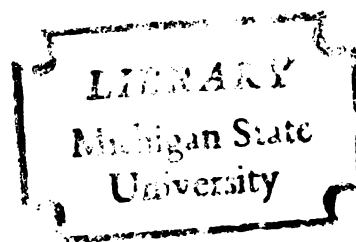
A GLOMERULONEPHRITIS ASSOCIATED
WITH ACUTE BABESIA RODHAINI
INFECTIONS OF RATS

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ABSTRACT

A GLOMERULONEPHRITIS ASSOCIATED WITH ACUTE BABESIA RODHAINI INFECTIONS OF RATS

by Gloria M. Iturri

It has been known for many years that kidney disease is frequently associated with malaria, especially chronic quartan malaria caused by Plasmodium malariae, and blackwater fever which is associated with Plasmodium falciparum infections. A similar kidney disease has been associated with Babesia canis infections of dogs and Babesia equi or Babesia caballi infections of horses.

Experiments have been undertaken to determine whether a similar kidney disease is associated with Babesia rodhaini infection of rats and to determine the nature of the changes in kidney tissues associated with this infection.

The experiments indicated that there was a nephritis associated with B. rodhaini infection. It appeared that the disease represented a clear-cut glomerulonephritis uncomplicated by cellular exudate, thrombi or marked hemorrhage.

Changes in the nephron were detected as early as 2 days after infection and were maximal prior to the peak of parasitemia or anemia which occurred on the 12th to the 14th day.

The severity of glomerulonephritis was, however, correlated with the titers of agglutinins for trypsin-treated normal rat erythrocytes in the serum of the rats at the time of necropsy. These

agglutinins were detected for the first time in the sera of rats exsanguinated on the 4th day after infection and persisted through the 16th day.

The presence of agglutinins for trypsinized autologous or homologous erythrocytes has been associated with the presence of autoantibody in hemolytic anemias and other immunologic diseases. It is suggested that the presence of agglutinins in the serum of rats with acute B. rodhaini might be indicative of an autoimmune mechanism for the observed glomerulonephritis.

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Gloria M. Iturri

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To my husband, Sergio

To my parents

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I. INTRODUCTION

Kidney disease has been known for many years to accompany malaria, especially in post-acute quartan malaria, blackwater fever, and acute malignant tertian malaria (Maegraith, 1948).

Though it is not well documented, there is also kidney disease associated with acute babesiosis, especially in canine and equine infections (Malherbe and Parkin, 1951; Sippel, 1962).

The kidney disease of both malaria and babesiosis has been described as acute or chronic glomerulonephritis. It has been suggested that various factors, such as anoxia following blood loss, parasite toxins, blood-parasite thrombi, and mechanical blockage of the tubules of the kidney were the mediators of the disease (Maegraith, 1948; Malherbe and Parkin, 1951). Though Maegraith (1948) does give a short comment, the role of immunologic mechanisms as mediators of kidney disease in malaria has received but little attention until recent times. Immunologic activities have been related to the nephritis associated with chronic quartan malaria in African children (Gilles and Hendrickse, 1963) and also among adults (Giglioli, 1930; Kibukamusoke et al., 1967).

Dixon (1966) has suggested that malarial antigens and their antibodies form complexes which act on kidney tissues to produce glomerulonephritis. That glomerulonephritis is in general mediated by immunologic mechanisms has come to be rather well accepted and is fairly well discussed in standard textbooks on medical immunology

(Raffel, 1961; Humphrey and White, 1963; Dixon and Humphrey, 1966).

Recently it has been found that anemias of both malaria and babesiosis are more relatable to immunologic mechanisms than to the destruction of erythrocytes by parasites (Zuckerman, 1960; McGhee, 1960; 1964; Cox et al., 1966; Schroeder et al., 1966). In addition to a relationship between anemia and autoantibody-like substances, it has been shown that there are substances in the sera of animals with acute malaria or babesiosis that will cause anemia when they are injected into normal animals (Cox, 1966; Corwin and McGhee, 1966; Schroeder, 1966; Sibinovic et al., 1967a, 1968).

With the assumption that immunologic mechanisms might be responsible for anemia in these infections, it seemed reasonable to suspect that the same mechanisms might be associated with glomerulonephritis in acute malaria or babesiosis. Based on these thoughts, experiments were undertaken to determine whether there was glomerulonephritis associated with acute Babesia rodhaini infections of rats, whether it might be associated with immune mechanisms that have been related to anemia and to study this host-parasite system as a model for studying kidney disease associated with other erythrocytic infections.

II. LITERATURE REVIEW

1. BABESIA PARASITES, GENERAL

Protozoan species of the genus Babesia are blood parasites of many vertebrates including domestic animals important to man. They are intra-erythrocytic parasites and have been placed in the class Sporozoa, subclass Hemosporidia, order Babesiida and suborder Piroplasmae. This suborder is represented by two families, Babesidae, Foche, 1913, and Theileridae, du Toit, 1918. (Neitz, 1956; Levine, 1961).

Babes (1888) was the first to observe Babesia parasites. He found parasites in the red blood cells (RBC) of African cattle which showed signs of hemoglobinuria. Babes named them Hematococcus bovis since he thought he had successfully cultured them in vitro.

Starcovici (1893) changed the name given by Babes to Babesia bovis, and thus established the generic name. Smith and Kilborne (1893) studied Texas fever of cattle and recognized the role of ticks as biologic vectors of the causative agent. This is the first record of arthropod transmission of a protozoan parasite and the first record of transovarian transmission of infections in arthropods. These authors recognized the protozoan nature of the agent and proposed the name Pyrosome bigeminum which was later changed to Babesia bigemina to conform with the classification of Starcovici (1893).

The family Babesidae includes the non-pigmented parasites of the erythrocytes of mammals which reproduce asexually within these

cells by division into two or more pear-shaped daughter individuals (Starcovici, 1893). They are transmitted by hard ticks belonging to the family Ixodidae. Ticks of the genera Rhipicephalus, Ixodes, Boophilus, Haemaphysalis, Hyalomma, and Dermacentor have been incriminated as vector hosts. A sporogonous reproductive cycle for B. bigemina in ticks of the genus Boophilus has been described. Parasites taken with a blood meal undergo gametocytogenesis in the hind gut. The zygote invades the reproductive organs and multiplies. They infect the embryo within the eggs and subsequently undergo extensive multiplication, migrating to all of the tissues of the developing embryo. Some of the parasites enter the salivary glands and are transmitted by the seed (larval) ticks when they feed. The adult ticks do not transmit the infection (Smith and Kilborne, 1893; Dennis, 1932). In the vertebrate host the parasites are known to multiply only in the RBC (Levine, 1961). Although about twenty species of Babesia have been named, many of these are probably not valid and only a few are important parasites of domestic animals. These include six species in cattle: B. bigemina, Babesia cosmopolitan and Babesia argentina in South America; Babesia bovis in Europe, and Babesia barber and Babesia major in North Africa. Two species are found in sheep and goats: Babesia ovis and Babesia motasi in Europe and Africa. There are two species in pigs: Babesia trautmani in eastern Europe and Africa and Babesia perroncitoi in Sardinia. Two species infect horses: Babesia caballi, in Central America and around the Mediterranean and Babesia equi in Europe and Africa. Cats are hosts to a single species, Babesia felis in Africa

while in dogs Babesia canis is found in Europe, Asia, Africa and the Americas and Babesia gibsoni in Asia and Africa. (Chandler and Read, 1966).

2. BABESIA RODHAINI

B. rodhaini was found in the blood of an arboreal rat, Thamnomys surdaster surdaster, in Katanga, province of the Congo, by Van den Berghe and co-workers (1950).

Infections of laboratory white mice and rats, splenectomized cotton rats and Syrian hamsters have been established (Rodhain, 1950). Repeated passages in white mice yielded a strain which developed severe infections with nearly one hundred percent mortality occurring 8 to 11 days after infection (Beveridge, 1953; Colas-Belcour and Vervent, 1953).

Wright's stain shows the parasite within the erythrocyte to be a simple blue ring with a pink laterally situated nucleus. The organism appeared to multiply by budding or fission (Van den Berghe et al., 1950; Beveridge, 1953).

Electron microscopic studies made on infected mouse erythrocytes showed the organism to be surrounded by a limiting membrane. The parasite contained a nucleus and food vacuoles. Intracytoplasmic structures composed of concentric membranes (possibly primitive mitochondria) have been reported. The formation of a food vacuole involves invagination of the protoplasm of the parasite to form a vacuole which contains hemoglobin from the cytoplasm of the erythrocyte, a process called "phagotrophy". The process of digestion of hemoglobin within the vacuole appeared to be complete since no pigment was formed

(Flewett and Fulton, 1959; Rudzinska and Trager, 1960, 1962).

3. ANEMIA IN ERYTHROCYTIC INFECTIONS

Blood of malarious patients and destruction of erythrocytes have been studied since the early 19th century. Bright in 1831 recognized that "graphite" like pigment in tissues was of blood origin and was related to malaria. Meckel in 1847 also recognized the blood origin of black pigment in the brain and related it to malaria. In 1876, Joseph Jones, Professor of Chemistry and Clinical Medicine at the University of Louisiana, stated that he had studied blood changes in malaria for twenty years and could definitely differentiate the blood of malarious and non-malarious humans (Russell, West and Manwell, 1946).

The earliest indication of the mechanisms involving blood loss was the record of Christophers and Bentley (1908), who observed the presence of large numbers of normal RBCs in the macrophages of the spleen of patients dying from blackwater fever. They were the first to postulate that RBCs were destroyed by mechanisms other than direct parasite attack. Others have since described this phenomenon in the spleen of animals with acute malaria, but have placed more emphasis on the number of infected cells also phagocytized. These later observations have given rise to the so-called cellular immunity mechanism for malaria (Taliaferro and Mulligan, 1937; Coggeshall, 1943).

The earliest evidence that blood removal or loss in malaria or blackwater fever might have an immunological basis was furnished by Oliver Gonzalez (1944) in his reports of cold agglutinins for human RBCs in serum of patients suffering from blackwater fever.

Gear (1946) postulated that an autoimmune-like mechanism might be responsible for blood loss in malaria.

It remained for Zuckerman (1960) to furnish the first evidence that anemia which was inconsistent with parasitemia in rats infected with Plasmodium berghei might be due to autoimmunization. However, she has since reported that the positive direct Coomb's test on erythrocytes of rats with P. berghei anemia also pertained in rats made anemic by repeated bleeding, or by treatment with phenylhydrazine hydrochloride (Zuckerman and Spira, 1961).

McGhee (1960, 1964) postulated that the anemia of Plasmodium lophurae infections of ducks might have an autoimmune basis since ducks with quinine-suppressed infections became as anemic as did ducks with full blown P. lophurae infections. He later reported that the anemia was accompanied by extensive phagocytosis of uninfected RBCs by macrophages of the bone marrow (McGhee and Corwin, 1964). Corwin and McGhee (1966) found that there were factors present in the plasma of ducks with acute P. lophurae which would cause anemia when injected into normal ducks.

Further evidence of an autoimmune basis for anemia of malaria was furnished by the work of Cox et al. (1966). These workers demonstrated that the anemia of P. berghei infections of rats was accompanied by extensive phagocytosis of uninfected erythrocytes by macrophages of the spleen and bone marrow. Correlated with this anemia and erythrophagocytosis was the presence of agglutinins for trypsinized rat RBCs in the sera of these rats. The presence of anemia-inducing factors in the serum of animals with acute malaria was confirmed by

the demonstration that globulins from monkeys with acute Plasmodium knowlesi would upon injection cause anemia in rats (Cox, 1966).

It appeared that similar mechanisms were associated with the anemia of acute babesiosis. The anemia of acute B. rodhaini infections of rats is not consistent with parasitemia and is also accompanied by phagocytosis of uninfected RBCs by macrophages of spleen and bone marrow which is, in turn, correlated with the presence of agglutinins for trypsinized rat erythrocytes (Schroeder et al., 1966).

The presence of anemia-inducing substances in the sera of animals (dogs and rats) with acute babesiosis (B. canis and B. rodhaini) has also been demonstrated (Sibinovic et al., 1967b). Sibinovic et al. (1968) demonstrated that substances in the sera of such animals quickly combined with RBCs after injection and caused the cells to be sequestered in the spleen within 24 hours. In as much as substances present in the serum during acute malaria and babesiosis were found to give serological cross reactions it has been postulated that the anemia of acute malaria and babesiosis might be mediated by identical or similar substances (Cox et al., 1968; Cox and Milar, 1968).

Evidence has been presented that the severe anemia associated with anaplasmosis of cattle might be mediated by similar mechanisms (Mann and Ristic, 1963; Kreier et al., 1964; Schroeder and Ristic, 1965, 1965a).

4. RENAL DISEASE IN MALARIA AND BABESIOSIS

It has been known for many years that nephritis has been associated with cases of malaria. Reports by Atkinson (1884), Thayer

(1899), Marchiafava and Bignami (1900) have described glomerulonephritis in quartan malaria. Craig (1909) reported some form of nephritis in 3% of all cases of malignant tertian. James (1910) described acute nephritis associated with edema during Plasmodium malariae infections. Clarke (1912) and Deeks (1916) found that nephritis was the commonest complication of malaria occurring in the Canal Zone. Goldie (1930) and Giglioli (1930) also found an association of malaria with nephritis. Jansco and D'Angel (1931) in Hungary registered 28 cases of kidney disease where malaria parasites were present in the peripheral blood.

Giglioli (1932) described advanced glomerular changes in P. malariae infections, but only in chronic or recurrent cases. He made histologic studies of 5 fatal cases of malarial nephritis and suggested that the renal lesions found in acute Plasmodium falciparum, and occasionally in P. malariae infection, may progress to a cure, cause a fatal disease, or develop into chronic kidney disease. In P. malariae infections, the process appears to be more gradual. These observations were confirmed by James in 1939 with similar clinical studies from New Guinea and the Solomon Islands.

Carothers (1934) reported 15 cases of subacute nephritis in children in E. Africa; 10 of such cases were infected with P. malariae.

Maegraith and Findlay (1944) studied the pathologic changes in the kidneys in malaria and found that the lumen of tubules were frequently filled with material usually described as "casts" varying in appearance from desquamated epithelium and red blood cells, to reddish-brown granules and spherules, the composition of which was

uncertain. The material within the lumen was most abundant in the distal convoluted tubules, ascending loops of Henle and the collecting tubules. The contents of the lumens also changed from the proximal to the distal extremities of the nephron. In the proximal and distal tubules the contained material was often more obviously cellular in nature, consisting of desquamated cells and portions of cells in various stages of degeneration; in the ascending loops of Henle and the collecting tubules the granules were larger and may reach the size of an erythrocyte. Spitz (1946) found hypercellular glomeruli and swelling of the tuft of the glomerulus in 9 of a series of 50 cases of malignant tertian malaria; he also found changes in the different tubules of the kidney.

Gilles and Hendrickse (1960) reported 80 cases of nephrosis in children in Nigeria where P. malariae was found in 89.6% of the cases. They also indicated the close association between P. malariae and renal disease.

Giglioli (1962a,b) reviewed the evidence of a causal relationship between malaria, particularly quartan malaria and nephritis. He pointed out the dramatic decrease in the incidence of nephritis followed the eradication of malaria, particularly quartan malaria.

Kibukamusoke et al., (1967) also suggested that there was a causal relationship between P. malariae and nephrotic syndrome, and that the mechanism involved might be a sensitization phenomenon, based on the presence of circulating antigen-antibody complexes. A similar mechanism was suggested by Gilles and Hendrickse (1963).

Malherbe and Parkin (1951) studied the atypical symptomatology

of B. canis infection of dogs and found that the kidneys were deeply involved in babesiosis and that renal damage usually followed some time after damage to the liver. They suggested that lesions were due partly to the anemia and partly to an intoxication of some sort. They showed that kidney damage ranged from an acute to a chronic nephritis and postulated that there was a possibility of blockage of glomerular tufts by sludged parasitized red blood cells, or that anemia itself may have further damaged the kidney. They also reported that edema was common in various parts of the body.

Siebold and Bailey (1957) found that B. canis parasites were more numerous in smears of kidney than in the peripheral blood. They also found a marked deposition of hemoglobin within the epithelium of the proximal convoluted tubules and that the lower renal tubules were blocked by casts composed of a mixture of both amorphous and crystalline hemoglobin. The blocked tubules showed damage of the epithelium which they suggest was due to the presence of casts. The epithelium of proximal convoluted tubules was swollen and contained some hemosiderin. They concluded that since the dog had survived the parasitemia stage the actual death was due to nephrosis. Severe blood destruction was also evident.

Sippel (1962) found parasitized RBCs in smears made from kidneys of horses infected with B. caballi. The kidneys were swollen, pale yellow and some of them had hemorrhagic areas.

Maurer (1962) found nephritis in kidneys of horses infected with B. equi, and felt that B. caballi was more pathogenic than B. equi. He found that edema was limited to the head, especially to the supraorbital areas.

III. MATERIALS AND METHODS

The B. rodhaini strain used in this research was obtained from Dr. Paul E. Thompson, Parke, Davis and Company, Ann Arbor, Michigan. It is maintained by blood passage in mice and rats in this laboratory.

Wistar male rats weighing 405-560 grams were obtained from Harlan Industries, Cumberland, Indiana. They were housed in cages containing not more than five rats. Control rats were housed in separate cages. There were five groups of rats in the experiment; a) ten rats were infected with B. rodhaini to be used for base line data such as erythrocyte counts and parasitemia; b) eight rats were not infected with the parasite and were used to obtain data for uninfected control rats throughout the experiment; c) five rats were necropsied prior to the experiment; d) 32 rats were infected for blood and necropsy samples; e) seven uninfected rats were used for control sera and for control necropsy material. The rats to be used for necropsy and the base line data were inoculated intraperitoneally with 1×10^6 parasitized erythrocytes from infected rats.

At two or three day intervals after infection RBC counts were made on each rat of the infected control group, the uninfected controls and the rats that were selected for necropsy. Blood films to be stained with Wright's stain were taken on each rat of the infected control group and on each rat of the necropsy group at the same intervals. Blood for preparing films and RBC counts was obtained by snipping the tip of the tail of the rat with scissors. Blood for the

counts was collected in red blood cell hemocytometer pipettes and diluted with Hayem's solution. Counts were made microscopically using a Spencer hemocytometer counting chamber.

On day 0, 5 uninfected rats were sacrificed by exsanguination and autopsied. At two day intervals after infection 3 infected rats and 1 uninfected necropsy control rat were sacrificed, with the exception of the 6th day when 4 infected rats were taken, the 9th when 2 infected rats but no control rat were sacrificed and the 12th, 14th, and 16th days when 6, 1, and 7 rats, respectively, were brought to necropsy.

Necropsied rats were etherized and exsanguinated by cardiac puncture. The collected blood was mixed with 1 ml of heparinized saline (100 units per ml of 0.85% NaCl). The plasma was removed after centrifugation at 800 G for 10 minutes and the plasma of each rat was stored at -18°C . The abdominal and thoracic cavities of the sacrificed rats were opened for examination and the kidneys were removed.

Each kidney was cut longitudinally so that one half represented the convex and the other the concave side of the kidney. The tissues were placed in Bouin's fixative for 6 hours. Afterwards they were washed repeatedly in changes of 70% ethanol to remove excess picric acid and were then transferred and stored in 70% ethanol. Dehydration was accomplished with an autotechnicon programmed to give successive treatments of 1 hour in 70% ethanol, 1 hour in 95%, 2 hours in absolute ethanol, 2 hours in xylol for clearing and 1 hour in Paraplast paraffin at 57°C . Each piece of tissue was then oriented and mounted

in a block of paraffin so that the desired sections could be cut with a microtome.

Sections of the blocks were cut 5-6 μ in thickness. The glass slides on which tissue sections were mounted were covered with Mayer's egg albumin. The sections were stained with either hematoxylin-eosin, Giemsa or with Mallory's stain. Pictures were taken with American Optical Microstar equipment using B High Speed Ektachrome ASA 125 transparency films and Plus x Pan Kodak ASA 125 prints.

In histologic study of the kidneys the severity of renal damage was evaluated by a scoring system to give a quantitative measurement. A hundred nephrons were studied and each was scored as 4-3-2-1 or 0. Glomeruli and adjacent tubules in a "very acute" stage were graded as #4 (Fig. 4a and b). Glomeruli and adjacent tubules in an "acute" stage were graded as #3 (Fig. 5a and b). Glomeruli and adjacent tubules in a "sub-acute" stage were graded as #2 (Fig. 6a and b). Near normal nephrons were scored as #1 (Fig. 11) while normal glomeruli with normal adjacent tubules were considered as #0 (Fig. 3a and b). The sum of individual scores was used as an estimate of the severity of disease score (SDK).

Hemagglutination (HA) tests with trypsin-treated normal rat erythrocytes were made on plasma of each of the necropsied rats following modified techniques developed by Morton and Pickles (1947). A solution of trypsin was prepared by adding 0.25 mg of reagent grade trypsin to 100 ml of 0.85% saline. Erythrocytes were obtained from normal rats by cardiac puncture. Two ml of this blood mixed

with 1 ml of heparinized saline and were sedimented at 800 G for 10 minutes. Plasma was removed and the cells were washed once by resuspension in 5 ml of saline. The saline was extracted after recentrifugation and to 0.5 ml of the packed washed cells 4.5 ml of 0.25% trypsin were added. The mixture was incubated for 20 minutes in a water bath at 25°C. The treated cells were then washed 3 times with 5 ml of saline for 10 minutes followed each time by centrifugation at 800 G for 10 minutes to remove the saline. Then a 2% saline suspension of the cells was prepared. In conducting the test 0.2 ml of this RBC suspension was added to an equal quantity of serial twofold dilutions of serum and the test was incubated for 4 hours in a water bath at 25°C. A positive agglutination reaction was represented by formation of a single clump which did not break up readily upon mild agitation of the tube. A negative test reaction was characterized by the uniform resuspension of erythrocytes in serum following mild agitation; aggregates that remained intact after agitation was considered to be 4+, and less tenacious aggregates were graded as 3+, 2+, or 1+. Since trypsinized RBC were excessively sensitive to agglutination, estimates of 1+ were considered doubtful.

IV. RESULTS

Data on the RBC counts and parasitemia of the 10 infected rats used for base line data are shown in Table 1. Data on erythrocyte counts taken at the same intervals on the 8 uninfected rats from the same stock housed in cages adjacent to the infected rats are presented in Table 2.

It may be noted that in Table 1 there was a decrease in the erythrocyte counts as time progressed. Patent parasitemia in the infected rats was first observed after 6 days of infection and there was a rapid increase thereafter until the 10th day. On the 14th day parasitemia had declined in most rats. The peak of parasitemia and its rapid decline was accompanied by severe anemia on the 10th and 12th days (Fig. 1).

In Table 2 it is apparent that there was a gradual but progressive reduction in RBC counts in the uninfected control (monitor) rats which reached its lowest level on the 14th day. However, at no time during the course of the experiment was anemia as prominent in the uninfected rats as it was in the infected rats. The RBC counts of the two groups are compared in Fig. 2.

Gross examination of infected animals necropsied during the 10th to the 14th day revealed that hemoglobinuria was common. Edema and petechial hemorrhage in the periorbital areas was also observed. Small amounts of bloody discharge were often seen about the nostrils.

TABLE 1 Erythrocyte counts ($\text{RBC} \times 10^6$) and percentage of parasitized erythrocytes (%PE) on rats infected with B. rodhaini estimated at 2 day intervals after infection.

Days after infection:

	2		4		6		8		10	
Rat #	%PE	RBC	%PE	RBC	%PE	RBC	%PE	RBC	%PE	RBC
1	-	8.60	-	7.97	-	6.30	4	6.27	1	6.60
2	-	10.16	-	6.54	-	2.14	8	4.16	10	3.59
3	-	12.80	-	8.94	-	6.12	+	6.59	3	3.80
4	-	9.00	-	8.83	-	6.64	+	6.11	1	7.50
5	-	7.13	-	7.59	10	6.85	1	5.84	3	4.06
6	-	4.31	-	6.45	6	6.00	+	6.30	6	4.00
7	-	9.45	-	8.49	++	7.53	6	7.88	47	5.70
8	-	9.70	-	8.99	-	6.17	6	6.17	6	5.79
9	-	9.32	-	5.35	2	5.90	10	3.30	47	3.34
10	-	6.60	-	6.71	-	6.60	3	6.70	10	4.90
$\bar{X}$	-	8.70	-	7.50	1.8	6.24	3.8	5.93	13.4	4.93

++ Less than one percent parasitized erythrocytes.

TABLE 1 (Continuation)

Days after infection:

	12		14		16		18	
Rat #	%PE	RBC	%PE	RBC	%PE	RBC	%PE	RBC
1	1	5.62	-	5.00	-	4.79	-	6.37
2	1	2.59	-	5.27	-	6.23	-	6.29
3	6	2.16	3	5.69	2	5.29	-	3.74
4	20	7.09	5	1.61	dead			
5	3	4.01	1	5.11	-	5.68	1	3.89
6	20	2.74	1	1.69	1	2.16	-	3.87
7	10	2.54	2	1.20	1	2.22	-	3.00
8	-	3.89	2	3.67	-	4.43	-	4.76
9	3	2.01	1	3.39	1	1.81	-	4.43
10	2	3.47	1	3.46	+	2.48	-	4.76
$\bar{X}$	6.6	3.61	1.6	3.60	0.5	3.89	0.1	4.58

Days after infection:

	20		23		25		27	
Rat #	%PE	RBC	%PE	RBC	%PE	RBC	%PE	RBC
1	-	6.27	-	7.31	-	7.93	-	7.49
2	-	4.68	-	4.31	-	5.81	-	6.85
3	-	3.27	-	3.95	-	6.94	-	6.99
5	1	5.69	-	5.81	-	6.76	-	6.93
6	-	5.32	-	5.60	-	6.44	-	6.17
7	-	4.88	-	4.75	-	4.71	-	6.29
8	-	4.40	-	6.10	-	6.59	-	6.95
9	-	4.43	-	4.69	-	5.84	-	6.88
10	-	6.00	-	6.98	-	6.90	-	6.80
$\bar{X}$	0.1	4.99	-	5.50	-	6.43	-	6.81

TABLE 2 Erythrocyte counts ($\text{RBC} \times 10^6$) at 2 day intervals on uninfected (monitor) rats housed in cages adjacent to infected rats of the experiment.

Days after infection:

	0	2	4	6	8	10	12
Rat #							
1	10.00	10.00	9.70	7.16	10.54	10.00	10.00
2	6.75	11.99	8.31	7.43	7.07	7.76	7.80
3	7.52	8.80	9.24	7.28	7.95	8.00	7.90
4	8.09	11.34	7.63	7.46	9.70	7.50	7.00
5	8.64	7.00	6.45	7.40	7.31	7.00	6.20
6	8.83	7.67	9.82	7.53	7.02	7.24	6.50
7	8.09	10.36	6.80	7.07	6.16	6.59	6.78
8	8.86	10.00	7.53	6.26	6.52	7.00	7.40
$\bar{x}$	8.31	9.64	8.18	7.14	7.78	7.63	7.44

Days after infection:

	14	16	18	20	23	25
Rat #						
1	7.29	8.00	7.17	7.31	8.30	8.33
2	6.90	8.30	8.50	7.80	8.09	7.40
3	7.63	8.57	7.30	7.10	8.31	7.43
4	8.04	7.29	7.10	7.50	7.80	7.99
5	6.55	6.74	6.78	6.53	7.81	7.31
6	6.40	8.32	7.80	6.99	7.80	7.61
7	6.64	8.24	7.90	8.00	8.64	7.63
8	7.29	6.28	6.90	7.80	8.10	7.47
$\bar{x}$	7.00	7.71	7.43	7.37	8.10	7.77

Fig. 1 Average RBC counts and average percent parasitized erythrocytes
at 2 day intervals in the infected control rats.

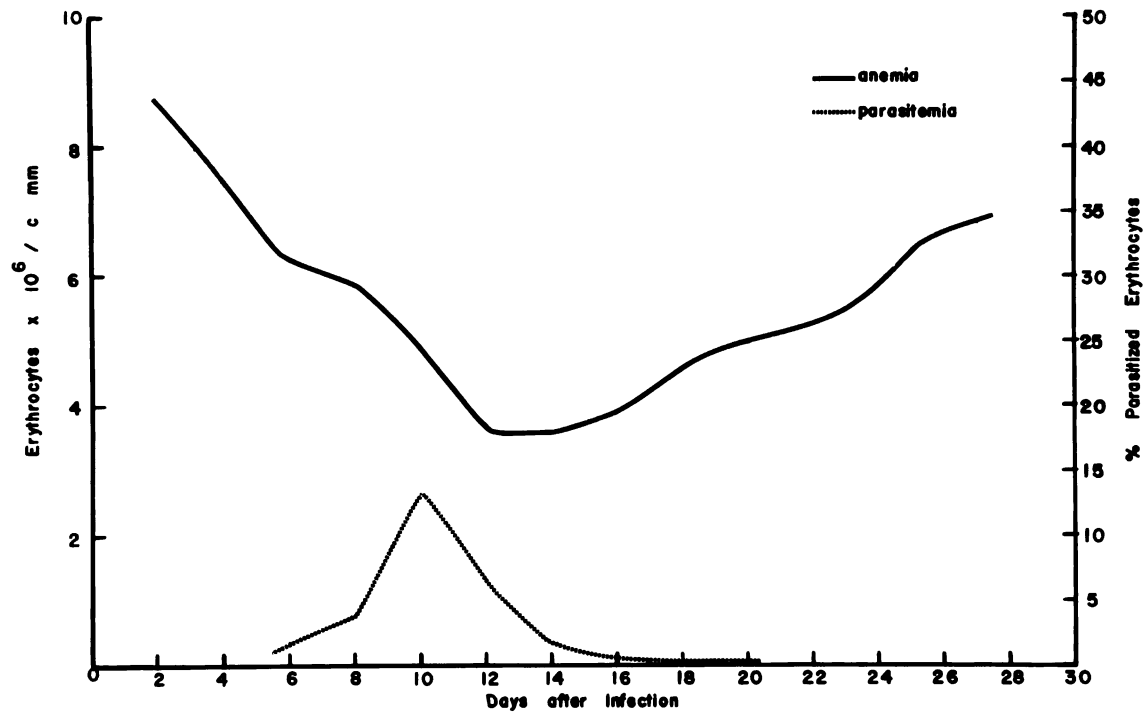
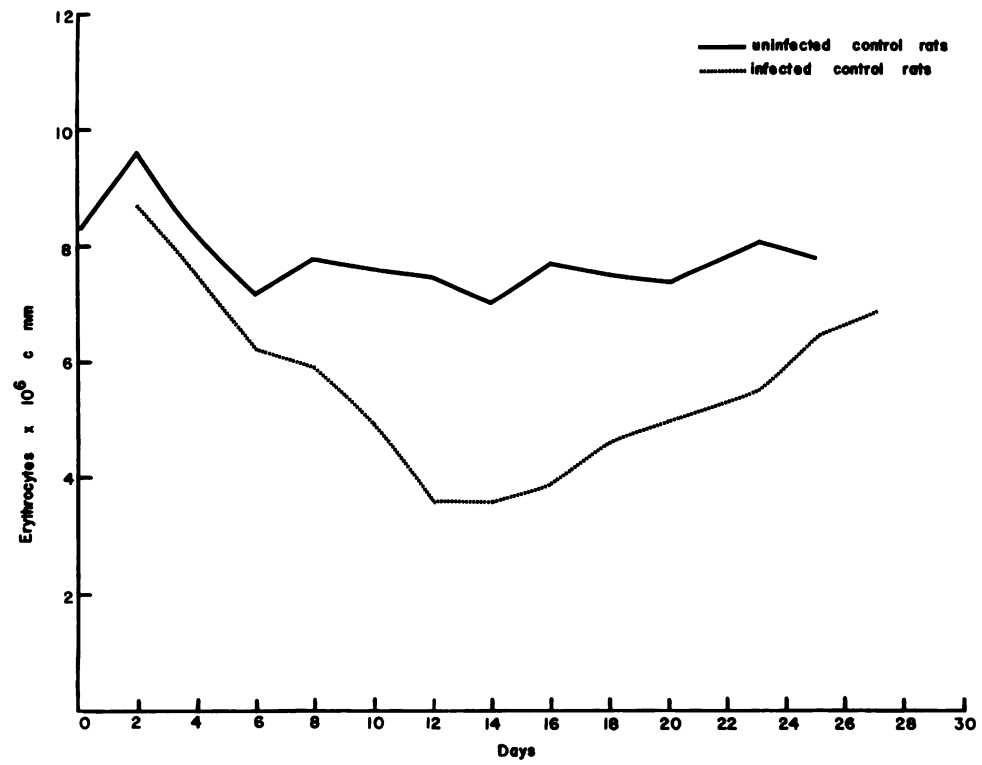


Fig. 2 Average RBC counts at 2 day intervals for infected control rats and uninfected control rats of the experiment.



The kidneys appeared to be swollen and very dark during the same period. Initially, the discoloration was uniform but shortly scattered brownish speckling of the cortices was seen. Some of the kidneys were yellow to white or gray in color. The renal tissue bulged from the cut surface and the cortex was cloudy. The liver and the spleen were also enlarged during the same period.

Histologic examination of the kidneys of the rats sacrificed at the various intervals during the course of the experiment showed that there were marked changes in the structure of the glomeruli. Accompanying these changes there was swelling in the walls of proximal and distal convoluted tubules which in many cases appeared to close the lumen. In kidneys taken during the later period of the experiment hyaline casts became prominent in the distal tubules. Blood pigment (hemosiderin) became prominent on the 14th day in the cells of the convoluted tubules adjacent to the blood vessels. Thrombi and hemorrhages were not remarkably evident. However, in some kidneys taken after the 10th day, extravasated RBCs were found in the interstitial spaces of the cortex adjacent to the capsule. RBCs were not observed in the tubules themselves. Shedding of the epithelium of the tubules accompanied by necrosis was observed in later stages of infection.

Figures 3a and 3b are photographs of a glomerulus and adjacent tubules, respectively, from rats not infected with B. rodhaini. Glomeruli and tubules appearing in this condition were considered as normal and assigned a score of zero.

Figures 4a and 4b show a glomerulus and adjacent tubules which

Fig. 3a Section of a glomerulus of a kidney from an uninfected rat. Hematoxylin and eosin. (H. and E.), x 45.

Fig. 3b Section of convoluted tubules of a kidney from an uninfected rat. The epithelial lining is intact and the lumens of the tubules are patent. H. and E., x 45.

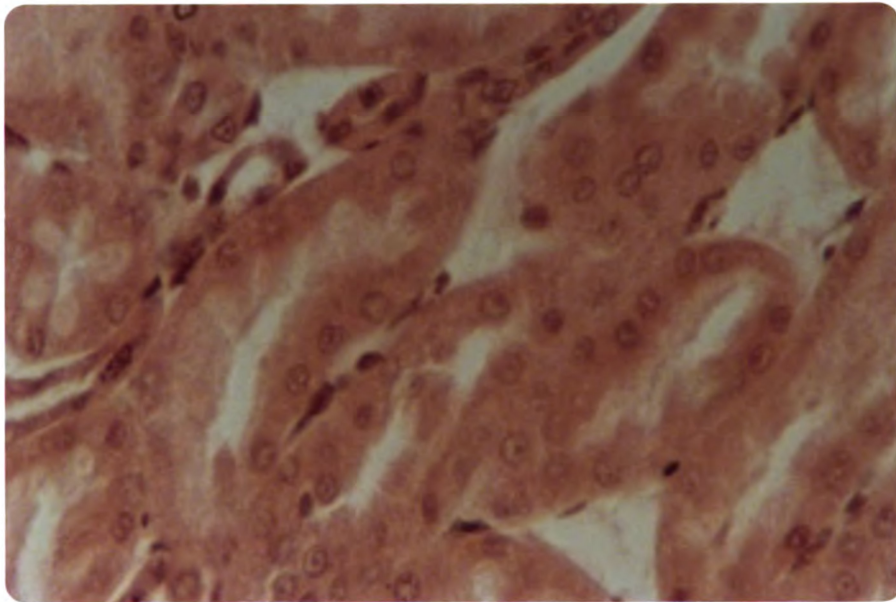
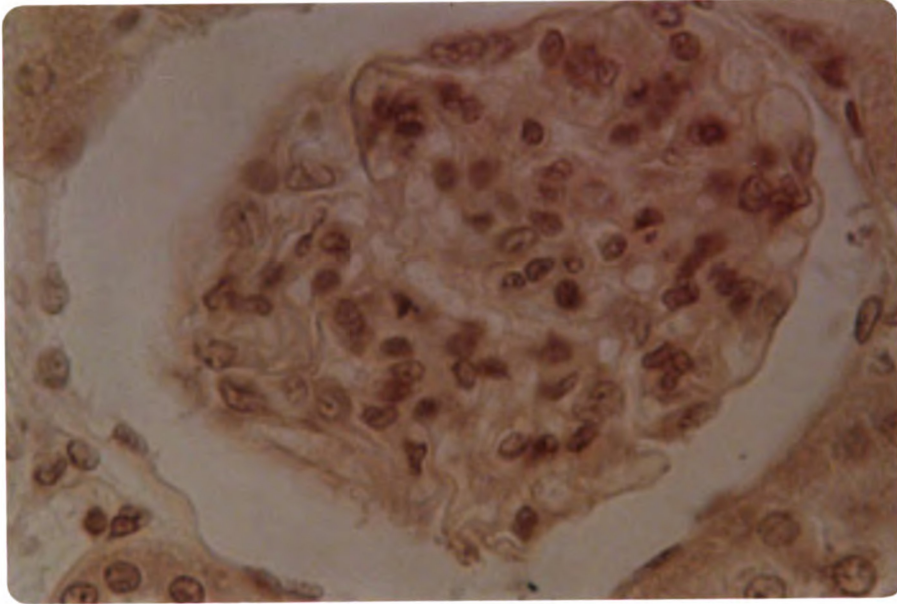
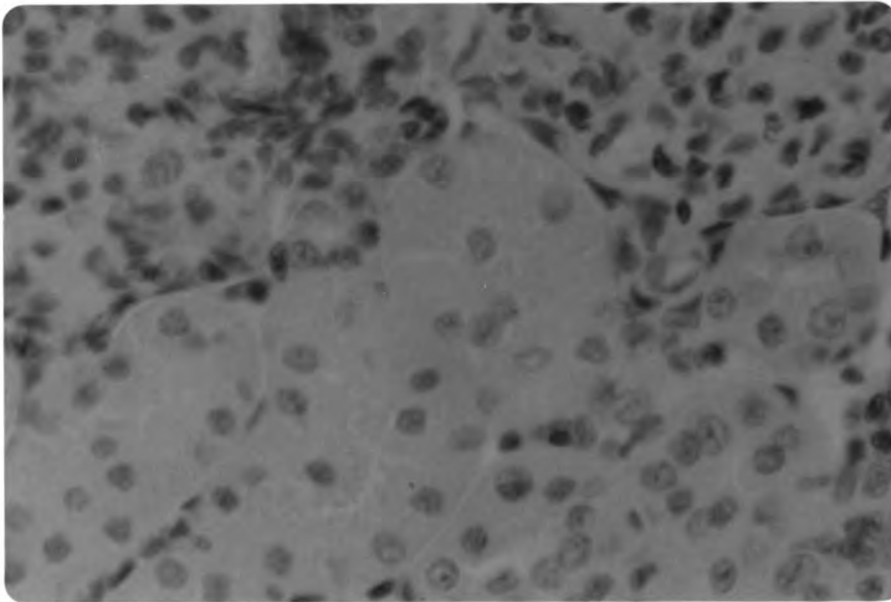
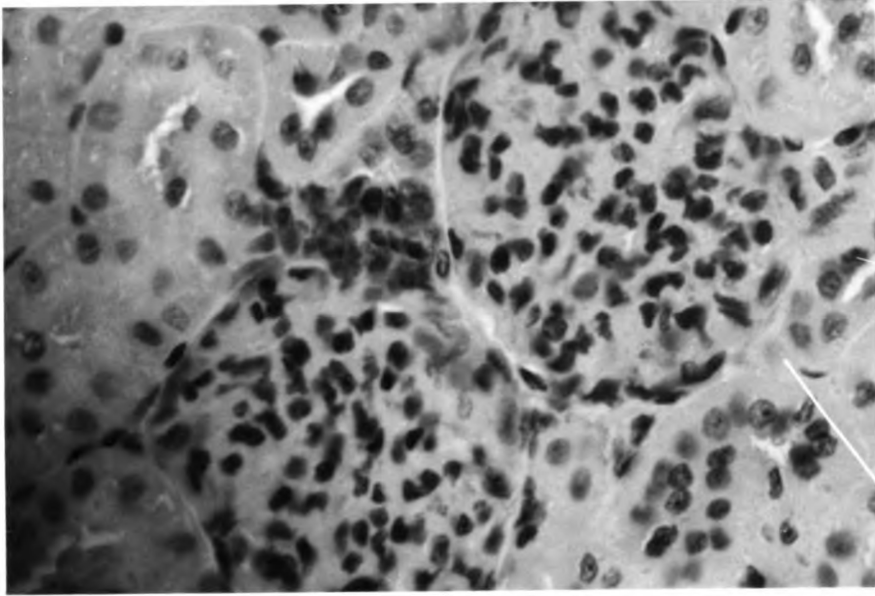


Fig. 4a Section of a glomerulus of a kidney from a rat infected with B. rodhaini. There is evidence of hypercellularity and swelling of endothelial cells that fills Bowman's space. H. and E., x 45.

Fig. 4b Section of convoluted tubules of a kidney of a rat infected with B. rodhaini. Tubular epithelium is swollen and the lumen is closed. H. and E., x 45.

* Glomeruli and tubules found in this condition were graded as "very acute" and given an evaluation of 4.



were considered to be "very acute" and were graded as 4. It will be noted that the structural integrity of the glomerulus has been changed. Bowman's space appears to be filled with disorganized cells of the glomerulus, which is due to hypercellularity and swelling of the endothelial cells. The lumen of the tubules has been obliterated from swelling of the tubular epithelium.

Figures 5a and 5b show a glomerulus and adjacent tubules which were considered to be "acute" and were graded as 3. The Bowman's space was not as compressed by the enlarged glomerulus as it was in the "very acute" stage. The adjacent tubules were swollen but the lumens were not as closed as in a "very acute" stage.

Figures 6a and 6b represent a glomerulus and adjacent tubules which were considered to be "sub-acute" and were graded as 2. The glomerulus appears to be segregated into semilunar cellular masses. Formation of "semilunar crescents" results in partial obliteration of Bowman's space. Thickening of the capsule which also tended to obliterate Bowman's space is evident in Mallory's stained sections (Fig. 6a). The tubules associated with such glomeruli presented desquamated and necrotic epithelium. The lumen of the tubules contained cellular debris (Fig. 6b).

Fig. 7 represents a glomerulus corresponding to an intermediate condition between "acute" and "sub-acute" glomerulonephritis. A thickening of Bowman's capsule with intensive staining may be noted. Intensive staining of the capillary endothelium and the basement membrane of the convoluted tubules is evident.

Figure 8 shows convoluted tubules most of which contain hyaline casts. Interstitial edema is also evident. This was a consistent

Fig. 5a Section of a glomerulus of a kidney of a rat infected with B. rodhaini. There is evidence of hypercellularity and swelling of endothelial cells that nearly fills Bowman's space. H. and E., x 45.

Fig. 5b Section of convoluted tubules and part of a glomerulus of a kidney of a rat infected with B. rodhaini. Tubular epithelium is swollen but there is slight patency of the lumen of the tubules. H. and E., x 45.

* Glomeruli and tubules found in this condition were considered as "acute" and given an evaluation of 3.

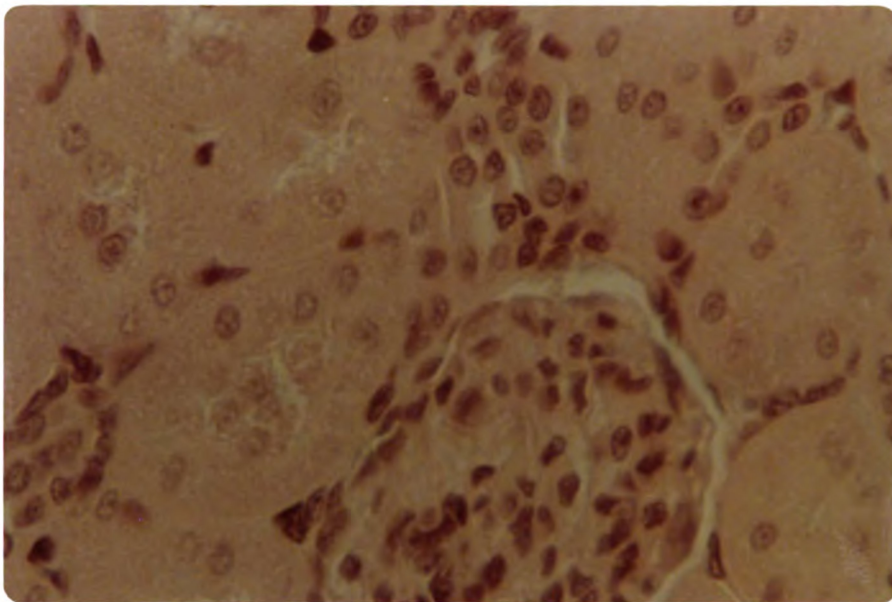
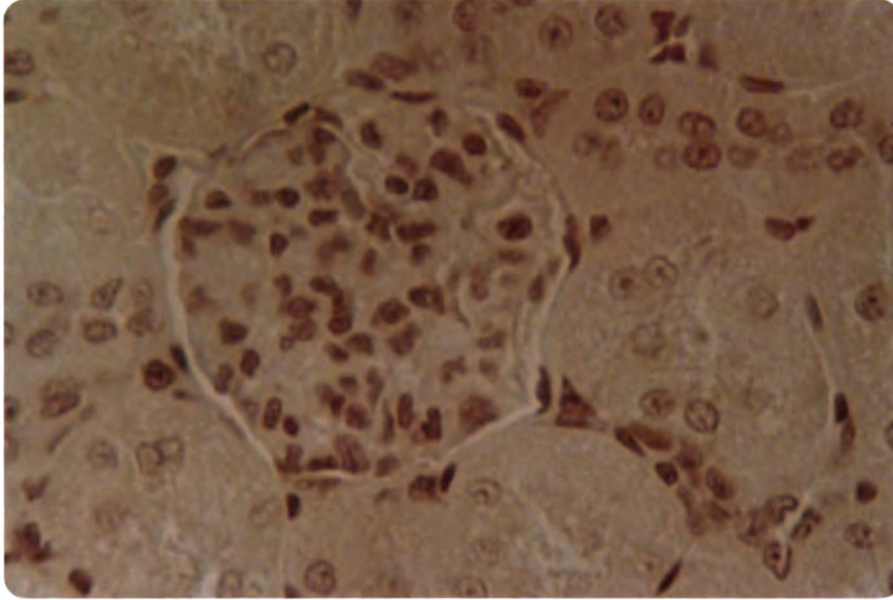


Fig. 6a Section of a glomerulus from the kidney of a rat infected with B. rodhaini. The glomerulus has become lobulated to form semilunar crescents part of which adhere to Bowman's capsule. Mallory's stain, x 45.

Fig. 6b Section of convoluted tubules from the kidney of a rat infected with B. rodhaini. The epithelium of the convoluted tubules is necrotic and the lumens are open. Giemsa stain, x 45.

* Glomeruli and tubules found in this condition were considered as "acute" and given an evaluation of 2.

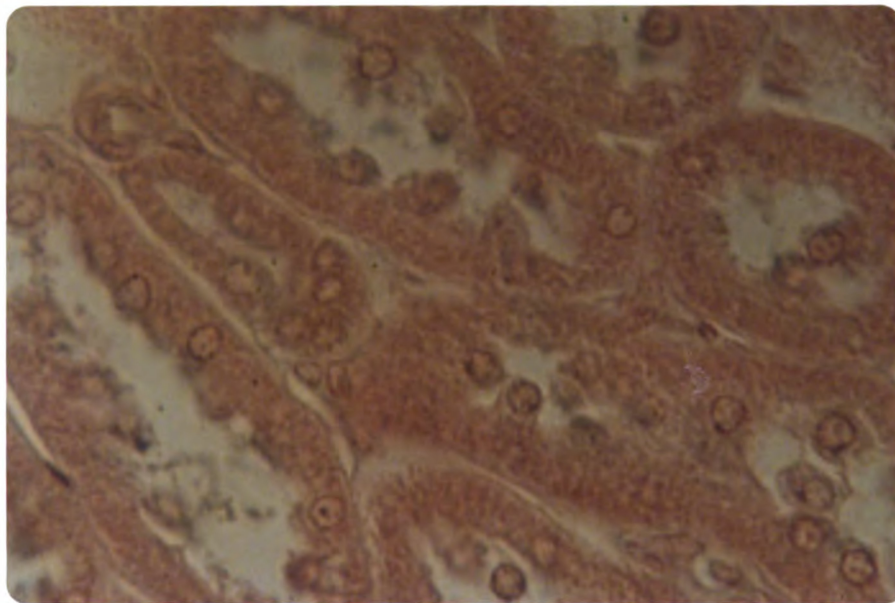
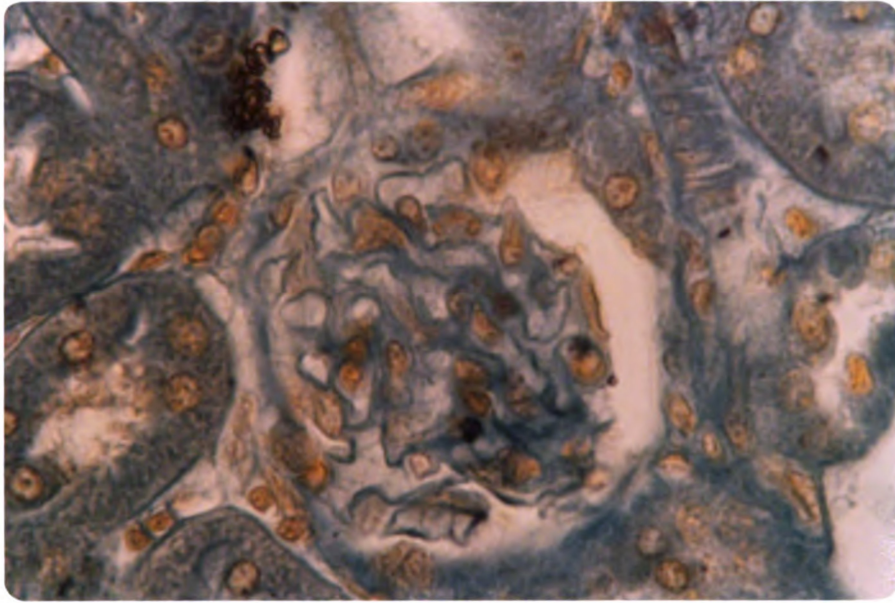


Fig. 7 Section of a glomerulus of a kidney of a rat infected with B. rodhaini showing a condition intermediate to acute and sub-acute. A thickening of Bowman's capsule with intensive staining may be noted. Intensive staining of the capillary endothelium and the basement membrane of the convoluted tubules is evident. Mallory's stain, x 45.

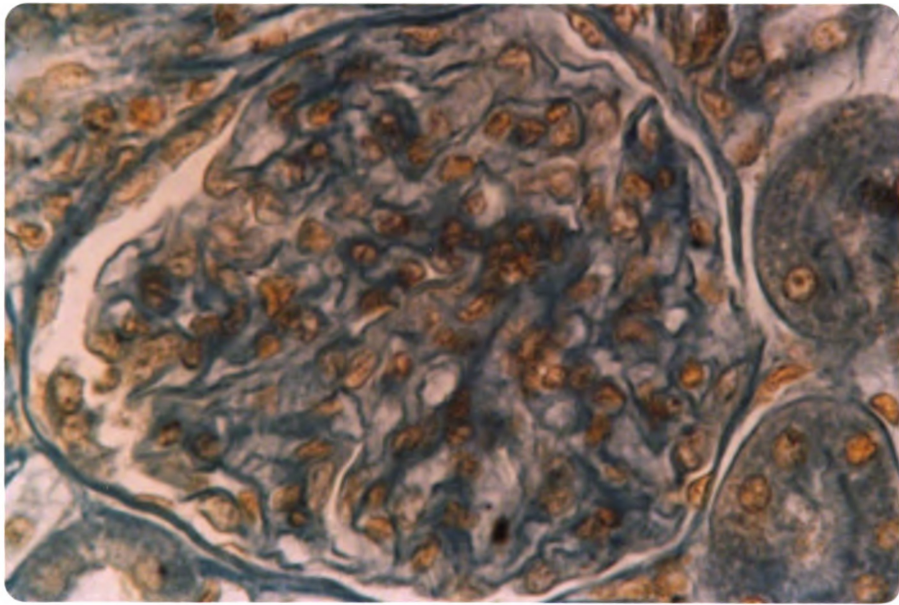
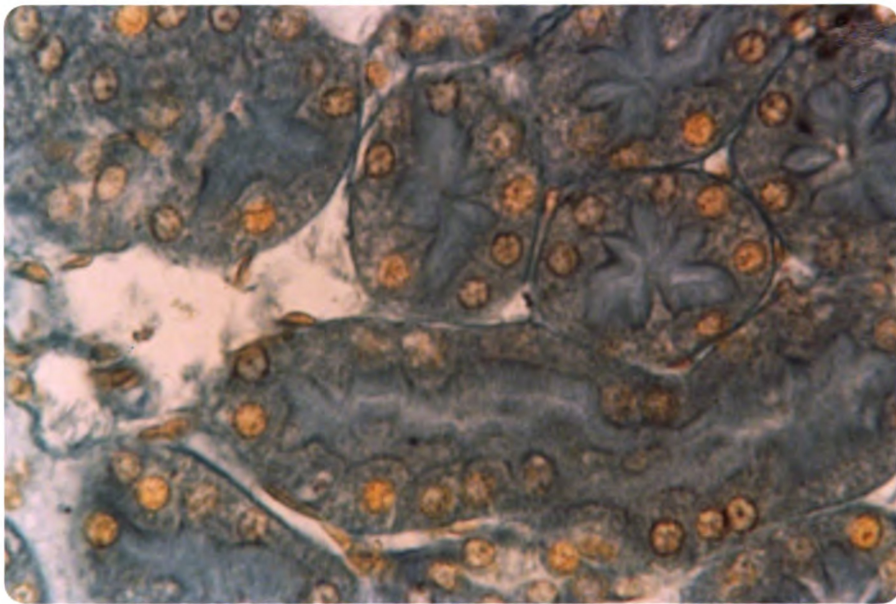


Fig. 8 Section of convoluted tubules of a kidney of a rat infected with B. rodhaini sacrificed after 12 days of infection. The tubules are filled with hyaline casts. There is edema in the interstitial space. Mallory's stain, x 45.



finding in rats sacrificed 12 to 14 days after infection. Figure 9 illustrates an example of the extravasated RBCs in the interstitial spaces of the cortex near the capsule in rats necropsied after the 10th day of infection. Deposits of hemosiderin seen in tissues adjacent to the tubules after the 12th day of infection are shown in Figure 10.

Figure 11 represents a glomerulus as seen during the period in which rats were recovering from acute infection. The glomerulus and tubules approximate those observed in normal rat kidney and were scored as 1. In the study of the sections, it was found to be difficult to determine that a score of 1 was preferable to a value of 2 or 0. Therefore judging of damage less than 2 but greater than 0 was abandoned.

The data on RBC counts, parasitemia, HA titer, and evaluation of kidney section slides (SDK) from each rat brought to necropsy are summarized in Table 3. It can be noted that rats in the earlier days of experiment which had reduced RBC counts tended also to have scorable kidney damage. Significant damage accompanied by reduced RBC counts was evident as early as 2 days after infection. In all of the rats including some of the controls, scores indicating significant degrees of glomerular damage were obtained. The highest mean score of kidney damage was obtained on the 9th day after infection. Scores of kidney damage in the rats sacrificed on the 10th, 12th, and 14th days after infection indicated that the condition of the kidney tended to subside from an "acute" to a "sub-acute" stage.

To test the possibility that significant degrees of kidney damage were related to the presence of autoantibody-like substances in the serum the data from the rats presented in Table 3 were separated

Fig. 9 Section of the peripheral cortex of a kidney of a rat infected with B. rodhaini sacrificed after 12 days of infection. Interstitial space is filled with extravasated erythrocytes. H. and E., x 45.

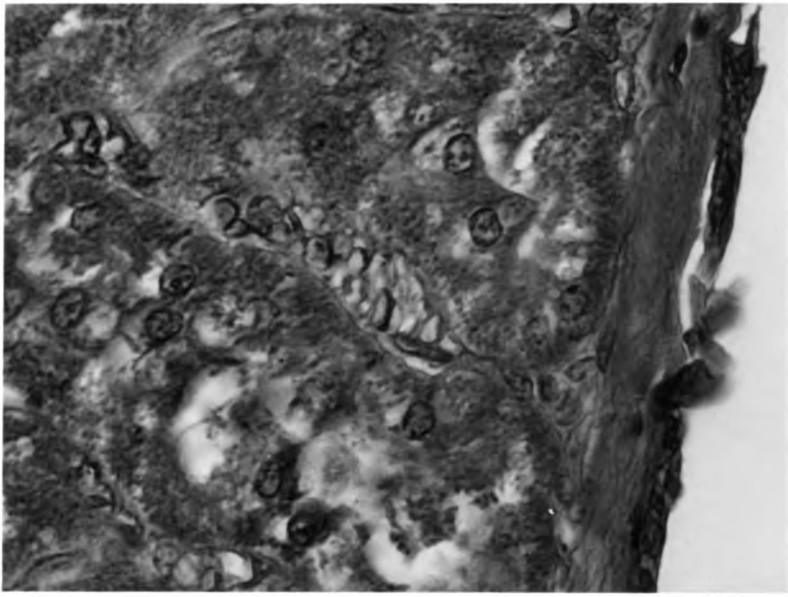


Fig. 10 Section of convoluted tubules of kidney of a rat infected with B. rodhaini sacrificed after 12 days of infection. Deposits of hemosiderin are seen in the tissues. Giemsa stain, x 45.

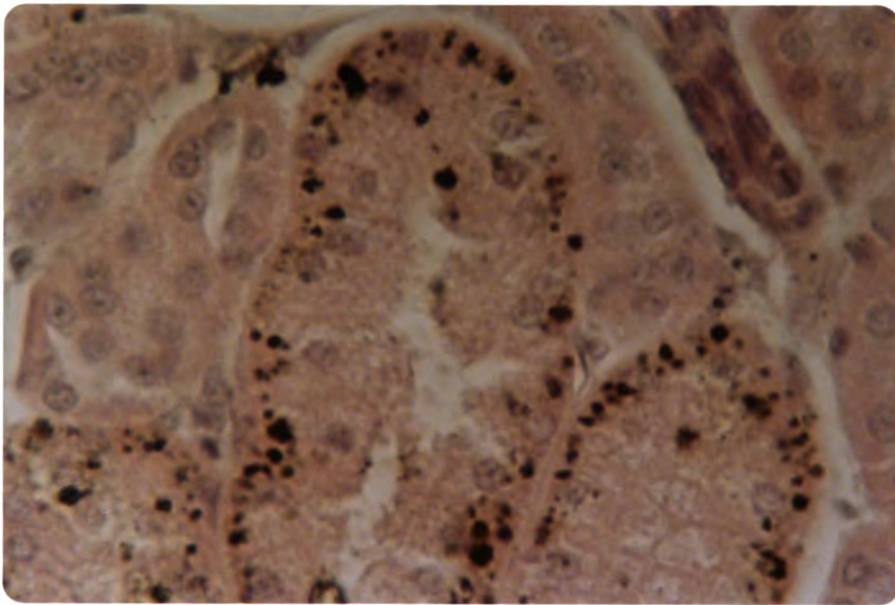


Fig. 11 Section of a glomerulus from the kidney of a rat infected with B. rodhaini after the rat has recovered from acute infection. Deposits of hemosiderin are seen in the tissues. Giemsa stain, x 45.

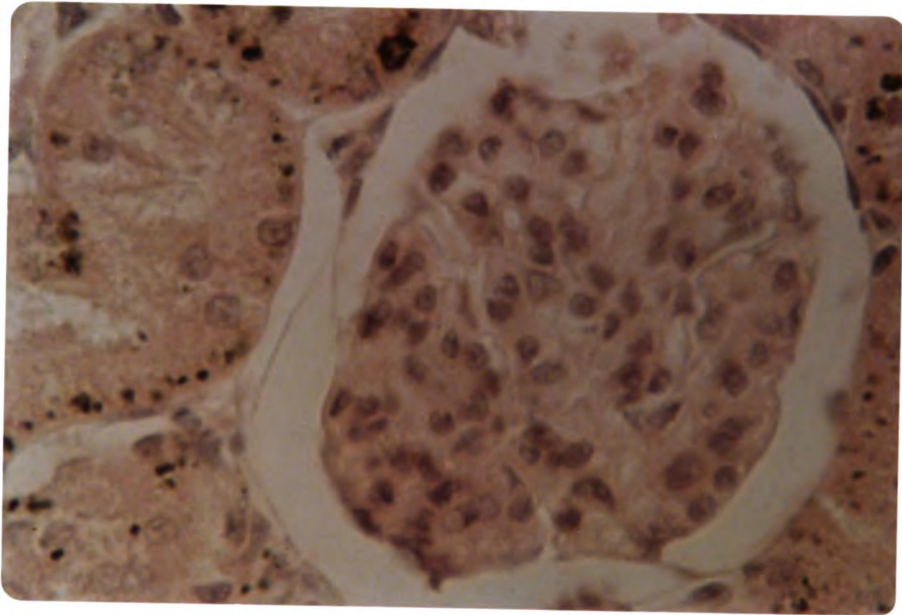


TABLE 3 Erythrocyte counts ($\text{RBC} \times 10^6$), percentage of parasitized erythrocytes (%PE), reciprocals of titers for agglutinins for trypsinized rat erythrocytes (HA), and evaluation score for kidneys (SDK) of all the rats necropsied during the experiment.

Days	Rat #	RBC	%PE	HA	SDK
0	1	8.64	-	-	0
0	2	7.05	-	-	0
0	3	7.04	-	-	0
0	4	9.40	-	-	0
0	5	7.75	-	-	0
2	1	8.08	-	-	0
2	2	6.85	-	-	250
2	3	5.61	-	-	160
2 c*	4	6.24	-	1:4	40
4	1	6.63	-	1:16	290
4	2	7.13	-	-	90
4	3	6.78	-	-	150
4 c	4	7.05	-	1:4	42
6	1	8.16	-	1:16	280
6	2	5.12	-	1:64	300
6	3	6.00	-	1:32	210
6	4	7.26	+	1:16	180
6 c	5	6.86	-	-	100

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* Glomeruli and tubules found in this condition were considered as "acute" and given an evaluation of 2.

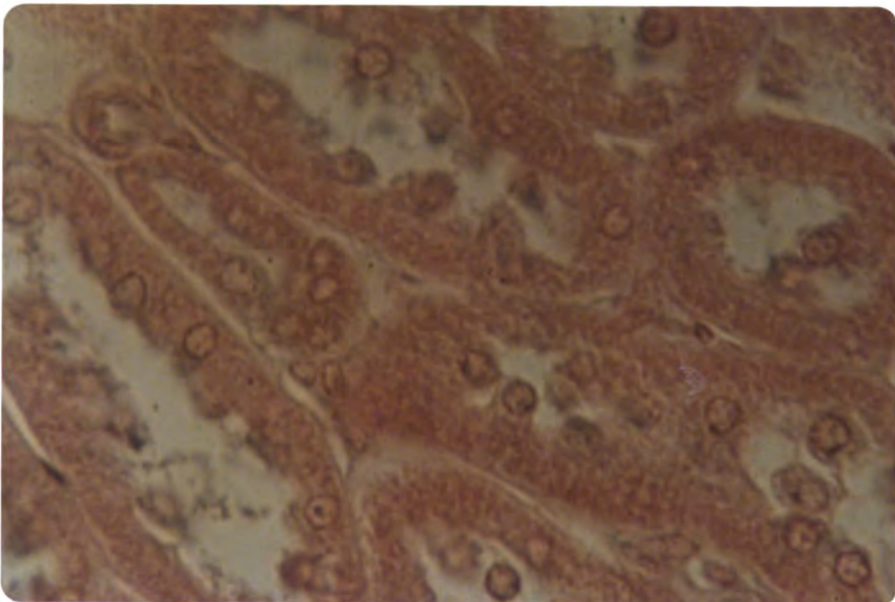
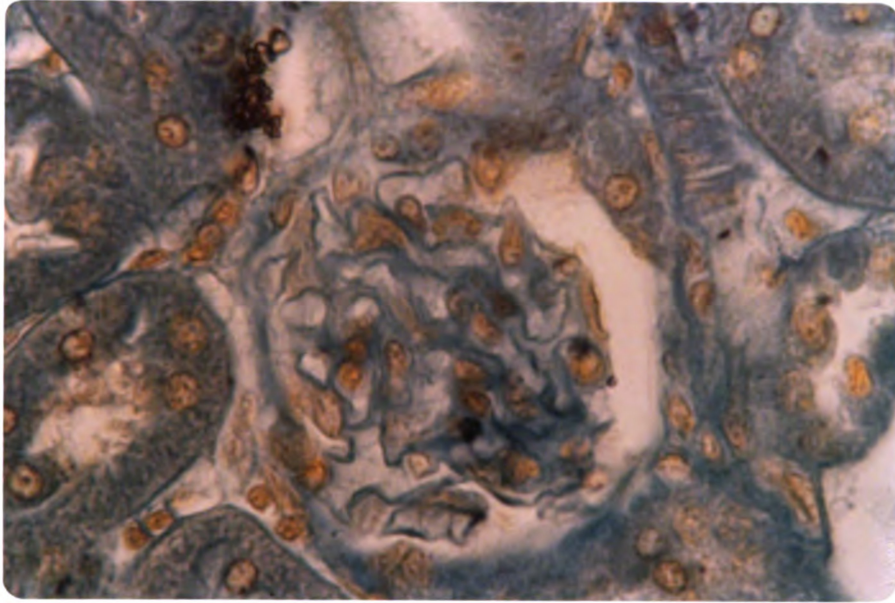


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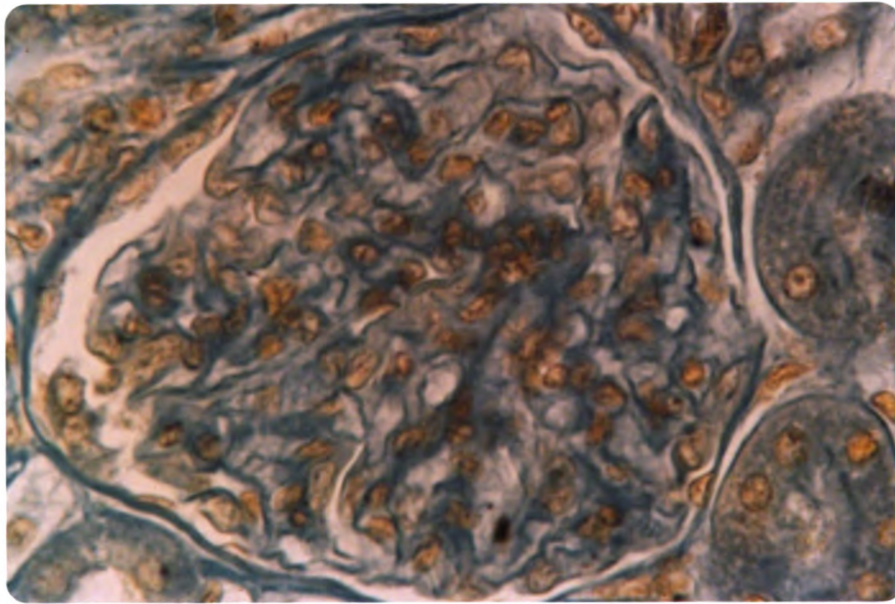
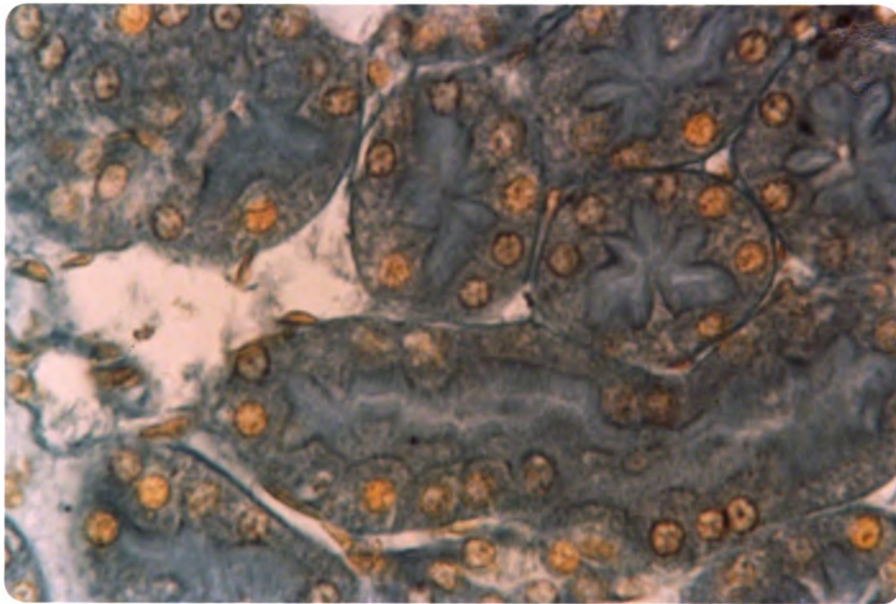


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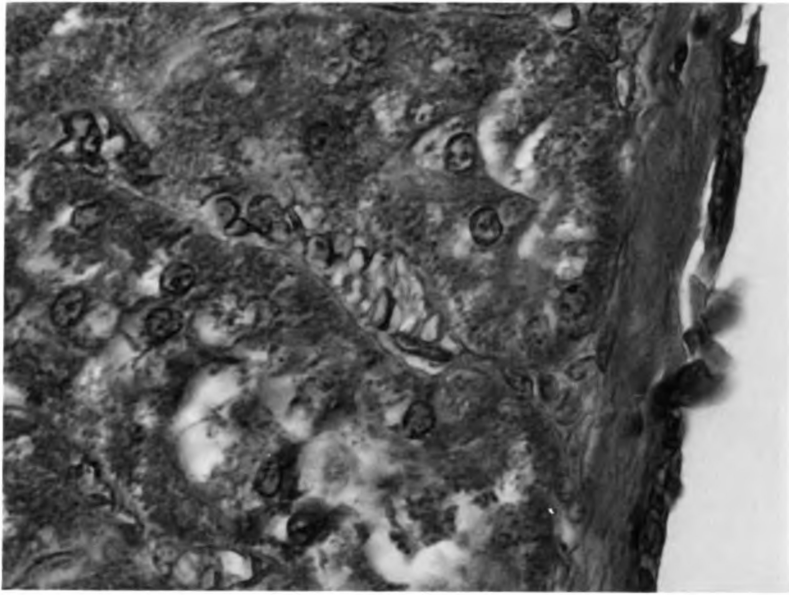


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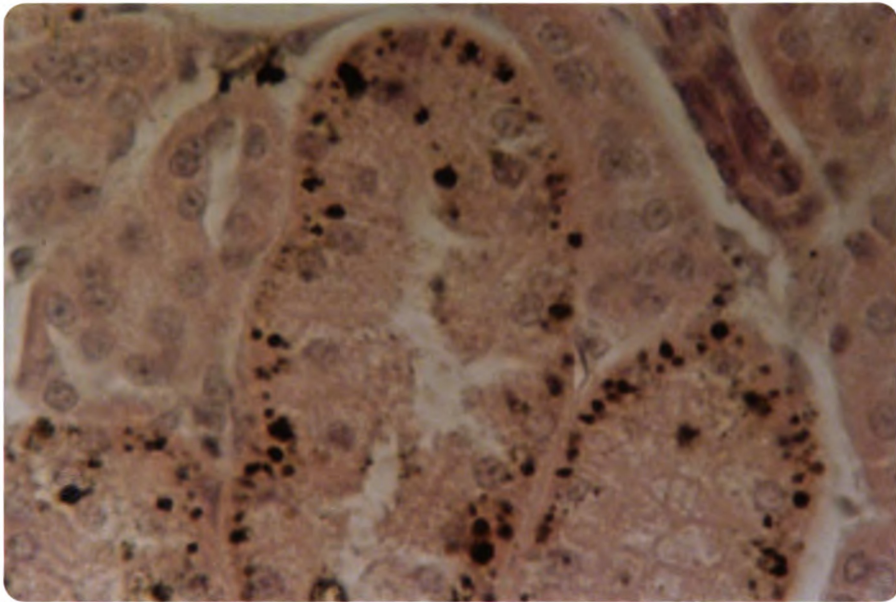


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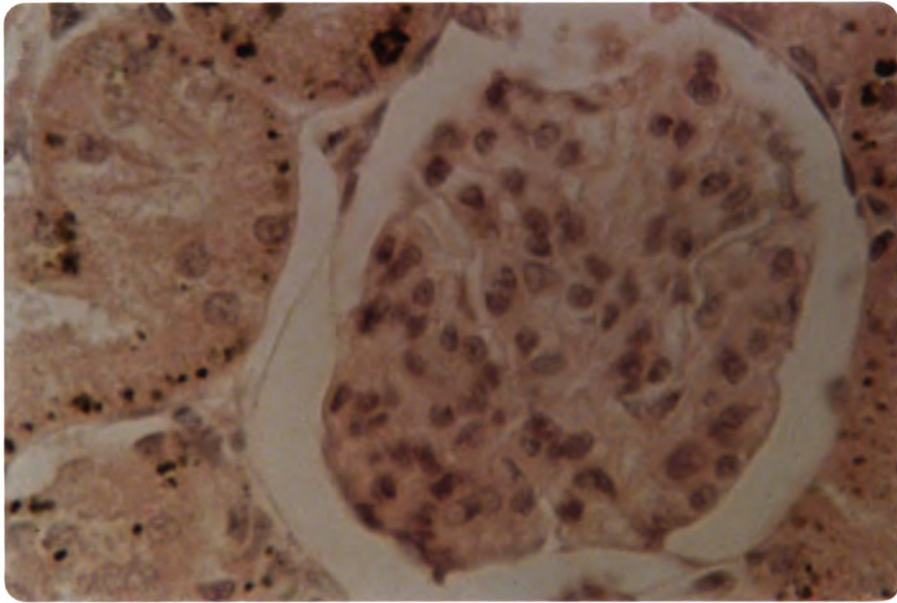


TABLE 3 Erythrocyte counts ($\text{RBC} \times 10^6$), percentage of parasitized erythrocytes (%PE), reciprocals of titers for agglutinins for trypsinized rat erythrocytes (HA), and evaluation score for kidneys (SDK) of all the rats necropsied during the experiment.

Days	Rat #	RBC	%PE	HA	SDK
0	1	8.64	-	-	0
0	2	7.05	-	-	0
0	3	7.04	-	-	0
0	4	9.40	-	-	0
0	5	7.75	-	-	0
2	1	8.08	-	-	0
2	2	6.85	-	-	250
2	3	5.61	-	-	160
2 c*	4	6.24	-	1:4	40
4	1	6.63	-	1:16	290
4	2	7.13	-	-	90
4	3	6.78	-	-	150
4 c	4	7.05	-	1:4	42
6	1	8.16	-	1:16	280
6	2	5.12	-	1:64	300
6	3	6.00	-	1:32	210
6	4	7.26	+	1:16	180
6 c	5	6.86	-	-	100

TABLE 3 (Continuation)

Days	Rat #	RBC	%PE	HA	SDK
8	1	4.92	4	1:16	290
8	2	5.39	-	1:16	300
8	3	6.24	1	1:32	300
8 c	4	6.00	-	1:32	200
9	1	5.90	+	1:16	270
9	2	5.06	4	1:64	400
10	1	5.84	2	1:16	300
10	2	dead	17	1:16	330
10	3	5.08	-	-	130
10 c	4	6.90	-	1:8	122
12	1	5.00	47	1:16	200
12	2	6.50	1	1:16	160
12	3	3.00	27	1:8	220
12	4	3.67	10	1:32	300
12	5	6.18	4	1:64	310
12	6	4.38	4	1:64	300
12 c	7	6.00	-	1:4	88
14	1	4.82	8	1:8	200
14	2	6.26	1	1:16	250
14	3	5.90	1	1:4	200
14	4	7.76	3	1:32	300
14	5	6.70	10	1:64	202
14	6	3.32	27	1:8	240
14	7	6.07	1	1:8	200
14 c	8	7.87	-	1:4	60
16	1	6.24	-	1:8	200

c* uninfected control rats

on the basis of whether the HA tests were negative or positive. The data of rats with negative HA tests, rats with titer of 1:8 or less, 1:16, 1:32, and 1:64 are shown in Table 4 and presented as a graph in Figure 12.

The SDK assigned to the kidneys of the 6 infected rats with negative HA tests were considerably lower than those obtained for infected rats with positive tests. There was a general correlation with the higher HA titer and higher SDK values. Rats with a titer of 1:8 or less had an average score of 211, those with 1:16, 259; those with 1:32, 277, and rats with titers of 1:64 had an average score of 302 (Fig. 12).

The data obtained on uninfected control rats brought to necropsy are shown in Table 5. It can be seen that the rats sacrificed on day 0 had moderately constant RBC counts, the HA tests were negative and kidney damage was not evident. There was however, a persistent fall in the red blood cell counts among those rats that were sacrificed thereafter. Anemia was accompanied by both positive HA titer and increase in kidney damage score. However, after the 10th day SDK values of the uninfected rats tended to subside.

TABLE 4 Day of necropsy, erythrocyte counts ($\text{RBC} \times 10^6$), percentage of erythrocytes parasitized (%PE) and evaluation scores of kidney damage (SDK) of necropsied rats infected with B. rodhaini with a negative test for agglutinins for trypsinized rat erythrocytes (HA test), rats with an HA test titer of 1:8 or less, rats with titer of 1:16, 1:32 and 1:64.

Negative HA test:

Days	RBC	%PE	SDK
2	8.08	-	0
2	6.85	-	250
2	5.61	-	160
4	7.30	-	90
4	6.78	-	150
10	5.08	-	130
$\bar{x}$	6.60	-	130

HA test with titer of 1:8 or less:

14	5.90	1	200
12	3.00	27	220
14	4.82	8	200
16	6.24	-	210
14	3.32	27	240
14	6.07	1	200
$\bar{x}$	4.89	10.6	210

TABLE 4 (Continuation)

HA test with titer of 1:16:

Days	RBC	%PE	SDK
4	6.63	-	290
6	8.16	-	280
8	4.92	4	290
8	5.39	-	300
10	dead	17	330
10	5.84	2	300
14	6.24	1	250
12	5.00	47	200
12	6.50	1	160
9	5.90	+	270
6	8.26	+	180
$\bar{x}$	6.26	6.5	259

HA test with titer of 1:32:

6	6.00	-	210
8	6.24	1	300
14	7.76	3	300
12	3.67	10	300
$\bar{x}$	5.90	3.5	277

HA test with titer of 1:64:

6	6.20	-	300
12	6.18	4	310
12	4.38	4	300
14	6.70	10	202
9	5.06	4	400
$\bar{x}$	5.70	4.4	302

Fig. 12 The relationship of SDK and reciprocals of the HA titers
in B. rodhaini infected rats brought to necropsy.

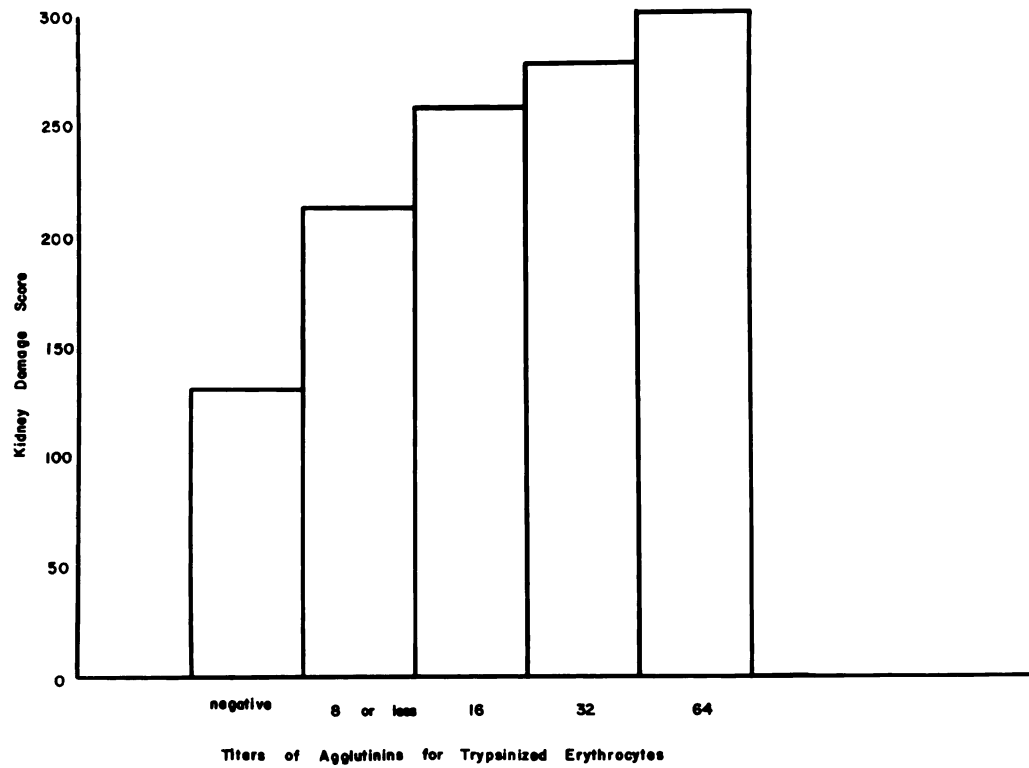


TABLE 5 Erythrocyte counts ($\text{RBC} \times 10^6$), percentage of parasitized RBC (%PE), titer for agglutinins for trypsinized rat erythrocytes (HA) and evaluation score for kidneys (SDK) of normal rats necropsied prior to the experiment and at 2 days intervals.

Days	Rat #	RBC	%PE	HA	SDK
0	1	8.64	-	-	0
0	2	7.05	-	-	0
0	3	7.40	-	-	0
0	4	9.40	-	-	0
0	5	7.75	-	-	0
2	1	6.24	-	1:4	40
4	2	7.05	-	1:4	42
6	3	6.86	-	-	100
8	4	6.00	-	1:32	200
10	5	6.90	-	1:8	122
12	6	6.00	-	1:4	88
14	7	7.87	-	1:4	60

DISCUSSION

Results of the studies presented indicate that a kidney disease is associated with acute B. rodhaini infection of rats. It appears that the kidney disease represents a clear-cut glomerulonephritis uncomplicated by cellular exudate, thrombi or marked hemorrhage. Changes in the nephron were detected as early as 2 days after infection and were maximal on the 8th to the 10th day. Thereafter glomerulonephritis passed from "acute" to "subacute" stage.

Nephritis was detected prior to the appearance of patent parasitemia and became maximal prior to the peak of parasitemia or anemia, which occurred on the 12th to the 14th day. Severity was, however, correlated with the titers of agglutinins for trypsin treated normal rat RBCs in the serum of the rats at the time of necropsy. These agglutinins were detected for the first time in the sera of rats exsanguinated on the 4th day after infection. The higher titers (1:32 or 1:64) were observed between the 8th and 14th day of the infection. The highest agglutinin titers in the sera of individual rats did not seem to be well correlated with the peak of parasitemia. Neither were they correlated with more severe anemia, though all of the rats that presented severe anemia at the time of necropsy did have positive agglutination tests.

It was noted that the most severe cases of glomerulonephritis tended to precede the period of severest illness which was manifested by marked anemia, periorbital edema with petechial hemorrhage and

red urine. The copious amounts of blood or fresh hemoglobin in the urine apparently did not originate in the kidney. There was little evidence of hemorrhage in histologic studies and blood cells were not observed in the convoluted or collecting tubules of the kidney.

It was found that there was marked swelling in the epithelium that appeared to close the lumen of the convoluted tubules which were scored as "very acute" glomerulonephritis. This observation raises the possibility that renal function might have dropped to a low level or ceased at this stage of the disease. An experiment in which renal function tests are performed throughout the course of acute rodent babesiosis seems to be indicated.

It was noted that there was a mild reduction in RBC counts throughout the experiment among the uninfected controls and rats brought to necropsy along with the infected rats. Signs of glomerulonephritis were present in some of the control rats that had reduced counts at the time of necropsy. Some of these rats also had low titers of agglutinins for trypsinized rat RBC in the sera taken at the time of necropsy. However, the degree of renal damage, anemia and the HA test titers of none of the uninfected rats became as acute as found in those with B. rodhaini infection.

Since it was found that Hemobartonella muris is enzootic in the stock rats used for this experiment, it is suspected that this erythrocytic infection might have accounted in part for the observed anemia and the slight glomerulonephritis of the uninfected rats.

Cox and coworkers have investigated H. muris infections. They found that splenectomy of Wistar rats, from the same stock as used

for the present experiments, invariably resulted in the exacerbation of latent H. muris infections with profound anemia and death of the rats. Splenectomized rats with acute H. muris also had agglutinins for trypsinized rat erythrocytes. Intact rats given a massive infection with blood of moribund splenectomized rats also developed moderate to severe anemia and after recovery had antibody to antigens associated with malaria or babesiosis (Nelson et al., 1968). The signs of anemia and the presence of autoantibody-like hemmagglutinins make it reasonable to suspect that kidney damage might also be associated with acute H. muris infections.

Renal disease is known to accompany erythrocytic infections. This has been established in cases of acute malignant tertian malaria, blackwater fever (P. falciparum), and chronic quartan malaria (P. malariae) (Maegraith, 1948). It is also clear that nephritis is a complication of babesiosis at least in the cases of dogs and horses (Malherbe and Parkin, 1951; Sippel, 1962).

The changes in the kidneys associated with human malaria are degenerative. Degenerative and necrotic lesions have been described in the convoluted tubules rather than in the glomeruli in blackwater fever. The lumens of those tubules and those of the distal convoluted tubules were irregularly filled with "casts" composed of hyaline material. Renal lesions of chronic, recurrent or repeated quartan malaria have some similitudes to those found in blackwater fever. The glomeruli are little affected, the necrotic and degenerative convoluted tubules contained desquamated cells, cell debris and hyaline material. In P. falciparum (malignant tertian) malaria, Goldie (1930)

found degeneration of the epithelium of the convoluted tubules in acute infection. Spitz (1946) found enlarged and hypercellular glomeruli accompanied by swelling of the tuft endothelium in several cases of acute malignant tertian malaria.

Kidney disease described for human malaria differed from the condition found in the present experiments. However, it is pointed out that in the first place the laboratory rat may respond differently to mediators of kidney disease and secondly, the presently reported observations were made on necropsied animals, starting soon after infection. None of the animals could truly be considered to be post mortem cases. In contrast to the kidney disease of human malaria there were marked changes in the glomeruli which were accompanied by extensive swelling of the cells of the tubular epithelium. Casts observed in the present work were all hyaline and presumably proteinaceous in nature. Casts consisting of cell debris, blood pigments, pus or other particulate matter were not seen. Desquamation of tubular epithelium was present but occurred late in the infection when the glomeruli had the appearance of "subacute" glomerulonephritis. The blood pigment, hemosiderin, was present in the tissues late in infection, but was not seen in the lumen of the tubules.

Glomerulonephritis has been related to a variety of infectious agents which have been associated with rheumatic fever. Recently it has been found to be well correlated with rejection diseases involving transplanted kidneys in humans. In kidney rejection the severity of nephritis was directly correlated with the number of rejection episodes suffered by the recipient and with the degree of lymphocyte incompat-

ibility of the donor and recipient (Porter, 1966).

It is now generally accepted that glomerulonephritis is in general an immunologic or allergic disease; however, there are different schools of thought about how the disease is mediated. One thought is typified by the concept held by Dixon (1962, 1963, 1966) (Dixon and Humphrey, 1966) and Weigle (1964). This concept is based on the observed activities of antigen-antibody complexes, such as cytotoxicity, and changes in capillary permeability resulting in vascular leakage and edema. Another concept involves autoimmunization or activity of antibodies to self or near self. These thoughts have a basis in the well recognized demyelinating encephalitis which might accompany Pasteur treatment for rabies, or in some of the various idiopathic hemolytic anemias (Dacie, 1962). This concept postulates the formation of antibodies which react with cells and tissues of the animal mediating the antibody. This seems to be the case in erythroblastosis fetalis where antibody to erythrocytes of an Rh+ fetus can be detected in the cord blood. The test employed in this work, for agglutinins for trypsinized erythrocytes, was developed by Morton and Pickles (1947) to demonstrate nonagglutinating antibody to Rh+ cells. It has had broader applications since it is frequently a positive finding in serum from cases of hemolytic anemia (Dacie, 1962). The Coomb's test for erythrocyte bound globulin (direct test), or for circulating erythrocyte binding globulin, is also thought to indicate the presence of autoantibody to erythrocyte (Dacie, 1962).

Cox and associates have given some attention to the presence of autoantibody-like substances in the serum of animals with acute

erythrocytic infections. Agglutinins for trypsinized cells were found in the sera of rats with acute P. berghei (Cox et al., 1966), rats with acute B. rodhaini (Schroeder et al., 1966), and cows with acute Anaplasma marginale (Schroeder, 1966). It was also found that anemia inducing substances which apparently act as opsonins were present in sera of monkeys with acute P. knowlesi (Cox, 1966), dogs and rats with acute babesiosis (Sibinovic et al., 1967a; 1967b; 1968), and ducks with P. lophurae (Corwin and McGhee, 1966).

Though the evidence is indirect, it is suggested that there is ample indication that anemia in erythrocytic infections might be mediated by autoimmune mechanisms.

In the present study, the severity of glomerulonephritis associated with acute B. rodhaini of rats was correlated with the titer of autoantibody-like substances. On the basis of these findings it is suggested that there might be a causal relationship between the two observations.

SUMMARY

A kidney disease has been shown to be associated with acute Babesia rodhaini infection of rats. The disease represented a clear-cut glomerulonephritis uncomplicated by cellular exudate or hemorrhage. Changes in the nephron were detected as early as two days after infection, were maximal on the 8th to the 10th day and thereafter subsided to a subacute state. The maximal changes preceded the peak of parasitemia and anemia, which occurred during the 12th to the 14th day of infection.

The acute stage was characterized by the presence of hypercellularity of the glomeruli due to proliferation and swelling of the endothelial cells. There was edema between the capillaries of the glomeruli which narrowed their lumens. Bowman's space was compressed by the enlarged glomerulus. The epithelium of the adjacent convoluted tubules was swollen and the lumens appeared to be closed. In the later stages the glomeruli appear to be segregated into semilunar crescents some of which were seen adhering to the wall of Bowman's membrane. The convoluted tubules appeared open but presented evidence of necrotic epithelium. Hyaline casts were frequently observed in the later stage when deposits of hemosiderin were also present in tissues of kidney. Hemorrhage was detected only in the later stage of infection in the interstitial spaces in certain areas of the cortex near the capsule.

While the severity of the nephritis was not well correlated

with acute parasitemia or anemia, it was, however, well correlated with the titer of agglutinins for trypsinized erythrocytes in the serum of the rats at the time of necropsy. Since the presence of these agglutinins has come to be accepted as evidence for the presence of functional autoantibodies in immunologic diseases, it is suggested that the glomerulonephritis of acute B. rodhaini might have been caused by autoimmune mechanisms.

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