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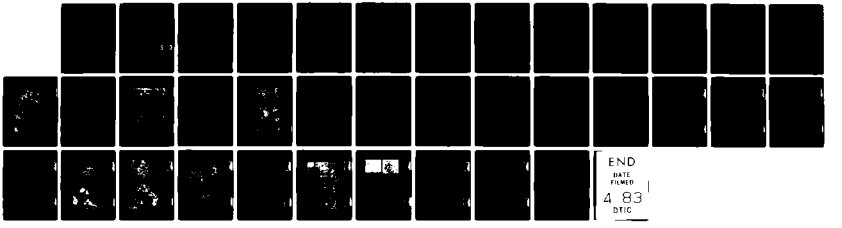

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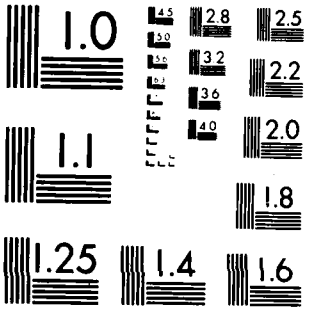

Pathogenesis of African Trypanosomiasis

Final Report

Raymond B. Nagle, Ph.D.

October 1980

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I. BACKGROUND

African trypanosomiasis is characterized by persistent but variable parasitemia. The level of the circulating parasite varies as a result of the interplay of host immunologic responses and parasitic adaptation resulting in changing antigenicity. Previous studies have indicated that the level of parasitemia is at times very high, and specific antitrypanosomal antibodies develop (1). It is hypothesized that this situation leads to periods of antigen excess and it is probable that circulating antigen-antibody complexes are formed. Early studies with Rhesus monkeys infected with Trypanosoma rhodesiense (Strain 1886) indicated pathologic changes within the glomeruli associated with increased serum creatine and in some cases hypoalbuminemia (1). A closer analysis of these renal lesions revealed thickened and duplicated basement membranes associated with mesangial proliferation. These changes resemble the glomerular pathology seen in human membrano-proliferation glomerulonephritis and was associated with persistent hypocomplementemia (2). These initial studies in a small group of animals related depression of serum complement with glomerular change and deposition of IgM and complement components (2). The early findings indicated participation of the alternate pathway of complement activation (4). The first nine months of our present contract has been largely spent on completion and extensions of this work in the Rhesus and is summarized in the following section.

II. RESUME OF PROGRESS TO DATE

A. The first objective of the present contract was to describe in detail the sequence of morphologic and immunohistochemical events in the renal lesions of the Rhesus infected with Trypanosoma rhodesiense (Strain 1886).

1. LIGHT MICROSCOPY

A total of 50 open renal biopsies and terminal biopsies were processed for light microscopy, electron microscopy, and immunohistochemical studies utilizing standard techniques (2).

The sections for light microscopy were cut at 3-4μ and stained with hematoxylin and eosin (H&E), periodic acid-Schiff (PAS) and Masson's trichrome (MT). The biopsies were evaluated and graded on a scale of 0 (no change) to 4+ (severe change) with regard to twelve morphologic parameters. The biopsies were pooled into four groups: controls, 14-15 days, 22-38 days, and greater than 38 days post infection based on earlier observations (2). The results of this analysis are

summarized in Table I. Half of the normal monkeys revealed focal mild glomerular hypocellularity and sclerosis and minimal interstitial change. The earliest changes seen by light microscopy in biopsies obtained at 14-15 days following intravenous injection consisted of swelling of the endothelial cells. In the biopsies, obtained from 22-38 days there was more marked endothelial cell swelling, marked mesangial hypercellularity, capillary basement membrane thickening, mesangial sclerosis, and marked focal interstitial infiltrates of mononuclear and plasma cells. An interesting finding in this time period was the small number of biopsies showing exudation of polymorphonuclear leukocytes 4/25 (16%). Five animals surviving greater than 38 days revealed more pronunced hypercellularity, progressive basement membrane thickening, and mesangial sclerosis. The degree of interstitial infiltration seems to correlate with the degree of glomerular damage (See Table I).

2. ELECTRON MICROSCOPY

Forty biopsies were analyzed by electron microscopy for glomerular ultrastructural changes. In each case four thick sections were made. An average of ten electron micrographs from each case were obtained and analyzed for the presence of the fourteen parameters. These changes are summarized in Table II and shown graphically in Figures 1-5. Figure 6 is taken from a normal control and illustrates minimal basement membrane thickening. Dramatic ultrastructural changes were not seen until 22-38 days at which time there was extensive swelling and vacuolation of endothelial cells, presence of endothelial (38%) and intramembranous electron-dense deposits (67%), mesangial hypercellularity and sclerosis with mesangial deposits (81%). (See Figures 7 and 8). In the four animals which survived greater than 38 days, in addition to these changes, three animals revealed subepithelial electron dense deposits. (See Figure 2) and (Figure 12 of accompanying reprint). This late change is particularly interesting since this resembles the changes seen in rabbits given repeated daily dosages of protein antigen (chronic serum sickness model) whereas the earlier change of hypercellularity and endothelial swelling resemble one shot serum sickness (3). Another interesting late change was the presence of capillary basement membrane duplication (Figure 5) which resembles the changes of human membrano-proliferation glomerulonephritis associated with chronic hypocomplementemia. (4).

3. IMMUNOHISTOCHEMICAL CHANGES

Renal biopsies were tested using the direct immunofluorescence technique for the immunoglobulins, IgA,

TABLE I

MORPHOLOGIC GLOMERULAR CHANGES IN RENAL BIOPSIES

	CONTROL						14-15 DAYS (14)						22-38 DAYS (25)						>38 DAYS (5)					
	0	1	2	3	4	%Δ	0	1	2	3	4	%Δ	0	1	2	3	4	%Δ	0	1	2	3	4	%Δ
CELLULAR SWELLING	6	0	0	0	0	0	9	4	1	0	0	35	12	8	4	0	1	52	3	0	2	0	0	40
HYPERCELLULARITY	3	3	0	0	0	50	7	7	0	0	0	50	2	14	5	1	3	92	0	0	2	1	2	100
PRESENCE OF POLPS	6	0	0	0	0	0	14	0	0	0	0	0	21	1	3	0	0	16	3	0	1	1	0	40
THICKENING OF BASEMENT MEMBRANE	6	0	0	0	0	0	11	3	0	0	0	21	4	13	8	0	0	84	0	0	3	0	2	100
MESANGIAL SCLEROSIS	3	3	0	0	0	50	6	8	0	0	0	57	3	11	9	2	0	88	0	0	2	1	2	100
TUBULAR CASTS	6	0	0	0	0	0	8	5	0	1	0	43	17	4	3	1	0	32	1	1	2	0	1	80
TUBULAR NECROSIS	6	0	0	0	0	0	14	0	0	0	0	0	0	0	0	0	0	0	2	2	1	0	0	60
TUBULAR ATROPHY	6	0	0	0	0	0	13	1	0	0	0	7	23	2	0	0	0	8	2	2	1	0	0	60
INTERSTITIAL INFILTRATES	5	1	0	0	0	17	9	4	1	0	0	35	3	12	8	2	0	88	0	0	0	1	4	100
INTERSTITIAL FIBROSIS	5	1	0	0	0	17	12	2	0	0	0	14	23	1	1	0	0	8	2	1	2	0	0	50
VASCULAR SCLEROSIS	6	0	0	0	0	0	14	0	0	0	0	0	25	0	0	0	0	0	5	0	0	0	0	0
VASCULAR THROMBI	6	0	0	0	0	0	14	0	0	0	0	0	24	1	0	0	0	4	5	0	0	0	0	0

*Each parameter on the left was graded for each biopsy on a scale of 0 (no change) to 4+ (severe change). Each number in the table indicates the number of biopsies at a give time post infection. % > - listed in the right column for each time group indicates the percentage of the biopsies in that group showing changes (1+ to 4+).

TABLE II

GLOMERULAR ULTRASTRUCTURAL CHANGES IN RENAL BIOPSIES

EXPRESSED AS PER CENT SHOWING CHANGE

	CONTROLS (4)		14-15 DAYS (11)		22-38 DAYS (21)		MORE THAN 38 DAYS (4)	
Endothelial Swelling	0	(00)	1	(9)	16	(76)	4	(100)
Endothelial Vacuolation	0	(00)	2	(18)	16	(76)	4	(100)
Subendothelial Deposits	0	(00)	0	(00)	8	(38)	3	(75)
Intramembranous Deposits	0	(00)	1	(9)	14	(67)	2	(50)
Subepithelial Deposits	0	(00)	0	(00)	0	(00)	3	(75)
Polymorphonuclear Leukocytes	0	(00)	0	(00)	5	(24)	3	(75)
Mesangial Cellular Hypertrophy	0	(00)	1	(9)	15	(71)	3	(75)
Mesangial Sclerosis	1	(25)	4	(36)	15	(71)	3	(75)
Mesangial Granular Deposits	0	(00)	1	(9)	17	(81)	2	(50)
Mesangial Vesicular Deposits	0	(00)	4	(36)	16	(76)	3	(75)
Proliferation of SER in Endothelial Cells	3	(75)	3	(27)	8	(38)	3	(75)
Focal BM Thickening	3	(75)	3	(27)	8	(38)	3	(75)
Membrane Duplications	0	(00)	0	(00)	4	(19)	4	(100)
Trypanosomes Present in Capillary Lamina	0	(00)	0	(00)	2	(10)	0	(00)

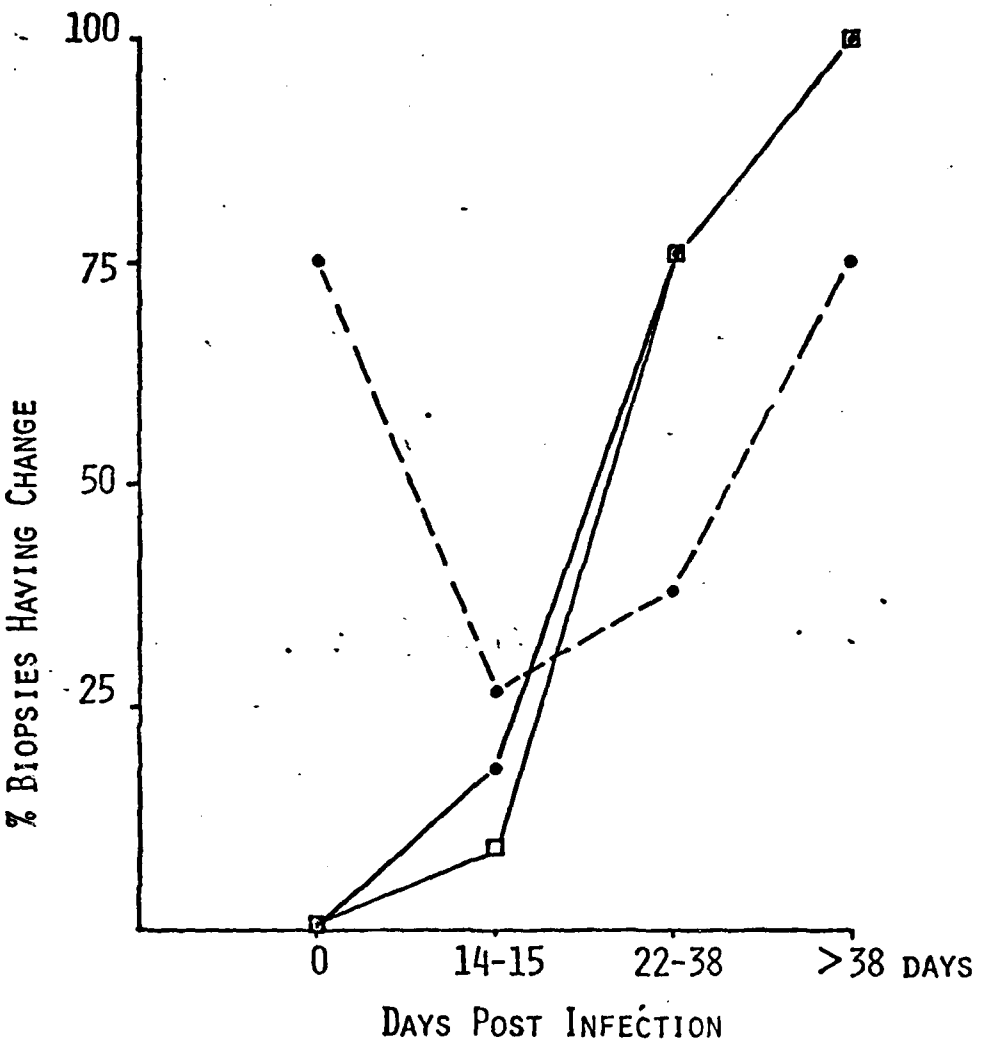


FIGURE 1

ULTRASTRUCTURAL
ENDOTHELIAL CHANGE

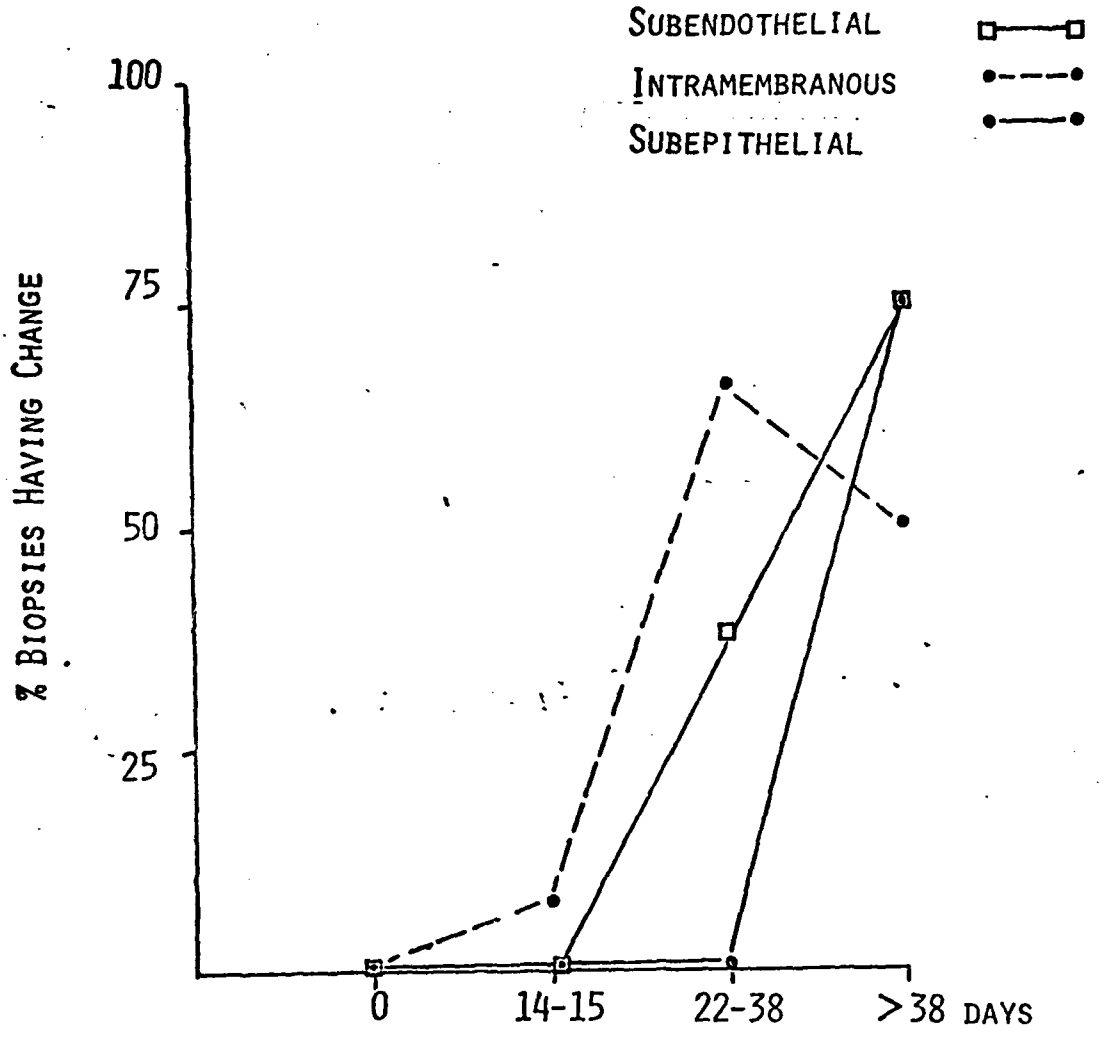


FIGURE 2

ELECTRON DENSE
CAPILLARY DEPOSITS

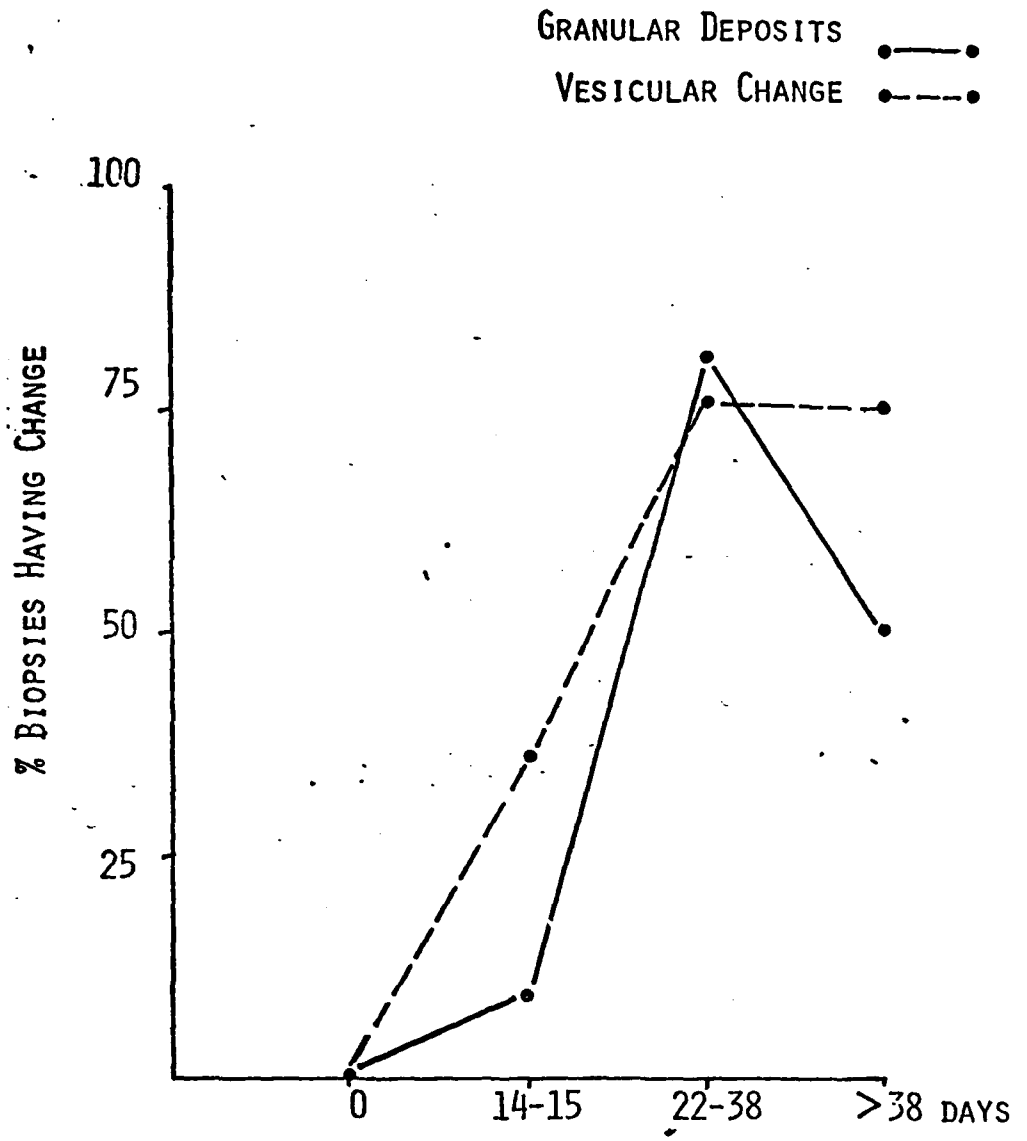


FIGURE 3

MESANGIAL DEPOSITS

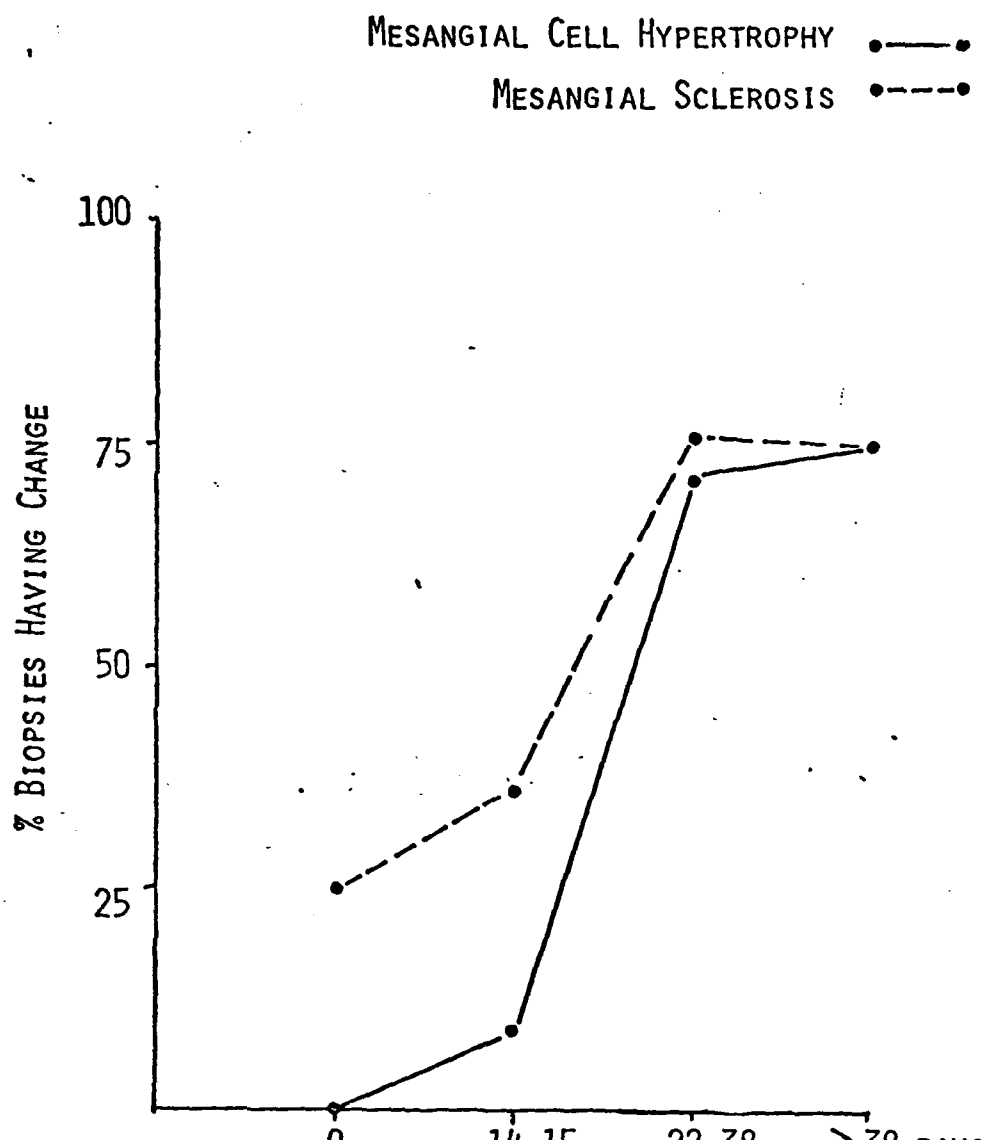


FIGURE 4

ULTRASTRUCTURAL
MESANGIAL CHANGES

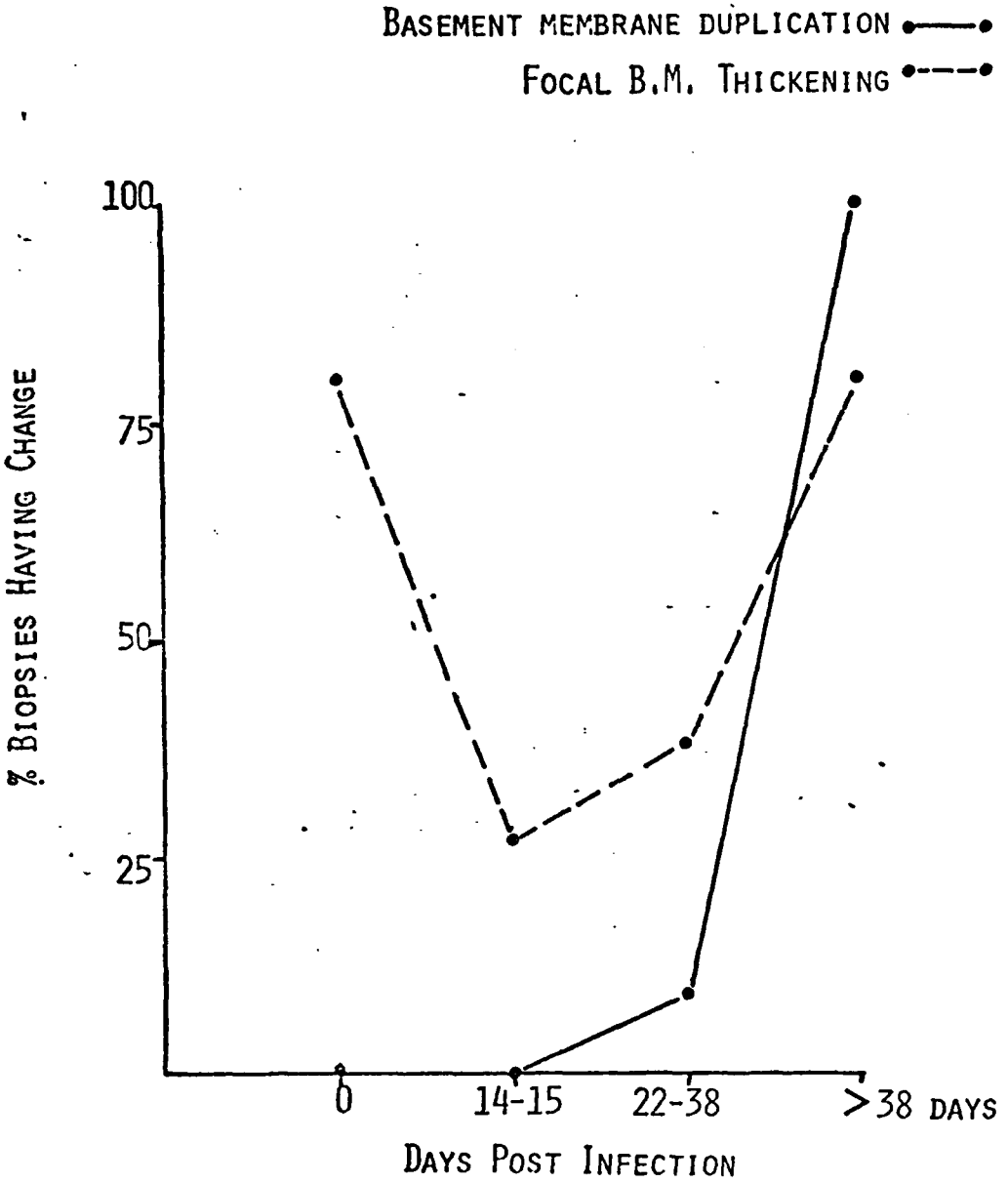


FIGURE 5

CAPILLARY BASEMENT MEMBRANE CHANGES

FIGURE 6

Renal glomerulus from preinfection control biopsy #976 showing minimal changes of mild focal mesangial sclerosis and focal basement thickening (arrow). X 4,300.

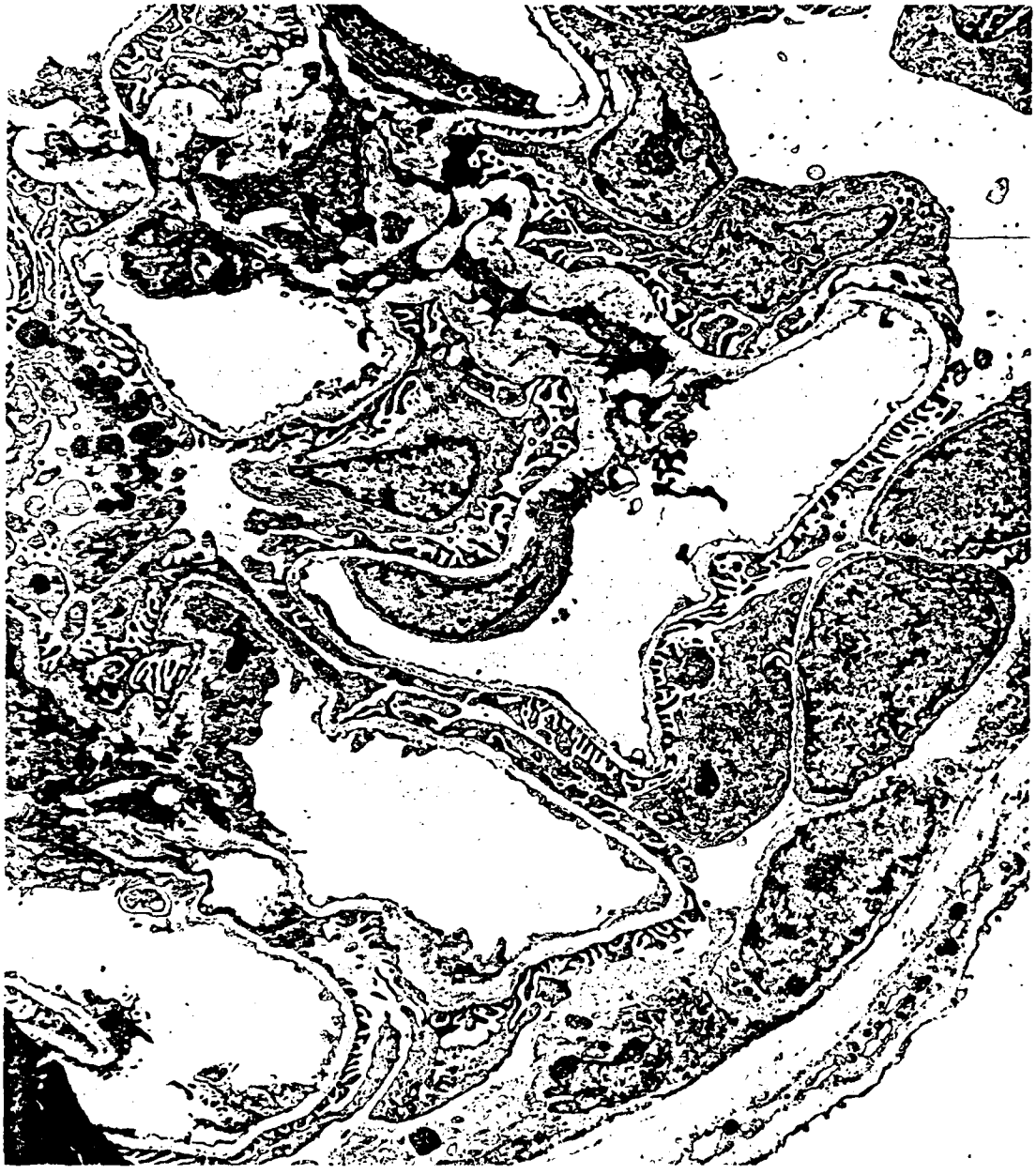


FIGURE 7

Portions of glomerulus from Rhesus #M029 infected 28 days. Note endothelial swelling with obliteration of capillary lumen. Marked granular deposits are present in the perimesangial region (arrow). Also note the vesicular deposits in the same areas. X 8,300.

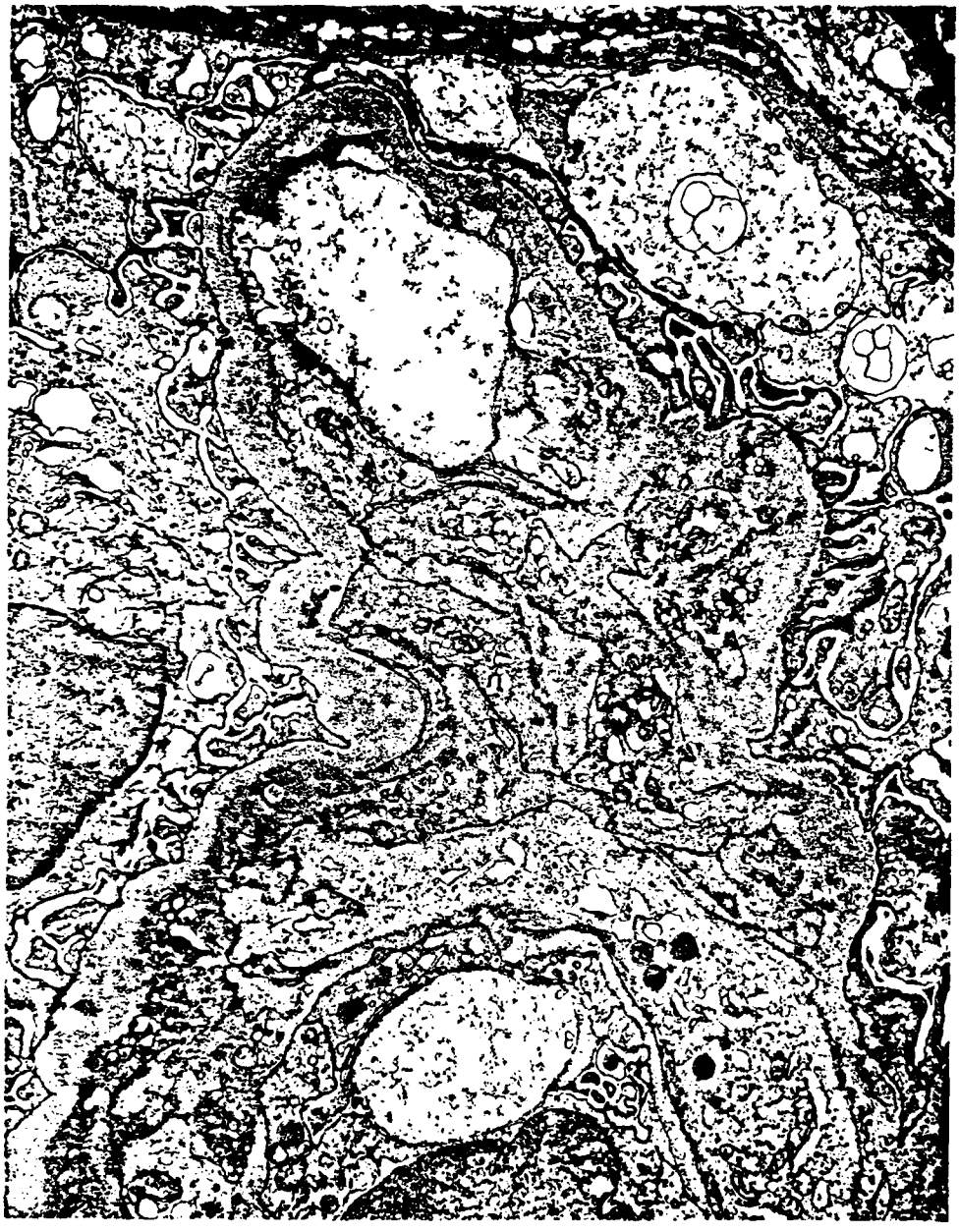
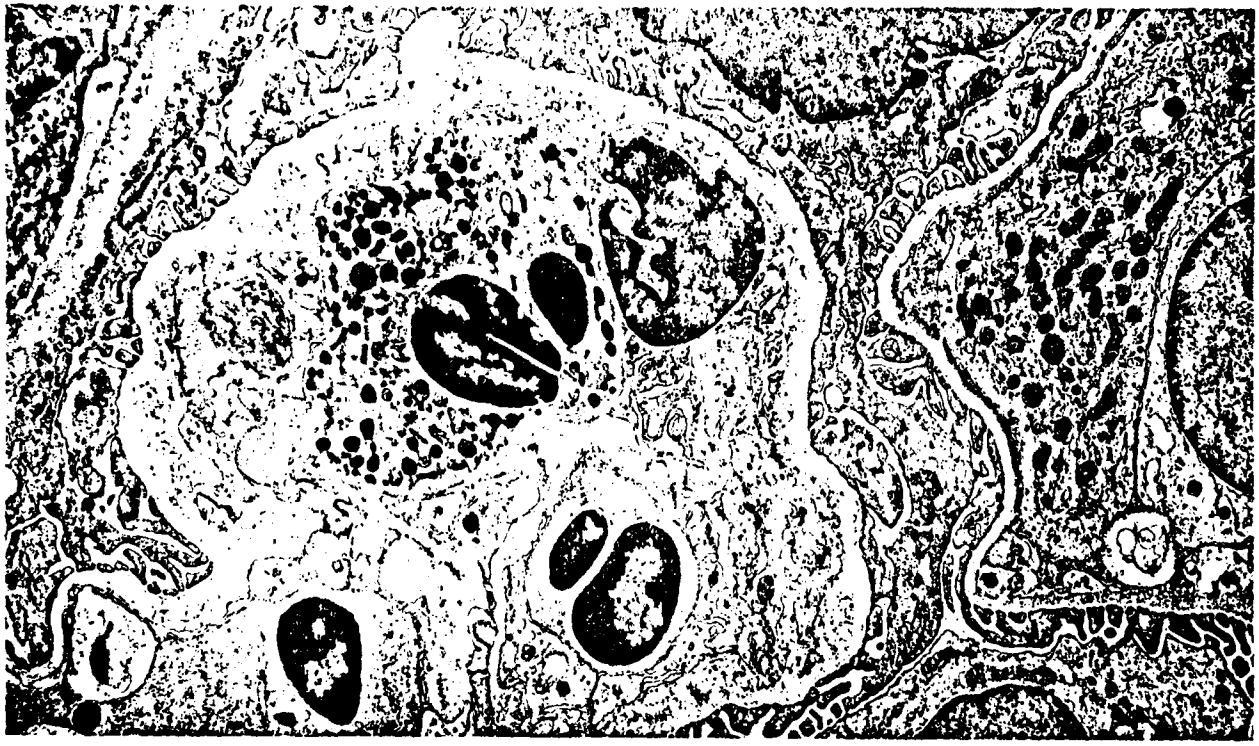


FIGURE 8

Portion of glomerulus from Rhesus #966 infected 28 days. Note complete obliteration of capillary lumen with endothelial swelling and margination of polymorphonuclear leukocyte. The endothelial cells are focally detached and there is an irregular disruption of the lamina rara interna. X 6,600.



IgG, and IgM and the complement components C3 and C4. Indirect immunofluorescence was used to localize properdin, a reactant of the alternate pathway (5). These findings are summarized in Table III and shown graphically in Figures 9 and 10. It can be seen that the four control animals studied reveal no evidence of either immunoglobulins or complement components. The early biopsies (14-15 days post infection) showed deposits of IgM, C3, and properdin in approximately 1/4 of the biopsies with only two of eleven showing C4. This correlated with the early endothelial cellular swelling and hypercellularity described above. The period from 22-38 days represented the peak of the immunologic activity with 14/19 animals showing mesangial IgM deposits, 12/20 showing C3 deposits, and 15/20 showing properdin deposits. Only 2/15 showed IgG and 3/13 showed C4 deposits. This data correlates nicely with the severity of the morphologic changes described above and suggest the presence of both the classic and alternate pathway of complement activation. The late biopsies (greater than 38 days) indicate the persistence of properdin (4/4) and C3 (5/6) deposition with diminution of IgM. If Figure 10 showing the percentage of biopsies showing C3 and properdin is compared with Figures 2 and 3 showing the presence of electron dense deposits in the basement membrane and mesangium,one can see a strong correlation. The distribution of the deposits as seen by immunofluoresence also correlate with the distribution of the deposits seen by electron microscopy. This data strongly suggests that the lesion represents immune-complex glomerulonephritis similar to that described in Plasmodium (6,7,8) and Babesial (9) protozoan diseases.

B. The second objective was to search for evidence of specific antigen in the renal lesions. These studies are presently in progress. Preliminary tests with immune sera labelled directly with anti sera have failed to reveal the presence of trypanosomal antigens although this sera reacts strongly with whole trypanosomes in blood smears. This may be due to the fact that all of the antigenic sites are bound by specific antibody. We are now in the process of setting up an experiment in which we will first attempt to elute off the antibodies with glycine-HCl buffer (pH 3.2) and then stain with the fluorescence labelled antitrypanosome sera.

A total of 15 kidneys from infected animals and two from controls have been tested for the presence of specific antitrypanosomal antibody, using the antibody elution technique described by Oldstone (10). Table III shows that when these eluates were tested by immunodiffusion, only four were shown to contain IgG and none were positive for IgM. The lower yield of IgM is probably a result of the diffuculty in elution of this molecular

TABLE III

IMMUNOHISTOCHEMICAL FINDINGS IN RENAL GLOMERULI

STRAIN EATRO 1886

	CONTROLS	14-15 DAYS	22-38 DAYS	GREATER THAN 38 DAYS
IgA	0/4 (00)	0/6 (00)	0/4 (00)	ND
IgG	0/4 (00)	0/10 (00)	2/15 (13)	0/3 (00)
IgM	0/4 (00)	4/14 (29)	14/19 (73)	1/5 (20)
C3	0/4 (00)	5/12 (24)	12/20 (60)	5/6 (83)
C4	0/4 (00)	2/11 (18)	3/13 (23)	0/1 (00)
Properdin	0/4 (00)	5/17 (29)	15/20 (75)	4/4 (100)

WELCOME STRAIN

IgA	ND	ND	ND	-- --
IgG	0/2 (00)	2/3 (67)	2/3 (67)	-- --
IgM	ND	ND	ND	-- --
C3	0/2 (00)	2/3 (67)	2/3 (67)	-- --
C4	ND	ND	ND	-- --
Properdin	0/2 (00)	2/3 (67)	2/3 (67)	-- --

*Percentage given in parantheses.

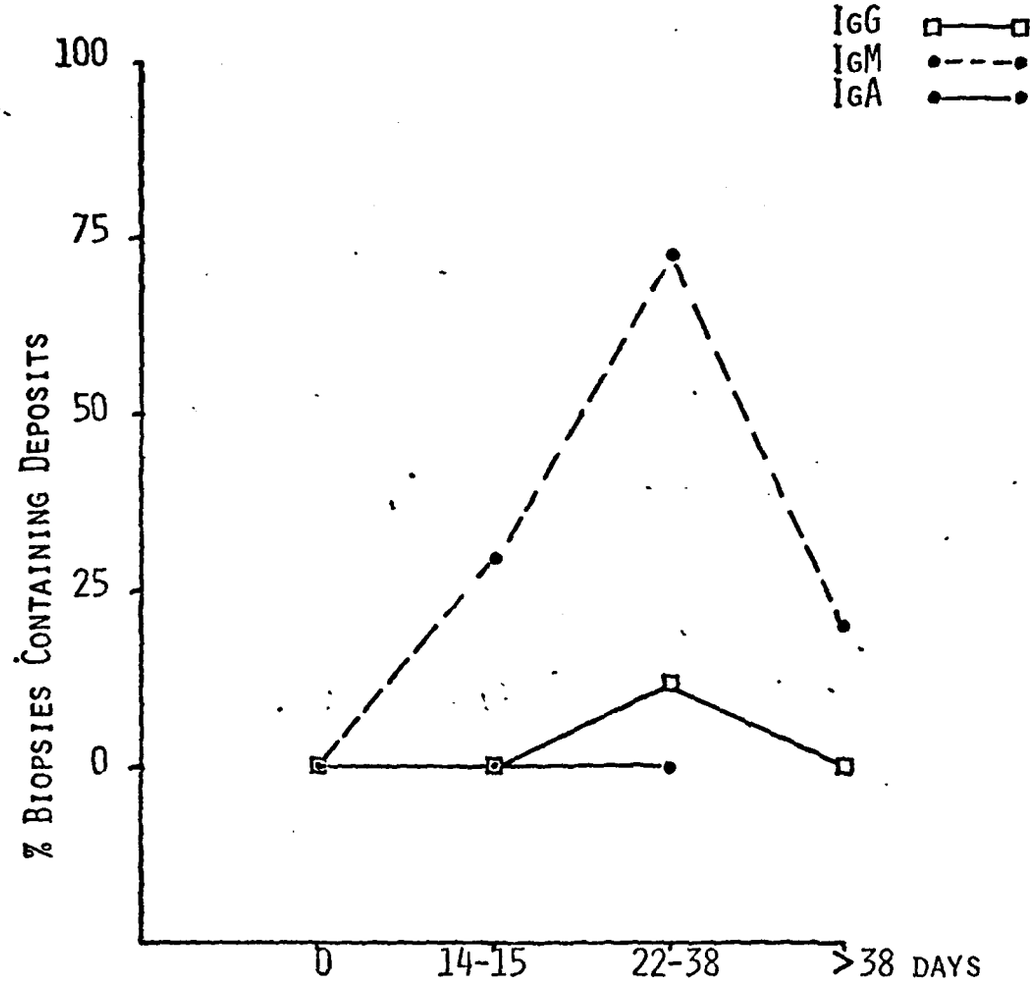


Figure 9

Immunohistochemical Localization
Of Immunoglobulin Deposits

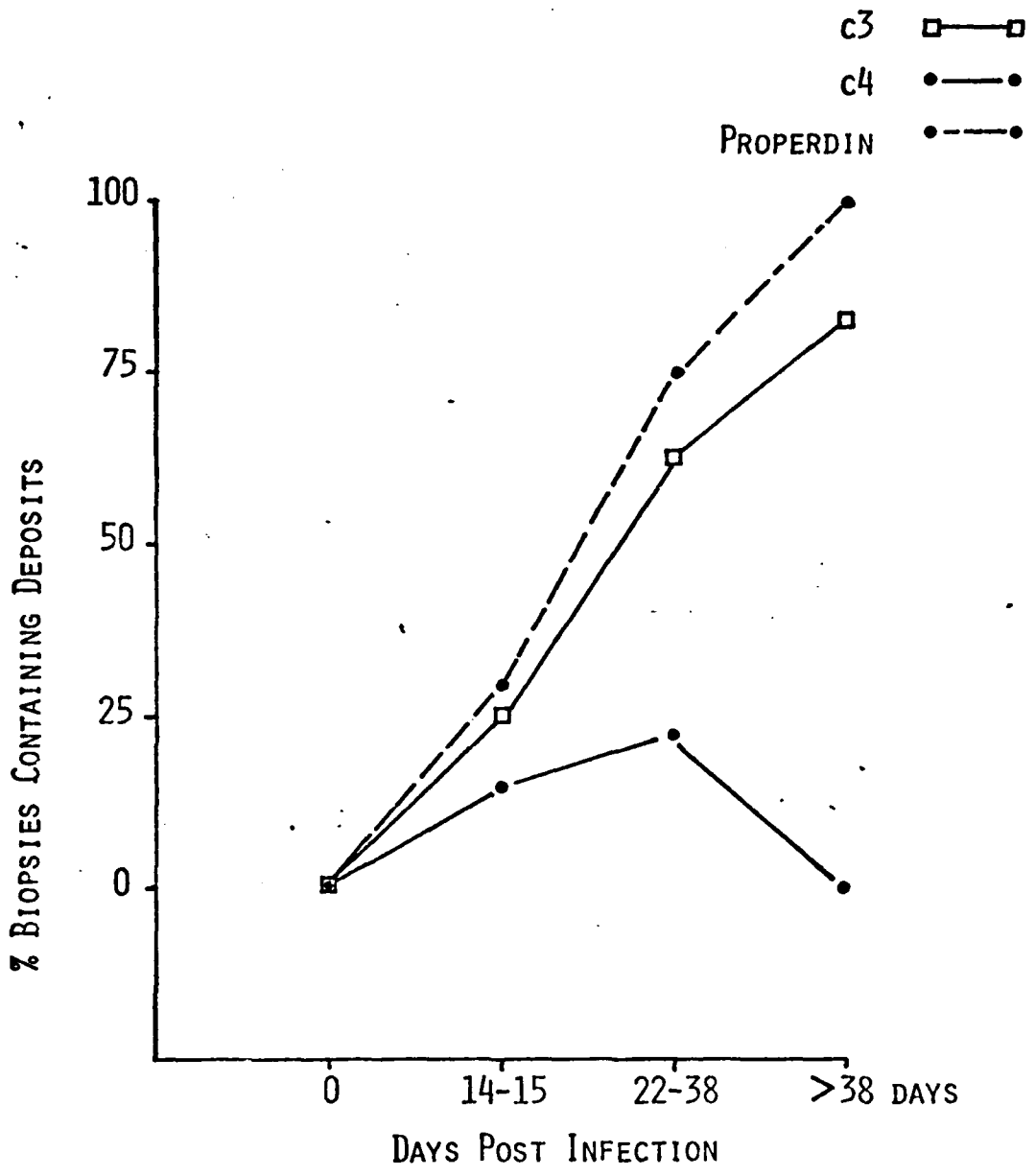


FIGURE 10
IMMUNOHISTOCHEMICAL LOCALIZATION
OF COMPLEMENT COMPONENTS

TABLE IV

DETECTION OF ANTIBODY IN ELUATES OF RENAL CORTEX

	Immunodiffusion against antiIg		IIF tested with whole trypanosomes	
*Infected (15)	IgG	IgM	IgG	IgM
Positive	4	0	5	1
Negative	11	15	10	14
Control (2)				
Positive	0	0	0	0
Negative	2	2	2	2

*Kidneys obtained from Rhesus infected 22-64 days

species. Testing against whole trypanosomes reveal five eluates positive for IgG antibodies and one positive for IgM.

C. The third objective was to test strains other than EATRO 1886 for their potential to produce glomerulonephritis. Three Rhesus infected with Welcome strain at WRAIR were shown to produce a similar glomerulonephritis. The immune histochemical findings of these animals is shown in Table III. It is noteworthy that it seems that a greater proportion of these animals revealed the presence of IgG than in animals infected with Strain 1886 although the number of animals was small.

D. The fourth objective of the present contract was to attempt to localize deposits of C3 and properdin at the ultrastructural level with the use of peroxidase labelled antibodies.

Goat anti-rabbit antibodies IgG fractions were obtained by passing whole serum through sephadex G200 columns. These fractions were conjugated with horse radish peroxidase using the techniques of Avrameas (11). Renal tissue was tested by IFF with rabbit anti-C3 and anti-properdin in the following ways:

1. Tissue was frozen and sectioned on a cryostat. The immunohistochemical reactions were carried out on glass slides, followed by reaction with 3-3' diaminobenzidine, osmication, and subsequent dehydration. Embedding was accomplished by inverting gelatin capsules containing Epon over the glomeruli areas. Thin sections were cut and analyzed with the electron microscope for the peroxidase reaction product.

2. Fresh tissue was obtained from a Rhesus infected at WRAIR. The kidney was minced and fixed by immersion in cold 2% formaldehyde, 1% glutaraldehyde in phosphate buffer for one hour. Individual glomeruli were teased out under a dissecting microscope and all subsequent reactions were carried out by transferring the glomeruli to the various anti sera and reagents. The glomeruli were finally individually embedded and sectioned.

The first technique proved unsatisfactory for although there was excellent staining of the glomerular deposits by light microscopy, when these sections were examined by electron microscopy, it was obvious that the freezing destroyed the ultrastructural features. It was also seen that the conjugated antibodies did not penetrate the tissue and therefore only superficial parts of the specimens were reacted.

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EXPERIMENTAL INFECTIONS WITH AFRICAN TRYPANOSOMES

VI. GLOMERULONEPHRITIS INVOLVING THE ALTERNATE PATHWAY OF COMPLEMENT ACTIVATION*

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Abstract. Rhesus monkeys infected with *Trypanosoma rhodesiense* developed a proliferative glomerulonephritis associated with glomerular deposits consisting of the third component of complement (C3), properdin, and IgM. None of the glomerular deposits contained IgG or IgA. The pattern of deposits as revealed by immunofluorescence was granular. Sera from animals with glomerulonephritis were hypocomplementemic; by radial immunodiffusion some animals showed depression of C3 but not C4 levels. These findings suggest that the glomerulonephritis associated with trypanosomal infections in monkeys is related to deposition of immunologically important serum proteins, two of which represent components in an alternate pathway of complement activation. Trypanosomiasis in the rhesus monkey appears to be a valuable model for studies on the pathogenesis of glomerular injury.

Immune complex glomerulonephritis in man is thought to be an immunologic complication following a variety of infections, such as nephritogenic streptococcal infections,[1,2] quartan malaria,[3-5] acute staphylococcal endocarditis,[6,7] and secondary syphilis.[8,9] Immunoglobulins and components of complement have been found in renal glomeruli of patients who developed glomerulonephritis as a result of such infections. Experimental work has implied that antigens interact with specific antibodies forming complexes in the circulation which are deposited in the glomerular capillaries. These complexes activate complement through the classic pathway leading to the release of mediators some of which have the capacity to cause local inflammation which results in glomerular injury.[10] Endogenous antigens may also react with antibodies and lead to the development of glomerulonephritis. Thus, in the glomerulonephritis seen in patients with systemic lupus nephritis, antinuclear antibodies have been found in the kidneys,[11] and host immunoglobulins, nuclear antigens and complement have been demonstrated in a characteristic lumpy pattern in the glomeruli.[12] Recently properdin, a component of the alternate pathway of complement activation, has also been demonstrated in the glomeruli of patients with acute post-streptococcal glomerulonephritis, membranoproliferative glomerulonephritis, and the glomeruli of lupus erythematosus patients, implying that activation of complement and subsequent damage may be initiated by other less-understood mechanisms.[13,14]

Immunopathologic studies of protozoan diseases have proven to be of considerable interest in regard to the pathogenesis of glomerulonephritis in man and monkeys infected with *Plasmodia*,[3-5,15] and in rats infected with *Babesia*.[16] In malaria and babesiosis, the glomerulonephritis is associated with glomerular deposits containing immunoglobulins, antigens originating from the infective agent, and complement proteins. This is compatible with the concept that in each case the glomerulonephritis is induced by immune complexes entrapped from the circulation.

The present studies were begun after finding that monkeys infected with *Trypanosoma rhodesiense* developed chemical and histologic evidence of renal failure and glomerulonephritis.[17] Morphologic changes similar to those described in human cases of membranoproliferative glomerulonephritis[18] were observed in the kidneys of some of the infected animals.

Accepted 4 August 1973.

* This work was supported in part by NIH Grant No. AI09651.

MATERIALS AND METHODS

Animals. Nineteen young male monkeys (*Macaca mulatta*) originating from India were used in this study.* After a 30-day quarantine and period of conditioning, the animals were placed in individual cages and given a diet of Purina Monkey Chow, fresh fruit and water. Sixteen monkeys were each inoculated intravenously with approximately 10,000 *Trypanosoma rhodesiense* (EATRO No. 1886 strain) contained in 0.5 ml of phosphate-buffered saline-glucose (PSG) solution. Three control animals received 0.5 ml of phosphate-buffered saline-glucose solution only. The EATRO No. 1886 strain was isolated from a human patient in Uganda in 1971 and has since been maintained in the laboratory either by storage at −70° C or by passage in laboratory rats. The trypanosomes for the inocula were separated from infected rat blood in a DEAE-cellulose column,[19] and washed twice by centrifugation in PSG solution. Fresh preparations of blood obtained by ear puncture were examined 5 times each week to determine the course of parasitemia. Blood for serum collection was taken from the femoral veins of the monkeys after they had been sedated with intramuscular Sernylan (Bio-Ceutic Laboratories, Inc.) (0.5 mg/kg). The sera were stored at −70° C until used.

Biopsies. Renal wedge biopsies were taken at 14, 30 and 50 days following inoculation of trypanosomes. Uninfected control monkeys were subjected to biopsy at the same time intervals. For biopsy, the animals were anesthetized with intravenous pentobarbital (50 mg/kg) and an incision was made through the flank using sterile operating procedures. Wedges of renal cortex were removed and immediately divided into three parts. One portion was fixed for electron microscopy (EM) by dicing the piece into 1 mm cubes and immersing them in cold one-half strength Karnovsky's fixative.[20] A second portion was quick-frozen for immunohistochemical studies by placing the tissue in isopentane quenched in liquid nitrogen. A third portion was fixed for light microscopy (LM) in 10% neutral buffered formalin.

Morphologic studies. Paraffin sections were cut at 3–4 μ and stained with hematoxylin and eosin (H&E), periodic acid Schiff (PAS), and Avallone's modification of the methenamine silver stain (MS). Tissues from seven animals were studied by EM. Pieces of renal cortex were fixed for 4 hours in one-half strength Karnovsky's fixative at 4° C and then washed overnight in 0.1 M cacodylate buffer (pH 7.2) containing 7.5% sucrose. The tissues were treated in osmium tetroxide, dehydrated in graded alcohols, and embedded in Epon.[21] Sections cut with glass knives at 1 micron were stained with toluidine blue. Thin 100-mμ sections were cut with a diamond knife, doubly stained with uranyl acetate[22] and lead citrate,[23] and examined with an electron microscope (RCA EMU-3G).

Immunofluorescent studies. Fresh frozen renal cortical tissue was processed according to earlier descriptions.[3, 15] Gel double diffusion tests showed antibodies to human IgG, IgA, IgM and C4 properdin gave a strong cross-reaction to the heterologous monkey serum protein. Accordingly, antibodies to the human proteins were used for immunofluorescence studies. The only exception was the use of antibodies to monkey C3 prepared in rabbits as previously described.[15] In IF studies the direct technique was employed for all proteins except properdin. The usual controls to insure specificity of reactions were employed.[3, 15] For the detection of properdin the indirect IF technique was employed. The tissue sections were first incubated with rabbit antibody to human properdin, then washed and incubated with fluorescein tagged sheep antibody to rabbit IgG.[15] Additional details of the technique of IF are given elsewhere.[3, 15]

Serologic measurements for CH_{50}, C3 and C4. Serum CH_{50} levels were assayed with sensitized sheep red cells according to the technique of Kent and Fife.[24] Assays of complement components C3 and C4 were based on the cross-reactions of antibodies between human and monkey proteins. The single radial immunodiffusion technique of Mancini et al.,[25] as modified by Yount et al.[26] was used. Human antibodies and standards for C3 and C4 were obtained from Hyland Laboratories (Los Angeles, Ca.) and Meloy Laboratories (Springfield, Va.). Serum albumin was deter-

* In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

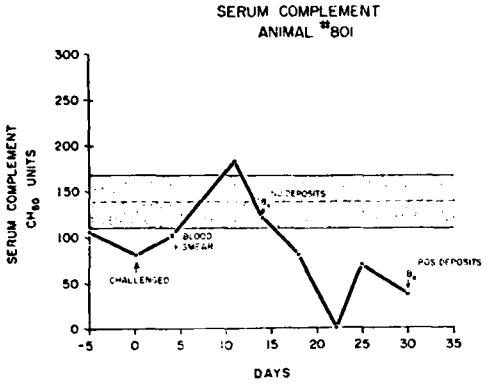


FIGURE 1. Sequential serum complement levels in animal No. 801. Arrows indicate time of renal biopsy (14 and 30 days post-inoculation of trypanosomes) and immunohistochemical results. Interrupted line and shaded area indicates mean ± S.E. of the control values for serum complement (CH_{50}).

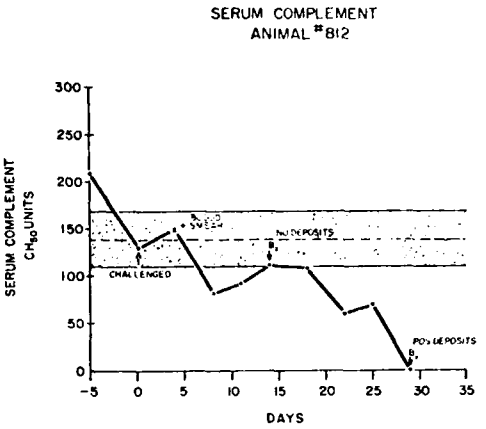


FIGURE 3. Sequential serum complement levels in animal No. 812. Arrows indicate time of renal biopsy (14 and 29 days post-inoculation of trypanosomes) and immunohistochemical results. Interrupted line and shaded area indicates mean ± S.E. of the control values for serum complement (CH_{50}).

mined by protein-electrophoresis in cellulose acetate.

RESULTS

Serum Complement and Renal Deposits in Non-Injected Monkeys

The serial serum complement levels in three control animals had a mean value of 138 ± 28 CH_{50} units. Renal biopsies from the control animals obtained at three different intervals (days 14, 30, 50) failed to show the presence of any protein deposits in the kidneys

Serum Complement and Renal Biopsies in Infected Monkeys

The details of serum complement as well as the normal range in each of four infected animals are given in Figures 1–4 (Numbers 801, 997, 812 and 970 respectively). Between 20 and 30 days after inoculation with the infective agent (15–25

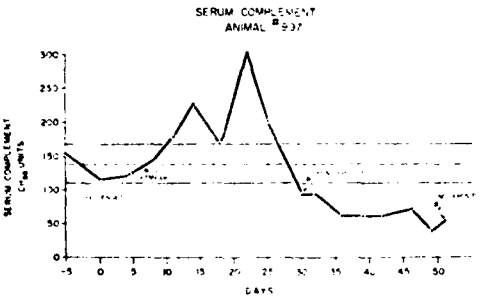


FIGURE 2. Sequential serum complement levels in animal No. 997. Arrows indicate time of renal biopsy (30 and 51 days post-inoculation of trypanosomes) and the immunohistochemical results. Interrupted line and shaded area indicates mean ± S.E. of the control values for serum complement (CH_{50}).

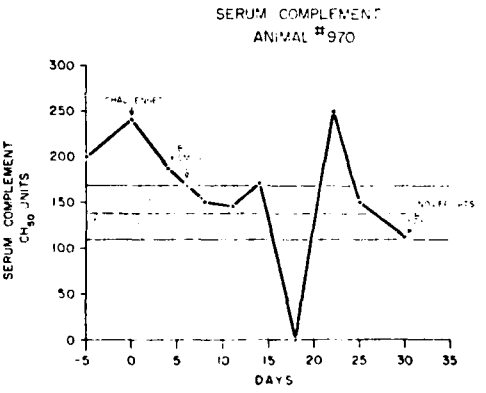


FIGURE 4. Sequential serum complement levels in animal No. 970. Arrows indicate the time of renal biopsy (30 days post-inoculation of trypanosomes) and immunohistochemical results. Interrupted line and shaded area indicates the mean ± S.E. of the control values for serum complement (CH_{50}).

days after detectable parasitemia), serum complement levels began to fall in each of the animals. In animals 801, 812 and 970 there was no detectable hemolytic activity on at least one occasion during this period. The CH_{50} levels in monkey No. 997 persisted at a 50% level for at least 10 days (Fig. 3). There was a persistent parasitemia during this time. In one infected animal (No. 970) (Fig. 4), the CH_{50} level fell to undetectable levels at 18 days, but then returned to hypernormal or normal range. Also demonstrated in Figures 1 to 4 are the findings of the renal biopsies. Positive deposits consisting of one or more of the proteins listed above were found at some time during the hypocomplementemic period.

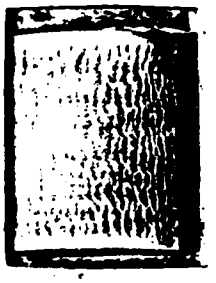
Morphologic Changes in Glomeruli

Renal biopsies obtained from the three control animals revealed normal cellularity without evidence of exudation, proliferation, or sclerosis (Figs. 5, 6).

Fifteen of the 16 infected animals showed glomerular abnormalities. The common feature consisted of mesangial cell proliferation, endothelial swelling, the presence of few to many polymorphonuclear leucocytes, variable mesangial sclerosis, and focal basement membrane thickening and duplication. In four infected animals in which serologic studies were done, three (801, 997, 812) showed glomerular changes associated with depression of serum complement and protein deposits within the glomeruli. The specific pathologic changes of each of these animals are presented below:

Animal 801 was first subjected to biopsy on the 14th day (Fig. 1). The LM observations of this biopsy were essentially normal except for rare focal areas of mesangial sclerosis. EM observations confirmed this finding and revealed essentially normal capillary loops without evidence of abnormal deposits. The second biopsy obtained on the 30th day, at a time when the serum CH_{50} was 41% of the pre-infective level, showed definite focal mesangial hypercellularity with increased mesangial sclerosis and focal basement membrane thickening (Fig. 7). Electron microscopy revealed widening of the central mesangial areas which contained increased numbers of mesangial cell processes (Fig. 8). Surrounding the mesangial cells, the mesangial matrix was characterized by numerous electron lucent defects. Within these areas of rarefaction were irregular electron dense deposits (Fig. 8). Focally this change extended out into the basement membrane of the capillary loops at a point where the capillaries attach to the central mesangium.

Animal 997 was first biopsied on the 30th day at a time when the CH_{50} level was depressed to 68% of preinfection values (Fig. 2). This biopsy revealed diffuse glomerular hypercellularity, numerous polymorphonuclear leukocytes in the capillaries, and obliteration of the capillary lumina (Fig. 9). EM studies revealed focal swelling of the endothelial cells, and plugging of the glomerular capillary lumina with polymorphonuclear leukocytes, mononuclear cells, and occasional macrophages containing secondary lysosomes. The mesangial areas were widened and contained electron lucent areas similar to those described in animal 801. A second biopsy obtained on the 51st day following 15 days of depression of the CH_{50} serum levels exhibited a persistent hyper-



FIGURES 5 and 6. **5.** Biopsy from control rhesus monkey showing normocellular glomerulus with open capillary loops limited by delicate basement laminae. PAS, × 520. **6.** Electronmicrograph of normal control. Note appearance of mesangial areas containing mesangial cells (arrows) surrounded by a compact mesangial matrix with an electron density similar to that of the basement membrane. × 1,500

FIGURES 7 and 8. Renal biopsy of animal No. 801 taken 30 days post-inoculation of trypanosomes. **7.** Note general hypercellularity of the glomerular lobules and obliteration of capillary lumens. 3 μ section stained with PAS, ×550. **8.** Note rarefraction of mesangial matrix in areas surrounding mesangial cells. Numerous fine cytoplasmic processes of the mesangial cells extend into these areas. In addition, numerous electron dense, punctate material is seen within the mesangial area. × 2,900.

FIGURES 9 and 10. Renal biopsy of animal No. 997. **9.** Taken 30 days post-inoculation of trypanosomes, at time of depressed serum complement. Note marked hypercellularity of glomerular tufts, polymorphonuclear leukocytes, and obliteration of capillary lumina. PAS, × 500. **10.** Taken 51 days post-inoculation of trypanosomes. Note thickened and duplicated basement membranes (arrow), mesangial sclerosis, and obliteration of capillary lumina. Note also the focal interstitial inflammatory infiltrates. PAS, × 500.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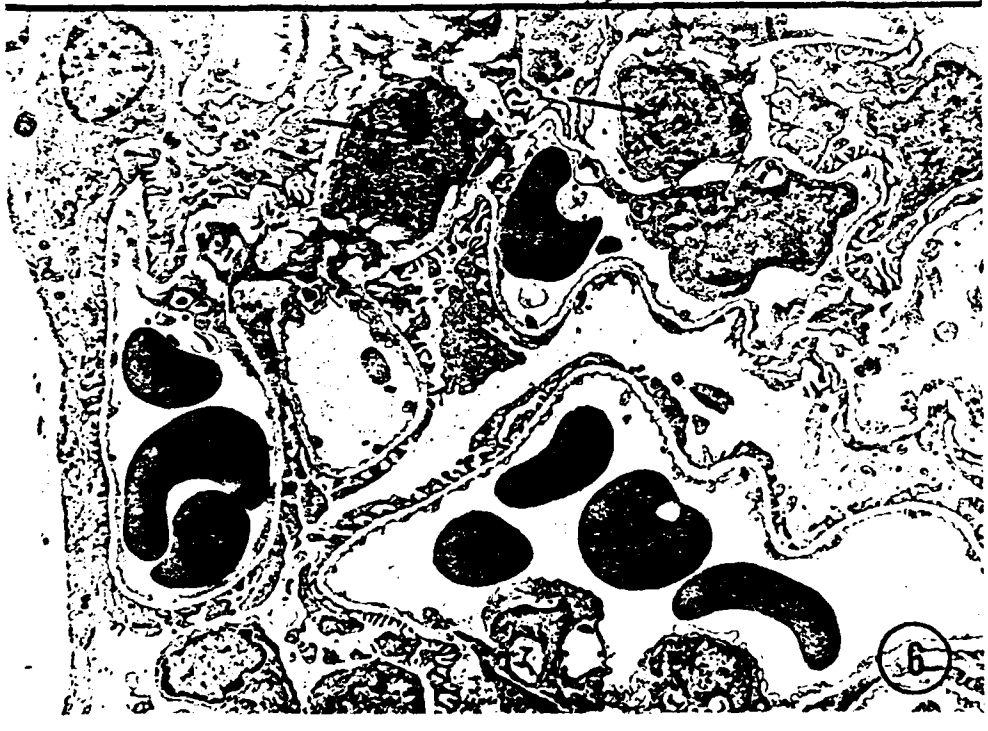
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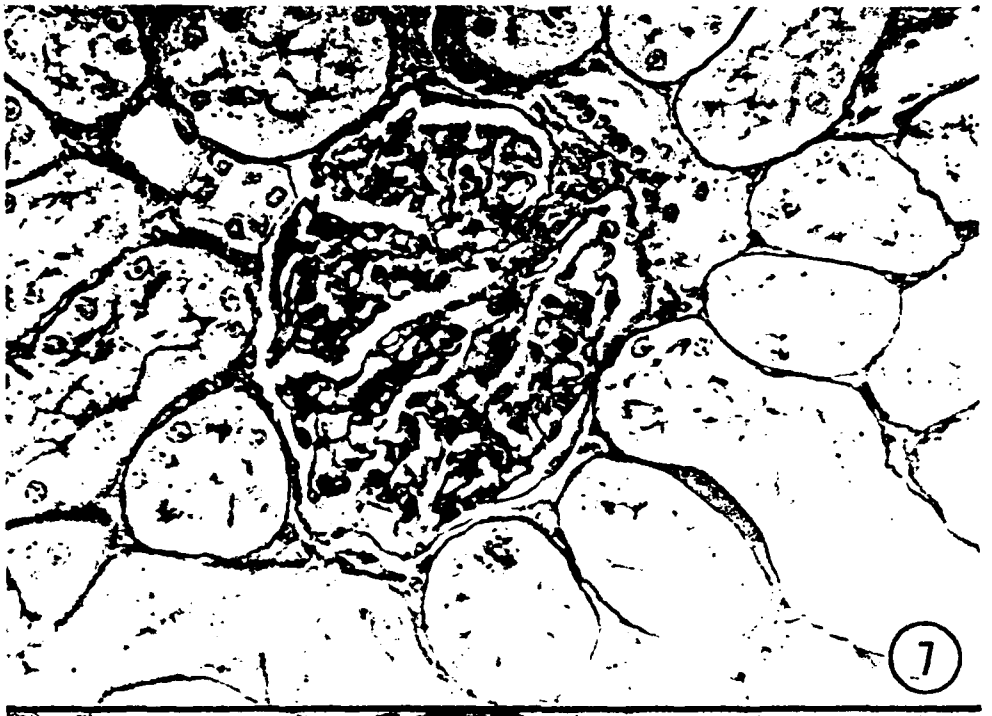

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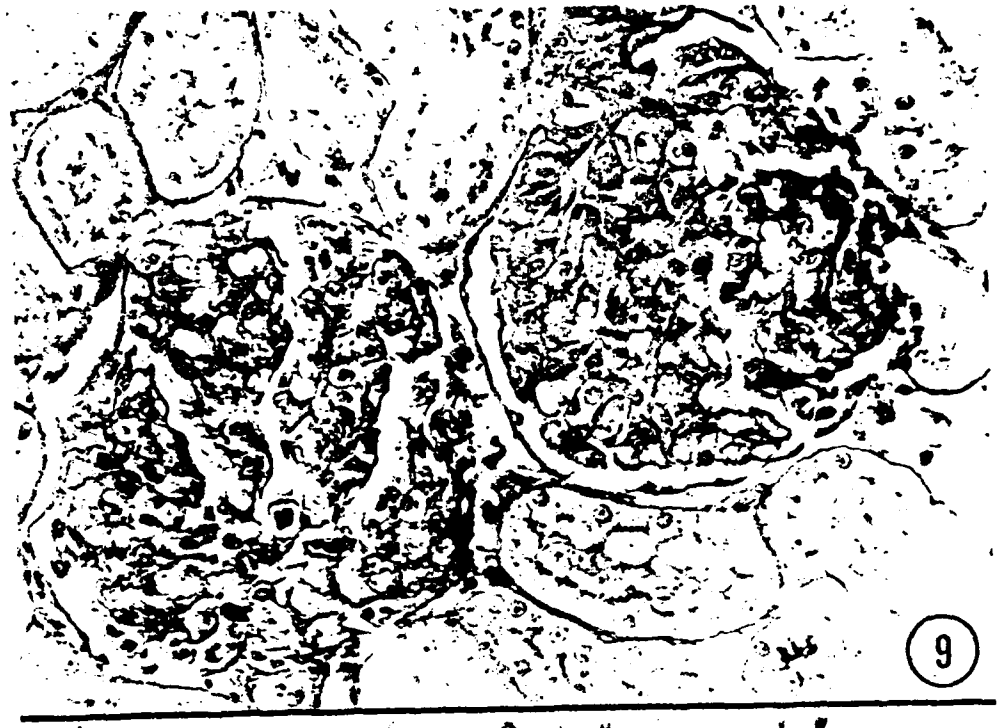

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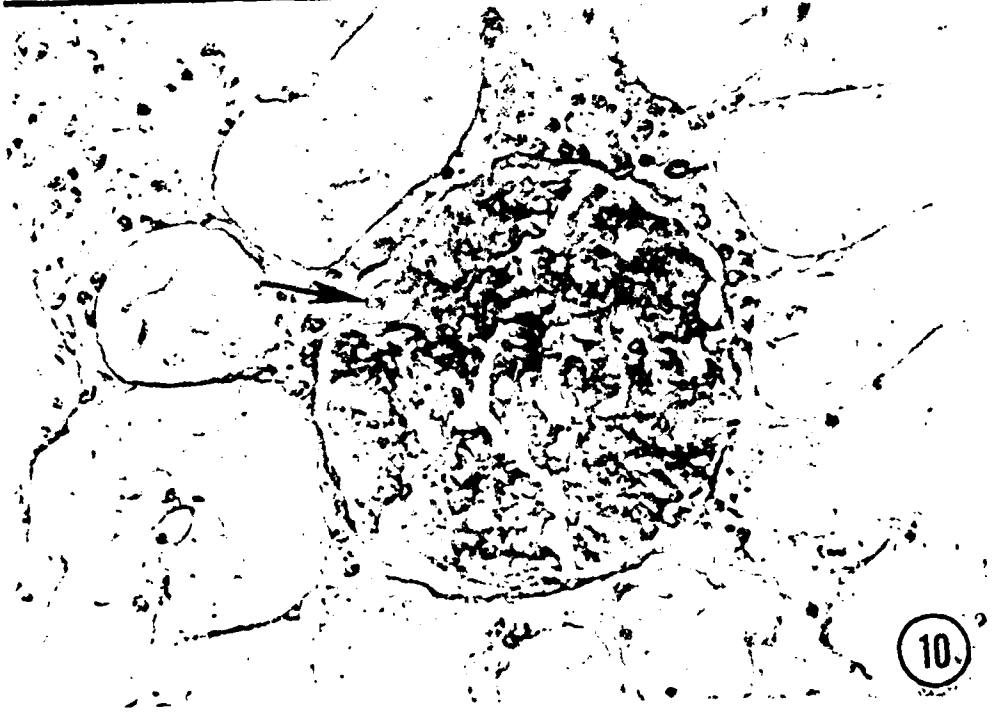

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TABLE 1

Percent changes in selected serum proteins and deposits in glomeruli 30 days after inoculation

Animal no.	Serum				Renal deposits					
	Albumin (%)*	CH_{50} (%)*	C4 (%)*	C3 (%) *	Properdin	C3	C4	IgG	IgM	IgA
801—infected	46	41	135	45	4+	3+	0	0	Trace	0
997—infected	49	68	122	27	4+	3+	0	0	2+	0
812—infected	51	12	19	22	4+	3+	0	0	2+	0
970—infected	62	57	29	72	0	0	0	0	0	0
952C—noninfected	91	135	208	115	0	0	0	0	0	0
966C—noninfected	84	104	95	98	0	0	0	0	0	0
976C—noninfected	ND†	95	112	106	0	0	0	0	0	0

* Percentage of preinfection value.
† ND, not determined.

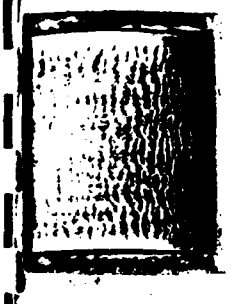
cellularity of the mesangium which, in addition, showed increased PAS positive mesangial mature material (Fig. 10). Methenamine silver and PAS stains showed that the capillary membrane was thickened and focally duplicated. Many capillary loops appeared obliterated (Figs. 10, 11). EM observations disclosed focal areas of capillary wall thickening with processes of the mesangial cells extending out into the peripheral capillary wall (Fig. 11). In these areas, there was production of new basement lamina which corresponds to the picture of peripherally duplicated basement membranes seen with the PAS and MS stains by LM. The capillaries were focally occluded by swollen endothelial cells, mononuclear cells and an occasional polymorphonuclear leukocyte (Fig. 11). Occasional electron dense subepithelial deposits were seen (Figs. 11, 12) as well as rare subepithelial deposits.

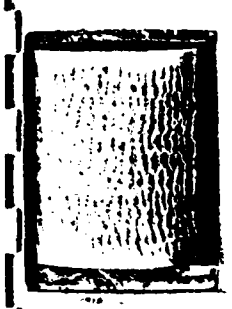
The first biopsy obtained from animal 812 on the 14th day at a time when the CH_{50} level was in the normal range showed a rare focus of mesangial sclerosis but no evidence of active disease. A second biopsy obtained on the 29th day when the CH_{50} level had been depressed for 7 days, revealed a definite, diffuse mesangial hypercellularity and wrinkling and focal thickening of basement membrane. Electron microscopy confirmed widening of the mesangial regions and increased numbers of mesangial cellular processes. In addition, the mesangial matrix contained electron lucent areas which contained irregular electron dense material.

A biopsy obtained on the 30th day of infection from animal 970 revealed essentially normal glomeruli with focal areas of slight mesangial hypercellularity. The CH_{50} level was within the normal control range at this time although it was 72% of this animal's preinfective value. No glomerular deposits were seen.

Correlated Studies of Serological and Renal Biopsy Observations

Four infected monkeys and three uninfected animals were studied in detail with respect to serological changes of whole complement, levels of C3 and C4 as determined by radial immunodiffusion, and protein deposits in renal glomeruli. The data are summarized in Table 1. The four infected animals were biopsied on the 30th day (after inoculation with trypanosomes) at which time their CH_{50} values were 41%, 68%, 12% and 72% of the pre-infection values determined in the same animals. Three uninfected control animals sampled also on the 30th day had complement levels of 135%, 104% and 95% of pre-infection values. The antigenic assay for C3 indicated severe depression in the C3 levels (45%, 27% and 22%) in three infected animals (Table 1). This was correlated with depressed CH_{50} levels and C3 deposits in the glomeruli as determined by immunofluorescence. Two animals showed depres-





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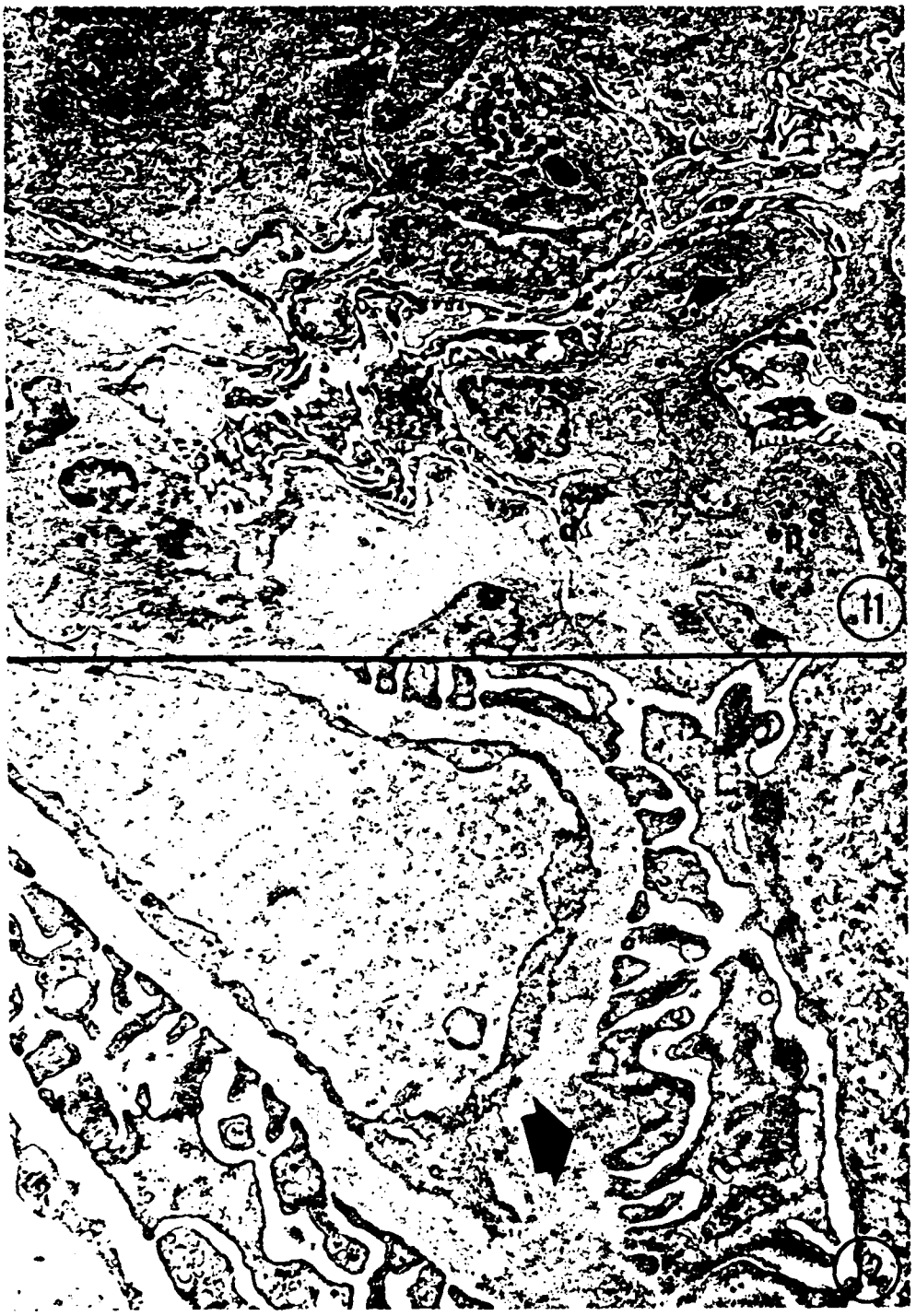
FIGURES 11 and 12. Renal biopsy obtained in animal No. 997 51 days post-inoculation with trypanosomes. **11**. Note obliteration of capillary lumina, some of which contain polymorphonuclear leukocytes (p). There is focal reduplication of basement membrane (arrow). There is swelling of podocytes with loss of filtration sites focally and rare focal subepithelial deposits (d). × 1,700. **12**. Arrow indicates subepithelial deposit in area of slightly tangential section of capillary wall. × 22,000.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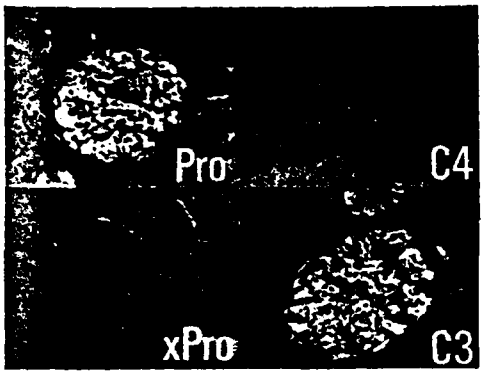

FIGURE 13. Immunohistochemical findings in a renal biopsy from animal No. 997, 30 days post-inoculation of trypanosomes. Upper left panel reveals a 4+ reaction with indirect technique using rabbit serum containing anti-properdin antibody (Pro). Lower left shows blockage of the reaction by prior absorption with purified human properdin (xPro). Upper right shows negative staining in C4. Lower right reveals 3+ staining for C3.

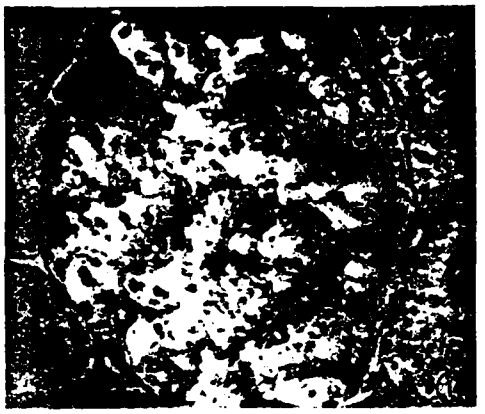

FIGURE 14. Renal biopsy from animal No. 997 showing deposits of IgM in a granular distribution.

sion of C4 levels but C4 deposits were not present in the glomeruli. The C3 and C4 levels in the uninfected controls were close to the preinfection values.

Immunofluorescent Patterns of Glomerular Deposits

The various proteins detected in glomerular deposits have been summarized in Table 1. The pattern of fluorescence was one of a diffuse stain, limited to glomeruli, involving both glomerular capillary loops and mesangial areas. In some cases discrete granular patterns of fluorescence were seen. There was no evidence of smooth, linear fluorescence. Typical patterns of fluorescence are shown in Figure 13; the properdin reaction (Pro) was completely blocked (xPro) by previous absorption of the antiserum with purified human properdin. Deposits of IgM were seen in one monkey (Fig. 14). The granular appearance of the deposits was particularly apparent. Considerable deposition in mesangial areas was evident.

DISCUSSION

Glomerulonephritis develops in the course of experimental trypanosomiasis in monkeys. The earliest lesions are characterized as proliferative and show variable degrees of increased numbers of mesangial cells, swelling of endothelial cells, and variable margination of polymorphonuclear leukocytes. Later in the evolution of the process there is mesangial sclerosis and duplication of capillary basement membranes which in several cases clearly resemble a membranoproliferative type of glomerulonephritis.[18]

Glomerulonephritis has been implicated in at least two other parasitic diseases. Kibukamusoke reported a high incidence of diffuse proliferative and membranoproliferative glomerular lesions in adults and children with nephrotic syndrome in Uganda and provided extensive evidence suggesting a malarial etiology in many of the cases.[27] A spectrum of glomerular abnormalities has been found in selected patients with hepatosplenic schistosomiasis resembling the lesions of membranoproliferative glomerulonephritis.[28] It should be emphasized, however, that the pathogenesis of schistosomal nephropathy is not yet understood and that the possible role of immune complexes in schistosomiasis is still unresolved.[29]

The immunohistochemical evidence presented in this study points to an immunopathologic basis for the glomerulonephritis. The granular pattern of immunofluorescence would indicate the likelihood of an immune complex-type of nephritis rather than a nephrotoxic mechanism. The great rise in total serum IgM levels on human infections with African trypanosomes is well documented. Therefore, additional studies were conducted to determine the presence of anti-trypanosomal IgM antibodies in infected monkeys. The results which will be published elsewhere show a consistent increase in specific IgM antibodies during the

time in which the glomerular deposits develop. The presence of properdin and C3 in the glomerular deposits of all animals studied suggested that the alternate (properdin) pathway was activated. The serologic data indicated that the basis for the acquired hypocomplementemia is in some animals related to a change in the C3 but not the C4 levels. The explanation for a low C4 in two animals but absence of C4 deposits in the glomeruli in these animals was not apparent. A second study has indicated that in at least some animals C4 deposits were in the glomeruli (unpublished). It is probable that both the classic and alternate pathway operate in the pathogenesis of this lesion. The agent(s) responsible for activating complement has not been defined. Indeed, it is not evident if the activating agent resided in the glomerular deposits, or if the complement pathway was activated within the blood stream with subsequent deposition of altered proteins in glomeruli. On the basis of these studies it would seem that trypanosomal infection in monkeys is particularly predisposed to initiating activation of the alternate pathway of complement.

In the context of glomerulonephritis in man it should be pointed out that there are two general situations in which reactant proteins of the alternate pathway (properdin) have been demonstrated in glomerular deposits. On one hand, a combination of reactant proteins of both the classical (immunoglobulin and C4) and the alternate (properdin deposits) pathways have been activated. This agrees with the current concept that activation of the classical pathway can result in activation of the alternate pathway through release of the C3b fragment from C3 and sequential interactions of complement proteins beyond this point.[30-33]

A second disease reported in humans which seems relevant to these studies is the syndrome of hypocomplementemic glomerulonephritis. This disease is associated with a membranoproliferative glomerulitis, deposits of properdin in renal glomeruli, and persistently low C3 levels in serum with relatively normal levels of C1, C4 and C2.[17, 34-36] Most recent investigations suggest that this disease may be a unique syndrome insofar as the renal disease appears to be associated with a more or less specific activation of the alternate complement pathway.[36, 38] There is, as yet, no information that would pinpoint the reaction product responsible for the morphologic and functional alterations in the glomeruli.

T. rhodesiense infection in monkeys results in glomerulonephritis which in certain respects resembles the hypocomplementemic glomerulonephritis of humans. In view of these findings it would be of interest to carefully study the renal structure and function of patients infected with African trypanosomiasis. Likewise this experimental infection could prove useful in studying the evolution and pathogenesis of glomerulonephritis involving the alternate pathway (properdin) of complement activation.

ACKNOWLEDGMENTS

Drs. J. Minta and I. H. Lepow generously provided the antisera for the detection of properdin and C4. The authors greatly appreciate the valuable technical assistance of Eugene F. Bernard, David A. Stroupe and Margaret A. King. Special thanks are due to Ralph E. Duxbury, who prepared the inocula and monitored the parasitemias.

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