EXPERIMENTAL GLOMERULONEPHRITIS

The Pathogenesis of a Laboratory Model Resembling the Spectrum of Human Glomerulonephritis*, ‡, §

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There is much to suggest an immunologic basis for some kinds of human glomerulonephritis. The frequent history of streptococcal infection preceding the acute form of this disease with an associated host antistreptococcal response (1), the chronology of clinical events (2), and the usual low levels of serum complement in the acute stage (3), are all consistent with an immunologic disorder. However, in spite of these facts, little is known of the pathogenesis of this disease; *i.e.*, the way in which an immunologic response or responses might lead to kidney injury.

One possible pathogenesis is suggested by the experimental disease, nephrotoxic (antikidney) serum nephritis (4). In this model, heterologous antikidney antibodies fix in the host's glomeruli, probably in the basement membranes, and may produce injury immediately. The subsequent antibody response of the host to the heterologous serum presumably is accompanied by reaction of the host's antibody with the heterologous serum protein molecules fixed in the kidney, causing delayed injury, which, together with the immediate injury, produces prolonged renal disease not unlike that seen in human glomerulonephritis. The obvious pathogenetic implication of this model for human glomerulonephritis is that some foreign substance, perhaps a product of the preceding streptococcal infection (5), may combine with renal tissue and elicit a host antibody response either to the foreign substance alone, or to a combination of it plus the host's renal tissue. An alternative might be for the kidney alone to serve as an auto-antigen. In either situation, renal injury would be caused by the ensuing antibody reaction with kidney-fixed antigens. However, there has been little evidence obtained from clinical or experimental observations to support this thesis in the human

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disease. In clinical material, the search for antikidney antibodies which might be correlated with and help explain the human disease has not yielded convincing results (3, 6). In animals many attempts have been made to induce glomerulonephritis by immunization with a variety of bacterial and kidney preparations alone or in combination. A few of these attempts have resulted in renal injury (7), but they have not produced lesions resembling the spectrum of changes seen in human glomerulonephritis. While these disappointing results do not rule out the pathogenesis proposed above, they certainly speak against it, especially since identical procedures have yielded evidence for an "autoimmune" process in thyroiditis, encephalomyelitis, lupus erythematosus, and some of the anemias, leukopenias, and thrombocytopenias (8).

A second experimental model for glomerulonephritis is serum sickness nephritis which follows the injection of foreign serum proteins. In most instances this disease has been induced in rabbits by a single large injection of foreign whole serum or a purified protein fraction thereof (9). A severe but short lived acute proliferative glomerulonephritis involving predominantly endothelial cells (10) with heavy proteinuria develops at the time the host's first antibody reacts with circulating antigen, forming antigen-antibody complexes in the circulation (11). A prominant role of these antigen-antibody complexes in the pathogenesis of the disease has been suggested by demonstration of the nephritogenic effects of injection of complexes *per se.* (12), and the identification of antigen and probably antibody in the injured glomeruli (13). The feature of this experimental model which does not compare with the course of the human disease is the rapidity with which it subsides after the elimination of circulating antigen. Experiments employing small daily injections of foreign serum have produced chronic glomerular lesions, but have not afforded an understanding of the role of immunologic factors in the pathogenesis (14).

It was the purpose of the present studies to induce glomerulonephritis in rabbits by daily injections of various foreign serum proteins and to establish the immunologic factors important in the etiology and pathogenesis of the disease. This treatment resulted in acute proliferative and exudative glomerulonephritis in some rabbits and chronic membranous and proliferative glomerulonephritis in others, both remarkably similar, functionally and morphologically, to human glomerulonephritis. In this laboratory model there is no known affinity or toxicity of the antigens alone for the kidney. Rather, the recurrent interaction of antigens with antibody in the circulation appears to produce glomerular injury either by systemic liberation of nephrotoxic materials or by localization in the glomeruli of circulating antigen-antibody complexes, which injure the glomeruli directly. Since this renal injury apparently is not dependent on an immunologic reaction against antigens in or of the kidney, this model provides an alternative explanation for the pathogenesis of human glomerulonephritis to that suggested by antikidney serum nephritis.

Materials and Methods

160 albino male rabbits weighing 2.5 to 3 kg. received daily intravenous injections (6 times per week) of one of the serum protein antigens, bovine serum albumin (BSA), human serum

albumin (HSA), bovine gamma globulin (BGG), or human gamma globulin (HGG). The resultant prolonged constant or intermittent antigen-antibody interaction with complex formation in the circulation was calculated to provide an immunologic insult likely to produce chronic hypersensitivity lesions. The doses of antigens were varied in individual rabbits in an attempt to match the amount of antibody formed with the antigen injected and keep a ratio of antigen to antibody in the circulation near equivalence or slight antigen excess. In order to neutralize all of the antibody formed by the best responders, daily injections of 100 to 200 mg. of antigen and even more were needed. These injections were associated with anaphylactic symptoms and sometimes fatal anaphylaxis which removed the best responders from this experiment.

All rabbits were bled weekly 24 hours after a preceding antigen injection and the amount of antigen or antibody in the circulation was determined. The antigen in the serum was estimated by adding to a given volume of serum a fixed amount of antiserum and recording the amount of precipitate formed as 1 to 4+. The serum antibody was determined by quantitative precipitation (15). One day of each week, each rabbit was kept in a metabolism cage and the 24 hour urine output was collected. Proteinuria was determined by sulfosalicylic acid precipitation and visual comparison with standards and recorded as mg. protein/100 ml. urine. Hematuria was measured by a guaiac test which gave values of 1+ = 1:20,000 dilution of blood and a 4+ = 1:1,000 dilution. Blood urea nitrogen, serum cholesterol values, and serum protein concentrations were obtained on some of the rabbits. At appropriate times, 31 of the rabbits, particularly those with proteinuria, were subjected to open renal biopsy using a clean but not sterile technique and local anesthesia in order to follow the course of morphologic changes and correlate them with renal function. Biopsy consisted of a wedge of renal cortex approximately 10 × 4 × 3 mm. The renal defect was closed by cotton mattress sutures. With the technique employed, each kidney could be biopsied at least twice without complications. In order to find the degree of reversibility or irreversibility of the various morphologic changes, antigen injections of some of the rabbits were stopped after biopsy and the course of functional and morphologic changes followed.

Tissues taken at biopsy and autopsy were: fixed in 1 per cent osmic acid and embedded in methacrylate or vestopal W (Martin Jaeger, Geneva, Switzerland) for study in the electron microscope, quick frozen in liquid nitrogen for study by the fluorescent antibody technique, and fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin and periodic acid stain for study by light microscopy. The fluorescent antibody technique of Coons *et al.* (16), was employed to detect specific antigen and antibody as well as host's gamma globulin, albumin, and fibrinogen. Since the sandwich staining for antibody is difficult to interpret with antigen in the tissues, a blocking procedure for antibody staining was used (13). Sections stained directly with fluorescent anti-host gamma globulin were compared with adjacent sections first flooded with unlabeled specific antigen and then stained with fluorescent anti-host gamma globulin. A specific reduction in staining after use of antigen was interpreted as indicating the presence of antibody which had been in part covered by non-fluorescent antigen, thereby blocking the reaction with fluorescent anti-gamma globulin. The appropriate antibody protein solutions were conjugated to fluorescein isothiocyanate using a concentration of 0.05 mg. of fluor per mg. of protein. The technical procedures employed in conjugation and immunochemical staining of sections as well as the optical equipment photography and controls were similar to those described previously (17). Prior to immunohistochemical staining, the frozen sections were routinely fixed in alcohol ether and 95 per cent alcohol. Occasionally duplicate sections were washed in buffered saline (two changes) for 5 minutes prior to alcohol fixation. This latter procedure did not essentially change the detection of immunologic reactants in the tissue sections when compared to the unwashed sections.

The individual experiments are outlined in Table I.

BSA Experiments.—Six different initial schemes of BSA injection (daily doses of 0.5, 5,

TABLE I

Outline of Experiments and Results

Injections		No. of rabbits			Antibody responses and chronic glomerulonephritis					
Antigen	Initial dose	Total	Glomerulo-nephritis		Antibody excess		Equivalence		Antigen excess	
			Ac	Chr	Total	Glomerulo-nephritis	Total	Glomerulo-nephritis	Total	Glomerulo-nephritis
	mg.									
BSA	0.5	12	0	3	6	1	4	2	2	0
BSA	5	3	—	2	2	1	1	1	0	0
BSA	10	42	10*	12	34	5	7	7	1	0
BSAx	10	13	0	2	6	1	2	1	5	0
BSA	25	4	—	2	4	2	0	0	0	0
BSA	50	8	—	3	5	2	1	1	2	0
Totals.....		82‡	10	24	57	12	15	12	10	0
HSA	10E	11	4	1	10	0	1	1	0	0
BGG	10	10	0	5	7	2	3	3	0	0
BGGx	10	15	0	5	3	2	3	3	9	0
BGG	25	11	1	5	6	4	1	1	4	0
Totals.....		36	1	15	16	8	7	7	13	0
HGG	10	9	0	2	1	0	3	2	5	0
HGGx	10	10	0	2	6	1	2	1	2	0
HGG	25E	12	1	1	5	1	0	0	7	0
Totals.....		31	1	5	12	2	5	3	14	0
Total all groups..		160	16	45	95	22	28	23	37	0

x, 150 r whole body x-ray 1 to 2 days before beginning of injections.

E, 10 μg. *E. coli* endotoxin (Difco) given intravenously with first injection of antigen to increase antibody response.

* Two of these rabbits also developed chronic glomerulonephritis late in course after period of normal urine and increase in dose of antigen.

‡ 20 of these 82 rabbits were not observed for proteinuria during first 4 weeks of injections

10, 10 mg. preceded 2 days by 150 r whole body x-ray,[1] 25, and 50 mg.) were employed in six experiments involving a total of 82 rabbits. However, the doses of antigen given individual rabbits within each experiment were varied as the experiment progressed according to the individual antibody responses observed. For the 10 rabbits developing transient proteinuria

[1] Radiation given by a 220 kv., 15 ma. Picker installation with ¼ mm. Cu and 1 mm. Al added filtration which at 85 cm. delivered a dose of 18 R.P.M. measured in the middle of a paraffin rabbit phantom.

during the first 2 weeks of injections and for the 24 rabbits developing chronic glomerulonephritis, the doses of antigen employed at time of development of proteinuria are given on Tables II and III. The experiments varied from 6 to 12 months in duration. The interval between initiation of injection and development of proteinuria is given for each rabbit in

TABLE II

Acute Glomerulonephritis

Rabbit	Antigen	Dose	Antibody responses after 2–3 wks. injections*	Proteinuria		
				Onset, wk. of injection	Duration	Maximum‡
		mg.			*wks.*	
78-42	BSA	10	+++	2	1	350
78-43	BSA	10	++	2	3	600
78-50§	BSA	10	+++	2	1	400
78-52	BSA	10	++++	2	1	150
78-56	BSA	10	++++	2	2	1500
78-58	BSA	10	++++	1	1	650
78-66§	BSA	10	+++	2	1	100
78-68	BSA	10	++++	1	2	1300
78-72	BSA	10	++++	2	1	150
81-75	BSA	10	++++	2	1	55
83-05	HSA	10E	+++	1	1	5000
83-10	HSA	10E	+++	1	1	1500
83-13	HSA	10E	++++	1	1	500
83-14	HSA	10E	++++	1	1	1100
83-01	BGG	25	++++	1	1	200
82-79	HGG	25E	++++	1	1	2800

E, 10 μg. *E. coli* endotoxin (Difco) given intravenously with first injection of antigen to increase antibody response.

* ++++, best 25 per cent responders; +++, second best 25 per cent; ++, third best 25 per cent.

‡ mg. protein/100 ml. urine 24 hour specimen.

§ Also developed chronic glomerulonephritis late in course after period of normal urine and increase in dose of antigen.

Tables II and III. Data concerning those rabbits not developing renal disease are omitted from the tables for simplicity of presentation.

HSA Experiment.—Eleven rabbits were started on daily injections of 10 mg. HSA with 10 μg. *Escherichia coli* endotoxin[2] given intravenously with the first dose of antigen to enhance the antibody response. Doses of antigen used in the 5 rabbits showing renal disease appear on Tables II and III.

BGG Experiments.—Three experiments were carried out using a total of 36 rabbits. The

[2] *E. coli* endotoxin Lot O111:B4 supplied by Difco.

TABLE III

Chronic Glomerulonephritis

Rabbit	Antigen	Dose		Mos. of injections before proteinuria	Wks. of equivalence before proteinuria	Degree proteinuria*	Morphologic changes‡			
		Initial	At proteinuria				Basement membrane thickening	Glomerular proliferation	Inflammation	Scarring
		mg.	*mg.*							
80-07	BSA	0.5	1	4.75	5	4+	4	2	2	0
80-12	BSA	0.5	0.5	3.25	1	1+	1	1	0	0
80-14	BSA	0.5	1	7	5	4+	3	4	2	0
76-87	BSA	5	15	7	4	3+	4	3	2	1
76-90	BSA	5	5	<3	4	Not done	4	2	2	1
76-92	BSA	10	10	<3	7	Not done	2	2	2	4
76-93	BSA	10	50	7	2	4+	4	3	2	2
76-94	BSA	10	50	7	3	3+	4	3	3	4
78-50§	BSA	10	50	3	2	3+	3	1	3	2
78-55	BSA	10	10	2	0	2+	1	2	0	0
78-64	BSA	10	10	3.5	3	3+	4	2	2	1
78-66§	BSA	10	50	4.5	1	3+	3	3	2	0
78-69	BSA	10	10	2	1	4+	3	3	2	0
78-71	BSA	10	10	1.5	3	2+	3	3	2	0
78-74	BSA	10	50	4.25	3	1+	2	2	1	0
78-75	BSA	10	10	2	1	3+	—	—	—	—
78-80	BSA	10	10	2	4	3+	3	3	2	0
81-64	BSA	10	25	4	9	3+	2	3	2	4
81-71	BSA	10	50	3	2	3+	3	3	3	4
76-97	BSA	25	200	7.5	2	3+	4	3	4	1
76-98	BSA	25	50	7.5	1	4+	4	3	4	4
77-69	BSA	50	50	<2.5	2	Not done	3	3	4	3
77-92	BSA	50	100	4	1	2+	4	1	1	1
77-95	BSA	50	50	<1.5	0	2+	1	3	1	0
83-03	HSA	10E	25	2.75	4	3+	3	4	3	4
81-99	BGG	10	50	3.25	2	2+	2	1	0	0
82-00	BGG	10	25	4	2	1+	—	—	—	—
82-04	BGG	10	10	2.75	9	2+	1	2	2	1
82-05	BGG	10	10	3.75	4	2+	2	2	2	1
82-08	BGG	10	10	4	2	4+	3	3	3	2
81-85	BGGx	10	25	3	2	4+	2	2	2	2
81-86	BGGx	10	10	3	5	4+	2	3	2	0
81-87	BGGx	10	10	3.75	11	2+	2	1	1	0
81-89	BGGx	10	50	3.5	4	2+	2	2	1	0
81-94	BGGx	10	10	4	3	4+	3	4	4	1
82-94	BGG	25	50	2	1	4+	3	3	4	4

TABLE III—*Continued*

Rabbit	Antigen	Dose		Mos. of injections before proteinuria	Wks. of equivalence before proteinuria	Degree Proteinuria*	Morphologic changes‡			
		Initial	At Proteinuria				Basement membrane thickening	Glomerular proliferation	Inflammation	Scarring
		mg.	*mg.*							
82-95	BGG	25	10	1	2	2+	2	1	1	0
82-96	BGG	25	50	1.5	1	4+	—	—	—	—
82-97	BGG	25	50	1.75	2	3+	3	2	2	2
82-98	BGG	25	50	3	4	±	1	3	3	0
80-78	HGGx	10	10	4	8	2+	—	—	—	—
80-80	HGGx	10	25	6.5	14	2+	2	1	1	0
80-84	HGG	10	10	3.5	6	3+	3	2	1	0
80-89	HGG	10	10	4	14	4+	3	2	3	0
82-80	HGG	25E	50	2	1	4+	4	4	1	4

x, 150 r whole body x-ray 1 to 2 days before beginning of injections.

E, 10 μg. *E. coli* endotoxin (Difco) given intravenously with first injection of antigen to increase antibody response.

* ±, less than 20; 1+, 20–100; 2+, 100–500; 3+, 500–1000; 4+, more than 1000 mg. protein/100 ml urine for 2 or more weeks.

‡ Each morphologic change graded from 0 (normal) to 4 (most severe).

§ Also developed acute glomerulonephritis.

first employed 10 mg. BGG daily, the second 10 mg. BGG preceded 2 days by 150 r whole body x-ray to reduce the antibody response, and the third 25 mg. BGG. For those rabbits developing renal disease, the size and duration of injections before onset of renal disease are given in Tables II and III.

HGG Experiments.—Three experiments utilized 31 rabbits. The first experiment began with 10 mg. HGG daily. The second was initiated with 150 r whole body x-ray followed 2 days later by 10 mg. HGG daily. The third used a single injection of 10 μg. *E. coli* endotoxin intravenously with the first of daily 25 mg. HGG injections. The doses of HGG and duration of injections prior to development of renal injury are listed on Tables II and III.

RESULTS

The morphological and functional renal changes caused by the four different antigens were identical in kind, although they occurred with varying frequency with the different antigens. In view of this similarity, the following descriptions pertain to the events following the administration of any of the antigens.

Antibody Responses.—The antibody responses of all 160 rabbits to the initial level of daily injections of antigen have been divided into three groups (Table I). They were termed *antibody excess* if the rabbits made more antibody than could be neutralized by the daily injections of antigen and free antibody was always present in the circulation. In these rabbits, the intravenously injected antigen was almost immediately removed from the circulation. They were classed as *equivalence* if the amount of antibody in the circulation was neutralized by the daily injections of antigen so that the immunologic reaction in the circulation varied

from an excess of antigen, shortly after injection of antigen, to excess of antibody, sometime prior to the next injection. In these equivalence rabbits complexes of antigen and antibody were present in the circulation much of the time. One hour after injection more than one-half the antigen was present in complexes in the circulation, 6 hours after about one-third, and by 24 hours, just prior to the next injection, free antibody had reappeared in the serum of most rabbits. They were classed as *antigen excess* if there was always excess antigen in the circulation and little or no evidence of antibody formation.

At the initial dose levels, 95 of the 160 rabbits made antibody in excess of that needed to neutralize and eliminate the injected antigen (Table I). In almost all the 95 rabbits, this level of antibody was maintained many months, as long as injections were given. Some of the highest levels of precipitating antibody observed in this laboratory as a result of any immunization scheme (> 3 mg. Ab N/ml. serum) were seen in some rabbits receiving 10 to 50 mg. antigen daily. In only a few rabbits receiving the gamma globulin antigens did the rate of antibody formation decrease with time and then only after several months of injections. During the course of the experiments, the antigen doses were increased for the rabbits in antibody excess in an attempt to reach an equivalence state. Daily injections of as much as 200 mg protein were given without eliminating the excess antibody in extreme cases. Many of these rabbits died in anaphylaxis as a result of the large injections of antigen and others with extremely high levels of antibody were removed from the experiment. In 21 of these 95 rabbits it was possible to reach and maintain an equivalence situation by increasing the amount of antigen injected. Twenty-eight of the 160 rabbits responded to the initial level of daily injections with antibody in the equivalence range and their doses of antigen were not altered except in the case of one rabbit which, after 2½ months of injection of BGG began to make less antibody, and then the dose of BGG was reduced. Thirty-seven of the rabbits made no detectable antibody during several months of antigen injections.

Acute Glomerulonephritis.—As shown in the accompanying tables, 16 of 140 rabbits developed acute glomerulonephritis; *i.e.*, significant proteinuria during the first 2 weeks of injections. The acute glomerulonephritis was seen in a higher incidence in the animals receiving the albumin antigens (BSA—16 per cent, HSA—36 per cent) than in the animals receiving gamma globulins (BGG-3 per cent, HGG-3 per cent). The rabbits developing the early proteinuria were among the better antibody responders. Ten were in the upper ¼ of antibody responders, five were in the second ¼, and only one was among the third ¼. However, ⅓ of the best responders did not develop early proteinuria (Table II). Of the 38 rabbits x-rayed prior to initiation of injections in order to diminish and delay their antibody response none showed an early proteinuria. The proteinuria appeared by the end of the 1st week in half of the 16 animals and during the 2nd week in the other half. The proteinuria was heavy, reaching levels above 1000 mg./100 ml. urine in 6 rabbits. In general, those rabbits developing proteinuria earliest had the highest proteinuria, and among the BSA rabbits, those with the most prolonged proteinuria had the highest protein levels in the urine. Those rabbits in which the antibody responses would be expected to have been earliest, *i.e.* those receiving globulin antigens and those receiving endotoxin with albumin antigens, developed their proteinuria within the 1st week. In all rabbits, at the time of appearance of proteinuria, there was evidence of antibody formation by the host, either in the form of rapid elimi-

nation of antigen or presence of circulating antibody. In 13 of the 16 rabbits, the early proteinuria was brief, lasting about 1 week; in two the proteinuria lasted 2 weeks; and in one the proteinuria persisted for 3 weeks, at which time the rabbit died. Once the urines cleared, all but 2 of the 16 rabbits remained normal for the rest of the experiment. These two rabbits, Nos. 78-50 and 78-66, lost their acute proteinuria and were functionally normal for several months, and then with an increase in dose of antigen, which matched or slightly exceeded their ability to form antibody, they developed chronic glomerulonephritis.

The morphologic changes in the kidneys accompanying this early, transitory proteinuria were observed in weekly biopsies in several of the rabbits. The renal changes corresponded to those described in classical or "one-shot" serum sickness (9, 10). There was a marked proliferation of the glomerular capillary endothelium seen in both light and electron microscopy (Figs. 1 and 2). This proliferation obliterated most of the capillary lumina and caused the glomeruli to fill or distend Bowman's space. There was also a moderate accumulation of polymorphonuclear leukocytes in the glomeruli. There was no consistent change in the glomerular basement membranes and few, if any, capsular adhesions. The morphologic picture was quite comparable to that seen in acute proliferative glomerulonephritis in the human. Immunohistochemical stains for antigen showed it to be present in small granules scattered throughout the glomeruli without obvious anatomical localization as previously described in "one-shot" serum sickness (13). Only rarely were deposits observed in the electron microscope which might have corresponded with the fluorescent granules. In these instances the material was present along the capillary basement membrane on the luminal side (Fig. 3). This lesion resolved rapidly with glomerular structure returning to normal in 1 to 2 weeks (Fig. 4).

Chronic Glomerulonephritis.—The most consistent immunologic characteristic of the rabbits developing chronic glomerulonephritis was that most of these rabbits were in the equivalence range when the proteinuria developed; *i.e.*, the doses of antigen they were receiving were sufficient to neutralize their circulating antibody. Thus, after each injection of antigen there were free antigen and antigen-antibody complexes which persisted in the circulation for several hours and then were replaced by an excess of antibody prior to the next daily injection. Of the 45 rabbits with chronic glomerulonephritis, 43 were in the equivalence range when proteinuria developed. The remaining rabbits (Nos. 77-95 and 78-55) were in antibody excess when mild proteinuria and atypical glomerular capillar endothelial proliferation developed (Table III). For each of the antigens, the incidence of chronic glomerulonephritis appeared to be independent of the amount of antigen given (Table I). Those rabbits responding in equivalence at each dose level were susceptible to the same renal disease, morphologically and functionally.

Of the 28 rabbits which made an antibody response in the equivalence range

to the initial level of antigen injections, 23 had well developed chronic glomerulonephritis (Table I). The remaining 5 rabbits in this equivalence group had slight, inconstant proteinuria and demonstrable microscopic glomerular abnormalities which were of similar kind but not as severe as those seen in the other 23 rabbits. These 5 probably can best be considered to have minimal glomerulonephritis. There were no obvious immunologic features of these 5 rabbits with minimal glomerulonephritis which would set them apart from the 23 with more advanced disease. Of the 95 rabbits initially responding with an excess of antibody, 22 developed chronic glomerulonephritis after increases in their doses of antigen. Twenty-one of 95 rabbits originally in antibody excess were brought to equivalence by increasing the dose of antigen, and of these, 20 developed chronic glomerulonephritis. The remaining 2 rabbits in the antibody excess group with chronic glomerulonephritis developed mild proteinuria while in antibody excess. The other 72 of the 95 initial antibody excess group could not be brought to an equivalence state and did not develop kidney disease in spite of prolonged courses of antigen injection. None of the 37 rabbits failing to make detectable antibody developed kidney disease in spite of continued injections over many months.

The bovine antigens appeared to be more effective in producing chronic glomerulonephritis than did the human antigens. Twenty-nine per cent of the rabbits receiving BSA and 42 per cent of those receiving BGG developed chronic glomerulonephritis, while only 9 per cent of the HSA and 16 per cent of the HGG rabbits developed chronic glomerulonephritis. One further difference between antigens was that in all the BSA animals, chronic glomerulonephritis, once it developed, progressed as long as antigen injections were maintained, while in 4 of 10 BGG animals with chronic glomerulonephritis after several months the rate of antibody synthesis decreased and the glomerulonephritis improved. Attempts to manipulate the immune response by giving x-radiation prior to antigen or endotoxin with the first injection of antigen did not make any significant change in the incidence of chronic glomerulonephritis.

In Table III are listed for each rabbit developing chronic glomerulonephritis the initial doses of antigen given, the dose employed at onset of proteinuria, the total period of injections before the onset of proteinuria, the time during which the antigen-antibody ratio in the circulation was at equivalence prior to onset of proteinuria, the greatest degree of proteinuria attained for at least a 2 week period, and the morphologic changes in the kidney seen at autopsy with the light microscope. The morphologic changes recorded are the degree of basement membrane thickening, the proliferation of glomerular epithelial and endothelial cells, the inflammatory reaction evidenced by leukocytes, largely polymorphonuclear, in the glomeruli, and scarring as evidenced by crescent formation, adhesions across Bowman's space, fibroblastic obliteration of glomerular tufts, and hyalinization.

Correlations among most of the parameters observed are not striking. In general it appears that the larger the dose of antigen given at onset of proteinuria, the shorter the period of equivalence prior to proteinuria. The degree of proteinuria and the extent of morphologic damage to the kidney (especially thickening of the basement membrane) were also positively correlated. Among the BSA rabbits there appeared to be a correlation between the number of inflammatory cells in the glomeruli and the size of the dose of antigen. Probably better correlations of morphologic changes and other observations would have been possible if the morphologic grading could have been based on biopsies early in the course of the disease and not on autopsy tissue.

Morphologically, the renal changes developing in these rabbits were very similar to those seen in human subacute and chronic glomerulonephritis. The earliest and most frequent alteration observed in the light microscope was a diffuse hyaline, amorphous thickening of the glomerular capillary basement membrane (Fig. 5). The thickened membranes were strongly eosinophilic and PAS-positive. In some instances this change, even in an advanced state, was seen without other severe glomerular damage and then resembled the membranous or nephrotic type of glomerulonephritis. More commonly, however, advanced basement membrane thickening was associated with lobulation of glomerular capillaries, inflammation, proliferation of glomerular epithelial and endothelial cells, and scarring and obliteration of the glomeruli (Figs. 6, 7). These advanced lesions resembled the changes seen in the proliferative form of human chronic glomerulonephritis. There were no consistent or significant morphologic changes in the extra renal tissue of the rabbits with chronic glomerulonephritis except for mild to moderate cardiac hypertrophy.

The thickened basement membranes stained strongly and uniformly for the specific antigen, the host's gamma globulin, and presumably specific antibody when treated with appropriate immunohistochemical reagents. The stainable material in all three reactions was distributed identically throughout the entire glomerulus and in all glomeruli in the form of discrete, granular precipitates that in places coalesced to form homogeneous, although irregular, bands along the capillary walls (Figs. 8 to 10). The degree of antigen staining was independent of the dose or kind of antigen injected and was related primarily to the degree of thickening of the basement membranes. In no instance was stainable antigen found in kidneys that showed no basement membrane thickening. Characteristically, when a glomerulus showed more advanced changes, such as capsular proliferation and fibrosis, these scarred regions were devoid of stainable antigen in contrast to the brightly stained thickened basement membranes in unscarred regions (Fig. 11). Present in the glomerular capillaries of some animals with a significant inflammatory component to their glomerulonephritis, were hematogenous leukocytes, both polymorphonuclear and mononuclear, containing granular antigen presumably in complex form. This appearance

suggested that the leukocytes had phagocytized the immunologic complexes either within the kidney or elsewhere in the circulation. The extra glomerular components of the kidneys contained no detectable antigen except for a dust-like sprinkling of the convoluted tubular epithelial cells in a few rabbits. The only extra renal localization of antigen found was a fine dust-like deposit in the walls of splenic arterioles in animals with chronic glomerulonephritis similar to that seen in serum sickness (13). There was no morphologic abnormality associated with this deposition of antigen.

With electron microscopy, the most frequent alteration observed in the kidneys of rabbits with chronic glomerulonephritis was the deformation of the glomerular basement membrane. Examination at moderately high magnifications revealed a dense deposit on the epithelial side of the lamina densa (Figs. 12, 13). The precise localization of this dense deposit varied; in some instances it was clearly distinguishable from basement membrane and in other instances, it blended with the lamina densa. Presumably this material was precipitated antigen-antibody complexes, and corresponded to the distribution of specific complexes as visualized by fluorescent microscopy. Closely applied to these deposits were sheets of epithelial cytoplasm rather than foot processes. For the most part the capillary loops were patent and their outlines somewhat angulated. More frequently in the intercapillary area three or four cells were seen, an increase over the one or two usually found in control kidneys. No constant abnormalities in mitochondria, Golgi element, or endoplasmic reticulum of either epithelial or endothelial cells could be observed. The more advanced and extensive glomerular lesions (Fig. 14) were characterized by the presence of numerous varying sized masses of dense material, beaded along the extra capillary surface of the basement membrane. These deposits were most likely antigen-antibody complexes which corresponded closely both in size and distribution to the fluorescent particles visualized on the fluorescent microscope. Over these deposits the foot processes were smeared or replaced entirely by broad sheets of cytoplasm. In addition, the capillary loops were usually partly filled with proliferated endothelial cells and leukocytes. In the severest lesions, the lumina were completely obstructed by these cells. The capillaries were usually drawn together and formed a lobule of solid cellular elements. In the most advanced lesions (Fig. 15) it was difficult to distinguish epithelial from endothelial cells, the basement membranes were wrinkled, enfolded, or fragmented, and extensive synechiae were present, manifested by numerous nondescript cells massed in Bowman's space between glomerular capsule and capillaries. Rarely, fibrils with the periodicity of collagen were present in the scarred areas of severely injured glomeruli.

The glomerular alterations of the 2 rabbits developing proteinuria while in antibody excess (Nos. 77-95 and 78-55) were significantly different from those seen in the other rabbits with chronic glomerulonephritis. These 2 rabbits

showed principally a proliferation of the mesangial or stalk cells of the glomeruli with little evidence of basement membrane or other change. This proliferation was distinguishable from that seen in acute glomerulonephritis where there was diffuse proliferation of all endothelial cells with occlusion of capillaries. In the two antibody excess rabbits, the capillaries at the periphery of the tufts were widely patent except for occasional hyaline embolic material which reacted as antigen-antibody complexes in immunohistochemical staining. The basement membrane in these rabbits contained little or no stainable antigen or host's gamma globulin.

In order to determine the reversibility of the renal damage at various stages in its development, 19 rabbits with chronic proteinuria had renal biopsies and injections of antigen were stopped. The course of proteinuria was then followed with weekly urinanalyses and morphologic changes by periodic renal biopsy. Of the 19 rabbits, 14 were observed for more than 1 month after biopsy and cessation of injections of antigen. The other 5 died within 1½ to 3 weeks of biopsy of progressive renal failure which was advanced at time of biopsy. Of the 14 observed for more than 1 month, 5 had irreversible renal functional damage which was followed for from 5 to 19 weeks. Three of these died in uremia and 2 are alive 16 and 19 weeks after cessation of antigen with severe proteinuria and variable hematuria. All of these rabbits had severe proteinuria prior to biopsy. Six of the 14 rabbits had disappearance of all but traces of proteinuria from 1 to 3½ months after cessation of antigen. These 6 rabbits had only mild proteinuria (100 to 300 mg. per cent) prior to biopsy. Three of the 14 have had their proteinuria only partially clear during an observation period of 5 months and their prebiopsy proteinuria was moderate to severe. The morphologic alterations at biopsy in those rabbits with irreversible damage included, in addition to severe basement membrane thickening, more proliferation, inflammation, and scarring than was found in those with reversible functional alterations. Those rabbits dying after cessation of antigen had progression of renal damage, particularly scarring, in absence of continued antigen injections. Thus, in these rabbits in which damage had already progressed to scarring, the process seemed progressive in the absence of further antigen injections. In the 6 rabbits in which proteinuria diminished greatly after cessation of antigen there was moderate basement membrane thickening, but lesser degrees of proliferation and inflammation and no scarring seen in biopsy.

Morphologic repair after cessation of antigen injections was inconspicuous in all rabbits as viewed with the light microscope. There was some reduction in the inflammatory reaction in the glomeruli after cessation of antigen injections, but the basement membrane thickening and proliferative changes did not diminish within 5 to 6 months in any animals regardless of the stage the disease had reached. Immunohistochemical observations, however, showed a decrease in the amount of stainable antigen in the glomerular basement mem-

branes of all rabbits once antigen was no longer injected (Figs. 16 and 17). After 6 months without antigen injections, the originally heavy antigen and host gamma globulin staining of the entire basement membrane had diminished to a light sprinkling of stain in the capillary wall. Observations with the electron microscope confirmed the findings of the light microscope. The basement membranes remained diffusely thickened in spite of cessation of antigen. However, there was the apparent breaking up of the antigen-antibody deposits. Two to 3 months after cessation of antigen, the deposits were no longer present as dense masses but were of irregular density and were found extending out into Bowman's space (Fig. 18). Cytoplasmic processes of epithelial cells adhered to the periphery of the material. In some instances a single globule of the deposit was contained within the cytoplasm of the epithelial cells at a distance from the basement membrane. The capillaries contained fewer cells and were more patent than they were in biopsies taken from the same animals before antigen injections were terminated. Further, foot processes of normal configuration and distribution returned and were seen covering relatively large segments of the capillaries. Complete return to normal architecture as well as complete disappearance of stainable antigen was not seen, however, in any of the biopsies even 6 months after cessation of antigen injections.

Rabbits with chronic glomerulonephritis had a number of other abnormalities. Those rabbits with most severe proteinuria became hypoproteinemic; there was a fall in serum albumin to negligible levels, and in gamma globulin to 50 per cent of normal, while the changes in alpha globulin were inconstant and the beta globulin levels remained normal. In the most hypoproteinemic rabbits, dependent subcutaneous edema and ascites developed. In rabbits with the most severe renal damage, uremia was observed. While blood urea nitrogens were not run on all animals at autopsy, in seven with severe proteinuria the blood urea nitrogens were between 132 and 504 mg. per cent. In most of the rabbits with only mild to moderate proteinuria, the blood urea nitrogens were within normal limits. Also, serum cholesterol values in those animals with most severe proteinuria, hypoproteinemia, and uremia were elevated to approximately twice upper normal values. A few rabbits with the most severe renal damage and uremia developed hypertension as judged by their cardiac hypertrophy. In these rabbits the heart weight/body weight ratio was increased 1.5 to two times normal. Borderline cardiac hypertrophy developed in most of the rabbits with moderate renal changes.

DISCUSSION

It is apparent from these observations that functional and morphologic alterations quite similar to acute, subacute, and chronic human glomerulonephritis develop in the kidneys of some rabbits receiving daily injections of any one of several foreign serum proteins. One of the critical factors determining whether

a rabbit will develop glomerulonephritis or not appears to be the amount of antibody formed in response to the antigen injected. In all rabbits in which the amount of antibody formed barely neutralized the antigen injected, some degree of chronic renal disease developed. Since no renal injury appeared in those animals injected with antigen but making little or no antibody, it appears that the antigens *per se* did not injure the kidneys. Since those animals making excessive amounts of antibody also showed no chronic kidney damage, the antibody response itself or an antigen-antibody interaction in an antibody excess environment did not appear to be nephritogenic. Therefore, it seems reasonable to conclude that the daily interaction of host's antibody with slightly excess amounts of antigen in the circulation was related to the development of chronic glomerulonephritis.

These studies give considerable information about the fate of antigen and antibody in the glomerulonephritic animals so that some postulations concerning pathogenesis may be made. However, even in this relatively simple model the pathogenetic factors are not entirely clear. In the rabbits developing chronic glomerulonephritis, the daily injections of antigen usually exceeded the circulating antibody so that for some time after injection there were excess antigen and antigen-antibody complexes in the circulation which were replaced by an excess of antibody within a 24 hour period. In the kidneys of all animals with proteinura and significant chronic morphologic renal alterations, easily demonstrable concentrations of antigen and host's gamma globulin were found in the glomerular capillary wall. Further, such deposits of antigen were never seen in animals which did not develop renal damage or in animals destined to develop chronic glomerulonephritis prior to onset of renal functional and morphologic changes. There was no vascular localization of antigen in any extra renal tissues of any rabbits except for a light deposit in the walls of morphologically normal splenic arterioles of some of those with chronic glomerulonephritis. Whether the antigen, presumably in complex form, lodges in the kidney and acts as the etiologic agent of renal disease, it is not possible to say with certainty; however, this is an attractive possibility. This possibility is supported by observations of others of the nephritogenic potentialities of preformed antigen-antibody complexes injected intravenously into animals (12). On the other hand, it is possible that the antigen-antibody reaction in the circulation might act systemically to liberate nephritogenic factors and that the localization of complexes in the glomeruli might be secondary to renal damage. However, the localization of antigen along the basement membrane as part of a non-specific deposition of plasma protein, perhaps related to proteinuria, seems unlikely since neither host albumin nor fibrinogen were found in the deposits.

Rather than mediating renal injury directly, it is possible that the antigen in complex form might lodge along the basement membrane and serve as an antigen depot with which the host's antibody, to the antigen itself or the

antigen-antibody complex, might then react and produce renal injury. This latter pathogenesis would resemble the chain of events presumed to occur in the delayed component of nephrotoxic serum nephritis where a foreign protein attached to renal glomerular basement membrane is made the target of the host's immunologic response. This possibility is not supported by the present study, however. Never was antigen seen concentrated along basement membrane prior to the development of glomerulonephritis. The gradual improvement in renal function seen in some rabbits after cessation of injections of antigen while concentrations of antigen in the glomeruli persisted would also seem to contradict this viewpoint.

There must be, however, other pathogenetic factors than merely the presence of an immune reaction or antigen-antibody complexes in the circulation. The lack of a strong correlation between the amount of reaction or circulating complexes, *i.e.* size of dose of antigen and duration at equivalence and degree of morphologic or functional renal abnormality, suggests additional pathogenetic factors. The type of tissue response, *i.e.* merely a deposition of material along the basement membranes or a proliferative, inflammatory or scarring reaction, may at least in part be determined by non-immunological conditions. The fact that severe renal disease could be produced by 0.5 to 1 mg. antigen/day suggests that much of the immunologic reaction occurring with larger injections was not essential to the pathogenesis of the disease.

The reason for the precise localization of the antigen-rich deposits along the outer surface of the glomerular basement membrane is unknown. Their presence in this site could result from specific, exclusive precipitation here, caused by unknown factors, or they might be more effectively preserved here than elsewhere. In any case, this region apparently does offer a protected site for the deposited antigen, because demonstrable antigen may persist here for as much as 6 months after cessation of antigen injections. In this site the deposit must be sequestered from phagocytic cells and proteolytic enzymes which are known to act promptly on antigen-antibody complexes in other situations (18). The persistence of antigen here is reminiscent of the long persistence of I^{131}-labeled heterologous antikidney antibodies localized in glomeruli (19) presumably attached to basement membranes (20).

In the case of the acute glomerulonephritis, a close relationship between disease and size of antibody response is also seen. Two-thirds of the rabbits with early proteinuria were among the very best antibody responders and all but one with acute glomerulonephritis made a large antibody response. During the 1st week or two of injections, before significant amounts of antibody are formed, the amount of antigen in the circulation increases, approaching levels such as those seen in classical "one-shot" serum sickness. Then with the onset of antibody formation the immunologic environment in the serum shifts from one of antigen excess to one of antibody excess. During this shift, antigen-anti-

body complexes of all types must be formed in the circulation. Those rabbits with the largest antibody response would have the highest concentrations of circulating complexes, albeit for the shortest period of time. The tissue change seen in this early phase is an endothelial proliferation in the kidney with small amounts of antigen and presumably complexes scattered diffusely throughout the glomeruli, apparently not concentrated along capillary basement membranes. Thus, the morphologic changes associated with proteinuria in this instance differ from those seen in the chronic disease. Another apparent difference in the acute reaction was the development of arteritic and endocarditic lesions seen in the active form in those rabbits sacrificed during the acute glomerulonephritis and seen as scars in coronary vessels at autopsy sometime after disappearance of the acute disease. In the rabbits with chronic glomerulonephritis, there was a conspicuous absence of vascular and cardiac lesions as noted also by previous workers (14). It may be that the higher concentrations or perhaps greater variety of complexes formed during the acute disease predispose to the cardiovascular damage, while smaller concentrations of complexes present over long periods do not cause cardiovascular damage.

How, then, does this experimental model of immunologically induced glomerulonephritis compare with human kidney disease? Obviously the mode of introduction of antigen in the experimental situation could not apply in the human, but prolonged contact with antigen in the human is a possibility and some host factors might operate similarly in both conditions. Several clinical aspects of the acute form of the experimental disease appear to resemble acute proliferative human glomerulonephritis. If one can consider the antigenic stimulus of the streptococcal infection preceding many cases of acute glomerulonephritis to be comparable to the foreign protein injected into the rabbit, there are several parallels. First, those children who develop acute nephritis following streptococcal infection usually are among those who make large antibody responses to the streptococcal antigens (1, 3) as is the case in the experimental disease. Second, acute glomerulonephritis, especially of childhood, is usually a self-limited disease followed by virtually complete recovery and, at least according to some, relative immunity to recurrence (1). This would appear to be the case also in the experimental disease. The morphologic changes in the experimental acute glomerulonephritis are almost identical with the changes seen in acute proliferative human glomerulonephritis.

The subacute and chronic experimental diseases may be more difficult to compare with their human counterparts, because this group of human diseases is itself not homogeneous or well understood. It may be significant that in neither the chronic experimental disease nor in many cases of chronic human glomerulonephritis is there evidence of a large immune response. However, in both clinical and experimental lesions, concentrations of host's gamma globulin can be found in glomerular capillary walls (21). In addition, morphologically

and clinically, the nephrotic stages of human glomerulonephritis closely resemble the majority of the instances of chronic disease in our rabbits. Primary basement membrane thickening and epithelial cell abnormalities dominate the morphologic picture of both entities. Proteinuria, hypoproteinemia, edema, and hypercholesterolemia are also common to both. Similarly, the proliferative, obliterative or scarring forms of human disease resemble the most advanced examples of the experimental disease. Here also, the morphologic correspondence is close—even to the osmophilic deposits on the outer surface of glomerular basement membranes seen in both human (22) and experimental chronic glomerulonephritis. These deposits are known to contain antigen and probably antibody in the experimental disease. The basement membrane changes, proliferation, inflammation, and scarring seen in the rabbits are identical with those seen in human disease. Proteinuria, hematuria, uremia, and hypertension, as well as hypoproteinemia and edema accompanied the advanced stages of human and experimental disorders.

The difficulties in translating what we know of the pathogenesis of this experimental glomerulonephritis to the human disease are obvious. The constant introduction of antigen is hard to visualize in most clinical situations unless the antigen were endogenous. The amounts of antigen used in the experimental disease are probably larger than most antigenic exposures resulting from infections, etc. However, since the smallest doses employed in these studies were as nephritogenic as the larger doses, we cannot conclude anything regarding amounts of antigen needed to produce the experimental disease.

Perhaps more profitable than trying to draw comparisons between an experimental disease in rabbits and a clinical entity in humans is considering the implications of this experimental disease. First, as has been shown for conventional serum sickness, this renal injury is precipitated by the administration of antigens with no demonstrable affinity for, or immunological relationship to, constituents of the kidney. Second, the acute form of the disease is associated with a hyperactive antibody response while the subacute and chronic forms are associated with relatively poor antibody responses. Thus, the magnitude of the antibody response to a given antigenic stimulus determines the type of disease, and severe hypersensitivity disorders can be associated with poor as well as with good antibody responses. Third, complexes of antigen and antibody localize in the kidney without any apparent immunologic reason, presumably as a result of anatomic or physiologic factors. Fourth, the antigen-antibody interaction in the circulation or the complexes themselves, neither of which is immunologically oriented against kidney, appear to be able to induce pathological changes which are quite similar to the changes produced by heterologous antikidney serum. If the complexes themselves mediate the injury after first localizing in the kidney, the important factor in both this experimental disease and in antikidney serum disease might be the focusing of an immunological

reaction, even though it be non-specific for kidney, in this anatomic site. It is possible, therefore, that antigen-antibody complexes in the circulation may act as potential immunologic pathogens capable of mediating tissue damage without immunologic specificity for the injured structure.

SUMMARY

Daily injections of any one of several foreign serum proteins produced in rabbits functional and morphological alterations similar to those seen in acute, subacute, and chronic human glomerulonephritis. The critical factor determining whether a rabbit would develop renal disease and the type of disease developed was the amount of antibody the rabbit formed. Those responding with much antibody were likely to develop an acute, self-limited glomerulonephritis and to be subsequently immune to further renal damage. Those responding with antibody barely sufficient to neutralize the antigen injected developed subacute and chronic glomerulonephritis. In the circulation of the rabbits with chronic glomerulonephritis, there was a daily recurring antigen-antibody reaction in the region of near antigen excess to near antibody excess which presumably led to the disease. Antigen apparently in the form of antigen-antibody complexes was deposited along the renal capillary basement membranes coincident with the development of subacute and chronic glomerulonephritis. Once developed, the morphologic stigmata of chronic glomerulonephritis persisted even after injections of antigen were stopped. However, in milder instances the renal function recovered in part after stopping antigen.

This experimental model has several implications: first, the renal injury is precipitated by antigens with no known affinity for, or immunologic relationship to, kidney; second, antigen antibody complexes localize in the kidney, apparently on the basis of non-immunologic factors, and may be an etiologic agent of renal injury; third, severe hypersensitivity disorders can be related specifically to relatively poor as well as to good antibody responses; and finally, the pathogenesis suggested here offers an alternative to that of nephrotoxic serum nephritis for the experimental approach to the study of human glomerulonephritis.

Addendum.—Similar experiments in which rabbits have been injected daily with varying doses of BSA have been carried out by Dr. Frederick G. Germuth of the Charlotte Memorial Hospital, Charlotte, North Carolina. Results quite comparable to the immunological and light microscopic observations reported here were obtained.

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EXPLANATION OF PLATES

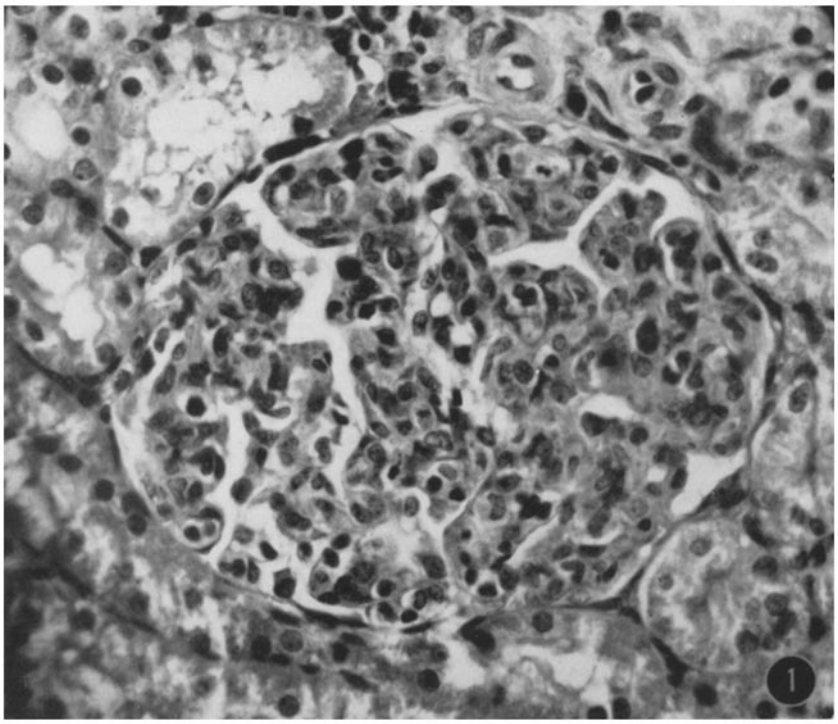
The following abbreviations are used in the electron micrographs:

BM, basement membrane	*F*, fibrin
BS, Bowman's space	*fp*, foot processes
Cap, capillary	*L*, leukocyte
CL, capillary lumen	*M*, mass
D, deposit	*Nu*, nucleus
En, endothelium	*RBC*, red blood cell
Ep, epithelium	*Sp*, splitting of basement membrane

Tissues for electron microscopy were embedded in methacrylate (Me) or vestopal W (Ve). All fluorescence micrographs were made from 7 micron thick frozen sections of rabbit kidneys which developed glomerulonephritis after prolonged daily injections of bovine serum albumin, stained with fluoresence anti-bovine serum albumin for the detection of the corresponding antigen.

PLATE 89

FIG. 1. Typical hypercellular glomerulus from rabbit with acute proteinuria during 2nd week of antigen injections. The capillary lumina are almost completely filled by endothelial proliferation. A few leukocytes are present in the occluded capillaries. Even though the glomerulus completely fills Bowman's space, there are no adhesions. × 300. Hematoxylin and eosin.

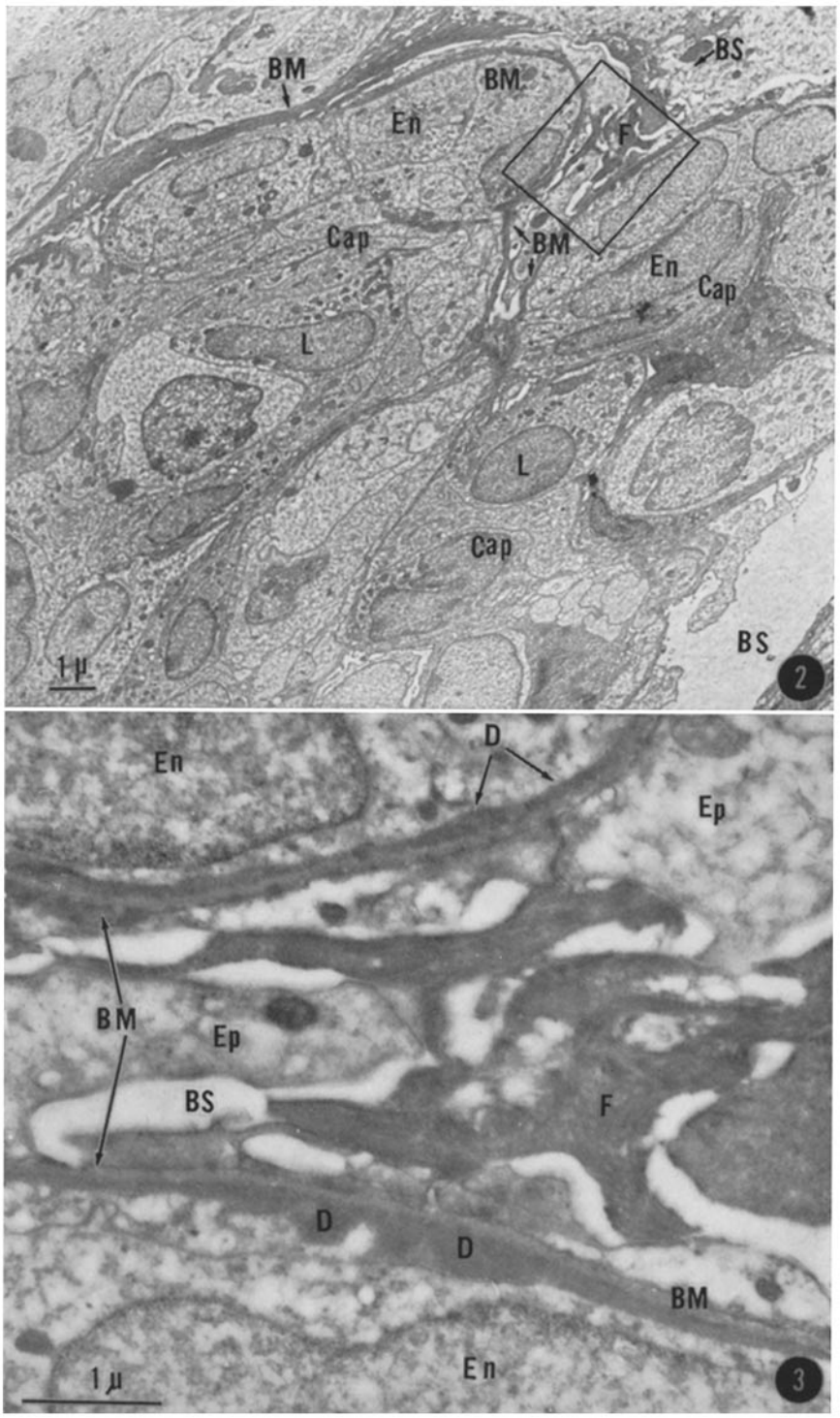


(Dixon *et al.*: Experimental glomerulonephritis)

PLATE 90

FIG. 2. Portion of glomerulus from a rabbit at peak of acute proteinuria. The capillaries (*Cap*) are distended and filled with cells, both endothelial (*En*) and leukocytic (*L*). Bowman's space (*BS*) is encroached upon. A dense mass (*F*) is present in Bowman's space, probably fibrin. Foot processes are replaced by sheets of epithelial cytoplasm covering the basement membranes (*BM*) which are somewhat irregularly thickened and difficult to trace. (See Fig. 1). Me. × 5800.

FIG. 3. A higher magnification of the area indicated in Fig. 2. Along the inner aspect of the basement membranes (*BM*) are dense amorphous deposits (*D*), possibly containing antigen-antibody complexes. It is unusual to observe this in acute glomerulonephritis. Endothelial cells (*En*) occupy the lumens completely. Epithelial cytoplasm (*EP*) is applied to the outer surface of the basement membranes in sheets rather than with foot processes. Dense material (*F*) in Bowman's space (*BS*) is fibrillar and most likely fibrin. Me. × 20,000.

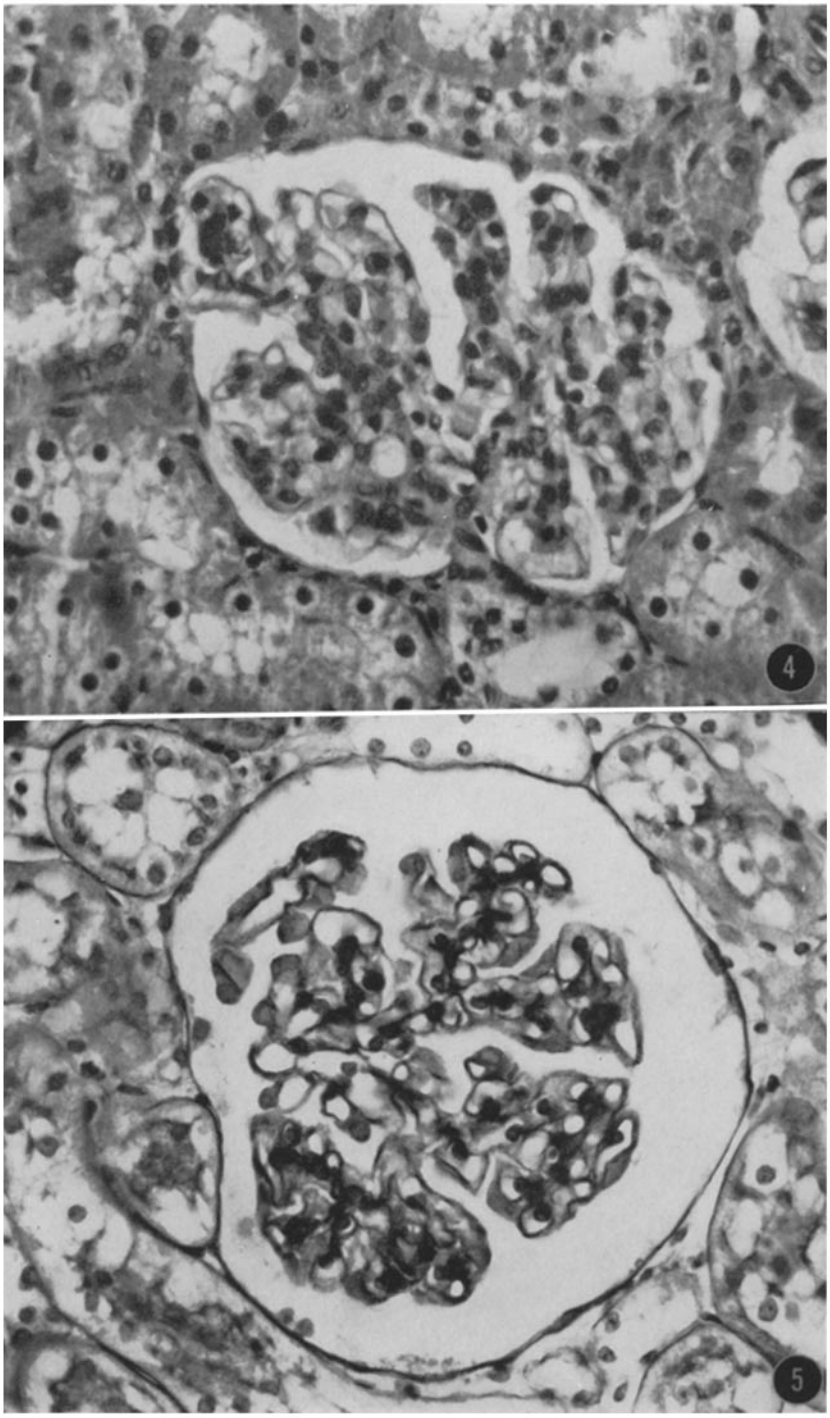


(Dixon *et al.*: Experimental glomerulonephritis)

PLATE 91

FIG. 4. Glomerulus from same rabbit as seen in Fig. 1 but biopsied during 3rd week of antigen injections when acute proteinuria was disappearing. There is a considerable reduction in cellularity and many glomerular capillaries are patent. Within one more week the kidneys of this rabbit returned to normal. Hematoxylin and eosin. × 300.

FIG. 5. Glomerulus from rabbit early in the course of chronic, severe proteinuria which developed during 2nd month of injections. It shows thickened basement membranes and lobulation of capillary tufts without significant cellular proliferation, inflammation or scarring. This lesion resembles that seen in the membranous form of human glomerulonephritis. Proteinuria associated with lesions of this type improved after cessation of antigen injections but basement membrane thickening persisted. Periodic acid stain. × 300.

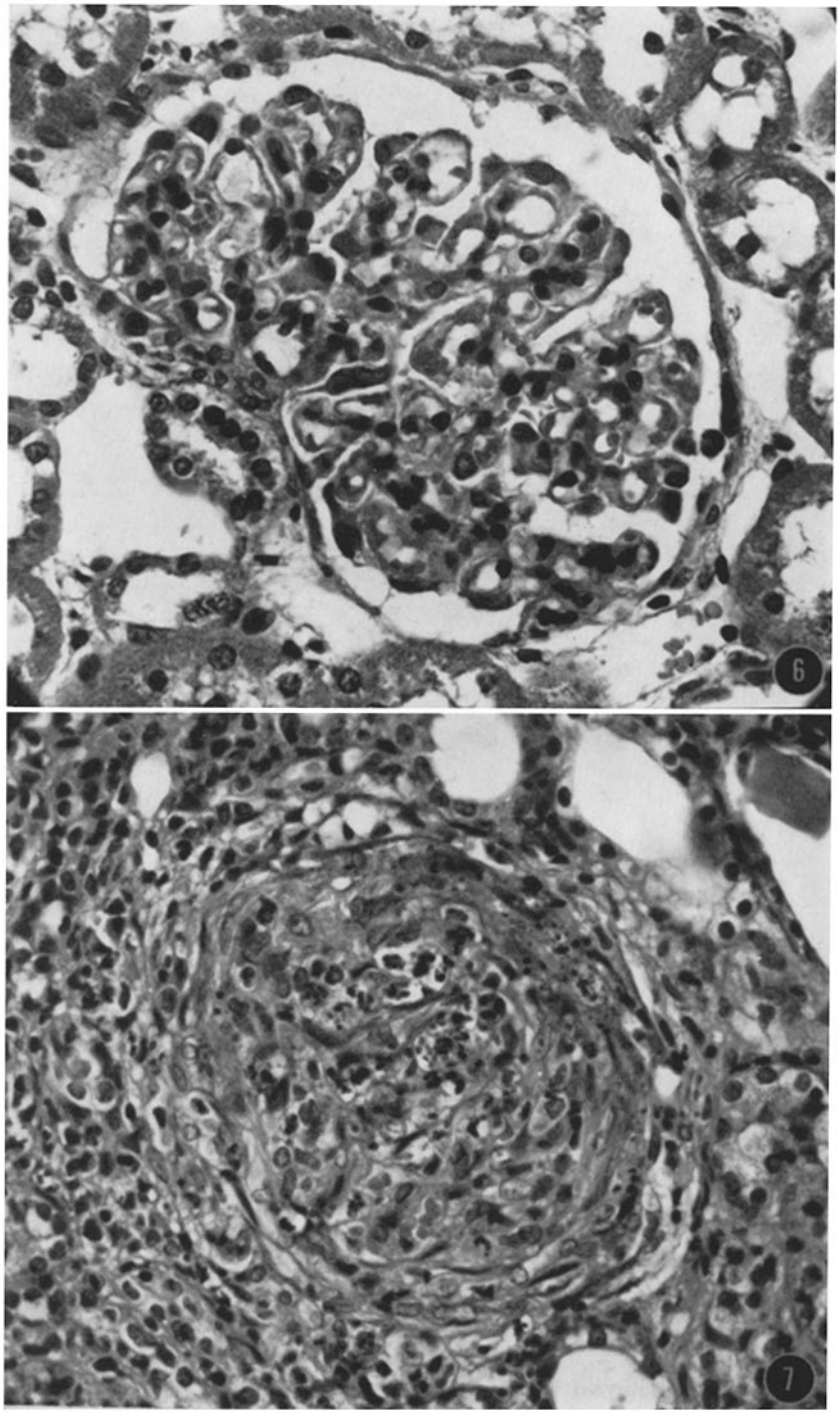


(Dixon *et al.*: Experimental glomerulonephritis)

PLATE 92

FIG. 6. Glomerulus from rabbit during 3rd month of chronic, severe proteinuria. Capillary basement membranes are markedly thickened. In addition, there are moderate proliferation of glomerular cells, two early adhesions of capillary tufts to Bowman's capsule, and some distortion of glomerular architecture. Cessation of antigen injections in rabbits with this degree of change was not followed by either complete functional or morphological recovery. Hematoxylin and eosin. × 300.

FIG. 7. Glomerulus from rabbit in the 2nd month of chronic proteinuria and uremia. Glomerulus has been obliterated by proliferation, inflammation, and scarring. In most of the rabbits dying of renal failure this degree of glomerular damage was commonplace. Hematoxylin and eoxin. × 300.

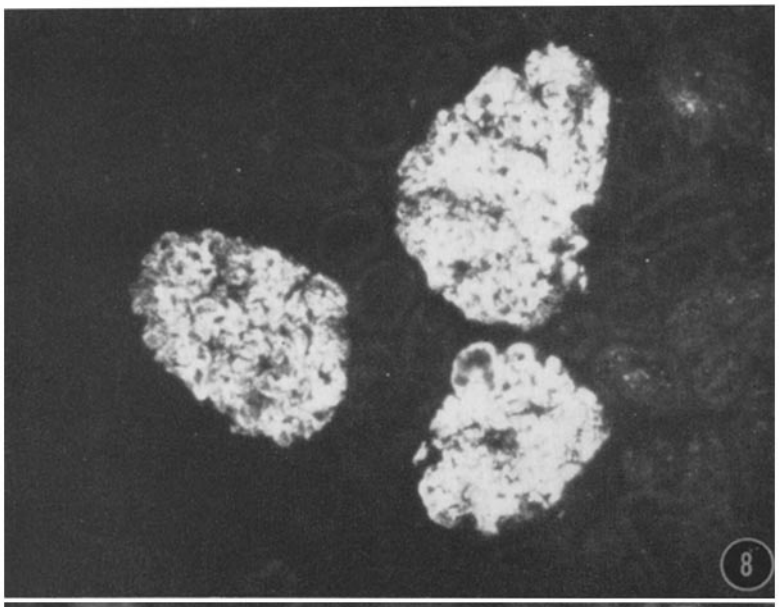


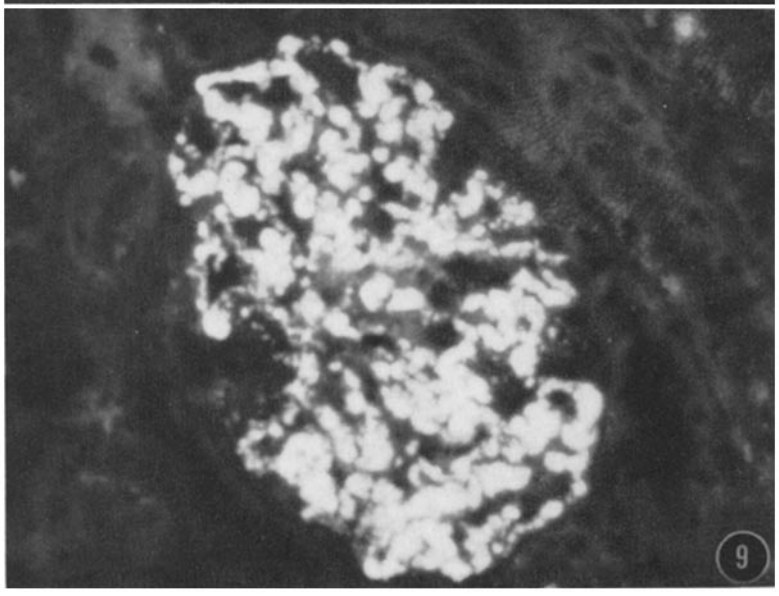
(Dixon *et al.*: Experimental glomerulonephritis)

PLATE 93

FIG. 8. Low magnification of a kidney section with three glomeruli showing bright specific fluorescence of antigen deposits throughout each entire glomerulus. The tubular structures in the background lack specific fluorescence. Fluorescence micrograph. × 120.

FIG. 9. A higher magnification of a glomerulus similar to those in Fig. 8 to show in more detail the bright fluorescence of antigen deposits. Note the "coiled" pattern of such deposits suggesting their localization along the capillary walls. Fluorescence micrograph. × 300.



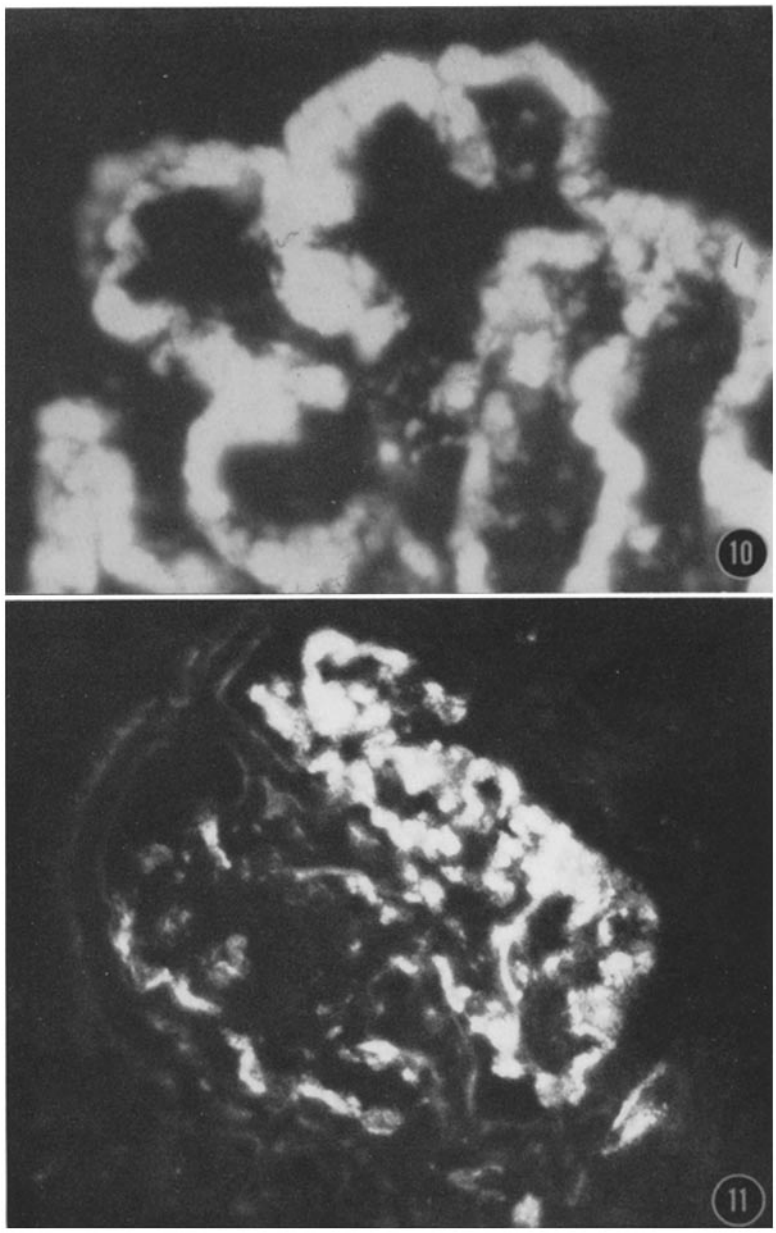


(Dixon *et al.*: Experimental glomerulonephritis)

PLATE 94

FIG. 10. A high magnification of a portion of a glomerulus similar to those in Figs. 8 and 9 showing in more detail the characteristics of antigen deposition in the walls of glomerular capillaries. Note the beaded appearance of specific fluorescent material in the capillary loops closely resembling the dense deposits seen by electron microscopy along the basement membranes of the capillary walls. The discrete, lumpy appearance of antigen deposits is sometimes obscured by superposition of many of such deposits due in part to the thickness of the preparation. Fluorescence micrograph. × 900.

FIG. 11. High magnification of a glomerulus which developed fibrosis and capsular proliferation. Note the specific fluorescence of antigen deposits in the upper right half of the glomerulus, similar to that seen in Figs. 9 and 10, corresponding to an area in which there are basement membrane changes but not fibrosis. By contrast, most of the lower left half of the glomerulus shows no specific deposits of antigen. These areas, which lack specific fluorescence, correspond to scarred sections of the glomerulus. Fluorescence micrograph. × 300.

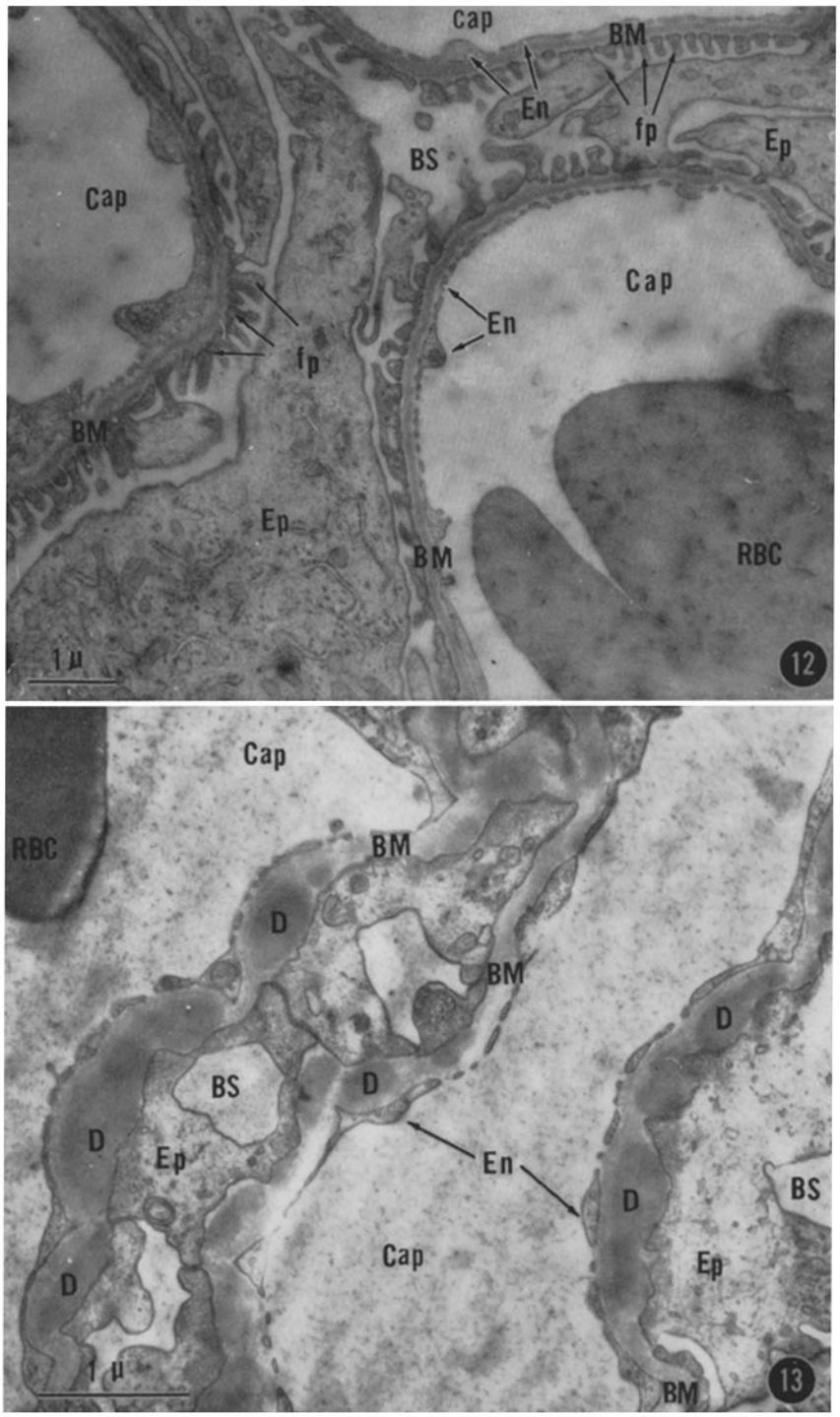


(Dixon *et al.*: Experimental glomerulonephritis)

PLATE 95

FIG. 12. Portion of a glomerulus from a control uninjected rabbit. Parts of three capillaries (*Cap*) are visualized and one contains red blood cells (*RBC*). The basement membrane (*BM*) is quite constant in thickness and shows a central lamina densa flanked on each side by a lamina lucida. Epithelial foot processes (*fp*) are regularly distributed and evenly spaced over much of the basement membranes. The endothelial cytoplasm (*En*) is thin, perforated, and closely applied to the inner lamina lucida. Ve. × 14,000.

FIG. 13. Portion of a glomerulus from a rabbit with chronic proteinuria of 5 weeks' duration ("membranous glomerulonephritis"). Parts of two capillaries (*Cap*) are seen. The basement membranes (*BM*) are markedly thickened. Deposits (*D*), presumably containing antigen-antibody complexes, are present within the basement membranes and at their outer aspects. They are covered by sheets of epithelial cytoplasm (*Ep*). The capillary lumina are patent. A thin, perforated endothelial film lines the inner surface of the basement membranes. Compare with Figs. 5 and 12. Ve. × 22,000.

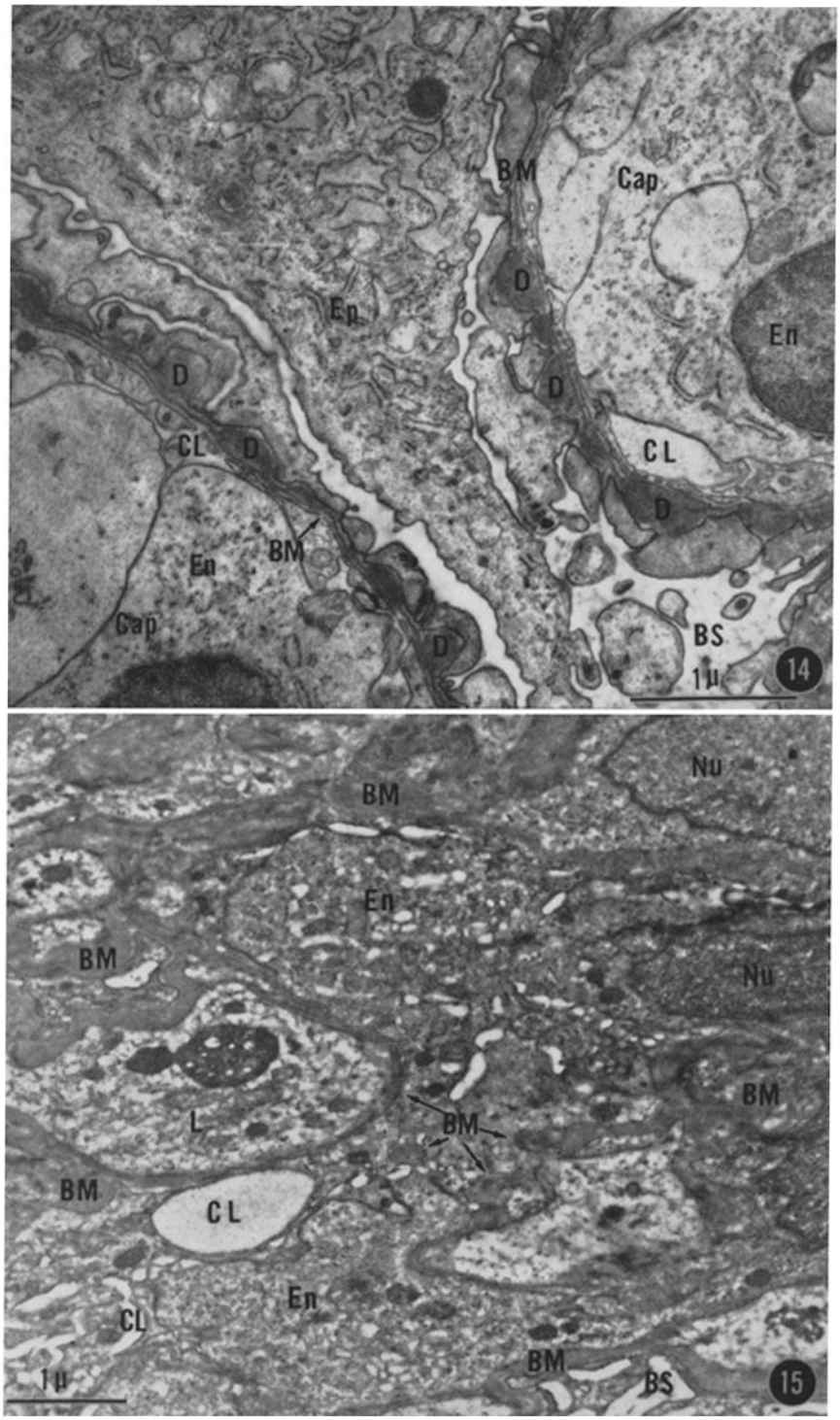


(Dixon *et al.*: Experimental glomerulonephritis)

PLATE 96

FIG. 14. Portion of a glomerulus from a rabbit with chronic proteinuria of 10 weeks' duration. Parts of two capillaries (*Cap*) are visible. The basement membranes (*BM*) are beaded with dense deposits (*D*), presumably containing antigen-antibody complexes. Epithelial cytoplasm (*Ep*) is smeared over the deposits. Endothelial cytoplasm (*En*) is swollen and nearly obliterates the lumina (*CL*) of the capillaries. See Figs. 6 and 10. The morphologic changes illustrated in this micrograph resemble those seen in human glomerulonephritis (reference 21). Ve. × 14,000.

FIG. 15. Portion of a glomerulus from a rabbit with severe glomerulonephritis and proteinuria of 6 weeks' duration. The architecture is effaced and intra- and extracapillary spaces are difficult to recognize. The basement membrane or basement membrane-like material (*BM*) is folded, fragmented, and of irregular density. An accumulation of cells (endothelial (*En*) and leukocytic (*L*)) almost completely fills the vascular lumina. Similar architectural alterations are found in advanced human glomerulonephritis. Me. × 9,500.



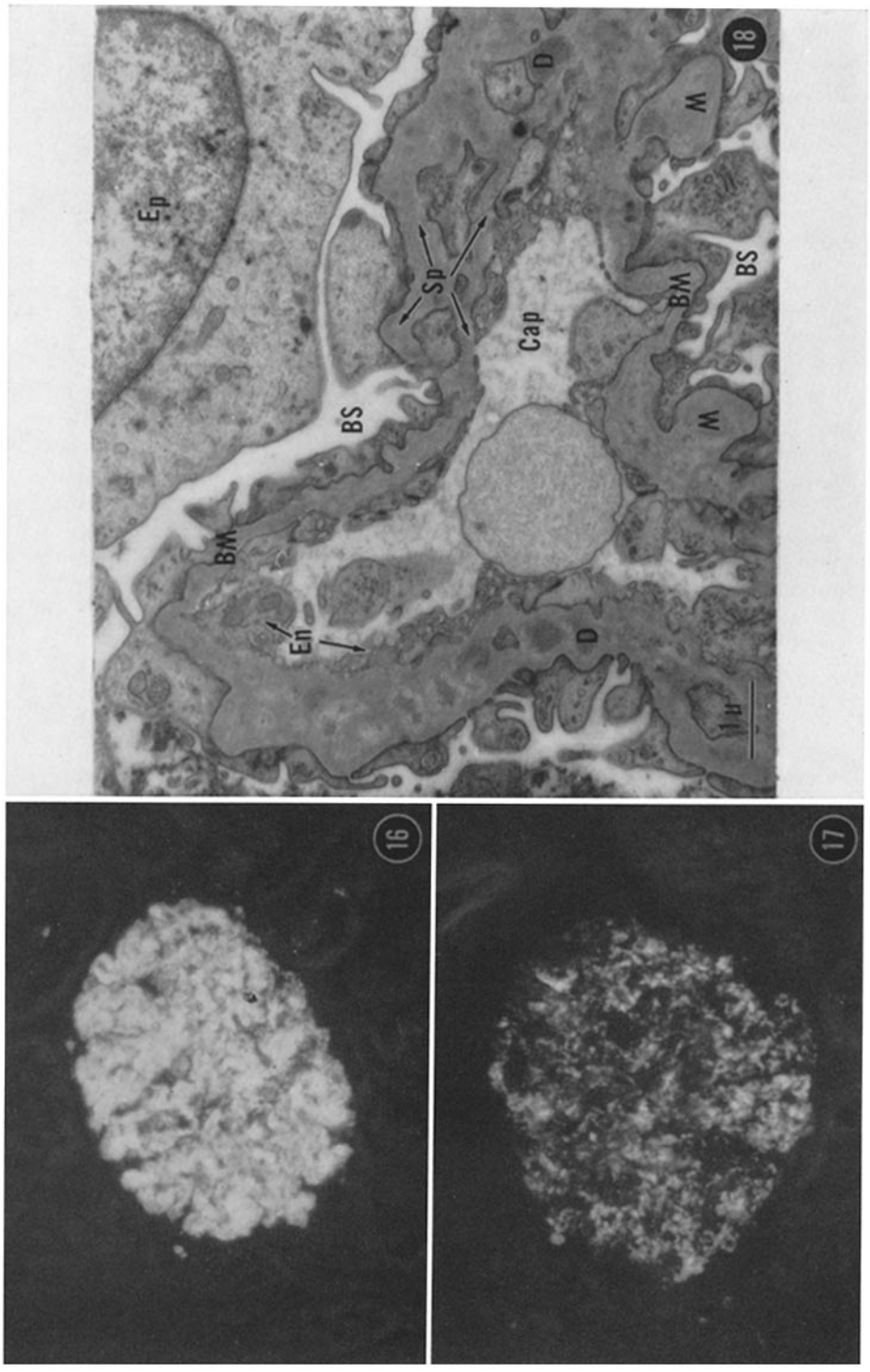
(Dixon *et al.*: Experimental glomerulonephritis)

PLATE 97

FIG. 16. A glomerulus from the first renal biopsy in rabbit 78-80 during antigen injections showing appreciable deposits of antigen throughout the glomerulus in a similar fashion to those seen in Figs. 8 and 9. Compare with Fig. 17. Fluorescence micrograph. × 200.

FIG. 17. A glomerulus from the second renal biopsy in rabbit 78-80, 32 days after daily injections of antigen were stopped. Note by comparison with Fig. 16, the appreciable diminution of specific fluorescence in this instance, indicating that although detectable antigen is still present along the capillary walls it is markedly reduced after the injections of antigen are discontinued. Fluorescence micrograph. × 200.

FIG. 18. A capillary (*Cap*) from a rabbit with severe proteinuria for 5 months. This biopsy was taken 3½ months after cessation of antigen injections. The capillary is angulated. Its basement membrane (*BM*) is variably thickened, frayed, and split (*Sp*). Irregular masses (*M*) project from the basement membranes into Bowman's space (*BS*) where they are surrounded by epithelial cytoplasm. Dense deposits (*D*) in the thickened wall presumably contain remnants of antigen-antibody complexes. Ve. × 11,000.



(Dixon *et al.*: Experimental glomerulonephritis)