THE PATHOLOGIC EFFECTS OF INTRAVENOUSLY ADMINISTERED SOLUBLE ANTIGEN-ANTIBODY COMPLEXES*

II. Acute Glomerulonephritis in Rats

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Plates 15 and 16

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In previous studies it was shown that acute glomerulonephritis can be produced passively in the mouse by the intravenous injection of soluble antigen-antibody complexes (1–3). The character and course of the histologic changes were investigated. Both antigen and antibody were demonstrated in glomeruli of these mice with glomerulonephritis produced in this fashion (4). Because the effect of these lesions on renal function could not be adequately investigated in mice, similar experiments were carried out in the rat. It was found that in rats also, glomerulonephritis occurred following injection of antigen-antibody complexes. Several aspects of renal function were studied in the course of the disease.

Materials and Methods

White Carworth W rats of both sexes weighing between 130 and 210 gm. were used. Antisera against 3 × recrystallized hen ovalbumin and against bovine serum albumin (BSA) were prepared in rabbits as previously described (3). Pooled antiovalbumin and anti-BSA were analyzed for antibody content by the quantitative precipitin technique (5). The following pooled antisera were used: rabbit anti-hen ovalbumin, pool 3 containing 3.5 mg. and pool 4 containing 7.8 mg. of antibody per ml.; rabbit anti-BSA, pool 2 containing 4.3 mg. and pool 3 containing 3.0 mg. of antibody per ml. The soluble complexes were prepared as follows: the amount of antigen calculated to precipitate antibody at equivalence was added to aliquots of antiserum and the precipitate was allowed to form overnight at 4°C. The precipitate was then centrifuged, washed twice with cold saline, and redissolved by the addition of 20 times the amount of antigen used for precipitation. The complexes were dissolved at room temperature usually within an hour and were immediately injected in the tail vein.

The animals were sacrificed by ether and sections were prepared of lungs, liver, spleen, and kidney and routinely stained with hematoxylin and eosin.

Blood urea nitrogen was measured by use of the Nessler reagent. When the studies on the urine were carried out, the animals were provided with food only from 10:00 a.m. to 4:00 p.m.,

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at which time they were placed in cages in which urine was collected from individual rats for the 18 hour period. Drinking water was provided continuously. The urine volume was measured daily for the 18 hour period and the protein concentration determined by the biuret method. Samples of urine were examined microscopically. In the animals receiving BSA anti-BSA complexes or BSA alone, the urine was tested for the presence of BSA by ring tests with undiluted anti-BSA serum, pool 1 (3). Similar tests were not done routinely with the animals injected with ovalbumin antiovalbumin complexes because ovalbumin is excreted in the urine by normal rats.

EXPERIMENTAL

The soluble complexes for each system were injected in 3 equal doses within 24 hours. Group I consisted of 9 rats treated with soluble ovalbumin antiovalbumin complexes. Three of these received 10 mg. of antibody per injection and the other 6 received 15 mg. of antibody per injection. Group II consisted of 6 rats given BSA anti-BSA complexes. Three of this group

TABLE I

Incidence of Glomerulonephritis in Rats Given Redissolved Antigen-Antibody Complexes

Material injected	Amount per injection		Incidence of glomerulonephritis
	Antibody	Antigen	
	mg.	*mg.*	
Ovalbumin antiovalbumin complexes*	10–15	30–40	9/9
BSA anti-BSA complexes*	15–20	60–80	4/6
Ovalbumin antiovalbumin complexes	10	30	
followed by BSA anti-BSA complexes‡	20	80	6/6
Ovalbumin*		93	0/6
BSA*		80	0/3
None			0/3

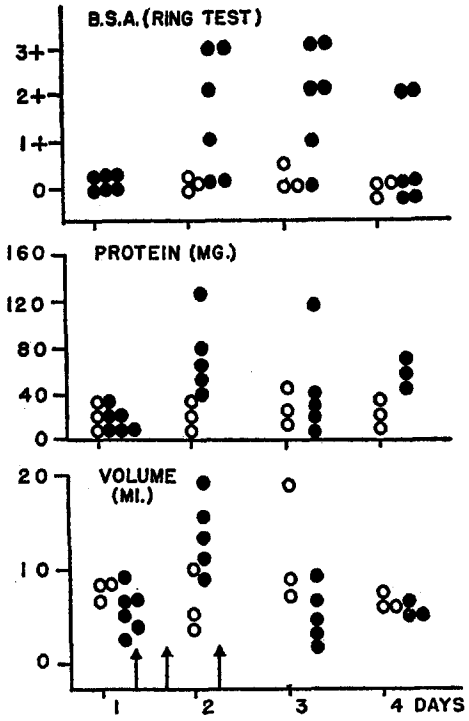
* Three injections given in 24 hours.

‡ Three injections of ovalbumin antiovalbumin in 24 hours and then 6 days later 3 injections of BSA anti-BSA in 24 hours.

received 15 mg. of antibody per injection and the other 3 received 20 mg. per injection. Group III consisted of 6 rats treated first with ovalbumin antiovalbumin complexes, 10 mg. per injection, and 6 days later with BSA anti-BSA, 20 mg. of antibody per injection. As controls, 3 rats (group IV) received 3 injections of 93 mg. of ovalbumin per injection in 24 hours and 9 rats (group V) were given 3 injections of 80 mg. of BSA in 24 hours. Group VI consisted of 3 control rats which received no treatment. The animals were sacrificed between 24 and 48 hours after the last injection, except for 1 animal in group I and 1 animal in group II which died after the second injection. All of the rats receiving complexes showed some signs of distress which were regarded as mild anaphylactic shock. The reactions were less severe than in mice given comparable amounts of antigen-antibody complexes (3).

Pathologic Findings.—No abnormalities were seen on gross inspection at autopsy. Histologically, none of the control rats in groups IV, V, or VI showed any pathologic changes (Fig. 1). The chief abnormality seen histologically in the rats given complexes was acute glomerulonephritis, the incidence of which is shown in Table I. The glomeruli in the rats affected were enlarged, hypercellular, and relatively bloodless (Fig. 2). The glomerular endothelial cells

were swollen. In most of the rats, these changes involved almost all glomeruli to a fairly uniform extent. In general, there was relatively slight neutrophile infiltration in glomeruli, but in 3 of the 6 animals in group I, which received 15 mg. of antiovalbumin per injection, there was a moderate number of neutrophiles in glomeruli (Fig. 3). In 6 animals, the sections of kidneys were stained



TEXT-FIG. 1. Urinary findings in rats following intravenous injections of soluble BSA rabbit anti-BSA complexes. Controls (group V, open circles) received 3 injections of BSA. Rats in groups II and III (black circles) received 3 injections of soluble BSA rabbit anti-BSA complexes containing 15 to 20 mg. antibody. Injections were given on the 1st and 2nd days of the experiments at the times indicated by the vertical arrows on the abscissa. Urinary volume and protein are recorded as total amount for 18 hour period.

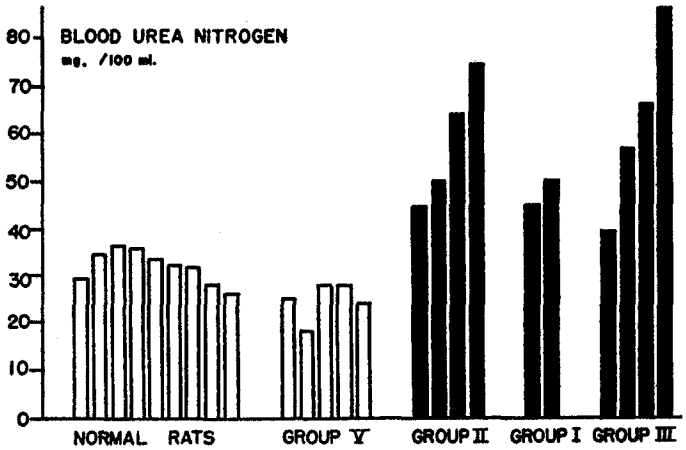
by the PAS and azocarmine methods, and no thickening of the basement membranes was found.

Examination of sections from heart, lung, liver, and spleen revealed no abnormality.

Studies on Renal Function

Urinary Findings.—It was shown by ring tests using antiovalbumin serum that rats given ovalbumin or ovalbumin antiovalbumin complexes excreted large amounts of ovalbumin. It is known that ovalbumin is normally excreted in the urine. Therefore, only the data concerning those animals given BSA

anti-BSA complexes (groups II and III) and those given BSA alone (group V) are recorded. The results are shown in Text-fig. 1. In comparison with animals given BSA alone, those given complexes showed a variable increase in proteinuria, most marked on the 1st and 3rd days. Abnormal protein excretion was also demonstrated by the presence of BSA in the urine detected by ring tests using anti-BSA serum and was present in one or more urine samples from each of 6 rats; 9 of 12 specimens examined during the first 48 hours were positive.



TEXT-FIG. 2. Changes in blood urea nitrogen in rats following administration of soluble antigen-antibody complexes. Blood urea nitrogen was measured 48 hours after the third injection of BSA alone in group V, of BSA anti-BSA in group II, and of ovalbumin anti-ovalbumin in groups I and III. The rats in group III also received BSA anti-BSA complexes 1 week before the ovalbumin antiovalbumin.

Only one of the control animals given BSA alone showed one specimen with a questionable positive ring test. There was some increase in urine output in the 18 hour collection period following the second injection of soluble complexes. Microscopic examination of the urine was performed in 15 rats in groups I and III after 2 injections, and in 9 of these animals on the day following the last injection. Of these, 2 rats showed abnormalities in the form of casts, red cells, and white cells. In all 15 animals, urine obtained before injections showed no abnormalities microscopically.

Blood Urea Nitrogen.—In several animals from each experimental group (I, II, and III) and in those given BSA alone (Group V) blood urea nitrogen was measured before and after the series of injections. There was an elevation of the blood urea nitrogen in every animal given soluble antigen-antibody complexes (Text-fig. 2). Elevated levels were observed only in samples obtained from animals which received injections of soluble complexes. The highest value obtained was 87 mg./100 ml.

DISCUSSION

These experiments show that acute glomerulonephritis can be produced passively in the rat by the injection of soluble antigen-antibody complexes, using the same schedule which has been found to cause passive serum sickness in mice. The amounts of antibody injected were of the same order of magnitude on a weight basis required to produce glomerulonephritis in mice. The incidence of renal lesions was high and did not differ significantly from that in mice.

In contrast to mice, the rats did not show either arteritis or endocarditis. Because of the focal nature of these lesions and because of the comparatively small number of rats studied, these negative findings do not exclude the possibility that they do in fact occur in rats. The glomerulonephritis was characterized histologically by enlargement of glomeruli, swelling of endothelial cells, hypercellularity, and bloodlessness. The glomerular changes were less severe than those generally seen in mice following similar treatment. In particular, there was relatively slight neutrophile infiltration in most cases in contrast to the exudative character of the lesions usually seen in mice. However, in keeping with the observations in mice, the most severe lesions were seen in the rats injected with the ovalbumin rabbit antiovalbumin system. This may be due to the more rapid excretion of ovalbumin leading to increased formation of aggregates which are then arrested in glomeruli.

All of the affected animals showed renal functional abnormalities. In every case there was abnormal proteinuria. Two animals had casts, red cells, and white cells in the urine. In all of the animals investigated, there was increase in the blood urea nitrogen.

It is relevant to compare the renal functional and histologic changes which occur following the injection of antigen-antibody complexes with those seen following the injection of anti-kidney antibody (Masugi nephritis). The character and course of this type of experimental nephritis in rats, which has been extensively investigated by Seegal and her associates (6, 7), is variable and depends upon the source, potency, and quantity of anti-kidney serum used. The administration of a potent anti-kidney serum to the rat often results in the rapid development of the full blown nephrotic syndrome, with marked proteinuria (up to 6 gm./100 ml.), edema, and hypercholesterolemia. Such severe protein loss with its associated changes was not seen in the rats given antigen-antibody complexes. The severe protein loss seen in Masugi nephritis may be a reflection of the involvement of the basement membrane, which is thought to be the structure primarily affected in this condition. In the nephritis caused by antigen-antibody complexes, on the other hand, the primary site of involvement appears to be the glomerular endothelial cells (4). Histologically, the early glomerular changes, which have been described in rats given anti-kidney serum, consist of swelling and haziness of basement membranes, enlargement of glomerular cells, occasional fibrin thrombi and slight leucocytic infiltration

consisting chiefly of lymphocytes (7). In contrast, in rats given soluble antigen-antibody complexes, there appears to be more marked hypercellularity of glomeruli (representing in part endothelial proliferation), there is more leucocytic infiltration and no apparent change in the basement membrane. While Masugi nephritis usually has a prolonged course, often leading to death in renal failure, the nephritis caused by antigen-antibody complexes resolves soon after the injections are discontinued (2, 3).

SUMMARY

The intravenous administration to rats of soluble antigen-antibody complexes in antigen excess resulted in acute glomerulonephritis. This occurred with both rabbit antiovalbumin and rabbit anti-BSA systems.

The rats so treated regularly showed proteinuria and elevation of blood urea nitrogen.

These findings are compared with those reported in rats injected with anti-kidney serum.

BIBLIOGRAPHY

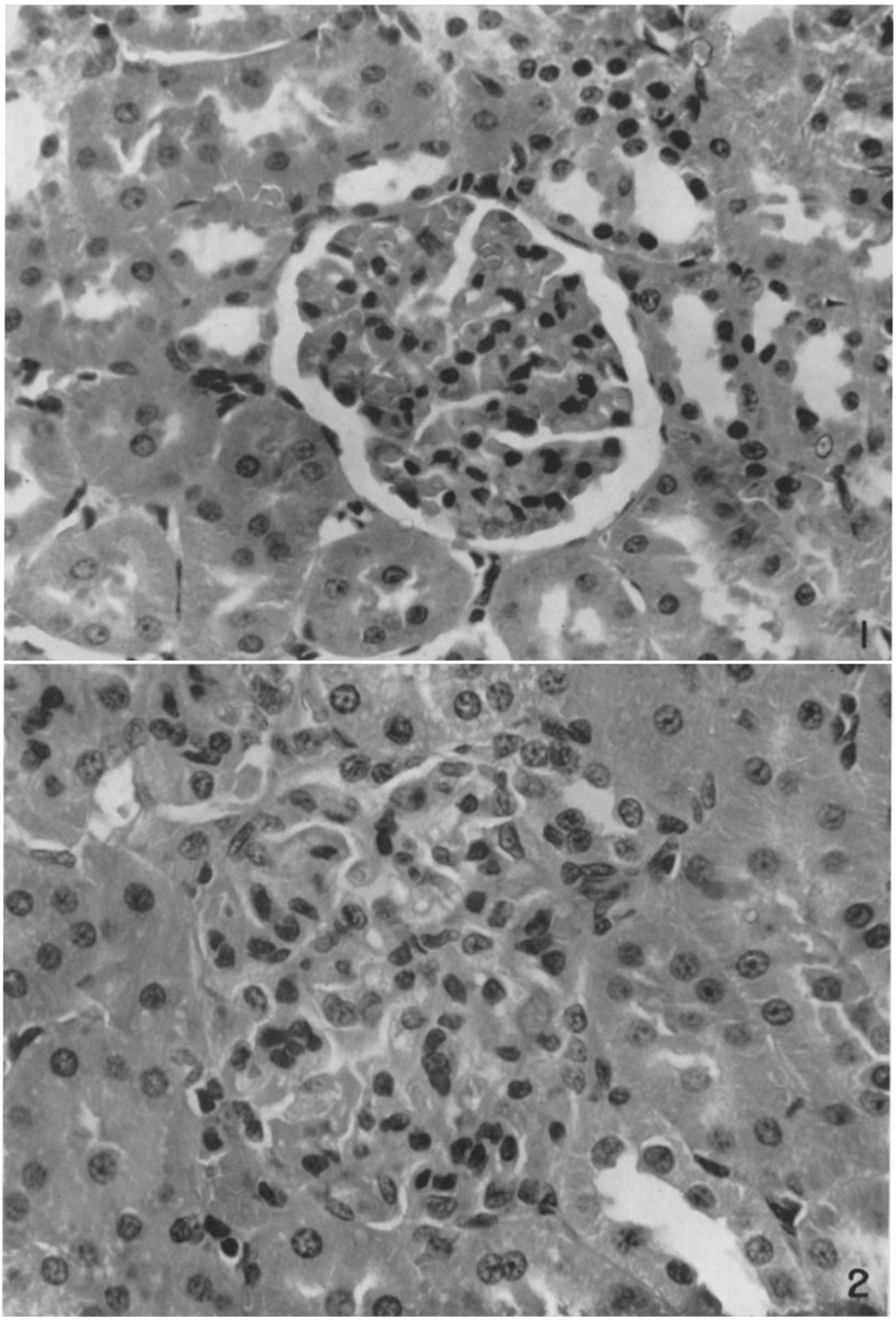
1. McCluskey, R. T., and Benacerraf, B., Localization of colloidal substances in vascular endothelium. A mechanism of tissue damage. II. Experimental serum sickness with acute glomerulonephritis induced passively in mice by antigen-antibody complexes in antigen excess, *Am. J. Path.*, 1959, **35,** 275.
2. McCluskey, R. T., Benacerraf, B., and Potter, J., Acute glomerulonephritis produced by soluble antigen-antibody complexes, *Fed. Proc.*, 1959, **18,** 2295 (abstract).
3. McCluskey, R. T., Benacerraf, B., Potter, J. L., and Miller, F., The pathologic effects of intravenously administered soluble antigen-antibody complexes. I. Passive serum sickness in mice, *J. Exp. Med.*, 1960, **111,** 181.
4. Cooper, N. S., McCluskey, R. T., Benacerraf, B., and Potter, J. L., Pathologic effects of intravenously administered soluble antigen-antibody complexes. III. The fate and distribution of antigen and antibody, in preparation.
5. Kabat, E., and Mayer, M. M., Experimental Immunochemistry, Springfield, Illinois, Charles C. Thomas, 1948, 567.
6. Seegal, B. C., *in* Proceedings of the Ninth Annual Conference on the Nephrotic Syndrome, J. Metcoff, editor, New York, National Nephrosis Foundation, Inc., 1958.
7. Hasson, M., Bevans, M., and Seegal, B. C., Immediate or delayed nephritis in rats by duck anti-rat-kidney serum, *Arch. Path.*, 1957, **64,** 192.

EXPLANATION OF PLATES

PLATE 15

FIG. 1. Kidney of normal rat. Hematoxylin and eosin stain. × 410.

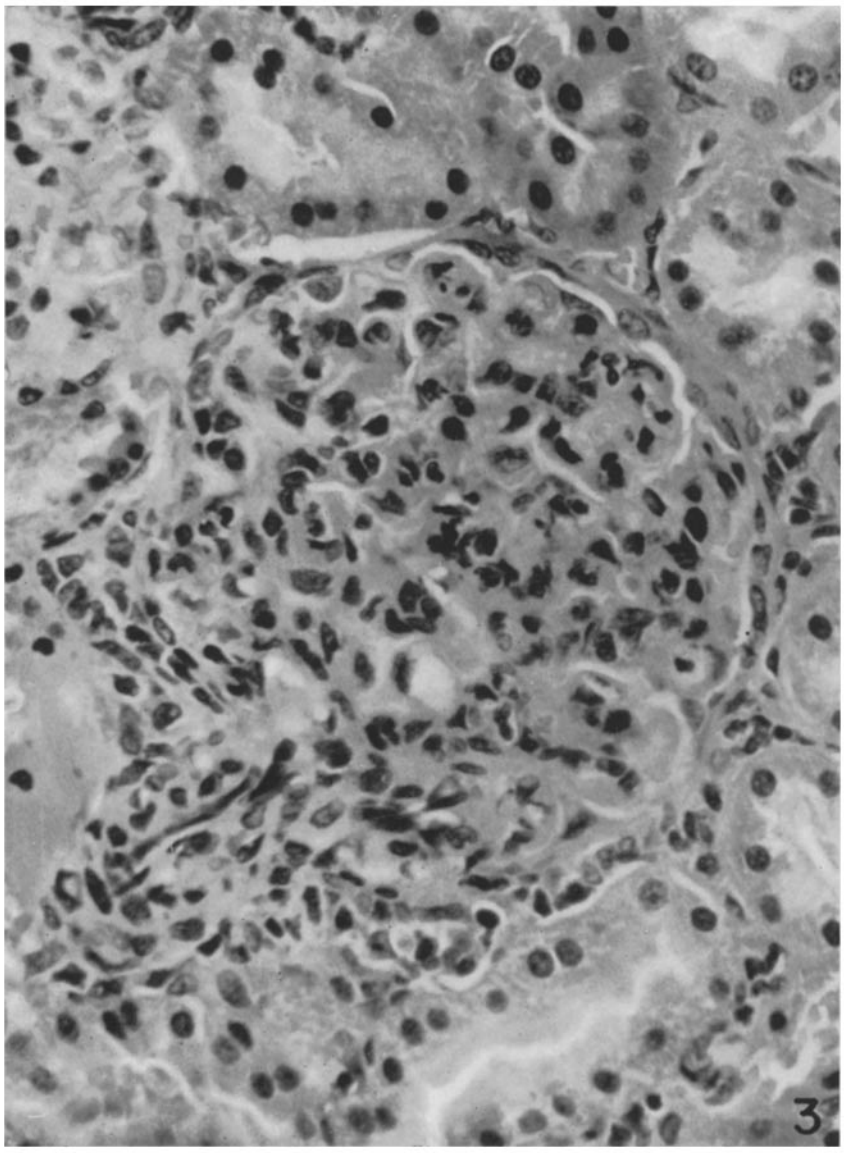
FIG. 2. Kidney of rat given BSA anti-BSA complexes (group II). The glomerulus fills Bowman's space, the glomerular cells are swollen and the glomerular capillaries contain very few red cells. Hematoxylin and eosin stain. × 455.



(Benacerraf *et al.*: Acute glomerulonephritis in rats)

PLATE 16

FIG. 3. Kidney from rat given ovalbumin antiovalbumin complexes (Group I, 15 mg. antiovalbumin per injection). The glomerulus is swollen, relatively bloodless, and is infiltrated with leucocytes. Hematoxylin and eosin stain. × 450.



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