DIFFUSE GLOMERULONEPHRITIS PRODUCED IN RABBITS BY MASSIVE INJECTIONS OF BOVINE SERUM GAMMA GLOBULIN*

By ROBERT H. MORE, M.D., and DOUGLAS WAUGH, M.D.,C.M.‡

(From the Department of Pathology, Pathological Institute, McGill University, Montreal)

Plates 22 and 23

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In the present study, interest was directed toward three aspects of experimental acute diffuse glomerulitis. In the first place, an experimental technique was sought which would produce pathological changes morphologically analogous to those of human glomerulonephritis. In the choice of a method the main objective was to employ one which would give a high incidence of well developed lesions. It was also desired to use a technique as uncomplicated as possible, in order that the relation between lesions and treatment might be more easily perceived and assessed. Secondly, it seemed important to examine, in an experiment wherein neither renal substance nor antirenal serum was used, the possible rôle of antikidney antibodies (autoantibodies) in the development of experimental acute diffuse glomerulitis. The third point in this investigation was concerned with the possibility that changes in blood coagulability might play a rôle in the development of diffuse glomerulitis. This report is accordingly divided into three parts, dealing with each of these aspects separately.

1. Production of Experimental Glomerulonephritis

Treatment of experimental animals by a variety of methods has produced renal lesions resembling, to a greater or lesser degree, those of human glomerulonephritis. Nephritis has been produced by injection of various bacteria or their products into suitable animals (1–7). Diffuse glomerular damage has also been evoked experimentally by the injection of non-bacterial foreign protein (8–10). A considerable amount of experimental work has been done using the method of Masugi (11–13), whereby diffuse glomerulitis is produced by injection of specific antirenal antibodies (14–21). The renal lesions produced in this way seem to be the closest morphological equivalent to human glomerulonephritis yet produced.

More recently Hawn and Janeway (22) reported acute diffuse glomerular

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‡ Graduate Medical Research Fellow, National Research Council of Canada.

damage in rabbits following intravenous injection of a single massive dose of purified bovine serum gamma globulin (fraction II). In their animals glomerular damage was most marked and acute 1 week after injection. "Healing" lesions were found in animals killed later than this. Unfortunately the illustrations of glomerular lesions in their publication are not such as to constitute convincing evidence of an injury closely resembling human glomerulonephritis.

It was apparent that if this experimental technique could be modified in such a way as to produce more severe renal lesions it would constitute a valuable contribution to the experimental study of glomerulonephritis. The advantage of such a method over others in the experimental study of nephritis lies in the fact that it involves the use of but a single, purified antigen, whereas all other techniques employ complex antigen mixtures; *e.g.*, horse serum (8–10), duck serum (11, 12), bacteria (1–7), bacteria and kidney mixtures (23–26). The use of the single antigen method would reduce considerably the number of factors to be evaluated in understanding the pathogenesis of this type of nephritis.

Method.—Twenty-eight rabbits were used in all. Of these, eighteen were treated with bovine serum gamma glubulin,[1] and ten were used as controls. Animals of different strains and both sexes were used. They had an average weight of about 2,500 gm. at the time treatment was instituted. Before receiving any treatment all animals were unilaterally nephrectomized. This was done because of the frequent observation that unilateral nephrectomy increases the susceptibility of the remaining kidney to damage (12, 27). Nephrectomies were done from 3 to 6 weeks before globulin treatment was begun. The animals were given two massive intravenous injections of globulin instead of the single large dose used by Hawn and Janeway. There was a 12 day interval between the two massive injections. Possible fatal anaphylactic reactions were avoided by desensitizing the animals with a small intravenous injection of globulin about 18 hours before they were to receive the second massive dose.

The animals were injected intravenously with 1 gm. per kilo of bovine serum gamma globulin, as a 10 per cent solution in normal saline. Eleven days later they were slowly injected intravenously with the desensitizing dose, consisting of 1.0 cc. of the concentrated solution diluted to 5 or 10 cc. total volume in normal saline. On the following day, a second massive intravenous dose was given, equalling in amount the initial dose.

Blood urea nitrogen estimations were made on eight animals before treatment was begun, and again immediately before the animals were killed. Similar estimations were done on two other animals before killing only. All animals were killed by intravenous air injection 1 week after the second large dose of globulin. Autopsies were performed immediately, and tissues were fixed in Bouin's and Helly's fluids. Histological sections were cut at 3 to 4 μ thickness, and stained with hematoxylin and eosin, hemalum-phloxin-saffranin, Mallory-Haidenhain, Mallory's phosphotungstic acid hematoxylin, and according to McManus' periodic acid routine (28).

Controls.—Ten animals comprised the control group. All were unilaterally nephrectomized.

[1] The supply of bovine serum gamma globulin (fraction II) used in these experiments was kindly supplied to us through the courtesy of the Biochemical Sales Research Department, Armour and Company, Chicago.

Two developed postnephrectomy infections and were killed and autopsied 1 month after operation. Three animals of the control group were allowed to survive nephrectomy for 7 months, in order to control the possibility of spontaneous appearance of renal lesions following this treatment alone. The remaining five animals were treated in exactly the same manner as those of the experimental group, with the exception that instead of injections with bovine serum gamma globulin, they received equivalent amounts of normal saline.

All control animals were killed and autopsied by the same technique as was used in the globulin-treated group, and histological preparations from these animals formed the basis for estimation of the degrees and types of damage found in the treated group.

Results.—Fourteen (77.8 per cent) of the eighteen globulin-treated animals showed gross and microscopic evidence of diffuse glomerulitis in the remaining kidney. It was noted in the gross that the organ was enlarged. However, this increase in size was not significantly greater in the globulin-treated animals than in the nephrectomized controls. Kidneys from the treated animals were yellower and paler than normal. On section most kidneys showed a distinct cortical pallor. In those that proved on microscopic examination to have severe lesions, it was noted that the fine red peppering of the cortex corresponding to the glomeruli, which is normally visible in the gross, was no longer distinguishable. Two of these kidneys showed fine pitting of their external surfaces.

Among the fourteen animals with glomerulitis, the histological changes varied from slight to very severe. Three showed very severe diffuse glomerulitis (++++), two were moderately severe (+++), and nine showed less marked, but nonetheless definite lesions (+ and ++). The outstanding feature was the diffuse distribution of the changes. Almost all glomeruli were altered to a greater or lesser extent. In some, the degree of glomerular damage was of about equal severity in all glomeruli, while in others there was considerable variation from one glomerulus to another.

In the kidneys showing the mildest degree of damage, there was glomerular enlargement, and considerable cellular proliferation. Both endothelial and epithelial cells appeared to participate in this. Enlargement of both epithelial and endothelial cells was also striking, the former tending to fill the capsular space, while the latter in many instances almost occluded the glomerular capillaries. The resulting picture was one of reduction in the number of patent glomerular capillary loops. Polymorphonuclear leucocytes were occasionally seen in these glomeruli. Basement membrane changes were only occasionally met with and consisted merely of slight fragmentation of the basement membrane. Homogeneous acidophilic protein material was occasionally seen in Bowman's spaces, and in the tubles, but this was an inconstant feature. Lesions of this type were graded as + and were found in four animals (Fig. 5).

Glomerular lesions of the next grade in severity were characterized by the above changes, which, in the over-all picture, were more marked. Homogeneous acidophilic protein material was seen here with greater frequency, within the glomerular capillary lumina and the capsular space. Cellular proliferation was more marked, and in many of the glomeruli there was fusion of one or more of the glomerular tufts. There was some thickening of the epithelial cells of Bowman's capsules, suggestive of early proliferative changes. Slight thickening and

shredding of the basement membranes into two or three layers could also be seen in some of these glomeruli. Polymorphonuclear leucocytes were seen with about the same frequency as in those of grade +. This type of lesion was graded as ++, and was found in five animals (Fig. 6).

Lesions classed as severe (+++) were seen in two animals. Here there was marked reduction in glomerular capillarity, so that in many glomeruli, only a few patent capillary loops could be seen. Swelling and proliferation of the glomerular endothelial and epithelial cells were marked, and proliferation of the capsular epithelium with beginning crescent formation was commonly encountered. Polymorphonuclear leucocytes were seen in many glomeruli. Thickening and shredding of the glomerular basement membrane was a common feature in these glomeruli, and in many the basement membranes appeared to be discontinuous; *i.e.*, fragmented. This type of change is illustrated in Fig. 7.

The most severe lesions were found in three animals (++++). In these all the above changes were very conspicuous (Figs. 1 and 2). Capsular proliferation was so advanced as to produce well marked epithelial crescents, partial or complete fusion of glomerulus and capsule, and compression of the glomerulus. Proliferative changes within the glomerulus made it impossible to distinguish the individual tufts. Partial or complete obliteration of the glomerular capillaries was conspicuous. This was due in some instances to plugging of the capillary lumina with protein material, and in others to swelling and proliferation of the capillary endothelium. Shredding and fragmentation of both the glomerular and capsular basement membranes were very marked and contributed to the narrowing of the glomerular capillaries in most of these glomueruli (Fig. 3).

Of the four remaining animals in the treated group, two showed no renal lesions, one showed pyelonephritis, and one showed an interstitial nephritis. In both the latter animals, the lesions had none of the characteristics of the diffuse glomerulitis which was ascribed to globulin treatment in the other fourteen animals.

In none of the kidneys of the control animals, nor in the kidneys removed from the treated animals before globulin therapy, was diffuse glomerulitis of the type described above encountered. Lesions of typical, focal, pyelonephritis were seen in kidneys of two of the control animals. Other control animals showed no renal alteration other than the expected changes of hypertrophy (Fig. 4).

It is also of interest that one of the globulin-treated animals showed evidence of renal functional impairment, with the blood urea nitrogen level reaching 187 mg. per cent. At autopsy this animal showed very severe renal damage (Figs. 2 and 3). In the remainder of the animals tested, normal blood urea nitrogen levels were found both before and after treatment.

Focal granulomatous infiltrations were found in the heart valves and valve rings in nine of the globulin-treated animals, and form the basis of a separate report (29). In addition, two animals showed lesions of a proliferative arteritis in the coronary arteries, of the type commonly associated with experimental foreign protein sensitivity.

2. Immunological Studies

It has recently been suggested (23–26, 30) that acute nephritis might be the result of the products of streptococcal infection acting upon renal substance in such a way as to render it antigenic. Antibodies to this renal substance

were thought then to give rise to the lesions of nephritis as a manifestation of local organ-specific, antigen-antibody reaction.

Schwentker and Comploier (23) brought experimental evidence to support such a conception. Using the highly sensitive collodion particle technique they demonstrated antikidney antibodies in the sera of rabbits injected with ground rabbit kidney extract mixed with streptococci. They were unable to show antikidney antibodies when kidney extract or streptococci alone were injected. These findings were confirmed in a series of similar experiments by Cavelti and Cavelti (24–26). Unfortunately the precise relationship between the appearance of antirenal antibodies and the production of renal lesions is not clear in the last mentioned series of articles.

An attempt was therefore made in the present experiments to determine whether antikidney antibodies might play a rôle in the development of the renal lesions in our rabbits. Such investigation seemed important, since in this case, the experimental technique did not involve administration of renal substance, antikidney serum, or streptococci.

Method.—Blood was drawn from ten of the eighteen globulin-treated animals on three occasions: before the first globulin injection, before the desensitizing dose of globulin on the 11th day of the experiment, and finally just before the animals were killed. The blood was centrifuged and the serum drawn off. A simple qualitative ring test technique, similar to that employed by Hawn and Janeway (22) was used in all tests. In this technique a quantity of test antigen is carefully layered over the serum in a small test tube (ours were 3 × 40 mm.). The tubes were incubated at 37° C. for 1 hour and promptly read. The formation of a clear cut white ring at the interface between the serum and antigen was recorded as positive. Reactions were roughly graded from zero to four plus. Tests were made on these ten sera for the following:—

1. Antibodies to bovine serum gamma globulin. 0.05 per cent globulin solution was used as test antigen.
2. Antibodies to rabbit kidney. The serum of each rabbit was tested against saline extract of perfused ground rabbit kidney. Twenty per cent, 10 per cent, and 2 per cent kidney extracts were used. The kidneys removed from the animals prior to globulin therapy had been washed free of blood by perfusion with normal saline at the time of removal. They were then stored at −20°C. until required for serological testing. The serum of each rabbit was tested then against extract of the rabbit's own kidney, as well as against the pooled extracts of several kidneys.
3. Antibodies to rabbit liver. This served as a control of test 2 above. Twenty per cent, 10 per cent, and 2 per cent extract of ground perfused rabbit liver was used as test antigen.

In addition to these tests, each lot of bovine gamma globulin was tested against the individual and pooled rabbit kidney extracts, and also against rabbit liver extract and sera of all the animals before treatment was initiated. This step was necessary in order to rule out the possible presence of antibodies to rabbit organs and serum in the bovine gamma globulin. All serological tests were controlled by corresponding tests with normal saline.

Intradermal skin sensitivity tests were carried out in all animals just before killing. For this purpose, a shaved area on the back of the animal was injected intradermally with 0.1 cc. of each of the following substances: (*a*) bovine gamma globulin (10 per cent solution), (*b*) rabbit kidney extract (20 per cent saline suspension of rabbit's own kidney), (*c*) rabbit liver

extract (20 per cent saline suspension), and (*d*) normal saline. In ten of the animals this test was done 2 days before killing, and the tests were read at the time of killing. In the other eight globulin-treated animals the remaining kidney was surgically removed before skin testing. It was hoped thus to eliminate the possibility that the remaining kidney might be absorbing any kidney antibody that may have been present, rendering it undetectable by skin test. Unfortunately these animals failed to survive the second nephrectomy for a long enough period to allow accurate reading of the tests.

Results.—The results of the serological and skin tests, and their relation to the presence of renal lesions are shown in Table I. Preliminary tests showed no demonstrable anti-rabbit–organ antibodies in any of the lots of bovine serum gamma globulin. Sera from eight of the ten animals on which serological studies were done gave moderately strong reactions with bovine globulin 11 days after the initial globulin injection. The reactions of the remaining two animals were weak or doubtful at this time. All the animals showed antibodies to bovine gamma globulin in sera drawn just before killing.

The sera of two animals gave positive reactions with extracts of their own kidneys 11 days after the first globulin injection. One of these animals showed diffuse glomerulitis of the most severe degree at autopsy (Figs. 2 and 3). The other showed rather severe focal pyelonephritis which was quite different morphologically from the lesions in other treated animals, and which was considered spontaneous. Less marked reactions were obtained between the sera of three animals and the pooled kidney extract. However, in only one of these did a corresponding reaction with the rabbit's own kidney appear. All reactions with both liver and kidney extracts were negative in sera drawn just before the animals were killed.

All animals skin-tested showed some degree of skin sensitivity to bovine gamma globulin. These tests were graded according to severity. The most marked reactions were classed as ++++, and consisted of well marked areas of edema, erythema, and central necrosis. Reactions graded as + consisted of a zone of edema only. Some slight reaction was noted at the site of injection with kidney extract in three animals. These reactions consisted of edema only. Two of these three animals had histologically normal kidneys at autopsy, whilst the third showed lesions of diffuse glomerulonephritis.

In general, then, it may be stated that no correlation could be noted between the results of immunological tests for antikidney antibodies done in this experiment, and the presence or severity of renal lesions. It should also be pointed out that although all animals showed antibodies to the injected antigen (bovine gamma globulin), in four of them the lesions of diffuse glomerulonephritis were lacking.

3. Blood Coagulation Studies

A frequent observation in both human and experimental nephritis in the acute stage, is the presence of fibrin or protein coagula in the glomerular capil-

TABLE I

Relation of Immunologic Reactions to Presence of Renal Lesions

Animal No.	Time of test	Serologic reaction to				Intradermal test			Glomerulonephritis
		Own kidney extract	Pooled kidney extract	Liver extract	Globulin	Kidney extract	Liver extract	Globulin	
	Control*	0	0	0	0				
2	11 days	0	+	0	++				
	17 days	0	0	0	++	+	0	++	0
	Control	0	0	0	0				
4	11 days	++	+	0	++				
	17 days	0	0	0	++	0	0	+++	++++
	Control	0	0	0	0				
5	11 days	++	0	0	++				
	17 days	0	0	0	++	0	0	++	Focal interstitial nephritis only
	Control	0	0	0	0				
7	11 days	0	0	0	++				
	17 days	0	0	0	++++	+	0	++	++
	Control	0	0	0	0				
8	11 days	0	+	0	++				
	17 days	0	0	0	++++	0	0	++	++
	Control	0	0	0	0				
9	11 days	0	0	0	+				
	17 days	0	0	0	++	0	0	+	++
	Control	0	0	0	0				
11	11 days	0	0	0	++				
	17 days	0	0	0	++	0	0	+	Spontaneous pyelonephritis only
	Control	0	0	0	0				
12	11 days	0	0	0	++				
	17 days	0	0	0	++	0	0	++++	+++
	Control	0	0	0	0				
13	11 days	0	0	0	±				
	17 days	0	0	0	++	+	0	+	0
	Control	0	0	0	0				
14	11 days	0	0	0	++				
	17 days	0	0	0	++++	0	0	+	++++

* Sera obtained 24 hours before initial globulin injection.

laries (31–33). Since this phenomenon is one of the earliest morphologically detectable changes in nephritis, it was thought that some alteration of blood coagulability might be found during the period in which nephritis could be presumed to be developing. In this connection, Silfverskiöld (34) was able to prevent, by preliminary injection with heparin, the urinary changes of experimental horse serum nephritis in rabbits.

Morphological changes in the kidneys of these animals are only briefly dealt with by Silfverskiöld, but he states that "the kidneys of the heparinized animals seemed to be less involved than the non-heparinized controls." It is interesting that in a similar experiment by Silfverskiöld with nephrotoxic nephritis in rats, heparin failed to have this effect. Silfverskiöld's work has not been repeated, and examination of blood coagulability in experimental nephritis does not appear to have been carried out before.

TABLE II

Changes in the Blood Coagulation Time during the First 7 Days after a Massive Bovine Gamma Globulin Injection

	No. of readings	Mean coagulation time	Standard deviation
		min.	
18 globulin-treated animals	168	3.90 ± 0.104	1.35
18 nephrectomized controls*	34	5.42 ± 0.215	1.25
5 saline-injected controls‡	50	5.68 ± 0.16	1.16

* These observations were made on the same 18 animals that were subsequently injected with globulin. They were recorded 3 to 6 weeks after unilateral nephrectomy, within the 5 days preceeding the initial globulin injection.

‡ These observations were made during 7 days following injection with 10 cc. of normal saline per kilo of body weight.

Method.—Coagulation time observations were made on globulin-treated and control animals. The capillary tube technique was used in all determinations, and times were recorded to the nearest 1/10th of a minute. In this technique blood from a fresh cut in a small ear vein was drawn into a glass tube of capillary bore. The tube was mounted upright in a piece of plasticine, and small bits of it broken off every few seconds. The coagulation time was recorded as the time at which, when the tube was broken, a thin strand of clot pulled away with the broken end. Daily observations were made in most instances, and hourly recordings were made on several occasions. Estimation of the normal coagulation time was based on thirty-four observations made on the eighteen animals which were subsequently injected with globulin.

Results.—The results of the coagulation time observations on globulin-treated and control animals are shown in Table II. The individual variation in the readings was such that there was no uniformity of coagulation time in one particular animal from day to day, nor in all animals on any particular

day of the experiment. The results do indicate, however, that during the 1st week after globulin injection there is an increase in blood coagulability, as shown by a shortening of the mean coagulation time during this period. This change was not noted during the 6 days following the second globulin injection.

The normal mean coagulation time of the eighteen animals, as estimated from determinations made within the 5 days immediately before the initial globulin injection, was 5.42 minutes, with a standard deviation of 1.25. The mean coagulation time of the animals during the first 7 days after the first globulin injection was only 3.90 minutes, with a standard deviation of 1.35. Analysis of this difference in mean coagulation times shows that the increase in blood coagulability is statistically significant. During the 6 days following the second globulin injection the mean coagulation time did not differ significantly from the normal time, the mean being 5.39 minutes. No significant alteration in blood coagulability was observed in the five control animals on which coagulation studies were done following injection with normal saline (see Table II).

On the 8th day after the initial globulin injection, eight of the eighteen animals showed a slight prolongation of coagulation time (to 7.5-12 minutes). Although it was considered that this might be a phenomenon associated with the disappearance of antigen from the blood stream (as was shown by Hawn and Janeway to occur at about this time), further observations will have to be made to confirm this incidental finding. Several of the animals also showed marked prolongation of coagulation time within $\frac{1}{2}$ hour following the desensitizing injection of globulin on the 11th day of the experiment. Absence of this well known transient feature of anaphylaxis (35) in the other animals was probably due to the fact that all observations on this date were not made at exactly the same interval after globulin injection.

DISCUSSION

There can be little doubt that the high incidence of diffuse glomerulitis in our animals was the result of their treatment with bovine gamma globulin. The experimental injury does not resemble any spontaneous renal lesion in rabbits, and similar lesions did not occur in any of the control animals. Preliminary nephrectomy, together with the use of two massive globulin injections, appears to have markedly increased the severity of the nephritis in comparison to that produced by Hawn and Janeway (22) with a single injection in the intact animal. The morphological appearance of the lesions closely resembles that of human glomerulonephritis. The features of glomerular enlargement, cellular proliferation, shredding of basement membranes, protein deposits in the glomerular capillaries and Bowman's spaces, and the more advanced changes of crescent formation, and fusion of the glomerular tufts are characteristics common to these experimental lesions and those of human

glomerulonephritis. A further similarity is the diffuse involvement of practically all glomeruli in both instances. The appearance of the lesions in the more severely damaged kidneys is such that chronicity and progression of the pathological changes in the absence of further treatment might be presumed (Figs. 1, 2, and 3). This constitutes an added feature common to human nephritis and experimental globulin nephritis. The total morphological picture in the kidneys of these animals appears, in fact, identical with that of human diffuse glomerulonephritis in its acute and subacute phases.

The experimental method employed in these experiments appears to us to be equal or superior to other techniques in its capacity to produce in high incidence, a close counterpart of human diffuse glomerulonephritis. It should be possible, through study of the various features of globulin nephritis and their integration with those of other varieties of experimental nephritis and the human disease, to draw some conclusion regarding the etiology and pathogenesis of the lesions.

The currently favored hypotheses of the etiology and pathogenesis of nephritis assign a fundamental rôle to the state of hypersensitivity (23–26, 30). In the human disease the association between hypersensitivity and the development of nephritis is suggested by the time relation between the onset of an upper respiratory infection (usually streptococcal) and the appearance of renal symptoms. In the various forms of experimental glomerulonephritis which can be said to resemble the human disease, hypersensitivity invariably accompanies the development of the lesions. The mechanism by which hypersensitivity produces nephritis has been the stumbling block in most theories causally relating the two conditions.

Recently Cavelti and Cavelti (24–26) demonstrated experimentally that kidney tissue can be rendered antigenic for homologous species by the addition of streptococci to an extract of perfused, ground kidney. This had been shown earlier by Schwentker and Comploier (23) who, in addition, demonstrated antibodies to kidney in sera of human nephritics. In both these pieces of work, the use of the highly sensitive collodion particle agglutination technique was necessary for consistent demonstration of kidney autoantibodies. These results suggested to the Caveltis and to Schwentker and Comploier that in streptococcal infection, the bacteria or their products may act upon the kidney in such a way as to render it antigenic, and that nephritis ensues as the result of the action of antikidney antibodies on renal tissue *in situ*.

In our experiments neither kidney antibodies, streptococci, nor renal substance was injected. Nevertheless, the resulting morphological picture was indistinguishable from that produced by one or another of these agents. It seems reasonable to assume, therefore, that the mechanism by which the lesions were produced must also be similar, if not identical. It was not, however, possible to demonstrate that the development of nephritis in this instance was mediated, or even accompanied by the formation of antikidney

antibodies. While the simple ring test and intradermal sensitivity tests used here are certainly less sensitive than the collodion particle agglutination technique used by the above investigators, they are also less likely to give non-specific cross-reactions.

These results seem to cast some doubt upon the kidney autoantibody hypothesis, in the pathogenesis of this type of nephritis. Indeed, this entire concept is brought into question by the recent publication of Humphrey (36). Using the Cavelti technique, he was unable to reproduce their results, either morphologically or immunologically, in a single animal.

It is more difficult to assess the rôle of hypersensitivity in general in the development of globulin nephritis. Antibodies to the injected globulin were demonstrated in all animals tested. Hypersensitivity can thus be said to have accompanied the development of the renal lesions in every instance. However, critical consideration of the evidence from this experiment and that of others makes it clear that no cause-and-effect relationship between hypersensitivity and nephritis has been unequivocally established. In view of this, it seemed advisable to examine other possible factors. One such factor which was investigated was that of possible changes in blood coagulability during the time of development of globulin nephritis. There was, indeed, a significant increase in blood coagulability during the first 7 days after the initial globulin injection. Moreover, the appearance of the more severe renal lesions in these rabbits is consistent with their origin at about this time. This alteration in coagulability was inconstant from one animal to another, and in the same animal from day to day, but showed up clearly as a depression of the mean coagulation time in the treated animals during the first 7 days of treatment. Although the changes in individual animals were not such as to allow us to predict which of them would show renal lesions, the alteration does constitute evidence of a physicochemical alteration in the blood following massive globulin injection. It is possible that the formation of fibrin thrombi in the glomerular capillaries may be related to an alteration of blood coagulability. The work of Silfverskiöld cited above, indicating an amelioration of one type of experimental nephritis by heparin, adds some support to this suggestion. There is sufficient evidence from both Silfverskiöld's work and our own to justify closer examination this possibility. It seems equally possible that the glomerular capillary plugs are protein coagula resulting from a decrease in the colloidal suspension stability of the plasma protein molecules. For instance, it is known that hyperglobulinemia is associated with an increase in red blood cell sedimentation velocity. It seems possible that a large quantity of globulin may produce physicochemical alteration sufficient to bring about precipitation of protein in the glomerular capillaries where the blood is concentrated about 25 per cent through fluid loss. It seems essential to consider such possibilities in experiments like the present, in which large amounts of

protein are injected, before more complex mechanisms are accepted as the basis of the lesions produced.

It should be emphasized that although the most striking morphological lesion produced by massive dosage with bovine gamma globulin is a diffuse glomerulitis, the injection of purified antigen did not in our experiments produce this single type of lesion alone. Nine of the globulin-treated animals showed focal granulomatous lesions in the heart valves and valve rings, and two showed coronary arteritis (29). It should be further noted that cardiac and renal lesions did not always coexist in the same animals. This confirms the findings of Hawn and Janeway (22), who also found both cardiac and renal lesions in their globulin-treated animals. It appears that if the lesions of focal cardiac granulomata and diffuse glomerulonephritis are based upon hypersensitivity, that the specificity of the antigen is not the important factor in the production of one lesion or the other.

SUMMARY AND CONCLUSIONS

A high incidence of acute diffuse glomerulitis was produced in unilaterally nephrectomized rabbits by injection with two successive doses of purified bovine serum gamma globulin (fraction II). This experimental nephritis is morphologically analogous to human acute and subacute diffuse glomerulonephritis. The technique described is advanced as a valuable experimental method in the study of the pathogenesis of glomerulonephritis.

Qualitative immunological investigations produced no evidence that the pathogenesis of experimental globulin nephritis is mediated by kidney autoantibodies. The rôle of hypersensitivity in the pathogenesis of nephritis is discussed.

During the 1st week of the development of experimental globulin nephritis there is a significant increase in blood coagulability, as shown by a lowering of the mean coagulation time in globulin-treated animals during this period. This observation has not been reported previously. The possible relation of this increased blood coagulability to the formation of coagula in the glomerular capillaries is discussed.

The injection of a single purified antigen (bovine serum gamma globulin) produced three distinct types of lesion, diffuse glomerulitis, focal granulomata of the heart valves and valve rings, and coronary arteritis.

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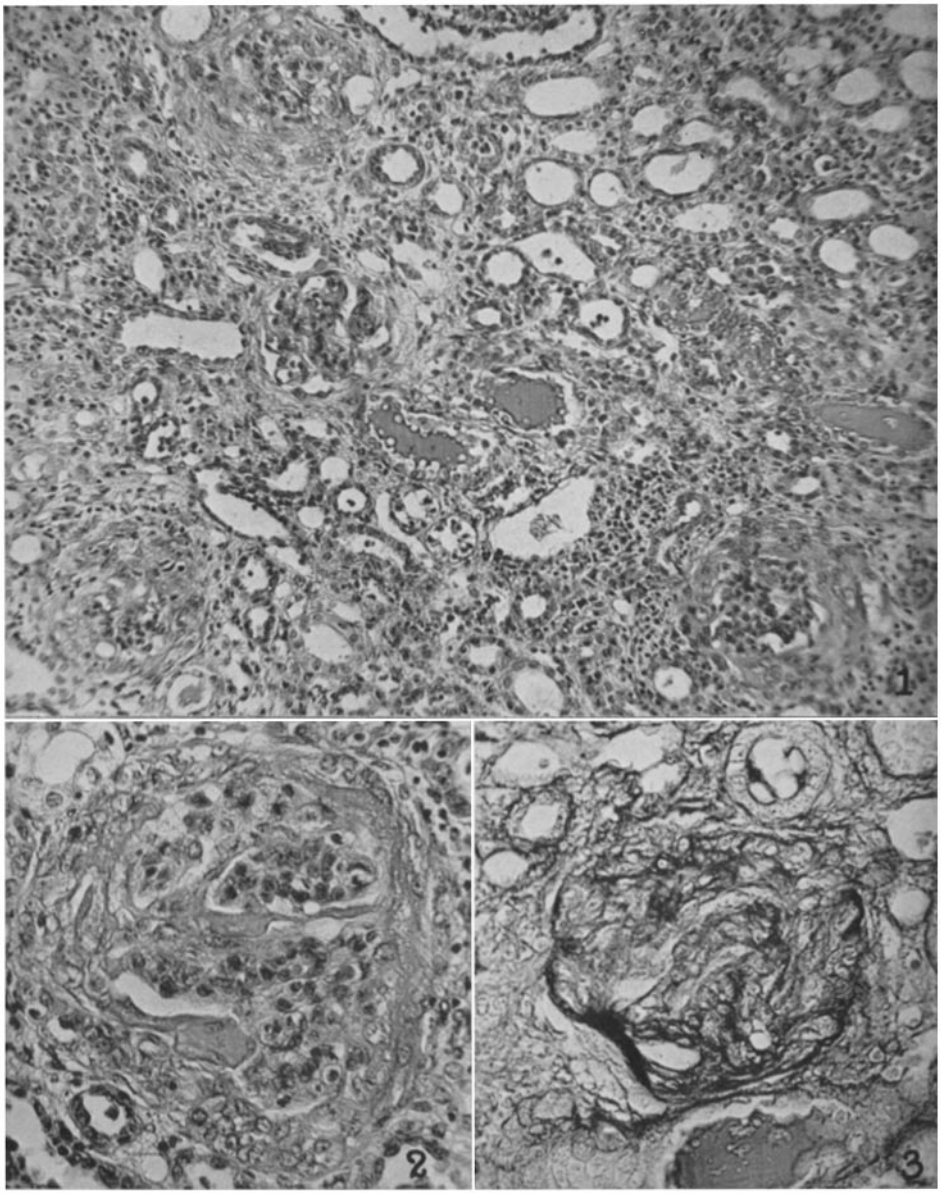
EXPLANATION OF PLATES

PLATE 22

FIG. 1. Diffuse glomerulonephritis, grade ++++ in animal 4 which had uremia. The diffuse distribution of the lesions is shown. In addition to marked proliferative changes in four glomeruli, a diffuse increase in interstitial connective tissue, atrophy of tubules, and tubular casts are to be seen. Hematoxylin and eosin. × 137.

FIG. 2. Diffuse glomerulonephritis, grade ++++; glomerulus from same kidney as shown in Fig. 1. There is marked cellular proliferation, forming almost complete synechia between glomerulus and capsule, and compressing remnants of glomerular tuft at center. A protein coagulum is seen near the center. Hematoxylin and eosin. × 300.

FIG. 3. Diffuse glomerulonephritis, grade ++++; same kidney as in Fig. 1 and 2. There is irregular thickening and shredding of both glomerular and capsular basement membranes. Mallory-Haidenhain. × 300.



(More and Waugh: Globulin glomerulonephritis in rabbits)

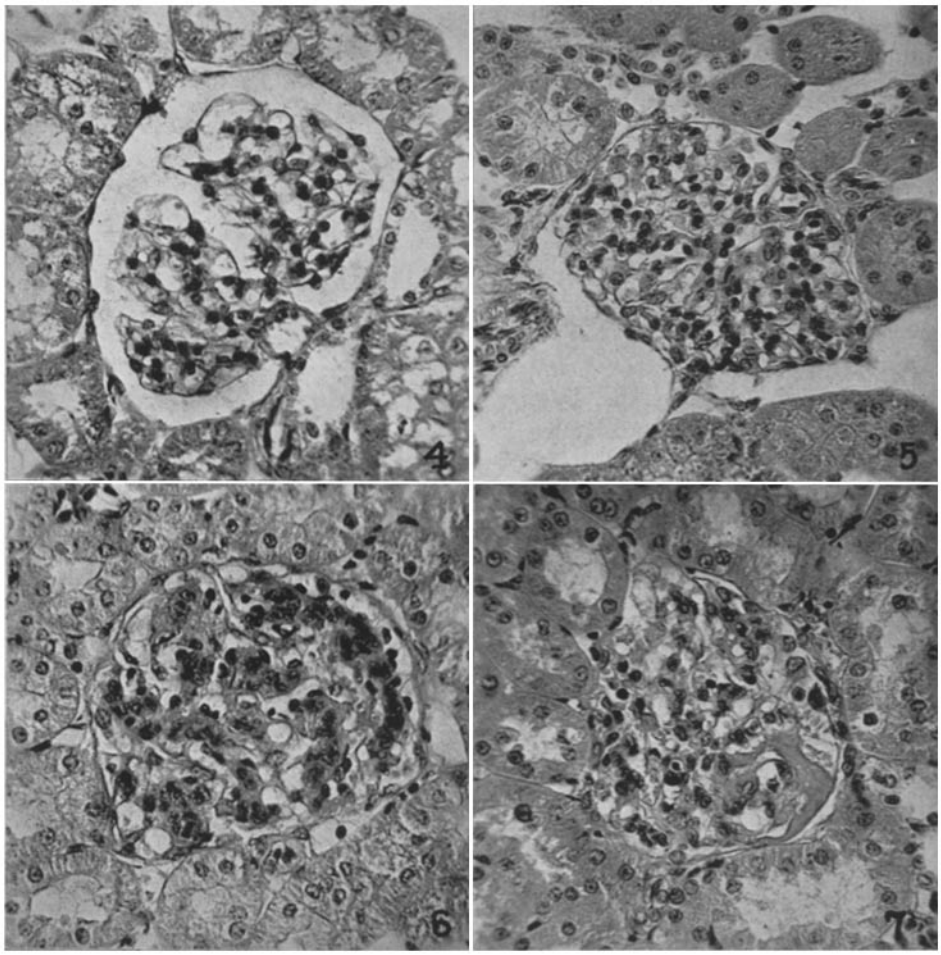
PLATE 23

FIG. 4. Normal glomerulus from nephrectomized control animal. Glomerular tufts are discrete, and capillary loops delicate and patent. Hematoxylin and eosin. × 300.

FIG. 5. Diffuse glomerulonephritis, grade +. There is conspicuous increase in glomerular cellularity and reduction in size of capillary lumina. The more darkly staining nuclei are those of endothelial cells. Hematoxylin and eosin. × 300.

FIG. 6. Diffuse glomerulonephritis, grade ++. Here the reduction in the capillary lumina is more marked. This is due to proliferation and swelling of endothelial cells into the capillaries. Thickening of the epithelium of Bowman's capsule suggests early crescent formation. Hematoxylin and eosin. × 300.

FIG. 7. Diffuse glomerulonephritis, grade +++. Proliferative changes have led to early crescent formation. Hyalin protein exudate in Bowman's space. Hematoxylin and eosin. × 300.



(More and Waugh: Globulin glomerulonephritis in rabbits)