EXPERIMENTAL GLOBULIN GLOMERULONEPHRITIS IN RABBITS

MORPHOLOGICAL AND FUNCTIONAL CHANGES*

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In a previous publication it was shown that acute diffuse glomerulonephritis develops in rabbits injected intravenously with large quantities of purified bovine serum gamma globulin (1). Other investigators have produced experimental glomerulonephritis by giving animals intravenous injections of either normal horse serum (2–6), normal duck serum (4), a number of "antikidney sera" (7–15), antiplacenta serum (16), or horse (17) or bovine (1, 18) serum gamma globulins. The number of these substances, and the variety of technics employed in their use, at once suggest that if a single pathogenetic explanation is valid for the glomerulonephritis in all cases, it must bring into harmony the differences in experimental procedures.

The results to be reported here indicate that the acute glomerulonephritis induced in rabbits by massive injections of purified bovine serum gamma globulin is similar both in its signs and morphologically to that resulting from massive injections of horse serum, or "nephrotoxic" duck serum, and also closely resembles human diffuse proliferative glomerulonephritis (extracapillary glomerulonephritis of Fahr (19), or Ellis' Type I nephritis (20)). Findings in the present study, together with those from other studies in experimental nephritis, suggest that the quantitative antibody turnover may have greater pathogenetic importance than antigen specificity.

Methods

A total of 77 unilaterally nephrectomized albino rabbits of both sexes were used. Left nephrectomy had been done 2 to 6 weeks prior to the first globulin injection, by which time most animals weighed between 2.0 and 2.5 kilos. The animals were kept in individual cages and were fed either Purina dried chow or Ogilvie miracle chow, and water *ad libitum*. Unilaterally nephrectomized control animals were kept simultaneously with all groups of globulin-injected animals. The following groups of animals were subjected to the procedures indicated.

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Group I—Twenty-four animals were injected on two occasions (10 to 12 days apart) with 10 cc. per kilo of 10 per cent purified bovine serum gamma globulin[1] solution in physiological saline. Fatal anaphylactic reactions on the occasion of the second injection were avoided by preliminary intravenous injection of 5 to 10 cc. of 1 per cent bovine globulin solution given slowly. This desensitizing injection was given 3 to 18 hours before the second large injection. The animals were killed 6 or 7 days after this second injection.

Group II.—Sixteen animals were placed in a refrigerated room 4 days before the first globulin injection. Otherwise these animals were treated exactly as those of group I. The animals remained in the cold throughout the remainder of the experimental period at ambient temperatures varying between −9.4°C. and +4.4°C., but generally below freezing.

Group III.—Five animals kept at room temperature were given a single intravenous injection consisting of 10 cc. per kilo of 10 per cent solution of bovine serum gamma globulin. All were killed 8 days after this injection.

Group IV.—(Controls, room temperature). 20 unilaterally nephrectomized rabbits were kept at normal room temperature for the same period as those of group I. Except for the substitution of physiological saline for the bovine gamma globulin injections, these animals were subjected to exactly the same procedures (bleeding, urinalysis, etc.) as those of group I.

Group V.—(Controls, refrigerated). 12 unilaterally nephrectomized animals were kept in the cold room as controls for those of group II. Except for the substitution of physiological saline for the bovine globulin injections, they were subjected to the same procedures as the animals of group II.

Antemortem Studies.—The number and variety of observations made during the course of these experiments were changed from time to time as was suggested by preliminary pilot experiments. For this reason all observations were not carried out on each animal of every group. Sufficient observations were made in all groups, however, to ensure validity of the data recorded.

Urinalysis was carried out daily on 24 hour urine specimens collected *via* a screen grid and funnel bottom in individual cages. Urinary protein was estimated by the addition of 3 per cent sulfosalicylic acid and comparison with Kingsbury-Clark turbidity standards. pH was roughly estimated with Fisher alkacid paper (c). In addition, 24 hour output was measured, and microscopic examination was done on uncentrifuged specimens.

Blood urea nitrogen was estimated in some animals of group I at the end of the experimental period. Similar determinations were carried out on animals from all groups on days 0, 7, 10, and 16 of the experiment, with day 0 indicating the day of first globulin injection. In all determinations, the photelometric technic outlined by Hoffman (21) was employed.

Serum protein estimations were carried out on 6 animals in each of groups I, II, IV, and V, using blood drawn on the same occasions as urea nitrogen determinations were made. Additional determinations were made on animals of groups II and IV the day before they were placed in the cold room. In all serum protein studies, a modification of the technic of Wolfson *et al.* (22) was employed. As originally proposed, this method was intended to give, by salting-out procedures, results equivalent to those obtainable by electrophoretic analysis. The principal modification introduced in the present work was that of separating the precipitated globulin fractions by filtration rather than by centrifugation. To this end, surface-active agents (ether, span 20) were omitted from the procedure. Furthermore, in the precipitation of the gamma globulin fraction, NaCl was omitted from the ammonium sulfate solution, and precipitation was carried out over a 24 hour period at 37°C. This precipitate was then quantitatively estimated using the phenol reagent of Folin and Ciocalteu (23), after redissolving in 0.05 N NaOH. Substitution of this reagent for biuret (Weichselbaum) was necessary, since

[1] Obtained from Armour & Co., Chicago.

it was found that the ammonium sulfate used in precipitation gave a high, and variable blank reading with biuret, but not with the phenol reagent. The difference in color reagents was controlled by carrying out simultaneous estimations of total serum protein with both. All other features of the original technic were retained. Duplicate analyses were usually made, and tests were repeated when there appeared to be any reason to suspect technical error. Every effort was made to ensure standard strength of reagents and to carry out all analyses at a constant rate of speed. As reported elsewhere, the technic in its modified form gave results closely approximating those obtained by electrophoresis in both normal and immune sera (24).

Hematological studies included estimation of sedimentation velocity (Wintrobe (25)) and recording of relative volumes of packed red and white blood cells after centrifugation for 30 minutes at 1200 R.P.M. Immunological investigations were limited to the demonstration of the presence or absence of antibodies to the injected globulin by the simple qualitative ring test used in earlier experiments (1). These tests were done (*a*) shortly before the first globulin injection, (*b*) daily on 6 animals of group II until antibodies appeared, and (*c*) on the 7th, 10th (immediately prior to the second globulin injection), and 16th days after the first injection on 6 animals each from groups I and II.

Morphological Studies.—The animals were killed either by air embolism or by a blow on the back of the neck. Gross and microscopic observations were recorded on tissues from all groups. At autopsy the weights of kidney, heart, spleen, and thymus were recorded, in addition to body weight. These organs, and specimens from other tissues, were fixed in Zenker-formol for 8 to 18 hours, washed in running water for 24 to 48 hours, dehydrated, and embedded in paraffin. Histological sections were cut at 5μ and stained in a routine manner with hemalum-phloxin-saffron. In addition, kidney sections were stained with Mallory-Heidenhain, Masson's trichrome, McManus' periodic acid-Schiff's stains, and according to Lendrum's method for basement membranes (26). Sections from tissues of control animals killed at the same time as treated groups, formed the basis for estimations of degrees and types of damage found in the treated animals. In addition, sections of all kidneys surgically removed before the start of the experiments were examined to rule out possible spontaneous renal disease.

RESULTS

During the experimental period of 16 or 17 days all animals gained weight, although there was considerable individual variation. The relation between rates of weight gain in the different groups is shown in Table I. Among the globulin-injected groups, no relationship was apparent in individual animals between the amount of weight gain and the presence, or severity, of glomerulonephritis.

Weight of the Organs.—The weights of the remaining kidney, heart, spleen, and thymus were expressed as per cent of body weight at the time of autopsy as shown in Table I.

Kidney weight in control animals kept at room temperature (group IV) ranged from 7.5 gm. in a 2.3 kilo rabbit to 16.0 gm. in a 3.8 kilo animal, the average kidney weight being 12.35 gm. The average kidney weight in control animals kept in the cold (group V) was slightly higher (13.78 gm.), the most striking weight increase being a kidney of 17.2 gm. in a 2.46 kilo animal. Of the 6 globulin-treated animals of group I, whose organ weight data were recorded, 4 developed glomerulonephritis and had moderately enlarged kidneys, whilst

TABLE I

Relation of Organ Weight to Body Weight in Bovine Globulin-Injected and Control Groups of Rabbits

Group*	No. of animals	Body weight	Average daily weight gain	Kidney		Heart		Spleen		Thymus		No. of animals with glomerulonephritis
				Weight	Per cent of body weight	Weight	Per cent of body weight	Weight	Per cent of body weight	Weight	Per cent of body weight	
		kg.	*gm.*	*gm.*								
I	6	2.66	24.8	14.32	0.555 (±0.0529)	7.10	0.268 (±0.0110)	2.15	0.080 (±0.0104)	4.40	0.164 (±0.0396)	4
II	15	2.33	25.3	17.77	0.784 (±0.0749)	8.36	0.361 (±0.0178)	2.43	0.106 (±0.0110)	4.39	0.187 (±0.0153)	15
III	4	2.84	—	13.38	0.468 (±0.0294)	7.85	0.273 (±0.0258)	3.98	0.140 (±0.0315)	3.78	0.130 (±0.0322)	Minimal glomerulitis in all
IV	10	2.81	25.8	12.35	0.431 (±0.0160)	7.54	0.260 (±0.0012)	1.89	0.062 (±0.0075)	5.63	0.199 (±0.0192)	0
V	10	2.51	26.2	13.78	0.549 (±0.0285)	7.81	0.310 (±0.0152)	1.62	0.062 (±0.0253)	5.33	0.199 (±0.0146)	0

* Group I, two massive intravenous injections 10 days apart, kept at room temperature.
Group II, same as group I, but kept in cold room throughout treatment period.
Group III, killed 8 days after single massive bovine globulin injection, kept at room temperature.
Group IV, room temperature controls.
Group V, cold room controls.

those which did not have nephritis, had kidneys of normal size. Although all animals treated in the cold developed nephritis, only 9 of the 16 showed renal enlargement beyond that seen in controls. The largest kidney in this group was one weighing 28.0 gm. in a 2.35 kilo animal. In the average values, the increase in renal weight is statistically significant. The kidneys of animals which had severe nephritis were usually yellower and paler than normal in addition to being enlarged (Fig. 1).

There were no striking alterations in heart weights in any of the groups, although globulin-treated animals kept in the cold showed a slightly higher average heart weight (8.36 gm.) than corresponding controls (7.81 gm.). These same animals usually showed some slight increase in spleen weights, but in other groups, neither spleens nor thymuses varied beyond the normal range. Alterations detectable in the gross in organs other than kidneys were not encountered, except for occasional instances of mild coccidiosis in the liver.

Morphological Observations.—Microscopically, changes of acute diffuse glomerulonephritis were easily recognizable in animals of groups I and II. In group I (treated at room temperature) nephritis was present in 18 (75 per cent) of the 24 animals. All animals of group II (refrigerated) had the disease. Lesions were graded according to severity from 0 to 4 plus, as in previous experiments (1). The higher incidence in group II was accompanied by an increase in the severity of the nephritis. Apart from these differences, however, there was no alteration in the character of the renal lesions between these two groups.

Glomerulonephritis of the mildest type (+) was found in 4 of the 24 animals in group I, and in 2 of the 16 in group II. Sections of these six kidneys showed definite, diffuse glomerular enlargement together with an increase in glomerular cellularity. Glomerular tufts were usually discrete, but swollen and more club-shaped than usual. Basement membrane stains showed swelling and proliferation of both endothelial and epithelial cells. The capillary space enclosed by the glomerular basement membrane was enlarged, but the effective capillary lumen was somewhat reduced by endothelial swelling and proliferation. Swelling was even more prominent in the epithelial cells. Polymorphonuclear leucocytes were much more numerous in glomerular capillaries than in kidneys of control rabbits. Alterations in the basement membrane itself were present, but mild in these animals, consisting only of slight nodularity and irregular thickening. Acidophilic protein material was occasionally present in Bowman's spaces and in the tubules. When present, this usually appeared as a loose granular acidophilic mesh but in some instances was deeply staining and hyalin in texture. Deeply acidophilic "colloid" droplets were occasionally found in the cytoplasm of cells lining the proximal convoluted tubules. Casts, when seen, were present in both proximal and distal tubules, as well as in collecting tubules.

More severe (++) renal lesions were found in 7 of the 24 animals of group

I and in 5 of the 16 in group II. The glomerular changes in these kidneys were of similar character to those just described, but of greater intensity (Fig. 2). Enlargement of the tufts was generally more marked, and this appeared to result from more advanced endothelial and epithelial proliferation. In some instances the latter was extensive enough to have brought about partial or complete fusion of two or more tufts. Irregular thickening and splitting of the glomerular basement membranes were more readily demonstrated in this group. Occlusion of the capillary lumina by swelling and proliferation of endothelial cells was also more marked. The glomerular changes were readily apparent when sections were compared with those from nephrectomized control animals (Fig. 3). Bowman's capsular epithelium occasionally showed swelling. Protein exudate was seen in the capsular spaces with greater frequency, and usually was homogeneous and deeply acidophilic. In some kidneys of this class, protein casts in the tubules were extremely numerous, whilst in others they were few. Small interstitial infiltrations with lymphocytes, plasma cells, and large mononuclear cells were sometimes found in the region of the corticomedullary junction.

Renal lesions classed as severe (+++) glomerulonephritis occurred in 4 of the 24 animals in group I, and in 7 of the 16 in group II. These lesions were considerably more severe than either of the foregoing classes. In most there was extreme occlusion of the glomerular capillaries by endothelial increase, with only relatively few patent capillaries remaining distinguishable (Fig. 5). Fusion of the glomerular tufts was present in about half the glomeruli, and more or less marked crescent formation was seen in about the same proportion. Tubular casts were usually prominent, and, as a rule, interstitial edema and cellular infiltrations were more marked. Thickening and shredding of the basement membranes were usual, and in many, fragmentation of the membrane was pronounced. In kidneys of several of these animals, in which the tubular protein content was considerable, rounded, spherical masses of homogeneous or spongy, deeply acidophilic protein material could be seen in the epithelial cytoplasm, apparently bulging into the lumen of the tubule. The cells in which these blobs of protein were present showed no sign of degenerative changes (Fig. 4).

The most severe glomerulonephritis (++++) was seen in 3 of 24 animals in group I and in 2 of the 16 in group II. Proliferative changes in the glomeruli were usually so marked as to render impossible the distinction of the individual tufts. The occasional glomerular capillary lumina which could be distinguished at all were frequently plugged with acidophilic protein material. Many glomeruli appeared reduced in size through compression of excessive crescent formation. In addition, there was invariably a marked increase in basement membrane material, which appeared as a complex, laminated structure of great irregularity. Proliferation of the epithelium of Bowman's capsule had

often led to partial or complete encirclement of the glomerulus, with which it was usually fused. In these kidneys distortion of the renal architecture was marked. There were considerable interstitial edema and fibrous proliferation, associated with more or less diffuse round cell infiltration.

Lesions of acute or healing arteritis were seen in only 2 globulin-treated animals and in only 1 of these was a renal vessel involved.

In the animals of group III, which were killed 8 days after a single injection of bovine gamma globulin, changes presumably representing the early manifestations of glomerulonephritis were found. Generally, there was slight, but definite, glomerular enlargement, as estimated by comparison with kidneys from control animals killed at the same time, and fixed in the same manner (Fig. 6). Glomerular tufts were prominent, and in some instances definitely swollen and club-shaped. Basement membrane stains showed moderate distension of the glomerular capillary lumina, most of which were widely patent and filled with blood. A slight degree of swelling of the glomerular epithelium was sometimes evident, but endothelial swelling could not be distinguished at this time. The glomerular capillaries sometimes contained masses of protein material, in addition to red blood cells. The inflammatory nature of the process was further suggested by the finding of a six- to tenfold increase in glomerular content of polymorphonuclear leucocytes. This was estimated by enumerating the leucocytes in 50 consecutive glomeruli under high magnification. In control animals this leucocyte count varied between 3 and 10 per 50 glomeruli, whilst in globulin-treated animals the number was between 30 and 60 for every animal in the group. It is unlikely that this increase could be accounted for solely on the basis of generalized leucocytosis, or by variation in the thickness of the sections examined. Casts and loose protein coagula were occasionally seen in proximal and distal convoluted tubules, and accumulations of lymphocytes and plasma cells in intertubular spaces were sometimes seen.

Diffuse glomerulonephritis was not encountered in any of the kidneys surgically removed prior to treatment with globulin, nor were such lesions seen in any of the unilaterally nephrectomized control animals of groups IV and V. Mild focal interstitial nephritis and pyelonephritis were seen occasionally in both treated and control animals. These lesions were always easily distinguishable from the diffuse glomerulonephritis which occurred in the globulin-treated animals.

Urinary Findings.—In all experimental groups there was considerable individual variation in 24 hour volume of urine output. The mean daily output for control animals kept at room temperature (group IV) was 164 cc. (75 to 222 cc.), and for cold room controls (group V) was 123 cc. (87 to 174 cc.). Average daily urine and urinary protein outputs of groups I and II are shown in Table II. It was found that even during the period of control observations

on these animals, mild proteinuria occurred from time to time. This usually amounted to no more than 10 to 15 mg. per cent, and might be ascribed to

TABLE II

Average Daily Urine and Urinary Protein Output in Refrigerated and Non-Refrigerated Rabbits Injected with Bovine Serum Gamma Globulin

Day	Group I 6 animals		Group II 11 animals	
	Quantity	Protein	Quantity	Protein
	cc.	*mg./100 cc.*	*cc.*	*mg./100 cc.*
−7	140	0	189	1
−6	154	0	119	2
−5	161	0	119	3
−4	161	0	188	11
			Placed in cold room	
−3	140	7	156	16
−2	193	0	134	8
−1	153	0	137	0
0	154	2	139	0
	First massive bovine globulin injection			
1	125	11	129	0
2	144	4	119	6
3	177	5	140	3
4	195	6	150	2
5	165	1	120	6
6	193	2	123	8
7	204	1	113	18
8	139	588 (3/6)*	129	285 (5/11)
9	137	590 (3/6)	136	276 (6/11)
10	67	793 (2/6)	163	100 (5/11)
	Second massive bovine globulin injection			
11	280	89 (3/6)	138	100 (5/11)
12	203	18 (0/6)	149	40 (2/11)
13	244	10 (0/6)	138	30 (2/10)
14	244	10 (0/6)	105	407 (5/10)
15	160	52 (1/6)	111	331 (6/10)
16	160	40 (2/6)	153	429 (6/9)

* The fraction in brackets indicates proportion of animals in group showing conspicuous proteinuria (50 mg./100 cc. or more). Changes in the denominator in cold room group are result of elimination of 2 animals from this series, 1 of which died of uremia on day 15, the other having been killed on day 13.

contamination with feces or in some cases with blood oozing from an ear vessel on the days the animals were bled.

In the animals of groups I and II, conspicuous proteinuria (more than 50 mg. per 100 cc.) made its appearance on the 8th day after the initial injection

with bovine globulin. In both groups this occurred in about half the animals, but in some of these proteinuria to the extent of 2 gm. or more per 100 cc. was recorded. Heavy proteinuria was present in both groups between the 8th and 10th days of the experiment, after which it gradually diminished. Most of the animals of group II (refrigerated) but not those of group I (room temperature) showed a secondary wave of heavy proteinuria between the 14th and 16th days of the experiment; *i.e.*, 3 to 6 days after the second injection of bovine globulin. Coincident with the initial massive proteinuria, most animals showed hematuria, usually of the order of 5 to 10 red blood cells per high power field. Two animals of group II showed gross hematuria at this time. Identification of casts was difficult in the strongly alkaline urine (pH

TABLE III

Time of Appearance of Antiglobulins after First Massive Injection

Group	No. of animals	No. of animals showing positive ring test										No. of animals with glomerulonephritis
		Days after injection										
		0	1	2	3	4	5	6	7	8	9 10	
Room temperature (group I)	6	0*							0		6 (+ to +++)	4
Cold room (group II)	6	0	0	0	0	0	0	0	0	6 (+ to +++)		6
Cold room (group II)	6	0							3 (+)‡		6 (+ to +++)	6

* Numbers in columns indicate number of animals in group showing precipitins on that day. No figure is entered on days when ring tests not done.

‡ Sign in parentheses indicates quantitative estimate of strength of positive ring tests.

7.5 to 9.0) produced by most animals. However, occasional acid samples showed large numbers of hyalin casts.

Immunological Studies.—The results of ring tests for antiglobulins are shown in Table III. In 6 animals treated at room temperature (group I) antiglobulins were not demonstrable on the 7th day after the first injection, but had appeared in all animals by the 10th day. In the 6 animals of group II which were tested daily for antiglobulins, all tests became positive on the 8th day after the first injection. Sera from these animals gave negative reactions in similar daily tests for 2 days after the second injection, but all showed precipitins on the 3rd day. Thus, in these animals of group II, after both first and second globulin injections, antiglobulins made their appearance in the sera on the same days as those on which the urinary changes of acute nephritis became most apparent. 6 other animals of group II were tested only on the 7th, 10th, and 16th days of the experiment. In these, positive reactions were recorded in 3 of the 6 on the 7th day, and in all animals on the remain-

ing two occasions. Thus, the time of appearance of antibodies in animals treated at room temperature was on either the 8th, 9th, or 10th day after the first injection, whereas in animals treated in the cold, 3 of 12 animals showed positive reactions as early as the 7th day. In both these groups therefore, the findings indicate a close correlation between the demonstrability of antiglobulins by ring test, and the urinary manifestations of acute glomerulonephritis.

Blood Chemistry.—(*a*) *Serum Proteins:* Table IV shows mean values and standard errors for serum protein fractions at various times during the experiment for animals of groups I and II. Both groups showed a rise in total protein at the end of the experimental period (16th day). By the 7th day after the first globulin injection, both showed a significant elevation of serum gamma globulin, this being rather more marked in 6 animals treated at room temperature. Gamma globulin levels on the 10th day remained unchanged in both groups, but 6 days later showed a further significant rise, again more striking in non-refrigerated animals. Apparently minor elevations of alpha and beta globulins also occurred during this period. It is considered possible, however, that beta globulin may have been relatively increased at the end of the experiment (0.62 gm. per cent) since we have found (24) that untreated rabbits usually show depression (to about 0.10 gm. per cent) in this fraction after 20 days' refrigeration. It is of interest that a significant elevation of total serum globulin was present in both groups by the 7th day after the initial injection; *i.e.*, before conspicuous proteinuria had occurred.

(*b*) *Blood Urea Nitrogen:* Table IV shows average values for blood urea nitrogen in groups I and II during the experiment. Although changes in average values are insignificant, individual animals in both groups showed distinct blood urea nitrogen elevation at the end of the experiment. Thus, 3 animals of group I showed levels of 29.1, 29.8, and 30.5 mg. per cent whilst blood urea nitrogen of 2 animals of group II was 33.0 and 52.5 mg. per cent.

Hematological Findings.—In control estimations the *sedimentation velocity* was usually between 0.3 and 1.6 mm. during the 1st hour, and in no instance was a control value above 2.0 mm. per hour. On the 7th and 10th days after the first globulin injection, 4 of 6 animals in group I showed accelerated sedimentation rates (beyond 4.0 mm. per hour). These same 4 animals showed lesions of glomerulonephritis at autopsy, whilst the 2 whose rates were normal had no nephritis. Similar, but generally less marked, elevations in sedimentation rate occurred on the 7th day only in 3 of 6 animals in group II. Nevertheless, all animals in this group showed lesions of acute glomerulonephritis when killed.

A moderate reduction in *erythrocrit*[2] level occurred in all groups of animals

[2] For convenience, the terms erythrocrit and leucocrit are used to designate volume of packed red blood cells and volume of packed white blood cells respectively, expressed as per cent of whole blood.

TABLE IV

Serum Proteins, Sedimentation Rates, Erythrocrit Level, Leucocrit Level, and Blood Urea Nitrogen in Rabbits with Experimental Globulin Nephritis

Time		Pre-nephrectomy	Post-nephrectomy		Group I, 7th day	Group II, 7th day	Group I, 10th day	Group II, 10th day		Group I, 16th day	Group II, 16th day
No. of animals		23*	23*		6	6	6	6		6	6
Serum proteins	Total protein, *gm./100 cc.*	4.40(±0.066)	5.56(±0.102)	First massive injection bovine globulin	6.42(±0.422)	5.69(±0.176)	5.84(±0.265)	5.46(±0.213)	Second massive injection bovine globulin	6.88(±0.165)	6.03(±0.366)
	Albumin, *gm./100 cc.*	2.81(±0.061)	3.62(±0.104)		3.92(±0.188)	3.03(±0.097)	3.20(±0.239)	2.91(±0.107)		3.15(±0.276)	3.20(±0.294)
	Globulin, *gm./100 cc.*	1.59(±0.052)	1.90(±0.055)		2.51(±0.269)	2.66(±0.162)	2.62(±0.207)	2.53(±0.214)		3.73(±0.419)	2.84(±0.265)
	α-Globulin, *gm./100 cc.*	0.41(±0.059)	0.37(±0.078)		0.46(±0.127)	0.63(±0.049)	0.56(±0.141)	0.75(±0.106)		0.60(±0.122)	0.68(±0.118)
	β-Globulin, *gm./100 cc.*	0.48(±0.067)	0.76(±0.058)		0.73(±0.172)	0.96(±0.172)	0.71(±0.172)	0.74(±0.311)		1.36(±0.173)	0.62(±0.088)
	γ-Globulin, *gm./100 cc.*	0.71(±0.049)	0.78(±0.035)		1.40(±0.104)	1.08(±0.043)	1.36(±0.161)	1.07(±0.057)		1.77(±0.222)	1.54(±0.171)
Blood urea nitrogen, *mg. per cent*		19.8 (±1.17)	18.4 (±1.34)		18.8 (±5.37)	20.7 (±1.59)	19.4 (±1.82)	22.2 (±1.30)		25.1 (±3.24)	29.6 (±5.38)
Sedimentation rate, *mm./hr.*		0.6 (±0.08)	0.5 (±0.08)		4.9 (±2.08)	3.0 (±1.30)	12.9 (±7.34)	1.0 (±0.19)		5.8 (±3.74)	1.4 (±0.45)
Erythrocrit, *per cent of blood volume*		41.7 (±0.71)	41.6 (±0.54)		38.1 (±1.89)	35.6 (±1.15)	38.1 (±1.82)	35.2 (±0.66)		34.9 (±1.74)	37.3 (±1.75)
Leucocrit, *per cent of blood volume*		0.8 (±0.18)	1.0 (±0.12)		2.0 (±0.26)	1.6 (±0.23)	2.2 (±0.22)	1.7 (±0.21)		1.9 (±0.47)	1.7 (±0.25)

* This figure includes animals subsequently separated as controls.

during the course of the experiment. Since the only procedure to which all groups were subjected was that of repeated taking of blood samples, it is likely that this anemia was a result of this factor, rather than being due to any effect of immunization or exposure to cold. Similarly, all groups showed a tendency to a rising volume of packed white blood cells. This was somewhat less marked in animals kept in the cold, but the difference does not appear significant.

DISCUSSION

The glomerulonephritis in the foregoing experiments emerges as a pathological process with distinct signs during life and morphological manifestations. The onset is usually abrupt, characterized by proteinuria, hematuria, and cylindruria. The appearance of these phenomena coincides with the development of precipitins in the blood, and a rising serum gamma globulin. The latter is progressive and most marked at the end of the experimental period 8 to 10 days after the first urinary manifestations. Failure of renal function, as indicated by rising blood urea nitrogen, occurred but was uncommon.

The sequence of morphological changes appears to begin with dilatation of glomerular capillaries and increased glomerular leucocyte content. This is followed by progressive endothelial and epithelial swelling and proliferation, capillary embarrassment, and finally basement membrane thickening, shredding, and fragmentation. Our findings are in agreement with those of Ehrich *et al.* (4) in suggesting that epithelial proliferation, glomerulocapsular adhesions, and crescent formation are more likely a manifestation of severity than of chronicity. Certainly those animals with lesions graded as ++++ had renal lesions which according to the usual criteria applied to human nephritis would have been called subacute, yet urinalyses indicate that these lesions had developed within a period of 10 days or less.

The characterization of globulin-induced glomerulonephritis thus far indicates a close similarity with the human disease and with experimental glomerulonephritis induced by other means. The type of onset, course, and serum gamma globulin elevation (27) are similar to those in acute nephritis in man (20, 28). Both the signs and morphological findings closely resemble those of Ellis' type I nephritis (extracapillary glomerulonephritis of Fahr (19)). Points of similarity include abrupt onset with hematuria, absent or slight edema, and histological evidence of predominantly, though not exclusively, extracapillary glomerular proliferation. The early changes of capillary dilatation and leucocyte accumulation have been described by Dunn (29) in cases of early nephritis in man, and by Gukelberger (30) in experimental nephrotoxic nephritis.

Comparison of experimental globulin nephritis with other varieties of ex-

perimental nephritis also discloses manifold similarities. In nephrotoxic nephritis in rabbits Ehrich *et al.* (12) noted an abrupt onset 7 days after injection of anti-rabbit-kidney (nephrotoxic) duck serum. The morphological changes in kidneys of animals treated in this way (7–15) and in those injected with horse serum (2–6), normal duck serum, and with horse serum gamma globulin are similar to, though generally less intense than, those reported here. In nephrotoxic nephritis the urinary manifestations have also been found to coincide with the appearance of anti-duck precipitins in the rabbits' sera (31).

The foregoing raises the question of whether we are dealing merely with similar end-points produced by diverse mechanisms, or whether these various experimental nephritides and human acute nephritis have a common pathogenesis. Logic favors the latter assumption, but must either explain or ignore the differences in initiating cause.

All varieties of experimental acute diffuse glomerulonephritis thus far described have been accompanied by the state of hypersensitivity, and there is considerable evidence that this also occurs in human acute nephritis (32). It has been suggested that human and experimental glomerulonephritis find their common basis through the medium of antikidney antibodies (31). The growing weight of evidence is against this. Such antibodies have only rarely been demonstrated in human nephritis (33) and their occurrence in experimental nephritis, apart from those experiments in which nephrotoxins are injected, has been denied (1, 34). In an earlier series of experiments we were unable to demonstrate antikidney antibodies in either bovine serum gamma globulin or in the sera of rabbits with nephritis induced in the manner reported here (1).

In contrast to the equivocal role of antikidney antibodies in the pathogenesis of nephritis, it seems highly probable that the experimental nephritides discussed above are mediated by hypersensitivity. All are produced by rather large intravenous injections of foreign protein. Inhibition of antibody production by x-ray (31) has been found to prevent nephrotoxic nephritis in rabbits. Similar inhibition also follows reduction of dietary protein (13). Other nvestigators have shown that such dietary restriction simultaneously inhibits antibody production (35). We have recently found it possible to completely prevent globulin glomerulonephritis in rabbits by slight over-all reduction in food intake (36). This was accompanied by a delay in the time of appearance of antiglobulins. The evidence just cited is in agreement with the suggestion that immunological specificity of the antigen used to induce nephritis is probably of less pathogenetic importance than is the total antibody response itself, in terms of both rate and quantity of production or turnover. This suggestion does not deny the possibility that *some* special quality of the antigen may still be of importance, since it is clear that many antigens which evoke profound antibody response do not produce nephritis.

There is, indeed, some evidence that factors which increase antibody production or rate of turnover may be associated with an increase in incidence or severity of nephritis. In radioisotope studies Schoenheimer *et al.* (37) found that the globulin and presumably antibody fractions of serum participate in general metabolic exchanges, at the same rate and to the same extent as do other serum and tissue proteins. In the present experiments animals exposed to cold presumably required increased food consumption in order to maintain normal rate of weight gain. They also showed an apparently significant increase in the severity of experimental globulin nephritis. The fact that they did not show higher serum globulin levels than their companions treated at room temperature need not argue against the assumption of increased antibody activity, since the rate of antibody turnover which is not known at present, may well have been greater.

This relation to rate of metabolic turnover might explain the tendency for acute nephritis in human beings to occur in young individuals. It could also be used to explain the tenfold increase in incidence of acute nephritis seen by Formijne (38) in Amsterdam during the first 6 months of dietary rehabilitation of a starved (39) population in 1945 following the cessation of World War II.

It seems probable that other, as yet poorly recognized, factors also play a role in the pathogenesis of glomerulonephritis. The work-load borne by the kidney at the time of immunological insult is probably one of these, since nephritis seems to be much more readily induced in unilaterally nephrectomized animals.

SUMMARY

Diffuse glomerulonephritis was induced in unilaterally nephrectomized albino rabbits by giving them two massive intravenous injections of purified bovine serum gamma globulin. Morphologically, these renal lesions were similar to those of human acute and subacute glomerulonephritis. Globulin nephritis in rabbits also closely resembles experimental nephritis induced by injection of other foreign proteins.

The course of this experimental disease was characterized by abrupt onset of proteinuria, hematuria, elevation of serum globulin, and reduction of albumin. Uremia developed in some animals. The manifestations of nephritis during life coincided with the development of demonstrable antibodies to the injected globulin.

In view of the similarities between experimental globulin nephritis in rabbits and human nephritis, together with common features with other experimental nephritis, it is suggested that all may have a common pathogenetic mechanism. It is concluded that quantitative antibody response may be of greater importance in the pathogenesis of nephritis than antigen specificity.

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EXPLANATION OF PLATE 55

FIG. 1. Globulin nephritis, gross appearance. The lower kidney is from an animal of group II and the upper from unilaterally nephrectomized control animal (group V) of the same body weight. The appearance is similar to that of the "large white kidney" of human glomerulonephritis. × 1.35.

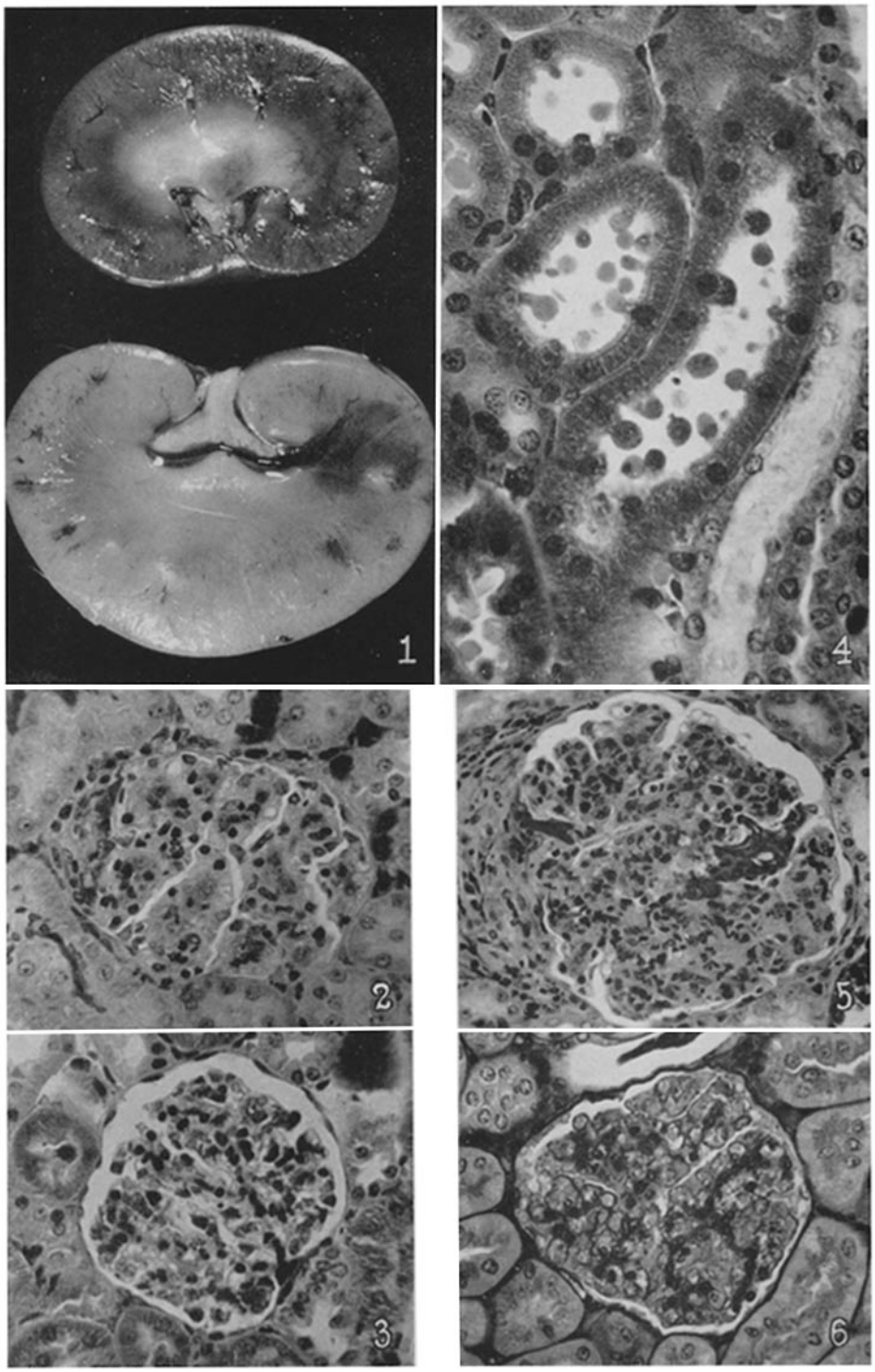
FIG. 2. Relatively mild (++) glomerulonephritis. There is thickening and enlargement of glomerular tufts, increased cellularity, and reduced capillary bed. Compare with normal (Fig. 3). Hemalum-phloxin-saffron. × 335.

FIG. 3. Normal glomerulus from unilaterally nephrectomized control animal. Glomerular tufts are discrete and delicate, capillaries patent. Hemalum-phloxin-saffron. × 335.

FIG. 4. Protein globules in proximal tubules of nephritic rabbit. Appearance suggests extrusion of cytoplasmic protein masses into tubule lumen. Hemalum-phloxin-saffron. × 335.

FIG. 5. Moderately severe (+++) glomerulonephritis. There is marked cellular proliferation, beginning fusion of tufts, and early crescent formation. Protein coagulum is seen between tufts. Hemalum-phloxin-saffron. × 320.

FIG. 6. Early glomerulonephritis 8 days after single globulin injection. Tuft enlargement due mainly to capillary distension with slight endothelial swelling. McManus' periodic acid–Schiff's, hemalum, metanil yellow. × 335.



(Waugh and More: Globulin glomerulonephritis in rabbits)