ACUTE EXPERIMENTAL GLOMERULAR NEPHRITIS IN RABBITS: A CORRELATION OF MORPHOLOGICAL AND FUNCTIONAL CHANGES*

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PLATES 32 TO 35

Of the two methods of correlating morphologic and functional changes in renal disease, clinical and experimental, the former has been repeatedly utilized with but moderate success, for when most patients die it is difficult to interpret the complex morphological alterations in the kidneys. The application of the experimental method, on the other hand, has been limited by our inability to reproduce the more important disorders. Necrotizing nephrosis, to be sure, has frequently been produced; but in this condition the lesions are so different from those of common renal disease that comparable conclusions are of little value. Only since the recent discovery of Masugi (1933) that glomerular nephritis can be produced by the injection of anti-kidney serum, could this avenue of approach be regarded as open. It may thus be attempted once more to correlate certain morphologic and functional changes.

In acute glomerular nephritis in man, as is well known, the tubules may be little if at all altered morphologically. It should be possible now to ascertain by experiment whether or not they are impaired functionally, or, in other words, whether glomeruli and tubules can function independently of one another. Another item which appears ready for investigation is the much disputed question as to whether the initial impairment of glomerular function in glomerular nephritis

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is due to spasms of the arterioles, as maintained by Volhard (1931), or to the anatomical changes of the loops. This question has been attacked already by Fahr (1934, 1935) and his pupils Hemprich (1935) and Weiss (1935–36), as well as by Tsuji (1936–37); but as their evidence is only morphologic, this question cannot be looked upon as settled. Finally, attempts may now be made to ascertain whether common clinical findings such as edema, albuminuria and hematuria can be correlated with histological changes.

Method

The following method (Ehrich, 1937) was used to produce glomerular nephritis: Peking ducks were immunized with 16 to 20 doses of 5 to 15 cc. of an emulsion which was prepared by suspending the blood free mash of the kidneys of a rabbit in 60 cc. of normal saline solution. 4 to 8 days after the last injection the ducks were decapitated, the blood collected and the serum separated and inactivated by heating to 56°C. for 30 minutes; 0.5 to 7.0 cc. were then injected intravenously into rabbits either once or repeatedly at intervals of 2 days. Most rabbits were Chinchillas weighing 1500 to 2000 gm.

Though there was a considerable variation in the response of different animals, it can be seen from Table I that the effect of the sera depended partly on the dosage with which the ducks were immunized. Serum 9, for example, was so toxic that a single dose of 1 cc. resulted in the death of one rabbit, whereas 7 cc. of sera 1 and 5 failed to kill. With repeated doses larger amounts could be given; but then a number of animals died in what appeared to be acute anaphylactic shock. This has been observed as early as 7 days after the first injection, in spite of the fact that the rabbits were injected every 2nd day.

The cause of the toxicity has not been detected. As the animals developed an acute anemia, the possibility cannot be excluded that the toxicity was due to substances other than the nephrotoxin. However, it might just as well be a matter of the strength of the serum, for Smadel (1936, 1937) found in rats a severe anaphylactoid reaction either as a result of giving a comparatively large amount of a relatively pure nephrotoxic serum or a smaller amount of serum rich in non-organ-specific anti-rat-tissue antibodies as well as in the more specific nephrotoxin.

The function of the kidneys was tested by water and dye tests, described in the preceding paper. Cyanol was used as a measure of filtration, azofuchsin I as a measure of secretion. 100 cc. of water were given by stomach tube, and the urine collected every 15 or 30 minutes. Usually, 1 hour after the ingestion of water, 2 cc. of a 0.1 per cent solution of cyanol or azofuchsin were injected intravenously, and thereafter the urine collected every 10 minutes. In addition, we determined the reaction of the urine, the presence of protein and in some animals the blood urea nitrogen.

In the microscopic sections special attention was paid to the size of the glomeruli

TABLE I

The Effect of Different Sera on Different Rabbits

Serum No.	Doses of kidney juice injected into ducks		Doses of serum injected into rabbits
	cc.	*times*	
1	5	16	3.5/1, 7.0/1
2	5	20	3.5/1, (7.0/1)
3	10	16	3.5/1, (7.0/1)
4	10	18	5.0/1
5	10	20	3.5/1, 7.0/1
6	15	15	2.5/1, 3.5/1, 4.0/1, 7.5/3, (7.5/3), (7.8/3)*
7	15	18	(5.0/1)
8	15	18	5.0/1
9	15	20	(1.0/1), (2.0/1), (3.0/1), (3.5/4)*, (3.5/4)*, (3.5/4)*, 4.0/5, (4.0/5), 6.5/6
10	Mixture of sera 4, 7 and 8		2.5/1, 4.0/1, (4.0/1), 5.0/1, 5.0/1, 5.0/1, (5.0/1), (6.0/1), 7.0/2, 9.0/3, 11.0/3 (3.0/1), (4.0/1), 5.0/1, 5.0/1, 5.0/1, (5.0/1), 7.0/2, 9.0/3, 9.0/3

( ) = died within 48 hours after the last injection.

Figure at left of the oblique line = total cc. of serum injected; figure to right of the line = number of injections given.

* Died in acute anaphylactic shock.

TABLE II

The Excretion of Water, Cyanol and Azofuchsin I during Different Periods of the Disease

Period	Animal No.	Serum injected No.	Serum injected cc.	Duration of disease days	Water given the day before	Amount excreted in 24 hrs. cc.	Cyanol excreted 20 min.	Cyanol 30 min.	Cyanol 60 min.	Cyanol 90 min.	Cyanol urine 20 min.	Cyanol urine 30 min.	Cyanol urine 60 min.	Cyanol urine 90 min.	Azofuchsin excreted 20 min.	Azofuchsin 30 min.	Azofuchsin 60 min.	Azofuchsin 90 min.	Azofuchsin urine 20 min.	Azofuchsin urine 30 min.	Azofuchsin urine 60 min.	Azofuchsin urine 90 min.
A	1-01	10	5	1	0	2.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
A	1-02	10	5	1	0	3.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
B	1-07	10	5	3	0	81.5	—	—	—	—	—	—	—	—	0.984	1.240	1.440	1.537	14.4	20.8	24.0	39.4
B	1-04	10	5	3	0	74.3	—	—	—	—	—	—	—	—	0	0	1.000	1.600	0.1	0.9	3.3	19.1
B	1-12	10	9	6	0	20.3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
B	7-0	8	5	7	0	75.8	0.292	0.380	0.498	0.563	18.0	26.8	40.0	50.4	—	—	—	—	—	—	—	—
B	1-11	4	5	7	0	118.2	—	—	—	—	—	—	—	—	0.480	0.819	1.308	1.502	15.7	27.0	51.0	76.2
C	1-12	10	9	7	+	6.4	0	0	0.180	0.225	1.2	1.7	4.1	4.9	—	—	—	—	—	—	—	—
C	1-21	10	7	8	0	7.8	0	0	0.111	0.329	1.2	1.7	4.1	8.0	—	—	—	—	—	—	—	—
C	1-21	10	7	9	+	4.2	—	—	—	—	—	—	—	—	0	0.016	0.216	1.085	1.4	1.6	2.2	3.5
C	1-20	10	7	9	0	16.2	0	0.020	0.244	0.289	1.4	2.4	7.1	12.6	—	—	—	—	—	—	—	—
C	1-12	10	9	10	+	39.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
C	1-20	10	7	10	0	37.7	—	—	—	—	—	—	—	—	0.950	1.070	1.230	1.348	11.1	14.1	20.1	31.5
C	9-0	10	11	11	0	30.6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
C	1-00	10	9	11	+	15.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
C	1-12	10	9	11	+	19.8	0.108	0.133	0.244	0.326	4.4	4.9	7.0	9.4	—	—	—	—	—	—	—	—
C	1-20	10	7	11	+	33.3	0.175	0.243	0.316	0.340	4.2	7.6	17.6	29.7	—	—	—	—	—	—	—	—
C	1-99	9	4	11	+	47.1	—	—	—	—	—	—	—	—	0.485	0.941	1.332	1.470	3.7	7.5	17.3	40.3

D	1-88	6	4	12	+	62.0	—	—	—	—	—	—	—	—	0.492	0.992	1.517	1.657	4.9	9.9	32.7	48.7
	1-99	9	4	12	+	62.2	0.125	0.214	0.384	0.477	6.9	10.6	23.1	38.6	—	—	—	—	—	—	—	—
	1-88	6	4	13	+	66.6	0.179	0.226	0.442	0.513	14.6	17.2	28.8	50.8	—	—	—	—	—	—	—	—
	1-74	6	7.5	14	+	77.8	0.184	0.226	0.282	0.311	16.6	23.6	36.4	47.0	—	—	—	—	—	—	—	—
	1-00	10	9	15	0	93.8	0	0.051	0.230	0.300	1.4	8.2	36.4	62.6	—	—	—	—	—	—	—	—
	1-86	6	5.5	20	+	47.3	0.032	0.084	0.299	0.396	2.6	5.2	22.2	36.1	—	—	—	—	—	—	—	—
	2-02	9	6.5	20	+	70.2	—	—	—	—	—	—	—	—	0.723	1.083	1.425	1.582	9.6	16.8	33.0	50.5
	1-86	6	5.5	21	+	56.8	—	—	—	—	—	—	—	—	0.758	1.108	1.518	1.661	8.2	13.2	27.6	44.6
	2-02	9	6.5	21	+	72.8	0.172	0.218	0.320	0.370	15.3	19.1	32.5	49.0	—	—	—	—	—	—	—	—
E	7-1	10	5	23	0	1.5	0	0	0	0.045	0.4	0.4	0.9	2.0	—	—	—	—	—	—	—	—
	1-00	10	9	23	0	6.8	—	—	—	—	—	—	—	—	0	0.036	0.436	0.709	0	1.2	4.3	9.9
	7-1	10	5	24	+	23.1	—	—	—	—	—	—	—	—	0	0.660	1.577	1.758	0.5	2.7	14.4	33.4
	1-00	10	9	24	+	16.5	0.120	0.210	0.338	0.367	3.5	5.5	10.6	17.4	—	—	—	—	—	—	—	—
	7-1	10	5	25	+	5.1	0	0	0.050	0.325	0.4	1.0	2.2	5.4	—	—	—	—	—	—	—	—
	1-00	10	9	25	+	15.8	—	—	—	—	—	—	—	—	0	0.135	0.570	1.121	2.0	3.8	6.7	14.4
F	1-74	6	7.5	28	+	32.2	0.048	0.110	0.265	0.332	3.2	5.8	19.3	28.4	0.766	0.991	1.394	1.602	5.7	8.2	13.4	25.0
	1-74	6	7.5	29	+	32.5	—	—	—	—	—	—	—	—	—	—	—	—	3.3	5.9	15.9	27.5
	1-02	10	5	31	0	50.5	—	—	—	—	—	—	—	—	0.600	0.990	1.384	1.525	—	—	—	—
	1-04	10	5	31	0	41.7	0.076	0.195	0.342	0.397	2.1	3.5	10.7	24.2	—	—	—	—	—	—	—	—
	1-02	10	5	32	+	12.7	0.126	0.252	0.312	0.355	3.1	5.9	8.0	15.8	—	—	—	—	—	—	—	—
	1-04	10	5	32	+	64.4	—	—	—	—	—	—	—	—	0.954	1.161	1.501	1.819	14.6	19.2	23.8	42.3
	1-74	6	7.5	43	+	99.9	—	—	—	—	—	—	—	—	0.990	1.140	1.486	1.635	17.9	22.9	42.9	71.4
	1-74	6	7.5	44	+	99.9	0.204	0.270	0.391	0.428	13.7	22.8	52.8	68.8	—	—	—	—	—	—	—	—
Normal average					+	59.7	0.218	0.267	0.410	0.502	—	—	—	—	0.889	1.089	1.393	1.504	—	—	—	—

and to the number of their nuclei. Both were determined by the method of Ehrich and Sommer (1933) by measuring the two largest diameters, which were perpendicular to each other, of 10 to 20 tufts the afferent or efferent vessels of which were met in the slides, and by counting the number of nuclei in the same tufts. Because the size of the central glomeruli is slightly larger than that of the peripheral ones (Peter, 1909), we proceeded as in the case of differential counts of leucocytes by moving the slides from capsule to medulla and *vice versa*. It should be noted that all glomeruli with fibrin or fibrosis were omitted in these counts.

RESULTS

1. The *excretion of water* has been tested in 41 experiments on 17 rabbits, from 1 to 44 days after induction of the disease. During the first week of the experiment, with the exception of the first day,[1] the diuresis was essentially normal, but thereafter it was markedly depressed (Table II). After the 11th to 25th day the diuresis was again found to be normal. It is true that during any one period the individual figures were quite as variable as in normal animals (Ehrich, Bartol and Wolf, 1938). However, if we compare different periods, we find that the differences are not caused by the normal variations alone, but by a shift in the whole range of variations. It should be noted that it made no difference whether or not the animals had been given water the day before the experiment, but that all animals behaved as if they were "wet" animals. The explanation for this may be found in the fact that they already had edema or were in a state of *Oedembereitschaft* (*i.e.*, ready to develop edema) (see page 777).

2. The *excretion of cyanol* has been studied in 19 experiments, in 13 rabbits, from 7 to 44 days after the injection of serum. With the exception of the first and of the last experiment, practically all figures are considerably lower than normal (Table II). Since the amount of cyanol excreted during a certain period depends in part on the urine volume (previous article), the two have been compared by plotting the concentration of the dye against the cc. of urine excreted in the corresponding periods (Text-fig. 1). It is seen that almost all the figures are either in the lowest region of the normal range or below it; or, in other words, that the diminution in cyanol excretion is greater than could be explained by the diminution in urine formation alone.

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[1] Three additional rabbits tested on the 1st or 2nd day excreted no urine whatsoever during the experiment.

3. The *excretion of azofuchsin I* has been studied in 16 experiments, in 14 rabbits, from the 3rd to the 43rd day of the disease. It has been found that in almost all instances it was normal at all times. There was no change in the amount excreted (Table II), nor in the concentration as compared with the urine volume (Text-fig. 2). There was but one exception, namely rabbit 1-00, which showed a decreased azofuchsin excretion on the two occasions at which this was measured. It will be seen later that this animal was the only one which showed a marked fatty degeneration of the tubules (see page 782).
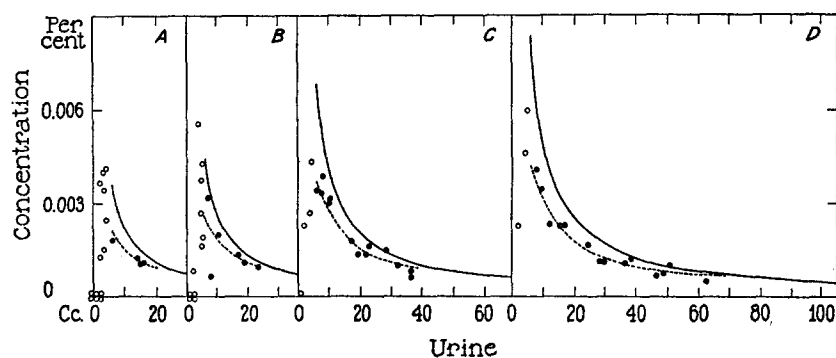
TEXT-FIG. 1. The excretion of cyanol from the 7th to the 32nd day of the disease. The concentration percentages of the dye during the first 20 (*a*), 30 (*b*), 60 (*c*) and 90 (*d*) minutes of the dye experiments as compared with the cc. of urine produced during these periods. ———— hyperbola calculated from the average amount of dye excreted normally; -------- lowest limit of normal range.

4. The *presence of protein* in the urine, as determined by boiling, was studied in 46 experiments in 21 rabbits. Whereas in the first week after the injection of the serum there was but a trace of protein in most experiments, in the 2nd week there was a marked proteinuria in most instances (Table III). After the 3rd week there was either no protein or but a trace, with the exception of rabbit 1-00 and perhaps rabbit 7-1, which are the same rabbits which showed a decrease in cyanol excretion even at this time. If we divide the experiments into periods, as in Table II, and if we compare the two tables, we find that both proteinuria and water and cyanol retention started at the same time; namely, 1 week after the injection of the serum.

5. The *reaction of the urine*, as determined by litmus paper, was studied in 20 experiments, in 12 animals. Although with a very small
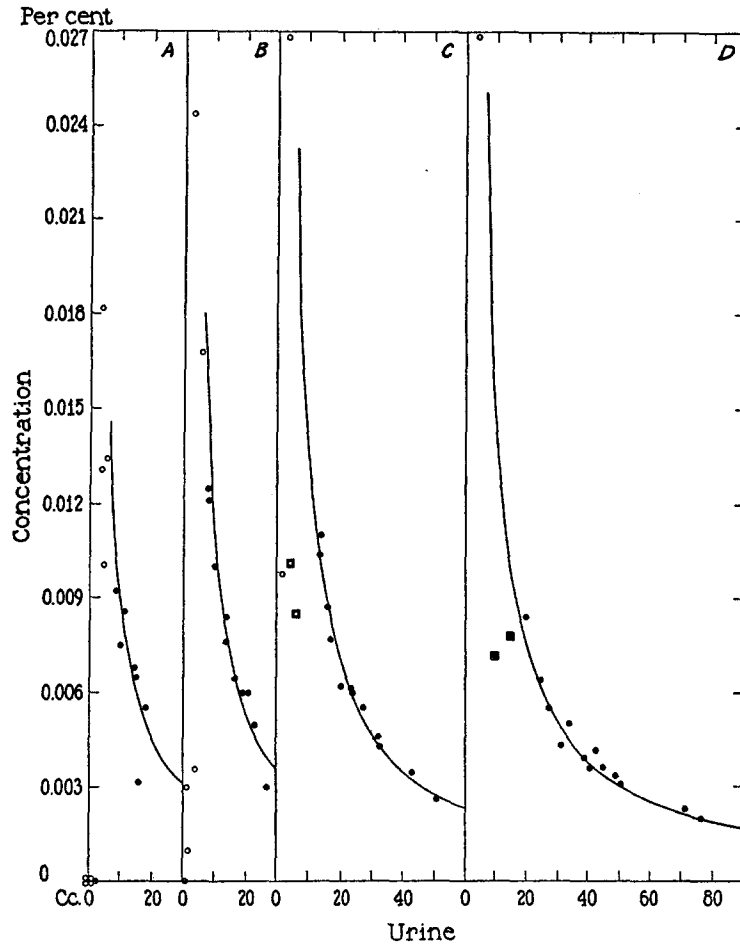
Per cent

Text-Fig. 2. The excretion of azofuchsin I in acute glomerular nephritis (all experiments). The concentration percentages of the dye during the first 20 (*a*), 30 (*b*), 60 (*c*) and 90 (*d*) minutes of the dye experiments as compared with the cc. of urine produced during those periods. ———— hyperbola calculated from the average amount of dye excreted normally; □ data from rabbit 1-00.

diuresis an acid reaction was found, and though with an acid reaction the diuresis tended to be smaller than with an alkaline reaction, this

was by no means constant. There was no distinct relation to the phases of the disease. However, in the cases which were protein-free the urine gave an alkaline reaction in most instances, whereas with proteinuria it invariably gave an acid reaction in all cases tested (Table III).

6. The *blood urea nitrogen* concentration in the blood was tested in 21 experiments, in 10 animals. In most instances the figures were well within the normal range (9 to 20 mg. per cent). However, of four tests made between the 6th and 10th day of the disease, two were slightly increased and two were as high as 60 and 105 mg. per cent.

7. As to the *gross changes* in the tissues of our animals, it should be noted that on the first day after the injection of the serum we frequently observed a marked edema of penis and scrotum; and on dissection, an increased amount of fluid in the abdominal and thoracic cavities. In the 2nd week and thereafter we frequently found ascites, hydrothorax and hydropericardium. The kidneys were not much changed during the first few days. However, in the animals killed on the 6th day and thereafter they were swollen, pale or yellowish brown in color, and showed fine blood dots on their surface. After the 4th week the blood dots were less conspicuous; and after the 6th week they were absent.

8. For the *histology* the kidneys of 30 rabbits were studied. The size of the glomeruli and the number of their nuclei increased during the first week to be more than doubled after 8 days (Figs. 1 and 2), and later they decreased again (Table IV). The number of granulocytes contained in the glomeruli varied a great deal; in the first 4 weeks of the disease, however, they were distinctly elevated in at least 70 per cent of the cases. Fibrin was present in the glomeruli (Fig. 2 *d*) from 8 to 12 days after the injection of the serum, in 7 of 8 animals; the number of glomeruli affected varied from 1 per cent to 53 per cent. Capsular adhesions, crescents or fibroses (Figs. 3 and 4) were observed from the 8th day onward; they were present in 15 of 23 animals, in 3 per cent to 92 per cent of the glomeruli. The amount of blood contained in the glomeruli varied greatly, partly because some animals died spontaneously, whereas the others were killed by a blow on the neck. But apart from this it can be seen that in the

TABLE III

The Excretion of Protein and the Reaction of the Urine in Different Periods after the Injection of the Serum

Period	Animal No.	Serum injected		Duration of disease	Protein in urine	Reaction of urine
		No.	*cc.*	*days*		
A	1-01	10	5	1	Trace	Acid
	1-02	10	5	1	"	"
	1-12	10	9	6	0	—
	9-4	3	3.5	6	+++	—
	7-0	8	5	7	Trace	Acid
	1-11	4	5	7	"	"
	9-3	1	7	7	+	—
B	1-21	10	7	8	+++	—
	1-20	10	7	8	+++	—
	1-20	10	7	9	+	—
	9-3	1	7	9	+	—
	9-4	3	3.5	9	+++	—
	1-12	10	9	10	+++	—
	9-0	10	11	11	+++	—
	1-00	10	9	11	+++	—
	1-12	10	9	11	++	—
	1-20	10	7	11	+++	—
C	1-99	9	4	11	0	Alkaline
	1-11	4	5	12	+++	—
	9-7	10	4	12	+	—
	7-0	8	5	12	++	—
	1-88	6	4	12	Trace	Acid
	1-99	9	4	12	0	"
	1-88	6	4	13	Trace	"
	1-00	10	9	15	++	"
	9-0	10	11	15	++	—
	1-86	6	5.5	20	0	Alkaline
	2-02	9	6.5	20	0	"
	1-86	6	5.5	21	+	Acid
	2-02	9	6.5	21	0	"
D	7-1	10	5	23	+	—
	1-00	10	9	23	++	—
	1-00	10	9	24	+++	—
	1-00	10	9	25	++	—

TABLE III—*Concluded*

Period	Animal No.	Serum injected		Duration of disease	Protein in urine	Reaction of urine
		No.	*cc.*	*days*		
E	1-74	6	7.5	28	+	Acid
	1-74	6	7.5	29	Trace	"
	1-02	10	5	31	0	—
	1-04	10	5	31	Trace	—
	1-74	6	7.5	43	0 '	Alkaline
	1-74	6	7.5	44	0	"
	3-3	5	7	47	+	—
	3-6	2	3.5	47	+	—
	9-4	3	3.5	61	0	—
	9-3	1	7	61	0	—
	9-4	3	3.5	63	+	—
	9-3	1	7	93	0	—

first week the glomeruli were hyperemic in most cases, that they then became anemic, and that after the 4th week they were well filled again in most instances (Table IV).

Tubular changes were chiefly evident in the lumina (Table IV). There were hyaline casts (Fig. 5) from the 2nd to the 7th week in 13 of 18 cases, and erythrocytes or blood casts in the tubules (or in the glomerular spaces or in both) (Fig. 6) in 12 of 18 cases. In the first week erythrocytes were seen in one instance only. After the 7th week they were absent with but one exception. As a result of the casts the tubules were frequently dilated (Fig. 7). Changes in the epithelial cells were observed in but 7 animals. In all these instances there were fatty changes (Fig. 8), and in two instances hyaline droplets as well (Fig. 9).

If we compare Table IV with Tables II and III, we find that the onset of oliguria, diminished cyanol excretion and marked proteinuria closely conforms to the period when proliferation and anemia within the tufts were at their peak. If we compare the tubular changes with those of the glomeruli, we see that the fatty changes closely parallel the deposition of fibrin or the adhesions, crescents or fibroses, whereas the hyaline casts or the erythrocytes or blood casts show but a partial correlation. However, if we compare the hyaline casts with the clinical findings, we find that they closely follow the proteinuria.

TABLE IV

Glomerular and Tubular Changes in Different Periods of the Disease

Animal No.	Weight		Serum injected		Duration of disease	Glomerular changes						Tubular changes			
	Beginning of experiment	End of experiment	No.	cc.		Size	No. of nuclei	No. of granulocytes	Glomeruli having		Content of blood*	Hyaline casts	Erythrocytes, blood casts	Fatty change	Hyaline droplets
									Fibrin	Adhesion crescents					
	gm.	gm.	No.	cc.	days	μ²			per cent	per cent					
1-01†	1840	1870	10	5	1	6940	92.5	10.2	0	0	+++	0	0	0	0
1-87	1800	1800	6	3.5	3	5502	89.3	0.8	0	0	++	0	0	0	0
1-15	1570	1100	10	9	6	6267	91.2	3.1	0	0	+	0	Medium	0	0
1-95†	1600	1620	9	3.5	7	7208	98.1	4.3	0	0	+++	0	0	—	0
1-96†	1880	1880	9	3.5	7	7337	111.5	3.7	0	0	++++	0	0	—	0
1-97†	1720	1610	9	3.5	7	6138	88.3	1.6	0	0	+++	0	0	—	0
1-19	2210	2160	10	7	8	13,478	180.5	0.9	53	30	+	Medium	Few	Slight	0
1-70†	1460	1520	6	8	8	9677	164.2	0.8	1	0	+	0	0	0	0
1-98†	1820	1740	9	3.5	9	7089	93.5	—	0	0	++	Few	Few	0	0
1-21	1910	1690	10	7	10	11,310	148.4	3.8	38	28	++	"	0	Slight	0
1-94†	1750	1660	9	4	11	6735	103.1	—	1	0	+	0	Medium	—	0
7-0	2180	2180	8	5	12	9161	129.3	3.1	14	35	+	Many	Medium	Moderate	Moderate
9-7	2200	2200	10	4	12	10,387	147.5	3.6	23	40	+	"	Few	Moderate	"
1-11	1940	2150	4	5	12	8866	141.3	2.6	7	20	+	Few	0	Slight	0
1-88†	1710	1560	6	4	19	7014	106.2	4.6	0	0	++	0	0	0	0
1-03†	1530	1360	10	5	21	6315	118.9	3.2	0	5	+++	—	—	—	—
1-86†	2000	2010	6	5.5	21	6113	120.9	2.5	0	0	+	0	Few	0	0
7-1	2610	2510	10	5	25	6648	108.4	2.9	0	5	+	Few	"	0	0

| | | | | | | | | | | | | Medium | " | Slight |
												"	Medium	Marked
9-0	3020	3020	10	11	25	10,751	147.0	3.8	0	61	+	Medium	"	0	0
1-00	2400	2040	10	9	25	8659	122.3	3.5	0	88	+	"	Medium	0	0
1-12	1760	1920	10	9	34	8091	107.4	0.8	0	0	++	0	Few	0	0
1-07	1820	2380	10	5	37	8659	130.1	1.4	0	3	++	Few	0	0	0
1-20	2040	2040	10	7	39	7284	105.4	0.3	0	0	++	"	Medium	0	0
3-3	2040	2160	5	7	47	8012	116.1	1.2	0	30	+	"	Few	0	0
3-6	1880	2080	2	3.5	47	9161	107.5	1.8	0	92	-	Many	"	0	0
1-02	1960	2330	10	5	53	7238	102.0	0.8	0	0	++	0	0	0	0
1-04	1720	2550	10	5	53	8659	123.8	2.3	0	20	++	0	0	0	0
9-4	1920	1920	3	3.5	63	8495	123.1	1.5	0	85	+	Medium	Few	0	0
9-3	2060	2480	1	7	93	6504	103.2	0.8	0	15	++	0	0	0	0
1-74†	1900	2080	6	7.5	149	6113	95.0	2.0	0	0	++	0	0	0	0
Controls	(4)‡	1505	—	—	—	5135	78	1.5	0	0	-	0	0	0	0
"	(40)	1815	—	—	—	5317	83	1.6	0	0	-	0	0	0	0
"	(11)	2135	—	—	—	6070	86	1.4	0	0	-	0	0	0	0

* + = 20 or less; ++ = 25 to 80; +++ = 90 or more erythrocytes per glomerular section.
† Died spontaneously.
‡ Number of animals studied.

Finally, it can be seen that the only rabbit which showed a decrease in the azofuchsin excretion (No. 1-00) is the only one which showed a marked fatty degeneration (see page 775).

<div align="center">DISCUSSION</div>

1. The Nature of the Disease.—In a preliminary report (1937) it was stated that the changes produced resembled not so much those of diffuse hemorrhagic glomerular nephritis as those of focal glomerular nephritis as observed in bacterial endocarditis. At that time we had studied a few rabbits only, Nos. 3-3, 3-6, 9-4 and 9-3 of the present series. It is evident from Table IV that in these cases distinctly focal changes, such as adhesions, crescents and fibroses, were the predominant lesions. From the same table it can be seen, however, that in other animals, in addition to the focal changes which may or may not be present, there are what appear to be diffuse lesions, namely enlargement of all or nearly all the glomeruli, increase in the number of their nuclei and decrease in their blood content. These lesions now appear to be invariably present; but at the time the former animals were studied they had mostly disappeared. The question whether in the milder cases these lesions were also focal, *i.e.*, whether there were glomeruli or loops which were not affected (non-embolic focal glomerular nephritis), or whether the differences in our results were mainly differences in degree, some kidneys being more affected than others, we were unable to decide.

If we compare our findings with those obtained in man, we find a close resemblance. If we consider only such early cases in which the diagnosis has been secured anatomically as well as clinically, we discover that in man there are also two types of changes: (*a*) enlargement of the glomeruli, increase in the number of their nuclei and decrease in their blood; and (*b*) deposition of fibrin and crescent formation. Also in man the glomerular loops are swollen and fused. The capillaries contain a "protoplasmic" substance with an increased number of nuclei therein. The tufts are anemic. This change has been observed as early as 3 days after the clinical onset of the disease (Fahr, 1926). It appears to be typical of glomerular nephritis and invariably present (Gross, 1919; Fahr, 1925, 1926; Koch, 1927; McGregor, 1929; Volhard, 1931; Ehrich and Sommer, 1933; Fish-

berg, 1934; Bell, 1936, 1937). A deposition of fibrin, however, has been observed in some cases only, and if it was present, it was observed only in some glomeruli or in some isolated loops. Thus Gross saw hyaline thrombi but rarely. Fahr (1926) found no fibrin in his case. McGregor found fibrin occasionally in a few glomerular loops. Bell (1937) observed thrombi or crescents in 26 of 51 cases, but it appears that at least 5 of these cases should not be classified as glomerular nephritis.

Turning to the functional changes, we wish to stress the close conformity of our findings with the earlier data obtained in rabbits and rats. In our rabbits oliguria was present beginning with the 7th day; Weiss (1935–36) and Tsuji (1936–37) observed it in the 2nd week and thereafter. In our rabbits, marked proteinuria was present from the 7th day onward; Masugi (1933–34) observed it after the 6th to 8th day, Hemprich (1935) after the 5th to 9th day, Weiss (1935–36) after the 7th to 12th day, and Tsuji (1936–37) after the 5th to 7th day. In our animals, blood was found in the tubules from the 6th to the 63rd day; Masugi (1933, 1934) found hematuria regularly; Tsuji (1936–37) observed it as early as in the first week, and in some animals a long time after the acute phase of the disease. It is true that in rats Smadel and Farr (1936, 1937) found no significant hematuria, if they injected a pure nephrotoxin; but in these cases the histological appearance was not typical, since according to their own description proliferation and infiltrative changes were practically absent. Nitrogen retention was found in the 2nd week in our rabbits. Masugi (1934) found it also in rabbits which recovered later. Since all these findings conform very closely, not only in their character, but also in their time of occurrence, we feel entitled to believe that our data are not casual findings, but represent a true cause and effect. Since it has further been demonstrated (Masugi, 1934; Smadel and Farr, 1937) that these animals may also show hypertension, fall in blood urea clearance, fall in the plasma proteins, lipemia and lipuria, it appears that, anatomically as well as functionally, our disease is the same as human diffuse hemorrhagic glomerular nephritis.

2. Pathogenesis.—Turning to the pathogenesis of the disease, it should be noted that there was, as partly observed by Masugi (1933),

Hemprich (1935), Weiss (1935–36) and Tsuji (1936–37), a period of latency of about 1 week before the clinical symptoms became manifest. It is true that there was in some cases edema and oliguria the day following the introduction of the serum; but the diuresis quickly returned to normal and in most cases tested during this period there was little or no protein in the urine. We believe, therefore, that this primary reaction was the result of a general disturbance, perhaps resulting in a greater capillary permeability and consequent edema and oliguria. That such a latency may also occur in man, is mentioned by Fahr (1925) and Volhard (1931). The latter also cites Kylin's observation that in cases of scarlatina a rise in capillary pressure occurred, even several days to 1 week before the nephritic symptoms became manifest.

It should be noted also that in 4 of our 5 animals tested between the 3rd and 7th days, the urine volume was conspicuously above the normal average. Furthermore, and this has also been observed by Fahr (1934), Hemprich (1935) and Tsuji (1936–37), in most of the animals which died or were killed during this period there was hyperemia of the glomeruli. On the other hand, the glomeruli were anemic only from the 6th day onward when the glomerular changes were already well developed. It must therefore be concluded that nephrotoxic glomerular nephritis in rabbits does not start with arteriolar spasm, but that the glomeruli become anemic only when the loops are clotted with protoplasmic material.

The first morphologic changes observed were, as has already been stated, an enlargement of the glomeruli and an increase in the number of their nuclei. This increase was fully developed on the 7th to 8th day (Table IV). Fahr (1935) states that he saw the endothelial proliferation as early as on the 4th to 5th day, and Tsuji (1936–37) on the 4th to 8th day, while Masugi (1933) claims that it can be found in rats after 24 hours. It appears, therefore, that proliferation starts soon after the injection of the serum and reaches a visible or measurable amount in the second half of the first week. Since, just as in man (Gross, 1919; McGregor, 1929) we found abundant mitoses during this period, it is evident that the increase of cells was due to a multiplication of local cells. The question whether these cells were endothelial cells, as maintained by most authors, or

whether they were elements outside the capillaries (MacCallum, 1934) or blood cells, as has been suggested, is one that we could not decide.

As to the cause of the multiplication of the cells, it is true that we found pyknotic nuclei in the glomeruli of a few rabbits during the first week, as did Tsuji (1936–37) in rabbits, and Gross (1919), Fahr (1925), Koch (1927) and McGregor (1929) in man. But as they were present in only a few instances, it appears unlikely that necrohormones were the sole cause of the proliferation. We believe rather that the leucocytes present, which incidentally have been observed by all students of acute glomerular nephritis, were the main source of the growth promoting substances.[2] This view seems to be supported by the frequent observation of disintegrating polymorphonuclear leucocytes within the tissue of the loops.

The second lesion, namely the deposition of fibrin, was in our series first observed 8 days after the injection of the serum. Hemprich (1935) observed it in rabbits on the 7th day, and Masugi (1933) in rats as early as the first day. As to the location of the fibrin, we were unable to convince ourselves that there were intracapillary thrombi. In our experience, the fibrin is located outside of the capillaries, either between the loops or between them and the capsule. It can be seen from Table IV that the fibrin disappears gradually within the 2nd week, and that adhesions, crescents and fibroses develop in its place. It is also evident that adhesions, crescents and fibroses develop only in those glomeruli in which fibrin had previously been deposited. It follows, therefore, that crescents are not an essential feature of glomerular nephritis, but that, as in focal embolic nephritis or malignant nephrosclerosis, they represent a complication. That this holds also in man is a common experience. It is for this reason that Fahr (1925) distinguishes between intra- and extracapillary nephritis. But if the formation of crescents is merely a complication, we can no longer look upon it as being pathognomonic of the subacute phase of the disease, as is commonly done. That this is an erroneous idea is obvious also from the fact that in man (Koch, 1927), as in rabbits, crescents are found as early as in the 2nd week of the disease.

[2] The increase in leucocytes has also been demonstrated clinically (Addis and Oliver, 1931; Murphy, Grill and Moxon, 1934, and others).

The cause of the deposition of fibrin has not been detected. It is evident from Tables I and IV that it is not due to the introduced serum or to the size of the dose alone. Rabbits 1-00 and 1-12, for example, both receiving the same dose of the same serum, reacted very differently in this respect, rabbit 1-00 showing that 88 per cent of the glomeruli contained fibrin, rabbit 1-12 none. In rats Masugi (1933) found fibrin only when he injected large doses, and Smadel (1936, 1937), also using rats, found it either as a result of giving a large amount of a relatively pure nephrotoxic serum or a smaller amount of serum rich in non-organ-specific anti-rat-tissue antibodies as well as in the more specific nephrotoxin. At least in rabbits, then, the kind or amount of serum is not the sole factor, but individual susceptibility appears to play an important rôle. Whether the deposition can be taken for an anaphylactoid reaction, as Smadel suggests, cannot as yet be decided. If this were true, we must conclude that such a reaction occurs also in man, since fibrin and crescents are also seen in human glomerular nephritis. However, it appears certain that this lesion amounts to much more than proliferation alone. The old idea of Loehlein, which is held also by Fishberg (1934), seems to be correct, namely that crescents are an indication not so much of the subacute stage, as of a stormy course of the disease.

Concerning the outcome of our disease, it appears that Masugi (1933) is right when he assumes that glomeruli that are simply enlarged and have an increased number of nuclei can return completely to normal. The question whether destruction of the glomeruli is a result of fibrin deposition only, or whether there is also a hyalinization of the loops following pure proliferation, cannot be answered from our material.

As to the tubular changes, it has been demonstrated (page 779) that the fatty changes follow the deposition of fibrin. Both can therefore be attributed to the same factor, as has been maintained by Fahr (1925). It should be noted that after the 4th week no more fat was seen (Table IV). Instead, we found granules in the epithelial cells the nature of which could not be detected.

3. Correlation of Morphology and Function.—The attempt to correlate certain functional changes with the lesions found in our kidneys produces some definite relationships. It has been demonstrated

already (page 779) that the onset of marked proteinuria and oliguria occurs at the time when the glomerular changes have reached full development. It has further been demonstrated (Table IV) that during the period of proteinuria and oliguria the output of the glomerular dye, cyanol, is markedly reduced, whereas the excretion of the tubular dye, azofuchsin, with one exception was not changed.

The proteinuria seems without doubt to be caused by the glomerular damage. The initial oliguria may be said to be due to extrarenal factors, both because edema was present and because at this time there were no glomerular or tubular changes which would explain a diminished diuresis. The oliguria in the 2nd week and thereafter, however, can hardly be explained by such an assumption, as there had been a period of good diuresis or polyuria in between. As the filtering membrane was very much thickened during the period of oliguria, and as there was a marked anemia of the glomeruli at this time, and as in rabbits diuresis is largely regulated by the glomeruli (Kaplan and Smith, 1935), it appears that Fahr (1925) was probably right when he concluded that oliguria in acute glomerular nephritis (other than the initial oliguria) is due rather to glomerular damage than to extrarenal factors.

As to the decrease in cyanol excretion, it has been demonstrated (page 774) that it cannot be explained by the oliguria alone. Nor did the dye escape into the edema fluid, as in no case was any dye seen therein. It is also unlikely that some dye diffused back from the lumina of the tubules into the blood or lymph stream through possibly damaged epithelial cells, as in this case it would be difficult to understand why the excretion of azofuchsin was not diminished. However, it might be possible that the proportion of the water which is excreted extrarenally was increased in these animals, and that some dye left the plasma with this water. It might also be that a larger amount of dye was adsorbed by the thickened filtering membrane. However, as water diuresis in rabbits appears to be both a glomerular and a tubular function, we favor the explanation that the oliguria was mainly caused by hindering the glomerular contribution to diuresis. If the tubules functioned more or less normally, it should be expected that the decrease in cyanol output was greater than the decrease in diuresis. Though we are unable to

prove at present that this explanation is correct, it is clear from our findings that the retention of cyanol parallels the oliguria and the morphological changes in the glomeruli. It appears, therefore, that, if the oliguria is mainly a result of glomerular damage, the retention of cyanol is due to the same cause.

In the only test performed during the first week of the experiment the diuresis and the dye excretion were both increased. As the amount of dye excreted corresponded to the degree of diuresis, it appears that filtration was actually increased during this time.

The azofuchsin excretion, as has been pointed out (page 775), with but one exception was undisturbed. In all these animals the tubular epithelial cells were little if any changed, and the only animal (rabbit 1-00) which showed a diminished azofuchsin output was also the only one which showed a marked fatty change. It therefore appears obvious that the fundamental question which has been mentioned in the introduction, namely whether glomeruli and tubules may function independently of each other, must be answered affirmatively.

SUMMARY AND CONCLUSIONS

It has been shown in this paper that structural and functional changes in acute glomerular nephritis in rabbits produced by nephrotoxins by the method of Masugi are the same as those found in human glomerular nephritis. The morbid anatomy is characterized by glomerular cell proliferation, and in some cases by deposition of fibrin and crescent formation of the glomeruli and by fatty changes of the tubules. The functional changes are: oliguria, proteinuria, hematuria, cylindruria, edema, rise in blood urea, and according to Masugi (1933, 1934) and Smadel (1936, 1937), rise in blood pressure, lipuria, and fall in urea clearance and plasma proteins. As we are unaware of any discrepancies between the experimentally induced disease and human nephritis, the conclusion follows that the two so closely resemble each other that they appear to be identical.

As to the pathogenesis, it has been shown that the disease begins with a period of latency. This is characterizied anatomically by hyperemia of the glomeruli; and functionally, in at least a number of cases, by an increased diuresis. It follows, therefore, that the theory of Volhard, according to which glomerular nephritis is caused by

arteriolar spasms, can no longer be maintained. It has further been demonstrated that proliferation of glomerular cells is the typical lesion, and that deposition of fibrin and crescent formation occur only in certain cases, and in these only in a widely varying number of glomeruli. As crescents are found as early as the proliferation itself, it follows that they should not be regarded as pathognomonic of the subacute phase, but that they represent a complication which probably aggravates the disease.

As to correlation of morphological and functional changes, it has been demonstrated that oliguria, marked proteinuria and diminished excretion of cyanol appear at the time when the glomerular changes are at their peak. Evidence has been presented that the oliguria and the decrease in cyanol excretion in acute glomerular nephritis are chiefly the result of the glomerular damage. It has further been demonstrated that the excretion of azofuchsin was unchanged, except for a diminution in the rabbit which at autopsy showed a marked fatty change of the tubules. We regard these observations as evidence, that, in acute glomerular nephritis in rabbits, glomeruli and tubules may function independently of each other.

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EXPLANATION OF PLATES

PLATE 32

FIG. 1. Rabbit 1-70. Diffuse glomerular nephritis of 8 days' duration. The glomeruli are much enlarged and rich in nuclei. Hematoxylin-eosin. × 110. (Compare with Fig. 2c, the magnification of which is more than double that of Fig. 1.)

FIG. 2. (a) Rabbit 1-19; nephritis of 8 days' duration; and (b) Rabbit 1-21; nephritis of 10 days' duration. Glomeruli showing increase in size and in the number of their nuclei. Hematoxylin-eosin. × 240. (c) Rabbit 1-92. Normal glomeruli. Hematoxylin-eosin. × 240. (d) Rabbit 1-21; nephritis of 10 days' duration. Glomeruli showing deposition of fibrin between loops and capsule. Azur II-eosin. × 240.
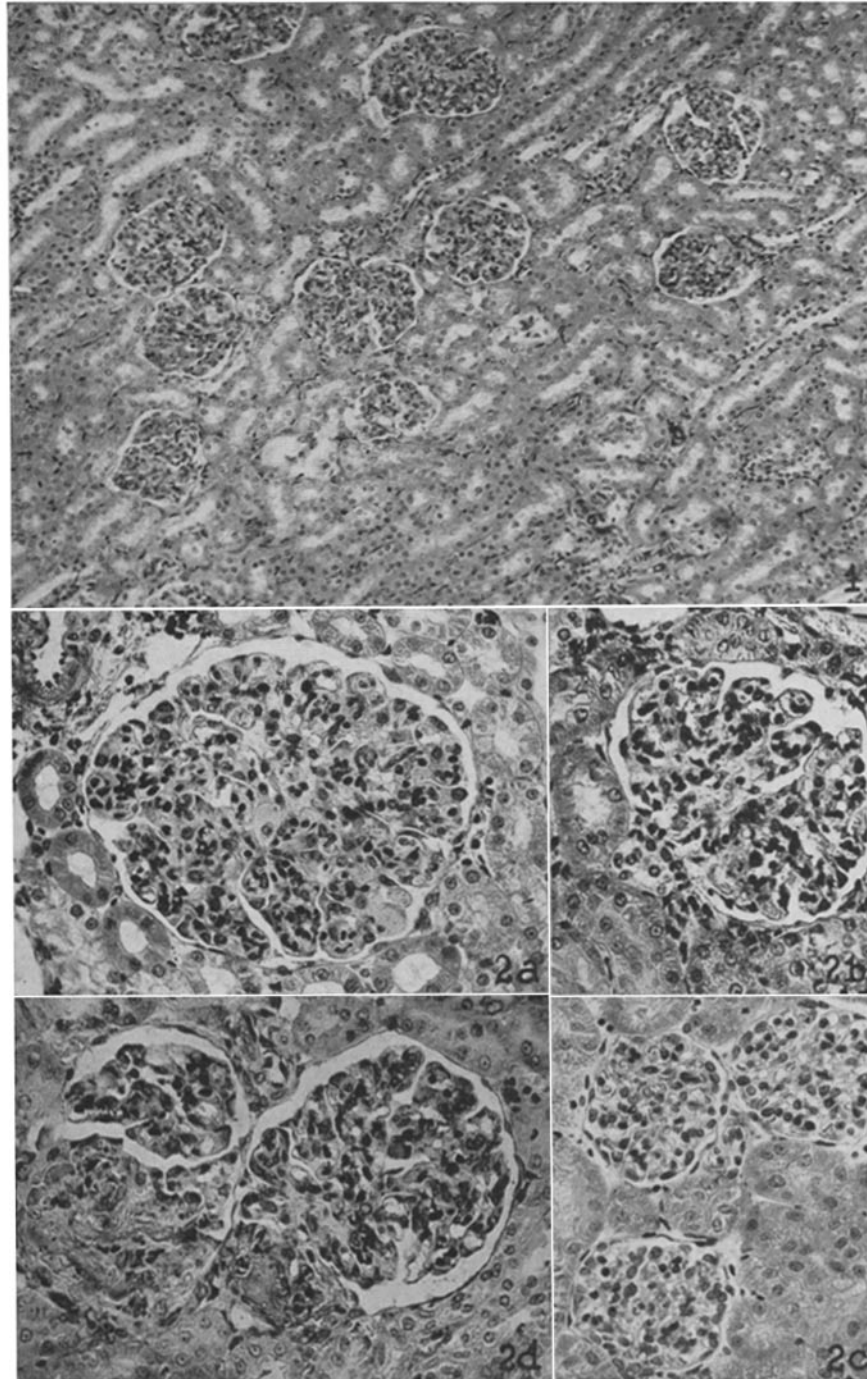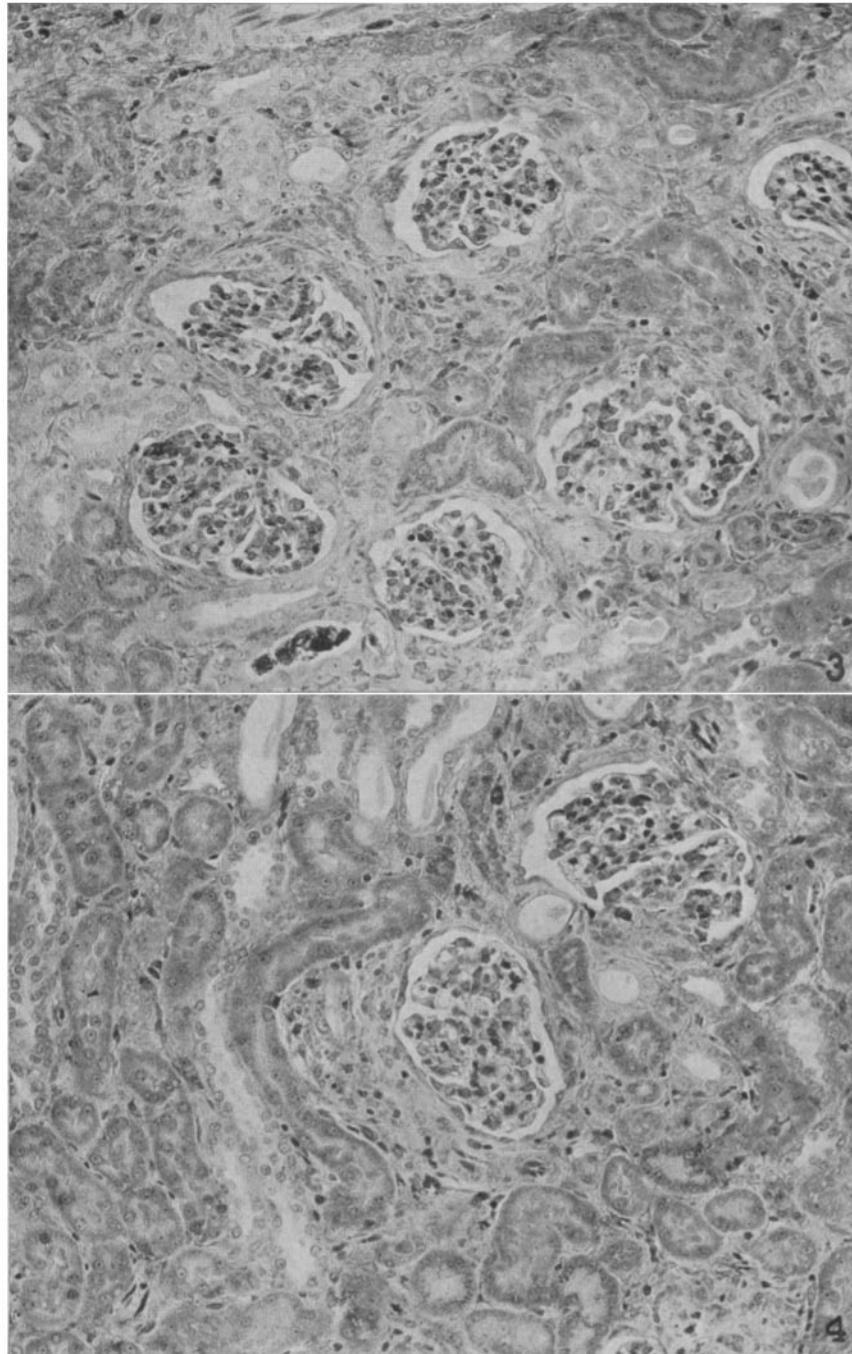
(Ehrich *et al.*: Acute glomerular nephritis)

PLATE 33

FIG. 3. Rabbit 1-00. Nephritis of 25 days' duration. Capsular adhesions and crescents. Azur II-eosin. × 240.

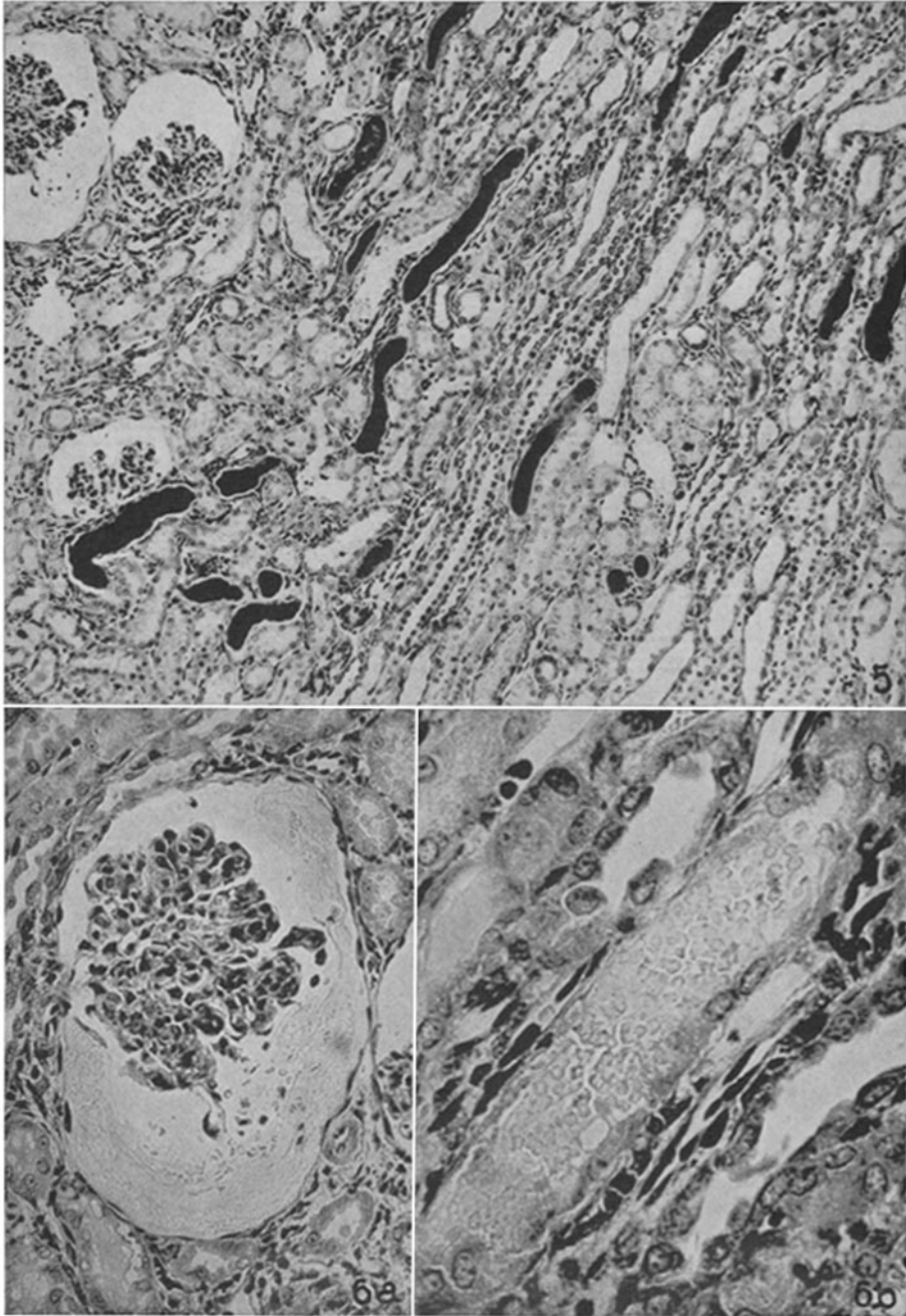FIG. 4. Rabbit 1-00. Nephritis of 25 days' duration. Glomerulus showing typical crescent. Azur II-eosin. × 240.

(Ehrich *et al.*: Acute glomerular nephritis)

FIG. 5. Rabbit 7-0. Nephritis of 12 days' duration. Hyaline casts with dilatation of capsular spaces. Azur II-eosin. × 110.

FIG. 6. Rabbit 7-0. Nephritis of 12 days' duration. (*a*) Erythrocytes and protein in capsular space. × 240. (*b*) Erythrocytes in tubule. × 480. Azur II-eosin.
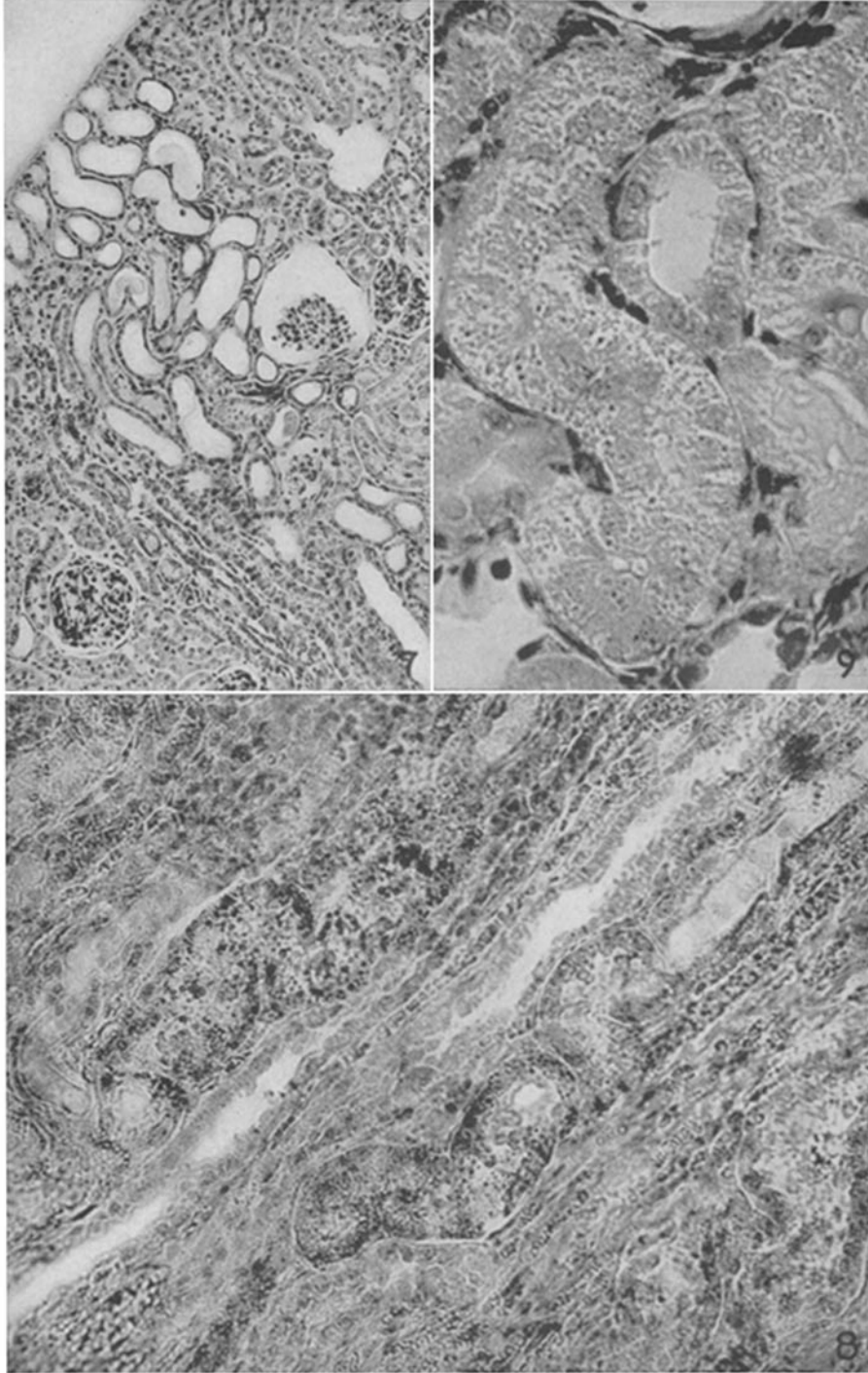
(Ehrich *et al* : Acute glomerular nephritis)

PLATE 35

FIG. 7. Rabbit 7-0. Nephritis of 12 days' duration. Dilatation of tubules and glomerular spaces. Note the proliferation in the glomeruli. Azur II-eosin. × 110.

FIG. 8. Rabbit 1-00. Nephritis of 25 days' duration. Marked fatty change of the distal portion of the proximal convoluted tubules. Hematoxylin-Sudan. × 240.

FIG. 9. Rabbit 9-7. Nephritis of 12 days' duration. Hyaline droplets in convoluted tubules. Azur II-eosin. × 480.

(Ehrich *et al.*: Acute glomerular nephritis)