EXPERIMENTAL NEPHRITIS IN RATS INDUCED BY INJECTION OF ANTI-KIDNEY SERUM

II. Clinical and Functional Studies*

By JOSEPH E. SMADEL, M.D., and LEE E. FARR, M.D.

(From the Hospital of The Rockefeller Institute for Medical Research)

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The glomerulonephritis induced by anti-kidney serum has been the subject of extensive study. Recent workers in the field have emphasized the close similarity of this experimental disease to human nephritis. Our purpose in this paper is: (*a*) to present clinical and functional data on a group of rats which developed nephritis after treatment with anti-rat-kidney serum, and (*b*) to point to functional evidence of the progressive nature of the induced disease. Pathological observations on the majority of the animals reported here are given elsewhere (1).

Anti-kidney serum was first used by Lindemann (2) in 1900 and was studied by Pearce (3) in 1904. The earlier workers were primarily interested in the immunological and pathological aspects of the problem. Rats have been previously employed in experiments with nephrotoxic serum only by Masugi (4, 5), who limited his clinical studies of renal damage to urinalysis.

Moderate elevation of the blood pressure some time after treatment with anti-kidney serum has been recorded in dogs by Lüdke and Schüller (6) and in rabbits by Masugi (7), Arnott, Kellar, and Matthew (8), and Koráni and Hámori (9). Pearce (3) observed no immediate effect on the blood pressure of dogs and rabbits injected with nephrotoxic sera. Takeda is quoted by Masugi (5) as having found in rabbits a distinct rise in the non-protein nitrogen of the blood. In three of six rabbits Masugi (7) observed a steady rise of the blood non-protein nitrogen from the time of injection with nephrotoxic serum until the animals died or were sacrificed about 4 weeks later. One animal (No. 6) showed an increase in the blood non-protein nitrogen of from 50 mg. per cent, before injection, to 224 mg.

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per cent several days prior to death. Kashiwabara's (10) data on the various non-protein nitrogen fractions of the blood and urine of rabbits, during a period of 2 weeks' observation after receiving anti-kidney serum, showed no significant change.

Methods and Materials

Anti-rat-kidney serum was prepared in rabbits by immunization with suspensions of perfused rat kidney. The preparation of nephrotoxic serum 4138, used throughout these experiments, has already been described (11). Young black and white hooded rats of both sexes from an inbred strain were used. The animals were given bread and dog biscuit daily, lettuce or cabbage and carrot twice weekly, and water *ad lib.* Vitamins were supplied in the form of wet baker's yeast and cod liver oil once a week.

Urea clearances were done by the procedure described by Farr and Smadel (12). Urine and blood for other studies were obtained by the methods used in the clearance determination. Blood urea nitrogen values were determined gasometrically by the hypobromite micro method of Farr (13). Quantitative urine albumin values were obtained by the method of Shevky and Stafford (14). The guaiac test for occult blood was done as routine on urine specimens and the urinary sediment was examined microscopically for formed elements. Plasma proteins were determined gasometrically by Van Slyke's micro Kjeldahl method (15). Plasma lipid values were obtained by the gasometric method of Kirk, Page, and Van Slyke (16). The gasometric micro procedure of Van Slyke and Sendroy was followed for blood calcium (17). Urinary chloride was determined by a modified Volhard-Harvey titration, as described by Peters and Van Slyke (18). The hemoglobin content of the blood was found by Sahli's method. Blood pressure was estimated with the apparatus of Moberg (19) but several changes in his technique were instituted. Pressures were determined on unanesthetized rats, for it was found that, after training, the animal remained quietly in the hands of an assistant throughout the procedure. The blood pressure was recorded at the point where blood again flowed through the previously compressed ear arteriole. This technique gave readings closely approximating the diastolic blood pressure. The pupil was dilated by homatropine for eyeground examination.

EXPERIMENTAL

A group of 18 young rats, weighing between 50 and 75 gm., was observed for a control period during which urea clearances, blood hemoglobin and plasma protein determinations, and urinary examinations were carried out. They were then divided into three subgroups as follows:

Group 1.—Ten rats. Eight of these (N-36 to N-43, inclusive) were treated with a total of 0.3 cc. per 100 gm. body weight of anti-kidney serum, given in divided amounts over a period of several weeks. Two other rats (N-78 and N-79) received a total of 0.45 cc. per 100 gm. body weight of serum in two injections on consecutive days.

Group 2.—Two rats (N-74 and N-75) were treated with a total of 0.65 cc. per 100 gm. body weight of anti-kidney serum, given in four divided amounts at 4 day intervals.

Group 3.—Six control rats. Of these, two (N-80 and N-81) were untreated; two (N-82 and N-83) received the serum of a rabbit immunized with rat serum; and the remaining two (N-76 and N-77) received anti-kidney serum completely absorbed by rat kidney or liver.

A fourth group of 29 rats had no chemical studies before treatment with nephrotoxin, and was subjected to less intense study after the induced disease was established. Rats N-21, N-22, N-24, N-25, and N-57 were of especial interest.

The examinations made during the control period were repeated at intervals after injection until the animals became moribund or were sacrificed. Plasma lipids and urinary chlorides were determined in a few instances. Blood pressure readings were obtained on a number of animals.

Urea Clearance.—The administration of anti-kidney serum in moderate amounts, that is, just sufficient to induce a marked albuminuria, cylindruria, and anasarca, usually had no immediate significant effect on the urea clearance, even though it became depressed at a later date. This is illustrated by rats N-37, N-39, and N-79 (Charts 1, 2, and 3) that received a series of small injections of anti-kidney serum, and by rat N-21 (Table I) that was given approximately the same total amount of nephrotoxin in a single injection.

The urea clearance fell rapidly and the animals died in apparent renal failure within a few weeks if a relatively large amount of nephrotoxin was given in several divided doses at short intervals (rats N-74, Chart 3, and N-75, Table III). The kidney damage in these animals was attributed to nephrotoxin alone, whereas another factor was present in rat N-78, Table III, which showed a similar rapid depression of the clearance. This rat had an anaphylactoid reaction following the first injection of serum and showed glomerular fibrin thrombi postmortem. In other studies (1, 11) severe anaphylactoid reactions were frequently associated with subsequent development of glomerular thrombi. Clearance studies were not done on these animals.

A progressive irreversible decline in the urea clearance resembling that in chronic active nephritis of man, was observed in certain animals that survived the acute experimental nephritis. Four rats (N-21, N-36, N-37, and N-39) became moribund, with apparent renal failure, from 84 to 313 days after treatment. The terminal clearance value on rat N-21 (Table I) was 3.6 cc. on the 84th day, while rats N-37

and N-39 (Charts 1 and 2) which died on the 313th and 240th days respectively, had final clearances of only 0.9 cc. per square meter per minute (normal 10.9 ± 3.1 cc. per square meter per minute (12)). Although a clearance value was not obtained terminally on rat N-36, its blood urea was 97 mg. per cent. Definitely progressive glomerular

TABLE I

Blood Urea Nitrogen, Urea Clearance, and Plasma Protein Values of Two Rats with Chronic Progressive Nephritis

Rat	Date	Blood urea N	Urea clearance	Plasma protein
	1935	*mg. per 100 cc.*	*cc. per sq.m. per min.*	*gm. per 100 cc.*
N-21	Feb. 20	6.7	33.8*	
Injected with:	Mar. 9	12.4	20.7	4.53
Anti-kidney serum	Apr. 10	16.6	11.2	
0.25 cc. Feb. 2	" 26	55.7	3.6	3.18
Total = 0.25 cc. per 100 gm.	" 27	Died		
N-36	Mar. 6	9.1	11.9	
Injected with:	" 12	9.0	17.2	
Anti-kidney serum	" 17	10.3	20.3	8.43
0.10 cc. Mar. 5 and 27	" 29	21.2	10.8	
0.025 cc. Apr. 23	Apr. 10	9.8	25.9	
0.075 cc. Apr. 24	" 19	9.3	15.6	
Total = 0.30 cc. per 100 gm.	" 29	12.3	13.9	
	May 12	40.9	6.0	
	" 19	10.1	16.0	
	" 30	25.7	6.8	5.46
	June 6	18.3	8.0	
	July 21	97.0		

Anti-rat-kidney serum, used throughout, was administered intravenously. The dosages are in cc. per 100 gm. of rat body weight.

* This clearance was done while the technique of determining the urea clearances was being developed. These high values became rarer with experience. For possible explanation of these variations, see Reference 12.

involvement was observed on histological examination of the kidneys of these four animals, and is described elsewhere (1). Seven other rats, N-38, N-41, N-43 (Table I), N-22, N-24, N-57 (Table II), and N-79 (Chart 3), that survived a severe acute nephritis, were followed for an average of 220 days, and were sacrificed from 171 to 225 days

after injection. In all of this group the urea clearances fell within the normal range when the observations were discontinued, although six animals still showed marked albuminuria and cylindruria.

TABLE II

Blood Urea Nitrogen, Urea Clearance, and Plasma Protein Values of Two Rats with Chronic Nephritis

Rat	Date	Blood urea N	Urea clearance	Plasma protein
	1935	*mg. per 100 cc.*	*cc. per sq.m. per min.*	*gm. per 100 cc.*
N-43	Feb. 27	10.5	15.5	5.89
Injected with:	Mar. 16	6.2	46.0	
Anti-kidney serum	" 12	10.5	12.0	
0.19 cc. Mar. 15	" 17	8.4	22.9	
0.035 cc. Mar. 27	" 30	16.6	13.4	5.73
0.05 cc. Mar. 28	Apr. 10	20.0	14.3	
Total = 0.275 cc. per 100 gm.	" 19	15.9	12.9	
	" 26	11.8	17.7	
	May 12	35.2	8.1	
	" 19	39.1	6.0	
	" 30	23.4	9.6	6.51
	June 6	16.9	10.1	
	Aug. 5	18.0	11.4	6.82
	" 20	16.3	9.6	
	Sept. 5	30.5	6.4	
	" 18	12.0	13.3	6.39
	Oct. 11	20.6	11.4	
	Nov. 14	17.1	10.8	
N-57	June 7	11.4	14.2	
Injected with:	Aug. 6	14.2	8.2	5.74
Anti-kidney serum	" 20	14.6	7.5	
0.52 cc. Apr. 20	Oct. 11	13.3	10.3	
Total = 0.52 cc. per 100 gm.				

These animals failed to show a depression of clearance although albuminuria and cylindruria continued throughout the course of the disease.

The control rats in group 3 maintained normal clearance values throughout the period of observation, from 6 to 14 months (rat 80, Chart 4).

Blood Urea Nitrogen.—The animals in group 1, that received 0.3 cc. of anti-kidney serum, developed clinical signs of nephritis but did

not show significantly elevated blood urea nitrogen during the acute phase. On the other hand, the two rats in group 2 that received larger amounts of anti-kidney serum and died during the acute phase showed a rapid rise in the blood urea nitrogen; in rat N-74 (Chart 3) it reached 144 mg. per cent terminally. The blood urea nitrogen values rose as the urea clearance fell in the animals with chronic progressive lesions. The highest blood urea nitrogen value recorded in these studies was 312 mg. per cent, obtained terminally on rat N-37 (Chart 1). An unexpected lability of the blood urea nitrogen was encountered in certain rats which showed marked transient elevations of urea. These elevations were apparently due, not to acute renal crises but to such extrarenal factors as diarrhea (12), prostration, or circulatory insufficiency. The wide fluctuations of blood urea clearance during the last few months of life in rat N-37 (Chart 1) may have been due to vascular accidents with transient circulatory disturbance, since microscopic examination revealed degenerative lesions of different ages in the heart and other viscera (1).

Proteinuria.—Proteinuria became manifest within a few hours after the injection of anti-kidney serum, and for a time usually maintained a level of about 40 mg. per cc. Some animals, *i.e.*, rat N-79 in Chart 3, showed as much as 60 mg. of protein per cc. During the early phase of the acute nephritis a marked oliguria was present. This oliguria usually disappeared after several days; the protein in the urine remained, but its concentration fell to lower levels of 20 to 30 mg. per cc. Rats with chronic nephritis, both progressive and latent, as judged by the urea clearance and histological studies, generally excreted during 12 hours about 1.5 to 2.0 cc. of urine when water alone was given. The protein content of this urine was about 20 mg. per cc. Rat N-79 (Chart 3) was an exception to this generalization, as its urine became normal 6 weeks after injection. Rat N-37 (Chart 1), with slowly progressing nephritis, had a definite polyuria during the last months of life, averaging 6 to 7 cc. of urine for 12 hours. The protein concentration in this urine was less (5 to 10 mg. per cc.) during the terminal phase, but the total protein loss per day was approximately the same as earlier in the course.

Urinary Sediment.—Casts appeared in the urine about the 3rd day, usually in very large numbers. Hyalin casts were generally observed

first and were followed within a few days by granular and cellular casts. Cylindruria later became less intense, but persisted until death, or, in those animals which apparently recovered, until the proteinuria ceased. In only one rat, N-78, was there sufficient increase in erythrocytes in the urine to give a positive guaiac test; for the serum was administered in a manner to avoid, as far as possible, glomerular thrombosis and hematuria (1, 11). Doubly refractile globules were searched for on several occasions but were not encountered.

Edema.—Subcutaneous edema and ascites appeared about the 5th day after the injection of an adequate dose of nephrotoxic serum. The anasarca rapidly reached a maximum and remained severe for 4 or 5 days, although in certain instances it persisted for as long as 3 weeks. The majority of the animals then developed severe diarrhea, which was followed by complete elimination of edema fluid. The remaining animals with anasarca lost their edema after a transient period of diuresis. Lipemia usually appeared when the edema was marked. Two edematous rats had total blood lipid carbon values of 2589 mg. per cent and 964 mg. per cent, respectively, whereas the corresponding determinations in two normal animals were only 556 mg. per cent and 562 mg. per cent. Ascitic fluid from the first animal contained 63.5 mg. per cent of lipid carbon. There was no apparent failure to excrete chloride during this phase, since two rats with edema eliminated NaCl at the rate of 7.5 gm. per liter of urine, while a normal rat on the same diet excreted 9.25 gm. per liter.

Anemia.—Rats with acute nephrotoxic nephritis maintained a normal blood hemoglobin value of 70 to 80 per cent (Sahli). Only one animal with chronic nephritis, N-37 (Chart 1), developed anemia, and this did not appear until 8 months after injection. The hemoglobin rapidly fell throughout the last 6 weeks of life to a terminal value of 20 per cent. No other rat with chronic progressive lesions survived as long as this one; hence it is impossible to state whether anemia would have developed frequently in prolonged cases.

Hypertension.—Elevated blood pressure occurred only in the rats which developed chronic progressive nephritis. Early in the work several methods for determining the blood pressure were used without success, and only later was Moberg's technique employed. With it rats N-37 and N-39 (Charts 1 and 2) were shown to develop hyper-

tension in the 8th month of the disease. The recordings in these two rats steadily rose from a normal range of 50 to 60 mm. of Hg to slightly above 100 mm. where they remained until just prior to death, when a moderate drop was noted. Other animals of the same age, both controls and rats with latent nephritis, failed to show a rise in blood pressure. Hypertension was not observed during the initial phase of this induced disease (rat N-74, Chart 3).

TABLE III

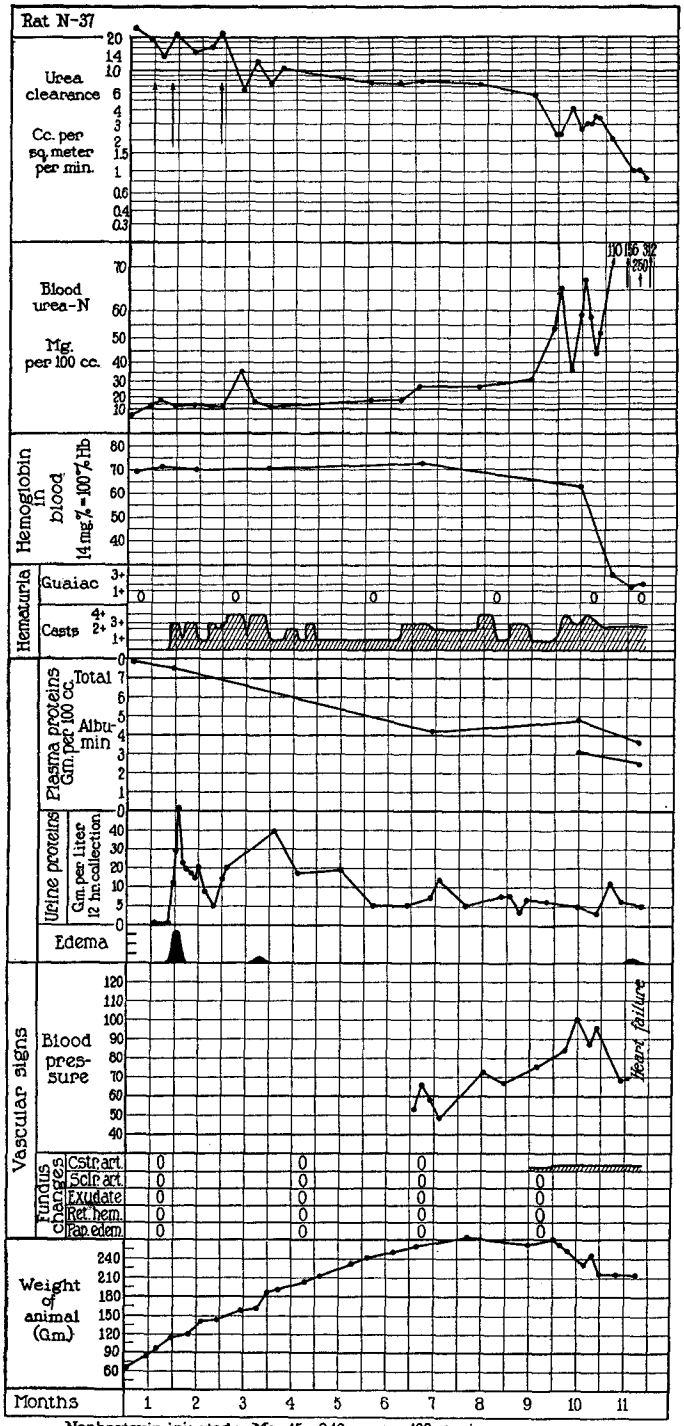
Blood Urea Nitrogen, Urea Clearance, and Plasma Protein Values of Two Rats Dying with Acute Nephritis

Rat	Date	Blood urea N	Urea clearance	Plasma protein
	1935	*mg. per 100 cc.*	*cc. per sq.m. per min.*	*gm. per 100 cc.*
N-75	May 12	8.6	38.5	
Injected with:	" 17	16.9	19.4	
Anti-kidney serum	" 22	19.3	15.0	
0.30 cc. May 8	" 26	47.7	5.8	
0.15 cc. May 23 and 27	" 30	36.2	6.7	
Total = 0.60 cc. per 100 gm.	June 3	68.3	2.7	
	" 4	165.3		6.81
N-78	May 17	12.4	23.0	
Injected with:	" 22	28.0	9.1	
Anti-kidney serum	" 25	24.6	8.9	
0.30 cc. June 10	June 3	17.6	13.3	
0.15 cc. June 12	" 17	50.5	5.3	4.63
Total = 0.45 cc. per 100 gm.	" 23	142.6	0.9	

N-75 illustrates a typical severe acute nephrotoxic response. The chemical and functional data on N-78 are similar; this animal, however, had in addition an anaphylactoid type of reaction with hematuria and thrombosis of glomerular capillaries.

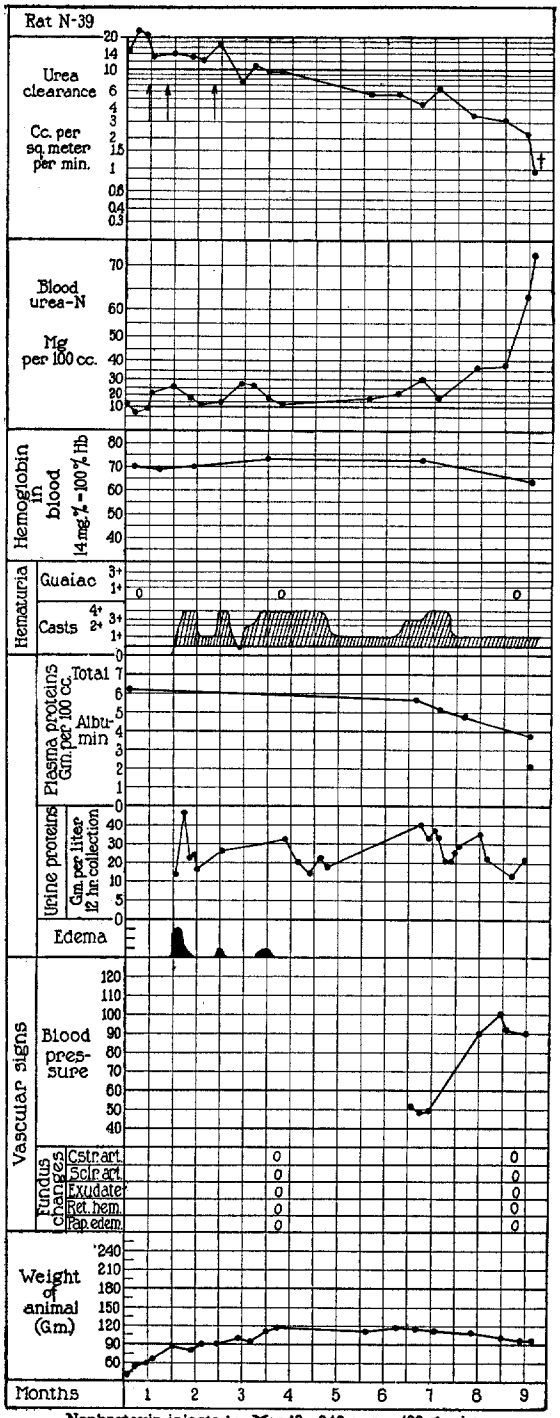
Retinopathy.—Neither hemorrhage, exudate, nor papilledema was seen in any of the animals. Some constriction of the retinal arteries seemed to be present in rat N-37 throughout the last month of life. The marked anemia at that time, however, made the accurate visualization of the retinal bed difficult.

Plasma Proteins.—Although no systematic study of the plasma proteins was carried out, sufficient observations were made to indicate the general trend. Early in the acute phase of the induced disease, when the animals were losing large quantities of protein in the urine,



Nephrotoxin injected: Mar. 15 0.10 cc. per 100 gm., i.v.
" 27 0.10 " " " " "
Apr. 23 0.025 " " " " "
" 24 0.075 " " " " "
Total 0.30 " " " " "

CHART 1



Nephrotoxin injected: Mar. 18 0.10 cc. per 100 gm., i.v.
" 27 0.07 " " " " "
" 28 0.08 " " " " "
Apr. 23 0.025 " " " " "
" 24 0.05 " " " " "
Total 0.325 " " " " "

CHART 2

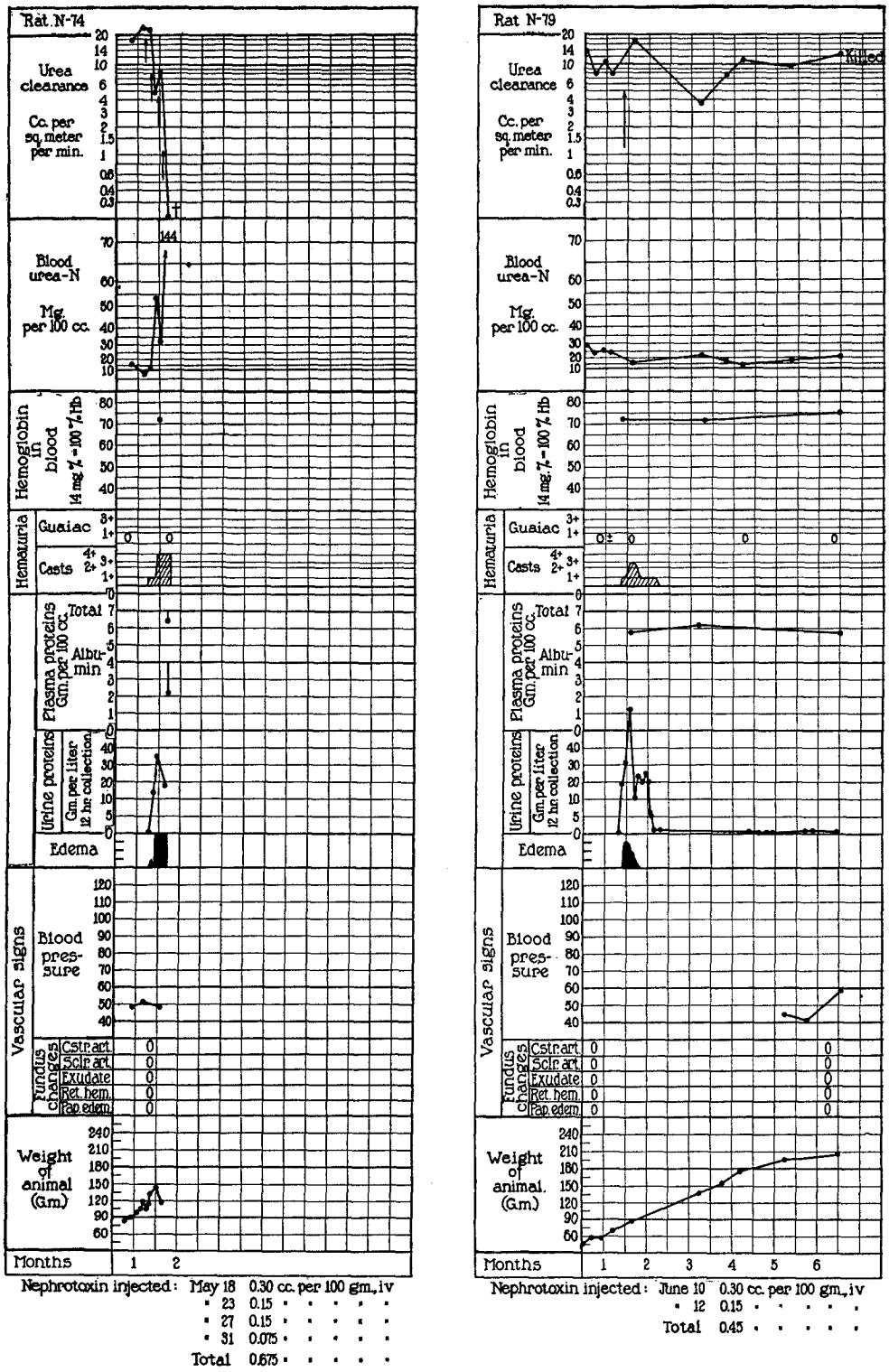

Rat N-74
Urea clearance
Cc. per sq. meter per min.
Blood urea-N
Mg. per 100 cc.
144
Hemoglobin in blood
14 mg. % = 100 % Hb
Hematuria
Guaiac
Casts
Plasma proteins Gm. per 100 cc.
Total
Albumin
Urine proteins
Gm. per liter 12 hr. collection
Edema
Vascular signs
Blood pressure
Fundus changes
Cstr. art.
Sclr. art.
Exudate
Ret. hem.
Pap. edem.
Weight of animal (Gm.)
Months
Nephrotoxin injected: May 18 0.30 cc. per 100 gm., iv
" 23 0.15 " " " "
" 27 0.15 " " " "
" 31 0.075 " " " "
Total 0.675 " " " "
Rat N-79
Killed
Nephrotoxin injected: June 10 0.30 cc. per 100 gm., iv
" 12 0.15 " " " "
Total 0.45 " " " "

CHART 3

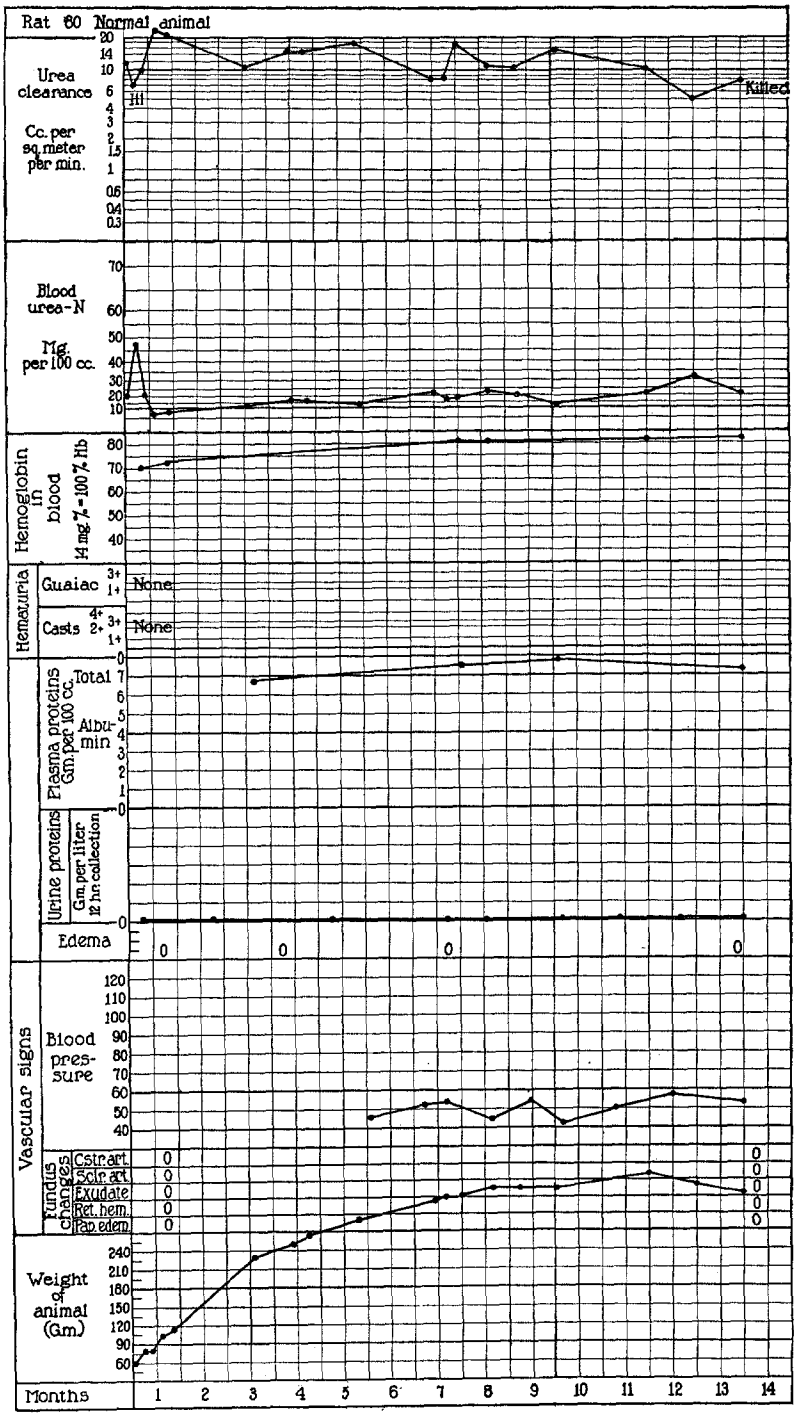

Rat 60 Normal animal
Urea clearance
Cc. per sq. meter per min.
20
14
10
6
4
3
2
1.5
1
0.5
0.4
0.3
Killed
Blood urea-N
Mg. per 100 cc.
70
60
50
40
30
20
10
Hemoglobin in blood
14 mg. % = 100 % Hb
80
70
60
50
40
Hematuria
Guaiac
3+
1+
None
Casts
4+
3+
2+
1+
None
Plasma proteins Gm. per 100 cc.
Total
Albumin
7
6
5
4
3
2
1
0
Urine proteins
Gm. per liter 12 hr. collection
0
Edema
0
0
0
0
Vascular signs
Blood pressure
120
110
100
90
80
70
60
50
40
Fundus changes
Cstr art.
Scle art.
Exudate
Ret. hem.
Pap. edem.
0
0
0
0
0
0
0
0
0
0
Weight of animal (Gm.)
240
210
180
150
120
90
60
Months
1
2
3
4
5
6
7
8
9
10
11
12
13
14

CHART 4

the plasma proteins were decreased from the normal of 6.5 per cent (20) to 4.51 per cent and 4.63 per cent in rats N-38 and N-78 (Table III), respectively. This fall in plasma protein was not a constant feature of the early disease, since N-74 when moribund on the 14th day had a total protein of 6.4 per cent with 2.2 per cent albumin. Animals with chronic nephritis had normal plasma proteins 2 months after treatment. Protein determinations were obtained during the terminal phase on two of the four rats with progressive nephritis (N-37 and N-39) and in both instances a depression was noted. Total plasma protein values of 3.58 per cent and 3.93 per cent were found, with albumin fractions of 2.5 per cent and 2.1 per cent, respectively. Animals that failed to develop progressive nephritis (N-22, N-24, N-38, N-41, N-43, N-79) had normal amounts of plasma protein when sacrificed after 6 to 8 months.

While plasma protein deficit depended in part, at least, on the large amount of protein excreted in the urine, synthesis of proteins also appeared important, since many of the rats which maintained normal plasma proteins lost as much protein in the urine as did those which developed a plasma protein deficit.

Growth.—Growth was permanently retarded only in certain of the animals with chronic progressive nephritis (rat N-39, Chart 2). In the initial phase, just after edema fluid had been eliminated, the animals usually appeared malnourished, and often weighed less than before the injection of anti-kidney serum. After a variable delay, growth was resumed and attained a normal range in most animals.

The cause of the retardation or the cessation of growth in the rats with severe renal involvement was not apparent. The possible occurrence of a process similar to renal rickets was considered; and rat N-39 was investigated with this in view. Blood calcium and phosphorous determinations, x-ray examination of epiphyses of long bones, and histological study of these bones revealed no significant abnormalities. The terminal weight loss observed in rats N-39 and N-37 was the result of malnutrition dependent upon marked anorexia.

SUMMARY

The glomerulonephritis induced in rats by nephrotoxin was characterized clinically during its initial phase by severe albuminuria, cylindruria, and anasarca, but not by hematuria.

Rapidly fatal nephritis was produced by injecting relatively large amounts of anti-kidney serum at frequent intervals. In such cases the blood urea mounted rapidly; the urea clearance fell; and death occurred within about 2 weeks.

A milder nephritis of the chronic type was induced by giving smaller quantities of anti-kidney serum in either single or divided doses. In these instances there was no immediate alteration of the urea clearance. Lipemia and plasma protein deficit appeared with the development of anasarca. The majority of rats which survived the initial stage of this experimental nephritis continued to show marked albuminuria with casts until they died or were sacrificed months later. Some of these animals showed retardation of growth and a progressive fall of the urea clearance. Terminally there developed marked retention of urea, plasma protein deficit, anemia, and hypertension.

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