Spontaneous Nephrosis, with Proteinuria, Hyperglobulinemia, and Hypercholesterolemia in the Rat.* (30199)

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Nephrosis is a spontaneous disease of laboratory rats which has been described under various names in animals over 500 days old (1,2,3,4,5,6). Spontaneous proteinuria has been observed in young rats(7,8,9,10). In the present study, parallel observations are made on urine proteins, serum proteins and serum cholesterol in relation to renal lesions in rats from time of weaning to 640 days of age.

Materials and methods. Male Sprague-Dawley rats bred from a colony started in 1945 were used. The animals were pathogen-free and were raised in air-conditioned quarters equipped with uniform temperature, humidity and lighting(11). Observations on renal lesions and related urine and blood findings were made in rats of 5 different ages (Tables I and II) starting at 4 weeks when they were weaned. In addition, 8 rats not included in the Tables were examined at 240 days of age for renal changes. The diet consisted of commercially prepared pellets† without added vitamin D, and contained 24.3% of crude protein, 4.0% of fat and 54.2% of carbohydrate. Rats were kept singly in metabolism cages for 24 hours to determine water intake and urine output. No food was provided during this period. After intervals of 5 to 7 days, food consumption was measured for 5 to 7 days and the daily intake was calculated from the total amount eaten. The rats were killed with sodium pentothal‡ injected intraperitoneally and complete autopsies were performed immediately after death. Tissues were fixed in Zenker's fluid, imbedded in paraffin, and prepared for microscopic examination by staining sections with hematoxylin and eosin. Sections of kidney tissue were also stained with the periodic acid-Schiff reagent and hematoxylin. Urine proteins were isolated by precipitation with tungstic acid and colorimetrically assayed with the biuret reagent. Ames paper reagent§ was used to detect trace amounts of protein. Total serum protein was determined by the biuret reagent. Globulins were precipitated with 22% Na_2SO_4, separated from the albumin fraction with the aid of ether, and albumin was determined by the biuret reagent. Globulins were calculated by the difference from the total protein. Total serum cholesterol was determined by the method of Carr and Drekter(12). The method described by Carr was used for blood urea nitrogen. Electrophoresis of serum and urine protein fractions was carried out for a period of 14 hours in Owen buffer, pH 8.9, at 220 volts on Whatman #3MM chromatography paper, using bromphenol blue as the stain. In interpreting the results of identification of urinary proteins by electrophoresis, the possible irreversible effect of their denaturation by large amounts of urea cannot be excluded.

Results. The kidneys showed no gross abnormality except for a faint brownish tint in older rats. Microscopically, the basement membranes of the glomerular capillaries stained deeper in the kidneys of weanlings than in older animals, and the tufts appeared more cellular. In 70- and 135-day-old rats, an occasional glomerular tuft showed capillary basement membrane thickening or lobular hyalinization. In some instances, adjoining such glomeruli were a few dilated convoluted tubules containing casts. At 240 and 370 days, lesions occurred with increasing frequency and the histologic changes corresponded to the findings in nephrosis. Glomeruli were involved to a much greater extent than tubules, and lesions were more ad-

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† Rockland "D-free" Pellets, A. E. Staley Manufacturing Company, Decatur, Illinois.

‡ Nembutal, Abbott, North Chicago, Illinois.

§ Combistix, Ames Company, Elkhart, Indiana.

TABLE I. Daily Food Intake, Urine Volume, Urinary Protein Excretion, and Severity of Glomerular Lesions in Male Rats at Different Ages.

No. of rats	Avg age, days	Food, g*	p‡	Water, ml*	p‡	Urine Vol, ml*	p‡	Urine Protein, mg*	p‡	Renal lesions
7	30					2.5 ± .28†		<1.0 ‖		None
8	60	9.5 ±.33†		12.1 ± 1.66†		9.0 ±1.13		14.7 ± 1.39†		Occasional
6	120	5.8 ±.08	<.001	6.0 ± 1.02		3.8 ± .64		14.6 ± 1.26		"
6	360	4.1 ±.14	<.001	2.1 ± .36		1.7 ± .22	.02	27.6 ± 7.75	ns	Moderate
9	620	4.2 ±.08		3.0 ± .39	ns§	2.7 ± .33	.05	44.1 ± 5.32	ns¶	Severe

* g, mg, or ml per 100 g body wt. † Mean values ± S.E. ‡ P value for difference between means at 2 successive ages. § ns = difference not significant. ‖ Approximate value. ¶ P = <.01 for difference between means at 120 days and 620 days.

vanced in some animals than in others. Glomerular changes included capillary basement membrane thickening, hyalinization and shrinkage of lobules or entire tufts, thickening and fraying of Bowman's capsule, and adhesions between tufts and capsules. Changes in the tubular system occurred in proximal convoluted segments adjacent to altered glomeruli. As a rule, the convoluted tubules were dilated, filled with casts, and lined by flattened epithelium. Groups of shrunken tubules lined by epithelial cells in varying degrees of degeneration and having wrinkled, often split basement membranes were also noted. Lymphocytic infiltration was observed occasionally in areas of tubular atrophy. Collecting ducts were frequently dilated and contained casts. At 640 days, incidence and severity of lesions was greater than in 370-day-old rats. In some of the older animals, the tubules showed little alteration whereas the glomeruli were involved extensively.

Food intake, water intake and urine output decreased up to 360 days (Table I). After this age, urine volume increased. Water consumption, urine output, and protein excretion varied considerably among rats of the same age. A trace amount of protein, too small for accurate measurement, was found in the urine of weanlings. Total urinary protein excretion at 120 days was 14.6 ± 1.26 mg/100 g, as compared with 44.1 ± 5.32 mg/100 g at 620 days. In 360-day-old rats, the variations in protein excretion were too large for meaningful comparison with other ages. Microscopically, the urine was free from casts, epithelial cells, leucocytes or red blood cells, in rats of all ages. Electrophoretic examination of urinary proteins showed mobilities similar to serum α 2 globulin and β globulin in 60- and 120-day-old animals. A mobility resembling serum albumin appeared when the protein concentration was about 20 mg/100 g. With increasing proteinuria in older rats, the albumin fraction predominated, and the electrophoretic pattern approached that of the serum proteins.

Except for a lower value at time of weaning, the level of serum protein (Table II) was constant and in accord with the values reported by others(13). Serum albumin was slightly lower in 370- and 640-day-old rats than in younger animals, whereas serum globulin increased markedly with advancing age. The A/G ratio was inversely proportional to the increase in globulin. The electrophoretic pattern of the serum proteins was characterized by a predominant β component and lighter α 1 and α 2 components(14-17). An inconstant γ component was also noted.

An increase of borderline significance was observed in the serum cholesterol of 370-day-old rats. At 640 days, the level of cholesterol was nearly twice as high as the value for young animals. There was no significant change with age, in blood urea nitrogen (range, 17.5 ± 0.82 mg/100 ml-22.1 ± 1.55 mg/100 ml), or in hematocrit values (range, 37 ± 1.1%-44 ± 0.8%).

Body weights and kidney weights increased up to 370 days. After this age no further increment was observed. Cessation of body weight increments is related to onset of disease(18,19).

Discussion. The higher excretion of urinary proteins by old rats as compared with young ones can be explained on the basis of

greater frequency and severity of microscopically observed renal lesions. In young rats, however, the histologic changes seen with the light microscope are insufficient to account for the proteinuria. Alterations in the glomerular capillaries in young rats have been described with electron microscopy(20), but the findings are of doubtful significance. The nature of the agent responsible for the initial changes in the capillary wall is obscure. Nutrition appears to be a factor, in that level of food intake influences age of onset of lesions(5,21,22), and level of dietary protein affects proteinuria(7,10,23). The predominance of globulins in the urine of young rats is in agreement with observations by Sellers *et al*(24).

TABLE II. Body Weight, Kidney Weights, Serum Proteins and Serum Cholesterol in Male Rats at Different Ages.

No. of rats	Avg age, days	Body wt, g*	Kidney wt, g*	p†	Serum proteins							Serum cholesterol, mg/100 ml*	p†
					Total, g/100 ml*	p†	Albumin, g/100 ml*	p†	Globulin, g/100 ml*	p†	A/G ratio		
7	30	80 ± 3.1	.72 ± .04		5.3 ± .19		3.8 ± .11		1.5 ± .11		2.6	83 ± 3.8	
8	70	294 ± 11.0	2.75 ± .07		5.7 ± .07	ns	3.9 ± .14		1.8 ± .07	ns	2.2	79 ± 2.5	
6	135	404 ± 7.3	3.25 ± .03		6.2 ± .20	ns§	4.0 ± .12		2.2 ± .08	.02	1.9	86 ± 1.6	
6	370	493 ± 8.6	4.06 ± .10		6.3 ± .12		3.5 ± .08	<.001	2.9 ± .12	.001	1.2	105 ± 6.5	.06 ‖
9	640	480 ± 14.3	4.37 ± .27	ns‡	6.3 ± .17		3.3 ± .10	ns	3.0 ± .17		1.1	157 ± 8.7	.001

* Mean values ± S.E. † P value for difference between means at 2 successive ages. ‡ ns = difference not significant.
§ P = .01 for difference between means at 30 days and 135 days. ‖ Borderline significance.

The slight decrease in serum albumin with coexisting severe poteinuria suggests an adaptive mechanism that compensates for the large amount of protein excreted in the urine. By contrast, serum globulin increases markedly with age. This may represent greater synthesis of globulins or slower rate of degradation in response to loss of globulins in the urine.

The glomerular lesions, proteinuria, and hypercholesterolemia are consistent with the findings in nephrosis. However, edema and hypoalbuminemia, characteristic of nephrosis in humans, are absent in the rodent. In the advanced stage of the disease, hypertension and cardiac hypertrophy develop in the rat (25), and in the terminal phase, hydrothorax, ascites and uremia ensue(5).

It is evident from the present study that nephrosis may exist in presumably healthy rats raised in an especially favorable laboratory environment, and that the disease starts in very early life. The question arises whether this condition exists in other rat strains, and to what extent it affects experimental results. Thus far, spontaneous renal lesions similar to those described here have been reported in Sprague-Dawley(5), Osborne-Mendel(1,2), and Wistar(9) rats, and proteinuria has been observed in Sprague-Dawley(9,10,23), Slonaker(7,24), and Wistar(9) rats.

Summary. A form of spontaneous nephrosis characterized by proteinuria, hyperglobulinemia and hypercholesterolemia occurs in the Sprague-Dawley rat. Proteinuria exists at weaning, and microscopically observed changes appear at 60 days of age. Degree of proteinuria corresponds with severity of lesions, and both increase with age. In young rats, globulin is the chief protein component excreted in the urine. In older animals, the

albumin fraction predominates. Serum globulin increases with age, but serum albumin shows little change. Hypercholesterolemia is observed in 640-day-old rats.

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Neutralization *in vitro* of Mouse Leukemia Virus by Specific Immune Serum. Importance of Virus Titration.* (30200)

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After it was demonstrated that leukemia in mice is caused by a filterable and transmissible virus(1), and the experimental conditions were determined under which a leukemogenic virus could be isolated from spontaneous mouse leukemia, a large number of individual isolations of leukemogenic viruses in mice have been reported from tissues of mouse donors with spontaneous(2), or radiation-induced(3,4) leukemia, and also from transplanted mouse tumors(5). Most of these viruses induce in mice, and also in rats, essentially the same disease; they also have similar biological and physical properties. The question has been raised whether the many leukemogenic viruses isolated from leukemic mouse organs, and from transplanted mouse tumors during the preceding decade represent individually distinct viruses, or whether they represent isolations from different sources of the same mouse leukemia virus, or one of its close variants(2).

This study deals with an attempt to identify one of the relatively potent mouse leukemia virus strains recently isolated from non-leukemic mouse tissues. Following the

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