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Renal Clearance of Human and Rat Albumins in the Rat (38666)

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Much evidence from clinical studies suggests a relationship between the molecular size or molecular radius of plasma proteins and the rates at which they are cleared into the urine (1–4). This relationship is not a simple one, for molecular size does not appear to be the only factor that influences transfer of proteins into the urine (2, 5). Some proteins of similar molecular size have different clearance rates. Amylase is excreted by glomerular filtration but is not reabsorbed by the tubules (6, 7); whereas light chain dimers, of similar molecular weight, are filtered by the glomerulus and almost completely reabsorbed by the tubules (8). Also, the clearances of three molecules of similar molecular size, IgG, IgA, and ceruloplasmin, differ considerably in patients with proteinuria (5).

Albumin was selected as the model protein for analysis of its renal clearance because: (a) the endogenous protein is the most abundant plasma protein and is easily measured in serum and urine; (b) it occupies a key role in understanding glomerular and nephron protein homeostasis; (c) it is the only protein thus far measured by nephron puncture which is filtered by the glomerulus and substantially reabsorbed by the tubules (9, 10); (d) its metabolic removal from serum in rats is well studied; and (e) it is well characterized structurally and chemically.

Studies on the renal handling of proteins pose at least two major problems: First, unlike polysaccharide marker molecules, the protein studied is being metabolized; and second, many methods which must be used to trace proteins require procedures such as iodination which produce alterations in the molecule and consequent profound effects on its metabolism after injection into a homologous animal (11–14). Radioiodination and other chemical procedures result in a heterogeneous population of molecules; their catabolism is a function of several different catabolic rates, and this heterogeneity complicates analysis of data from animal models.

To circumvent this latter problem, a heterologous unaltered albumin molecule was chosen for study. Human albumin is similar in size, conformation, shape, and charge to rat albumin, but differs from it chemically (15). In the present study, the removal from serum and renal handling of human albumin were investigated in the rat and compared with the renal handling of endogenous rat albumin.

The experiments were carried out in normal rats and in those with experimental glomerular disease and proteinuria induced by one intravenous injection of puromycin aminonucleoside. A major objective was to determine whether the renal handling of the heterologous and homologous species of albumin was similar or different in a model in which their catabolic rates might well differ.

Methods and Materials. Young Sprague–Dawley female rats, weighing 150–200 g, were housed in metabolic cages. They were fed a commercial rat pellet diet (Purina), and had free access to water. Urine collections were made in flasks containing thymol crystals and a layer of mineral oil. The 24-hr urine volume was measured; the urine was centrifuged, and the oil layer was removed. It was then divided into several aliquots, which were frozen and stored until the time of assay.

Blood was drawn from the marginal tail vein under light anesthesia, and collected into capillary tubes. The sealed capillary tubes were centrifuged in an International micro-capillary centrifuge. An aliquot of serum was diluted 1:100 into a 1% solution of bovine serum albumin and stored frozen. The remaining serum was transferred into fresh capillary tubes, sealed, and frozen until time of assay (9).

To study human albumin metabolism in

normal rats, five rats were placed in metabolic cages and two 24-hr urine collections were made. Zero time (48 hr later) 0.8 ml (40 mg) of human plasma albumin (USP 5%, Metrix) was injected into the marginal tail vein. Serum samples were obtained at 0 time, 10 min, 1, 4, 12, and 24 hr after injection, and daily thereafter for a total of 7 days. Urine collections were made 12 and 24 hr after injection, and daily thereafter for the duration of the experiment.

To study human albumin metabolism in rats with aminonucleoside nephrotic syndrome, six rats were placed in metabolic cages and two 24-hr urine collections were made. Aminonucleoside nephrosis was induced by a single injection of the aminonucleoside (6-dimethyl aminopurine-3-amino-*d*-ribose) of puromycin (AMN), 10 mg/100 g body wt in a 2% solution in the marginal tail vein (16). Serum and urine samples were obtained at 24 and 48 hr. Forty mg of human albumin was injected into the tail vein 48 hr after the injection of AMN. Serum and urine samples were collected for 6 days after AMN injection.

The levels of human and rat albumin in serum and urine were assayed by a specific solid-phase radioimmunoassay. The samples were thawed and appropriate dilutions were made into 1% bovine serum albumin. All samples were measured at two dilutions in replicates of five. The average coefficient of variation for the estimate was 12% (17).

In each time period, the results were calculated as the geometric mean ± one standard deviation. This was accomplished by first converting the concentration values to logarithms. The slopes of albumin excretion ($U_{alb}V$) vs time were calculated by a linear regression procedure, using all the concentration values for each time period.

To calculate the clearance of human albumin in the normal rat and of rat and human albumin in the experimental AMN animal, it was necessary to estimate a mean value for the serum concentration. Since the albumin levels were changing, the concentration used was the geometric mean of 2 successive days.

Results. Fate of human albumin in the normal rat. In the normal rat injected with human albumin, the geometric mean serum concentration of rat albumin was 38.0 (40.3, 35.9) mg/ml[1]; it did not change significantly during the course of the experiment (Table I; Fig. 1a). Significant levels of human albumin were detected in serum 10 min after injection of human albumin. To allow for mixing, values 1 hr after injection were used as the first point in analyzing the decay. The serum concentration of human albumin decreased steadily; the half-life was 15.8 hr, and the calculated slope (b) was −0.457 using the formula $\log y = a + bx$, where y = serum albumin concentration and x = time in days (Table II).

The excretion rates of human and rat albumin are summarized in Table I and Fig. 1b. Rat albumin was excreted at a constant rate over the period of study; it did not change after injection of human albumin, nor did this injection cause an increase in endogenous albuminuria. The excretion of rat albumin was 76.2 (112.0, 51.7) ng/min, and the clearance was 0.0019 (0.0044, 0.0008) μl/min. Human albumin excretion declined rapidly, and little could be found after the fourth day. On the first day, excretion was 3.6 (7.70, 2.64) ng/min; it decreased with a slope of −0.370 until it was undetectable (Table II). The slope of the human albumin excretion was not significantly different from that of the protein in serum, indicating that the clearance of human albumin was a function of the serum level. Over a 4-day period the human albumin clearance was 0.0026 (0.0038, 0.0017) μl/min—a value similar to that of the endogenous protein (Table I).

Fate of rat albumin in the nephrotic rat. The rat serum albumin level was first decreased on the third day after the injection of AMN (Table I; Fig. 2a). It declined slowly until day 5 with a slope of −0.078. Between days 5 and 6 there was a much more rapid decline in the serum albumin level, from 21.4 to 5.69 mg/ml. An increase in rat albumin excretion was first noted on the second day (Table I; Fig. 2b). Thereafter the albumin excretion increased until, on the sixth day, it was 195 × 10^3 ng/min,

[1] The values in parentheses are one SD above and one SD below the geometric mean.

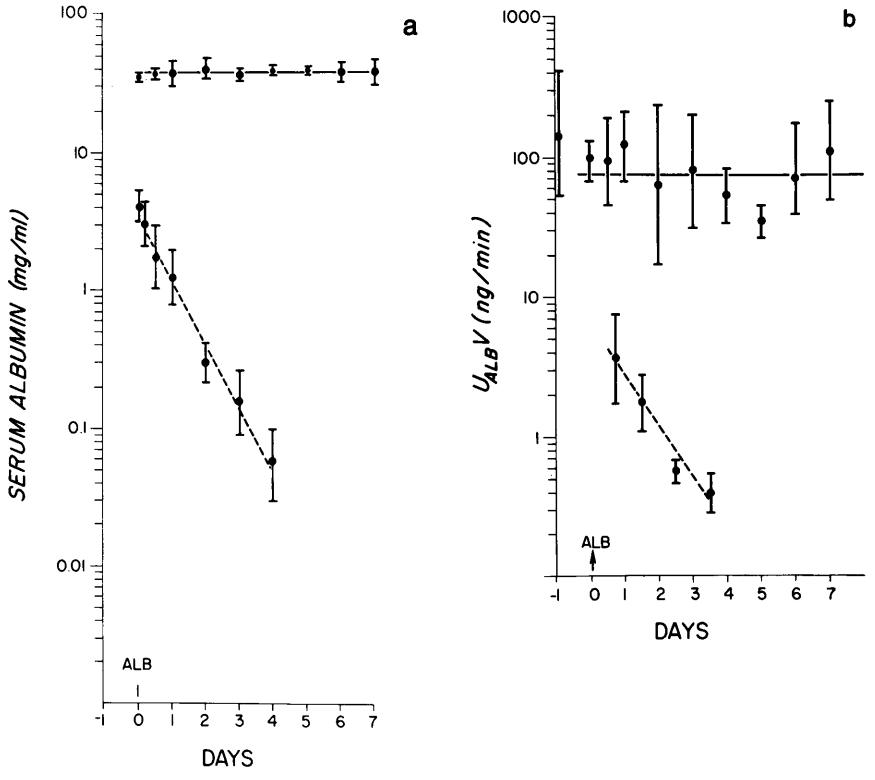

FIG. 1. Serum (a) and urine (b) albumin levels in normal rats injected with 40 mg of human albumin. The solid lines give the rat albumin serum level and excretion rate ± one SD; the interrupted lines, those for human albumin.

or about 1000-fold greater than the normal excretion. The slope of rat albumin excretion with time was +0.701. The rat albumin clearance increased from an average of 0.0047 to 15.82 μl/min on the sixth day.

Fate of human albumin in the nephrotic rat. Human albumin was injected 2 days after the administration of AMN. The human serum albumin concentration was 3.58 (4.94, 2.58) mg/ml 1 hr after it was injected. The human albumin level decreased steadily thereafter until day 5, with a slope of −0.410, and a half-life of 17.6 hr. The decrease in the human albumin level was greater from day 5 to day 6. In contrast to the data on days 2–5, the rate of change of the human and rat serum albumin levels was similar between days 5 and 6. The human albumin excretion rate, measured 12 hr after injection, was 19.8 (50.9, 7.6) ng/min and increased steadily to 729 ng/min on the fifth day; there was no further increase on the sixth day. The slope of the increase was +0.622. The human albumin clearance increased from 0.0076 to 9.00 μl/min on the sixth day. Analysis of the data from the individual rats showed that the clearances of human and rat albumin were consistently similar.

Discussion. Earlier observations have demonstrated that molecular weight or molecular size of the plasma protein is not the only factor which determines its handling by the kidney and appearance in the final urine. Although the function of the glomerular capillary wall is of major importance in determining which proteins appear in urine, as yet unknown factors must explain why some proteins are reabsorbed by the tubules, whereas others of similar molecular size are not. To explore the roles of glomerular filtration and of tubular reabsorption, it is desirable to investigate proteins that are important physiologically and are amenable to the induction of systemic changes in molecular structure. Changes in

TABLE I. DATA ON HUMAN ALBUMIN AND RAT ALBUMIN IN NORMAL RATS AND IN RATS WITH PROTEINURIA INDUCED BY AMINONUCLEOSIDE.

Time Days	Rat albumin			Human albumin			
	Serum[a]	Excretion ($U_{alb}V$)	Clearance	Serum[a]		Excretion ($U_{alb}V$)	Clearance
	mg/ml	ng/min	μl/min	mg/ml		ng/min	μl/min
			Normal animals				
0	35.5	95	0.0027	4.04	—	—	—
0.5	37.2	93	0.0025	1.74	—	—	—
1	37.2	121	0.0033	1.26	(1.47)	3.6	0.0025
2	40.5	63	0.0016	0.31	(0.62)	1.7	0.0028
3	36.7	79	0.0022	0.16	(0.22)	0.56	0.0027
4	42.4	54	0.0013	0.06	(0.10)	0.39	0.0042
5	40.0	35	0.0009	—	—	—	—
6	38.6	80	0.0021	—	—	—	—
7	38.3	109	0.0028	—	—	—	—
Mean[b]	38.0 (40.3, 35.9)	76.2 (112.0, 51.7)	0.0019 (0.0044, 0.0008)				0.0026 (0.0038, 0.0017)
			Animals injected with aminonucleoside				
0	36.4	208	0.0063	—	—	—	—
1	37.2	156	0.0041	—	—	—	—
2	36.9	152	0.0045	3.58	—	—	—
2.5	39.3	1060	0.022	1.74	(2.58)	19.8	0.0076
3	24.7	2040	0.104	1.39	(1.62)	36	0.023
4	28.8	7730	0.201	0.50	(0.82)	125	0.100
5	21.4 (25.0)	63700	2.61	0.20	(0.30)	729	1.870
6	5.69 (10.7)	195200	15.82	0.023	(0.059)	627	9.00

[a] In these columns the values in parentheses are the geometric mean of the values at the time indicated and of the preceding time period.

[b] Values given are the geometric means and, in parentheses, one SD above and one SD below the geometric mean.

TABLE II. RATES OF ALBUMIN DISAPPEARANCE FROM SERUM AND URINARY EXCRETION IN NORMAL RATS AND IN RATS WITH PROTEINURIA INDUCED BY AMINONUCLEOSIDE.

	Number of observations	Slope (b)		$t_{1/2}$
	Normal animals			
Human albumin				
Serum disappearance days 0.5–4	29	−0.457	0.953	15.8 hr
Urine excretion days 0.5–4	16	−0.370	0.861	
	Animals injected with aminonucleoside			
Rat albumin				
Serum disappearance days 2–5	21	−0.078	0.54	
Urine excretion days 2–5	20	+0.701	0.874	
Human albumin				
Serum disappearance days 2–5	26	−0.410	0.957	17.6 hr
Urine excretion days 2–5	20	+0.622	0.89	

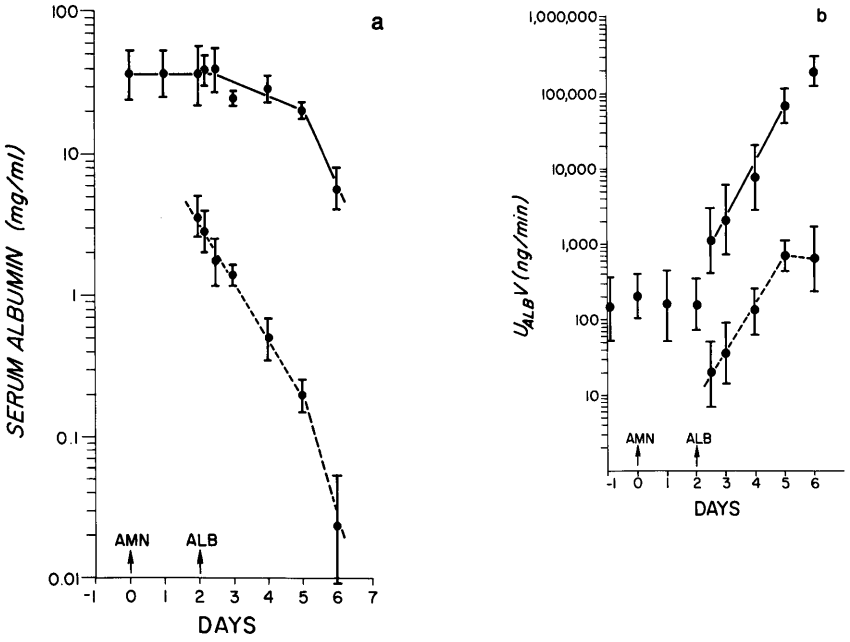

FIG. 2. Serum (a) and urine (b) albumin levels in rats injected with 10 mg/100 g body wt of aminonucleoside (AMN) on day 0, and with 40 mg of human albumin on day 2. The solid lines give the rat albumin serum level and excretion rate ± one SD; the interrupted lines, those for human albumin.

structure are liable to so affect protein catabolism that, in the experimental animal, the altered protein will behave differently from the unaltered or endogenous protein.

Human albumin was chosen as the model protein for initial study; its molecular size and structure are similar to that of endogenous rat albumin, but its primary sequence and immunologic characteristics differ. Its rate of removal from serum might also be expected to differ from that of endogenous rat albumin. Under these circumstances, it is important to demonstrate that the renal clearance of the heterologous protein is a function only of its serum concentration, and is independent of the catabolism and distribution of the heterologous molecule.

Discrepancies might well occur if two techniques, such as tracer radioactivity and radioimmunoassay, were used to measure the endogenous and heterologous species of albumin. In the present experiment the radioimmunoassay was used to measure both rat and human albumins, thereby eliminating any protein heterogeneity which might have been introduced by isotopic labelling procedures.

As anticipated, the removal of the heterologous human albumin from serum was much faster ($t_{1/2}$ = 15.8 hr) than that measured by Katz *et al.* (12) for endogenous rat albumin ($t_{1/2}$ = 46 hr). Over a 4-day period, as a consequence, the serum concentration and urine excretion of human albumin changed over a 12- to 17-fold range—changes that were well within the range of sensitivity of the radioimmunoassay. The renal clearance of human albumin did not vary significantly over this range. This finding is consistent with the hypothesis that the clearance of human albumin is a function of the serum level. The clearance of human albumin was identical to that of endogenous rat albumin. Thus, these two structurally and immunologically different species of albumin appear to be handled by the normal kidney in the same manner.

The methods used to measure rat albumin excretion were reproducible, for in these experiments the excretion rate and clearance of rat albumin were virtually identical to those measured previously in normal animals (18). The animals injected with aminonucleoside were studied under conditions similar to those in an earlier experiment;

the excretion rates and clearances of endogenous rat albumin were virtually the same as those reported (18). The endogenous albumin excretion first increased on the second or third day after injection of the aminonucleoside, and the increase was almost 1000-fold by the sixth day. Human albumin was removed rapidly from serum ($t_{1/2}$ = 17.6 hr) in the period preceding the rapid loss of serum protein in the urine; the renal clearance of human albumin was not significantly different from that of the endogenous rat albumin in each time period.

Although the fractional rate of albumin catabolism increases in the nephrotic rat, the absolute rate of albumin catabolism expressed as g albumin/kg/day is decreased. The slower rate of degradation of the human albumin between days 2–5 in these animals is consistent with these findings. Between day 5 and 6 when there was a large change in albumin distribution, the data were more difficult to interpret. Another effect, but very difficult to interpret, is the direct effect of the aminonucleoside on protein metabolism. Studies have shown that the drug is a powerful inhibitor of rRNA synthesis (19). Whether this in turn affects protein catabolism is not known.

Summary. Experiments were done to investigate whether molecular discrimination occurred in the renal handling of two species of serum albumin. Human albumin, 40 mg, was infused into rats; it was removed from serum ($t_{1/2}$ = 15.8 hr) more rapidly than previously reported measurements of removal of endogenous rat albumin ($t_{1/2}$ = 46 hr). Human albumin was cleared by the rat kidney at a constant rate of 0.0026 μl/min—a value virtually identical to that of rat albumin (0.0020 μl/min). In rats with proteinuria following the single iv injection of puromycin aminonucleoside, human albumin was removed from serum with a half-life of 17.6 hr. During the development of the nephrotic syndrome, the renal clearances of human and rat albumin increased proportionately.

Despite the difference in the serum concentration and rates of removal of the two species of albumin, renal handling of the two species was similar. Thus the kidney did not appear to discriminate in its handling of these two proteins.

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