Alterations in Albumin Metabolism after Serum and Albumin Infusions *

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The intravenous administration of whole serum or of serum albumin results in a temporary increase in the concentration of circulating protein; the rate of return toward preinfusion levels depends upon the state of protein depletion or pool size and upon the rates of synthesis and degradation of albumin (1-6). Much of the infused protein can be accounted for by increased nitrogen excretion in the urine (2-4); the remainder has been assumed to be stored in intravascular or extravascular areas (1-6). The rate of disappearance of tracer-labeled homologous and heterologous albumin from plasma has been observed to increase during the infusion of whole serum or of serum albumin (6-7). Thus, although there is an increase in protein degradation associated with at least a temporary storage of protein, observations on the changes in endogenous protein synthesis and degradation that might follow such infusions are not available. The effects of prolonged infusions of pooled homologous serum or salt-poor human serum albumin on albumin metabolism in rabbits tolerant to human albumin are reported in the present study.

Methods

Female rabbits were used in all studies and were fed a standard Rockland rabbit ration. The rabbits were kept in metabolism cages, and complete urine and stool collections were made daily. Endogenous albumin metabolism was measured with rabbit albumin labeled with I^{131} in all studies. Lugol's solution (2 to 3 drops) was added to the animals' drinking water daily to inhibit the thyroidal uptake of I^{131} released from degraded protein.

The procedures for separating rabbit albumin from pooled rabbit serum and labeling with I^{131} have been described previously (8-9). Two groups of rabbits were studied. The first group of 12 rabbits received whole rabbit serum.[1] After the intravenous injection of 30 to 300 μc of rabbit albumin I^{131} (RSA-I^{131}), 0.6 to 0.8 ml of heparinized blood was obtained from the ear opposite to the side of injection 6 and 10 minutes later and daily thereafter for a control period of 10 to 12 days. Daily infusions of 20 to 30 ml of pooled serums containing 530 to 935 mg of albumin (equivalent to 105 to 207 mg of albumin per kg body weight) were then started and continued for 18 to 24 days while observations on plasma, urine, and stool radioactivity were continued. At this time, and while the infusions were continued, a second injection of albumin I^{131} was made to remeasure albumin distribution and pool size. Plasma samples were obtained just before the serum infusion for that day.

A second group of 6 rabbits, tolerant to human serum albumin, was also studied. Seventy-five to 100 μc of rabbit albumin I^{131} was injected intravenously, and control values were obtained for 7 to 10 days as described above. The rabbits then received between 125 and 150 mg of human albumin per kg body weight per day for 12 to 14 days, and plasma, urine, and stool I^{131} was measured daily. A second injection of rabbit albumin I^{131} was then made, and while the infusions continued, observations on plasma, stool, and urine I^{131} were continued for another 8 days. During this period there was no significant change in the exchangeable albumin space or the serum albumin levels. All plasma samples were obtained just before the albumin infusion and 24 hours after the previous infusion. These rabbits had been exposed to human serum albumin [2] before birth by infusing 250 mg of human serum albumin into the mothers 24 to 48 hours before delivery and thereafter by injecting 15 to 25 mg intraperitoneally for the first 45 days following birth. Fifteen to 50 mg of albumin was then infused intravenously every 2 to 8 weeks for the next 18 months. Agar diffusion studies failed to reveal precipitin lines between the sera of these rabbits and human serum albumin (10). More indicative of the tolerant state was the observation that these rabbits handled I^{131} human serum albumin and

[1] Baltimore Biological Laboratories, Baltimore, Md.
[2] E. R. Squibb & Sons, New York, N. Y.

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either homologous or autologous rabbit albumin I^{131} in an identical fashion (11).

All lots of albumin I^{131} were tested in normal control adult rabbits to assure against the use of preparations that contained significant amounts of denatured protein. The urine was checked for protein by precipitation with 10% trichloroacetic acid.

Plasma protein concentrations were determined by a micro-Kjeldahl procedure and protein partition by moving boundary electrophoresis in a Kern microelectrophoresis unit (12).

The methods for calculating albumin distribution and metabolism have been described previously (8, 9, 13). Plasma volume was determined from the 6- to 10-minute space of distribution of the injected albumin I^{131}; total exchangeable albumin space (TEAS) from the space of distribution of albumin I^{131} at distribution equilibrium; total exchangeable albumin (TEA) from the product of this space and the plasma albumin concentration. Albumin partition was determined from the ratio: plasma albumin/TEA. Albumin degradation during both the control and experimental periods was determined by the metabolic clearance procedure that has been shown to be valid in nonsteady states (14). In view of the serum and albumin infusions, however, this technique was modified. In calculating the daily metabolic clearance of albumin during the control period the mean plasma I^{131} level was used, and this value was obtained from the mid-point of the plasma decay curve connecting two successive daily determinations. The infusion of 30 ml of serum or 600 mg of human serum albumin, however, resulted in an immediate increase in the plasma volume and a decrease in plasma albumin specific activity (Figure 1). The plasma concentration of I^{131} thereafter was nearly constant for the next 24 hours. Thus, the value used in all of the experimental calculations was obtained 24 hours after the serum infusion, since this value more closely approximated the mean plasma I^{131} level. The plasma I^{131} value derived from the plasma decay curve would have been erroneously high (Figure 1). Microcuries I^{131} excreted per day/mean albumin specific activity = (microcuries I^{131} excreted per day/microcuries I^{131} per milliliter 24-hour plasma) × serum albumin concentration = grams degraded per day.

Albumin synthesis during the control period was assumed to equal albumin degradation, since the plasma
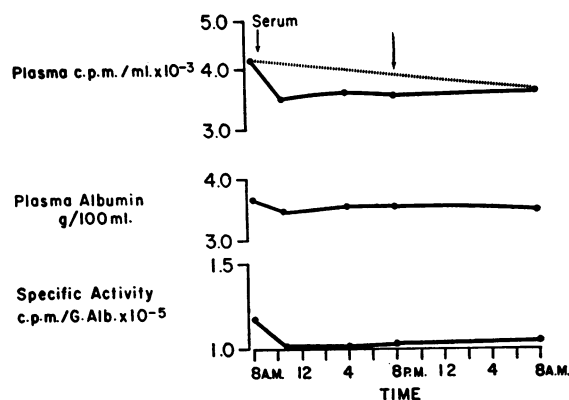

FIG. 1. ALBUMIN SPECIFIC ACTIVITY. After the infusion of 30 ml of pooled rabbit serum there was a rapid decrease in plasma radioactivity (upper curve), plasma albumin concentration (middle curve), and albumin specific activity (lower curve), and these values then remained stable during the rest of the day. Plasma I^{131} remained below the mean value (arrow at 8 p.m.), and the plasma concentration of I^{131}, 24 hours after the serum infusion, was not different from the measured mean value.

albumin level and the space of albumin distribution remained constant. Albumin synthesis during the experimental period was calculated from the sum of albumin degraded and the change in total exchangeable albumin less albumin infused.

Results

Data concerning serum protein distribution during the infusion of whole rabbit serum are shown in Table I. The rabbits' weights did not change significantly during the period of study. After 18 to 24 days of continued serum infusions, the mean plasma volume increased by only 3% (determined 24 hours after the last infusion). The total exchangeable albumin space and albumin partition likewise were not significantly altered.

TABLE I

Albumin distribution in rabbits receiving pooled rabbit serum*

	Weight		Plasma volume		TEAS		Albumin partition % intravascular	
No. of studies (12)	C	E	C	E	C	E	C	E
	kg		*ml/kg*		*ml/kg*		%	
Range	3.7–6.7	3.7–6.7	25–41	28–36	72–120	78–120	32–43	30–40
Mean	4.7	4.7	31	32	83	90	37	36
± SE of mean			1	1	4	3	1	1

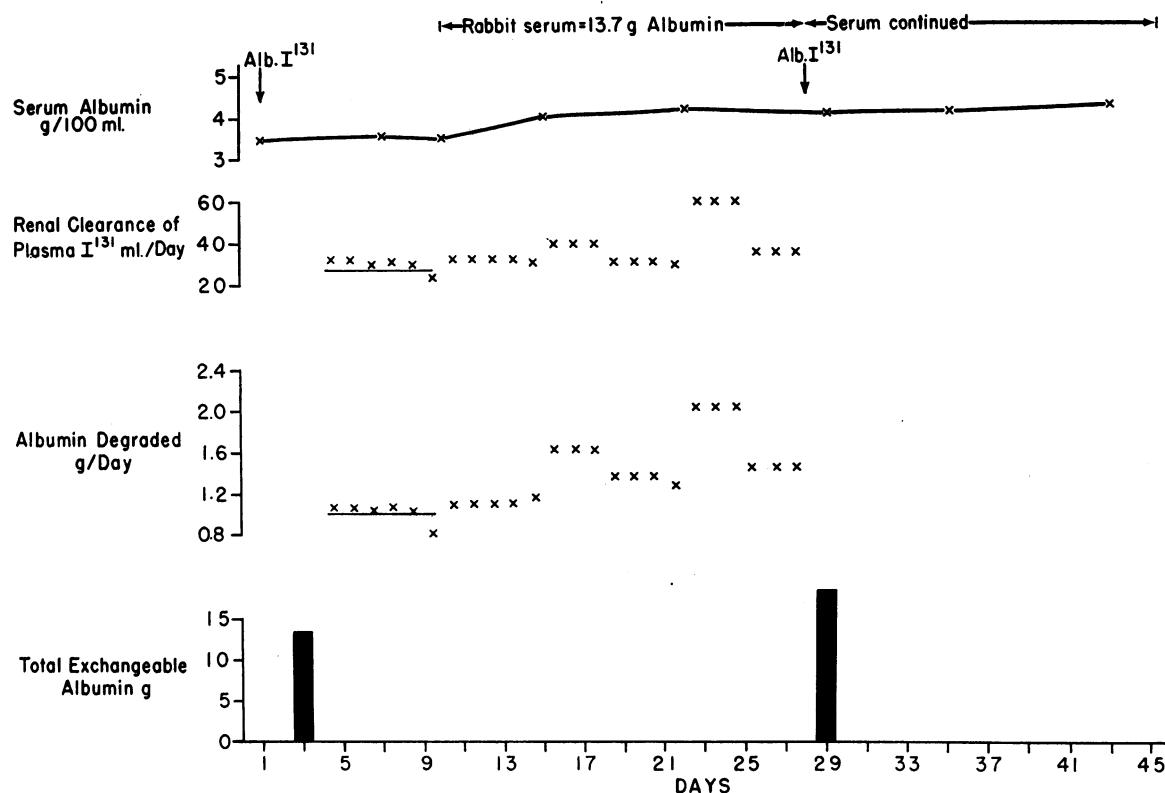* C = control; E = experimental; TEAS = total exchangeable albumin space.

FIG. 2. TYPICAL RABBIT STUDY, POOLED RABBIT SERUM. The serum albumin levels rose slightly (upper curve). The renal clearance of plasma I^{131} was not markedly affected (middle curve). Albumin degradation rose steadily (lower curve), and the total exchangeable albumin pool (bar graphs) increased by about 3 g.

The data concerning albumin metabolism are shown in Table II and Figure 2. During the serum infusions the total protein increased from 7.0 ± 0.2 to 7.6 ± 0.2 g per 100 ml due both to an increase of 0.3 g per 100 ml in the serum albumin level and to an increase of 0.3 g per 100 ml in the serum gamma globulin level. Albumin degradation increased by 46% to account for 81% of the infused albumin while the total exchange-

able albumin pool rose by 20%. Since the intravascular-extravascular albumin partition did not reveal any significant changes, the retained albumin was evenly distributed in these areas. Mean albumin synthesis was unaffected.

The data for albumin distribution in the 6 human albumin tolerant rabbits are shown in Table III. Albumin distribution was evidently not influenced by the exogenous supply of human albu-

TABLE II

Albumin metabolism in rabbits receiving pooled rabbit serum*

No. of studies (12)	Total protein		Albumin		Albumin degradation		TEA		Excess albumin degraded	Albumin infused	Net albumin synthesis
	C	E	C	E	C	E	C	E			
	g/100 ml		*g/100 ml*		*g/kg/day*		*g*		*g*	*g*	*g*
Range	6.0–7.6	6.9–8.6	3.3–4.5	3.4–5.0	0.19–0.37	0.28–0.49	11.5–17.4	13.3–24.7	5.4–16.2	11.3–18.8	−7.9–+4.2
Mean value	7.0	7.6	3.9	4.2	0.28	0.41	14.8	17.8	12.3	15.2	+0.1
± SE of mean	0.2	0.2	0.1	0.1	0.02	0.02	0.6	0.9			
Per cent change		+9		+8		+46		+20			
p value						<0.01		<0.01			

* C = control; E = experimental; TEA = total exchangeable albumin.

TABLE III

*Albumin distribution in rabbits receiving human serum albumin**

No. of studies (6)	Weight		Plasma volume		TEAS		Albumin partition % intravascular	
	C	E	C	E	C	E	C	E
	kg		*ml/kg*		*ml/kg*		*%*	
Range	2.8–4.6	2.8–4.2	29–38	26–36	72–92	68–97	36–42	30–45
Mean	3.7	3.7	33	32	82	82	39	39
± SE of mean	0.2	0.3	2	1	3	5	1	2

* C = control; E = experimental; TEAS = total exchangeable albumin space.

min, since the mean values for plasma volume, total exchangeable albumin space, and albumin partition were unaltered. The plasma volume was not significantly altered in either study. The rapid increase in plasma volume that occurred immediately after the infusions returned to preinfusion levels by the following day. After a few days of infusions, there was even a more rapid return towards preinfusion levels, and at the end of this study, no change in plasma volume was noted even though the serum albumin levels were higher than before the infusions. The data for albumin metabolism are shown in Table IV and Figure 3. The serum albumin levels rose by a mean of 15%, while albumin degradation increased by a mean of 39%. The mean total exchangeable
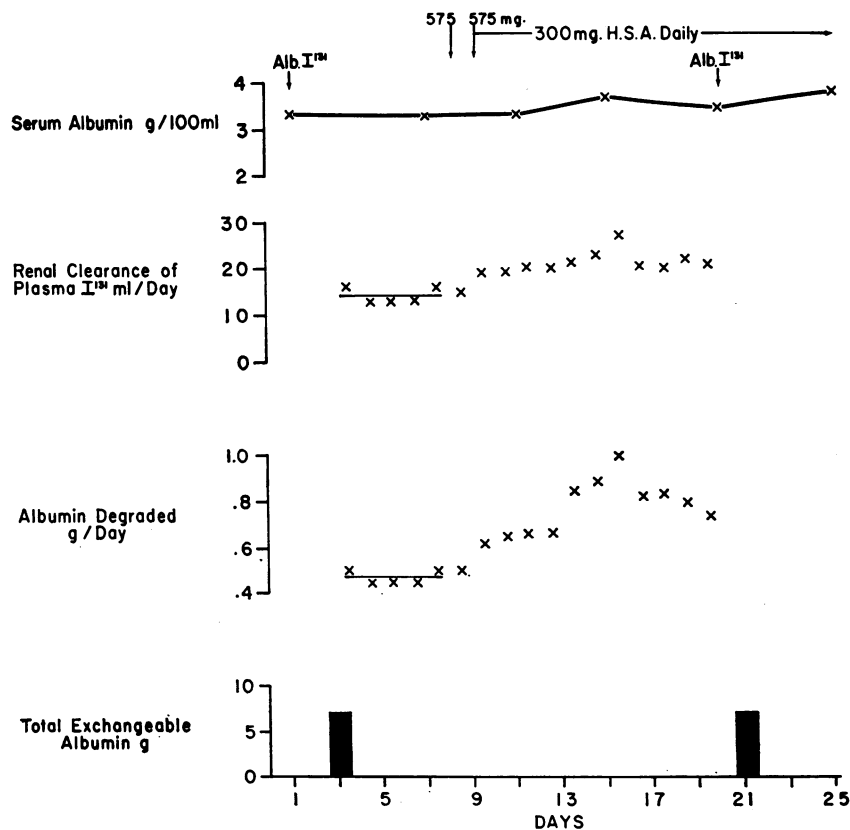

FIG. 3. TYPICAL RABBIT STUDY, HUMAN SERUM ALBUMIN. The serum albumin levels rose slightly (upper curve). The renal clearance of plasma I[131] was not markedly affected (middle curve). Albumin degradation rose steadily (lower curve), and the total exchangeable albumin pool (bar graphs) did not change.

TABLE IV

*Albumin metabolism in tolerant rabbits receiving human serum albumin**

No. of studies (6)	Total protein		Albumin		Albumin degradation		TEA		Excess albumin degraded	Albumin infused	Net albumin synthesis
	C	E	C	E	C	E	C	E			
	g/100 ml		g/100 ml		g/kg/day		g		g	g	g
Range	6.0–6.8	6.5–6.9	2.9–3.7	3.6–4.1	0.17–0.27	0.30–0.36	7.2–12.2	7.9–14.0	2.8–6.9	4.2–9.1	−5.9-+1.5
Mean value	6.3	6.7	3.3	3.8	0.23	0.32	10.2	11.3	4.1	7.2	−2.0
± SE of mean	0.1	0.1	0.1	0.1	0.02	0.01	0.2	0.9			
Per cent change		+6		+15		+39		+11			
p value				<0.01		<0.01					

* C = control; E = experimental; TEA = total exchangeable albumin.

albumin pool increased by only 1.1 g, and 58% of the infused albumin could be accounted for by the increase in degradation. Although albumin synthesis decreased in 5 of the 6 rabbits, the mean value (189 ± 16 mg per kg per day) was not significantly lower than the control rate (227 ± 17 mg per kg per day) (p value between 0.1 and 0.2). Human albumin I^{131} was reinjected into these rabbits after the completion of the study, and no loss of tolerance could be demonstrated.

The fractional turnover rate of albumin I^{131}, calculated from the ratio, albumin degraded per day/mean value of the control and experimental TEA, was increased above the control rates in both studies. During the serum infusions the turnover rate rose from 8.9 to 11.8% per day, and during the albumin study from a mean of 8.3 to 11% per day.

Discussion

The intravenous administration of homologous serum containing an amount of albumin equivalent to about one-half or more of albumin synthesized normally was continued for 5 to 6 weeks without any untoward reaction. Since the labeled albumin was obtained and separated from the same lots of serum that were infused, data could be obtained concerning the development of antibodies against the infused albumin. Repeated injections of RSA I^{131} after the period of study failed to show either a primary or an accelerated immune response as might have been expected if the infused albumin had been foreign (11). Although the fractional rate of loss of I^{131} albumin was increased during the infusion period, the rates did not approach those usually observed after the development of antibodies (11). The slight in-

crease in gamma globulin levels during the serum infusions reflects either antibody production towards some constituent of the serum other than albumin or retained gamma globulin. The lack of an immune type of response after the reinjection of human serum albumin I^{131} in the human albumin tolerant rabbits indicated that the previous injections with iodinated proteins had not broken the tolerance. Weigle demonstrated that modified heterologous proteins could break the tolerant state (15), but in this study the iodination apparently did not alter the albumin enough to affect the immune mechanism.

During the infusion of serum, albumin degradation increased rapidly and achieved a rate sufficient to prevent more than a 20% increase in total exchangeable albumin pool. The increase in albumin degradation may be related to the even greater transient increases in the albumin pool that must have exceeded the value determined 24 hours after the last infusion. In addition, the increase in degradation may be related to the increase in nitrogen intake represented by the infused serum, for an increase in protein turnover has been observed after increased nitrogen intake by mouth (16, 17). The administration of human serum albumin to rabbits made tolerant to this protein resulted in a similar increase in albumin degradation that accounted for most of the infused albumin.

During the administration of serum and human albumin, albumin synthesis was not significantly lower than the control rate. These results agree with what has been noted at low albumin levels, namely, that albumin synthesis does not respond to changes in albumin concentrations per se (12, 18, 19). It had been proposed that albumin syn-

thesis may be controlled by a regulatory system responding to changes in colloid osmotic pressure; however, the infusions were isosmotic with the plasma, and a change in colloid osmotic pressure probably did not occur (12, 19). Apparently, therefore, when albumin concentrations or pool size are increased, as in the present study, or diminished as in proteinuria (18), changes in albumin degradation are predominant. In contrast, the administration of dextran or the production of persistently elevated gamma globulin levels results in a reduction in the rate of albumin synthesis rather than a change in the rate of degradation (12, 19). In the presence of an exogenous source of serum protein, albumin levels are maintained primarily by an increase in albumin degradation, indicating that albumin degradation and synthesis change independently of each other.

Summary

Albumin distribution and metabolism were studied in 12 rabbits receiving pooled rabbit serum and in 6 rabbits, tolerant to human serum albumin, receiving pooled human serum albumin equivalent to one-half to three-fourths of the rabbits' daily albumin synthesis.

Albumin distribution was not significantly influenced by either the serum or albumin infusions.

Albumin degradation increased 46% during the serum infusions to account for 81% of the albumin contained in the infused serum. The infusion of human serum albumin into tolerant rabbits resulted in a 39% increase in albumin degradation, accounting for 58% of infused albumin. In both situations albumin synthesis was not significantly lower than the control preinfusion rate.

Albumin synthesis and degradation are not interdependent. In the presence of an exogenous source of albumin, serum albumin levels are maintained primarily by changes in albumin degradation.

Acknowledgments

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