17251. Mechanism of Proteinuria. II. Identity of Urinary Proteins in the Rat Following Parenteral Protein Injection.

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In considering the proteinuria produced by Addis and associates[1] in the rat by intraperitoneal injections of protein, it seemed logical to conjecture that the urinary protein might be identical with the injected protein. Thus, after injections of bovine albumin, it was supposed that the urinary protein, appearing after large doses and some lapse of time, was largely bovine albumin. On the other hand, after injections of egg white and Bence-Jones protein, it was conjectured that the small molecules might more easily and quickly permeate the glomerular membrane, so that protein appeared in the urine rapidly and after smaller dosage.

Doubt was cast upon this desirably simple hypothesis by work recently reported[2] which showed, by the use of hemoglobin as an indicator, that administration of bovine albumin, under the conditions used by Addis, not only saturated the tubular capacity for reabsorbing hemoglobin, but also altered the glomerular permeability to hemoglobin. In contrast,[3] administration of egg white affected hemoglobin excretion in a very different manner. In this study it was possible to demonstrate a similar functional difference between the effects of bovine albumin and egg albumin upon rat serum protein excretion.

Methods. Rabbit antisera to bovine albumin, egg albumin and rat serum were prepared according to the general plan of Goettsch and Kendall.[4] Each female Belgian rabbit received 1.0 mg of antigen intravenously in a volume of 0.1 cc, 3 times a week for 6 weeks. The rabbits were then exsanguinated and the active antisera against a given antigen were pooled and stored in the frozen state. Small portions for current use were stored at 0-2°C.

For the precipitin test, 1 cc of antiserum was added to 2 cc antigen diluted with 0.85% sodium chloride. Results were read after 2 hours at room temperature (25-28°C) followed by refrigeration at 0-2°C for 17 hours. There was no cross precipitation between the antisera and heterologous antigens.

For the quantitative determinations, known amounts of antigen protein measured by the gravimetric method of Barnett, Jones and Cohen,[5] were mixed with antiserum. The precipitates which formed in several tubes with a given antigen and antiserum were pooled and washed repeatedly with 0.85% sodium chloride solution at 0-2°C. Precipitation was performed in the range of marked antibody excess, determined by the addition of antigen to the supernatant fluid and reincubation.

The washed precipitate was drained, then dissolved in a known volume of 4% sodium hydroxide. Ultraviolet light absorption of this solution was determined in a Beckmann spectrophotometer at 280 mμ.†[6] For the latter purpose, serial dilutions were prepared with 4% sodium hydroxide.

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† Use of this procedure was suggested by Dr. Erwin Haas.

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TABLE I.
Precipitin Tests Performed upon Rat Urine after Parenteral Injection of Bovine Albumin.

Rat No.	Total protein excretion, mg/24 hr	Urine dilution	Precipitin tests: Bov. alb. antiserum 0 to 4 +	Precipitin tests: Rat serum antiserum 0 to 4 +
62	842	1:24	4	4
35	1440	1:40	1	2
61	859	1:24	2	2
80	320	1:8	2	2
53	750	1:16	2	2
00	106	1:20	3	1
02	202	1:50	2	2
04	404	1:100	3	2
08	270	1:50	4	2
15	1197	1:200	4	1
17	414	1:100	3	2
21	274	1:50	4	1
25	151	1:20	3	2
30	548	1:100	4	1
35	358	1:50	3	2
50	529	1:100	3	2
65	468	1:100	3	2

TABLE II.
Precipitin Tests Performed upon Rat Urine after Parenteral Injection of Egg Albumin.

Rat No.	Total protein excretion, mg/24 hr	Urine dilution	Precipitin tests: Egg alb. antiserum 0 to 4 +	Precipitin tests: Rat serum antiserum 0 to 4 +	Urine dilution	Precip. test rat serum antiserum 0 to 4 +
38a	151	1:20	3	0		
26	158	1:20	2	0		
29	168	1:20	3	0		
31	145	1:20	3	0		
32	139	1:10	3	0		
59	188	1:6	4	0		
25	258	1:250	3	0	1:10	0
38b	88	1:100	2	0	1:10	0
67	234	1:250	4	0	1:10	0
64	195	1:200	3	0	1:10	0
51	80	1:100	4	0	1:10	0
72	192	1:200	3	0	1:10	1
20	208	1:200	4	0	1:10	1
92	139	1:150	3	0	1:10	1
24	90	1:100	3	0	1:10	1
77	18	1:20	3	0	1:10	0
52	184	1:200	4	0	1:10	0
22	225	1:200	3	0	1:10	1

As the light absorption coefficient is specific for each protein, it is necessary to determine the coefficient for each protein or mixture in constant ratio that is used. It was found that the degree of absorption for bovine albumin and egg albumin bore a linear relation to concentration, in accordance with Beer's Law. Highly variable results were obtained with the precipitates formed with rat serum antigen. This was attributed to inhomogeneity of the antigen, and this antiserum was not used for quantitative determinations.

Urine sample were tested qualitatively by precipitation with the appropriate antiserum in the zone of marked antibody excess. For quantitative determinations, precipitation was performed as described, the precipitate was dissolved in 4 cc of 4% sodium hydroxide, after washing and draining, and the light

TABLE III.
Protein Excretion in Rats after Parenteral Bovine Albumin Injections.

Rat No.	Total prot. excretion, mg/24 hr	Total prot. excretion, mg/min.	Bovine alb. excretion, mg/min.	Ratio of bov. alb. to total prot. excreted
35	1440	1.000	0.738	0.74
53	750	0.521	0.369	0.71
61	859	0.597	0.366	0.62
62	842	0.585	0.408	0.70
80	320	0.222	0.167	0.75
45	634	0.441	0.245	0.56
47	918	0.638	0.351	0.55
71	884	0.614	0.398	0.65
74	737	0.512	0.293	0.57
98	1044	0.726	0.508	0.70
00	1160	0.806	0.534	0.66
Mean	872			0.66 ± 0.05

TABLE IV.
Protein Excretion in Rats after Parenteral Egg Albumin Injections.

Rat No.	Total prot. excretion, mg/24 hr	Total prot. excretion, mg/min.	Egg alb. excretion, mg/min.	Ratio of egg alb. to total prot. excreted
3	158	0.1101	0.1000	0.91
9	168	0.1163	0.1257	1.08
31	145	0.1009	0.0923	0.92
32	139	0.0967	0.0822	0.85
38	151	0.1048	0.1090	1.04
59	188	0.1303	0.1081	0.83
95	266	0.1847	0.1712	0.93
96	201	0.1399	0.0782	0.55
07	174	0.1210	0.0939	0.78
08	288	0.2002	0.1880	0.95
10	252	0.1750	0.1782	1.02
14	310	0.2153	0.2295	1.06
Mean	203			0.91 ± 0.10

absorption measured. All determinations were performed in duplicate, and many in quadruplicate on more than one occasion. Total protein concentrations in the urine were determined by the biuret method.[7]

In studying the proteinuria following bovine albumin injections, we followed the same routine described by Addis and used in our previous work. Female rats weighing about 200 g received intraperitoneal injections of 6% bovine albumin in 0.85% sodium chloride solution. The injections, each of 16 cc, were given at 9:30 A.M., 4:30 P.M, and 9:30 A.M. on the next day. At the time of the last injection they were taken from stock diet and given 10% dextrose solution in 0.4% sodium chloride, in order to promote diuresis. Urine was collected from 10:00 A.M. to 3:00 P.M.

Because of differing dose-time relationships, in the experiments with egg albumin it was necesary to use other conditions in order to collect urine at the height of proteinuria. Female rats of similar size were taken from stock diet at 3:30 P.M. and were given a single intraperitoneal injection of 6% egg albumin in 0.85% sodium chloride solution. These rats were then placed on the dextrose-salt solution diet and urine was collected until 9:30 A.M. the next morning. These rats appeared edematous but otherwise well, with large urine volumes.

Results. The results are given in Tables I to IV. It is readily seen that, after injections of bovine albumin, appreciable amounts of rat serum protein are also excreted. On the contrary, after injections of egg albumin, nearly all of the urinary protein is egg albumin, so that qualitatively weak positive reactions to rat serum antiserum are encoun-

[7] Kingsley, G. R., *J. Biol. Chem.*, 1939, **131**, 197.

tered only in very low dilutions, and, quantitatively, the amounts of rat serum protein appearing (calculated by difference) are within the order of magnitude for normal protein excretion.

The data presented here cannot answer whether rat serum protein is excreted along with bovine albumin as the result of diminished tubular reabsorption or the result of increased glomerular permeability or filtration rate. Such information might be obtainable from further investigation under different conditions, varying the rates of protein excretion over a wider range. However, the data given do demonstrate a gross difference in the mechanism of proteinuria following administration of bovine albumin and egg albumin, proteins that differ widely in structure. This difference is consistent with differences found in the effect produced by intraperitoneal injections of these proteins upon hemoglobin excretion in the rat,[2,3] and is of special significance in that the data given here were obtained by an independent experimental approach.

Summary. 1. Parenteral injection of egg albumin in the rat produces proteinuria composed of egg albumin, with no appreciable excretion of rat serum protein.

2. After parenteral injection of bovine albumin, large quantities of rat serum protein are excreted in addition to bovine albumin.

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17252. Some Observations on Growth Factors Required by *Leuconostoc citrovorum*.*

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The presence of a growth factor for *Leuconostoc citrovorum* 8081 in various natural materials was reported by Sauberlich and Baumann.[1] They noted that the organism would respond to thymidine but it was concluded that some other active factor was present in a liver concentrate. The "citrovorum factor" in liver extract was differentiated[2] from a factor active for *Lactobacillus leichmannii* in liver extract by observing that the two factors migrated in opposite directions in an electric field. In the present report, further observations of the characteristics of the "citrovorum factor" are described.

Experimental. Lactobacillus leichmannii 313 (ATCC 7830) and *Leuconostoc citrovorum* 8081 were used in this study. Methods for the use of *Lactobacillus leichmannii* have been previously described;[3-5] the assay technic with *Leuconostoc citrovorum* followed that of Sauberlich and Baumann.[1]

The data of Table I indicate the response of the test organism to concentrated liver extract (15 U.S.P. units per cc), vitamin B_{12} and thymidine before and after heating with alkali. *Lactobacillus leichmannii* gave heavy growth with only 0.03 μl of untreated liver extract, but after treatment with alkali much higher levels of liver extract were required to promote good growth of the organism. As noted elsewhere this alkali treatment destroys the growth-promoting action of vitamin B_{12}

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