Proteinuria after Albumin Infusion in Patients with Renal Disease* (33465)

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The pattern of urinary protein excretion has recently been studied with increased accuracy by an immunoprecipitation technique (1). In the present study, this technique was used to evaluate the results of intravenous albumin infusion on the proteinuria of patients with renal disease. The results were similar to earlier studies using electrophoretic methods (2), and these results have been interpreted in the light of recent evidence in an attempt to define the role of the glomeruli and tubules in the excretion of protein in patients with renal disease.

Methods. The study was carried out in nine patients with the nephrotic syndrome whose kidney biopsies were interpreted as glomerulonephritis of the membrane type (four patients), the minimal type (three patients), proliferative type (one patient), and amyloidosis (one patient). Twenty-five g of salt-poor human serum albumin was given into a forearm vein in 1–2 hr. The urine collection periods were begun before the infusion and continued for up to 24 hr. Heparinized blood samples were obtained at the end of each urine collection period.

Total urine protein was determined by a modification of the method of Hiller *et al.* (3). The immunoprecipitation technique was performed as described by Soothill (1) and previously reported in detail from this laboratory (4). The ratio of urine concentration to plasma concentration (U/P) of six protein fractions was determined using specific antisera to the following proteins: orosomucoid, albumin, transferrin, seven-S, gamma-A, and alpha-2-M. The clearance for each protein fraction was expressed as percentage of transferrin, as there was less variability in the immunological behavior of this protein and antisera (5). Further, by comparing one clearance to another the rate of the urine flow (V) is canceled, leaving U/P ratios, i.e., the concentrations of the test proteins to transferrin. When the renal clearances of these several protein fractions relative to transferrin were plotted on log–log paper against their molecular weights a linear relationship was found and a straight line was calculated by the method of least squares. The slope (K) of this line is an index of the selectivity of the kidney in excreting proteins and is expressed as the angle made by this line with the abscissa and is expressed in degrees or theta (θ). If the relative clearances of the small protein fractions predominate over the heavy molecular weight proteins, the selectivity of protein excreted is said to be high; if heavy molecular weight proteins predominate the slope is low, the angle shallow and the renal filter is considered nonselective (Fig. 1).

In three patients serum concentrations of transferrin, seven-S, gamma-A, and gamma-M were determined in mg/100 ml by measuring and comparing the diameter of the diffusion area in agar plates impregnated with antisera to a semilog calibration curve. This was drawn by using known amounts of the pure protein (Hyland Lab.). Human serum albumin from Pentex Inc. was used as standard for the serum albumin determination.

Results. In Table I, the angle of selectivity or theta and total urine protein excretion before and after intravenous albumin infusion are shown with the co-efficient of correlation (r) between the molecular weight and the relative protein clearances in any one patient. The maximum excretion of total pro-

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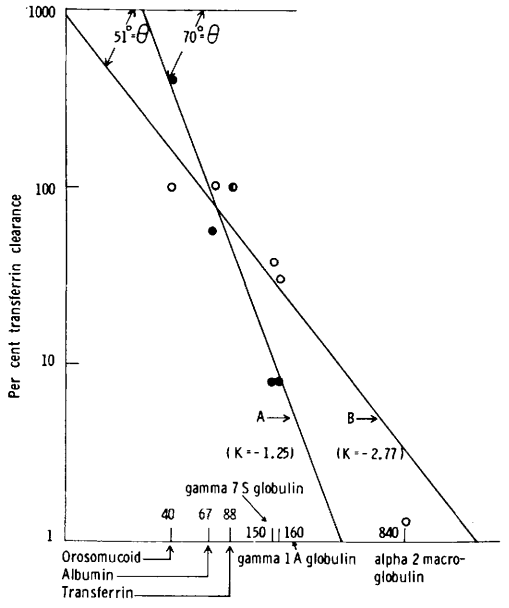


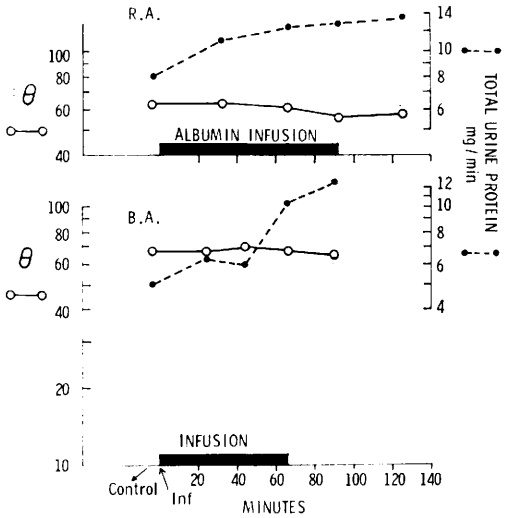
FIG. 1. The urine protein clearances as percentage of transferrin clearance plotted on a log-log graph against their respective molecular weights. The angle (θ) made by the integration line of the open circles with the abscissa (51°) shows a low renal selectivity for proteins. The solid dots reveal a high selectivity (70°).

tein after the infusion of albumin was significantly higher ($p<.01$) than the control values, and there was no difference in r ($p<.8$) or the selectivity pattern ($p<.8$) of the protein excreted. In all patients the clearance of each of the four protein fractions making up the selectivity pattern did not vary between pre-/and postalbumin infusion. For example, orosomucoid: $p<.4$; albumin: $p<.5$; seven-S: $p<.7$; and alpha-2-M: $p<.7$.

In Figs. 2 and 3 the constancy of the selectivity pattern with variation in the total protein excretion is shown in three patients, before, during and after the albumin infusion. Table II shows the increased albumin concentration in plasma after its infusion, while the concentration (mg/100 ml) of transferrin, seven-S, gamma-A and gamma-M did not change significantly.

Discussion. In the studies reported here, the selectivity pattern of urinary proteins did not change by acutely increasing the serum albumin concentration. Further, there was an increase in all protein fractions excreted the urine following intravenous albumin ad ministration.

Proteinuria has been attributed to an increased glomerular permeability and/or failure of tubular reabsorption of proteins filtered at the glomerulus. Other possible causes of proteinuria include abnormal proteins, proteins arising in the lower genitourinary tract and abnormally increased concen-



FIGS. 2 and 3. These figures show the constancy of the renal selectivity of specific protein excretion during and after albumin infusion irrespective of changes in total urine protein loss.

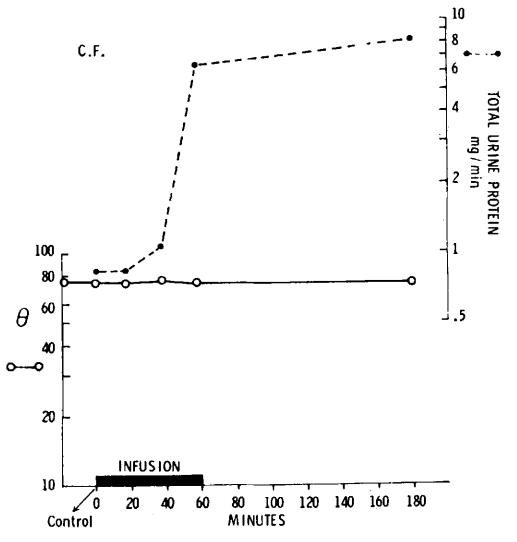


TABLE I. Values before and after Intravenous Albumin Infusion.[a]

No.:		1	2	3	4	5	6	7	8	9	
Patients:		RA	MS	GU	PE	RL	BA	DK	CP	CF	p values
θ (°)	Pre	63	61	57	52	54	67	61	64	75	$p < .8$
	Post	61	63	56	58	51	65	60	61	74	
r	Pre	—.98	—.99	—.89	—.75	—.98	—.98	—.95	—.93	—.98	$p < .9$
	Post	—.94	—.97	—.91	—.96	—.97	—.89	—.96	—.90	—.95	
U prot V	Pre	8.0	13.0	7.5	3.5	8.8	5.2	5.2	13.4	.84	$p < .01$
(mg/min)	Post	12.0	25.0	15.3	7.1	30.4	12.4	10.3	23.6	7.20	

[a] Pre = prealbumin infusion values; Post = postalbumin infusion values; θ = renal selectivity for protein excretion expressed in degrees; r = correlation coefficient for the log–log relationship between clearance of proteins as percentage of transferrin clearance and their molecular weights; U prot V = total urine protein excretion (mg/min).

trations in the plasma of normal plasma proteins.

The theory of increased glomerular permeability is based on: (i) the observation of "pores" in the basement membrane of the glomerular capillaries, although this is not generally accepted (6); and (ii) the inverse relation of renal excretion of Dextrans of varying molecular weights (7), i.e., the higher the molecular weight the less the excretion.

In support of the theory of tubular rejection of proteins as a cause of proteinuria there are several lines of evidence: (i) micropuncture data of proximal tubular fluid in normal and uranium proteinuric rats (8, 9) revealed no difference in protein concentrations; (ii) perfusion of the rabbit kidney with cooled serum allowed proteinuria presumably by inhibiting transfer processes (10); (iii) infusion of serum proteins into animals and normal subjects results in proteinuria possibly due to overloading a transport mechanism (11, 12); (iv) in man, if we concede that proximal tubular urine contains as little as 5 mg/100 ml protein, then in 24 hr approximately 7.5 g would be filtered and almost quantitatively reabsorbed to account

TABLE II. Plasma Protein Concentrations (mg/100 ml) before and after Infusion of Albumin.

Time after inf. of alb.	(mg/100 ml) Alb.	Transf.	7S	A	M	U prot. V (mg/min)
Patient						
Control	490	40	600	205	135	7.5
6 hr 25 min	1568	40	600	205	105	14.6
11 hr	1960	28	600	154	105	15.3
24 hr	2355	40	600	145	115	12.8
Patient C.P.						
Control	1176	84	195	178	115	13.4
6 hr	1568	76	143	165	100	23.6
30 hr		105	200	178	105	15.8
42 hr	1958	105	200	178	105	10.4
Patient C.F.						
Control	1568	80	115	100	190	.84
17 min	1960	80	88	80	190	.83
37 min	1960	72	115	74	190	1.07
57 min	3136	64	100	70	175	6.45
3 hr	2038	64	110	86	190	7.20

for the normal of less than 100 mg/day excreted in normal man. This calculation is based on the work of Dirks *et al.* (13) who using immunochemical methods to determine protein concentrations in glomerular filtrate of dogs, noted concentrations of less than 8 mg/100 ml.

If the theory of glomerular pores is valid, we would expect after increasing the serum albumin concentrations to find a similar increase in urine albumin without a change in the heavy molecular weight proteins in the urine. This was not the case, however, as heavy molecular weight proteins were excreted.

However, in Table II there is no statistical difference ($p>.8$) between the urine protein selectivity before and after the infusion of albumin. Moreover the total protein excretion after the infusion of albumin is significantly higher ($p<.01$) than before the infusion, which is due to an increase in all proteins, both low and heavy molecular weight (Table II). As the serum concentration of albumin increased without a similar increase in the globulin concentration it is unlikely that the increased urinary excretion of heavy proteins was due to an increase in the "pore" size of the glomerulus.

In 1952, Chinard (14) drew conclusions based on thermodynamic considerations, that the concept of flow through glomerular pores is not applicable to the passage of proteins through glomerular "pores" and that *diffusion* across the capillary wall is more likely. Electronmicroscopic studies support this concept (15, 16).

If proteins pass through the glomerulus by diffusion, they must be in a fixed pattern and similarly reabsorbed by the tubules so that changes in plasma protein concentration do not influence the relative concentration of proteins in the glomerular filtrate. Further, this is not changed by the tubular reabsorption, but the increased excretion of total protein is the result of a change in glomerulo–tubular balance.

Summary. Studies were carried out in nine patients with massive proteinuria. The selectivity of the kidney in excreting six protein fractions was determined by an immunoprecipitation technique using specific antisera to orosomucoid, albumin, transferrin, seven-S, gamma-A, and alpha-2-M. These patients were given an intravenous infusion of salt-poor albumin and while the total amount of protein in the urine did increase, the protein selectivity pattern was constant before, during, and after the albumin infusion. While the exact mechanism of this proteinuria is not understood, possible explanations are given, with the net effect probably resulting from a critical glomerular–tubular balance. This balance has to do with glomerular loss of protein and tubular reabsorption or rejection of the filtered protein.

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