Hyperoncotic Albumin Infusion in Experimental Glomerulonephritis in Rats: A Micropuncture Study

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The effect of infusion of hyperoncotic (25 g% salt-poor bovine plasma albumin) (0.06 g% of body weight) has been studied using clearance and micropuncture techniques in animals with two forms of immunologically induced experimental glomerulonephritis and in age-matched control rats.

In control rats the significant natriuresis and diuresis after hyperoncotic albumin were associated with decreased fractional and absolute proximal reabsorption, decreased calculated efferent oncotic pressure, and a transitory rise in efferent arteriolar hydrostatic pressure. These findings, however, do not necessarily indicate a predominant role for peritubular "physical factors" in the control of proximal reabsorption.

In glomerulonephritic rats a smaller diuresis and an insignificant natriuresis were found after hyperoncotic albumin, especially in anti-GBM nephritis, when no change in SNGFR or proximal absolute reabsorption occurred. In AICN there was a rise in SNGFR and a fall in absolute reabsorptive rate, especially in those nephrons with high filtration rates. There was no evidence that any alteration in efferent arteriolar hydrostatic pressure or calculated efferent oncotic pressure had occurred.

Clinicians have long been familiar with the ability of hyperoncotic salt-poor albumin infusion to result in a significant, though transient, natriuresis and diuresis in patients with hypoproteinemia and edema (1–3). An initial rise in the plasma albumin concentration has been found to accompany this diuresis, the largest increases in albumin concentration being seen in those patients having the largest diuresis (2). At first sight such an observation might seem to be at variance with current concepts regarding the role of "physical factors" (oncotic and hydrostatic pressure) in determining the rate of tubular reabsorption (4, 5). Thus, a rise in peritubular oncotic pressure might be expected to be associated with an increase in proximal tubular reabsorption and an antidiuretic effect.

Therefore, we have studied the acute effects of infusion of salt-poor hyperoncotic albumin in rats with two forms of immunologically induced experimental glomerulonephritis and in control animals, using clearance and micropuncture techniques. The largest diuretic and natriuretic effect was found in the control rats, where it was associated with a fall in both fractional and absolute proximal tubular reabsorption, a fall in calculated efferent oncotic pressure, and a small rise in efferent arteriolar hydrostatic pressure. No such changes were found in efferent ar-

teriolar oncotic or hydrostatic pressure in either form of glomerulonephritis, although a significant diuretic response was seen in both disease states, and in AICN a significant fall in proximal absolute reabsorptive rate was also found. Hence, there was no evidence that peritubular "physical factors" were important in the response to hyperoncotic albumin infusion in glomerulonephritis.

METHODS

Autologous immune complex or Heymann's nephritis (AICN) (6–8) was induced in 10 male Wistar rats by repeated injections of partially purified rat renal tubular antigen emulsified in complete Freund's adjuvant (9). Antiglomerular basement membrane (GBM) antibody induced glomerulonephritis was produced in five male Wistar rats by intravenous injection of 200–275 μg of rabbit antirat GBM antibody (9). The AICN rats were studied between 5 and 20 months after the first injection of antigen, whereas the anti-GBM group was studied 10–38 days after injection of the heterologous anti-GBM antibody. Eight large male Wistar rats, age matched with the AICN animals, and three smaller animals of similar age to the anti-GBM group were used as controls.

Clearance and micropuncture studies were carried out for approximately 2½ hr before, during, and 1½ hr after infusion of hyperoncotic (25 g%) salt-poor bovine plasma albumin (0.06 g% of body wt) (Armour Pharmaceutical Co., Chicago, Ill. 60690).

All animals were deprived of food but not of water overnight before study and were anesthetized by intraperitoneal injection of sodium pentobarbital, 60 mg/k body wt. They were prepared for micropuncture as previously described (10), isotonic saline 0.5% of body wt being given during surgery to replace fluid losses. Both ureters were cannulated with PE 10 polyethylene tubing. Blood pressure was recorded from the carotid artery using a Statham P 23 Db transducer (Statham Instruments, Inc., Oxnard, California) and a Beckman Dynograph Recorder (Beckman Instrument, Inc., Fulton, California).

Intravenous infusion was commenced using modified Ringer's solution (NaCl 0.8 g/100 ml; KCL 0.042/100 ml; $CaCl_2$ 0.025 g/100 ml; $NaHCO_3$ 0.02 g/100 ml), given intravenously at the rate of 0.4 ml/100 g body wt/hr, together with d-aldosterone (Ciba Pharmaceutical Co., Summit, N.J.), vasopressin (Parke, Davis and Co., Detroit, Michigan), H^3 methoxy inulin (International Chemical and Nuclear Corp., Burbank, California), and in some instances C^{14} p-aminohippurate (PAH) (New England Nuclear Corp., Boston, Mass.). In those rats in which intrarenal pressure measurements were made, a priming dose of 3 μg of d-aldosterone, 60 μc H^3-inulin, and 2 μc PAH was followed by infusion of d-aldosterone, 2.4 μg/hr, vasopressin 0.03 μg/hr, C^{14} PAH 5–7 μc/hr, and H^3-inulin at the rate of 20 μc 100 body wt^{-1} hr^{-1}. When proximal tubular fluid (F/P) inulin levels were to be measured, the latter was usually increased to 40–80 μc H^3-inulin body wt^{-1} hr^{-1}.

Three consecutive timed urine collections, each lasting approximately 45–60 min, were made from both kidneys. The intravenous infusion was then changed to one containing 25 g% salt-poor bovine plasma albumin (sodium concentration 120.4 ± 11.9 SD mM/liter) but otherwise identical to the previous infusion in terms of calcium, bicarbonate, d-aldosterone, vasopressin, H^3-inulin, and C^{14} PAH content. This solution was infused at the rate of 0.4 ml 100 g body wt^{-1} hr^{-1} until a total of 0.06 g% of body wt of albumin had been given, generally over 30–40 min. A fourth urine collection was obtained during this period. The original infusate was then recommenced, and two 45–60-min collections made. There were thus a total of six

consecutive timed urine collections, three before, one during, and two following hyperoncotic albumin. Blood (less than 30 μl) was obtained from the carotid artery and left renal vein at approximately 45-min intervals for measurement of hematocrit, protein, H^3-inulin, and C^{14} PAH levels. Clearances were determined as previously described (11). Glomerular filtration rate (GFR) was calculated separately for the left and right kidneys, renal plasma flow (RPF) for the left kidney only.

In those rats not given C^{14} PAH, the left RPF was calculated as follows: RPF = $[V(U - R)]/(A - R)$, where V = urine flow rate and U, A, and R = inulin concentration in urine, arterial plasma, and renal venous plasma, respectively. In those rats given C^{14} PAH, RPF was calculated as follows: $(C\ PAH)/(E\ PAH)$, where C = clearance and E = extraction.

Two groups of micropuncture experiments were carried out. In the first series (five AICN, five anti-GBM, and six control animals), the kidney was bathed with mineral oil heated to 36 ± 1°C. The last loop of a proximal tubule visible on the surface of the kidney was identified by following the passage of lissamine green-colored saline injected through a sharpened siliconized pipette, external tip diameter 4–6 μm, inserted into a superficial proximal convolution. The last convolution was then punctured using a sharpened siliconized pipette, external diameter 11–12 μm, and a mineral oil block 4–5 tubular diameters in length was injected. Tubular fluid was then collected, controlled suction being used to keep the oil block in place and the tubular diameter constant if at all possible. The sample was pulled into the straight part of the collection pipette using toluene, the length and diameter measured using a micrometer and the volume calculated. The whole sample was then discharged into counting fluid (PCS, Amersham/Searle, Arlington Heights, Ill.) and counted using a Packard Tricarb liquid scintillation counter.

The following calculations were made:

$$SNGFR = F/P\ \text{inulin} \times \text{tubular flow rate in nanoliters/minute},$$

$$\%\ \text{reabsorption to the late convolution} = \{1 - [1/(F/P\ \text{inulin})]\} \times 100,$$

$$\text{absolute reabsorption} = \text{fractional reabsorption} \times SNGFR\ \text{in nanoliters/minute}.$$

In the second group (five AICN, five control rats), the kidney was bathed with isotonic saline 36 ± 1°C in order to allow the use of an electronic servo-nulling device for measurement of hydrostatic pressure as previously described (12). Sharpened siliconized glass pipettes, external tip diameter 3–5 μm, filled with 2 M NaCl, were used to measure hydrostatic pressure in proximal tubules during free-flow, in the large efferent arteriolar "stars," in intermediate vessels, which are large-diameter straight vessels and which often arise from stars, and in the larger peritubular capillaries.

Serial plasma protein concentrations were measured before, during, and at the end of each experiment by an adaptation of the Lowry technique (13) using a rat serum protein standard. These values were used to calculate oncotic pressure by the Landis and Pappenheimer equation (14):

$$\pi a = 2.1\ Pa + 0.16\ Pa^2 + 0.009\ Pa^3.$$

This equation holds for rat protein concentrations estimated in this laboratory and has been validated by measuring directly the serum oncotic pressure (G. Navar, University of Mississippi Medical Center, Jackson, Mississippi, and W. Arendshorst, University of North Carolina, Chapel Hill).

Efferent arteriolar protein concentration was calculated using the whole kidney filtration fraction (FF):

$$\text{efferent arteriolar protein concentration} = \text{afferent arteriolar protein}$$
$$\text{concentration}/(1 - \text{whole kidney FF}).$$

Blood was taken terminally for blood urea nitrogen determination (BUN), measured on a Technicon autoanalyzer (Technicon Corp., Ardsley, New York) by a modification of the carbamidodiacetyl fraction as applied to the determination of urea nitrogen and cholesterol. Urine plasma electrolytes were measured using an IL direct-reading flame photometer (Instrumentation Laboratories, Inc., Watertown, Mass.). Fractional electrolyte excretion was calculated without use of the Donnan factor. Determination of 24-hr urine protein concentration measured immediately before study was made using either the Biuret technique or salicylsulphonic acid.

Results have been analyzed on a per rat basis using the paired Student's t test and by analysis of variance.

Blood Volume Measurement

After the last clearance period was completed, the blood volume of the rat was measured in seven AICN animals, four anti-GBM animals, and six control rats using sodium chromate Cr 51-labeled red blood cells (Rachromate 51, Abbott Radiopharmaceutical, N. Chicago, Ill. 60064). Blood was obtained from a male Wistar rat, anticoagulated with A-C-D solution (Abbott Radiopharmaceutical), and incubated with chromium 51 at room temperature for 30 min. The red cells were then washed twice and resuspended at a hematocrit of 50% in isotonic saline. An accurately measured volume of this solution was then injected intravenously, and a blood sample was obtained after a further 30 min. The sample was counted in a gamma counter, and the blood volume was calculated knowing the relative specific activities of the samples and the hematocrit.

IMMUNOPATHOLOGIC STUDIES

At the end of each experiment, both kidneys from each animal were weighed and bisected along the anterior–posterior axis. The halves were either fixed in Bouin's solution for histological study or snap frozen (in liquid nitrogen or dry ice-alcohol bath) for immunofluorescence studies as previously described (9). The immunofluorescence studies utilized fluorescein isothiocyanate-conjugated antiserum specific for rat IgG and C_3 also as previously described (9). The histologic sections were examined and graded for the following categories: glomerular hypercellularity, necrosis, sclerosis, crescent formation; GBM thickening; overall architectural derangement; interstitial infiltration; tubular atrophy, luminal cells, casts; and thickening of tubular basement membrane.

RESULTS

Structural and functional changes in the kidneys of the animals with glomerulonephritis were similar to those previously described by us in a similar group of animals (9). Histologically, AICN was typified by uniform thickening of the glomerular basement membrane (GBM), minimal glomerular hypercellularity, and granular deposits of IgG and C3 diffusely along the GBM (Fig. 1). Anti-GBM nephritic kidneys showed increased glomerular cellularity, polymorphonuclear leucocyte infiltration, and crescent formation together with linear deposits of IgG and C_3 along the GBM (Fig. 1). Although there was some individual variation among
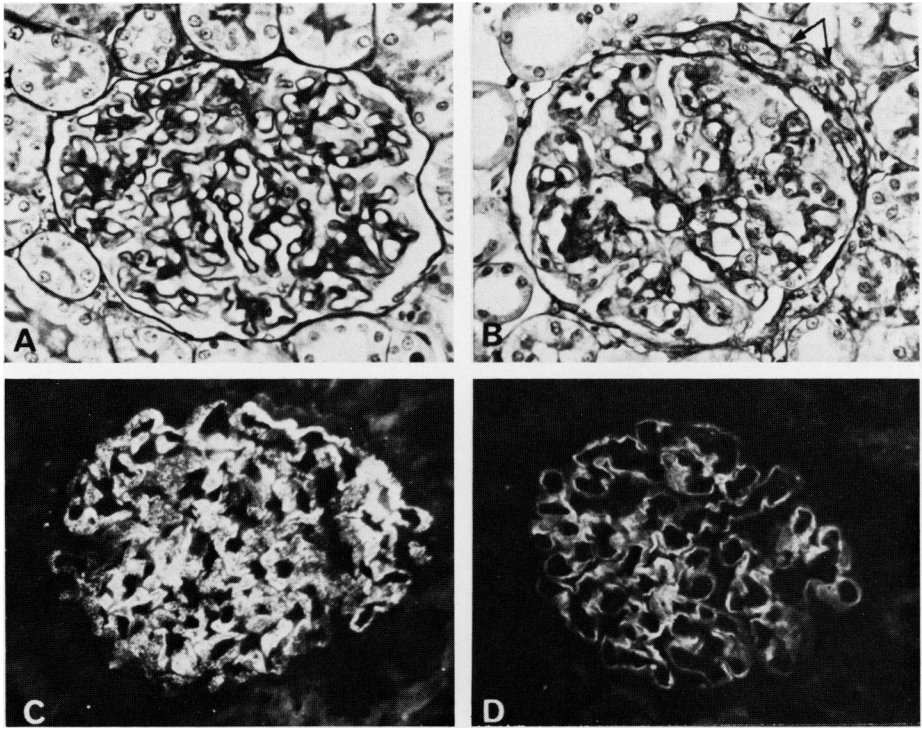
FIG. 1. (A) Uniform thickening of the GBM in the absence of proliferative changes typified the membranous glomerulonephritis that characterized the glomerular lesions of the AICN rats. (B) Proliferation of endothelial and epithelial cells characterized the proliferative glomerulonephritis induced in the anti-GBM rats. An area of proliferation of the epithelial cells of Bowman's capsule, so-called crescent formation, is indicated by the arrows. (C) Innumerable fine granular deposits of rat immunoglobin (accompanied by complement C3) were observed uniformly along the GBM of the AICN rats. (D) Linear accumulations of rat immunoglobulin (accompanied by complement C3 and rabbit immunoglobulin) were observed outlining the GBM several days after the anti-GBM rats received rabbit anti-rat GBM antibodies.

Figure 1A, 1B periodic acid-Schiff (PAS) stain, original magnification ×320. Figure 1C, 1D direct immunofluorescence using fluorescein isothiocyanate-conjugated rabbit anti-rat IgG antisera, original magnification ×250.

glomeruli, the severity of glomerular damage was uniform throughout the cortex of each individual kidney in both of these disease states.

The glomerular lesions in both forms of glomerulonephritis were accompanied by variable changes in the renal tubules and interstitium leading to different degrees of derangement of renal architecture. The overall severity of the renal lesion corresponded to the degree of glomerular involvement and was much slower in developing in the chronic membranous glomerulonephritis of the AICN rats than in the acute proliferative, often crescent-forming, glomerular lesions induced by anti-GBM antibodies (9).

Table 1 compares the 24-hr urinary protein excretion rate, initial plasma protein concentration, hematocrit, cholesterol, and BUN in 10 AICN animals, five rats with anti-GBM nephritis, and a total of 11 age-matched controls. The latter have been divided into two groups, comparable in age and weight to the AICN and anti-GBM nephritic animals. All the glomerulonephritic rats had very significant pro-

TABLE 1

Proteinuria, Initial Plasma Protein Concentration and Hematocrit, and Terminal Cholesterol and
BUN Concentrations in Control and Glomerulonephritic Rats (Mean ± SD)

	AICN $n = 10$	p	AICN controls $n = 8$	Anti-GBM $n = 5$	p	Anti-GBM controls $n = 3$
Body weight (g)	665	NS	658	275	NS	292
	±53		±91	±50		±48
Proteinuria (mg/24 hr)	440	<0.001	15.6	354	<0.001	18.0
	±211		±18	±141		±4
Plasma protein (g%)	5.2	NS	5.6	4.7	NS	5.0
	±0.4		±0.4	±0.9		±0.7
Hematocrit (%)	44.5	NS	46.8	42.9	NS	51.0
	±4.3		±2.5	±6.8		±4.3
Cholesterol (mg%)	194	<0.005	72.4	194	NS	82.3
	±78		±12.9	±92		±15
BUN (mg%)	30.6	NS	26.7	44.8	NS	36.7
	±8.9		±9.7	±17.8		±9.0

teinuria, but neither group was significantly hypoproteinemic. None was edematous. All had hypercholesterolemia. The mean BUN concentration was not significantly elevated in either group.

Clearance Studies

Clearance data are given for the left (L) kidney only, there being no statistical difference between the right (R) and (L) GFR.

Figure 2 shows the mean values ± SD for urine flow rate, sodium excretion, GFR, RPF, and FF during the three periods prior to albumin, during albumin infusion, and for the two periods immediately thereafter.

The mean values for urine flow rate, U/P inulin concentration, GFR, RPF, filtration fraction, and electrolyte excretion for the control and glomerulonephritic rats before albumin administration (Tables 2 and 3) are similar to those previously reported by us in a similar group of animals (9), with a significant fall in GFR and FF in both AICN and anti-GBM animals and a significant rise in RPF in the more acute anti-GBM nephritis.

All animals then received 0.06 g% body wt of albumin iv as a 25 g% solution during the fourth clearance period. Mean hematocrit fell 11.3% in large control rats, 6% in small control animals, 8.5% in AICN, and 7% in anti-GBM nephritis. Plasma protein concentration increased significantly in all groups during albumin infusion (Period 3 vs Period 4). However, the mean plasma protein concentration in the three periods before albumin infusion did not differ from that in the three periods during and after albumin infusion in any of the groups (Fig. 3). A significant fall occurred in plasma protein concentration in the large control rats and in animals with AICN during the initial pre-albumin-clearance periods. A corresponding significant fall in hematocrit was not observed.

In all control rats a significant increase in urine flow rate (average 185%) and in absolute and fractional electrolyte excretion occurred, the maximum effect being seen 45–90 min after starting the albumin infusion (Fig. 2). A similar, very significant rise in urine flow rate (average 129%) and a significant, although less marked, natriuretic response was found in rats with AICN. The smallest diuretic and natriuretic effects were seen in animals with anti-GBM nephritis, urine flow rate increasing on average by 103%.
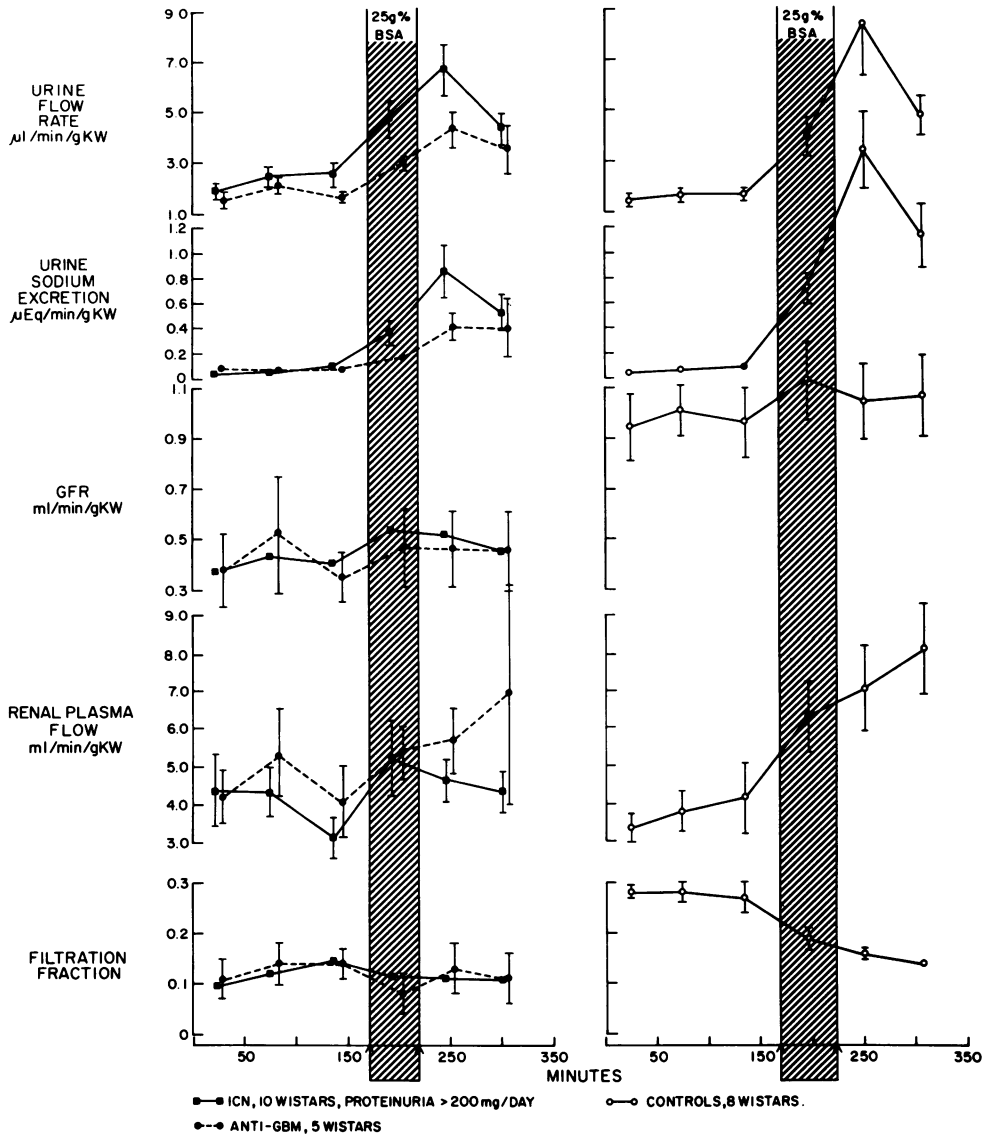
FIG. 2. Results before, during, and after infusion of bovine serum albumin.

There was no significant change in mean GFR after albumin in either the larger, older (AICN) or the smaller, younger (anti-GBM) control rats. Renal plasma flow increased in both, but this reached statistical significance only in the larger rats. Hence, whole kidney filtration fraction fell significantly only in this group of control rats. In AICN both GFR and RPF increased significantly after albumin, FF remaining unchanged. No significant change in GFR, RPF, or FF was seen in anti-GBM nephritis.

The extraction of PAH was measured in five out of 11 control rats and fell significantly after albumin from 0.87 ± 0.01 SD to 0.60 ± 0.10 SD ($p < 0.001$). A fall was also seen in rats with both AICN and anti-GBM nephritis (0.67 ± 0.12 SD to 0.56 ± 0.06 SD $n = 4, p < 0.025$).

TABLE 2

Clearance Data (Left Kidney) in Control and Glomerulonephritic Rats Before (B), and During and After (A) Hyperoncotic Salt-poor Albumin (Mean ± SD)

	Urine flow rate (μl min^{-1} g kidney wt^{-1})		U/P inulin		GFR (ml min^{-1} g kidney wt^{-1})		RPF (ml min^{-1} g kidney wt^{-1})		Filtration fraction	
	B	A	B	A	B	A	B	A	B	A
AICN	2.3	5.2[a]	223	116[a]	0.41	0.50[a]	3.89	4.84[b]	0.12	0.11
(n = 10)	±1.1	±2.2	±128	±47	±0.1	±0.1	±1.3	±1.4	±0.04	±0.03
AICN	1.6	5.5[b]	626	254[d]	1.04	1.20	3.94	8.34[c]	0.28	0.16[d]
controls (n = 8)	±0.5	±2.9	±73	±110	±0.2	±0.5	±1.2	±4.4	±0.04	±0.03
Anti-GBM	1.7	3.5[c]	220	134	0.42	0.46	4.54	5.77	0.13	0.11
(n = 5)	±0.4	±1.4	±138	±94	±0.3	±0.3	±0.7	±2.4	±0.07	±0.09
Anti-GBM	2.6	5.9[c]	387	171[c]	1.00	1.06	2.94	3.75	0.34	0.31
controls (n = 3)	±0.5	±1.3	±90	±96	±0.1	±0.1	±0.2	±1.6	±0.07	±0.09

[a] $p < 0.01$, statistically significant result.
[b] $p < 0.005$.
[c] $p < 0.05$.
[d] $p < 0.001$.

Systemic blood pressure increased after albumin in all animals studied (Table 4), but this did not reach statistical significance in the anti-GBM group.

Terminal blood volume, expressed as a function of body weight, was significantly higher in rats with anti-GBM nephritis (Table 5), i.e., in those animals showing the smallest diuretic response to hyperoncotic albumin.

Micropuncture Studies

As previously reported by us (9), marked functional heterogeneity of SNGFR and proximal tubular hydrostatic pressure was present in the diseased kidneys. Mean SNGFR, late proximal fractional reabsorption, and absolute reabsorption were

TABLE 3

Electrolyte Excretion (Left Kidney) in Control and Glomerulonephritic Rats Before (B), and During and After (A) Hyperoncotic Salt-poor Albumin (Mean ± SD)

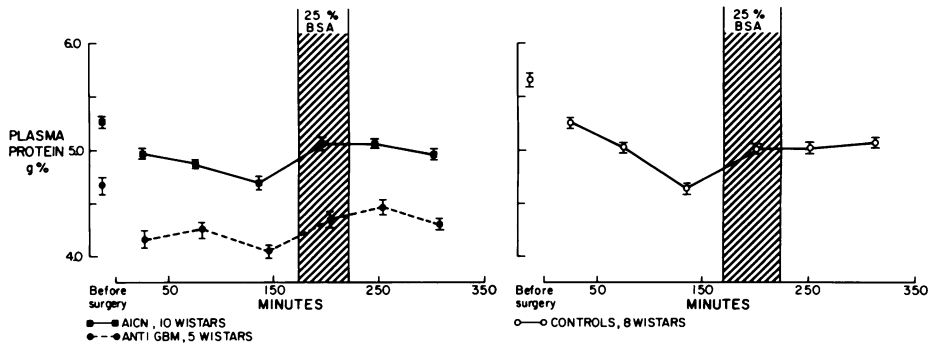|	Sodium excretion (μequiv min^{-1} g kidney wt^{-1})		Potassium excretion (μequiv min^{-1} g kidney wt^{-1})		Filtered load sodium excreted (%)		Filtered load potassium excreted (%)	
	B	A	B	A	B	A	B	A
AICN	0.09	0.58[a]	0.34	0.66[a]	0.11	0.81[a]	17.0	24.8
	±0.05	±0.41	±0.18	±0.31	±0.06	±0.63	±11.5	±12.6
Control	0.07	1.22[a]	0.37	0.65[a]	0.05	0.96[b]	5.7	11.5[b]
	±0.03	±0.69	±0.16	±0.19	±0.02	±1.02	±1.4	±5.2
Anti-GBM	0.07	0.32	0.32	0.66	0.15	0.50[b]	27.3	28.6
	±0.04	±0.26	±0.07	±0.37	±0.06	±0.19	±21.9	±15.4
Control	0.12	1.26	0.41	0.58	0.09	0.88[c]	7.2	9.5
	±0.08	±0.84	±0.12	±0.10	±0.05	±0.43	±1.8	±1.3

[a] $p < 0.005$.
[b] $p < 0.02$.
[c] $p < 0.05$.

FIG. 3. Systemic plasma protein concentration immediately after anesthesia (before surgery), during the control periods, and during and after albumin infusion. Values are means ± SE.

TABLE 4

Hydrostatic Pressure in Carotid Artery Before (B), and During and After (A) Hyperoncotic Albumin in Control, AICN, and Anti-GBM Rats (Mean ± SD)

	B (mm Hg)	p	A (mm Hg)
AICN	118.2 ± 12.0	<0.005	127.7 ± 11.3
AICN controls	105.8 ± 9.0	<0.01	115.0 ± 7.2
Anti-GBM	117.3 ± 17.0	NS	126.0 ± 7.3
Anti-GBM controls	110.6 ± 12.0	<0.025	123.4 ± 6.7

TABLE 5

Terminal Blood Volume in Seven AICN Rats, Six AICN Control Rats, and Four Animals with Anti-GBM Nephritis (Mean ± SD)

	(ml/100 g body wt)
AICN	4.24 ± 0.27
p	NS
AICN controls	3.93 ± 0.48
p	<0.01
Anti-GBM	5.27 ± 0.72

TABLE 6

Single Nephron Filtration Rate (SNGFR) and Late Proximal F/P Inulin Ratio (Mean ± SD) Before (B), and During and After (A) Hyperoncotic Albumin in Six Rats with AICN, Three Age-matched Controls, Five Anti-GBM Nephrotic Animals, and Three Age-matched Control Rats

	SNGFR (nl/min)			F/P inulin		
	B	p	A	B	p	A
AICN	47.8	<0.025	58.2	2.04	<0.05	1.48
	±18.5		±17.1	±0.4		±0.2
AICN controls	41.7	NS	45.4	2.71		1.78
	±7.2		±11.3	±0.8		±0.3
Anti-GBM	17.7	NS	22.9	1.81	<0.025	1.60
	±8.6		±8.1	±0.2		±0.1
Anti-GBM controls	31.2	NS	32.2	2.32		1.73
	±8.7		±9.3	±0.6		±0.5

TABLE 7

Absolute Reabsorption to Late Proximal Convolution and Calculated Efferent Oncotic Pressure in Five AICN Rats, Six Control Rats, and Five Anti-GBM Animals, and Efferent Arteriolar and Proximal Tubular Hydrostatic Pressure in Another Five AICN Rats and Five Control Rats Before (B), and During and After (A) Hyperoncotic Albumin Infusion

	Absolute reabsorption (nl/min)		Efferent oncotic pressure (mm Hg)		Efferent arteriole pressure (mm Hg)		Proximal tubular pressure (mm Hg)	
	B	A	B	A	B	A	B	A
AICN	24.4	16.8[a]	19.1	19.5	11.0	11.1	13.2	13.8
	±8.2	±8.8	±1.8	±1.5	±1.8	±1.3	±4.2	±5.3
AICN	25.0	18.4	26.5	20.5	13.0	13.5	11.9	12.1
controls	±7.3	±6.3	±2.2	±3.1	±1.0	±1.7	±1.1	±1.5
Anti-GBM	8.2	9.0	14.9	15.6	−	−	−	−
	±4.2	±3.8	±4.2	±5.1				
Anti-GBM	17.2	11.6	30.1	30.0	−	−	−	−
controls	±3.0	±2.8	±8.4	±8.5				

[a] $p < 0.005$.

significantly lower in the anti-GBM group than in the control animals (Tables 6 and 7). In animals with AICN, mean fractional but not absolute reabsorption was significantly reduced, mean SNGFR increasing slightly. Efferent arteriolar oncotic pressure, calculated using the whole kidney filtration fraction, was significantly reduced below levels found in control rats in animals with both forms of glomerulonephritis.

The changes that occurred in these measurements during and after hyperoncotic albumin infusion are shown in Fig. 4; mean values found are given in Tables 6 and 7. Mean SNGFR increased in each group (large controls, 9%; small controls, 3%; AICN, 22%; anti-GBM, 29%). These figures should be compared with the increases found in whole kidney filtration rate (large controls, 15%; small controls, 6%; AICN, 22%; anti-GBM, 9.5%). Proximal fractional reabsorption fell in all animals. Absolute reabsorption also fell in control and AICN rats but not in the anti-GBM group. In the large control rats, the fall in absolute reabsorption rate was associated with a fall in calculated efferent oncotic pressure and a small rise in measured efferent arteriolar hydrostatic pressure that reached statistical significance during the fifth urine collection period (13.0 mm Hg ± 1.0 SD to 14.8 mm Hg ± 1.2 SD, $p < 0.01$).

Despite the marked heterogeneity in SNGFR in the glomerulonephritic kidneys, absolute reabsorption to the late proximal convolution varied in direct proportion to the individual single nephron filtration rate both before and after hyperoncotic albumin (Fig. 5). In the AICN group, a significant change occurred in the shape of this relationship after albumin, the largest fall in proximal absolute reabsorption being seen in those nephrons with the highest rates of glomerular filtration.

DISCUSSION

In our study, infusion of hyperoncotic salt-poor bovine albumin into normal hydropenic Wistar rats given ADH and aldosterone resulted in expansion of plasma volume and a significant natriuresis and diuresis and was associated with a significant fall in proximal fractional reabsorption.

A review of the extensive literature concerning the effect of hyperoncotic albumin infusion in normal human subjects and in the experimental animal quickly reveals
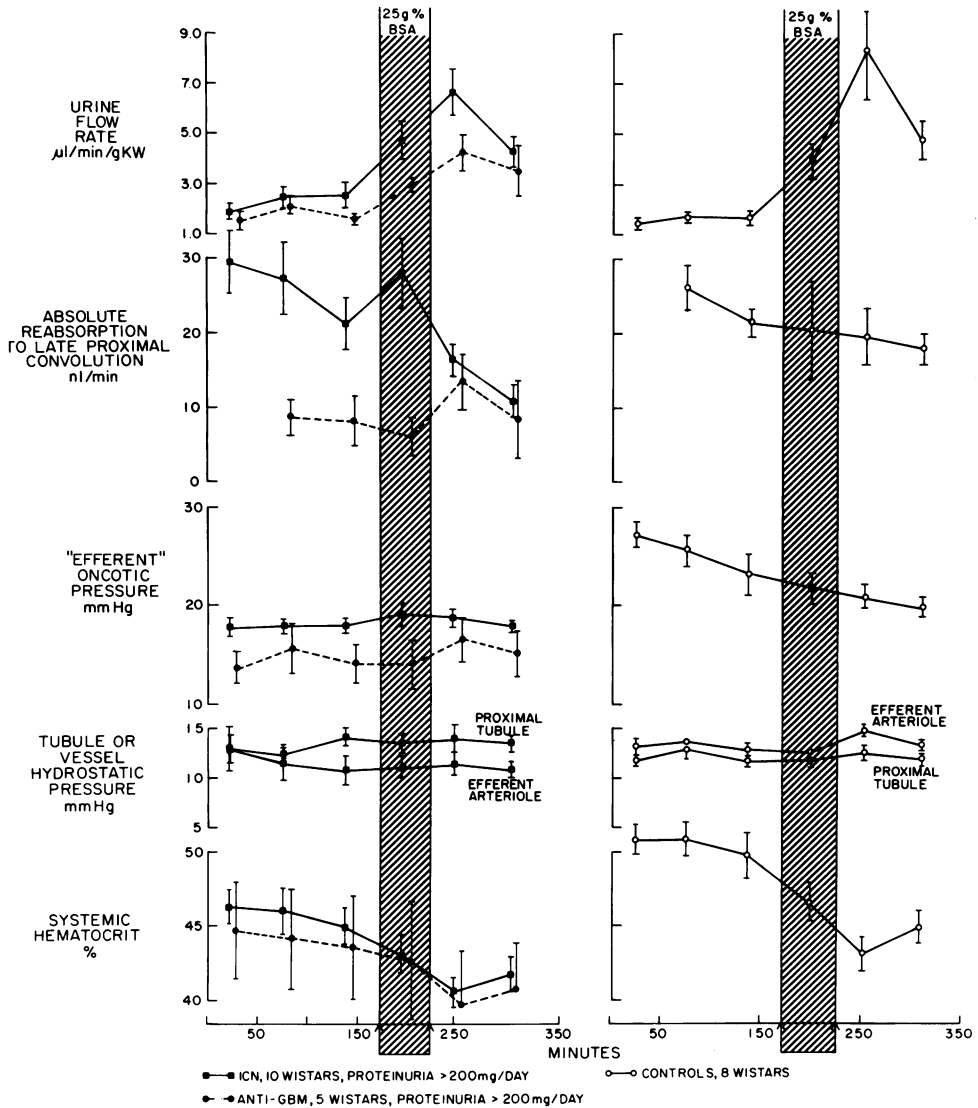
FIG. 4. Results before, during, and after infusion of bovine serum albumin.

the results to be extremely variable and apparently influenced by such diverse factors as the pre-existing state of hydration, the duration and quantity of albumin infused, the ionized serum calcium level, and the time of day (15–18). Thus, 24 yr ago Welt and Orloff observed that infusion of 25 g% salt-poor hyperoncotic albumin to themselves while undergoing a water diuresis had no effect on urinary sodium or chloride excretion, unless given at night when an increase was found. Similarly, clearance studies in the intact or isolated saline loaded dog kidney revealed enhanced sodium reabsorption after albumin (19, 20). Levy and Levinsky (21) found a rise in proximal fractional reabsorption in the dog during infusion of 30% bovine albumin or dextran, which coincided with a significant increase in systemic plasma protein concentration.

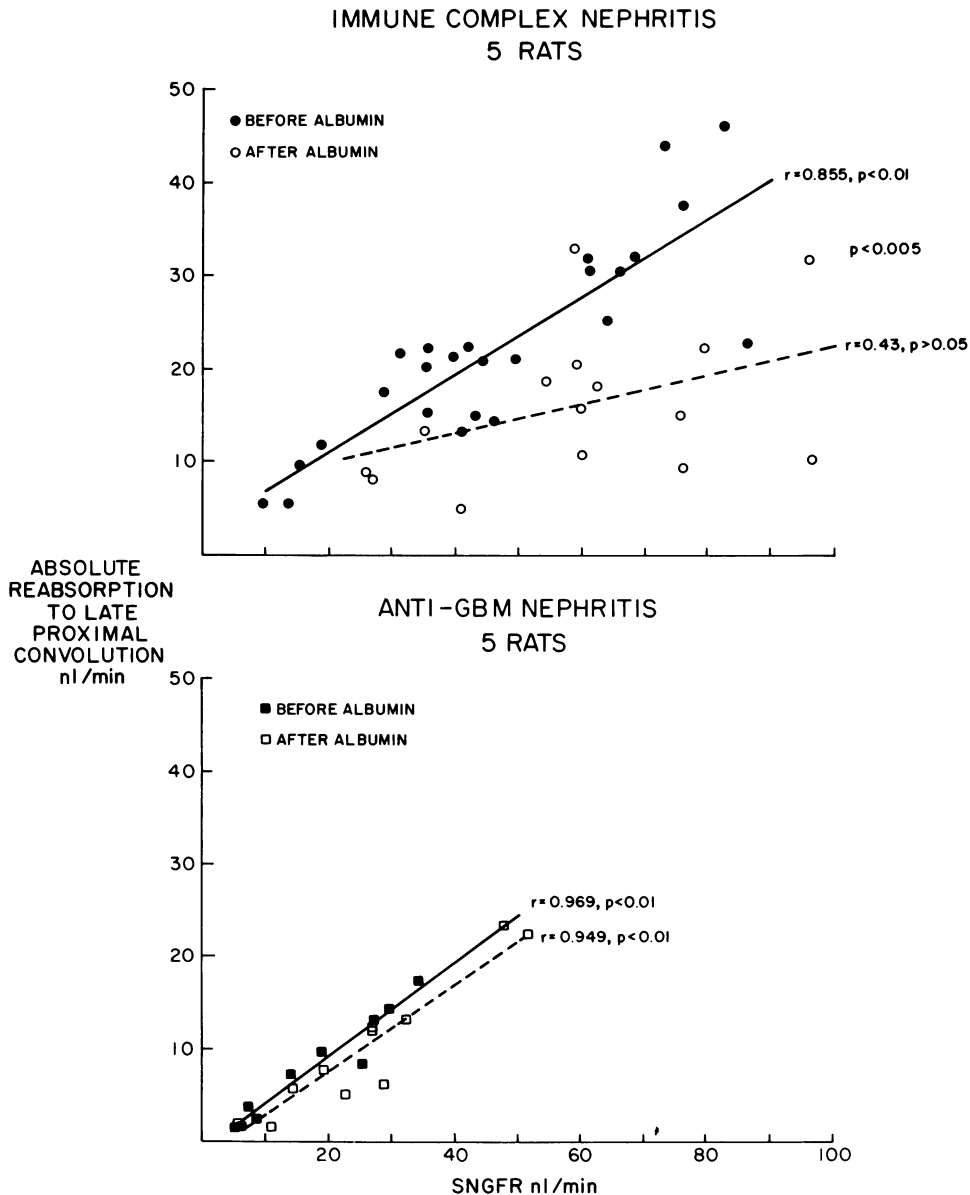In contrast, more recent micropuncture studies in hydropenic animals have

FIG. 5. Absolute rate of proximal reabsorption in rats with AICN and anti-GBM nephritis before and after albumin.

shown that following expansion of plasma volume with hyperoncotic albumin, there is a fall in proximal fractional reabsorption and a small, but significant, natriuresis (16, 22–25). Our observations in normal Wistar rats are in keeping with these findings, a significant rise in fractional sodium excretion being associated with a fall in proximal fractional reabsorption. Whole kidney and single nephron glomerular filtration rates rose slightly and insignificantly (kidney GFR by 6–15%, SNGFR by 3–9%), providing little evidence of intrarenal redistribution after hyperoncotic albumin. Others have reported significant large and parallel increases in both kidney GFR and SNGFR in the rat (22, 24, 26) after infusion of larger quantities of albumin.

The cause of the diminished fractional reabsorption observed after hyperoncotic albumin in normal rats remains speculative. A significant role cannot be ascribed to either aldosterone or ADH since in this as in other studies these substances were infused throughout the study. There is also little support for the suggestion that foreign albumin may have a direct "pharmacological" effect on the renal tubular cells (27). Volume expansion of rats using hyperoncotic rat albumin has been found to have similar effects.

In recent years there has been much discussion concerning the role of peritubular physical factors (hydrostatic and oncotic pressure) in the regulation of proximal reabsorption, which has been based on the observations of Earley, Windhager, and Brenner and their colleagues. In our studies, fractional and absolute reabsorption fell after hyperoncotic albumin infusion, although in the latter instance this did not reach statistical significance. Systemic blood pressure rose significantly. A small but significant increase in efferent arteriolar hydrostatic pressure was measured during the period immediately following the albumin infusion, which corresponded with the phase of maximum diuretic and natriuretic activity. Proximal intratubular hydrostatic pressure was unchanged; calculated efferent oncotic pressure fell simultaneously by approximately 6 mm Hg. Although these observations might at first sight appear to support a primary role for "physical factors," the validity of using whole kidney filtration fraction to calculate efferent oncotic pressure and the nature of the changes in interstitial oncotic and hydrostatic pressures after albumin are unknown. Whole kidney filtration fraction and systemic protein concentration can be used to calculate efferent oncotic pressure during hydropenia and after isovolaemic exchange transfusion (28, 29). A poor correlation, however, has been found following volume expansion or dehydration or both, and, hence, our calculated efferent oncotic pressure after albumin may be misleading. In a recent study by Knox *et al.* (23), direct measurements of peritubular capillary hydrostatic and oncotic pressures were made and revealed no significant changes following hyperoncotic albumin infusion. Fractional and absolute proximal reabsorption, however, were significantly reduced. Protein concentration in renal lymph was significantly increased, and Knox has suggested that this indicates a rise in interstitial oncotic pressure after albumin and, hence, a decrease in the Starling forces acting across the peritubular capillary wall for reabsorption. At the same time, however, they point out that a rise in oncotic interstitial fluid pressure might also be expected to increase reabsorption since this force could facilitate fluid movement across the basement membrane of the proximal tubules. Resolution of this problem awaits the development of techniques capable of measuring interstitial fluid hydrostatic and oncotic pressures.

An alternative explanation of the diminished proximal reabsorption has been the presumed release of a natriuretic factor liberated in response to an alteration in the systemic circulation, i.e., arterial pressure, central venous pressure, plasma volume, etc., following hyperoncotic albumin (30). More definite evidence exists for a second hormonal influence, namely parathyroid hormone. Knox *et al.,* in 1973 (17), found that the natriuretic and phosphaturic effects of hyperoncotic albumin infusion in rats were associated with an increase in parathyroid hormone excretion provoked by a fall in ionized calcium levels following albumin infusion. Addition of sufficient calcium to the infusate to prevent reduction in plasma ionized calcium levels resulted in a failure of the albumin infusion to decrease significantly sodium reabsorption by the proximal tubule. It is doubtful if the small quantity of calcium present in our infusate (0.024 g/100 ml) would be sufficient to prevent binding of plasma calcium by the infused albumin. The major natriuretic and diuretic effects were seen in the 45

min immediately following the end of the albumin infusion. Knox *et al.* have found this to coincide with the period of maximum phosphaturia, although systemic parathyroid hormone levels were falling by this time.

Distal tubular sodium reabsorption was not examined in the present study. Recently Stein *et al.* (24) and Knox and Gasser (25) demonstrated that in addition to diminished proximal tubular reabsorption after albumin, alterations occur in sodium reabsorption in some distal segments of the nephron that have been attributed to a medullary washout effect created by an increased plasma flow. Our finding of decreased extraction of PAH and increase in renal plasma flow after albumin would be in keeping with this hypothesis. Similar changes have been previously described in humans (31) and dogs (19) and have led to similar conclusions.

In addition, Knox and Gasser (25) found evidence of increased sodium reabsorption (with increased potassium excretion) from the distal nephron after hyperoncotic albumin. In keeping with this, in our study, absolute and fractional potassium excretion rose significantly following albumin.

The effect of hyperoncotic albumin infusion in the experimental animal with glomerulonephritis has not previously been reported. In 1950 Orloff, Welt, and Stowe (2) observed a diuretic and natriuretic response to hyperoncotic albumin infusion in patients with nephrotic syndrome. This they attributed to inhibition of ADH secretion and a rise in serum sodium concentration consequent to the ensuing water diuresis. In salt-loaded, volume-expanded nephrotic patients, however, Grausz, Lieberman, and Earley (32) found that the infusion of hyperoncotic albumin resulted in increased fractional sodium reabsorption.

In our study, a significant diuresis occurred in both forms of glomerulonephritis after hyperoncotic albumin; the pattern and magnitude of the response, however, varied in the two conditions and was less marked than in the control rats. Thus, in the more chronic AICN animals, there was a large rise in whole kidney and single nephron filtration rate and a fall in proximal absolute reabsorption. We found no alteration in either efferent oncotic or hydrostatic pressure after albumin in these animals. The presence of marked single nephron heterogeneity, however, makes it impossible to arrive at an accurate assessment of efferent oncotic pressure from whole kidney filtration fraction without direct measurement. Although variations in blood flow in individual glomeruli in chronic AICN rats almost certainly occur, nephrons with higher filtration rates may have had higher filtration fractions and, hence, higher efferent oncotic pressure. These nephrons also showed the largest fall in absolute reabsorptive rate after hyperoncotic albumin. The smallest diuretic and natriuretic responses occurred in the anti-GBM nephritic animals. In these rats no changes occurred in single nephron filtration rate or proximal absolute reabsorptive rate; hence, the small diuretic response observed must have been related to more distal effects.

It is interesting to speculate why a smaller diuretic and natriuretic effect was seen in the glomerulonephritic as compared to the control rat after similar quantities of albumin. It should be noted that with the exception of some of the anti-GBM nephritic rats, our animals were not, strictly speaking, "nephrotic" (i.e., proteinuria, hypoproteinemia, and edema). There was no evidence that they were avidly retaining sodium since their mean sodium excretion rate before albumin was not significantly reduced below that of the control animals. In both forms of glomerulonephritis there was evidence of diminished proximal fractional reabsorption before the administration of hyperoncotic albumin, and this was associated with a lower calculated efferent oncotic pressure. Distal tubular function was not measured, but

overall fractional sodium excretion was increased. Maintenance of sodium balance by increased fractional excretion of sodium is a well-recognized physiological adaptive response of the kidney to a variety of disease states (33). Although proximal fractional reabsorption fell still further following albumin infusion, its overall effects may have been somewhat attenuated by the prior diminished reabsorption related to glomerulonephritis.

ACKNOWLEDGMENTS

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