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Albumin Excretion by the Kidney: The Effects of Furosemide Diuresis[1] (40243)

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In previous experiments in the rat it has been shown that extracellular fluid volume expansion with normal saline (1) results in a significant increase in urine volume (V), glomerular filtration rate (GFR), fractional sodium excretion (FE_{Na}), and albumin excretion rate ($U_{alb}V$). It was also shown that mannitol-induced osmotic diuresis (2) results in a significant increase in V and FE_{Na}, but GFR and $U_{alb}V$ remain stable. The results of these experiments indicate that the main factor controlling $U_{alb}V$ is the filtered load of albumin in the proximal tubule, which in turn is dependent on GFR. In patients with proteinuria, the rate of protein excretion increases following intravenous furosemide administration (3); the percentage increase or decrease in protein excretion had a significant linear correlation with the percentage increase or decrease in GFR and V. In a number of well-controlled experiments, however, (4–6) furosemide administration has not been shown to result in a significant change in GFR. The present study was therefore designed to examine the effect of furosemide-induced diuresis on $U_{alb}V$ in normal rats in an attempt to elucidate the aforementioned discrepancies and to evaluate whether furosemide might affect albumin handling by the nephron by mechanisms other than those dependent on changes in GFR.

Materials and methods. Experiments were done on 10 female Sprague–Dawley rats obtained from a single source and weighing 200–250 g. They were placed in individual metabolic cages and fed rat chow (Purina) and water *ad libitum*. Before the study began, two 24-hr urine samples, collected under mineral oil with thymol added as a preservative, were analyzed for total protein and albumin.

On the day of the experiment, the rats were weighed and anesthetized intraperitoneally with sodium pentobarbital (50 mg/kg). They were then placed on a heated laboratory board; body temperature was maintained at 37°C by an external heat source and monitored with a rectal thermistor and telethermometer.

The trachea was exposed and cannulated with a 10-mm length of polyethylene (PE-100) tubing. Both femoral veins were then cannulated with PE-50 tubing. Using previously calibrated Braun syringe pumps, ^3H inulin solution was infused into one, the experimental solution into the other. A femoral artery was cannulated to collect blood samples and monitor blood pressure, using a Narco Biosystems Physiograph (Desk Model DMP-4B). The bladder was exposed by laparotomy and a funnel-shaped piece of PE-60 tubing was tied securely into the urethral orifice. Urine specimens were collected via capillary action from the exposed end of the PE-60 tubing; completeness of collection was insured by gentle direct bladder compression.

In each experiment, there was an initial period of 60 min for equilibration, during which normal saline was infused at the rate of 3.75 ml/hr (62.5 μl/min) to compensate for the extracellular fluid losses (1, 2). ^3H inulin was administered in a priming dose of 4.5 μCi, followed by a continuous infusion of 3 μCi in 0.5 ml of normal saline per hr. At the end of the 1 hr equilibration period, the bladder was emptied, and three 30-min urine collections were made. Using a previously calibrated Harvard syringe pump diuresis was then induced by the infusion of furosemide (10 μg/ml) in normal saline at 25 μl/min. At the end of the second equilibration period lasting 10 min, the bladder was again emptied, and three 10-min urine collections were made. Arterial blood samples (<200 μl) were obtained at the midpoint of each of the six urine collection periods. Replacement of urinary losses after furosemide administration was accomplished by the infusion of

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isotonic saline through the femoral vein catheter. Isotonic saline was administered over 5-min in amounts equal to the rate of urine formed during the previous 5-min as measured by weight. At the end of the experiment the rats were again weighed and then sacrificed.

The urine volume collected during each of the six collection periods was measured by weighing; 10 μl was used to measure ^{3}H inulin, the remainder frozen for later albumin and sodium determinations. To measure ^{3}H inulin, 50 μl of serum was used; the remainder was frozen for later albumin and sodium determinations.

Sodium was measured in an Instrument Laboratories flame photometer. GFR was measured by ^{3}H inulin clearance using a Packard liquid scintillation spectrometer for radioactive counts. Albumin concentration in serum and urine was measured in duplicate by radial immunodiffusion (7–9). The serum was diluted 1:300 in phosphate-buffered saline. Five microliters of test sample, either urine or plasma, was allowed to diffuse radially from a well in a uniform layer of agar containing rabbit anti-rat antibody. Preliminary tests revealed that the albumin concentration of the urine samples ranged from 12.5 to 200 μg/ml. Serial dilutions of a known amount of rat albumin from 50 to 200 μg/ml were made and included in each plate. Samples with lower concentrations of albumin, in the range of 12.5 to 50 μg/ml, were plated by the double injection method (8). Five microliters of the solution was introduced into the well and, after 5 min, another 5 μl was delivered. It has been shown that larger volumes of solution containing antigen form larger precipitates (8). The plates were incubated at room temperature and diameters measured after 48 hr, after which no further increase in diameter was observed. The diameter of each well was measured in the horizontal and vertical axis, using a microscope with a Vernier scale. For each plate, a graph was prepared, plotting the albumin concentration against the diameter squared (d^2) (9). The coefficient of variation was 5%.

The results were studied by analysis of variance. All data, except for the albumin concentration, $U_{alb}V$, and sieving coefficient for albumin ($C_{alb}/GFR \times 100$),[2] were expressed as arithmetic means. Previous observations in our laboratory have shown albumin concentrations and $U_{alb}V$ to be log normally distributed (10). The data pertaining to $U_{alb}V$ and the sieving coefficient underwent log transformation before analysis, and the data are expressed as geometric means.

Results. The results of a representative experiment are shown in Fig. 1; the results of experiments in all ten animals are summarized in Table I. In the control period, V was 25.00 μl/min, GFR was 4.19 ml/min/kg, $U_{alb}V$ was 1.36 μg/min, FE_{Na} was 1.10%, and the sieving coefficient for albumin was 0.00232. Following furosemide administration, V increased in all 10 animals; the mean increase was 52.34 μl/min, and the range from 23.13 to 90.17 μl/min. GFR increased slightly in seven animals and decreased slightly in three animals. The mean change was +0.38, and the range was from −0.24 to +1.79 ml/min/kg. $U_{alb}V$ increased slightly in seven animals and decreased slightly in three animals. The mean increase was 0.11 and the range from −0.55 to +0.52 μg/min. FE_{Na} increased in all 10 animals and rose to 5.83%. The sieving coefficient for albumin increased slightly in five animals and decreased slightly in five; the mean value did not change. Blood pressure remained stable throughout the experiment in all ten animals.

Discussion. Following the administration of furosemide there was a significant increase in V and FE_{Na} while GFR, $U_{alb}V$, and sieving coefficient for albumin remained constant. It has been reported that, following the administration of furosemide to proteinuric patients, there is a significant increase in protein excretion which shows a significant linear correlation with the increase in creatinine clearance and urine volume (3). However, in well-controlled experimental conditions, furosemide results in a decrease in GFR, as a result of contraction of the extracellular fluid volume (11); under conditions in which this drug-induced contraction of extracellular fluid volume is minimized or prevented by the simultaneous replacement of diuretic

[2] The term "sieving coefficient" as used herein reflects the net effects of glomerular filtration and tubular reabsorption of proteins, and is therefore a measure of protein handling by the whole nephron. The true glomerular sieving coefficient for albumin can only be estimated by measurement of the concentration of albumin in the first part of the proximal tubule.

fluid losses, GFR is well sustained (4–6). In the present study, replacement of urinary losses after furosemide administration was accomplished by volume-for-volume infusion of isotonic saline, and there was little change in GFR; V and FE_{Na} increased significantly, but neither $U_{alb}V$ nor the sieving coefficient for albumin changed significantly. This suggests that there was no increase in glomerular permeability to albumin while the GFR remained stable, and that the filtered load of albumin remained constant.

It has been shown that, in healthy female Sprague–Dawley rats, about 99% of the albumin filtered by the glomerulus is reabsorbed along the tubules, and that the protein absorption process in the tubules is saturable (12–14). Micropuncture data have revealed that the amount of albumin the tubules can reabsorb is only slightly in excess of that filtered by the normal glomerulus (12), and that most of the filtered albumin is reabsorbed in the proximal tubules (15). It is well established that the major site of furosemide action is in the loop of Henle (4, 16). In addition, furosemide decreases the tubular fluid: plasma (TF/P) inulin ratio in the proximal tubule (6, 11); similarly, a decrease in albumin concentration in the proximal tubule would be anticipated. However, a stable GFR, a stable filtered load of albumin, and an unchanged $U_{alb}V$, indicate that net reabsorption of albumin throughout the nephron was not altered following furosemide-induced diuresis. The results of this experiment confirm that the main factor controlling $U_{alb}V$ is the filtered load of albumin to the proximal tubule, which in turn is dependent upon glomerular filtration. Provided that diuretic-induced fluid losses are replaced and GFR maintained, furosemide does not affect albumin handling by the nephron.

Summary. $U_{alb}V$ in the female Sprague-Dawley rat was measured during a stable period of normal hydration and following

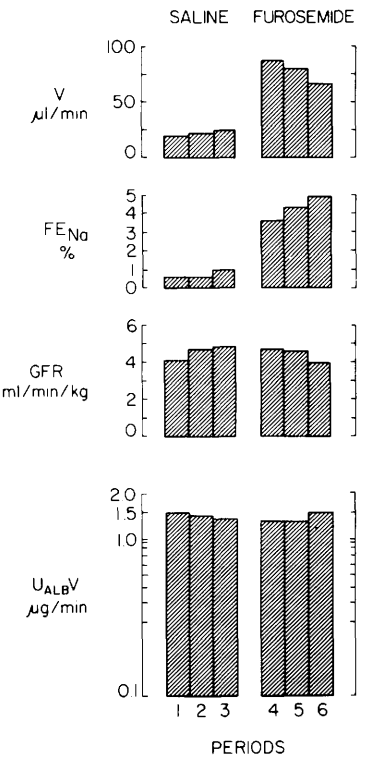

FIG. 1. Changes in urine volume (V), fractional excretion of sodium (FE_{Na}), glomerular filtration rate (GFR), and albumin excretion rate ($U_{alb}V$) during control periods and following furosemide administration in a representative animal.

TABLE I. SUMMARY OF RESULTS FOLLOWING INFUSION OF NORMAL SALINE AND 0.001% FUROSEMIDE IN NORMAL SALINE IN 10 RATS.

	Normal saline (62.5 µl/min)	Mean difference during infusion of normal saline (90 µl/min) with 0.001% furosemide in normal saline (25 µl/min)	*P*
Volume (µl/min)[a]	25.00 ± 13.35	52.34 ± 18.55	<0.002
Glomerular filtration rate (ml/min/kg)[a]	4.19 ± 0.90	0.38 ± 0.65	NS
Albumin excretion rate (µg/min)[b]	1.36 (1.11, 1.47)	0.11 (−0.19, 0.42)	NS
Fractional excretion of sodium (%)[a]	1.10 ± 0.44	4.73 ± 1.83	<0.002
Sieving coefficient for albumin (%)[b]	0.00232 (0.00147, 0.00332)	−0.00005 (−0.00041, 0.00031)	NS

[a] Values are expressed as the arithmetic mean ± 1SD.
[b] Values are expressed as the geometric mean, with the figures in parentheses 1SD below and above the mean.

furosemide-induced diuresis with the simultaneous replacement of diuretic fluid losses. Furosemide administration resulted in a significant increase in V and FE_{Na}, but GFR, $U_{alb}V$, and the sieving coefficient for albumin remained stable. The results suggest that, under physiological conditions, changes in albumin excretion are dependent upon changes in glomerular filtration rather than urine flow.

1. First, M. R., Sloan, D. E., Pesce, A. J., and Pollak, V. E., J. Lab. Clin. Med. **89,** 25 (1977).
2. First, M. R., Patel, V. B., Pesce, A. J., Bramlage, R. J., and Pollak, V. E., Nephron **20,** 171 (1977).
3. Pillay, V. K. G., Gandhi, V. C., Sharma, B. K., Smith, E. C., and Dunea, G., Arch. Intern. Med. **130,** 90 (1972).
4. Puschett, J. B., and Goldberg, M., J. Lab. Clin. Med. **71,** 666 (1968).
5. Dirks, J. H., and Seeley, J. F., Amer. J. Physiol. **219,** 114 (1970).
6. Burke, T. J., Robinson, R. R., and Clapp, J. R., Kidney Int. **1,** 12 (1972).
7. Fahey, J. L., and McKelvey, E. M., J. Immun. **94,** 84 (1965).
8. Mancini, G., Carbonara, A. O., and Heremans, J. R., Immunochemistry **2,** 235 (1965).
9. Berne, B. H., Clin. Chem. **20,** 61 (1974).
10. Gaizutis, M., Pesce, A. J., and Lewy, J. E., Microchem. J. **17,** 327 (1972).
11. Brenner, B. M., Keimowitz, R. I., Wright, F. S., Berliner, R. W., and Troy, J. L., J. Clin. Invest. **48,** 290 (1969).
12. Oken, D. E., Coates, S. C., and Mende, C. W., Kidney Int. **1,** 3 (1972).
13. Lewy, J. E., and Pesce, A. J., Pediat. Res. **7,** 553 (1973).
14. Straus, W., J. Cell. Biol. **12,** 231 (1962).
15. Cortney, M. A., Sawin, L. L., and Weiss, D. D., J. Clin. Invest. **49,** 1 (1970).
16. Suki, W. N., Hull, A. R., Rector, F. C., and Seldin, D. W., J. Clin. Invest. **44,** 1458 (1965).

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