

Furosemide effect on isolated perfused tubules

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BURG, M., L. STONER, J. CARDINAL, AND N. GREEN. *Furosemide effect on isolated perfused tubules*. Am. J. Physiol. 225(1): 119-124. 1973.—Proximal convoluted tubules, thick ascending limbs of Henle's loop, and cortical collecting tubules were dissected from rabbit kidneys, and perfused in vitro. The effect of the diuretic agent furosemide on electrolyte transport and electrical potential difference (PD) in these segments was determined. Furosemide in the lumen (10^{-5} to 10^{-6} M) inhibited active Cl transport in the thick ascending limbs of Henle's loop with resultant decrease in net NaCl absorption and electrical PD. Concentrations of furosemide as high as 10^{-4} M in the bath had little effect. Furosemide at 10^{-4} M also had no definite effect on proximal convoluted tubules and cortical collecting tubules whether placed in the bath and/or the lumen. We conclude that the principal renal action of this drug is to inhibit active Cl transport in the thick ascending limb of Henle's loop.

rabbit kidney; NaCl transport; electrical resistance; potential difference; Na and Cl permeability; thick ascending limb of Henle's loop; cortical collecting tubule; proximal convoluted tubule

FUROSEMIDE, A POTENT DIURETIC AGENT, has a rapid onset of action that is quickly reversible (4, 30, 36, 38). Its principal site of action as determined by clearance and micropuncture studies is the ascending limb of Henle's loop (4, 12, 14, 16, 26, 29, 30, 31, 36, 37). Inhibition of transport at this site by the drug results in impairment of urinary concentration and dilution. Furosemide also is thought to act in the proximal tubule (3, 11, 14, 25, 26, 28, 29, 31, 32), possibly by inhibiting carbonic anhydrase activity (31, 32); but some of the micropuncture results concerning this site of action are conflicting (16, 25), which complicates the interpretation (3, 11, 25).

It had not been previously possible to investigate the mechanism of the action of furosemide on the thick ascending limb of Henle's loop since this nephron segment is not accessible to micropuncture. In the present studies the action of furosemide on thick ascending limbs of Henle's loop, proximal convoluted tubules, and cortical collecting tubules has been directly studied using tubule segments dissected from rabbit kidneys and perfused in vitro.

METHOD

The method employed in these studies has previously been described in detail (6) and is summarized below with additions and modifications.

Fragments of renal tubules were dissected from rabbit kidney and were perfused at 37 C using concentric glass

pipets. The pipet system used (5) includes 1) a means of changing the perfusion fluid with the tubule in place, 2) Sylgard 184 liquid dielectric at the outside of the tubule at both ends, and 3) a volumetric constriction pipet for collection of tubule fluid. The bath solutions generally were bubbled with 95% O₂ + 5% CO₂ gas mixture before and during the experiment.

Proximal convoluted tubules were dissected in chilled rabbit serum (Microbiological Associates, Inc.) (9). The perfusate was an ultrafiltrate of rabbit serum prepared with Amicon XM-50 membranes. The osmolality of the ultrafiltrate and the serum used in the bath during the experiments was equalized by addition of water. Fluid absorption rate (J_v) was measured using iohalamate-¹²⁵I (Abbott Laboratories) in the perfusate as a volume marker. ¹²⁵I was also measured in the bath. The leak of fluid and/or iohalamate was negligibly small (mean 0.14 nl min⁻¹) and did not differ when furosemide was added. Mean perfusion rate was 12 nl min⁻¹.

Thick ascending limbs of Henle's loop (7) and cortical collecting tubules (18, 19) were dissected at room temperature in the saline buffer that was used in the bath during perfusion. It contains, in millimoles per liter: NaCl, 115; KCl, 5; NaHCO₃, 25; Na acetate, 10; CaCl₂, 1; MgSO₄, 1.2; NaH₂PO₄, 1.2; and glucose, 5 mM, plus 5% v/v rabbit serum. The perfusate contained, in millimoles per liter: NaCl, 150; K₂HPO₄, 2.5; CaCl₂, 1.0; and MgSO₄, 1.2; adjusted to pH 7.4 with HCl. For some experiments (Fig. 2) the perfusate was also used in the bath and was bubbled with 100% O₂. During these experiments, the NaCl concentration in the bath and lumen was decreased in some periods by substituting raffinose isosmotically for NaCl.

Perfusions were carried out either by gravity or with a Sage perfusion pump.

Na and K were measured using an Instrumentation Laboratory flame photometer (1) for bulk solutions and an Aminco helium-glow photometer (39) for microsamples of perfusate and collected fluid. Chloride was measured in bulk solutions using the Cotlove-Aminco chloridometer (13) and in microsamples of perfused and collected fluid using a modification (6) of the Ramsay method (33). Osmolality was measured in bulk solutions using the Bowman-Aminco freezing-point apparatus (2). The transepithelial electrical potential difference (PD) was measured between calomel cells connected to the fluids in the bath and perfusion pipet with bridges containing 0.16 M NaCl-agar (10). The PD was displayed on a Tektronix oscilloscope or Varian recorder connected via a Bak ELSA-3 or 4 or Transidyne MPA-6 amplifier.

The transepithelial electrical resistance was calculated by

cable analysis using the voltage change at both ends of the tubule that results from passage of current from the perfusion pipet to a ground connected to the bath (20). For experiments with proximal convoluted tubules, the current (2×10^{-7} amp) was carried through a platinum-black coating on the outside of the perfusion pipet (27). For thick ascending limbs of Henle's loop, the current (5×10^{-8} amp) was passed through the lumen of the perfusion pipet. The voltage artifact resulting from the passage of current through the perfusion pipet was nulled with a bridge circuit (20).

Ion fluxes in the thick ascending limb of Henle's loop were measured as previously (7) with radioisotopes using ^{36}Cl , ^{24}Na , and ^{22}Na (International Chemical & Nuclear Corp.).

Na flux from lumen to bath ($J_{\text{Na},\text{lb}}$) at a Na concentration in the lumen equal to that in the perfusate, $[\text{Na}]_o$, was measured by placing ^{24}Na in the perfusate. Then assuming that, since this tubule segment has an extremely low water permeability (7), there is no net water movement:

$$J_{\text{Na},\text{lb}} = [\text{Na}]_o \frac{V}{L} \ln \frac{[\text{C}]_o}{[\text{C}]_l}$$

where V is the perfusion rate, L is the tubule length, and C_o and C_l , the concentrations of isotope perfused and collected.

Na flux from bath to lumen ($J_{\text{Na},\text{bl}}$) was measured simultaneously by placing ^{22}Na in the bath and recording the amount collected from the lumen.

$$J_{\text{Na},\text{bl}} = \frac{[\text{Na}]_b[\text{C}]_l}{[\text{Na}]_o[\text{C}]_b} \frac{J_{\text{Na},\text{lb}}}{[1 - e^{-(J_{\text{Na},\text{lb}}L/V[\text{Na}]_o)}]}$$

where $[\text{C}]_b$ is the concentration of isotope placed in the bath.

Chloride flux from bath to lumen ($J_{\text{Cl},\text{bl}}$) was measured by placing ^{36}Cl in the bath and recording the amount collected in the lumen.

$$J_{\text{Cl},\text{bl}} = \frac{V[\text{C}]_l[\text{Cl}]_b}{L[\text{C}]_b}$$

^{22}Na and ^{24}Na were measured with a Packard well scintillation counter; ^{36}Cl was measured with a Packard liquid scintillation counter using Multisol fluid (Isolab, Inc.). The furosemide dilutions were prepared from Lasix injection ampules (Hoechst Pharmaceutical Co.). When furosemide was added to the bath surrounding cortical collecting tubules and thick ascending limbs, the 5% v/v rabbit serum was omitted from the bath to avoid possible binding of the drug by serum proteins. In control experiments no effect on the electrical PD was found from omission of the serum per se.

Results are given as means \pm standard errors with the number of tubules studied in parentheses (mean \pm SE (n)).

RESULTS

Thick ascending limb of Henle's loop. There is an electrical potential difference of approximately +5 mV (lumen positive) across the epithelium of the thick ascending limb. The PD, generated by active chloride transport from lumen to bath, is the major driving force for the associated transport of Na in the same direction (7). Low concentrations of furosemide (10^{-5} to 10^{-6} M) in the tubule lumen cause the PD to decrease (Figs. 1 and 2). The PD decreases to a steady value within a few seconds when the furosemide is present

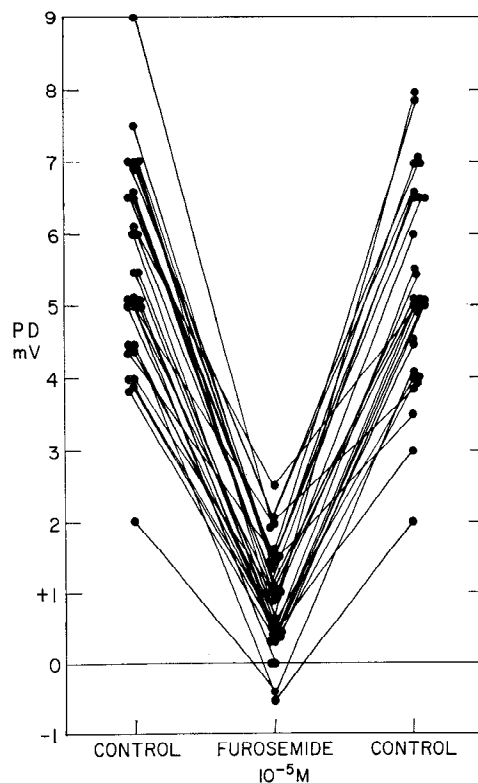


FIG. 1. Effect of furosemide in lumen on PD across thick ascending limbs of Henle's loop. Pre- and postcontrol values of PD did not vary significantly with time. After addition of furosemide, PD reached new level within a few seconds and remained steady as long as drug was present (up to 20 min).

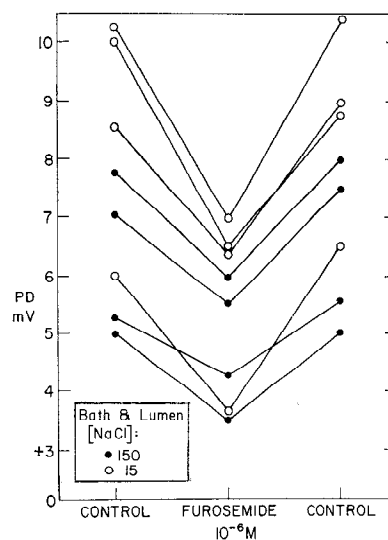


FIG. 2. Effect of furosemide in lumen on PD across thick ascending limbs of Henle's loop. Time course of change in PD was same as in Fig. 1.

in the solution in the lumen; and returns within a few seconds to the control value when the original solution containing no drug is reperfused. In contrast, there is no immediate change in the PD when a much higher concentration of furosemide (10^{-4} M) is placed in the bath (Fig. 3). Although in some tubules a delayed decrease in PD occurred with 10^{-4} M furosemide in the bath (see Fig. 4), the change was

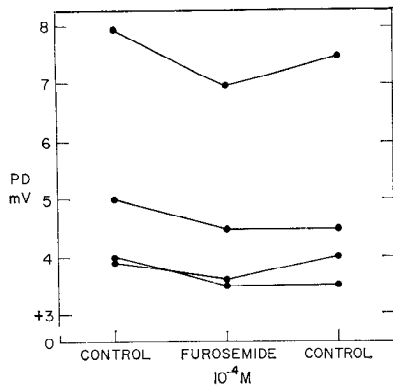


FIG. 3. Effect of furosemide in bath on PD across thick ascending limbs of Henle's loop. PD is mean value during 2-3 min after addition of furosemide.

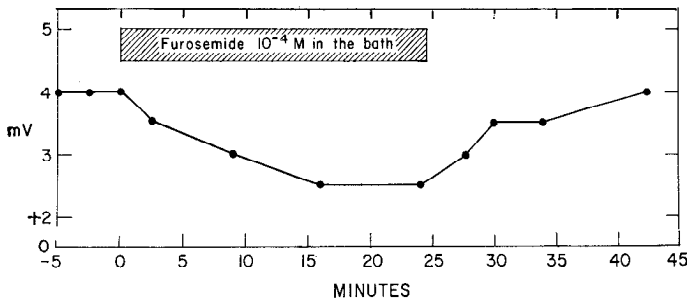


FIG. 4. Effect of furosemide in bath on PD across a thick ascending limb of Henle's loop. In this experiment there was a slow progressive decrease in PD during 15 min after addition of furosemide. Note that effect was slower and smaller than from a lesser concentration of furosemide in lumen (Fig. 1).

considerably less than that elicited by furosemide in the perfusate.

Reabsorption of NaCl from the lumen of the thick ascending limb results in a fall in lumen NaCl concentration (7). Furosemide, 10^{-5} M, in the lumen reduces the fall in NaCl concentration (Table 1) and reduces the net transport of Na (Table 2). Since the drug decreases both PD and net NaCl reabsorption, it must act primarily to inhibit active Cl transport which secondarily results in a fall in the PD and in the electrically coupled passive transport of Na.

When the thick ascending limb is perfused with a solution containing a lower NaCl concentration than in the bath, the electrical PD increases. This is a consequence of a diffusion potential across the epithelium, resulting from the imposed chemical concentration gradient (Na conductance exceeds Cl conductance) (7). All other things being equal, the magnitude of the diffusion potential depends on ratio of Na-to-Cl conductance. In the experiments in Table 3, tubules were perfused with solutions containing a normal (150 mM) or low (50 mM) NaCl concentration. The mean difference in PD with the two solutions was approximately the same whether furosemide was present in the lumen (17.2 mv difference) or absent (16.3 mv difference), indicating little change in the ratio of Na-to-Cl permeabilities with drug.

Furosemide (10^{-5} M in the lumen) caused the trans-epithelial electrical resistance to increase by 15%. In five tubules the mean electrical resistance was 3,550 ohm-cm (tubule length) in the precontrol periods (C1), 3,840 with furosemide

(10^{-5} M) in the lumen (E), and 3,290 in the aftercontrol periods (C2). The mean paired difference, $E - ((C1 + C2)/2)$, was 400 ± 116 , $P < .05$. Since the electrical resistance increased slightly without change in the diffusion PD for NaCl, it is likely that permeability of the epithelium to both of the major ions present, (Cl and Na) decreased slightly. This is confirmed by the radioisotope measurements which follow.

The effect of furosemide on Na and Cl fluxes was measured using radioisotopes (Tables 2 and 4). Interpretation of these studies is complicated by the decrease in J_{Na} and J_{Cl} between pre- and postcontrol periods. This occurs spontaneously with time and was previously noted to occur even in the absence of any experimental intervention (7).

In order to facilitate interpretation, the data from the pre- and postcontrol periods have been averaged and compared with those of the experimental periods with furose-

TABLE 1. Effect of furosemide (10^{-5} M) in lumen on NaCl transport by thick ascending limbs of Henle's loop

Exp	mEq liter ⁻¹ In Collected Fluid		Length, mm	Perfusion Rate, nl min ⁻¹
	Control	Furosemide		
	[Na]			
1	88	136	1.92	1.29
2	103	111	1.59	1.40
3	99	144	2.18	1.18
	[Cl]			
4	95	149	1.06	1.05
5	91	133	1.31	1.18
6	72	127	1.44	.97

Each value is the mean of three collection periods. Control periods preceded furosemide in experiments 4-6 and followed furosemide in experiments 1-3. Mean concentration of Cl was 153 mEq liter⁻¹ in the perfusate, 121 in the bath. Na was 142 in the perfusate, 151 in the bath.

TABLE 2. Effect of furosemide (10^{-5} M) in lumen on Na flux across thick ascending limbs of Henle's loop

	J _{Na, lb}	J _{Na, bl}	J _{Na, net}
	Eq cm ⁻¹ sec ⁻¹ × 10 ⁻¹²		
<i>Experiment 1</i>			
C1	43.1	21.9	21.2
E	26.0	20.9	5.1
C2	35.7	19.5	16.2
Difference	-13.4	+2	-13.6
<i>Experiment 2</i>			
C1	51.9	31.2	20.7
E	32.0	27.2	4.8
C2	33.2	20.4	12.8
Difference	-10.6	+1.4	-12.0
<i>Experiment 3</i>			
C1	67.4	35.9	31.5
E	36.3	31.2	5.1
C2	47.6	24.2	23.4
Difference	-21.2	+1.2	-22.4

Each value is the mean of three collection periods. C1 and C2 are pre- and postcontrol periods; E, furosemide, 10^{-5} M, in the lumen. Difference E is $-((C1 + C2)/2)$. The mean flow rate was 9.3 nl min⁻¹. The mean tubule length was 1.46 mm.

TABLE 3. Effect of furosemide and NaCl concentrations in lumen on PD across thick ascending limbs of Henle's loop

Perfusate [NaCl], mEq liter ⁻¹ Perfusate [Furosemide], M	50	150	50	150
	0	0	10 ⁻⁵	10 ⁻⁵
	PD, mv			
Experiment 1	20.0	5.6	16.8	1.0
Experiment 2	21.5	6.7	18.5	1.5
Experiment 3	23.3	7.8	17.0	2.0
Experiment 4	29.8	6.8	26.0	2.0
Experiment 5	20.3	6.5	15.8	1.3
Mean	23.0	6.7	18.8	1.6
Difference	16.3		17.2	

Each value of the PD is the mean of two readings taken 1 min apart, except with 150 mEq NaCl in the lumen and no furosemide, which is the mean of four readings (two at the beginning and two at the end of each experiment). The bath contained a solution identical to the 150 mEq liter⁻¹ NaCl perfusate without furosemide and was gassed with 100% O₂.

TABLE 4. Effect of furosemide (10⁻⁵ M) in lumen on Cl flux from bath to lumen (J_{Cl,bl}) of thick ascending limbs of Henle's loop

Exp	J _{Cl,bl} , Eq cm ⁻¹ sec ⁻¹ × 10 ⁻¹²		
	C1	E	C2
1	16.9	7.7	9.7
2	16.0	4.6	7.7
3	11.6	4.4	7.2
4	25.7	12.4	16.1
Mean	17.5	7.3	10.2
SEM	± 3.0	± 1.9	± 2.0

Each value is the mean of three collection periods. C1 and C2 are pre- and postcontrol periods; E, 10⁻⁵ furosemide in the lumen. The mean paired difference in P_{Cl,bl} with furosemide, $E - ((C1 + C2)/2)$, is $-6.7 \pm .8$ Eq cm⁻¹ sec⁻¹ × 10⁻¹² ($P < .01$). Flow rate was 20 nl min⁻¹. Mean tubule length was 1.63 mm.

mid. Furosemide caused J_{Cl,bl} to decrease 47% (Table 5). J_{Na,lb} decreased by 33% and J_{Na,bl} increased by 4% (Table 2).

Na and Cl fluxes may be altered by changes in the PD as well as by changes in the permeability of the epithelium. With 10⁻⁵ M furosemide in the lumen, the PD decreased on the average by approximately 5 mv (Fig. 1). Using the constant-field assumption (21), this change in PD alone (without any change in permeability) should have caused J_{Cl,bl} and J_{Na,lb} to decrease approximately 10%. The observed decrease in flux was greater than this, indicating that the permeability to Na and Cl, as well as the PD, had decreased. Similarly, the change in PD alone should have caused J_{Na,bl} to increase by 10%. The observed increase was less, also suggesting a small decrease in Na permeability.

Taking all the radioisotope and electrical measurements into account, we conclude that the changes in Na and Cl permeabilities which are induced by furosemide are relatively minor. The major effect of the drug evidently is to inhibit active Cl transport.

We attempted to test whether furosemide is a competitive inhibitor of Cl transport by varying the concentration of

chloride. If the drug competes with Cl, it might be more effective at a lower Cl concentration. In the experiments illustrated in Fig. 2, a low dose of furosemide in the lumen (10⁻⁶ M) was tested with 15 or with 150 mM of Cl in the lumen and bath. There was little difference in the base-line PD or in the fall in PD caused by furosemide at the different Cl concentrations. Therefore, this experiment is inconclusive as a test for competitive inhibition.

Proximal convoluted tubule. The proximal convoluted tubule, when perfused with an ultrafiltrate of serum, has a trans-epithelial PD which is oriented lumen negative, and is most likely caused by active Na transport (10). When furosemide 10⁻⁴ M is added for a period up to 35 min to the lumen of proximal convoluted tubules, there is no change in the electrical PD (Table 5). Since the proximal tubule reabsorbs Na salts isosmotically, fluid absorption is proportional to Na transport. There was only a small and statistically insignificant decrease in fluid (and Na) absorption (-8%) with the drug (Table 5). This result contrasts with that in the thick ascending limb in which a much lower concentration of furosemide (10⁻⁵ M) markedly inhibited both NaCl absorption and the electrical PD. Furosemide, 10⁻³ M, added to the bath did not alter either the electrical resistance or PD of proximal convoluted tubules. In three experiments the mean resistance was 955 ohm-cm (tubule length) in the control periods compared to 970 after up to 30 min with 10⁻³ M furosemide in the bath. The PD was -5.2 mv in the controls, compared to -5.3 mv with furosemide.

Cortical collecting tubule. The PD across the cortical collecting tubule is oriented lumen negative (8). There is net active absorption of Na out of the tubule lumen and net active secretion of K into the tubule lumen (19). Furosemide 10⁻⁴ M in the lumen and bath had no effect on the PD (Table 6). (The PD in these experiments is higher than that previously measured (8, 19, 20), because of the higher temperature used. Similarly, the decrease in Na concentration in the lumen and the increase in K concentration in the lumen caused by transport of these ions was not systematically affected by furosemide (Table 7). (The increase in Na concentration and fall in K concentration of the collected fluid in the aftercontrol periods were also present in control tubules which were not treated with the drug.)

TABLE 5. Effect of furosemide 10⁻⁴ M in lumen on fluid absorption and PD of proximal convoluted tubules

Exp	Fluid Absorption, nl mm ⁻¹ min ⁻¹			PD, mv		
	C1	E	C2	C1	E	C2
1	1.00 (2)	0.93 (3)	1.37 (3)	3.8	3.8	4.1
2	1.69 (6)	1.59 (6)	1.76 (7)	4.6	4.5	4.6
3	1.09 (3)	1.02 (3)	1.01 (3)	3.5	3.8	3.8
4	1.30 (3)	1.31 (3)	1.39 (3)	4.0	4.0	4.0
Mean	1.27	1.21	1.38	4.0	4.0	4.1

The number of collection periods taken over an interval of 10-35 min is in parentheses. C1 and C2 are pre- and postcontrol periods; E, 10⁻⁴ M furosemide in the lumen. The mean paired difference with furosemide, $E - ((C1 + C2)/2)$, is $-.11 \pm .06$ nl mm⁻¹ min⁻¹, $P > .10$. In experiment 4 furosemide, 10⁻⁴ M, was added to both bath and lumen simultaneously following C2. Fluid absorption was 1.59 (3) nl mm⁻¹ min⁻¹. C3 for this experiment was 1.45 (3).

TABLE 6. Effect of furosemide 10^{-4} M in the lumen and bath on PD of cortical collecting tubules

Exp	PD, mv		
	C1	E	C2
1a	-83	-85	-81
1b	-76	-74	
2	-56	-70	-87
3	-117	-127	-124
4	-48	-52	-50

Each result is the mean of two to five measurements over a 10- to 20-min period. Furosemide, 10^{-4} M, was added to the lumen (experiment 1a), the bath (experiment 1b), or both (experiments 2-4). C1 and C2 are pre- and postcontrol periods; E is with furosemide added.

TABLE 7. Effect of furosemide 10^{-4} M in lumen and bath on Na and K transport of cortical collecting tubules

Exp	[Na] in Collected Fluid, mEq liter ⁻¹			[K] in Collected Fluid, mEq liter ⁻¹			Length, mm	Perfusion Rate, nl min ⁻¹
	C1	E	C2	C1	E	C2		
2	135	139	138	16	17	10	1.75	2.0
3	108	112	120	27	20	17	3.10	1.4
4	132	135	139	18	16	10	2.60	1.9

Each result is the mean of three to five measurements over a 10- to 20-min period. C1 and C2 are pre- and postcontrol periods; E is furosemide, 10^{-4} M, in both the lumen and bath. [Na] in the perfused fluid and bath was 150 mEq liter⁻¹; [K], 5 mEq liter⁻¹. The experiments which are the same as in Table 6 are correspondingly numbered.

DISCUSSION

It is generally known that diuretics act by inhibiting salt and water absorption in the renal tubule. What has not been clear is how these drugs can inhibit electrolyte transport in the renal epithelium without disrupting the electrolyte transport and function of other tissues. In the case of furosemide, an answer to this question is provided by the present studies. In the thick ascending limb of Henle's loop, furosemide inhibits the active Cl transport which is responsible for NaCl reabsorption. Most likely it is some special feature of this transport system that renders it susceptible to the action of furosemide. Although active Cl transport is present in some other tissues such as the stomach (22, 34), it is not clear at this point that the Cl transport mechanisms are identical to that in the thick ascending limb of Henle's loop, or that they are inhibited by furosemide.

The fact that the principal site of action of furosemide is in the distal part of the nephron rather than the proximal may contribute to its diuretic efficacy. Although the proximal tubule reabsorbs the largest fraction of filtered salt and

water, there is evidence that inhibition of proximal absorption may be compensated by increased absorption in the distal nephron, when the latter is uninhibited (24). Since the thick ascending limb of Henle's loop is a major site of NaCl reabsorption in the distal nephron, inhibition of its function could lead to a greater diuresis than a comparable inhibition limited to the proximal tubule.

It has been problematical whether furosemide acts in the proximal tubule in addition to the thick ascending limb of Henle's loop (see the introduction). We are impressed by the major effect of low concentrations of furosemide in the lumen of the thick ascending limb in the present studies, compared to an equivocal effect of much higher concentrations in the proximal tubule. On this basis we believe that in the rabbit, at least, the most important effect of the drug is on the thick ascending limb.

Furosemide does not affect the distal nephron other than in the thick ascending limb. No definite effect was found on electrolyte transport in the cortical collecting tubule in the present studies, and none was found previously by micro-puncture in the distal tubule of the rat (14, 17, 28, 29). Also, K secretion which occurs in the distal convoluted tubule and collecting tubules is not directly affected by furosemide (17, 36, 37).

Furosemide probably reaches its site of action in the lumen of the thick ascending limb as a result of transport of the drug by the proximal tubule. The drug is bound to plasma proteins. Some furosemide is undoubtedly filtered at the glomerulus. Probably more important, however, is secretion by the proximal tubule (15). The effect of furosemide is blocked by competitive inhibitors of proximal tubule organic acid transport (23). Proximal tubule secretion of furosemide probably results in an elevated concentration in the tubule fluid, which then flows into the lumen of the thick ascending limb of Henle's loop where it acts.

The molecular basis for the action of furosemide remains unknown. It was attractive to speculate that the drug, as an anion, competitively blocks access of Cl to the active transport mechanism. The evidence for competitive inhibition is inconclusive however, in the present studies, (see RESULTS).

Furosemide was reported by Schmidt and Dubach (35) to inhibit "Na⁺-K⁺ activated adenosine triphosphatase" in ascending limbs of Henle's loop and distal tubules of rats given the drug. As previously discussed (7), it is unclear what relation, if any, there is between this ATPase and the active Cl transport. The same uncertainty applies to interpretation of the significance of the reported inhibition of the ATPase by furosemide.

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REFERENCES

- BOLING, E. A. A flame photometer with simultaneous digital readout for sodium and potassium. *J. Lab. Clin. Med.* 63: 501-510, 1964.
- BOWMAN, R. L., H. V. TRANHAM, AND P. A. CAULFIELD. An instrument and method for rapid, dependable determination of freezing point depression. *J. Lab. Clin. Med.* 43: 310-315, 1954.
- BRENNER, B. M., R. I. KEIMOWITZ, F. S. WRIGHT, AND R. W. BERLINER. An inhibitory effect of furosemide on sodium reabsorption by the proximal tubule of the rat nephron. *J. Clin. Invest.* 48: 290-300, 1969.
- BUGHORN, E., AND S. ANASTAS. Site and mechanism of action of furosemide on the distal nephron in man. *Klin. Wochr.* 42: 1127-1131, 1964.
- BURG, M. B. Perfusion of isolated renal tubules. *Yale J. Biol. Med.* 45: 321-326, 1972.
- BURG, M., J. GRANTHAM, M. ARBAMOW, AND J. ORLOFF. Preparation of furosemide from sodium furosemate. *J. Pharm. Med.* 1972: 1-4.

- tion and study of fragments of single rabbit nephrons. *Am. J. Physiol.* 210: 1293-1298, 1966.
7. BURG, M., AND N. GREEN. Function of the thick ascending limb of Henle's loop. *Am. J. Physiol.* 224: 659-668, 1973.
 8. BURG, M., L. ISAACSON, J. GRANTHAM, AND J. ORLOFF. Electrical properties of isolated perfused rabbit renal tubules. *Am. J. Physiol.* 215: 788-794, 1968.
 9. BURG, M., AND J. ORLOFF. Control of fluid absorption in the renal proximal tubule. *J. Clin. Invest.* 47: 2016-2024, 1968.
 10. BURG, M., AND J. ORLOFF. Electrical potential difference across proximal convoluted tubules. *Am. J. Physiol.* 219: 1714-1716, 1970.
 11. BURKE, T. J., R. R. ROBINSON, AND J. R. CLAPP. Determinants of the effect of furosemide on the proximal tubule. *Kidney Intern.* 1: 12-18, 1972.
 12. CLAPP, J. R., AND R. R. ROBINSON. Distal sites of action of diuretic drugs in the dog nephron. *Am. J. Physiol.* 215: 228-235, 1968.
 13. COTLOVE, E., H. V. TRANTHAM, AND R. L. BOWMAN. An instrument and method for automatic, rapid, accurate and sensitive titration of chloride in biological samples. *J. Lab. Clin. Med.* 51: 461-468, 1958.
 14. DEETJEN, P. Mikropunktionsuntersuchungen zur Wirkung von Furosemide. *Arch. Ges. Physiol.* 284: 184-190, 1965.
 15. DEETJEN, P. Micropuncture studies on site and mode of diuretic action of furosemide. *Ann. N.Y. Acad. Sci.* 139: 408-415, 1966.
 16. DIRKS, J. H., AND J. F. SEELY. Effect of saline infusions and furosemide on the dog distal nephron. *Am. J. Physiol.* 219: 114-121, 1970.
 17. DUARTE, C. G., F. CHOMETY, AND G. GIEBISCH. Effect of amiloride, ouabain, and furosemide on distal tubular function in the rat. *Am. J. Physiol.* 221: 632-640, 1972.
 18. GRANTHAM, J., AND M. BURG. Effect of vasopressin and cyclic AMP on permeability of isolated collecting tubules. *Am. J. Physiol.* 211: 255-259, 1966.
 19. GRANTHAM, J., M. BURG, AND J. ORLOFF. The nature of transtubular Na and K transport in isolated rabbit renal collecting tubules. *J. Clin. Invest.* 49: 1815-1826, 1970.
 20. HELMAN, S. I., J. J. GRANTHAM, AND M. B. BURG. Effect of vasopressin on electrical resistance of renal cortical collecting tubules. *Am. J. Physiol.* 220: 1825-1832, 1971.
 21. HODGKIN, A. L., AND KATZ, B. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol., London* 108: 37-77, 1949.
 22. HOGBEN, C. A. M. The chloride transport system of the gastric mucosa. *Proc. Natl. Acad. Sci. U.S.* 37: 393-395, 1951.
 23. HOOK, J. B., AND H. E. WILLIAMSON. Influence of probenecid and alterations in acid-base balance on the saluretic activity of furosemide. *J. Pharmacol. Exptl. Therap.* 149: 404-408, 1972.
 24. HOWARDS, S. S., B. B. DAVIS, F. G. KNOX, F. S. WRIGHT, AND R. W. BERLINER. Depression of fractional sodium reabsorption by the proximal tubule of the dog without sodium diuresis. *J. Clin. Invest.* 47: 1561-1572, 1968.
 25. KNOX, F. G., F. S. WRIGHT, S. S. HOWARDS, AND R. W. BERLINER. Effect of furosemide on Na reabsorption by proximal tubule of the dog. *Am. J. Physiol.* 217: 192-198, 1969.
 26. LEZOTTE, L. A., K. M. MAGAFFEY, E. W. MOORE, AND H. JICK. The effects of furosemide on renal concentration and dilution. *Clin. Sci.* 31: 371-382, 1966.
 27. LUTZ, M. D., AND M. B. BURG. A single microelectrode to measure resistance of the isolated perfused rabbit proximal convoluted tubule. *Federation Proc.* 31: 332, 1972.
 28. MALNIC, G., F. L. VIEIRA, AND H. ENOKIBARA. Effect of "Furosemide" on chloride and water excretion in single nephrons of the kidney of the rat. *Nature* 208: 80-81, 1965.
 29. MORGAN, T., M. TADOKORO, D. MARTIN, AND R. W. BERLINER. Effect of furosemide on Na and K transport studied by microperfusion of the rat nephron. *Am. J. Physiol.* 218: 292-297, 1970.
 30. MORRIN, P. A. F. The effect of furosemide, a new diuretic agent, on renal concentrating and diluting mechanism. *Can. J. Physiol. Pharmacol.* 44: 129-137, 1966.
 31. PUSCHETT, J. B., AND M. GOLDBERG. The acute effects of furosemide on acid and electrolyte excretion in man. *J. Lab. Clin. Med.* 71: 666-677, 1968.
 32. RADTKE, H. W., G. RUMRICH, E. KINNE-SAFFRAN, AND K. J. ULLRICH. Dual action of acetazolamide and furosemide on proximal volume absorption in the rat kidney. *Kidney Intern.* 1: 100-105, 1972.
 33. RAMSEY, J. A., H. J. BROWN, AND P. C. CROGHAN. Electrometric titration of chloride in small volumes. *J. Exptl. Biol.* 32: 822-829, 1955.
 34. REHM, W. S. A theory of the formation of HCl by the stomach. *Gastroenterology* 14: 401-417, 1950.
 35. SCHMIDT, U., AND U. C. DUBACH. The behavior of Na⁺K⁺-activated adenosine triphosphatase in various structures of the rat nephron after furosemide application. *Nephron* 7: 447-458, 1970.
 36. STASON, W. B., P. J. CANNON, H. O. HEINEMANN, AND J. H. LARAGH. Furosemide. A clinical evaluation of its diuretic action. *Circulation* 34: 910-920, 1966.
 37. SUKI, W., F. C. RECTOR, AND D. W. SELDIN. The site of action of furosemide and other sulfonamide diuretics in the dog. *J. Clin. Invest.* 44: 1458-1469, 1965.
 38. TIMMERMAN, R. J., F. R. SPRINGMAN, AND R. K. THOMS. Evaluation of furosemide, a new diuretic agent. *Current Therap. Res.* 6: 88-94, 1964.
 39. VUREK, G. G., AND R. L. BOWMAN. Helium-glow photometer for picomole analysis of alkali metals. *Science* 149: 448-450, 1965.