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Bicarbonate Reabsorption along Renal Tubules.* (24072)

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Montgomery and Pierce first demonstrated that in the frog kidney urine is acidified in the distal tubule(1). More recently it has been suggested that the mechanism of acidification is one involving an exchange of hydrogen for sodium. Such a system is also invoked in reabsorption of bicarbonate from tubular urine. Pitts(2) and Berliner(3) suggest that hydrogen exchanges for sodium along the entire nephron, *i.e.*, in proximal as well as distal tubules. Nicholson(4) demonstrated by histochemistry that the brush border of proximal tubules may be quite acidic, suggesting that hydrogen ions are secreted into urine by proximal cells as well as distal. Using the stop flow technic devised by Malvin, Wilde and Sullivan(5,6,7), we have located along the nephron the transport systems for bicarbonate.

Principle of stop flow analysis. Administration of an osmotic diuretic mannitol to a dog establishes such a high rate of urine flow that concentrations are changed to a new steady value. If during this period the ureter is clamped shut, intratubular pressure rises until filtration at the glomeruli ceases. During this interval of stopped flow urinary concentrations will be altered as static columns of tubular urine remain trapped in the various nephron segments. If glucosuria is developed before occlusion, such that glucose reabsorptive transfer maximum is exceeded, the occlusion will allow further time for lowering glucose concentrations along the proximal tubule. If para-aminohippurate (PAH) is included in the infusion, the occlusion will allow further time for increasing the proximal PAH concentrations. In the distal areas occlusion will allow the Na concentration to be lowered. On release of occlusion the diuretic flush washes the concentration pattern along each nephron. Serial urine samples segment this pattern into an ordered array, best pictured on a graph if concentration of each sample is plotted against accumulated volume of urine which has appeared before the particular sample since reinstatement of flow. Plots against time show a distortion due to variable flow rates. The first samples to enter the collector come from distal areas and show decreasing concentrations of Na. As proximal fluid begins to enter, the Na concentrations rise, while glucose

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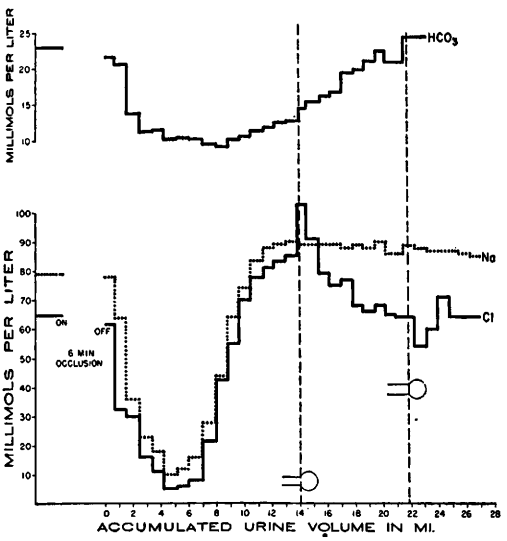

FIG. 1. Urinary stop flow patterns for bicarbonate, chloride and sodium.

concentrations fall. The lowered glucose concentration pattern overlaps that of rising PAH. Wilde and Malvin(7) have developed a graphical method of deriving the locus of transport segments along the nephron from these concentration patterns.

Methods. Six experiments were done on mongrel dogs weighing 12-16 kg, anesthetized with 35 mg/kg of sodium pentobarbital injected intraperitoneally. One ureter was exteriorized through flank incision and catheterized well into the pelvis with polyethylene tubing. A constant intravenous infusion was supplied at 10 ml/min. Infusions contained 15-20% mannitol plus PAH, creatinine and sufficient sodium bicarbonate, so that this anion appeared in all urine samples. All infusions were made up to volume with Ringer's solution. When urine flow stabilized at some high level the experiment was begun. Bicarbonate concentrations were determined using a Kopp-Natelson microgasometer. Chloride was determined using the method of Brun(8), creatinine by Bonsnes and Taussky(9), PAH according to Smith(10). Sodium was determined using Beckman direct reading flame photometer.

Results. Fig. 1 depicts a bicarbonate pattern typical of all 6 experiments. Urinary concentration along the ordinate is plotted against accumulated volume of urine which appeared after reinstatement of flow and before the particular sample. To the left of the break in each curve are plotted control concentrations of various urinary solutes during free diuretic flow. Solute concentrations to the right of break represent concentrations elaborated along the tubule during stopped flow as released in the new urinary washout pattern. The 2 vertical dashed lines bearing filter diagrams encompass that part of the volume scale over which more and more new filtrate mixes with the proximal stop flow pattern. The left line indicates the position of the filter of the shortest nephron; the right line represents the level of the filter of the longest nephron. The position of these filters can be located by the rising concentration of inulin injected after ureteral occlusion and which thus lies at the glomeruli ready to mark entry of new filtrate(6,7). More conveniently they were marked with PAH, the enormous proximal secretion of which forms a concentration peak at the first filter (14 ml on Fig. 1). A sample at this 14 ml level is most typical of true stop flow proximal fluid(7), being minimally contaminated with distal fluid from the longer nephrons or with new filtrate soon to enter the shortest nephrons.

Sodium (Fig. 1) reached its lowest concentration at 5 ml, representing delivery of samples from a maximum number of low sodium distal nephron segments. At the 14 ml landmark, sodium has already returned to the concentration plateau typical of free osmotic diuresis, indicating a uniquely weak proximal reabsorption(6,7) of sodium away from the water bulk held by mannitol.

Bicarbonate, as is sodium, is strongly reabsorbed at the distal 5 ml level but unlike sodium does not rise to the free flow plateau concentrations in the best proximal sample (14 ml). Its concentration is low here (14 ml) and then rises slowly as more and more new filtrate mixes with less and less of the low stop flow proximal concentration. It rises to free flow values at 22 ml just as the last of low-bicarbonate, stop flow proximal fluid from the longest nephrons is reaching the urine collector. This behavior proves that stop flow proximal concentrations of bicarbonate are

lower than new flow or free flow proximal values(7).

While chloride also is strongly reabsorbed at the 5 ml distal site, contrary to bicarbonate, it is relatively elevated in the proximal area. In 2 experiments Cl lies parallel to Na in the proximal area. In the 4 others, Cl concentrations rise to a peak at level 14 ml just as does PAH (Fig. 1). This is the criterion for secretion, or that stop flow chloride concentrations in the proximal are higher than new flow concentrations in this area(7).

In their micropuncture studies in rats and guinea pigs, Walker, Bott, Oliver and MacDowell(11) found proximal chloride concentrations elevated over those in plasma. They presumed that HCO_3 was preferentially reabsorbed.

Additional proximal reabsorption of $NaHCO_3$ by itself during stop flow should carry out water osmotically leaving the remaining chloride elevated over free flow urinary concentration by the same amount as the bicarbonate drop. Actually the chloride rise exceeds this. Part of the chloride rise may likewise result from reabsorption of sodium salts of phosphate, sulfate, etc., with water following to isotonicity.

In some experiments the mass of Cl associated with non-transportable creatinine in the proximal stop flow samples, exceeds that remaining with creatinine in the free flow samples, *i.e.*, our ratio $(U_{Cl}/U_{cr})/(P_{Cl}/P_{cr})$ is higher in proximal samples during stop flow (See definition in Ref. 6) than for urine of free flow. It seems possible that HCl is secreted into the proximal tubule during stop flow thus titrating $NaHCO_3$, phosphate and other buffers. A complete balance sheet for all ion species is required to test the various alternatives.

Summary. Using the stop flow technic in $NaHCO_3$ loaded dogs under osmotic diuresis we find that bicarbonate and chloride are reabsorbed avidly in a far distal area coincident with sodium. Compared to values in free flowing osmotic diuretic urine, concentration of bicarbonate developed in proximal tubules during stop flow remains low at the same point where chloride is relatively elevated. Reabsorption of proximal HCO_3 alone does not account for Cl elevation. Cl secretion into and removal of other anions from the proximal fluid is considered.

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