Stop-Flow Studies on Ion and Water Reabsorption in the Dog

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The stop-flow method has been used to study various aspects of renal tubular transport mechanisms. According to this method, aldosterone promotes the tubular reabsorption of sodium in the distal tubule. The method also indicates that the secretion of potassium, hydrogen and ammonium occurs in a very distal portion of the nephron. The substitution of anions such as ferrocyanide, sulfate and phosphate for chloride apparently increases the area of the nephron involved in the secretion of hydrogen and potassium. The stop-flow experiments support other evidence that the rate of flow of urine through the loops of Henle affects the countercurrent multiplier system which, in turn, determines the ability of the kidneys to elaborate a concentrated urine.

IT HAS been known for many years that the kidney reabsorbs a large part of the salts and water filtered at the glomerulus. However, only in recent years have some of the mechanisms controlling secretion and reabsorption been elucidated. The micropuncture technic as developed by Richards has been extremely useful in this respect, since it allows direct analysis of tubular urine. The stop-flow method, although not as precise as micropuncture, has also lent itself well to renal transport studies.

For the past 2 years, the authors, in collaboration with Drs. Lawrence Sullivan, Arthur Vander and Peter Abbrecht, have used the stop-flow technic extensively as a tool for investigating the transport of salt and water in the nephron. Because of the limitations of time, we have arbitrarily selected 3 areas of our research for discussion at this symposium: (1) the effect of aldosterone on the transport of sodium; (2) the effect of large impermeant anions on the transport of potassium; (3) the effect of osmotic diuresis on the renal concentrating mechanism.

Aldosterone

Recently, using the stop-flow technic, we demonstrated a distal site of action of aldosterone. The dashed curve in figure 1 illustrates a typical pattern of the concentration of sodium from a stop-flow experiment in a normal dog. The sodium concentration of free flow urine collected immediately before occlusion was 63 mM per liter. As distal fluid entered the collector, the concentration of sodium fell to a low of 5 mM per liter. As fluid from the loops and proximal tubules entered the collector, the concentrations of sodium rose to reach a plateau at the original free flow concentration. The peak concentration of para-aminohippurate (PAH), not shown in this figure, indicated the best stop-flow proximal sample to be at 10 ml.

In contrast to the normal dog, the lowest concentration achieved by the distal tubule of the adrenalectomized dog was only 24 mM per liter. The distal tubule was apparently unable to reduce the concentration of sodium to the minimum value achieved in normal dogs during stop-flow.

Occasionally, normally low concentrations of sodium were obtained from the distal tubular urine of the adrenalectomized animal. This seemed to occur only when the concentration of sodium in plasma was exceedingly low. The following series of experiments was designed, therefore, to study the relationship between the concentrations of sodium in the plasma and in the distal tubular urine. After the collection of normal stop-flow urine, the concentration of sodium in plasma was raised rapidly by the intravenous infusion of 2 to 3
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Figure 1
Comparison of the distal tubular concentrations of sodium during stop-flow in a normal and in an adrenalectomized dog. (Republized by permission of the Proceedings of the Society for Experimental Biology and Medicine.2)

Gm. of sodium chloride in 50 ml. of water, over a period of 5 minutes. After 10 more minutes, the occlusion was again performed, and another collection was made.

Figure 2 demonstrates the relationship between the concentration of sodium in the plasma and in the distal tubular urine during ureteral occlusion. It can be seen that during stop-flow, the distal tubule of the normal animal was capable of lowering the concentration of sodium almost to zero. Except possibly at extremely high values, the minimum concentration of sodium appears to be independent of the concentration of sodium in the plasma. This independence suggests that over the physiologic range, the maximal concentration gradient for sodium which the normal animal can develop and maintain between the plasma and the distal tubular urine has not been reached.

The effects of adrenalectomy upon this pattern are striking. Even at very low plasma concentrations of sodium, the distal concentrations during ureteral occlusion were abnormally high. During occlusion, there was a direct relationship between the concentrations of sodium in the plasma and in the distal tubule. This indicates that adrenalectomy has reduced the maximal concentration gradient for sodium which can be maintained across the distal tubular cells. As the concentration of sodium in the plasma rises, the minimal concentration of sodium also rises. Even if the duration of occlusion is prolonged from 4 to 6 minutes, the peak concentration of sodium in the distal tubular urine during stop-flow is not changed, indicating that maximal reabsorption of sodium by the distal tubules occurs within the first 4 minutes of ureteral occlusion.

The effects of aldosterone on the adrenalectomized dogs are demonstrated in figure 3. The administration of aldosterone restored the ability of the distal tubule to lower the concentration of sodium in the distal tubule even in the presence of an elevated concentration of sodium in the plasma.

Mode of Action of Aldosterone

Clearance methods indicate that aldosterone increases the tubular reabsorption of sodium. This action is manifested in stop-flow analysis by the ability of aldosterone to increase the maximal concentration gradient for sodium which can be developed between the plasma and the distal tubular urine. Since the mini-
maximal concentration of sodium attained in the distal tubule during ureteral occlusion is independent of the duration of occlusion, this concentration must be a steady state value; i.e., the concentration at which sodium movement out of the lumen is equal to sodium movement inward. Aldosterone could act, therefore, in 1 of 2 different ways: it could activate carrier systems responsible for sodium transport outward or it could decrease passive back-diffusion of sodium from interstitial fluid into distal tubular lumen.

A rough estimate of the rate of back-diffusion of sodium has been made in our laboratory, using the isotope Na\textsuperscript{24}. The ureteral occlusion stops filtration so that glomerular substances such as inulin will not enter the concentration pattern except as new filtrate. Na\textsuperscript{24} injected intravenously after the occlusion, by crossing the tubular epithelium transmurally, enters the urinary pattern ahead of inulin. Precise delineation of rates of movement into the stop-flow pattern is complicated by the continuing decline of the precursor Na\textsuperscript{24} in the blood plasma during the short 2-minute period allowed for the Na\textsuperscript{24} to enter the tubule lumen. The shape of the stop-flow curves for Na\textsuperscript{24} seems unchanged after adrenalectomy, with no suggestion of any increased rate of back-diffusion. These preliminary data coupled with the stop-flow data lead us to believe that aldosterone increases the maximal transport velocity by increasing the number of carrier sites available for the reabsorption of sodium.

**Potassium**

It has been established that potassium is reabsorbed and secreted by the nephron. The area involved in the secretion of potassium is located in a distal region of the mammalian nephron, which is also a site for the secretion of hydrogen.\textsuperscript{3} The secretion of ammonia is also thought to take place in the same distal area.\textsuperscript{4} Recently, Pitts, Gurd, Kessler and Hierholzer have shown that these secretory areas do exist at the same distal tubular site.\textsuperscript{5} On the basis of clearance studies, the secretion of eations is believed to be coupled with sodium reabsorption.\textsuperscript{6,7}

Using the stop-flow technic, we have obtained data which indicate that the secretion of potassium, hydrogen and ammonium occur in a very distal area of the nephron. Figure 4 shows a normal stop-flow curve on which sodium, potassium and hydrogen (titratable acidity) are plotted. The concentration of both potassium and hydrogen rose in the early distal samples and reached maximum values even before the concentration of sodium reached its minimum. Potassium concentrations then fell very sharply to values below those observed during free flow. This drop occurred in the area of low concentrations of sodium. As proximal fluid entered the collector, potassium concentrations returned to free flow values.

We believe that the increase in the concentrations of potassium in the early samples represents secretion of that cation in the collecting ducts and perhaps in some part of the distal tubule. This increase occurs in the first few samples, in urine which must have been trapped in the collecting ducts. This interpretation agrees with the findings of Ullrich et al., who demonstrated that the concentration of potassium may increase in the collecting ducts.\textsuperscript{8} Unfortunately, it is impossible for us to delineate more precisely the anatomic areas involved in the secretion of potassium. The secretory area may very well extend into the distal tubule itself.
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The minimum concentration of potassium always occurred in a distal area of the stop-flow pattern and was often below the concentration in plasma. We regard this as evidence for the reabsorption of potassium in an area of the distal tubule which also reabsorbs sodium to low levels.

Bott has demonstrated that throughout the proximal tubule of the amphibian nephron, the concentration of potassium remains at the level in the plasma. There is little doubt that the reabsorption of potassium against a chemical gradient does occur. Subjects in potassium deficiency excrete urine with concentrations of potassium lower than that in plasma. Since the reduction in the concentration of potassium in amphibia does not seem to occur in the proximal tubule, it must take place in a more distal area. It is not surprising, then, that the stop-flow patterns for potassium include a distal area of low concentrations of potassium.

The Effects of Impermeant Anions

Infusions of relatively impermeant anions, such as ferrocyanide, sulfate, thiosulfate and phosphate, retard the reabsorption of sodium in the proximal areas of the nephron because of their charge and their inability to accompany reabsorbed sodium across the tubular wall. Thus, it has been suggested that these anions increase the secretion of hydrogen and potassium because they deliver greater amounts of sodium to a distal mechanism.

Figure 4
Stop-flow pattern for sodium, hydrogen and potassium.
which reabsorbs sodium in exchange for secreted potassium and hydrogen. However, the data derived from the use of these anions in stop-flow analysis have led us to conclude that the effect of these anions upon the secretory mechanism is only part of their total influence upon hydrogen and potassium excretion.

The results of these stop-flow experiments show that the presence of impermeant anions within the tubule not only increased the secretion of hydrogen, ammonium and potassium, but also caused the points of maximum titratable acid and of maximum concentrations of potassium in the stop-flow patterns to be shifted proximally. The maximum concentrations of these substances then appeared in the area of the patterns which normally contained the minimum concentrations of potassium. An area of the nephron, much larger than was apparent in the control occlusions, seemed to be secreting hydrogen and potassium.

Figure 5 shows the results of 1 experiment in which a control occlusion was followed by a second after the infusion of phosphate so that the plasma phosphate level rose from 1.7 mM per liter to 14.3 mM per liter. The control patterns for potassium, ammonium and hydrogen all showed a distal secretory peak which was distal to the sodium minimum. After the infusion of phosphate, the maximum concentrations of potassium, ammonium and hydrogen were all increased. Their maximum concentrations were also all moved more proximally so that they coincided with the minimum for sodium. After infusion of phosphate, the distal reabsorptive area for potassium was no longer evident. Similar results are obtained if the dog is infused with any other relatively impermeant anion, e.g., thiosulfate, ferrocyanide or sulfate.

The stop-flow patterns indicate that, in the presence of the impermeant anions, the apparent secretion of hydrogen and potassium occurred along the full length of the distal nephron in which the strong sodium reabsorptive mechanism is located. Ordinarily, chloride follows the reabsorbed sodium at this site. However, in those animals which are infused with the sodium salt of the impermeant anions, more sodium than chloride is presented to the distal reabsorptive system, and the amount of sodium which is reabsorbed exceeds the supply of chloride. This excess of reabsorbed sodium over chloride cannot be accompanied by the infused anion because of its size and immobility. Thus, as sodium is removed, the anionic charge remaining in the tubule will attract other available cations to replace the sodium. The only relatively diffusible cations available in quantity are hydrogen and potassium, and they are drawn into the tubule from either the tubular cells or the interstitial fluid. Evidently the potassium reabsorptive mechanism cannot counteract this inward movement. In effect, then, a pseudo-exchange mechanism, created by the combination of the reabsorption of sodium and the presence of immobile anions, has been set up in a part of the tubule where one was not apparent in control experiments.

Countercurrent Multiplier System

It has been established that the ability of the kidneys to concentrate urine is dependent upon a countercurrent multiplier system. In this system, the interstitial osmotic pressure increases along the pyramids, being highest at the tip. In the presence of antidiuretic hormone (ADH) this gradient will cause water to be drawn out of the collecting ducts and so concentrate the urine. However, under certain conditions, urine osmolality may decrease even in the presence of maximum ADH. During osmotic diuresis urine osmolality decreases and approaches plasma osmolality as the diuresis becomes more severe.

This inability to concentrate during osmotic diuresis might be considered to result from increased rate of flow through the collecting ducts with concomitant decrease in time allowed for osmotic equilibrium. If this were true, one would expect urine osmolality to increase significantly during stop-flow as additional time is allowed for equilibration.

Figure 6 shows the results of a stop-flow experiment in which urine osmotic pressure was measured. Although the osmotic pressure
did rise in a distal area, the increase was only about 10 per cent of the control osmolarity (from 496 to 542 mOsm. per liter). These results suggest that the osmotic pressure of the interstitium is higher than urine osmolarity during osmotic diuresis, but only slightly so. Since urine osmolarity does increase during stop-flow, this is evidence that during osmotic diuresis the collecting duct urine does not reach osmotic equilibrium with the interstitium.

These results are consistent with the formulations of Hargitay and Kuhn that the rate of urine flow through the loops of Henle is 1 of the determinants of the magnitude of the countercurrent gradient which the kidney
may establish. There must be an optimum flow rate through the loops. Any increase in flow above that optimum would serve to carry off solute and lessen the osmotic gradient existing in the renal medulla.

Figure 7 shows the results of an experiment which indicates that this is true during osmotic diuresis. Catheters were placed in the left and right ureters and in the left renal artery. After a control period, 20 ml. of 20 per cent mannitol was injected into the left renal artery over a 1-minute period. One-minute urine samples were collected simultaneously from the 2 ureters, and the samples analyzed for their concentrations of sodium. The urine flow from the left kidney increased almost immediately, reaching a maximum during the third minute, then declined relatively slowly. The control sodium concentration in urine from the left kidney was 39 mEq per liter. As the urine flow increased, so did the concentration of sodium. In the second sample after injection, the concentration of sodium reached 117 mEq per liter and then declined.
to 23 mEq per liter. A similar pattern was seen on the right side: as the urine flow increased, so did the concentration of sodium. Again the concentrations of sodium fell off more quickly than urine flow. This experiment suggests that a sudden increase in flow rate through the nephron is able to sweep excess sodium out into the urine. This observation is consistent with the view that the rate of the flow of urine through the loop of Henle and the collecting ducts is a determinant of the countercurrent gradient which may be maintained by the kidney.

References
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