Ion Exchange Mechanisms in the Nephron

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Accumulating evidence favors the view that in biological systems, the active transport of strong electrolytes involves coupled exchanges of ions, usually of sodium for potassium. In the present paper, the author considers the renal exchange of potassium, sodium and hydrogen in this light, and reviews current concepts concerning the mechanisms involved in the secretion of these ions by the kidney. He indicates that new investigative techniques will be required to establish that the kidney behaves with respect to coupled ion exchanges as do nerves, red blood cells and frog skin. He concludes by cautioning against extrapolation of experimental results from one species to another.

The title "Ion Exchange Mechanisms in the Nephron" permits a more or less wide range of subject matter depending upon the interpretation of the phrase "ion exchange mechanisms." This presentation will be concerned with a few aspects of the processes involved in the excretion of acid and potassium, which are the processes which have been shown to result in the replacement of 1 ion in the urine by another. However, as knowledge increases concerning the transport of strong electrolytes generally, the evidence increasingly favors the concept that all such active-transport processes involve coupled exchanges—usually of sodium for potassium. At least such exchanges appear to be implicated in nerve, in red blood cell and in frog skin, and it seems reasonable to expect that when adequate investigative techniques have been devised for the more definitive study of renal tubular processes, similar mechanisms will be encountered. Indeed, the carrier mechanism now widely adopted as the model for ion transport implies ion exchange.

With respect to potassium excretion, no attempt will be made to review the entire field; certain aspects related to relatively recent work will be considered. One of these aspects concerns the site and extent of potassium reabsorption in the tubule. While very little is known about the mechanism concerned in this process and it is not, strictly speaking, one which can be identified with the ion exchange processes, some knowledge of the reabsorptive process is essential for the evaluation of the secretory sodium-potassium exchange, since only the difference between reabsorption and secretion can be measured. To quantify secretion, it is first necessary to assign some value to reabsorption. Gilman was the first to propose that reabsorption of potassium was equal to the filtration of potassium—that is, that the filtered potassium was completely reabsorbed before the potassium destined for excretion was added by secretion. At the time, 10 or 12 years ago, this seemed a rather unlikely possibility; but as time has passed and more data have been accumulated, it first became useful to adopt this as a working hypothesis, and then as a view for which there is a considerable body of experimental evidence.

The evidence for this view is based largely on 2 phenomena: first, the dependence of the excretion of potassium on the excretion of sodium and second, the lack of relationship between the glomerular filtration rate and the excretion of potassium when the excretion of sodium is maintained. Although earlier data illustrating the dependence of the excretion of potassium on the excretion of sodium will be considered later, the most striking demonstration of this phenomenon is to be seen in stop-flow studies.

Figure 1, taken from some recent studies by Jaenike and Berliner, shows the usual pattern of electrolyte concentrations in stop-flow studies. In this particular instance the first few samples collected, shown at the left of the figure, differ from the usual because in

From the National Heart Institute, National Institutes of Health, Bethesda, Md.
Figure 1
Stop-flow pattern obtained during mannitol diuresis in the dog. The dead space had been filled with mineral oil. First specimens collected appear at the left of the figure. (Republished by permission of the Journal of Clinical Investigation.)

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these studies the pelvic dead space was filled with oil. The first samples, therefore, differ from free flow because they are derived from the tubule system and have been modified during the period of stop-flow. This is not important for present purposes, but will explain the different appearance of these data from the figures of Vander, Sullivan, Malvin, and Wilde.\(^8\)\(^9\) With this exception, the pattern of potassium concentration seen here does not differ significantly from that previously published by others.\(^6\)\(^9\) The concentration of potassium shows a minimum in samples which, from their very low concentrations of sodium and chloride, are identified as having sojourned in distal tubules during the period of stop-flow. This low potassium concentration might be due to reabsorption of potassium at the indicated site. However, the samples from that site must traverse the remainder of the tubule system before they are collected and the more distal parts of the tubule certainly can and do secrete potassium.\(^9\)\(^10\) The low concentrations of potassium might also be interpreted as being due to failure to secrete potassium into those particular samples. There is a logical explanation for this possibility in that they contain virtually no sodium to be exchanged for potassium. These 2 explanations have been distinguished in experiments by Walker and Cooke and their associates at Johns Hopkins.\(^11\) The results of these experiments very clearly indicate that the latter interpretation is correct, that is, that the low potassium concentrations are due to inability of the more distal parts of the tubules to add potassium to the virtually sodium-free distal tubule samples. The experiments were done using a modification of the stop-flow technic devised by Murdaugh and Robinson.\(^12\) This modification consists of interrupting the stop-flow at the end of 4 minutes to permit samples from 1 site to move down to another. Flow is then stopped for an additional 4 minutes, following which the usual type of collection is made. If, during the interruption of stop-flow, about 3 or 4 ml. is permitted to escape from the tubules, most of the high potassium samples leave the tubules and the low sodium—low potassium samples are moved into the region in which potassium concentration was previously high. When the pattern from this second occlusion is examined, there is no distal potassium peak. Furthermore, if the secretion of potassium is markedly enhanced by the administration of the carbonic anhydrase inhibitor, acetazoleamide, the marked distal peak observed in the initial collection is still obliterated in the second part of the interrupted stop-flow. On the other hand, if chlorothiazide is used so that the minimal concentration of sodium in distal tubule samples is much higher, the potassium peak, although somewhat reduced, is still present. The question arises whether the failure to observe a potassium peak during the second occlusion does not stem from either damage to, or exhaustion of, the secretory mechanism during the prolonged period of stop-flow. This problem was tested by permitting 8 to 10 ml., rather than 3 to 4 ml., to escape between occlusions so that the low sodium samples could move out of the tubules; the results indicate that under this circumstance the potassium peak in the second occlusion was indistinguishable from the first.

Several conclusions can be drawn from these observations: (1) potassium can be secreted only into fluids which initially contain sodium; (2) the potassium minimum in stop-flow experiments owes its localization primarily to the absence of sodium in the same samples. Had the urine, in the moments preceding stop-flow, arrived at this site already freed of potassium but not of sodium, the result would be exactly that observed. Both more distal and more proximal samples would contain higher concentrations of sodium and therefore gain potassium in passing the secretory site after reinstatement of flow, while those around the sodium concentration minimum would remain free of potassium. It can be concluded, concerning the site of potassium reabsorption, that it may be at the region which is marked by the low concentrations of potassium in stop-flow patterns, but it may also be anywhere proximal to it. Furthermore, the concentration of potassium in these
ION EXCHANGE IN THE NEPHRON

low potassium samples reveals nothing about how much potassium was left in them by the reabsorptive process, since they subsequently gain potassium while passing the secretory site. If some intervention, such as the administration of chlorothiazide or the infusion of sulfate, results in a higher concentration of potassium in the low potassium samples, it does not warrant the conclusion that the reabsorption of potassium has been inhibited, 8,13 this reservation is particularly pertinent if the intervention increases the concentration of sodium in these samples, in which case it will enhance their capacity to accept potassium after flow is re instituted. As a matter of fact, data to be presented shortly, much of it derived after just such interventions, strongly support the view that even under these conditions all of the filtered potassium is reabsorbed.

It is apparent from the data already considered that in some tubule segments all, or nearly all, the potassium can be reabsorbed. However, this reabsorption, which occurred during the relatively prolonged stasis of stop flow, might not occur during free flow. The data of Davidson, Levinsky, and Berliner 14 show that the same extensive reabsorption of potassium does occur in free flow. The pertinent experiments involved the separate collection of urine from each kidney of the trained, unanesthetized dog which had been previously prepared by a bladder-splitting procedure. 15 At appropriate times the filtration rate in the right kidney was reduced by inflation of a cuff previously placed around the right renal artery and connected to the outside by a fine polyethylene catheter. 16 This made it possible to use the left kidney as a control for the right and thus to compensate for changes with time in the excretion of potassium from the control kidney. When no attempt was made to provide a high rate of sodium excretion, reduction of the filtration rate produced a sharp drop in sodium excretion and with it a marked, although lesser, drop in potassium excretion. This is illustrated in figure 2. Since sodium excretion frequently fell to quite low levels, the results were consistent with the interpretation that the observed effect was due, not primarily to the change in filtered potassium, but to the decrease in the sodium available for exchange with potassium in the more distal parts of the nephron.

Accordingly, measures of various sorts were taken to increase sodium excretion to such an extent that a high rate of sodium excretion was maintained even when glomerular filtration was reduced. The procedures which were used were the administration of the mercurial diuretic, salyrgan, the carbonic anhydrase inhibitor, acetazoleamide, and the poorly reabsorbed anion, sulfate, in the form of sodium sulfate. The result obtained, as shown in figure 3, was independent of the procedure used to maintain sodium excretion. Reduction of glomerular filtration rate by up to 35 per cent was entirely without effect on the excretion of potassium, although sodium excretion fell to an extent equal to or greater than the reduction of filtration rate. The excretion of potassium was maintained at the same level as in the control kidney whether that level represented only 15 to 20 per cent of the concurrent rate of filtration of potassium at the glomeruli, as was frequently the case when salyrgan was administered, or, as was usual when acetazoleamide was used, when the rate of excretion of potassium exceeded the rate at which it was filtered. The only reasonable interpretation of these data is that the filtered potassium makes no appreciable contribution to that excreted in the urine. If only a few per cent of the filtered potassium were excreted, the total excretion of potassium should have been detectably modified by the change in filtration rate. It should also be noted that the data can be explained only if the reabsorption precedes the secretion.

The data derived from micropuncture studies are the only ones which pertain directly to the localization of the site of potassium reabsorption. These data are conflicting. Wirz and Bott, 17 in the only micropuncture study of potassium in the mammal, found that in the rat the concentration of potassium fell markedly in the first half of the proximal tubule. At about the same time, Bott also found that the concentration of potassium was
markedly reduced in the proximal tubule of Necturus. But, subsequently, using different and probably more reliable methods, Bott did not find an appreciable reduction in the concentration of potassium in the proximal tubule of Necturus. Nevertheless, in view of the opposing electrical potential, even the maintenance of an unchanged concentration requires active transport of potassium (or a very considerable solvent drag effect); otherwise, if active transport were not involved, the potassium concentration should rise to almost twice that in plasma in order for the chemical gradient to balance the electrical gradient. It may be, then, that reabsorption of potassium is completed in some more distal segment, but for the reasons stated earlier, the site cannot be identified by the stop-flow technic.

The recent observations of Hilger, Klümper and Ullrich based on microcatheterization of the hamster papilla, have suggested, although they did not unequivocally establish, that potassium secretion occurs in the collecting ducts. Data from the stop-flow experiments of Jaenike and Berliner suggest that the same is true in the dog. However, it should be pointed out that the secretion of potassium cannot be confined to the collecting ducts but must, in large measure, be contributed by cortical portions of the nephron. It is not difficult to produce, and maintain over extended pe-
riods, potassium clearances twice the rate of glomerular filtration, which in the dog would be about half the renal plasma flow. Since, barring synthesis by the kidney, no substance can long be cleared at a rate exceeding the blood flow, this means that the potassium secretory site must receive a flow of blood which can supply the requisite potassium. Even assuming that there is no reabsorption of the filtered potassium, and disregarding the probable relative exclusion of potassium from the medulla by the countercurrent flow of the blood, the most generous estimates of medullary blood flow would not include enough potassium to supply the secreted potassium. Similar considerations probably also apply to the availability of ammonia precursors to the collecting ducts, but the necessary data for the calculation are less easily obtained.

There is little information concerning the mechanism by which the potassium-sodium exchange is accomplished. It appears not unlikely that potassium-sodium exchange underlies the sodium extrusion mechanism which characterizes virtually all animal cells. However, except for the red blood cell, potassium seems to be close to electrochemical equilibrium in those cells that have been studied. This may be due to a high permeability to potassium which enables potassium to be distributed in such a way that there is little departure from an electrochemical potential gradient of zero. The known low permeability of the red blood cell to cations will adequately explain its departure from the behavior of other cell types. In the case of membranes such as frog skin and renal tubule epithelium, which perform oriented transport, there is an additional requirement for a differential permeability of the 2 surfaces of the cell facing the exterior and interior of the body. This is essentially the model of Ussing and Koefoed-Johnsen,3 in which potassium is close to electrochemical equilibrium across the inward-facing surface of the cell, but not across the outward-facing surface which has a low permeability to potassium. The common sensitivity of all the sodium-potassium transport mechanisms, including that of the renal tubules, to strophanthidin and other cardiotonic steroids suggests, as pointed out by Orloff and Burg,24 that a similar sodium-potassium exchange process at the basal surface of the tubule epithelial cells underlies the monovalent electrolyte transport systems of the renal tubules. Whether or not any additional process is required to explain the secretion of potassium into the urine is uncertain, since the value of the electrical potential gradient between tubule lumen and peritubular fluid has not been measured at the right place and under the right conditions. However, the concentration ratio (urine to interstitial fluid) for potassium probably rarely exceeds 20 or 30 and the required 80 to 100 mV may well be present at these times. It should be noted that so long as the cell membranes are permeable to chloride, and chloride is present in appreciable concentration in the lumen, there would be a continuous flow of chloride from lumen to the peritubular fluid. This current of chloride ion would short-circuit the potential, keeping it relatively low and tending to suppress the accumulation of potassium in the lumen.

Although there is some virtue in simple hypotheses, the fact that a relatively simple model to explain net sodium-potassium exchange can be devised does not mean that nothing more is involved. One circumstance that makes this model seem incomplete arises in consideration of the problem of acidification. The ratio of hydrogen ion concentration in urine to that in plasma may reach 1,000 or more and a transtubular potential of 180 mV or more would be required to establish such a gradient passively. This suggests, although it does not prove, the existence of some more specific exchange mechanism, presumably at the luminal face of the cell as proposed in the model of Pitts9 (fig. 4). The addition of such an exchanger to the model would account for the exchange of potassium for sodium and explain the competitive relationship between potassium and hydrogen ion,6 a phenomenon which is a little more difficult to fit in with the simple model of a single pump.

In the further consideration of hydrogen ion exchange, a problem of definition exists. It has been fairly generally accepted that the
reabsorption of bicarbonate and the formation of titratable acid are effected by the secretion of hydrogen ion in exchange for sodium (fig. 5).\textsuperscript{4, 25, 26} The secreted hydrogen ion is assumed to combine with bicarbonate to yield carbonic acid which in turn yields CO\textsubscript{2} and water which may diffuse from the urine. There has been little direct evidence to support this view, but because this unitary hypothesis is simplest and there is no evidence to the contrary, it seems expedient to retain it. This view has, indeed, been somewhat strengthened by the recent findings of Gottschalk and Giebisch and their respective associates\textsuperscript{27, 28} that the pH of proximal tubule urine in the rat does not, as had long been considered to be the case, remain the same as that of plasma. Instead, it is lowered appreciably. Thus, one assumed difference between bicarbonate reabsorption in the proximal, and acidification in the distal tubule has been largely eliminated from consideration. One little-recognized consequence of the concept that bicarbonate reabsorption is effected by

Figure 3

Potassium (left) and sodium excretion (right) during reduction of the glomerular filtration rate in dogs with maintained sodium excretion from the experimental kidney. See legend figure 2. (Reproduced by permission of the Journal of Clinical Investigation.)\textsuperscript{14}
hydrogen-sodium exchange is that considerably more hydrogen ion is secreted in the proximal tubule where most of the bicarbonate is reabsorbed than in the more distal regions where the extremes of acidity are reached. This consequence is made all the more one-sided by the observation that bicarbonate concentration, as well as volume flow, is reduced in the proximal tubule.

One other very interesting consequence which may make possible the experimental verification of the hypothesis has been pointed out by Walser and Mudge.\textsuperscript{29} It can be calculated that if proximal bicarbonate reabsorption involves the formation of carbonic acid and its subsequent dehydration to carbon dioxide, as would be the case with hydrogen ion secretion, the latter step can proceed at a rate equal to the rate of bicarbonate reabsorption only if: (1) there is carbonic anhydrase in the surface of the proximal tubule cells so that the reaction of carbonic acid to \( \text{CO}_2 \) and water in the lumen is catalyzed; or (2) there is appreciable accumulation of carbonic acid in the fluid in the tubule—enough to bring the pH down approximately 1 pH unit below its equilibrium value. Unfortunately, the type of pH measurement which has been made, since it involves withdrawal or at least isolation of an element of fluid, does not exclude (although it does make it improbable) the possibility that the pH of the fluid \textit{in situ} is as low as required by hydrogen ion secretion in the absence of carbonic anhydrase. In the interval required to do the analysis, uncatalyzed dehydration of carbonic acid might have raised the pH to the observed value.

The third effect of hydrogen-sodium exchange is the accumulation of ammonia in the acidified urine. The process is believed to involve the formation of ammonia from appropriate precursors in the cell of the renal tubule and the diffusion of the nonionic ammonia, that is \( \text{NH}_3 \), into the urine; in the urine it combines with hydrogen ion to form ammonium ion to which the tubule cell is presumed to be relatively impermeable. There are those who prefer to believe that the excretion of ammonium ion involves direct ex-

change of ammonium and sodium ions.\textsuperscript{30, 31}

This view, however, fails to account for one of the most striking features of ammonia excretion (at least in man and in the dog)—its dependence upon the pH of the urine.\textsuperscript{32, 33} The argument advanced that glutamine hydrolysis yields, at the pH within cells, glutamate and ammonium ions\textsuperscript{31} is without cogency since even if free ammonia is formed, it must be considered to be instantaneously in equilibrium with its ionic form which greatly predominates at all physiologic pH's. This has, however, little bearing on the form in which the ammonia crosses the cell membrane. With regard to the latter question there is a very considerable body of evidence indicating that cell membranes are very much more permeable to nonpolar, nonionized substances than to their ions.

In closing, it seems warranted to mention a need for some caution in accepting data obtained in rats as quantitatively applicable to all mammals. While it is unlikely that similar mechanisms in different species differ in their qualitative nature, and indeed we have reason to hope that the fundamental similarities of transport mechanisms extend from tissue to tissue as well as from one animal type to another, nevertheless, it may be misleading to extend quantitative considerations from one to another. This is well illustrated in some of the disagreements concerning the determinants of ammonia excretion. These disagreements have certain of the features of the old story about the blind men and the elephant.
 Those who have studied ammonia excretion in the rat have been impressed with the marked and easily demonstrable increases in the glutaminase activity of the renal cortex, which over any relatively extended period are highly correlated with the rate of ammonia excretion. However, adaptation to acidosis in the dog is not associated with any such change in glutaminase activity, and in this species the relationship of ammonia excretion to the pH of the urine is much more striking and reproducible. Furthermore, the increase in ammonia excretion, which occurs in rats along with an increase in glutaminase activity in response to carbonic anhydrase inhibitors, is absent in both dog and man.

It is important, therefore, to extend the basis of our knowledge over as wide a range as possible and, particularly with regard to quantitative aspects, to hold some reservations, until our investigations have been extended to the specific circumstances concerning which we wish to draw conclusions.

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ION EXCHANGE IN THE NEPHRON


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