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# Renal Tubular Potassium Transport[1]

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In honour of Prof. F. Brücke's 60th anniversary

The renal tubular epithelium is endowed with the capacity of both net secretion and net reabsorption of potassium ions. Until recently the contribution of various tubular segments to the process of potassium excretion had to be inferred from clearance experiments and stop-flow studies. Such experiments have supported the view that distal nephron segments are an important site for net secretion of this ion species into the tubular fluid [1, 2, 4, 5, 21, 37, 47, 49]. However, since the concentration of potassium in the final urine may, under certain metabolic conditions, fall significantly below that of plasma [24], it is certain that the tubular pattern of potassium transfer may vary, and that even the direction of net movement may change along the most distal nephron segments. The very marked dissociation which can be shown to exist, under appropriate experimental conditions, between the rate of potassium excretion and the rate at which potassium is filtered, has led to the thesis that even under conditions of extensive net reabsorption, urinary potassium is derived from distal tubular secretion [2, 4]. This thesis of Berliner implies that reabsorption of potassium is virtually complete within the proximal tubular system.

Several factors have been recognized to affect the rate of urinary potassium excretion. It has been suggested that secretory potassium movement is dependent on sodium balance [2, 24]. This is demonstrated by the suppression of potassium excretion subsequent to sodium depletion [24] and the precipitous fall of potassium excretion when urinary sodium concentrations and/or excretion rates are depressed during acute reduction of the glomerular filtration rate [8, 16, 25]. Second, it has been suggested that not only potassium, but also hydrogen ions are secreted into the distal tubular lumen competitively, a view based on the well-known inverse

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relationship between potassium excretion and urinary acidification [2, 5]. In addition, it is well known that the nature of the anion excreted affects urinary potassium loss [2, 27, 28, 41], poorly reabsorbable anions like sulfate, ferrocyanide or phosphate being powerful kaliuretic agents. Finally, endocrine factors, the adrenal cortex in particular, are known to modify urinary potassium loss [18, 24]. Pre-treatment of animals with exogenous potassium loads also has a profound effect upon the pattern of urinary potassium excretion [2, 4, 24].

## Segmental Distribution of Potassium Transport

The contribution of various tubular segments to different excretion patterns and the mechanism of tubular potassium transport have only recently been studied in single nephrons. Such studies have been largely confined to the rat and the dog, but there is probably little qualitative difference among mammalian species in respect to the mechanism of potassium excretion [2]. The most extensive micropuncture studies of potassium transfer have been done in the rat, but recently, work on the canine kidney has made considerable progress [50].

Concerning the distribution of transtubular concentration gradients across various nephron segments, it has been a consistent observation that the general pattern across the proximal tubular epithelium varies but little in mammalian kidneys [6, 23, 26, 27, 28, 30, 50]. Over a wide range of excretion, varying from 3% of the filtered potassium to as much as 150%, in animals in which potassium secretion was stimulated, proximal tubular fluid/plasma ratios in the rat are unity or slightly below. Although the concentration gradients which are established across the proximal tubular epithelium are small or even absent, extensive fluid reabsorption accounts for the bulk of the filtered potassium to be reabsorbed along the proximal tubule. This also holds for situations in which the amounts of potassium in the final urine significantly exceed those filtered. Accordingly, with some exceptions to be discussed, modifications of proximal reabsorptive transport do not contribute importantly to variations in urinary potassium output.

Consideration of distal tubular data emphasizes a number of points. In general, early distal tubular concentration gradients for potassium ions are steeper than those found across the terminal part of the proximal convolution [27, 28, 30]. As the tubular fluid passes along the distal nephron segment, there is a trend for potassium tubular fluid/plasma concentration ratios to increase. Taking into account the extent to which inulin is concentrated, it can be calculated that some 80 to 95% of the filtered potassium are reabsorbed under almost all experimental conditions by the time the fluid has reached the beginning of the distal tubule. Analysis of fluid collected from the tip of Henle's loop leads to the conclusion that it is the ascending limb of the loop of Henle which establishes these steeper concentration gradients at the beginning of the distal tubule [53]. The rather

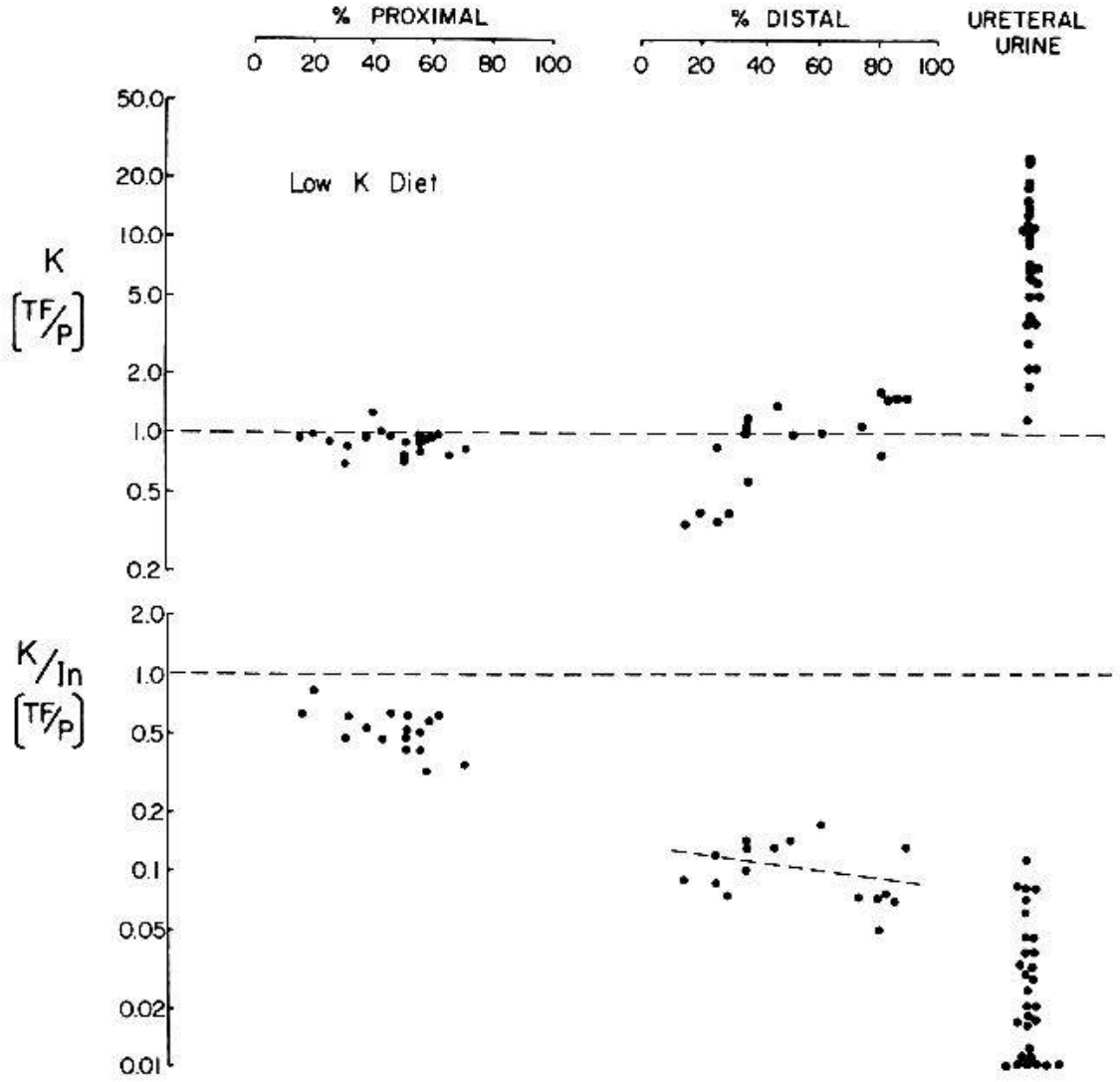


Fig. 1. Summary of potassium and potassium/inulin concentration ratios from rats on a low-K diet. – *Upper part:* tubular fluid/plasma concentration ratios of potassium as function of tubular length. – *Lower part:* potassium/inulin concentration ratios as function of tubular length [27].

obligatory reabsorption of such a large fraction of filtered potassium by the proximal tubule and the loop of Henle underscores the fact that the widely divergent urinary excretion patterns for potassium are the result of a fixed reabsorptive activity located proximally, and a greatly varying secretory or reabsorptive activity in the terminal parts of the nephron.

Fig. 1 and 2 show this functional pattern in two extreme conditions of potassium metabolism, demonstrating, in particular, the great range of transport facilities of the distal tubular epithelium.

The upper part of Fig. 1 shows tubular fluid/plasma concentration ratios of potassium plotted as a function of tubular length. These data have been obtained in animals maintained for several weeks on a low potassium diet. This is reflected in the plasma by potassium levels of about 2 mEq/l and bicarbonate levels ranging from 35 to 40 mEq/l. Inspection of the upper graph shows that proximal tubular fluid/plasma ratios are unity or slightly

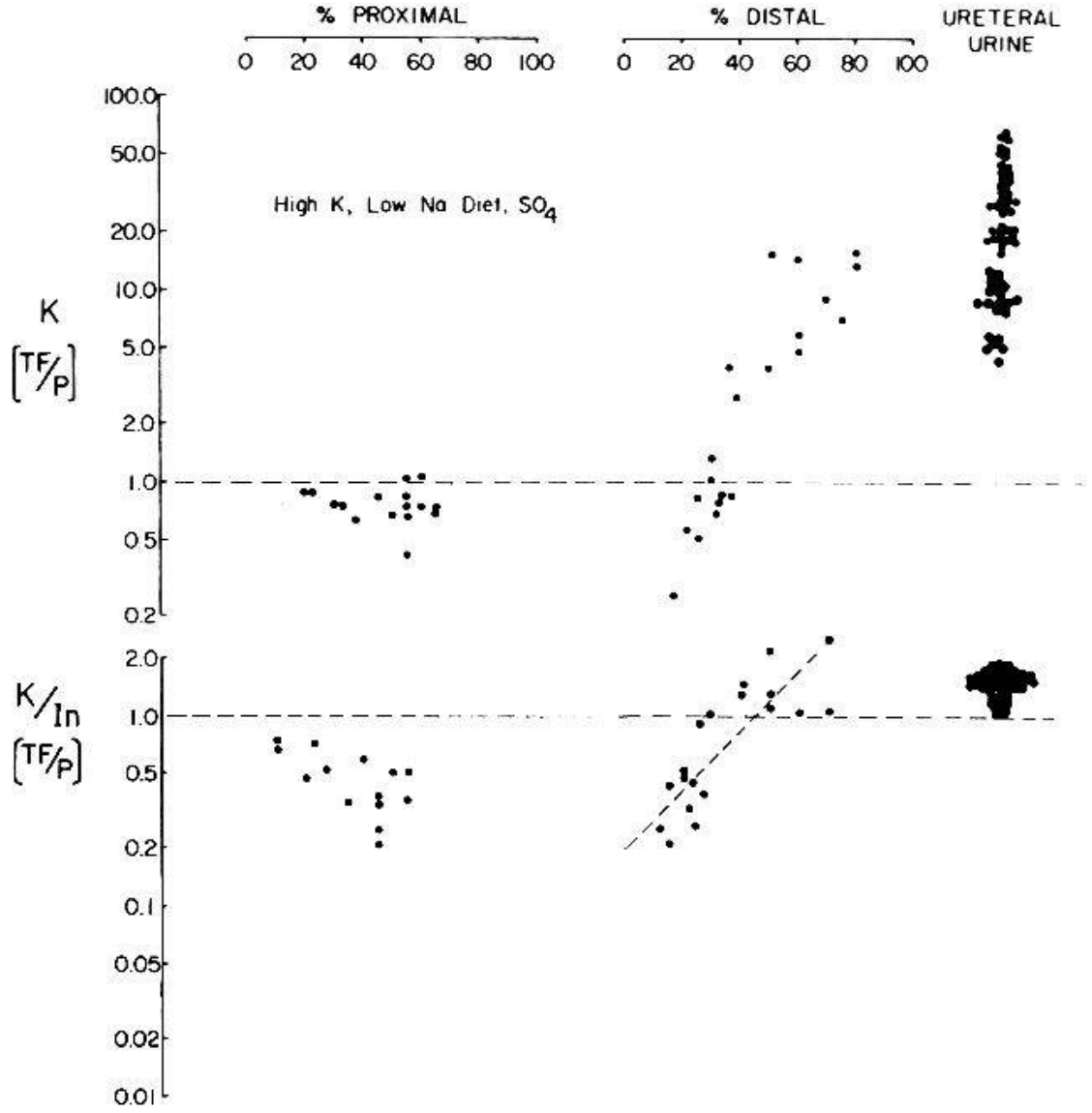


Fig. 2. Summary of potassium and potassium/inulin concentration ratios as function of tubular length from animals on high-K, low-Na diet, receiving sodium sulfate and potassium chloride plus dichlorphenamide [27].

less, while early distal concentration ratios are significantly less than unity. Distal transtubular concentration ratios tend to increase moderately as fluid passes along this nephron segment, but it is the collecting duct which maintains the steepest potassium gradients. To evaluate these data in terms of transtubular net movement, potassium concentration ratios are divided by the corresponding inulin ratio. The lower part of Fig. 1 shows such ratios plotted again as a function of tubular length. A negative slope indicates net reabsorption, a positive one, net secretion. It is obvious from inspection of the lower part of Fig. 1 that proximal reabsorption is extensive, that it continues along Henle's loop, and that some 85 to 90% of the filtered load has been reabsorbed by the time fluid issues from the loop. In essence, this process of net reabsorption continues along the distal tubule and the collecting duct, and leads to the very extensive removal of potassium from the urine.

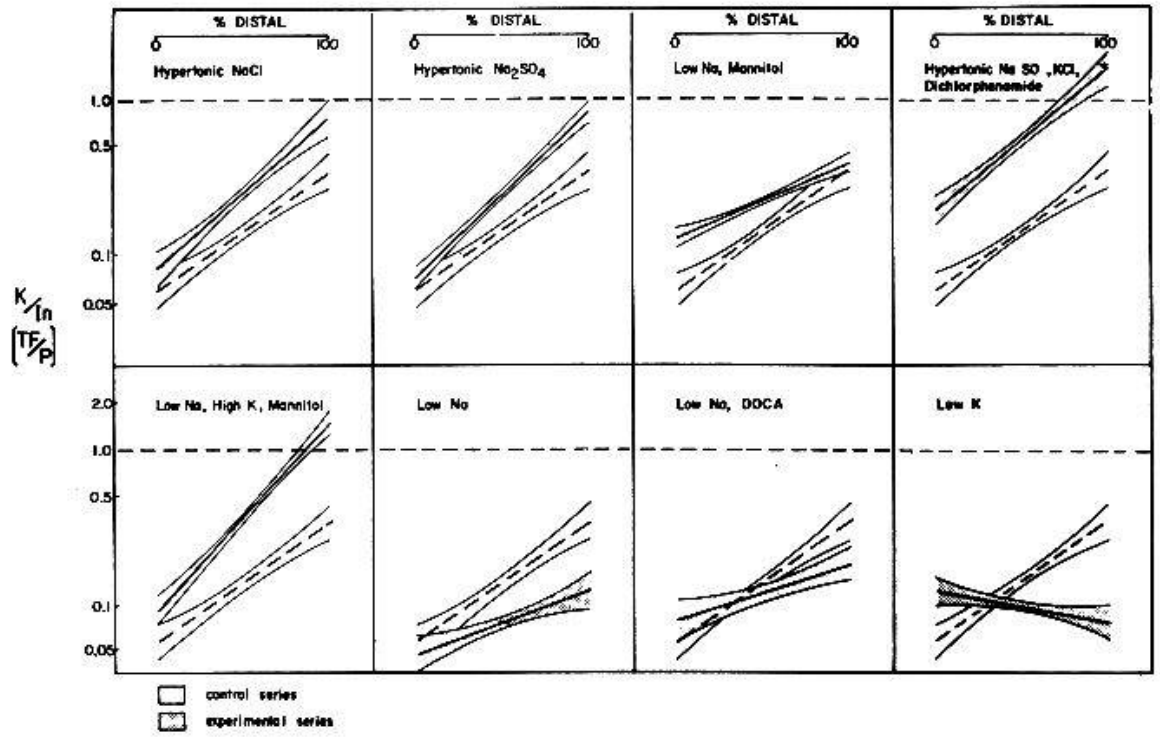


Fig. 3. Comparison of distal tubular potassium transfer under various experimental conditions. Regression lines were obtained by the least squares method. Shaded areas represent ± one standard error. Values between 0 and 20% distal tubular length are extrapolated [28].

In Fig. 2, the results are shown of an experimental condition in which potassium excretion was maximally stimulated, and which resulted in a mean potassium/inulin clearance ratio of 1.51. Along the proximal tubule, the situation is not markedly different from that found in potassium-deprived animals. It is along the distal tubule that the potassium concentration rises steeply. The lower section of Fig. 2 summarizes the conditions after correction for water movement. Proximal tubular data indicate that even when the mean fractional excretion is as much as 151%, some 80% of the filtered potassium have been reabsorbed by the time fluid reaches the early distal tubule. This could, conceivably, be an underestimate of reabsorption since it is impossible to collect from the very beginning of this tubular segment. However, in marked contrast to the state of potassium depletion, massive distal addition of potassium is evident, and it can be calculated that a minimum of some 90% of urinary potassium may have entered the lumen by way of tubular secretory transfer. Even under thees conditions of maximally stimulated potassium excretion, tubular reabsorption in the proximally located nephron segments continues at a high rate. Accordingly, net influx of potassium across the distal tubules accounts almost exclusively for the excretory capacity of the kidney [14, 27, 28]. It is only under conditions of brisk osmotic diuresis, induced by the intravenous administration of mannitol that diminished proximal tubular reabsorption contributes significantly to urinary excretion [28].

Table 1

Summary of tubular segmental contribution to urinary potassium excretion

| Experimental condition | Diet | % filtered K | |
|---|---|---|---|
| | | Early distal tubule | Final urine |
| Antidiuresis ..... | Control | 5 | 17 |
| Antidiuresis ..... | Low K | 10 | 3 |
| Antidiuresis ..... | Low Na | 6 | 4 |
| Antidiuresis, DOCA ..... | Low Na | 7 | 4 |
| 10% Mannitol, i.v. ..... | Low Na | 15 | 30 |
| 4% NaCl, i.v. ..... | Control | 8 | 48 |
| Hypertonic $Na_2SO_4$, i.v. ..... | Control | 7 | 60 |
| Dichlorphenamide (DCP) i.v .... | Control | 10 | 60 |
| 10% Mannitol, Isotonic KCl, i.v. | Low Na | 9 | 91 |
| $Na_2SO_4$, KCl, DCP, i.v. ..... | High K, Low Na | 20 | 151 |

The extent of distal tubular secretion is variable and clearly depends upon the metabolic situation. Fig. 3 summarizes the pattern of distal tubular potassium transfer under various experimental conditions [28]. A comparison of the moiety of potassium present at the beginning of the distal tubule with that in the final urine permits an estimate of the extent to which urinary potassium is derived from distal nephron activity (Table 1) [27, 28]. It is evident that a mean of 75% of urinary potassium can be accounted for by distal tubular secretion under non-diuretic conditions. Even higher fractions can be shown to be of distal secretory origin whenever potassium secretion is stimulated by potassium loading, administration of carbonic anhydrase inhibitors, or after sodium-chlorid or sodium sulfate infusions. Potassium secretion is absent or diminished in the potassium- and sodium-depleted state. It should be realized that the very early distal tubule is not accessible for micropuncture and that, consequently, the fraction of filtered potassium may be even less at the very beginning of the distal tubule than that calculated on the basis of potassium and inulin concentration ratios at some 20% of distal tubular length. Consequently, the fraction of potassium calculated to derive from distal tubular secretion represents a minimum value. The view of BERLINER [2] of a predominantly secretory origin of urinary potassium holds for all those conditions in which there is an adequate dietary intake of potassium and sodium. Due to the inability to obtain fluid samples from the ascending limb of Henle's loop, it is presently not possible to decide whether the small fraction of potassium, present at the beginning of the distal tubule under conditions in which distal tubular secretion is absent, is of secretory origin or whether it represents potassium which has escaped reabsorption along more proximally located nephron segments.

It is apparent from these considerations that, in the rat, secretory capacity resides mainly within the distal convoluted tubule, the collecting duct epithelium contributing only to a smaller extent [17, 27, 28]. On the other hand, significant reabsorptive movement has been observed occasionally along the collecting duct under conditions of normal potassium intake and after dietary sodium and potassium deprivation. It is thus apparent that the collecting duct epithelium can contribute significantly to the reduction of urinary potassium excretion. Both diminished or absent distal tubular secretion *and* reabsorption along the collecting duct are responsible for the low excretion rates of potassium in the sodium- and potassium-depleted state and under those control conditions in which the fraction of excreted potassium is less than 5%. Under certain conditions, particularly in rats on a normal potassium intake at low urine flow rates, the net movement of potassium occurs in three sequential steps: 1. net reabsorption in the proximal tubule and Henle's loop, 2. secretion in the distal tubule and 3. final net reabsorption in the collecting duct system.

One reservation to these conclusions regarding the role of the collecting duct system should be noted. An exact assessment of the role of these very terminal nephron segments is difficult to obtain by micropuncture methods. This is due to the fact that tubular samples are obtained from cortical nephrons only, whereas the final urine specimen is an admixture of fluid derived from nephrons of both short and long loops. Since these may contain different amounts of potassium, the composition of the final urine may be modified to an unknown degree. Despite these uncertainties, the conclusions reached by comparing late distal with final urinary values are in principal agreement with results obtained by more direct methods. The presence of either net secretory or net reabsorptive potassium movement along the collecting duct epithelium is demonstrated directly by collecting duct catheterization and comparison of the composition of fluid samples obtained at different distances from the papillary tip [17, 19].

Micropuncture studies in which the concentrations of sodium and of potassium as well as that of inulin were measured in distal tubular samples under a variety of experimental conditions permit some assessment of the role of distal tubular sodium reabsorption in the secretory process of potassium [14, 28]. In general, an evaluation of the quantities of sodium reabsorbed and those of potassium secreted along the distal tubule and the collecting ducts indicates that whereas the distal tubule is the main site of potassium secretion, it is in the collecting duct where the lowest sodium concentrations are achieved. This dissociation of the main nephron site for potassium secretion and that for the establishment of maximal sodium gradients is of importance in considering the possibility of potassium secretory capacity being critically dependent upon the amounts of sodium available for exchange [2, 8]. Within this context, it has frequently been suggested that the distal tubular capacity to secrete potassium may be brought into action by the delivery to it of more adequate amounts of

Table 2

Summery of tubular segmental contribution to sodium reabsorption

| Experimental condition | % filtered Na remaining | | |
|---|---|---|---|
| | End of proximal tubule | Early distal tubule | Final urine |
| Antidiuresis ..... | 34 | 10 | <1.0 |
| Antidiuresis, low Na diet . | 34 | 5–6 | ≪1.0 |
| Isotonic NaCl ..... | 70 | 12 | 6–8 |
| Isotonic NaCl, low Na diet | 63 | – | 6–8 |
| Hypertonic NaCl ..... | 70–34 | 10–15 | 5–10 |
| Mannitol (10–20%)..... | 70–60 | 10–15 | 2–5 |
| Dichlorphenamide ..... | 60 | 12–14 | 5 |
| Sodium sulfate ..... | 70–60 | 15–18 | 14 |
| Acute reduction GFR .... | 34 or less | 10 | ≪1.0 |
| Perfused rat kidney ..... | 64 | 24 | 8 |

sodium. Such considerations demand that, if a one-to-one exchange of potassium for sodium were the sole mode of potassium secretion [8], the reabsorption of sodium be almost complete prior to the site of potassium secretion, i.e., by the time the tubular fluid reaches the early part of the distal tubule. Only then would increased delivery of sodium be expected to promote potassium secretion. On the other hand, under experimental conditions in which sodium deprivation reduces potassium excretion, or renders the administration of desoxycorticosterone (DOCA) ineffective in producing kaliuresis [20, 40, 42], almost complete sodium reabsorption should have occurred by the beginning of the distal convoluted tubule [2, 20, 24].

The main conclusions to be drawn from studies on the distal transfer of sodium and potassium are the following: Normally, the amounts of sodium reabsorbed along the distal tubule greatly exceed the amounts of potassium secreted. Accordingly, the sodium supply is normally not limiting for potassium exchange. A summary of the fraction of sodium being presented to the distal tubular epithelium under widely varying experimental conditions is given in Table 2 [28]. There is no reason to believe that the kaliuretic effect of sodium chloride or sodium sulfate loading is due to the delivery of a more adequate supply of sodium, in the sense of providing previously inadequate substrate for a carrier-mediated ion exchange. The mechanism by which any supraoptimal distal sodium load may enhance potassium secretion must be different from its role in the proposed limited-supply hypothesis.

A similar conlusion is reached when experiments dealing with dietary sodium depletion are considered. Dietary sodium deprivation in the rat [28, 42], as in the dog [24] and man [9, 24, 40] reduces potassium excretion. Similarly, it protects rats from potassium depletion by DOCA [20, 24]. It has long been assumed that the enhanced tubular reabsorption of sodium subsequent to sodium deprivation occurs proximal to the site of sodium-for-

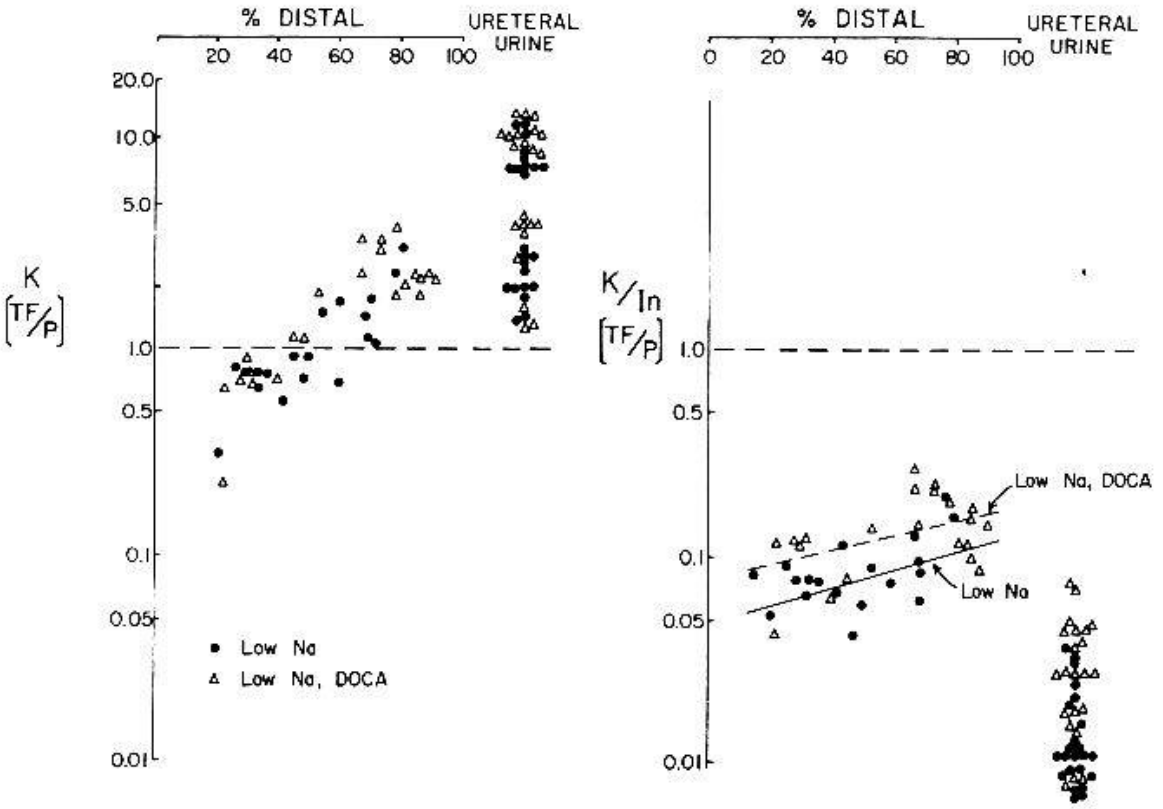


Fig. 4. Summary of potassium and potassium/inulin concentration ratios as function of tubular length from animals on a low-Na diet. One group of animals pre-treated with DOCA [28].

potassium exchange and that potassium secretion might be limited by the lack of sodium ions available for exchange [2, 20]. Pertinent data on sodium and potassium transport in sodium-deprived rats and in similarly treated rats given DOCA, are summarized in Fig. 4 and 5. It is obvious that although the final urine was almost sodium-free, the fraction of filtered sodium entering the distal tubule still amounted to some 4 to 6%, and an estimate of the fraction of sodium reabsorbed along the distal tubule exceeded the amount of distally secreted potassium by at least twentyfold. Since the concentration of sodium at the beginning of the distal tubule exceeds that of potassium by an order of magnitude, it follows that, in the rat, neither the amount of sodium entering the distal tubule nor the intratubular sodium concentration were rate-limiting for potassium secretion. It is also apparent from inspection of Fig. 4 that potassium reabsorption along the collecting duct contributes importantly to the reduction in urinary excretion. In view of the obvious adequacy of the distal tubular sodium supply, decreased potassium excretion during sodium deprivation, achieved both by diminished distal tubular secretion and significant reabsorption along the collecting duct, is not the consequence of, and cannot be related to enhanced sodium reabsorption proximal to the site of potassium secretion. Preliminary results of studies in which glomerular filtration rate was acutely reduced by clamping of the renal artery revealed a very similar situation [25]: an increased rate of sodium reabsorption as reflected by very low

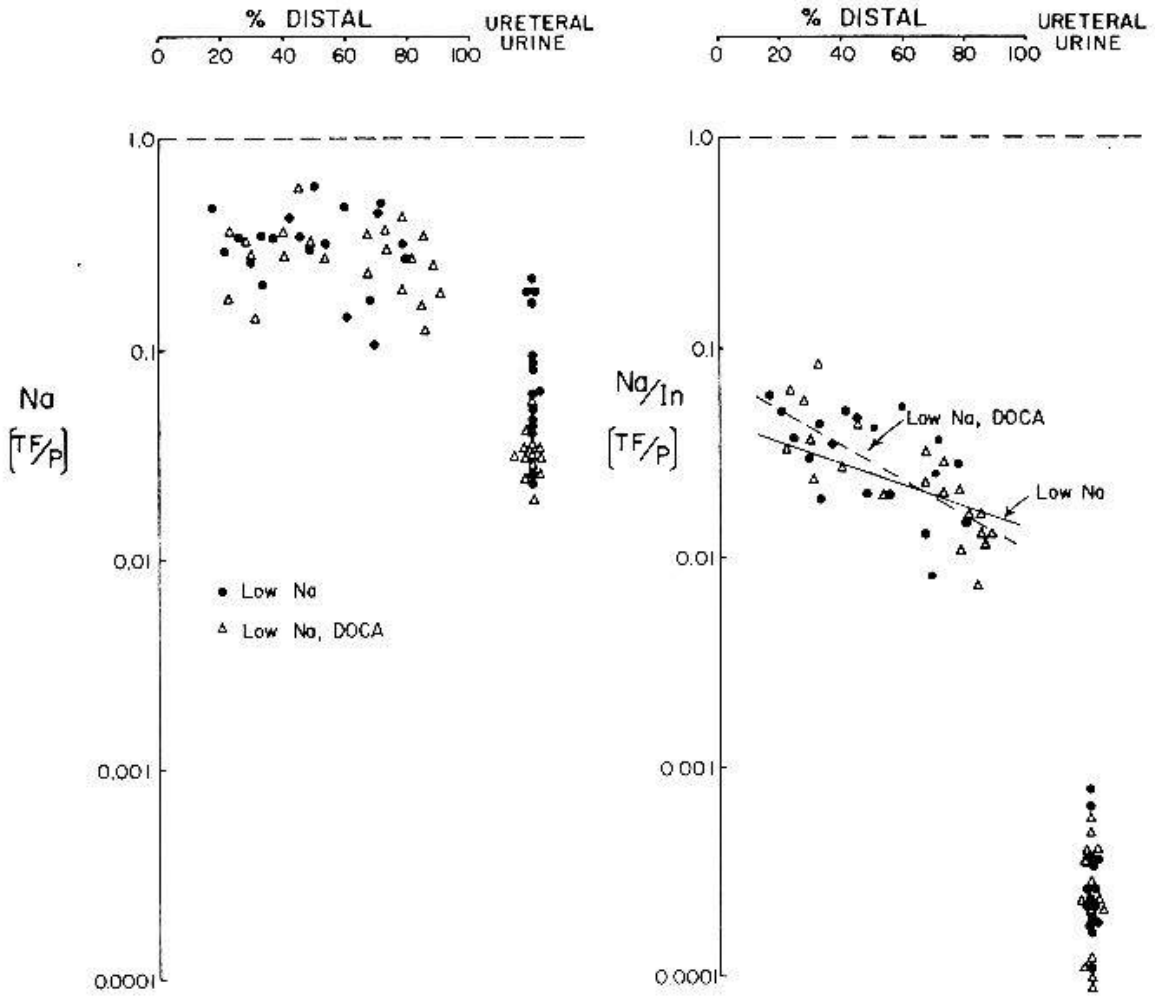


Fig. 5. Summary of sodium and sodium/inulin ratios as function of tubular length from animals on a low-Na diet. One group of animals pre-treated with DOCA [28].

urinary concentrations is mainly a function of the collecting duct epithelium, i.e., a nephron site beyond that where the bulk of potassium secretion takes place. An acutely reduced filtration rate leads to a drastic curtailment of urinary potassium excretion, due predominantly to enhanced reabsorption along the collecting ducts at a time when distal tubular sodium concentrations are not significantly reduced. Again, the fraction of sodium reabsorbed along the distal tubule greatly exceeds that of potassium secreted. Thus, in the rat, a reduced distal tubular supply of sodium is clearly not responsible for the dramatic suppression of potassium excretion when filtration rate falls.

Finally, it should be noted that there is no one-to-one, or any other, fixed exchange ratio between sodium reabsorption and potassium secretion. Such a fixed stoichometric relationship would be expected if carrier-mediated sodium for potassium exchange were importantly involved in distal tubular potassium secretion. Lack of a fixed coupling ratio is apparent from a comparison of the rate at which sodium and potassium are transferred across the distal tubular epithelium under widely varying metabolic situations [27, 28]. Indeed, distal tubular sodium reabsorption is most dramatically

reduced under those very experimental conditions (loading with poorly permeant anions, potassium chloride and administration of dichlorphenamide) in which a powerful stimulation of potassium secretion obtains [27, 28]. Absence of a fixed tubular exchange ratio between these two cationic species was also observed in the doubly-perfused frog kidney in which the effects on urinary potassium excretion of a step-wise reduction of the sodium concentration in the aortal perfusion fluid was studied [48]. While changes in sodium metabolism exert significant effects upon tubular potassium transport, the means by which such modifications are brought about appear to differ from the notion of sodium ions being required for a rigidly coupled exchange. Alternative modes by which sodium ions may affect potassium transport will be discussed subsequently.

## Mechanism of Tubular Potassium Transport

In both the rat and the dog the process of tubular potassium reabsorption occurs against an electrochemical potential gradient, and several groups of investigators have concluded that proximal tubular potassium transfer from lumen to peritubular fluid involves active transport [6, 23, 27, 28, 29, 30]. It is most reasonable to assume the luminal cell membrane to be the site of active potassium reabsorption [6, 11, 14, 30, 51, 52]. This is a consequence of the fact that the concentration gradient for potassium across this cell boundary is much greater than the opposing electrical gradient. This argument assumes a smaller electrical potential gradient across the luminal cell membrane than across the peritubular one. Were it not for active reabsorptive movement of potassium at this site, the smaller electrical potential gradient would be insufficient to prevent leakage into the lumen and a rise in proximal tubular potassium concentration above that of plasma. Stated differently, exclusive passive distribution of potassium ions across the luminal cell boundary without the presence of an active reabsorptive potassium pump would result in intratubular potassium concentrations greatly in excess of the observed values.

Potassium deficiency increases the capacity of the proximal convoluted tubule to reabsorb filtered bicarbonate and, thus, to secrete hydrogen ions [39]. Rector et al. [39] have pointed out that since potassium secretion does not occur across the proximal tubular epithelium, this effect of potassium deficiency cannot be due to decreased competitive inhibition at a common potassium-hydrogen pathway. Since proximal tubular potassium transfer is also rather insensitive to carbonic anhydrase inhibition, it appears safe to conclude no reciprocal relationship between hydrogen and potassium secretion is evident in the proximal tubule [39].

The question of the nature of distal tubular potassium transfer has been approached in a number of ways. In an effort to determine whether the distal tubular secretory process is mediated by active transport or by passive diffusion, studies were done in which the electrical potential gra-

Table 3

Maximal transtubular concentration ratios as calculated by the Nernst equation for the distal tubule, compared to the observed TF/P ratios, in free-flow experiments and in stationary microperfusion experiments.

| Free-flow experiments | | |
|---|---|---|
| Experimental condition | Calculated ratio | Observed peak ratio |
| Hypertonic NaCl | 8.3 | 2.4 |
| Hypertonic $Na_2SO_4$ | 11.2 | 5.4 |
| 10% Mannitol (low Na diet) | 5.4 | 2.4 |
| 10% Mannitol – KCl (low Na diet) | 7.6 | 7.0 |
| Low Na diet | 6.6 | 3.0 |
| Low Na diet – DOCA | 8.3 | 3.8 |
| **Stationary microperfusion experiments** | | |
| Experimental condition | Calculated ratio | Observed peak ratio |
| Raffinose, control diet | 6.6 | 3.90 |
| Raffinose, low-K diet | 6.1 | 2.35 |
| Raffinose, high-K diet, DCP | 7.7 | 5.00 |
| NaCl, low Na diet | 6.1 | 2.58 |
| $Na_2SO_4$, low Na diet | 21.3 | 5.25 |
| Choline Chloride (low Na diet) | 1.1 | 0.85 |

dients were evaluated with respect to the observed concentration ratios of potassium. Utilizing the Nernst equation, an assessment of the electrical potential gradient across the distal tubular epithelium indicates that the observed transepithelial potassium concentration ratios could be quantitatively accounted for by the transtubular electrical potential difference [27, 28, 30]. In a wide variety of experimental conditions including those in which maximal distal tubular potassium concentrations are achieved, it was a consistent observation that the concentration ratios were considerably lower than those which would be expected if potassium ions were to distribute passively according to the electrical driving force [27, 28]. Pertinent data are summarized in Table 3. Thus, under free-flow conditions, no evidence for active secretion of potassium into the distal tubular lumen could be obtained since potassium movement uniformly proceeded down an electrochemical potential gradient. It appears that the electrical driving force could be adequate to account for the observed extent of potassium accumulation in the distal tubule.

To gain some insight into the possible causes of the apparent disequilibrium of potassium ions across the distal tubular epithelium ($K_{tub} <$

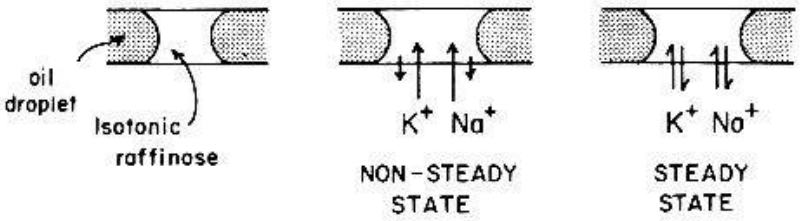


Fig. 6. Schematic presentation of stationary microperfusion method using a poorly permeant non-electrolyte solution as perfusion fluid. For details, see text.

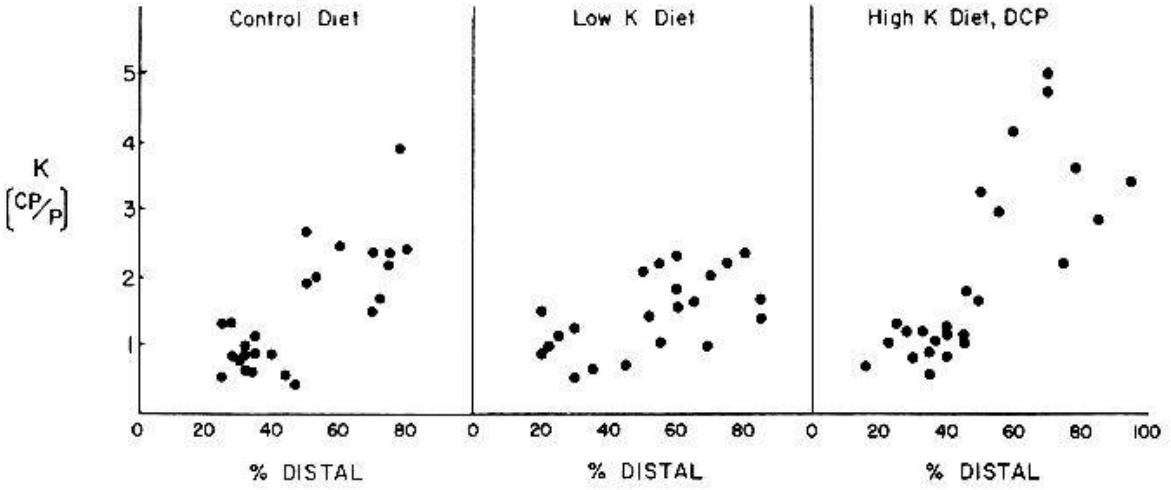


Fig. 7. Summary of distal transtubular potassium steady-state concentration ratios during isotonic raffinose perfusion [29].

$K_{expected}$), microperfusion experiments were carried out under conditions in which net fluid movement was almost completely abolished [10, 22, 29, 30]. As applied to the present problem, and as schematically shown in Fig. 6, this method consists in the deposition of a small fluid sample of a poorly penetrating non-electrolyte, separated by oil-columns, into the distal tubule and reaspiration and analysis after a variable time period. In such experiments ions move into the lumen until a limiting concentration difference is reached. At this point, active reabsorption, or secretory movement and passive leak balance each other such that a state of zero net flux of solutes and water is approached. Hence, the imposed reduction of net fluid movement reduces solute flux asymmetry and solvent drag effects and creates a situation in which steady state conditions obtain. It was a consistent observation that steady-state conditions are achieved after some 40 to 60 sec [29]. A comparison of concentration gradients with the electrical driving force is much simplified under these circumstances.

Fig. 7 summarizes collected perfusate/plasma concentration ratios of potassium, obtained under three experimental conditions, and plotted as a function of distal tubular collection site [29]. A number of points deserve comment. Concerning the steady-state concentration of potassium, it is apparent that late distal values exceed those established across the early part of this nephron segment. Furthermore, the extent to which potassium ions

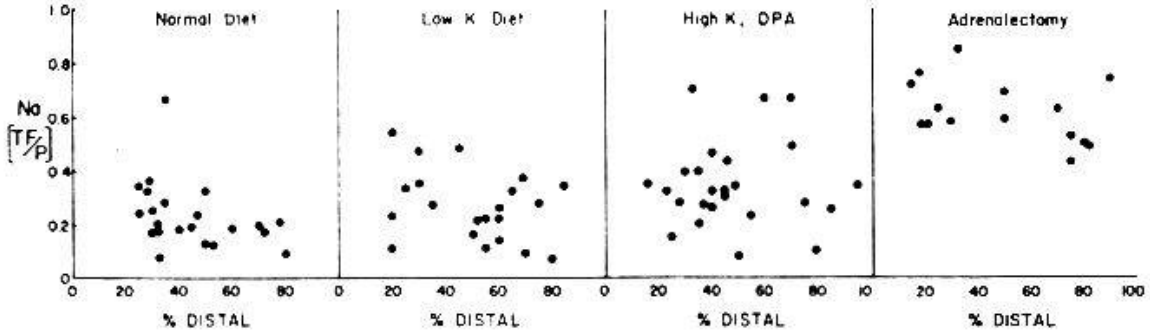


Fig. 8. Summary of distal transtubular sodium steady-state concentration ratios during isotonic raffinose perfusions [29, 18].

accumulate across the later part of the distal tubular epithelium clearly depends upon the metabolic state. In particular, pre-treatment with a low-potassium diet suppresses the capacity to establish transtubular concentration gradients for potassium. Comparison shows that steady-state concentration gradients do not differ significantly from those achieved under free-flow conditions. It follows that the observed disequilibrium cannot be due to the contact time of fluid being too brief to permit passive entry of potassium ions to reach equilibrium distribution. When transepithelial electrical potential differences are measured in these perfusion experiments, it is again apparent that the observed concentration ratios are considerably below those expected on the basis of passive distribution according to the electrical driving force (Tab. 3).

Comparable data for sodium are summarized in Fig. 8 [29, 18]. A trend will be noted for collected perfusate/plasma ratios to decline along the distal tubule in animals on either a control diet or a low-potassium diet. The ability of the distal tubular epithelium to lower the intratubular sodium concentration is reversibly depressed by adrenalectomy [18]. The general magnitude of the sodium concentration indicates 1. that the distal tubular epithelium is unable to lower the sodium concentration below some 15 mEq/l, and 2. that the sodium concentration in recollected samples generally exceeds that of potassium. This is evidence against the possibility that a low rate of leak of sodium into the distal tubule could in any way have limited secretory potassium movement.

Microperfusion methods also provide a means of evaluating whether a change in the electrical driving force affects the distribution of potassium ions across the distal tubular epithelium [29]. This consists in "chemically" clamping the transepithelial potential difference by the deposition of different solutions, known to alter the electrical potential gradient, and observing the effects upon the distribution of potassium ions.

Results of such a study are summarized in Fig. 9. It is clear that there are significant differences with respect to both electrical and ionic concentration gradients in the three series of perfusion experiments. Compared to isotonic sodium chloride perfusions, the electrical potential difference is significantly higher during sodium sulfate perfusions. Concomitantly, steady-

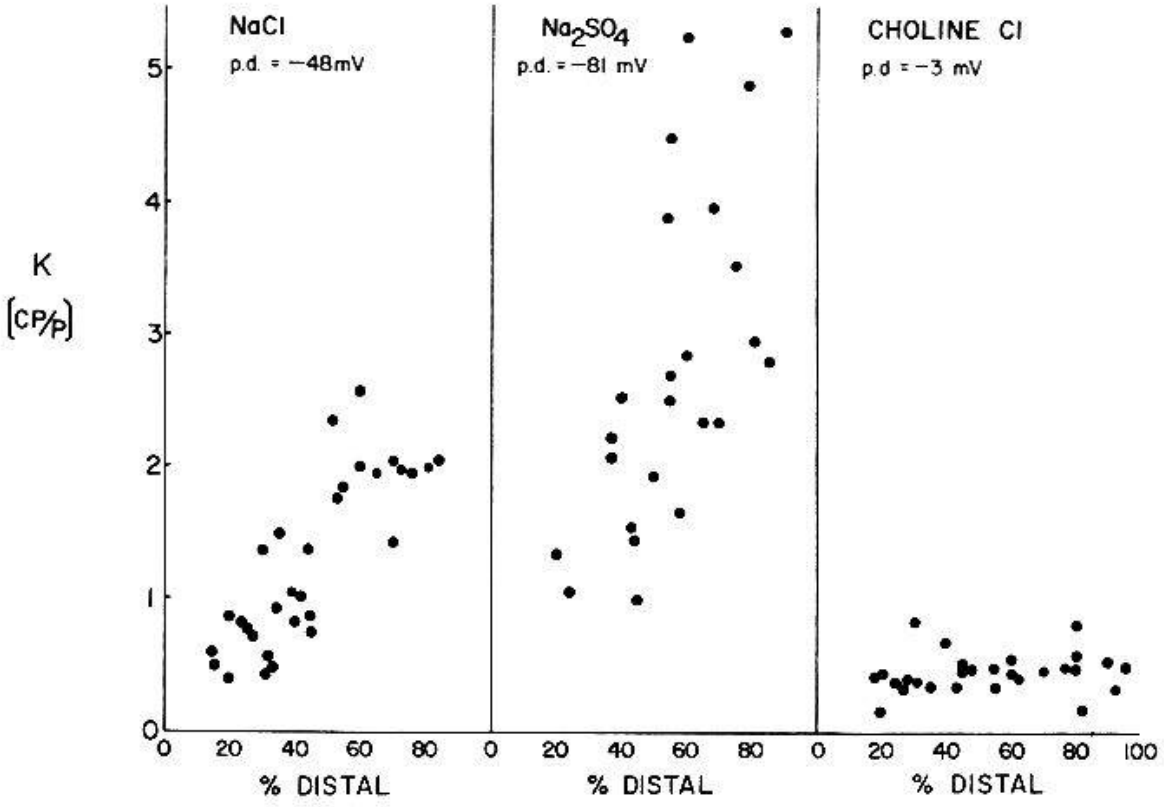


Fig. 9. Summary of distal transtubular potassium concentration ratios during perfusions with isomotic NaCl, $Na_2SO_4$ and choline chloride [29].

state concentration gradients for potassium ions are augmented along the whole distal nephron length. On the other hand, during perfusions with isotonic choline chloride solutions, tubular entry of potassium is greatly reduced as reflected by the time-independent low concentration gradient for potassium. In the almost complete absence of an electrical driving force, the potassium concentration is significantly less than unity. (It should be noted that the concentration of sodium during choline chloride perfusions exceeded that of potassium.) The general conclusion to be drawn is that the transepithelial electrical potential gradient is a powerful determinant of distal transtubular potassium movement.

From a number of considerations it appears certain that electrochemical equilibrium for potassium ions across the distal tubular epithelium is prevented by a component of active reabsorptive transfer out of the lumen. Such a mechanism opposes passive inward movement of potassium along the electrochemical potential gradient and would result in the observed steady-state intraluminal concentration of potassium below that expected from electrochemical equilibrium. Accordingly, there is no need to assume any specific active transport of potassium into the lumen. On the contrary, active movement of potassium out of the lumen must be the means by which electrochemical equilibrium is prevented [14, 15, 29, 52]. Active net reabsorption of potassium is an inherent property of both the proximal tubule and the collecting duct, and has been demonstrated to occur across the distal tubule under some experimental conditions [27, 30]. Under the

majority of experimental conditions, it is opposed by more extensive inwardly-directed passive potassium movement. In essence, this proposed mechanism of distal tubular potassium transfer corresponds to many pump-leak systems described for a variety of other biological membranes [46]. Evidence to be considered subsequently is consistent with the view that the luminal cell membrane is the site of active reabsorptive potassium movement.

It should be emphasized that from the evidence presented the possibility of some carrier-mediated secretory movement of potassium into the lumen, possibly even in exchange for reabsorptive sodium transport, cannot be excluded. It should be realized, however, that the orientation of such sodium- and potassium-pumping would be opposite to that found in all other mammalian cells in which potassium is pumped *into*, and sodium pumped *out of* the cell interior [46]. An important contribution of a carrier-mediated, coupled pump-mechanism to total distal tubular potassium transfer seems remote, however. This is a consequence of the fact not only that the electrical driving force is adequate to account for net secretory movement, but also that the entry of potassium depends critically upon the electrical potential gradient. This is evidenced by the almost complete absence of potassium entry and the very low concentration gradients (less than unity) when the normal electrical potential gradient is abolished in choline-chloride perfusions (Fig. 9). Carrier-mediated secretory potassium movement would not be expected to cease in the absence of an electrical driving force. MOREL et al. [33, 34] have come to similar conclusions regarding the mode of distal tubular potassium transfer.

Before considering in more detail the factors upon which the extent of distal tubular potassium accumulation depends, a brief consideration of some electrical characteristics of distal tubule cells is in order. Recent investigations on single amphibian and mammalian distal tubule cells have demonstrated that the overall transepithelial potential difference is a consequence of two finite potential steps. A considerably smaller voltage drop obtains across the luminal cell boundary than across the peritubular one, making the cell interior asymmetrically negative with respect to tubular fluid and interstitial fluid [13, 31, 38, 45, 52].

The effect of a number of intratubular and peritubular ionic substitutions upon the distal transepithelial potential difference can be used to make some inferences with respect to the ionic permeability properties of distal tubule cell boundaries [13]. The approach is depicted in Fig. 10.

The results of a series of such experiments indicate the following permeability differences between peritubular and luminal cell membrane [13]. Judging by the great effectiveness with which local application of high potassium solutions depolarize the overall transtubular potential difference, it appears certain that the peritubular membrane of distal tubule cells is a cell boundary with dominant potassium-electrode character. This view is also supported by more direct studies on the larger distal tubule cells of

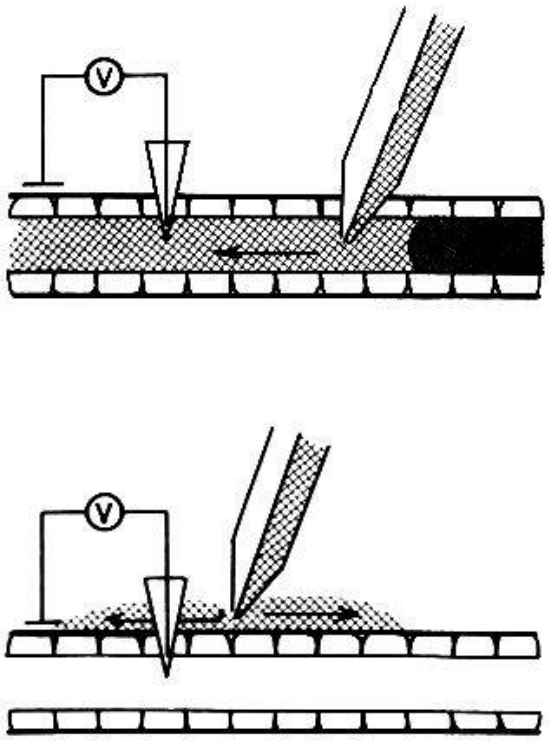

Fig. 10. Schematic presentation of methods of applying various perfusion fluids intratubularly and peritubularly [13].

amphibian species in which the changes of electrical potential differences across the peritubular cell membrane can be measured directly [31, 45]. In contrast, evidence is available to indicate considerable contributions of potassium, sodium and chloride to the potential gradient across the luminal cell membrane. This conclusion is based upon the following experimental evidence. Replacement of sodium and potassium by a less permeant cation such as choline in the distal tubular lumen depresses the transtubular potential gradient. Given finite permeabilities for sodium and potassium, a decrease in either of these cation concentrations would favor an increase in the potential difference across the luminal cell membrane, and thus reduce the overall transepithelial potential. This is a consequence of the direction of the respective concentration differences between cell interior (high K, low Na) and the tubular lumen (low K, variably low Na). The dependence of the potential difference upon these two ions makes it very likely that they participate in diffusion potentials contributing to the electrical asymmetries observed. Since the absolute magnitude of the distal transtubular potential difference bears a strong relationship to the sum of quite variable combinations of sodium and potassium concentrations (Fig. 11), it is likely that the relative permeabilities of the luminal cell membrane to sodium and potassium are about equal. This is also supported by the observation that quantitative replacement of potassium by sodium in tubular perfusion experiments does not change the transtubular potential difference. This is consistent with the view that these ion species contribute equally to the potential difference across the luminal cell boundary.

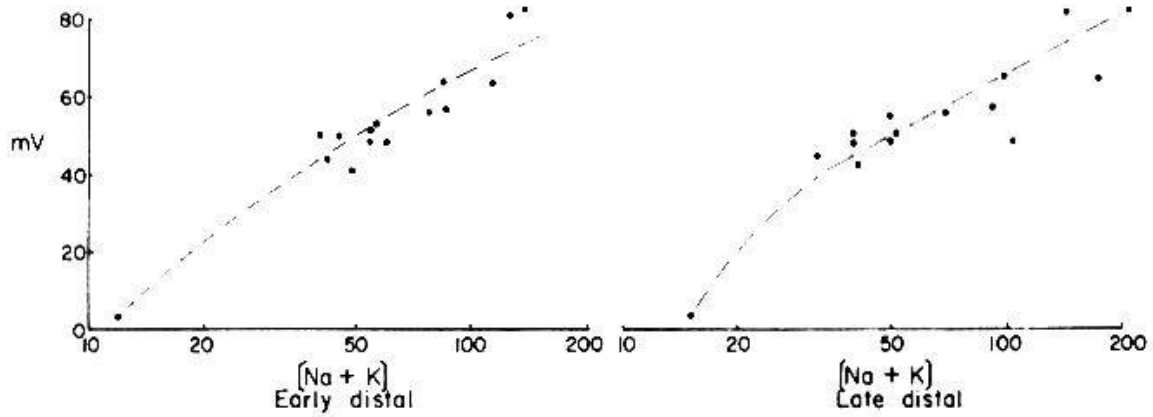


**Fig. 11. Relationship between distal transtubular potential difference and the logarithm of the sum of sodium and potassium concentrations during conditions of free-flow and of stationary microperfusion. The line indicates the least square fit. Data have been divided into an early and late distal tubular group. No corrections have been made for the relatively small change in activity coefficients of cations in the higher concentration range [13].**

Other evidence indicates that in addition to the demonstrated role of cations in the generation of the transtubular potential difference, anions also contribute to the electrical asymmetry. It is likely that reabsorptive anion movement, such as occurs when chloride is present in the distal tubular lumen, increases the luminal potential difference and thereby shunts the overall transtubular potential. Diminished diffusion of such poorly permeant anions as sulfate, ferrocyanide, bicarbonate and phosphate causes relative depolarization luminally and thereby increases the electronegativity of the lumen [7, 27, 28, 52].

The evidence thus presented supports the view that the potential difference across the distal tubular epithelium is generated, mainly or in part (no evidence bearing on directly electrogenic distal ion pumps is available), by two diffusion potentials in series, in conjunction with a peritubularly located sodium extrusion mechanism establishing a cell compartment with low sodium and high potassium concentration. At the peritubular cell membrane, a high potassium selectivity sets the potential difference close to the expected potassium equilibrium potential. The permeability of the luminal cell membrane is less selective, and it therefore maintains a much lower potential gradient. It is the different degree of cell polarization across the luminal and peritubular cell boundaries which establishes the overall transtubular potential difference.

A number of problems immediately arise from this situation. How is the apparent constancy of the transtubular electrical potential difference along the distal tubule [7, 13, 22, 27, 28, 29, 45, 52] explained in view of the considerable changes in the ionic concentrations known to occur with respect to the main tubular ions as fluid passes along this part of the nephron? How does the intratubular potassium concentration increase along the distal tubule in the presence of a uniform electrical potential gradient, in

view of the demonstrated very marked effect of the electrical driving force upon transtubular potassium distribution?

With respect to the first problem, an examination of the intraluminal concentrations of sodium and of potassium along the distal tubule shows that, frequently, the concentration of sodium declines whereas that of potassium increases [14, 27, 28]. These reciprocal concentration changes minimize alterations in the sum of total cationic concentrations along the distal tubule. It can be demonstrated that, given two properties, namely, 1. about equal relative permeabilities for sodium, potassium and chloride across the luminal cell boundary, and 2. relative constancy of total cationic concentrations along the distal tubule, no significant changes in the magnitude of the transtubular potential difference would be expected [13]. The relatively low degree of selective ion permeability of the luminal cell membrane to the main intratubular ion species represents a device by which changes in total cation concentration, were they to occur, result in only small alterations of the overall potential difference. These described permeability properties as well as the relative constancy of total cation concentration are the main factors responsible for the observed uniformity of the electrical potential difference along the distal tubule. A more extensive analysis and discussion of this complex problem has been attempted [13].

With regard to the mechanism underlying the gradual increase in the potassium concentration along the distal tubule, no complete evaluation is presently possible. It is, however, important to realize that the very significant difference between early and late distal tubular potassium concentration gradients which can occur in free-flow as well as in stationary perfusion experiments, cannot be attributed to differences in the electrical potential difference since the latter is uniform along this part of the nephron. Differences in potassium concentration along the distal tubule must be due to a gradual shift of balance between the strength of the reabsorptive potassium pump and net passive leak into the lumen. In the presence of a uniform electrical driving force, this could be the consequence of either a gradual increase in passive potassium permeability or in intracellular potassium concentration, or a continuous decrease in the electromotive force of the reabsorptive potassium transfer mechanism [14, 29]. Although there are, at present, no conclusive data to permit a decision between these alternatives, some experimental results lend themselves to the interpretation that a gradual increase in potassium permeability might be responsible for the increase in potassium concentration along the distal tubule. This argument is based on the observation that the steady-state concentration of potassium in the absence of an inwardly-directed electrical driving force (stationary microperfusion experiments using choline chloride as perfusate, Fig. 9) does not increase along the distal convoluted tubule. Since a uniform potassium gradient is established along the whole length of the distal tubule, it is likely that there is no progressive change in the strength of the reabsorptive transfer mechanism. However, direct measurements of the potassium per-

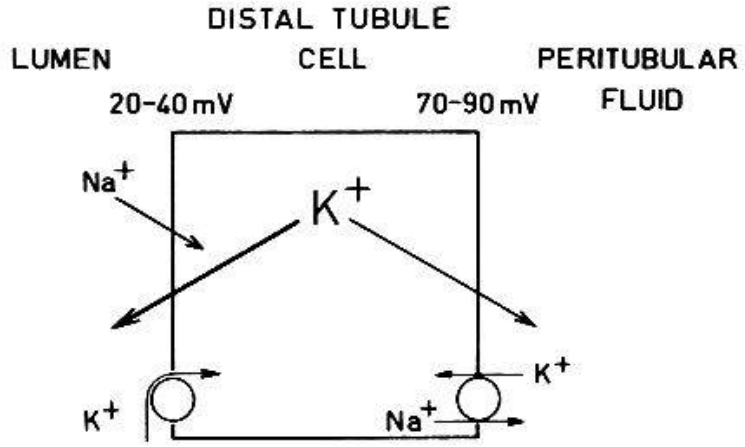


Fig. 12. Schematic presentation of some properties of distal tubule cells.

meability or its changes along the distal tubule are yet to be made, and would greatly contribute to a more complete evaluation of this problem.

According to our view, the extent of accumulation of potassium in the distal tubule depends importantly upon 1. the intracellular potassium concentration, 2. the electrical potential difference across the luminal cell membrane, 3. the passive potassium permeability across the luminal cell boundary, and 4. the strength (apparent electromotive force) of an active, reabsorptive potassium transfer mechanism across the luminal cell boundary. Our views on the properties of distal tubule cells are summarized in a schematic diagram (Fig. 12). It contains only those aspects of cell and membrane characteristics which are pertinent and have been part of the present discussion. It ignores hydrogen ion and chloride transport, the possibility of electrogenic ion transfer, the possibility that part of sodium reabsorption might be coupled to secretory hydrogen ion movement. It will certainly need modification in the future. Its main virtue may be that it facilitates the present discussion of those factors believed to determine the kinetics of distal tubular potassium movement.

Factors affecting intracellular potassium concentration, and thus the magnitude of the electrochemical potential gradient favoring potassium entry into the distal tubular lumen, might include 1. passive alterations in cell potassium concentration due to osmotically induced shifts by changes in body hydration [2, 35], 2. changes due to potassium uptake across the peritubular cell boundary. With regard to the latter, it is possible, but not proven, that a potassium pump at this site partly regulates the intracellular concentration of this ion [2, 11, 12, 13, 14, 51, 52]. If a fraction of such peritubular potassium uptake is active, changes in the transfer rate could affect intracellular concentration, and thus the electrochemical potential gradient across the luminal cell membrane. To this extent, active potassium transport would contribute to the overall secretory mechanism. Cardiac glycosides [36], mercurial diuretics [2, 32] and adrenal steroids [2, 18, 24] may elicit effects upon distal tubular potassium transfer by alterations in active uptake at this site.

Of the other factors affecting passive distal tubular potassium entry, the electrical potential difference is an important one. With an increase in intratubular negativity the accumulation of potassium in the distal tubular lumen and its urinary excretion rate rise markedly [27, 28]. Such an effect of the electrical driving force upon transtubular potassium distribution has also been suggested by others [2, 7, 14, 43, 44]. It would explain the observation that potassium is secreted more copiously at a given rate of total electrolyte excretion when the urinary anion is poorly reabsorbable and the distal tubular lumen maintains a higher degree of electronegativity. A higher potency of sodium phosphate or sodium sulfate [42] in promoting urinary potassium loss, compared to sodium chloride, is consistent with this view since both phosphate and sulfate are known to augment the distal transtubular potential difference [7]. In part, changes in the distal tubular anion load may also be involved in the inverse relationship between urinary potassium and hydrogen ion secretion [3, 29]. Substitution of chloride by bicarbonate in the distal tubular lumen and the collecting ducts, either by intravenous loading with bicarbonate or by depressing reabsorption at a more proximal site, may affect potassium transport along the distal tubule and the collecting ducts by means of an increase of the transtubular potential difference. The latter has been observed to be enhanced by the administration of dichlorphenamide [27] and by intravenous bicarbonate infusion [7]. Suppression of a possibly electrogenic hydrogen ion movement and subsequent augmentation of the distal intratubular negativity has been suggested as another possibility of increased potassium delivery into an alkaline urine [3].

Concerning the nature of the interrelationship between tubular sodium and potassium transfer, the following mechanism could be involved [28]. It has been pointed out that the presence of sodium ions is partly responsible for the maintenance of intratubular negativity [13]. If the extent of distal potassium accumulation is compared with the simultaneously existing transtubular potential difference, a correlation obtains in some, but not in all, experimental situations. For instance, the distal tubular lumen becomes more negative when experimental results in mannitol-loaded rats (mean –44 mV) are compared with sodium chloride-loaded (–56 mV) and sulfate-loaded (–64 mV) animals [28]. Accordingly, the enhanced rate of potassium excretion observed in the latter two situations could be mediated by an increase in the electrical potential gradient favoring net entry of potassium into the distal tubule.

If the electrical potential difference across the collecting duct, as across the distal tubular epithelium, were also partly dependent upon the concentration of sodium ions, reabsorptive loss of potassium from this nephron segment might be expected whenever the sodium concentration and the electrical potential difference fall strikingly along this tubular segment. A survey of data on potassium transfer across the collecting duct [27, 28] at low and intermediate urine flow rates shows that potassium reabsorption

is most significant under those very conditions when sodium reabsorption along this part of the nephron is almost complete, and the urinary concentration minimal. Potassium reabsorption is insignificant along the collecting ducts whenever the sodium concentration remains high. This striking relationship between sodium concentration and potassium reabsorption along the terminal collecting duct epithelium is quite consistent with the view that the extent of reabsorption of potassium from this part of the nephron is partly affected by the magnitude of the electrical potential gradient. Obviously, an exact evaluation of the nature of potassium transfer at this site must await a more precise knowledge of the electrochemical potential gradients obtaining under those conditions in which significant alterations in the potassium transfer rate have been observed.

One of the most interesting problems in the assessment of the factors determining distal tubular potassium transfer is the exact role played by changes in active reabsorption and in the passive permeability properties across the luminal cell boundary. As yet no evaluation has been made of possible changes of these two factors in contributing to the very dramatic modifications shown to occur in the pattern of distal tubular potassium transport. It would be of particular interest to explore these factors under those conditions in which changes in the transtubular electrical potential difference and changes in the intracellular potassium concentration are not the cause for the observed modifications in distal tubular potassium transfer.


## *Summary*

Numerous micropuncture studies in the rat indicate that potassium ions are always extensively reabsorbed along the proximal tubule and along Henle's loop. The distal tubular epithelium is the main site of secretion. This secretory process is variable. Under conditions of an adequate intake of sodium and potassium, and under various loading procedures, distal tubular potassium secretion accounts for a minimum of some 60 to 90% of urinary potassium. Distal tubular entry of potassium always occurs down an electrochemical potential gradient. In microperfusion studies it can be demonstrated that transtubular steady-state concentration ratios are always less than expected on the basis of passive distribution. Intratubular potassium concentration is strongly affected by the transtubular electrical potential gradient. It is believed that secretory entry of potassium is passive and that a component of active reabsorption prevents attainment of electrochemical equilibrium. At uniform electrical potential difference along the distal tubule, the observed progressive increase in potassium concentration along this nephron segment is believed to be due to a shift in the balance between active potassium reabsorption and passive potassium leak into the lumen.

*Zusammenfassung*

Zahlreiche Untersuchungen durch Nierenmikropunktionen an Ratten zeigen, daß der größte Teil des Kaliums im proximalen Tubulus und in der Henleschen Schleife reabsorbiert wird. Man weiß auch, daß es vorwiegend vom Epithel des distalen Kanals ausgeschieden wird, und daß dieser Sekretionsprozeß veränderlich ist. Bei ausreichender Natrium- und Kaliumzufuhr und bei verschiedenen Belastungen stellt die distale tubuläre Kaliumausscheidung 60% bis 90% des Kaliumgehaltes im Urin dar. Der distale Sekretionsvorgang findet längs eines elektrochemischen Gradienten statt. In Mikroperfusionsversuchen läßt sich zeigen, daß im steady-state der transtubuläre Konzentrationsquotient immer kleiner ist, als auf Grund einer passiven Verteilung erwartet werden kann; die intratubuläre Kaliumkonzentration wird durch den transtubulären Gradienten des elektrischen Potentials stark beeinflußt. Es wird angenommen, daß die Kaliumsekretion passiv ist und daß eine aktive Rückresorptionskomponente das Erreichen des elektrochemischen Gleichgewichtes verhindert. Wenn man längs des distalen Tubulus eine gleichbleibende elektrische Potentialdifferenz annimmt, kann die progressive Zunahme der Kaliumkonzentration durch eine Veränderung des Gleichgewichtes zwischen der aktiven Reabsorption und dem passiven Eintritt des Ions in das tubuläre Lumen erklärt werden.

*Résumé*

De nombreuses études par microponctions rénales chez le rat nous indiquent que la majeure partie du potassium est réabsorbée le long du tube proximal et de l'anse de Henle. On sait également que c'est à la hauteur du tube distal qu'il est principalement sécrété et que ce processus sécrétoire est variable. Lors d'apports suffisants en sodium et en potassium ainsi que lors de surcharges diverses, la sécrétion tubulaire distale de potassium représente au moins 60 à 90% du potassium urinaire. Le passage intratubulaire du ion a lieu le long d'un gradient électrochimique. Pourtant on peut démontrer, lors de microperfusions et dans des conditions de steady-state, que les rapports de concentration transtubulaire ont une valeur toujours trop basse pour n'être qu'uniquement le reflet d'une distribution passive; la concentration de potassium intratubulaire est fortement influencée par le gradient électrique transtubulaire. On estime que la sécrétion de potassium est passive mais que le déséquilibre électrochimique est dû à la présence d'une réabsorption active. Ainsi, pour une différence de potentiel électrique identique sur le trajet tubulaire distal, l'augmentation progressive de la concentration du potassium peut s'expliquer par une variation de l'équilibre entre la réabsorption active et la fuite passive du ion dans la lumière tubulaire.

*Riassunto*

Molti esperimenti sui ratti (effettuati mediante micropunzione renale) ci indicano che la maggior parte del potassio viene riassorbita all'altezza del tubolo prossimale e dell'ansa di Henle. È noto inoltre che è all'altezza del tubolo distale che avviene la secrezione più importante del potassio e che questo processo di secrezione è variabile. Quando l'apporto di sodio e potassio sono sufficienti e nel caso di diverse prove di carico, la secrezione tubolare distale del potassio rappresenta almeno il 60–90% del potassio urinario. Il passaggio intratubolare dello ione avviene sulla base di un gradiente elettrochimico. Nel caso di microperfusioni ed in condizioni cosidette «steady-state» si può dimostrare però che i rapporti di concentrazione transtubolare sono sempre troppo bassi per essere unicamente il risultato di una distribuzione passiva; la concentrazione del potassio intratubolare viene fortemente influenzata dal gradiente elettrico transtubolare. Si suppone che la secrezione del potassio sia passiva ma che lo squilibrio elettrochimico sia dovuto alla presenza di un riassorbimento attivo. In tal modo, supposta all' altezza del tubolo distale una differenza di potenziale elettrico identica, l'aumento progressivo della concentrazione del potassio può essere spiegata da una variazione dell'equilibrio fra il riassorbimento attivo e la perdita passiva dello ione nel lume tubolare.

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