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THE MECHANISM OF POTASSIUM REABSORPTION IN THE PROXIMAL TUBULE OF THE RAT *

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According to present concepts, renal excretion of potassium is mediated by a three-component system: complete filtration of plasma potassium at the glomerulus, virtually complete reabsorption in the proximal tubule, and active secretion in the distal portions of the nephron. Of these mechanisms, proximal reabsorption has been the most difficult to examine experimentally. Conventional clearance techniques have provided only indirect evidence for the existence and magnitude of this process. Berliner (1) cites the following experiments as support for essentially complete reabsorption of filtered potassium in the proximal tubule: *1*) in the dog infused with saline and given an organic mercurial diuretic, potassium excretion remains constant and independent of large variations in filtered potassium (*2*), and *2*) in the dog with a split bladder subjected to unilateral renal artery constriction, if sodium excretion is maintained by infusions of sodium salts or by diuretics, potassium excretion on the experimental side does not change despite a 30 to 35 per cent reduction in filtered potassium (3). Since these observations suggest that potassium excretion is independent of filtered load, the conclusion is drawn that either potassium reabsorption is adjusted exactly to filtered load, or, more likely, that filtered potassium is completely reabsorbed proximal to the secretory site and does not contribute to excreted potassium.

The stop-flow technique has been of definite value in localizing and characterizing distal potassium secretion. Since, however, proximal samples must pass through the secretory site before collection, potassium concentration patterns cannot accurately reflect proximal tubular events (4, 5). This approach has not been very informative regarding potassium reabsorption in the proximal convolution.

Attempts to clarify these problems by micropuncture have led to conflicting reports. Bott, using an ultramicro colorimetric method of potassium analysis, has reported concentrations in the proximal tubule significantly lower than in plasma in both *Necturus* (6) and rat (7). Subsequent studies from her laboratory, in which potassium analyses were done by direct flame photometry, failed to show a consistent concentration gradient between proximal fluid and plasma in either species (8). On the other hand, Oken and Solomon (9) have found the potassium concentrations of fluid collected from the most distal segment of the *Necturus* proximal nephron to exceed the simultaneous plasma concentration by about 50 per cent. Since inulin is concentrated to the same degree in this segment, no net movement of potassium either into or out of the tubular lumen was thought to have occurred.

The present study was undertaken to determine whether net potassium reabsorption occurs in the rat proximal tubule, and if so, whether the transport is active or passive. Simultaneous measurements of the transtubular potential difference, E_T, of potassium concentration in tubular fluid, $[K]_{TF}$, and of potassium concentration in plasma, $[K]_P$, have been obtained. The results indicate that potassium is reabsorbed across the proximal tubular cell against an electrochemical gradient by an active transport system at the luminal border.

METHODS

All experiments were performed on male Sprague-Dawley rats weighing 250 to 380 g. The animals were allowed free access to a diet of commercial rat pellets and tap water up to the time of experiment.

Each rat was anesthetized and prepared in the manner described previously (10, 11). To facilitate potential

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measurements, the renal capsule was stripped with a minimum of trauma from the ventral surface of the experimental kidney. Previous studies in this laboratory confirm the observation of Solomon (12) that this procedure has no significant effect on E_T.

Measurement of E_T was the same as previously outlined (10, 11), with the following modifications. *1*) The Pyrex glass microelectrodes were filled with a solution of 0.5 M KNO_3 in 2 M KCl. This solution has been found to reduce the incidence of unsatisfactory high tip potentials. *2*) Continuity on the reference side of the circuit was accomplished by immersing the clipped rat tail in a small beaker of physiologic saline, to which the Ag–AgCl reference electrode was connected by a 3 M KCl bridge. This procedure did not adversely affect measurements of E_T and provided more working area by eliminating abdominal placement of the reference electrode. *3*) Prolonged maximal readings of E_T could not be obtained in each instance. If a recording was stable for at least 30 seconds, was little affected by advancement, retraction, or lateral displacement of the electrode tip, and was characterized by rapid return to the baseline upon withdrawal of the microelectrode, it was considered satisfactory. Many of the recordings exhibited small increases or decreases in potential, presumably owing to variation in degree of "sealing in" of the electrode produced by respiratory movement and vascular pulsation of the kidney. These measurements were accepted if the variations were small and the resultant average potentials were not lower than the initial high value.

After each E_T measurement, the saline bathing the kidney surface was replaced by mineral oil. A sample of tubular fluid for potassium analysis was collected at the identical site in Pyrex micropipettes containing mineral oil previously equilibrated with deionized water. Studies by Clapp (13), subsequently confirmed in this laboratory, have demonstrated that this precaution prevents the gradual rise in osmolality, presumably by loss of water into the mineral oil, that may occur in samples stored in collecting pipettes for several hours. Simultaneously, blood for potassium analysis was obtained through a femoral artery catheter. The nephron was then injected with latex and the puncture site marked with nigrosin. Puncture sites were identified by microdissection of the injected nephrons (10, 11). Localizations are expressed as percentage of total proximal tubule length, measured from glomerulus to beginning of the thin segment.

Measurement of $[K]_{TF}$ was performed within 3 hours of collection by a direct flame method employing the Zeiss PMQ II spectrophotometer. Diluent solution and standards were prepared daily from 98 per cent redistilled acetone containing 0.3 mmole per L of CsCl and 0.1 mmole per L of $(NH_4)_2HPO_4$. Extensive preliminary investigations established that cesium enhanced potassium emission intensity and nullified the changes in potassium emission that ordinarily occur with varying sodium concentration in the biological samples. Similarly, the addition of excess phosphate was shown to swamp out the anion depressant effect over a wide range of bicarbonate, phosphate, and sulfate concentrations.

For analysis of tubular fluid, samples of 0.02 to 0.03 μl size were delivered from a Wigglesworth pipette (14) into 0.25 ml of diluent. The effective delivery volume of the Wigglesworth pipette was determined by calibration with KCl standards. Samples were then analyzed at 767 mμ by use of a hydrogen-oxygen flame and freshly prepared macrostandards containing 0.0002 to 0.0012 mmole per L potassium in acetone–CsCl–$(NH_4)_2HPO_4$. All analyses were performed in duplicate, and only those determinations with close agreement between duplicates were accepted.

Precision of the micromethod was ascertained by performing 12 replicate analyses on a sample of dilute bladder urine; the standard deviation was 3.1 per cent. Accuracy was checked by analysis of 10 different bladder urine specimens for potassium by both the micromethod and a macromethod using the Baird flame photometer. In a concentration range of 2.9 to 5.2 mmoles per L, the values obtained by the micromethod ranged from 92 to 110 per cent of those obtained by the macrotechnique.

Measurement of $[K]_P$ was performed on duplicate 100-μl samples with a Baird flame photometer with lithium as the internal standard. Accuracy within 2 per cent is obtained with samples of this size. $[K]_P$ values were not corrected for either plasma water content or Gibbs-Donnan equilibrium, since these factors tend to be cancelled. In addition, analytic error inherent in calculating the ratio of tubular fluid to plasma concentration, $[K]_{TF}/[K]_P$, far exceeds the combined correction factor.

RESULTS

Satisfactory E_T measurements, together with anatomical localization, were obtained in 58 instances. In 40 of these, simultaneous measurements of $[K]_{TF}$ and $[K]_P$ were obtained.

Transtubular potential differences. The frequency distribution of the 58 E_T measurements is presented in Figure 1. E_T ranged from -8 to -49 mV, with the lumen consistently negative to extracellular fluid. The distribution is asymmetrical. The mean E_T of -22 mV and median E_T of -18 mV compare favorably with values reported previously in the rat (11, 12, 15). A number, however, of stable potentials in excess of -35 mV were recorded in the present experiments.

That satisfactory low and high potentials can be obtained in the same animal is shown in rat 16, Table I. Consecutive E_T measurements of -16 and -45 mV, localized respectively at 38 and 33 per cent of proximal tubular length, were recorded within 15 minutes. Widely different values for E_T were frequently obtained from different nephrons in the same rat.

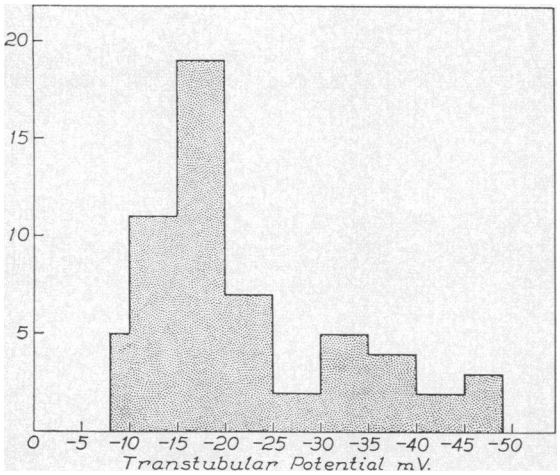

FIG. 1. FREQUENCY DISTRIBUTION OF PROXIMAL TRANSTUBULAR POTENTIAL DIFFERENCES IN NONDIURETIC RATS. The data represent 58 observations from 24 animals.

The geographic distribution of E_T is shown in the lower half of Figure 2. In the rat, the portion of the proximal convolution accessible to micropuncture extends from 10 per cent to somewhat over 60 per cent the distance from glomerulus to thin segment. E_T values in these experiments were recorded from segments ranging in location from 10 to 68 per cent of proximal tubular length. No correlation between magnitude of E_T and

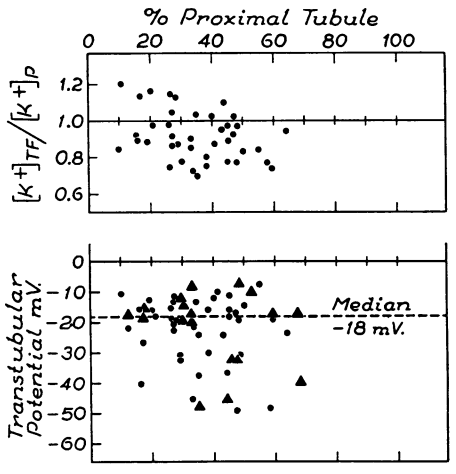

FIG. 2. GEOGRAPHIC DISTRIBUTION ALONG THE PROXIMAL TUBULE OF POTASSIUM CONCENTRATION RATIOS AND POTENTIAL DIFFERENCES. The upper figure shows 40 ratios from 20 animals. In the lower figure, the 40 potential differences for which simultaneous potassium data have been obtained are indicated by dots, and an additional 18 potentials without corresponding potassium determinations are indicated by triangles.

TABLE I

Tubular fluid and plasma potassium concentrations and transtubular potential differences in the proximal convoluted tubule

Rat no.	Site	$[K]_{TF}$	$[K]_P$	$[K]_{TF}/[K]_P$	E_T
	%	*mmoles/L*	*mmoles/L*		*mV*
2	50	3.8	4.6	.83	−14
3	20	5.1	4.4	1.16	−16
3	27	4.8	4.2	1.14	−12
3	33	3.7	4.4	.84	−22
3	41	3.6	4.1	.88	−10
5	29	3.2	3.6	.89	−32
5	38	2.7	3.6	.75	−30
7	27	4.3	4.7	.92	−20
7	48	3.3	4.2	.79	−31
9	35	2.3	3.3	.70	−38
9	47	3.1	3.3	.94	−49
10	27	4.2	4.0	1.05	−13
10	64	3.5	3.7	.95	−24
11	48	4.2	4.3	.98	−19
12	16	3.4	3.8	.90	−40
12	34	3.2	4.4	.73	−14
13	17	4.6	4.1	1.12	−26
14	44	4.5	4.1	1.10	−36
14	45	4.1	4.6	.89	−16
15	27	3.5	4.1	.85	−22
15	45	4.4	4.5	.98	−18
16	16	4.2	4.7	.89	−16
16	21	4.0	4.1	.98	−18
16	33	3.7	4.1	.90	−45
16	38	3.2	4.0	.80	−16
17	26	3.9	4.0	.98	−16
17	43	4.1	4.3	.95	−24
17	47	4.2	4.1	1.02	−17
18	35	4.5	4.3	1.04	−24
20	40	3.2	3.2	1.00	−12
20	59	2.3	3.2	.72	−19
21	11	4.8	4.0	1.20	−22
21	28	4.7	4.2	1.12	−18
21	30	3.7	4.7	.79	−31
22	55	3.7	4.4	.84	− 8
23	26	3.7	5.0	.74	−19
23	45	3.6	4.6	.78	−11
24	58	3.3	4.3	.77	−48
25	10	4.0	4.7	.85	−10
25	19	3.8	4.3	.88	−12

geographic location was noted. Values as high as − 35 to − 45 mV were found in all accessible segments of the proximal tubule.

Transtubular potassium concentration gradients. Simultaneous measurements of $[K]_{TF}$ and $[K]_P$, along with the calculated values of $[K]_{TF}/[K]_P$ and the corresponding E_T measurements, are listed according to geographic location in Table I. Values of $[K]_{TF}$ ranged from 2.3 to 5.1 mmoles per L, with a mean of 3.8 mmoles per L. The simultaneous values of $[K]_P$ ranged from 3.2 to 5.0 with a mean of 4.2 mmoles per L. Values of the ratio $[K]_{TF}/[K]_P$ ranged from 0.70 to 1.20; the mean was 0.92 ± 0.13 (SD). The individual ratios are plotted geographically in the upper half

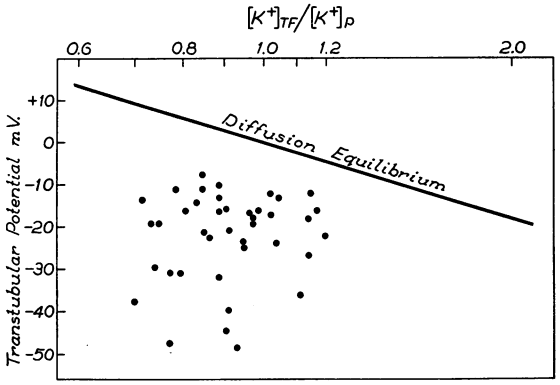

FIG. 3. RELATION BETWEEN POTASSIUM CONCENTRATION RATIOS AND SIMULTANEOUS POTENTIAL DIFFERENCES OBTAINED FROM THE RAT PROXIMAL TUBULE. The data consist of 40 observations from 20 animals. The oblique line, calculated from the Nernst equation, predicts the equilibrium potassium ratio for a given potential difference.

of Figure 2. All ratios were localized to sites ranging from 10 to 64 per cent of proximal tubular length. There was no definite correlation between magnitude of $[K]_{TF}/[K]_P$ and site. The mean ratio, 0.92, is significantly less than unity ($t = -4.0$; $p < 0.001$) by use of the statistic $\frac{\bar{X} - \mu_0}{S/\sqrt{N}}$ (16).

In Figure 3, log $[K]_{TF}/[K]_P$ is plotted against the corresponding E_T. The oblique line is drawn from the Nernst equation, $E_T = -61.5 \log [K]_{TF}/[K]_P$, and defines the value of $[K]_{TF}/[K]_P$ for a given E_T when thermodynamic equilibrium of potassium is present across the tubular cell. All experimental points fell below the oblique line, that is, the actual values of the ratio $[K]_{TF}/[K]_P$ were consistently lower than predicted from the corresponding E_T.

DISCUSSION

In numerous previous studies, an average potential gradient of approximately 20 mV, with the lumen negative to extracellular fluid, has been found across proximal tubular epithelium in both *Necturus* (17–21) and the rat (11, 12, 15). Only infrequently have E_T values greater than -35 mV been reported.

In the present experiments, 9 of 58 proximal E_T measurements fall between -36 and -49 mV. As shown in the lower half of Figure 2, potentials of this magnitude are found in all segments of the proximal nephron accessible to micropuncture. A similar wide range of E_T for each segment is obtained not only when all experiments are considered collectively but also, as shown in Table I, on repeated punctures of different nephrons in the same rat. This identical range of E_T values for each segment of the proximal convolution strongly suggests that the actual E_T does not change as the distance from the glomerulus is increased. It appears, therefore, that all portions of the proximal convolution generate the same transtubular potential differences. The spread of values for a given segment probably indicates that an uncontrolled factor in the technique of measurement is operative. Despite the mean E_T of about -22 mV, it is likely that the higher values are more nearly correct. Erroneous potential measurements are most often due to cellular injury and are almost always on the low side (22). When numerous measurements are made, a large proportion of the records show a high initial value followed immediately by a gradual fall to a lower, more stable reading. Such measurements are obviously unsatisfactory. It seems probable, however, that less obvious electrical shunting, owing to failure of the cell wall to seal completely about the microelectrode tip at the puncture site, is responsible for many low, stable potentials.

The results of the present experiments clearly establish that in the rat the bulk of filtered potassium is reabsorbed in the proximal tubule. This is in sharp contrast to the recent report by Oken and Solomon (9) that no net transport of potassium occurs during free-flow in the *Necturus* proximal nephron. Potassium reabsorption in the proximal tubule can be estimated from the reduction in the volume of filtrate and the transtubular concentration ratio of potassium. Gottschalk (23) has found tubular fluid to plasma inulin-C^{14} ratios of 3 to 4 at a point 60 per cent along the length of the proximal tubule in the nondiuretic rat. An inulin ratio of approximately 5 for fluid entering the distal tubule was estimated by backward extrapolation from the tubular fluid to plasma ratio of 6.7 found in the early distal convolution. Assuming that these ratios accurately reflect net water reabsorption, at least 66 per cent of the filtered water during antidiuresis is reabsorbed in the first two-thirds and about 80 per

cent by the end of the proximal tubule. In the present experiments, no estimate of net water reabsorption is available, but since experimental conditions were comparable to those in Gottschalk's studies, similar relationships may be assumed. Since $[K]_{TF}/[K]_P$ was found to be at or below unity at the end of the accessible proximal convolution, at least 66 per cent of filtered potassium is reabsorbed in the first two-thirds of the proximal tubule. Moreover, the behavior of potassium reabsorption is such as to produce approximately the same value of $[K]_{TF}/[K]_P$ in all accessible segments of the proximal tubule even though the volume of filtrate is drastically reduced. It is reasonable to conclude that a $[K]_{TF}/[K]_P$ of comparable magnitude (about 0.9) exists in the last third of the proximal tubule. If this is so, about 80 to 85 per cent of filtered potassium is reabsorbed in the entire proximal tubule, and the remaining 15 to 20 per cent escapes reabsorption in this portion of the nephron. Therefore, if filtered potassium is completely reabsorbed and all urinary potassium is derived from secretion, final removal of potassium must occur at a more distal site.

The present studies establish not only that the bulk of filtered potassium is reabsorbed in the proximal tubule, but that this movement occurs against an electrochemical gradient. Neglecting for the moment intracellular composition, an estimate of the relative electrochemical potentials of potassium in tubular fluid and in plasma can be made by the Nernst equation, $E_T = -61.5 \log [K]_{TF}/[K]_P$. By substituting measured values for E_T in this equation, values of $[K]_{TF}/[K]_P$ at thermodynamic equilibrium of potassium across tubular epithelium may be calculated. When the measured value for $[K]_{TF}/[K]_P$ is smaller than the value predicted from the simultaneous E_T measurement, potassium exists at a lower electrochemical potential in tubular fluid than in plasma. In the present studies, as depicted in Figure 3, all experimentally determined values of $[K]_{TF}/[K]_P$ were less than the predicted values. Consequently, an electrochemical gradient opposing movement of potassium from lumen to extracellular fluid exists. That tubular fluid potassium has a lower electrochemical potential than plasma potassium, however, cannot by itself establish that potassium is transported out of the lumen. Since potassium is completely ultrafiltrable (24) and must enter the tubule at approximately the same concentration as in plasma, time-dependent factors such as flow rate, surface-volume relationships, and membrane permeability might prevent complete equilibration in the proximal tubule, resulting in values of $[K]_{TF}/[K]_P$ lower than those predicted by the Nernst equation. However, when the existence of a higher electrochemical potential for potassium in plasma than in tubular fluid is considered in conjunction with the demonstration that most of the filtered potassium is reabsorbed, it follows that potassium is actively transported out of the lumen against an electrochemical gradient.[1]

The magnitude of the over-all electrochemical gradient opposing potassium movement across the tubular cell can be estimated from the difference between the actual transtubular potential and the potassium equilibrium potential as derived from the Nernst equation, using measured values of $[K]_{TF}$ and $[K]_P$. Use of the Nernst equation in this manner as a measure of electrochemical disequilibrium between tubular and extracellular

[1] The Nernst equation describes the relation between transtubular potential difference and the distribution of potassium only at thermodynamic equilibrium; hence its use as a criterion of active transport under circumstances where net reabsorption occurs might be questioned. Active reabsorption is defined as net movement from a lower electrochemical potential in the lumen to a higher level in the peritubular fluid. The minimal thermodynamic work (W_{Rev}) in potassium reabsorption is: $W_{Rev} = RT \ln ([K]_P/[K]_{TF}) - FE_T$. By definition, when W_{Rev} is positive the transport mechanism is active. At equilibrium, W_{Rev} is 0, and the equation above reduces to the Nernst equation: $E_T = -RT/F \ln ([K]_{TF}/[K]_P)$. The Nernst equation thus defines the value of $[K]_{TF}/[K]_P$ at which W_{Rev} changes from a negative to a positive value, and therefore can be used as the criterion for active transport.

When there is net transport, the Ussing equation for the total work of transport (W_T) has been used as a more rigid criterion of active transport: $W_T = W_{Rev} + W_{irr}$, where $W_{irr} = RT \ln (M_{out}/M_{in})$, and M_{out} and M_{in} are the unidirectional fluxes out of and into the lumen, respectively. If, however, net reabsorption against an electrochemical gradient (i.e., W_{Rev} is positive) can be demonstrated, then $M_{out} > M_{in}$, and W_{irr} is also positive.

In the presence of net reabsorption, therefore, W_{Rev} is always less than W_T. Nevertheless, a positive value for W_{Rev}, as delimited by the Nernst equation, constitutes a valid criterion for active transport, although the magnitude of the work involved is underestimated.

fluid, in a system in which the cell is interposed between these two compartments, has serious quantitative and conceptual limitations. The finding of an over-all electrochemical gradient for potassium provides no indication of the individual gradients that exist at the luminal and peritubular surfaces of the cell. Consequently, no statement can be made concerning the location and magnitude of the active transport system, since either or both cell surfaces may be involved. Therefore, an analysis of events within the tubular cell is required for an adequate description of potassium transport.

The over-all transtubular potential, E_T, is the difference between the potentials at the luminal (E_L) and peritubular (E_P) surfaces of the cell: $E_T = E_P - E_L$. The mean value for E_T is approximately -20 mV, with the lumen negative to extracellular fluid. E_P under nonequilibrium steady state conditions is given by a variation of the Goldman equation,[2]

$$E_P = -61.5 \log \frac{[K]_C + b_p [Na]_C}{[K]_P + b_p [Na]_P},$$

in which $[K]_C$, $[K]_P$, $[Na]_C$, and $[Na]_P$ refer to the concentrations of potassium and sodium in cell water and plasma, and b_p is the permeability coefficient for sodium relative to potassium for the peritubular membrane. $[K]_C$ is taken as 150 mmoles per L,[3] $[K]_P$ as 4.2, and $[Na]_P$ as 150 mmoles per L. The relative permeability coefficient of sodium for biological membranes has been estimated as 0.01 for frog muscle and squid axon (26), and 0.03 to 0.09 for *Necturus* tubular epithelium (27). In the following calculations, b_p is taken as 0.01. The expression $b_p [Na]_C$, is negligible compared with $[K]_C$ and may be ignored. An E_P of -87 mV, with cell interior negative to extracellular fluid, is obtained by substituting the values above in the Goldman equation. Since E_T is -20 mV and E_P -87 mV, it follows that E_L is -67 mV, with the cell interior negative to tubular fluid.

Figure 4 is a schematic representation of the electrical characteristics of the proximal tubular cell and of the individual electrochemical gradients involved in potassium movement from lumen to extracellular fluid. The upper portion of the figure depicts the electrical gradients. The extracellular fluid is arbitrarily assigned a potential of zero, and the axes are so adjusted that the over-all potential of -20 mV is portrayed as the difference between the height of the arrows, which represent E_P (-87 mv) and E_L (-67 mV).

These derived values for E_P and E_L are used to estimate the electrochemical potentials for potassium in tubular, cellular, and extracellular fluid that are depicted in the lower half of Figure 4; the manner of calculation is given in the legend to this figure. Reference to the scale of relative electrochemical potentials to the left of Figure 4 shows that when the electrochemical potential of extracellular potassium is arbitrarily set at zero, potassium in cell water exists at the higher energy level of + 198 cal per mole, whereas potassium in tubular fluid exists at the lower energy level of -522 cal per mole.

It is apparent from Figure 4 that the movement of potassium from cell to extracellular fluid is down an electrochemical gradient and could be passive. Therefore, the principal problem in accounting for potassium movement out of the lumen concerns the mechanism whereby potassium traverses the luminal membrane, where the major energy barrier exists. The dotted arrow in the diagram indicates that $[K]_{TF}$ of at least 12.2 mmoles per L is required for passive movement of potassium into the cell. This value of $[K]_{TF}$ may be an underestimate, since b_p was taken as 0.01 to calculate E_P and E_L. If b_p is actually as high as 0.03 (27), calculated E_P and E_L would be lower, and consequently $[K]_{TF}$ required for passive movement would be much higher than 12.2 mmoles per L. It is obvious that $[K]_{TF}$ actually required for passive diffusion is much higher than the value of 8.8 mmoles per L estimated from the application of Nernst equation, as depicted in Figure 4 by the dashed arrow, which

[2] Giebisch (19) has offered evidence indicating that the peritubular membrane is permeable to sodium to a degree that significantly influences E_P. Since the peritubular membrane is not a perfect potassium electrode (i.e., is permeable to cations other than potassium), the Goldman equation, which takes into account the influence of sodium as well as potassium, rather than the Nernst equation, is used.

[3] Derived from the following values: potassium, 72 mmoles per kg fresh rat kidney tissue (25); water, 770 g per kg fresh tissue (25); and extracellular fluid, 300 g per kg fresh tissue. An error in estimating the potassium concentration in cell water does not critically alter interpretation of the calculations made in the discussion.

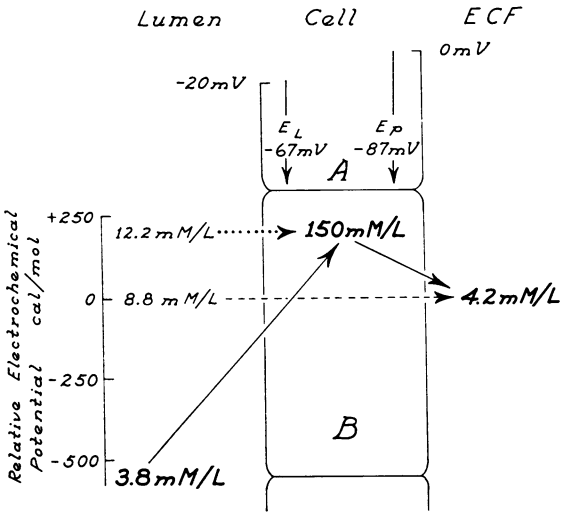

FIG. 4. CONDITIONS FOR PASSIVE AND ACTIVE POTASSIUM TRANSPORT IN THE RAT PROXIMAL TUBULE. A. ELECTRICAL GRADIENTS ACROSS THE TUBULAR CELL. The potential of the extracellular fluid is arbitrarily taken as zero. The over-all potential drop from extracellular to tubular fluid is 20 mV. The height of the arrows depicts the relative magnitudes of the luminal and peritubular membrane potentials, E_L and E_P. The values for E_L (−67 mV) and E_P (−87 mV) are derived in the text, by the Goldman equation. B. ELECTROCHEMICAL GRADIENTS FOR POTASSIUM ACROSS THE TUBULAR CELL. This schematic representation depicts the actual and theoretical electrochemical potentials of potassium in luminal, cellular, and extracellular fluids without stipulating the mechanisms responsible for establishing intracellular composition. Arbitrarily, the electrochemical potential of potassium in extracellular fluid is taken as zero. The electrochemical potentials of potassium in tubular fluid and cell water relative to that in extracellular fluid are calculated as the theoretical free energy change (ΔF) in calories per mole that is experienced during movement of potassium from lumen or cell to extracellular water, by the equation

$$\Delta F = RT \ln ([K]_2/[K]_1) + F (\Psi_2 - \Psi_1).$$

In this equation, R, T, and F have their conventional meanings, $[K]_1$ and $[K]_2$ refer to the initial and final concentrations of potassium, and Ψ_1 and Ψ_2 represent the electrical potentials of the initial and final compartments. The scale depicts the energy levels involved. Theoretical potassium concentrations and movements at various energy levels are given by the smaller numbers and interrupted lines, whereas empirically determined concentrations and movements to a different electrochemical potential are given by the larger number and solid lines. The dashed arrow depicts a hypothetical passive movement of potassium from lumen to extracellular fluid when the electrochemical potential of cellular potassium is neglected. As estimated from the Nernst equation, the concentration of potassium in tubular fluid must exceed 8.8 mmoles per L before passive movement can occur. The dotted arrow depicts a hypothetical passive movement of potassium from lumen to cell when the electrochemical potential for intracellular potassium is taken into consideration. A luminal potassium concentration greater than 12.2 mmoles per L is required. The solid arrows show the *actual* movement of potassium across the cell. It is clear that transport from lumen to cell is against a large electrochemical gradient, and therefore satisfies the criteria for an active process. The movement from cell to extracellular fluid is downhill, and could be passive. See text for discussion.

neglects intracellular composition. The actual movements of potassium are indicated in Figure 4 by the solid arrows. Potassium is moving from lumen to cell against a large electrochemical gradient.

These considerations contribute strong evidence that potassium movement into the cell is mediated by an active process. The objection might be raised, however, that the foregoing analysis neglects the role of solvent drag. During solvent flow through pores, frictional forces exerted on particles in solution may enhance solute flux in the direction of bulk flow and impede flux in the opposite direction (28). In the present experiments, bulk water reabsorption in the proximal tubule might conceivably result in passive potassium movement against the opposing electrochemical gradient. Two lines of evidence, however, argue against an important role for solvent drag in the present situation.

First of all, solvent drag as the only force effecting potassium movement against the existing gradient could maintain $[K]_{TF}/[K]_P$ approaching unity only if the epithelium were freely permeable to this ion. If, for example, water and potassium movement out of the lumen occurred via channels between cells, solvent drag could, at least theoretically, promote the passive reabsorption of large amounts of potassium. Recent evidence,[4] however, indicates that solvent drag

[4] Solvent drag has been demonstrated during the increased osmotic water flow produced by vasopressin in the anuran skin (29) and toad bladder (30). In these systems, vasopressin not only increases permeability to water and small solutes such as urea, but also augments active sodium transport (31, 32). Leaf (33) has reconciled these apparently diverse actions by postulating that the hormone increases the permeability to water, urea, and sodium of a porous diffusion barrier located in the cell membrane. Active sodium transport is augmented by an increased access of sodium to the transport mechanism via the intracellular water compartment.

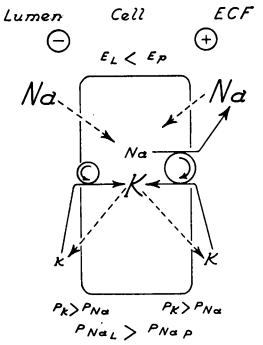

FIG. 5. MODEL OF ELECTROLYTE TRANSPORT BY THE PROXIMAL TUBULAR CELL OF *Necturus*, MODIFIED FROM GIEBISCH (18). The luminal and peritubular membrane potentials are represented by E_L and E_P, whereas the comparative permeabilities for potassium and sodium at these surfaces are indicated by P_K and P_{Na}.

traverses the intracellular water compartment. Thus, for solvent drag to produce potassium reabsorption comparable in degree to that of water, both the luminal and peritubular cell membranes would have to be equally permeable to potassium and water. Since potassium chloride solutions can exert a temporary osmotic effect across tubular cell membranes *in vitro* (27), and since the tubule cell is capable of maintaining a large potassium concentration gradient across both surfaces, such extreme potassium permeability is inconceivable.

More important, tubular fluid potassium concentrations lower than plasma cannot result from solvent drag, since this would imply that the physical drag force is capable of maintaining a higher concentration of potassium in reabsorbate than in tubular fluid. Yet values of $[K]_{TF}/[K]_P$ as low as 0.70 were found. For these reasons, it is concluded that the principal factor responsible for potassium reabsorption is an active transport mechanism located at the luminal surface of the cell.

In Figure 5, a model for proximal tubular electrolyte transport in *Necturus* as proposed by Giebisch (18) is depicted. It should be noted that two separate ion pumps are postulated. One pump, located at the peritubular surface of the cell, is responsible for the active transport of potassium into, and sodium out of, the cell. Such a pump could establish the higher electrochemical potential of intracellular potassium and at the same time accomplish net sodium transport from lumen to extracellular fluid. In addition, Giebisch recognized the possibility of a second pump at the luminal border, but owing to the conflicting data on the concentration of potassium in proximal tubular fluid, was unable to draw any conclusions as to its actual existence. The present studies, however, as shown in Figure 4, demonstrate that the electrochemical gradient at the luminal membrane will prevent passive movement into the cell. Thus the necessity for a second pump, located on the luminal surface, to transport potassium out of the lumen into the cell against its electrochemical gradient is established.

SUMMARY

Simultaneous measurements of transtubular potential difference, E_T, and of potassium concentration in tubular fluid and plasma, $[K]_{TF}$ and $[K]_P$, have been obtained from proximal tubules of nondiuretic rats. All puncture sites were localized by microdissection. E_T ranged from -8 to -49 mV. There was no correlation between magnitude of potential and location along the proximal tubule. In all segments of the proximal tubule, $[K]_{TF}$ tended to fall below $[K]_P$. The mean potassium concentration ratio, $[K]_{TF}/[K]_P$, of 0.92 $\pm$ 0.13 (standard deviation) was significantly less than unity. Since it may be presumed that extensive water reabsorption occurs in the proximal convolution during antidiuresis, these data indicate extensive net potassium reabsorption. In addition, $[K]_{TF}$ was invariably lower than that predicted for the corresponding E_T by the Nernst equation, demonstrating that the reabsorption of potassium must occur against an electrochemical gradient. It is concluded from an analysis of the electrochemical potentials of potassium in tubular fluid, cell water, and extracellular fluid that an active transport mechanism for potassium exists at the luminal cell membrane.

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