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EFFECTS OF K AND Na ON URATE TRANSPORT BY ISOLATED
PERFUSED SNAKE PROXIMAL RENAL TUBULES

by

Henry Walter Randle

A Dissertation Submitted to the Faculty of the
DEPARTMENT OF PHYSIOLOGY
In Partial Fulfillment of the Requirements
For the Degree of
DOCTOR OF PHILOSOPHY
In the Graduate College
THE UNIVERSITY OF ARIZONA

1973
I hereby recommend that this dissertation prepared under my direction by Henry Walter Randle entitled Effects of K and Na on Urate Transport by Isolated Perfused Snake Proximal Renal Tubules be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy.

William D. Darrell  April 11, 1973
Dissertation Director  Date

After inspection of the final copy of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:

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ABSTRACT

Previous work showed that with $2 \times 10^{-5}$ M urate in bath of control Ringer (3 mM potassium, 150 mM sodium) net urate transport occurred from bath to lumen against concentration gradient in isolated perfused snake (Thamnophis spp) proximal renal tubules. Cell water urate concentration was greater than that in bath or lumen, suggesting active uptake into cells and passive diffusion into lumen. Since potassium appeared important for urate uptake by snake kidney slices (Am. J. Physiol. 217: 1510-1519, 1969), its effect was studied in isolated tubules. Removal of potassium from bath led to significant depression of net urate transfer from bath to lumen in 20 minutes and to maximal depression in 60 minutes. In absence of potassium, cell water urate concentration was lower than that in bath but greater than that in lumen, suggesting active uptake into cells was blocked and movement from bath to lumen was by passive diffusion. In absence of potassium, unidirectional urate permeability from bath to lumen ($19.5 \times 10^{-6}$ cm sec$^{-1}$) was not significantly different from unidirectional permeability from lumen to bath in presence of potassium, suggesting that removal of potassium does not affect transepithelial permeability. When potassium was restored to bath, net urate transfer from bath to lumen increased significantly within 40 minutes and was restored to control levels in 60 minutes. Increasing potassium in bath to 40 mM
slightly depressed net urate transfer. Replacing sodium in bath with choline had no significant effect on net urate transfer over periods as long as 4 hours.
LITERATURE SURVEY

Development of the Concept of Renal Tubular Secretion

Renal tubular secretion is now accepted as one of the three fundamental processes (the others being glomerular filtration and tubular reabsorption) by which the kidney forms urine. This process refers to the addition of certain substances to the urine as it flows along the renal tubule. It is primarily by this mechanism that vertebrates excrete organic acids such as uric acid, para-aminohippuric acid, phenol dyes, and a number of drugs. Although the process of renal tubular secretion is now generally accepted, such acceptance only occurred after the first quarter of the present century.

Philosophers, writers, and piss-prophets often speculated about the mechanisms of urine formation, but the earliest experiments were performed by Galen, a physician in the second century AD, who cut the ureters and observed that the abdomen filled with urine, and who tied the ureters and observed that the bladder failed to fill with urine. He was thus the first to note that urine was, in fact, formed in the kidneys (Fulton and Wilson, 1966).

Renal science began to attract additional interest with the precise morphological studies of Bowman in 1842. In his classic paper Bowman described the structure of the glomerulus and its surrounding capsule and suggested purely on morphological grounds that the glomerulus would secrete only salt and water, and that the other components
of the urine (urea, uric acid, etc) would be added by the tubular epithelium in the manner of a true secretory gland. The water would serve to wash these secretory products down the tubule.

Simultaneously, Carl Ludwig (1844) was studying the microscopic anatomy of the kidney. He proposed that urine formation began with the ultrafiltration of blood in the glomerular capillaries and that the initial volume was then reduced by reabsorption as it passed along the renal tubule. Ludwig denied the concept of tubular secretion, maintaining the the volume of filtrate was sufficiently large to account for all solutes present in the urine.

Thirty years after Bowman proposed that urine formation was analogous to secretion in other glands, Heidenhain (1874) revived the idea from observations on the deposition of dyes in the tubule cells. He stressed that tubular secretion was a "vital" activity of the cells. The term "vital" was used to indicate an activity not readily susceptible to investigation. Thus, renal physiology came to be divided into two schools of thought represented by the Ludwigian filtration-reabsorption theory and the Bowman-Heidenhain secretion theory, respectively. No critical experimental test of either thesis was made for the next 50 years.

The modern era of renal studies began with the work of three pharmacologists: A. R. Cushny, A. N. Richards, and E. K. Marshall and their respective colleagues. Cushny wrote an extremely influential book in 1917, The Secretion of Urine, adopting Ludwig's concept (Cushny had studied with two of Ludwig's former students). In a letter to
E. H. Starling (Fulton and Wilson, 1966), Cushny wrote that his book should "serve as an advanced post from which others may issue against the remaining ramparts of vitalism... ." Direct support for the filtration hypothesis was provided by Richards in Philadelphia during the 1920s and early 30s. Using micropipets\(^1\) he collected and analyzed fluid from Bowman's capsule and thus demonstrated that the criteria of glomerular ultrafiltration were met in the amphibian, reptilian, and mammalian kidney (Richards, 1920-21; 1934-35). However, the hypothesis that filtration and reabsorption alone accounted for urine formation was challenged by E. K. Marshall as an over simplification. Marshall, who was at the Johns Hopkins Medical Center, had been interested in the very rapid excretion of phenol red, a dye being used as a clinical kidney function test. He observed that up to 35% of the total phenol red injected into a dog would soon be concentrated in the kidney. This suggested to him that phenol red was taken up by the renal cells prior to its release into the urine. Marshall also made assumptions about the quantity of renal blood flow and about the amount of unbound phenol red available for filtration and concluded that glomerular filtration alone could not deliver the amounts of phenol red which were actually excreted

---

1. A micromanipulator carrying needles for microdissection was first suggested by Purkinje about 1844 and a working model was first built by Schmidt in 1859. A micropipet was first constructed in 1907 by McClendon. The techniques of microdissection and microinjection were further developed over the period from 1912-1924 by Robert Chambers. In the spring of 1921 Richards observed a demonstration of Chamber's micromanipulator at a meeting of the American Association of Anatomists in Philadelphia. Richards who had had a long sustained interest in renal physiology, began the task of puncturing Bowman's capsule in the kidney of the living frog and analyzing the minute quantities of fluid collected in the micropipet (Pitts, 1968; Wearrn and Richards, 1924).
by the kidney. Hence, tubular secretion, at least in that case, had to account for a large fraction of the dye that was put out by the kidney (Marshall and Vickers, 1923). He was, however, unable to convince those within the renal heirarchy, since his evidence was essentially heuristic with no single conclusive experiment. In addition, it was argued that since phenol red was an unnatural product of the organic chemist's laboratory, its excretion was not related to normal renal function.

In 1926 Cushny died, still unconvinced about tubular secretion, a concept that always evoked something "vitalistic" to him. This conflict concerning tubular secretion continued for yet another decade between the groups in Baltimore and Philadelphia. The problem was eventually resolved when the investigators turned to comparative physiological studies. Over the years this approach has proved very fruitful in elucidating the basic principles of renal function.

In the mid 1920s, Marshall exploited the unusual agglomerular kidney of the goosefish. By demonstrating that this agglomerular kidney could excrete phenol red and a number of plasma constituents, Marshall was able to convince most workers that tubular secretion was indeed fundamental in urine formation. This study reportedly prompted Richards to remark at a 1930 Woods Hole seminar, "I am glad that at last Marshall has found an animal that fits in with his theory." Since this definitive demonstration of tubular secretion as a major renal function, investigators have attempted with modern quantitative techniques to provide an understanding of the mechanism of tubular secretion and of the specific requirements for the process.
Potassium Requirement

In 1948 Forster introduced the thin kidney slice as an *in vitro* preparation which would provide a simple means for observing events that would: 1) accurately reflect tubular secretion; 2) allow measurement of the metabolic activity necessary for tubular secretion; and 3) allow variation of conditions not possible *in vivo*. This technique was exploited by Taggart and his colleagues (Taggart, Silverman, and Trayner, 1953) for the study of the tubular secretion of organic acids. They demonstrated that when rabbit kidney cortex was sliced into thin sections (0.3 mm to 0.4 mm) and incubated in a medium containing a dilute organic acid, the concentration of organic acid in the slices would gradually rise. The ratio of organic acid concentration in the slices to that in the medium (S/M ratio) was used as a measure of the ability of the renal tubule cells to transport the organic acid against a concentration gradient. If the organic acid were distributed by diffusion alone, the expected S/M ratio would be about equal to one. With para-aminohippuric acid (PAH) in the bathing medium in a concentration of $1 \times 10^{-3}$ M, the ratio actually rose to about 20 as shown in Table 1 (Cross and Taggart, 1950). Although it was demonstrated that the tubule lumina are collapsed in kidney slices and that accumulation of substances by the slices probably represents transport across the peritubular cell membrane only (Engstrom and Josephson, 1953; Forster and Copenhaver, 1956) this accumulation was considered indicative of renal tubular secretion.
Taggart and his co-workers (1953) found that the experimental conditions required to achieve maximum S/M ratios were fairly critical. Maximal S/M ratios were consistently obtained only when potassium was present. They attempted to define the optimal ionic conditions for PAH transport in rabbit kidney slices (Table 2). When slices were leached for three hours in oxygenated isotonic NaCl (0 mM potassium) at 25°C, the intracellular potassium concentration fell from 70 to 29.5 mEq./cell water. These potassium-depleted slices exhibited a very limited capacity to accumulate PAH. Subsequent incubation in Mammalian Ringer solution restored cell potassium to normal levels and PAH uptake (S/M) reached the level ordinarily obtained with fresh slices.

In the ten years following Cross and Taggart's (1950) initial report demonstrating that there was a critical cation requirement for organic acid transport, a number of papers appeared in the literature describing a potassium requirement. These reports were largely descriptive, indicating only that potassium was necessary for organic acid accumulation.

In addition to introducing the slice technique, Forster (1948) demonstrated that the tubules of the flounder kidney could be used as an in vitro preparation for the study of active transport. The flounder tubules would separate with ease when shaken in Ringer's solution making it possible to microscopically observe organic dye secretion. Puck, Wasserman, and Fishman (1952) adapted this technique for the semiquantitative measurement of the secretion of phenol red. Under optimal conditions the dye concentration achieved in the lumina of the fish tubules
was 4,000-6,000 times as great as that in the bathing medium. In the absence of potassium no phenol red uptake into the cell or lumen was observed. In the absence of calcium the dye was concentrated in the cells but did not appear in the lumen. The accumulation of dye by the cells was directly proportional to the concentration of potassium in the medium (Figure 1). The above phenomena were explained on the assumption that potassium was required for the transport of phenol red across the peritubular membrane into the cell, and the movement from the cell to the lumen was dependent upon calcium.

In a more detailed study, Foulkes (1958), incubated rabbit cortical slices in cold (2° C) saline (0 mM potassium) for two hours rendering them potassium deficient and sodium enriched. The slices were then transferred to oxygenated Ringer (5 mM potassium) at 37° C where a subsequent exchange of potassium for sodium occurred. However, when protamine (a low molecular weight protein isolated from the sperm of certain fish) was added to the Ringer bath, the re-accumulation of potassium was completely blocked (Table 3). In addition, when control slices were suspended in warm Ringer, protamine depressed the uptake of \(^{14}\)C labeled PAH. Since the depression of PAH occurred prior to the measurable changes in cell potassium, the inhibition of the PAH transport was not attributed to a decrease in cell potassium. Foulkes inferred from these data that the protamine was blocking a carrier which was located in the peritubular membrane. This carrier was presumably responsible for the simultaneous transport of both potassium and PAH into the cell.
Table 1. Accumulation of PAH by rabbit kidney slices.--Adapted from Cross and Taggart, 1950.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>15</th>
<th>30</th>
<th>60</th>
<th>120</th>
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<tr>
<td>PAH (S/M)</td>
<td>5.1</td>
<td>8.7</td>
<td>13.5</td>
<td>20.5</td>
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Table 2. Effects of external potassium concentration on accumulation of PAH by rabbit kidney slices.--Adapted from Taggart et al, 1953.

<table>
<thead>
<tr>
<th>Bathing Medium</th>
<th>Slice [K]$_i$ (mEq/L)</th>
<th>PAH S/M</th>
</tr>
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<tr>
<td>150 NaCl</td>
<td>29.5</td>
<td>2.8</td>
</tr>
<tr>
<td>130 NaCl, 20 KCl</td>
<td>70.0</td>
<td>16.3</td>
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Table 3. Inhibition of potassium uptake by protamine.—Adapted from Foulkes and Miller, 1958.

<table>
<thead>
<tr>
<th>Incubation Medium</th>
<th>mEq/L Cell Water</th>
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<tr>
<td></td>
<td>[K]</td>
</tr>
<tr>
<td>cold saline</td>
<td>48</td>
</tr>
<tr>
<td>warm Ringer</td>
<td>114</td>
</tr>
<tr>
<td>Ringer + Protamine</td>
<td>49</td>
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</table>
Fig. 1. Effect of varying the potassium in a medium lacking calcium on the rate of concentration of phenol red in the tubule cells. The amount of dye accumulated at the end of 40 minutes has been used as the basis for comparison. Figure is redrawn from Puck, Wasserman, and Fishman (1952).
By 1962 it was well established that cardiac glycosides, as well as strophanthidin,² would decrease sodium reabsorption and diminish potassium secretion by renal tubules in vivo. In addition to inhibiting electrolyte transport, strophanthidin was noted to lower the clearance of PAH in chickens (Orloff and Burg, 1960). To determine if this were the result of an inhibition of PAH transport rather than a decrease in renal blood flow, Burg and Orloff (1962) investigated the effect of strophanthidin on the transport of PAH by rabbit renal slices. They observed that the incubation depressed the slice potassium as expected and also caused a decrease in the PAH S/M ratio. However, if the medium potassium were elevated above 20 mM, PAH would accumulate in the presence of strophanthidin. When the medium potassium was increased, the slice potassium rose from 90 to 190 mEq/L cell water. The PAH accumulation by the slices was found to vary directly with the cell potassium concentration. Even in the presence of strophanthidin and potassium, PAH S/M ratio varied directly with cell potassium (Figure 2).

Burg and Orloff (1962) also reported that maximal S/M ratios of PAH were seen in the presence of 4-5 mM potassium. Above this optimal concentration of potassium, there was a gradual decline in PAH accumulation (Figure 3).

² It has been shown in a variety of tissues, including the kidney, that the effects of the aglycone strophanthidin are qualitatively indistinguishable from those of the active cardiac glycosides (Strickler and Kessler, 1961).
Fig. 2. Effect of intracellular potassium concentration on PAH uptake by rabbit kidney slices.

Figure is redrawn from Burg and Orloff (1962).
Fig. 3. Influence of potassium in the medium on PAH uptake by rabbit kidney slices.

Figure is adapted from Burg and Orloff (1962).
Hoshi and Hayashi (1970) studied the time course of phenol red accumulation by goldfish renal tubules which were isolated and suspended in a bath. Significant depression of phenol red uptake only occurred after two hours of incubation in potassium-free medium. Chung, Park and Hong (1970) were unable to note a change in the PAH S/M ratio in rabbit kidney slices as a function of medium potassium concentration over a range of 0-40 mEq/L. Although the S/M ratio did increase from 4.2 (in 0 mM potassium) to 7.0 (40 mM potassium), the change was not statistically significant. Taggart et al. (1953) had observed PAH S/M ratios greater than 16 in control slices from the same species. There are a number of explanations why Chung and his colleagues failed to achieve such high values. First, Chung used slices cut approximately 0.4 to 0.5 mm thick, whereas the slices used in Taggart's study were 0.3 mm thick. In the latter case, the surface area exposed to the surrounding PAH would have been greater than in the former case. Second, when Taggart leached slices for three hours in a medium similar to that used by Chung, the slices showed little dependence on medium potassium (0 mM potassium = S/M of 4; 10 mM potassium = S/M of 4.5). This was in close agreement with Chung (0 mM potassium = S/M of 4.3; 4 mM potassium = S/M of 4.2). Taggart considered leached rabbit renal tissue to be depleted of endogenous substrate because when acetate was added the S/M reached 16. Thus, the system used by Chung could have been depleted of substrate which would have depressed the PAH accumulation. The removal of potassium in face of an already depressed transport system could not be expected to have considerable effect, as indeed it did not.
From the previous reports, it is apparent that potassium is important for the transport of organic acids by mammalian and fish renal tubules, although the specific role of potassium in those transport systems is not well understood. Attempts to further define the role of potassium in organic acid secretion have been made using a naturally occurring compound, i.e., uric acid, which is actively secreted by the kidney.

Berndt and Beechwood (1965) reported that urate uptake by rabbit renal slices was potassium dependent and that the maximum urate S/M ratio was reached at a medium potassium concentration between 10 and 40 mM/L (Figure 4). However, urate, which is a minor end product of nitrogen metabolism in mammals, shows a highly complex pattern of renal tubular transport which makes it difficult to interpret mammalian kidney slice data.

On the other hand, urate is the major end product of nitrogen metabolism in all birds, ophidian reptiles, and saurian reptiles. Tubular urate transport may be bi-directional in these animals, but tubular secretion must predominate since the clearance of urate consistently exceeds that of inulin (Dantzler, 1967). In addition, stop-flow studies on conscious water snakes (Natrix) gave no evidence of tubular reabsorption of urate (Dantzler, 1967). Recent studies with isolated perfused snake tubules have demonstrated conclusively that uric acid is secreted by both proximal and distal tubules (Dantzler, 1971; 1973).

Some of the factors regulating the tubular secretion of urate have been evaluated. Dantzler (1970b) noted that the addition of energy
Fig. 4. Influence of potassium concentration of the bathing solution on urate accumulation by rabbit kidney slices.

Figure is adapted from Berndt and Beechwood (1965).
sources, such as acetate, to the incubation medium did not influence the uptake of urate by slices from these animals. This contrasts with the studies mentioned previously on organic acid uptake by mammalian slices (Cross and Taggart, 1950).

The uptake of urate showed a definite requirement for potassium. The active uptake of urate by chicken slices was almost completely eliminated in the absence of potassium in the medium. Inhibition was less marked with slices from garter-snake kidneys and least marked with slices from desert spiny-lizard kidneys (Figures 5 and 6) (Dantzler, 1969; 1970a). These differences in sensitivity of urate secretion to decreases in potassium concentrations may be related to the normal variability in plasma ion concentrations. The plasma potassium concentrations of reptiles are much more variable than those of birds. Since urate is the major end product of nitrogen metabolism in reptiles, it appears important that its tubular secretion not be greatly reduced by natural decreases in plasma potassium (Dantzler, 1969).

Sodium Requirement

Observations suggesting that sodium influences the transport of other solutes across cell membranes occur throughout the early literature. In 1902 Reid implicated sodium as a stimulant of glucose absorption by the small intestine. This list was soon extended to include numerous sugars and amino acids and a host of other substances (see review by Schultz and Curran, 1970). Although sodium appears less important in influencing organic acid transport in the kidney than in
Fig. 5. Influence of potassium concentration in the incubation medium on urate S/M ratios for snake and chicken kidney slices.

Values are means ± SE. Figures are from Dantzler (1969).
Fig. 6. Influence of potassium concentration in the incubation medium on urate S/M ratios for spiny lizard kidney slices.

Values are mean ± SE. Figure is taken from Dantzler (1971).
other tissues, several reports indicate that this ion can influence the renal handling of certain organic compounds.

Vogel, Lauterbach, and Kroger (1965) utilized the isolated, experimentally perfused frog kidney (Rana ridibunda). With perfusion solutions containing a constant concentration of PAH (2.3 μM) and increasing concentrations of sodium (0, 5, 10 and 76.5 mM), they found that the quantity of PAH secreted by the renal tubules rose rapidly and then reached a plateau (Figure 7). The transcellular concentrations of sodium as well as the concentration of sodium in the perfusate appeared to be important. The maximum tubular PAH transport was observed when sodium was supplied to both the luminal and peritubular sides. Sodium supplied only to the peritubular side (via the renal portal system) resulted in a decreased transport of PAH (Vogel and Kroger, 1965).

The microperfusion experiments of Deetjen (1967) showed that a sodium concentration in the tubular lumen as low as 100 mEq/L did not influence the rate of PAH secretion in the rat. Berndt and Beechwood (1965) found that reduction of bathing medium sodium from 150 to 65 mM did not influence urate accumulation by rabbit cortical kidney slices. However, at a medium sodium of 15 mM the urate S/M ratio was markedly reduced (Figure 8).

Moller (1966) measured the excretion of urate during osmotic diuresis (mannitol) in the rabbit and concluded that the reabsorption of sodium did not influence urate reabsorption. Dantzler (1969) noted little change in the uptake of urate by snake kidney slices with reductions in the sodium concentration in the incubation medium. There was no statistically significant reduction in the S/M ratio until medium
Fig. 7. Secretion of PAH as a function of the sodium concentration in perfusion fluid on the isolated experimentally perfused kidney of *Rana ridibunda*.

Figure is adapted from Vogel, Lauterbach, and Kroger (1965).
Fig. 8. Effect of medium sodium concentration on urate accumulation by rabbit kidney slices.

Figure is adapted from Berndt and Beechwood (1965).
sodium was reduced from 150 mM to 25 mM. In all cases the osmolality of the bathing medium was maintained during reductions in sodium concentration by the addition of appropriate concentrations of sucrose.

**Proposed Mechanisms by Which Potassium and Sodium Might Affect Renal Tubular Transport of Organic Acids**

The following descriptions are intended to provide the salient features of the major theories concerning the influences of cations on organic acid transport and a number of the experimental criteria used to distinguish among them.

**The Potassium Requirement**

**Directly Coupled Transport at the Membrane.** The experimental findings of Foulkes (1958) suggest that potassium and PAH are directly coupled at the membrane for transport into the cell. Since protamine blocked both the uptake of potassium and PAH prior to changes in cellular potassium concentration, Foulkes maintained that the drug was simultaneously inhibiting the transport of potassium and PAH. Foulkes and Miller (1961) conducted further studies which they felt added support to the idea of a common carrier.

Rabbit kidney slices were rendered potassium deficient and then suspended in cold saline (0 mM potassium) plus PAH for 20 seconds after which time there was no accumulation of organic acid, see A in Table 4. However, when potassium-deficient slices were placed in warm Ringer (5 mM potassium) for 20 seconds the rate of transfer of PAH from interstitium to cell was the same as that for potassium-rich slices, see B in Table 4. The potassium-deficient slices would re-accumulate
Table 4. Potassium requirement for the rapid accumulation (20 seconds) of PAH by rabbit kidney slices.—Adapted from Foulkes and Miller (1961).

<table>
<thead>
<tr>
<th>SYSTEM</th>
<th>K DEPLETED SLICES</th>
<th>K DEPLETED SLICES</th>
<th>K RICH SLICES</th>
</tr>
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<tbody>
<tr>
<td>K IN MEDIUM</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>(mM)</td>
<td></td>
<td></td>
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<tr>
<td>PAH UPTAKE</td>
<td>0</td>
<td>100</td>
<td>100</td>
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<tr>
<td>(% CONTROL)</td>
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potassium from the Ringer medium with the intracellular potassium concentration approaching that of control slices in about 15 minutes. Since the maximal transport of PAH began within seconds after addition of potassium, the authors maintained that the accumulation of PAH was independent of slice potassium concentration. Yet, when they incubated potassium-rich slices for 20 seconds in cold saline (0 mM potassium), the rate of PAH uptake was 100% of the control, despite the fact that there was no potassium in the bath, see C in Table 4. These latter data appear to argue against the idea of simultaneous transport of potassium and PAH into the cells.

**Cellular Concentration of Potassium.** Burg and Orloff (1962) maintain that the effect of potassium on organic acid transport can be explained on the basis of a decrease in cell potassium alone. They based their conclusions on the fact that PAH uptake varied directly with cell potassium whether or not slices had been treated with a drug (strophanthidin) to block potassium uptake. That interpretation I find questionable. In red cells the inhibitory effect of cardiotonic steroids on cation transport can be competitively reversed if the medium potassium is elevated sufficiently (Glynn, 1957). This effect may be explained if it is supposed that the drug acts by interfering with the binding of potassium to sites on a cation exchange pump, thus preventing the first step in potassium transport. Addition of more potassium would then displace some of the bound steroid and reverse the block (Skou, 1965). In control studies, inhibition of cation transport by red cells by the removal of glucose from the medium cannot be reversed by addition of potassium (Glynn, 1957). Thus, in the presence of high medium potassium
with strophanthidin, potassium ions would occupy the potassium site on the Na-K-ATPase resulting in a normal amount of potassium transport into the cell and subsequently a normal intracellular potassium concentration. Strophanthidin affects both the transport of potassium and the level of potassium within the cell in a parallel fashion. So, as Burg and Orloff (1962) state, their data did not allow them to determine if the strophanthidin-induced inhibition of PAH transport was the result of a decreased transport of potassium or secondary to a depressed level of potassium within the cell.

The time course studies of Hoshi and Hayashi (1970) showed that significant depression of phenol red uptake by goldfish renal tubules did not occur in 0 mM potassium until after two hours of incubation. This delayed effect suggests that the transport of potassium is not directly coupled with organic acid uptake. Instead, since tissue potassium would have been depleted at this point, these data support the idea that a decrease in the cellular potassium concentration is indirectly responsible for the depression of transport observed in fish tubules.

If the secretion of organic acids depends upon the cellular potassium stores and the extracellular potassium is simply serving to maintain cellular potassium levels, then by what mechanism does the cellular potassium regulate organic acid transfer? It has been established that potassium can exert an activating or inhibiting effect (when potassium is too low or too high) on certain enzyme systems (Ussing, 1960). In experiments with the enzymes of brewers' yeast
maceration juice, it was observed that a brief dialysis of the juice against distilled water greatly decreased the ability of the juice to ferment glucose. This activity could be restored simply by the addition of potassium (Figure 9) (Muntz, 1947). The fact that potassium is necessary for alcoholic fermentation by a cell-free yeast extract suggests that the role of this ion in living yeast is not necessarily a membrane phenomenon.

Numerous other enzyme processes show a strong dependency upon the potassium concentration of the medium (Ussing, 1960). Thus, the depletion of cell potassium as a result of low medium potassium could have a direct effect on the enzymes necessary for energy-requiring transport processes such as organic acid secretion.

Another example of an enzyme that is inhibited by either low potassium or high potassium is fructokinase. This inhibition can be counteracted by the addition of magnesium and ATP. Hers (1952) advanced the following hypothesis to explain these experimental results.

The enzyme has two active centers of which one normally binds potassium and the other binds Mg-ATP:

\[
\text{Enzyme} \quad \overset{\text{Potassium}}{\longleftrightarrow} \quad \text{Mg-ATP}
\]

Excess potassium would be inhibitory to this system because it leads to an inactive complex:

\[
\text{Enzyme} \quad \overset{\text{Potassium}}{\longleftrightarrow} \quad \text{Potassium}
\]

It is clear from this hypothesis how changes in potassium concentration can alter enzyme activities. Similarly, organic acid
Fig. 9. Influence of potassium on the fermentation of glucose.

Glucose (40 micromoles) plus HDP (5 micromoles) were fermented with undialyzed and dialyzed maceration juice. Potassium (0.02 M) was added as the phosphate. Figure is redrawn from Muntz (1947).
transport is inhibited in media of high or low potassium concentration (Figure 3). It is possible that the depression of enzyme reactions by low or high potassium concentrations is indirectly responsible for the depression of organic acid transport observed in low or high potassium concentrations.

**Effect on Supply of Energy for Organic Acid Transport.** Most studies have attempted to determine if potassium is coupled to the movement of organic acids across the membrane or if the cellular concentration of potassium is indirectly influencing secretion. It was mentioned earlier in this paper that active transport requires energy. It is conceivable that potassium is affecting the energy supplies for the secretion of organic acids in some manner other than by directly influencing enzyme activity.

It has been recognized for many years that there is a relationship between metabolism and active transport. The emphasis has always been on the need of energy producing reactions for active transport. Conversely, one can examine the possible regulation of metabolism by the active movement of ions. Considerable evidence supports the idea that the active transport of potassium serves as a pacemaker of oxygen consumption in mitochondria (Blond and Whittam, 1964). Several studies demonstrated a positive correlation between medium potassium concentration and oxygen consumption by renal tissue in vitro (Figure 10). A similar correlation between organic acid accumulation and oxygen consumption by renal tissue has been observed (Taggart et al, 1953). Whittam and Willis (1963) proposed the following scheme to account for
Fig. 10. Effect of external potassium and sodium concentrations on the respiration of kidney slices.

Adapted from Blond and Whittam (1964).
the observed relationship between potassium and cellular energy production (Figure 11). The exchange of potassium for sodium at the peritubular membrane would stimulate the membrane bound ATPase. The ATPase would catalyze the hydrolysis of ATP to ADP which in turn would stimulate oxidative phosphorylation and oxygen consumption. The oxidative phosphorylation would generate ATP. The amount of potassium transported would determine the amount of ADP generated and thus the rate of oxidative phosphorylation, oxygen consumption, and ATP formation. This system might generate the ATP which in turn could be used to supply energy for organic acid transport.

**Effect on Membrane Structure.** In 1969 two pathologists, Bulger and Trump, published what some think could be the definitive explanation for the potassium dependence of organic acid transport. Using the electron microscope they were able to view marked structural changes in the morphology of flounder kidney tubule cells when potassium was omitted from the incubation medium. These potassium-deficient cells showed interruption of the peritubular plasma membrane. As noted earlier, the studies of Puck et al (1952) had indicated that potassium was required for the transport of phenol red into flounder tubule cells across the peritubular membrane. Since these morphological changes began to occur after 30 minutes in a potassium-free medium they could very possibly account for the lack of dye transport by the kidney tubules. The striking correlation between site of diminished physiological activity and anatomical alteration makes this explanation very attractive.
Fig. 11. Scheme for the coupling between active transport of potassium and sodium and the tissue respiration in slices of rabbit kidney cortex.

Figure is redrawn from Whittam and Willis (1963).
The Sodium Requirement

**Directly Coupled Transport at Membrane.** The interaction of sodium and various organic solutes has been well documented in a number of tissues and cells (Schultz and Curran, 1970). When strips of mammalian intestine are placed in a sodium-free bath the active transport of certain sugars and amino acids is usually abolished. The addition of sodium to the bathing medium causes an increase in the entry of the sugar into the cells, and similarly, the addition of sugar to the external solution causes an increase in sodium entry. The model that has been generally accepted to represent this interaction is the sodium-gradient or Crane model (Crane, 1962). The proponents of this model suggest that sodium binds to a membrane carrier and diffuses into the cell down its concentration gradient. The coupled solute is bound with the same carrier and is therefore simultaneously translocated into the cell. The solute then passively diffuses out of the cell at the opposite membrane. The requirement for this process is that the sodium gradient be maintained. This is continuously generated by the active extrusion of sodium at the serosal membrane by the well known sodium-potassium exchange pump which is located at that site (Figure 12).

This explanation may account for the interaction between sodium and glucose in the intestinal brush border and several other tissues. It is doubtful, however, that this coupling system can be assigned to the secretion of organic acids by the kidney since sodium transport in the kidney is in the opposite direction from organic acid secretion.
Fig. 12. Model for the interaction between the transport of sodium and organic solutes by isolated rabbit ileum.

Figure is redrawn from Schultz and Curran (1970).
Effect on Supply of Energy for Organic Acid Transport. The potassium sensitive ATPase which may regulate tissue metabolism is also sensitive to sodium (Skou, 1965). Thus, sodium may influence organic acid transport by indirectly controlling the production of energy as previously depicted in Figure 11.

Possible Effect on Cellular Enzymes. The alkali metal ions are known to activate certain enzyme systems. It is possible that sodium may influence organic acid transport via its direct effect on enzymes that catalyze energy producing reactions. The fact that sodium influences organic acid transport less than potassium is at least consistent with the observation that there is a predominance of potassium-activated enzymes over sodium activated enzymes (Ussing, 1960).
INTRODUCTION TO THIS STUDY

The importance of potassium in the transport of urate by mammalian kidney slices has been well documented (Berndt and Beechwood, 1965). However, the role of potassium in this transport system is not well understood. Urate, which is a minor end product of nitrogen metabolism in mammals, shows a highly complex pattern of renal tubular transport which makes it difficult to interpret mammalian kidney slice data.

On the other hand, urate is the major end product of nitrogen metabolism in ophidian reptiles, accounting for as much as 98% of the total nitrogen appearing in the urine (Khalil, 1948). It is freely filtered by the glomeruli of ophidian kidneys (Bordley and Richards, 1933) and, since the clearance of urate consistently exceeds that of inulin (Dantzler, 1967), it must be secreted by the renal tubules. Recent studies with isolated, perfused snake tubules have demonstrated conclusively that uric acid is secreted against a concentration gradient by both proximal and distal tubules (Dantzler, 1971; 1973). These studies have also indicated that secretion in the proximal tubule probably occurs by active transport of urate into the renal tubule cells across the peritubular membrane and subsequent diffusion into the tubule lumen (Dantzler, 1973).

Potassium has been shown to be important for urate uptake by snake kidney slices (Dantzler, 1969). Sodium appeared to be far less important (Dantzler, 1969). However, the slice technique only permits
evaluation of transport at the peritubular membrane in unperfused tubules. In order to evaluate the effects of cations on the steps in urate transport across the tubular epithelium directly, I studied the effects of variations in the potassium and sodium concentrations in the bathing medium on the secretion of uric-2-\(^{14}\)C-acid by isolated, perfused snake proximal tubules. I measured peritubular, cellular, and luminal concentrations of urate during these experiments. Since ouabain has been shown to inhibit urate uptake by mammalian kidney slices (Berndt and Beechwood, 1965), the effect of this cardiac glycoside on urate transport by snake tubules was examined. The effects of ethacrynic acid were also evaluated since this drug is known to inhibit sodium transport in kidney slices (Giebisch, Boulpaep, and Whittembury, 1971).

The results further support the current model (Dantzler, 1973) of urate secretion by snake proximal tubules and indicate that: 1) in the absence of potassium from the bathing medium urate secretion is markedly reduced apparently as a result of the inhibition of the active transport step across the peritubular membrane; 2) the active transport of urate into the cells and subsequent secretion into the lumen can be restored by restoring potassium to the bath; 3) the transepithelial permeability to urate is not significantly altered in the absence of potassium; and 4) sodium is not important for urate secretion.
METHODS

Animals and Dissection of Tubules

Garter snakes (Thamnophis spp) of both sexes, weighing 25-75 g (average weight: about 35 g for the 66 snakes used), were obtained from commercial suppliers in Wisconsin and North Carolina. They were housed in glass aquaria at 25 ± 2° C and fed a diet of raw fish. The animals were killed by decapitation and the kidneys quickly removed and placed in a dish of cold oxygenated Ringer solution (see below for composition of Ringer solution). Single kidney lobules were separated from the kidneys and transferred to another dish of oxygenated Ringer at room temperature. Using steel needles and microdissecting forceps (Dumont #7) the proximal tubules were dissected free under a stereo-microscope at 25-63 X magnification. The segments perfused varied in length from 0.7 to 1.6 mm (1.1 ± 0.11; mean ± SD). The mean length of the whole proximal tubule, determined in preliminary experiments with macerated tissue, is 4.5 ± 1.62 mm (mean ± SD on measurements of 31 tubules).

Ringer Composition

Various Ringer media were used in these experiments. For dissection, perfusion, and bathing the tissue, these media were variations of the following control Ringer medium and contained, in millimoles per liter: NaCl, 126; KCl, 3.0; NaHCO₃, 24; NaH₂PO₄, 0.72;
MgSO$_4$, 1.2; CaCl$_2$, 1.8. When the effects of changes in potassium concentration were studied, osmotic activity in the medium was maintained constant by appropriate changes in sodium concentration. In these studies and those with control Ringer, the media were bubbled continuously with a 95% O$_2$, 5% CO$_2$ gas mixture. This served to keep the supply of O$_2$ and pH constant and also to allow a constant gentle mixing of bathing medium. When the effects of reduction on sodium concentration were studied, osmotic activity was maintained by the addition of appropriate concentrations of choline chloride to the medium. In addition, the NaHCO$_3$ and NaH$_2$PO$_4$ were replaced with TRIS-HCl buffer (pH 7.4). In these studies the media were gassed continuously with compressed air.

Dantzler (1969; 1970b) previously demonstrated with snake kidney slices that the addition of possible exogenous energy sources, such as acetate, had no effect on urate accumulation. Therefore, no such exogenous energy sources were used in these experiments.

**Perfusion of Tubules**

The tubules were perfused *in vitro* by a technique essentially the same as that first described by Burg et al (1966) and modified by Dantzler (1973) for snake tubules. The dissected tubule was transferred in a drop of Ringer solution to a special lucite bathing chamber, containing 2 ml Ringer, and viewed through a stereo-microscope (16-100 X). The proximal end of the tubule was first drawn into an outer holding pipet with suction applied by means of a 20 ml syringe. This pipet had been prepared with a small constriction just above the tip to hold
the tubule and guide the inner pipet (Figure 13). As the tubule reached the constriction, the inner perfusion pipet was lowered into the tubule lumen and perfusion was begun (Figure 13). As soon as the tip of the inner perfusion pipet was well positioned in the tubule lumen and fluid was observed passing through the lumen, the distal end was drawn into the collecting pipet. This was again done by means of suction applied through the pipet holder with a 20 ml syringe. In order to prevent leakage of collected fluid into the bath, the tubule was sealed into the collecting pipet with an encapsulating resin (Sylgard 184, Dow Corning) (Burg et al, 1970). This material is biologically inert, but forms an exceptionally good seal between tissue and pipet. The collecting pipet was first pre-coated with polymerized Sylgard. This was done because the liquid Sylgard used to hold the tubule in place forms a better seal with hardened Sylgard than with glass. An amount of liquid Sylgard just sufficient to fill the tip of the collecting pipet was then drawn into it. As the distal tip of the tubule being perfused was drawn into the collecting pipet, some bathing medium and the liquid Sylgard at the tip were drawn further into the pipet. Since Sylgard had a density greater than that of the aqueous medium, it rolled down the sides of the collecting pipet to the tip where it formed the seal around the tubule shown diagrammatically in Figure 13. The pipets were mounted in specially designed holders similar to those used by Burg et al (1966) and the holders were attached to micromanipulators (Narishige) to position the pipets. These pipets were prepared using a vertical pipet puller (Narishige) and the tips were shaped with a Stoelting microforge.
Fig. 13. Arrangement for perfusing isolated snake proximal tubules.  

Pipets with tip diameters appropriate to the diameters of each tubule were selected. The outside diameter of the tubules, measured during perfusion with an ocular micrometer, varied from 50 to 80 μ with a mean of about 60 μ. The mean inside diameter measured at the same time was about 25 μ. A variable-speed syringe pump (Sage, Model 255-3) with a Hamilton 50 μl syringe fitted with 30 gauge hypodermic stainless steel tubing (0.012 in. O.D., 0.006 in. I.D., 0.003 in. wall; Small Parts) was used to deliver the perfusion fluid to the pipet.

Once the tubule was attached to the perfusing and collecting pipets, the bathing medium was replaced with one containing uric-2-14C-acid (Amersham/Searle Corp., specific activity 52-55 mC/mM) in a concentration of 2 X 10^-5 M. As shown previously (Dantzler, 1973), this concentration is well below that required to saturate the transport system. No urate was present initially in the perfusate. After approximately ten minutes of equilibration time, the perfusate which had accumulated in the collecting pipet was discarded and the experiment begun. Throughout the experiment, the bathing chamber was bubbled continuously with the appropriate gas and the temperature maintained at 25° ± 2° C.

The flow rate through the tubules varied from 0.7 to 9.0 nl min^-1 in different experiments. The single nephron glomerular filtration rate for one 66 g garter snake was reported to be about 5.6 nl min^-1 (Bordley and Richards, 1933). Thus, this range of perfusion rates seems reasonable. The perfusate was collected every 15-20 minutes in a pre-calibrated constant bore capillary (46.4 ± 0.65 μ I.D.; mean ± SE) and the volume determined by measuring the length of the fluid column.
The collected fluid was then added to 10 ml of scintillation fluid for radioactive counting in a scintillation counter (Nuclear-Chicago, Unilux II). A sample of the bathing medium was collected for counting after every two collection periods.

All tubules were initially prepared in control urate-free Ringer. When perfusion was established, the bath was changed completely to the appropriate Ringer solution (control, potassium-free, sodium-free, or high-potassium) with urate and the experiment begun. Some experiments were designed to study the recovery of urate transport after tubules were perfused in a potassium-free solution. After several collections were made from the tubule, the bath was changed completely to control Ringer containing urate. In some studies the tubules were perfused in a medium of control Ringer and then the bath replaced with potassium-free Ringer. In several secretion studies, following the initial preparation in control Ringer, the bath was replaced with control Ringer with urate plus either ouabain ($10^{-3}$ M) or ethacrynic acid ($10^{-3}$ M) and the experiment begun.

**Tissue Urate**

In several studies the tubules were recovered after perfusion to determine the concentration of urate in the cell water. The procedure involved removing tubules from the pipets with a minimum of contamination by bathing medium and luminal fluid. This was similar to the procedure of Burg et al., 1966 and Dantzler (1973).

The perfusion pipet was prefilled with mineral oil stained with Sudan Black B. A volume of control Ringer solution sufficient to allow
at least 60 minutes of perfusion time was then drawn up into the pipet, displacing the oil. Secretion studies were then performed as described above. Samples were collected in the usual fashion until the volume of Ringer in the perfusion pipet was exhausted and the oil filled the tubule lumen. Then the micromanipulator holding the perfusion pipets was rapidly elevated, pulling the tubule free of the collecting pipet and through the surface of the bathing medium. The tip of the perfusion pipet, still holding the tubule, was then rapidly immersed in a dish containing immersion oil (Cargille Type A). The time that elapsed between the filling of the tubule lumen with oil and the immersion of the whole tubule in oil was always less than 30 seconds. The tubule was then pulled free of the perfusion pipet under oil with a glass needle. The rapid immersion of the pipet with tubule attached in oil helped remove any possible contamination from the bath. The tubule was then transferred on the glass needle to 10 μl of 3% trichloroacetic acid under oil to precipitate tissue protein and extract the urate. After one hour the tubule was removed, and the trichloroacetic acid containing the extracted urate was added to 10 ml scintillation fluid for counting. Preliminary experiments showed that all of the $^{14}$C-labeled urate was extracted from the tissue by this procedure.

The tubule was allowed to dry on a glass slide and the oil was then extracted by immersion of the tubule in chloroform for one hour. Finally, the tissue was dried and weighed on a quartz fiber ultramicrobalance ("fishpole" balance). This balance was constructed according to the design of Bonting and Mayron (1961) using commercially available
quartz fibers (Bjorksten Research Laboratories, Madison, Wisconsin). In this balance, the deflection of the unanchored end of a quartz fiber upon addition of various weights is observed through a stereomicroscope and measured with an ocular micrometer. Each balance was calibrated with individual crystals of quinine hydrobromide. A single crystal was placed on the balance, and the deflection produced was measured. The crystal was then transferred to 1.0 ml of 0.1 NH$_2$SO$_4$. The fluorescence of this sample was measured with a Turner fluorimeter and compared with standard solutions of quinine hydrobromide to determine the concentration in the sample. The original weight of the crystal was calculated from its concentration in the sulfuric acid solutions. Quinine hydrobromide was used for calibration because its crystalline form makes it relatively easy to transfer to the balance and its fluorescence in sulfuric acid solutions made it easy to measure its concentrations. Since some tissue weight is lost during trichloroacetic acid and subsequent chloroform extraction, the final weight was multiplied by 1.249. This factor was obtained from a series of non-oil-filled tubules that were weighed before and after the same extraction procedure. The tissue water was 3.52 times the corrected dry weight. This value was determined by Dantzler (1973) using snake proximal tubules and Ringer containing tritiated water. A correction was also made by subtracting that fraction of the tubule length that was contained in the holding pipets.

**Measurement of Tissue Potassium**

The potassium concentration was measured in tubules in the absence of perfusion. The kidneys were removed and the tubules
dissected in a manner identical to that used in perfusion experiments. Six to eight tubules were incubated together in each experimental set. One control set of tubules was incubated for two hours in control Ringer. The other tubules were placed in potassium-free Ringer and sets of six to eight removed alternately after 20 minutes, 60 minutes, and 120 minutes of incubation. The tubules were then extracted under mineral oil for 4 hours in 10 μl (measured with a calibrated Lang-Levy constriction pipet) of 0.75 N HNO₃. Potassium was determined on a dilution of the extract using a Baird KY-3 flame photometer.

Analytical Methods
The activities of uric-2-¹⁴C-acid were determined by counting in a liquid scintillation spectrometer (Nuclear-Chicago, Unilux II). The scintillation solution for the studies was the same as that described by Truniger and Schmidt-Nielsen (1964). It contained toluene: ethanol 8:2, PPO (2,5-diphenyloxazole, 7.0 g/l) and POPOP (1,4-bis-2-(5-phenyloxazolyl)-benzene, 0.3 g/l). The quantity of urate in each sample was determined from its activity and the activity of urate of known concentrations counted in an identical system. Urate fluxes are expressed as moles per millimeter tubule length per minute (moles mm⁻¹ min⁻¹).

Linear regressions were determined by the method of least squares. The slope, y intercept, mean y, and correlation coefficients of the regression lines were calculated with a Hewlett-Packard 9100 Calculator and plotted with a Hewlett-Packard 9125 B Plotter. Mean, standard deviation, and standard error were computed for the slope,
y intercept, and mean y values and the levels of statistical significance determined by student's t test.
RESULTS

Urate Secretion in Control Medium

In a bath of control Ringer (3 mM potassium, 150 mM sodium) net secretion of urate from bath to tubule lumen occurred against a concentration gradient (Figure 14). The proximal tubules were suspended in a bathing solution containing uric-2-14C-acid (2 X 10^-5 M) and were perfused with urate free solution. Secretion from bath to lumen varied with flow rate (0.7 to 9.0 nl min^-1) in a linear fashion (Figure 15). The transfer of urate from bath to tubule lumen changed by about 16 X 10^-15 moles mm^-1 min^-1 for each one nl min^-1 change in flow rate.

Although there was considerable variation from tubule to tubule, Figure 16 shows that, in those control tubules in which the cell water urate concentration was measured, it was consistently greater than that in the bath or lumen. Moreover, the mean cell water urate concentration from all these tubules was significantly greater (0.005 < p < 0.01) than the mean luminal urate concentration (Figure 14) (Dantzler, 1973). As noted previously (Dantzler, 1973), this observation is consistent with active uptake of urate into the cells on the peritubular side and simple diffusion into the lumen.

Effects of Changes in Medium Potassium Concentration on Net Urate Secretion

In four tubules with flow rates maintained constant at 2 nl min^-1, the effect of changing the bathing medium from normal Ringer (3mM...
Fig. 14. Models for urate transport across snake proximal tubules in the presence and absence of potassium in the bathing medium.

Circle and solid arrow indicate active transport. Broken arrows indicate passive fluxes. Bars in upper part of figure indicate mean urate concentrations in bath, tubule fluid, and cell water at end of perfusion period. Vertical lines indicate SE. Numbers in parentheses indicate number of tubules. The control (3 mM potassium) data and control model are from Dantzler (1973).
Fig. 15. Relationship between flow rate in nanoliters per minute (nl/min) and net urate transport in moles per millimeter tubule length per minute (m/mm/min) in snake proximal tubules.

Tubules were perfused in a bathing medium of control Ringer (3 mM potassium, 150 mM sodium) containing $2 \times 10^{-5}$ M urate. No urate was present in perfusate entering tubule. Linear regression line was fitted by method of least squares.
Fig. 16. Comparison of concentrations of urate in tubule fluid and cell water of snake tubules bathed in a medium of control Ringer vs potassium-free Ringer.

Closed circles and solid lines represent control Ringer. Open circles and dashed lines represent potassium-free Ringer. Each pair of points connected by a line represents a separate tubule. Tubules were perfused at different flow rates varying from 0.4 to 9.0 nl min$^{-1}$. Tubule fluid concentrations are those found in collected perfusate. Some of data on tubules in control medium are from Dantzler (1973), but these studies were performed simultaneously with the present ones in potassium-free medium on tubules from the same animals.
Fig. 16. Comparison of concentrations of urate in tubule fluid and cell water of snake tubules bathed in a medium of control Ringer vs potassium-free Ringer.
potassium) to Ringer without potassium on net urate secretion was measured. The tubules were perfused in a medium of control Ringer and the bathing medium was then replaced with a potassium-free Ringer. The removal of potassium from the bath led to a significant depression of net urate transfer from bath to lumen within 20 minutes and to maximal depression within 60 minutes (Figure 17). In the absence of potassium, net urate secretion remained depressed despite large increases in flow rate (Figure 18). This depression of net urate secretion was highly significant (p < 0.001) at all flow rates. Even in the absence of potassium, however, there was a slight increase in net secretion with flow rate (about $2 \times 10^{-15}$ M mm$^{-1}$ min$^{-1}$ for each 1 nl min$^{-1}$ increase in flow rate) (Figure 18).

The concentration of uric-2-$^{14}$C-acid in the tubule cells was measured in perfused tubules bathed for at least 1 hour in potassium-free Ringer to determine if the cellular accumulation of urate were influenced by potassium. Although there was some variation, in the absence of potassium, the urate concentration in the bath tended to be greater than that in the cell water or the lumen (Figures 14 and 16). Moreover, the mean cell water urate concentration ($9 \times 10^{-6}$ M) in all these tubules was lower than that in the bath ($2 \times 10^{-5}$ M) but greater than that in the lumen ($7.6 \times 10^{-6}$ M) (Figure 14). This suggests that the active uptake of urate across the peritubular membrane was eliminated in the absence of potassium and that the movement of urate from bath to lumen occurred by simple diffusion (Figure 14).
Fig. 17. Effect of potassium on net urate transport when flow rate was maintained constant (2 nl min⁻¹).

At the end of 20 minutes, the bathing medium was changed from control Ringer (3 mM potassium) to potassium-free Ringer. Each point represents mean value for four tubules. Vertical lines indicate SE.
Fig. 18. Relationship between flow rate and net urate transport in snake proximal tubules perfused in control bathing medium (3 mM potassium, 150 mM sodium) and potassium-free medium.

Closed circles and solid line represent 3 mM potassium, 150 mM sodium). Open circles and dashed line represent potassium-free medium. Linear regression lines were fitted by method of least squares.
In order to determine if potassium affected the permeability of the whole epithelium to urate, the transepithelial permeability was calculated in the absence of potassium for comparison with previous calculations in the presence of potassium (Dantzler, 1973). The unidirectional permeability from bath to lumen (\(P_{B\rightarrow L}\)) was calculated from the net urate secretion rate and the urate concentration difference across the epithelium on the assumption that urate moved passively from bath to lumen in the absence of potassium. For this purpose, I used the following equation:

\[
P_{B\rightarrow L} = \frac{M_{B\rightarrow L}}{A_L (C_B - C_L/2)}
\]

In equation 1, \(M_{B\rightarrow L}\) is the net secretion of urate from bath to lumen, \(A_L\) is the surface area of the luminal membrane per unit length (for a mean luminal diameter of 27.5 \(\mu\), \(A_L\) is \(86.4 \times 10^{-5} \text{ cm}^2 \text{ mm}^{-1}\)), \(C_B\) is the urate concentration in the bath, and \(C_L\) is the urate concentration in the collected tubular fluid. Since no urate was present in the perfusate entering the tubule and since the urate concentration in the collected tubular fluid was the maximal level achieved, \(C_L/2\) was used as an approximation of the mean urate concentration in the tubule lumen.¹

¹ With a more elaborate analysis, Tune, Burg, and Patlak (1969) found that the mean PAH concentration in the lumen of perfused rabbit tubules only ranged from 0.40 to 0.49 times the concentration in the collected samples. Multiplying the urate concentration in the collected samples by values in this range did not significantly change the results from those obtained using \(C_L/2\). Thus, \(C_L/2\) appears to be a reasonable approximation of the mean luminal urate concentration.
The area of the luminal membrane was used to facilitate comparison with other measurements of transepithelial permeability and luminal membrane permeability (Dantzler, 1973). It was found that the unidirectional permeability from bath to lumen in the absence of potassium was $19.5 \pm 3.90 \times 10^{-6} \text{ cm sec}^{-1}$ (mean $\pm$ SE for 11 tubules). This is not significantly different from the unidirectional permeability from lumen to bath in the presence of potassium ($11.8 \pm 2.05 \times 10^{-6} \text{ cm sec}^{-1}$; mean $\pm$ SE for 6 tubules) (Dantzler, 1973). Thus, the absence of potassium does not seem to alter significantly the transepithelial permeability.

The movement of urate from the tubule cells into the lumen is consistent with a process of simple diffusion in both the presence and absence of potassium. It is possible, however, that in the absence of potassium the permeability of the luminal membrane to urate ($P_L$) is altered and that the rate of diffusion of urate from cell to lumen is also altered. In order to test this possibility, the effects of changes in bathing medium potassium concentration on $P_L$ were examined. $P_L$ was calculated for individual tubules from their net urate transfer rates and the concentration differences across the luminal membrane in a manner similar to that used by Tune, Burg, and Patlak (1969) and Dantzler (1973). For this purpose, I used the following equation:

$$P_L = \frac{M_{B-L}}{A_L(C_T-C_L/2)}$$  \hspace{1cm} (2)

In equation 2, $M_{B-L}$ is again the net secretion of urate from bath to lumen, $A$ is the surface area of the luminal membrane per unit length,
$\bar{C}_T$ is the mean urate concentration in the tissue water, and $C_L$ is the urate concentration in the collected tubule fluid. In the absence of potassium, this approach to the calculation of the luminal membrane permeability can be used only on the assumption that all urate moving passively from bath to lumen moves through the cells and not between the cells. In the presence of potassium, when transport from bath to lumen is against a concentration gradient, no movement between cells into the lumen would be expected. Since the transepithelial permeability was not altered by the removal of potassium from the bathing medium, it seemed unlikely that the luminal membrane permeability could be decreased if urate moved only through the cells. However, some increase in luminal membrane permeability could still occur under these circumstances. The permeability of the luminal membrane in five perfused tubules suspended in a bath of potassium-free Ringer was $46.3 \pm 23.40 \times 10^{-6}$ cm sec$^{-1}$ (mean ± SE). This is greater than that obtained previously ($7.5 \pm 2.61 \times 10^{-6}$ cm sec$^{-1}$; mean ± SE in 9 tubules) (Dantzler, 1973) in control Ringer. However, the two values are not statistically different ($0.10 < p < 0.20$). Thus, it appears unlikely that removal of potassium alters the permeability of the luminal membrane to urate.

Burg and Orloff (1962) found that strophanthidin, a cardiotonic steroid, decreased para-aminohippurate (PAH) accumulation by rabbit kidney slices. They explained this interference with PAH transport on the basis of a reduction in cell potassium. To determine if there were a correlation between cellular potassium concentration and urate secretion by proximal tubules, I compared the tissue water potassium
concentration of tubules incubated for 120 minutes in control Ringer with that of tubules maintained in a potassium-free medium for 20 minutes, 60 minutes, and 120 minutes. The potassium content of the tubules incubated in the potassium-free medium continued to decrease throughout the two hour incubation period when compared with the tubules incubated in the 3 mM potassium solution (Table 5). By the end of two hours the decrease in potassium was highly significant \((p < 0.001)\). On the basis of these data, it is possible to associate changes in urate secretion with changes in the cellular concentration of potassium, since removal of potassium from the bath resulted in decreased tissue potassium along with depressed urate transfer.

Bulger and Trump (1969) have presented electron microscopic evidence that the structure of the peritubular membranes of flounder renal tubule cells is altered by incubation in potassium-free medium. Earlier work (Puck, Wasserman, and Fishman, 1952) showed that the uptake of chlorphenol red across the peritubular membrane of the cells is also potassium dependent. If the absence of potassium had a similar effect on the structure of the peritubular membrane of renal tubule cells of the snake, then it appeared unlikely that urate transport across that membrane would recover after a prolonged incubation in potassium-free Ringer. To determine if the urate transport process would recover, four tubules were perfused at a constant rate \((3 \text{ nl min}^{-1})\) for 140 minutes in a bathing medium of potassium-free Ringer. Following this incubation, the bath was changed to control Ringer \((3 \text{ mM potassium})\) and the effect on net urate secretion measured. With potassium restored to the bath,
Table 5. Effect of variations in medium potassium and medium sodium concentrations on potassium content of snake proximal tubules.

<table>
<thead>
<tr>
<th>INCUBATION MEDIUM</th>
<th>INCUBATION TIME (min)</th>
<th>TISSUE K (mM/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Ringer</td>
<td>120</td>
<td>67.6</td>
</tr>
<tr>
<td>K-Free Ringer</td>
<td>20</td>
<td>60.9</td>
</tr>
<tr>
<td>K-Free Ringer</td>
<td>60</td>
<td>49.4</td>
</tr>
<tr>
<td>K-Free Ringer</td>
<td>120</td>
<td>38.5</td>
</tr>
<tr>
<td>Na-Free Ringer</td>
<td>120</td>
<td>53.6</td>
</tr>
</tbody>
</table>
net urate transfer from bath to lumen increased significantly within 40 minutes and was restored to control levels in 60 minutes (Figure 19).

The influence of a high concentration of potassium in the bathing medium is shown in Figure 20. In the presence of a 40 mM medium potassium concentration, urate transfer from bath to lumen varied with flow rate in a linear fashion. However, the mean transfer of urate was significantly \( p < 0.01 \) lower at all flow rates than that for control tubules bathed in Ringer containing 3 mM potassium.

**Effect of Changes in Medium Sodium Concentration on Net Urate Secretion**

The effect of removing sodium from the bathing medium was evaluated in the presence of control concentrations of potassium. An equivalent amount of choline was used to substitute for the sodium removed. After two hours in a sodium-free Ringer bath, the potassium content of the tubules was decreased but not to the extent it had been in the potassium-free medium (Table 5). As shown in Figure 21, the removal of sodium from the bathing medium failed to alter the net transfer of urate by perfused tubules \( p > 0.6 \). Under these conditions there was no significant effect on net urate transfer over periods as long as four hours (Figure 22). Some studies (Vogel, with associates, 1965, 1966) have indicated that the reabsorption of sodium from lumen to peritubular blood is important in regulating PAH secretion by the isolated experimentally perfused kidney of the frog, *Rana ridibunda*. However, when I removed sodium from the perfusion fluid (with 150 mM sodium in the bath) there was no significant change in urate secretion by perfused snake kidney tubules (Figure 23).
Fig. 19. Effect of potassium on net urate transport when flow rate was maintained constant (3 nl min⁻¹).

At end of 140 minutes, the bathing medium was changed from potassium-free Ringer to control Ringer (3 mM potassium). Each point represents mean value for four tubules. Vertical lines indicate SE.
Fig. 20. Relationship between flow rate and net urate transport in snake proximal tubules perfused in control bathing medium (3 mM potassium, 150 mM sodium) and high potassium (40 mM potassium) medium.

Closed circles and solid lines represent 3 mM potassium, 150 mM sodium. Open circles and dashed line represent 40 mM potassium medium. Linear regression lines were fitted by method of least squares.
Fig. 21. Relationship between flow rate and net urate transport in snake proximal tubules perfused in control bathing medium (3 mM potassium, 150 mM sodium) and in a bathing medium in which the sodium has been entirely replaced by choline.

Closed circles and solid line represent 3 mM potassium, 150 mM sodium. Open circles and dashed line represent choline. Linear regression lines were fitted by method of least squares.
Fig. 22. Effect of time on net urate transport at a constant flow rate (4 nl min⁻¹) in the absence of sodium from the bathing medium.

Each open circle represents a value from a separate tubule bathed in sodium-free medium. The solid line represents the mean net urate transport at 4 nl min⁻¹ for all tubules bathed in control (150 mM sodium) medium. Each dashed line represents one standard deviation from the control line.
Fig. 23. Relationship between flow rate and net urate transport in snake proximal tubules when sodium was removed from the perfusion fluid. All tubules were bathed in control medium (3 mM potassium, 150 mM sodium). Control tubules (closed circles and solid line) had 150 mM sodium in perfusion fluid. Experimental tubules (open circles and dashed line) had all sodium in perfusion fluid replaced by choline. Linear regression lines were fitted by method of least squares.
Effect of Ouabain and Ethacrynic Acid on the Net Secretion of Urate

Several authors (Giebisch, Boulpaep, and Whittembury, 1971) consider that there are two parallel sodium transport systems located on the peritubular border of the renal tubule cells. One of these is the classical sodium-potassium exchange pump which is sensitive to ouabain. This is considered to be responsible for the maintenance of selective cellular cation concentrations. The second pump is considered to be a sodium chloride pump which is sensitive to ethacrynic acid. Both of these may be involved in the transepithelial transport of sodium. The removal of potassium from the bathing medium would have inhibited the classical exchange pump, presumably without inhibiting the other pump. However, a few experiments on the effects of ouabain on urate transport were performed to determine if another inhibitor of the sodium-potassium exchange pump would reduce urate transport. Although removal of sodium from the bathing medium did not affect urate transport, a few experiments with ethacrynic acid were performed to determine if a presumed inhibitor of a second sodium pump would affect urate transport.

Ouabain, in concentrations of $10^{-3}$ M in the bathing medium containing 3 mM potassium, had no effect on net urate secretion (Figure 24). This is consistent with experiments on snake kidney slices in which ouabain ($10^{-3}$ M) had no statistically significant effect on the uptake of urate (Dantzler, 1969). Similarly, ethacrynic acid in concentrations of $10^{-3}$ M in the bath containing 3 mM potassium, failed to influence the net transfer of urate (Figure 25).
Fig. 24. Relationship between flow rate and net urate transport in snake proximal tubules perfused in control bathing medium (3 mM potassium, 150 mM sodium) and control bathing medium with ouabain (10^{-3} M) added.

Closed circles and solid lines represent 3 mM potassium, 150 mM sodium. Open circles and dashed line represent control bathing medium with ouabain (10^{-3} M) added. Linear regression lines were fitted by method of least squares.
Fig. 25. Relationship between flow rate and net urate transport in snake proximal tubules perfused in a control bathing medium (3 mM potassium, 150 mM sodium) and control bathing medium with ethacrynic acid ($10^{-3}$ M) added.

Closed circles and solid line represent 3 mM potassium, 150 mM sodium. Open circles and dashed line represent control bathing medium with ethacrynic acid ($10^{-3}$ M) added. Linear regressions were fitted by method of least squares.
DISCUSSION

In the present experiments it has been possible to perform physiological studies on isolated fragments of single reptilian nephrons. Since virtually all of the individual segments can be dissected from the kidney, more of the nephron is available for study than with conventional in vivo micropuncture techniques which are limited to those portions of the tubule which appear at the kidney surface. That the isolated tubules remain viable is supported by the fact that potassium concentration gradients are maintained by the cells and by the demonstration of urate secretion against a concentration gradient in proximal convoluted tubules for at least four hours. This preparation is preferable to the usual techniques for studying organic acid transport (kidney slices or tubule suspensions) since it permits measurement of net transtubular transport and allows comparison between different segments of the nephron. It also removes the uncertainty as to the locus of the observed transport, i.e., whether the active step in the transport process occurs at the luminal or peritubular border of the cells.

With control Ringer (3 mM potassium, 150 mM sodium) in the bathing medium and perfusion fluid, transport of urate from bath to tubule lumen occurred against a concentration gradient and the concentration of urate in the tissue was consistently greater than that in the bath and tubular fluid. These findings are consistent with the model for urate secretion during control conditions shown in Figure 14.
(Dantzler, 1973). In this model, urate is actively transported into the cells across the peritubular membrane and then diffuses down a concentration gradient into the tubule lumen. To this point, this model for urate secretion by snake proximal tubules is the same as that proposed by Tune, Burg, and Patlak (1969) for PAH secretion by rabbit proximal tubules.

It may be assumed in these studies, that the urate recovered from the tissue is unbound and in free solution in the cell water. This assumption is supported by measurements of tissue urate concentration made during studies of urate efflux from tubule lumen to bath (Dantzler, 1973). In these studies the urate in the tissue was only 20% of that in the perfusion fluid. Since more than 50 times the amount of urate in the tissue crossed the tubular epithelium during these studies, a tissue concentration greater than that in the perfusion fluid should have existed if significant tissue binding occurred. Thus it appears likely that the measured tissue urate concentration was representative of the free intracellular transport pool.

Net urate secretion varied with flow rate through the tubule. This is similar to previous data obtained with snake proximal tubules (Dantzler, 1973). However, this pattern was not observed for PAH transport in rabbit proximal tubules (Tune, Burg, and Patlak, 1969). Such a relationship between flow rate and net urate secretion would be expected if the transepithelial permeability for urate were sufficiently great that a fraction of that reaching the lumen would diffuse back out again through the cells. Previous data (Dantzler, 1973) have shown that the
concentration attained in the lumen varies inversely with flow rate. However, at the lowest flow rate at which the cellular urate concentration was measured (about 0.3 nl/min) the concentration in the lumen did not reach that in the cells (Dantzler, 1973). The amount of urate lost from the tubule by back diffusion would depend on the concentration in the tubule lumen and, thus, on flow through the tubule. At high flow rates, when the urate concentration in the lumen was low, this back-diffusion would be relatively small and the net secretion of urate correspondingly high. The opposite would be true at low perfusion rates when the luminal concentration of urate was high. Therefore when the flow of tubule fluid was reduced there might be a decrease in net urate transport without any change in the active-transport rate across the peritubular membrane. In the present experiments the rate of net transport decreased approximately 62% when flow rate was decreased 75%. This could be explained by variable backflux. In addition, Dantzler (1973) observed significant efflux from lumen to bath in snake proximal tubules. This efflux varied inversely with flow rate as expected. Moreover, Dantzler (1973) proposed, on the basis of the similarity between the transepithelial and luminal membrane permeabilities, that a significant fraction of the backflux could occur between cells. This is shown by an appropriate arrow and question mark in the control model (Figure 14). In respect to the significant backflux and the possible intercellular path for such backflux, the control model (Dantzler, 1973) differs from that described by Tune, Burg, and Patlak (1969) for PAH secretion by rabbit proximal tubules.
In the present study, the secretion of urate by isolated, perfused snake proximal renal tubules showed a requirement for potassium. The removal of potassium from the bathing medium was followed by a marked depression of net urate secretion at all flow rates. During maximum depression of urate transport, the cell water urate concentration was lower than that in the bath but greater than that in the tubule lumen. These findings are consistent with the model for urate transport in the absence of potassium shown in Figure 14. In this model, the removal of potassium has completely eliminated the active uptake of urate into the cells and the movement from bath to lumen is entirely by a process of passive diffusion. The inhibition of cellular accumulation of urate in this model would also agree with previous data on the effects of potassium on urate uptake by rabbit, chicken, and snake kidney slices (Berndt and Beechwood, 1965; Dantzler, 1969). The steady-state slice-to-medium (S/M) ratios of 1.0 or slightly above in the absence of potassium in these earlier slice studies probably reflected the inability of the urate that had diffused into the cells to move into the collapsed tubule lumina.

In the present study, although net transepithelial transport of urate was depressed at all flow rates following the removal of potassium from the bathing medium, it increased slightly with increasing flow rates ($2 \times 10^{-15}$ m mm$^{-1}$ for each 1 nl min$^{-1}$ increase in flow rate). This observation is also compatible with the model of completely passive diffusion from bath to lumen. With more rapid flow through the tubule, the urate concentration in the lumen would be reduced (from $1.1 \times 10^{-5}$...
moles $l^{-1}$ at flow rate of $1 \text{ nl} \ min^{-1}$ to $0.3 \times 10^{-5}$ moles $l^{-1}$ at $9 \text{ nl} \ min^{-1}$) and a steeper gradient for diffusion from bath to lumen would be maintained.

The calculated transepithelial permeability coefficient for the tubules bathed in potassium-free Ringer did not differ significantly from that determined previously (Dantzler, 1973) for proximal tubules perfused in normal Ringer. In the present study, this was a unidirectional permeability calculated from the urate movement from bath to tubule lumen on the assumption that all such movement was passive in the absence of potassium. In the former study (Dantzler, 1973), the transepithelial permeability value was calculated from the passive efflux of urate from tubule lumen to bath. In making this calculation (Dantzler, 1973), it was assumed that the urate efflux was independent of fluid reabsorption from the tubule lumen. The similarity between the values from both studies supports this assumption. This similarity also indicates that the removal of potassium from the bath does not significantly alter the transepithelial permeability and further supports the model (Figure 14) in which the primary effect of the removal of potassium is the elimination of active transport of urate into the cells across the peritubular border.

The permeability of the luminal membrane calculated in the absence of potassium was slightly greater than that calculated previously (Dantzler, 1973) in the presence of potassium, although the difference was not statistically significant. This suggests that the removal of potassium probably has no effect on the luminal membrane
permeability. However, this calculation is only valid in the present study if all the urate moving passively from bath to lumen moves through the cells. Since, as noted above, the previous study (Dantzler, 1973) suggested that some urate could move between cells, it seems possible that in the present study this also could occur. This is further suggested by the fact that the calculated luminal membrane permeability is quite variable and tends to be higher in the absence of potassium than in the presence of potassium. This passive intercellular movement from bath to lumen is indicated by an appropriate arrow and question mark in the model for urate movement in the absence of potassium (Figure 14).

If some urate did move passively between the cells in the present study, then the calculated luminal membrane permeability is not a true permeability, and it can not be said with certainty that it does not change in the absence of potassium.

Bulger and Trump (1969) found that the morphology of the basal or peritubular side of flounder renal tubule cells was drastically altered following incubation in a potassium-free medium for as short a period as 30 minutes. The tubule cells also lost their ability to concentrate phenol red from the bath. It was suggested that the morphological changes were responsible for the loss of ability to transport the phenol red. The severity of the morphological changes suggested that the integrity of the peritubular membrane structure would not readily be restored when potassium was restored to the bath, but Bulger and Trump (1969) did not attempt to determine if these changes were reversible.
When potassium was restored to the bath of snake tubules that had been perfused in a potassium-free medium for 140 minutes, net urate transport from bath to lumen increased significantly and was restored to control levels in 60 minutes. The fact that transport was restored to control levels after prolonged depression indicates that the transport mechanism in these tubules is not irreversibly damaged by the potassium-free Ringer. It also suggests that the severe morphological changes observed by Bulger and Trump (1969) in the peritubular membrane of flounder tubules may not have occurred in these snake renal tubules.

Burg and Orloff (1962), in a study on PAH uptake by rabbit kidney slices, found a correlation between the tissue potassium concentration and the tissue PAH levels attained. In the present study, a similar correlation between net urate transfer from bath to lumen and tissue potassium concentration was seen. Removal of potassium from the bathing medium led to a significant depression of net urate secretion in 20 minutes. The tissue concentration of potassium fell more than 10% during that time. At the end of 60 minutes the net urate transfer was maximally depressed and the tissue potassium was 73% of that in tissue incubated in control Ringer. The tissue potassium continued to fall and at the end of two hours was only 57% of control at which time the urate transport was depressed by about 75%. It must be noted however, that these data do not allow me to determine if the inhibition of urate transport resulted solely from a decreased concentration of potassium within the cell, a decreased rate of potassium transport into the cell, or a combination of these.
Although tissue sodium concentrations were not determined in the present study, they would be expected to increase with the removal of potassium from the bathing medium. This has been shown previously for rabbit, chicken, and snake kidney slices (Berndt and Beechwood, 1965; Dantzler, 1969) and for separated, non-perfused rabbit proximal renal tubules (Burg and Orloff, 1966). Since the tissue concentration of both of these cations is altered when potassium is removed from the bath, it is not possible to associate with absolute certainty changes in urate transfer with changes in the tissue concentration of any particular cation. It appears, however, that the decrease in secretion of urate correlates better with the fall in tissue potassium than with the rise in tissue sodium, since the medium sodium concentration appeared to be unimportant in the urate transport process.

Changes in cellular potassium concentrations would alter the intracellular electronegativity. These electrical potential differences across the tubule epithelium or the individual cell membranes were not considered in this study. But the greatly depressed cellular potassium resulting from removal of potassium from the bath would decrease the intracellular electronegativity. This should enhance urate influx into the cells from the bath since over 98% of the uric acid (pKa = 5.7) is in the anionic form at pH 7.4. In these studies the urate influx into the cells was actually depressed. However, no electrical potential measurements have been made in these studies, and the intracellular pH is not known precisely enough to determine the degree of urate dissociation in the cells.
Sodium-potassium-adenosine-triphosphatase (Na-K-ATPase) has been implicated in electrolyte transport in many tissues (Skou, 1965). If this enzyme is important in regulating the energy supply for urate transport in snake kidneys, it is possible that potassium may, in part, exert its effect on urate secretion directly through its effect on Na-K-ATPase. A feedback stimulation of tissue respiration by potassium transport via Na-K-ATPase has been described for the mammalian kidney (Whittam and Willis, 1963). This enhanced tissue respiration could have a role in regulating urate transport. This enzyme, which is located on the peritubular membrane, can be isolated in highest specific activity in the heavy microsomal fraction of snake kidney tissue (Dantzler, 1972).

Dantzler (1972) found that incubation of snake kidney slices in a potassium-free medium had no residual effect on the heavy microsomal Na-K-ATPase activity. However, there are several criticisms of this type of study. First, the potassium content of the slices was measured in whole tissue, but the Na-K-ATPase activity was studied in the heavy microsomal fraction. Also, the assay only indicates the activity of the enzyme during the assay and not during the period of prior incubation when the potassium depletion was occurring.

The effects of ouabain on urate secretion by isolated perfused tubules did not clarify the relationship of potassium to urate transport. The sodium-potassium exchange pump is generally ouabain sensitive. In the studies with isolated tubules, however, ouabain (10^{-3} M) failed to influence urate secretion, supporting the suggestion that urate transport is not associated with the sodium-potassium exchange pump.
Yet these studies do not rule out the possibility of such a relationship. The failure of ouabain to alter urate transport could be due to the insensitivity of Na-K-ATPase in snakes to ouabain. Dantzler (1969; 1972) found that both the tissue potassium concentration and the heavy microsomal Na-K-ATPase activity in snake kidneys were insensitive to ouabain in concentrations as high as $10^{-3}$ M. Similar results have been reported for the Na-K-ATPase in rat tissues, in which there is a lack of formation of a ouabain-ATPase complex (Allen, with associates, 1969; 1971).

Numerous enzyme processes show a strong dependency upon the potassium concentration of the medium (Ussing, 1960). The potassium concentration needs to be optimal for certain enzymatic reactions since it exerts an inhibitory effect when either too low or too high. In addition, there appear to be more potassium dependent enzyme systems than sodium dependent enzyme systems (Ussing, 1960). It is of interest that in the present study net urate transport from bath to tubule lumen followed this same pattern and was depressed in potassium-free media and slightly depressed in media with an elevated (40 mM) potassium concentration. A similar relationship between the medium potassium concentration and the steady-state PAH slice-to-medium ratio has been reported for rabbit kidney slices (Burg and Orloff, 1962). Moreover, in the present study, variations in the medium sodium concentration (0 and 150 mM) had no effect on net transepithelial urate transport.

When the potassium was elevated in the medium to 40 mM, the sodium was reduced from 150 mM to 113 mM to maintain a constant osmolality. It appears unlikely that a change in medium sodium content
could have influenced significantly the results observed with an increasing medium potassium concentration. This conclusion is strengthened by the observation that removal of all sodium from the bathing medium had no effect on net urate transfer from bath to lumen over periods as long as 4 hours.

Although sodium has been clearly implicated in the transport of organic molecules across cell membranes (Schultz and Curran, 1970) it appears to be less important in influencing organic acid transport in the kidney than in other tissues. As already noted, sodium in the bathing medium does not appear to be necessary for urate transport by isolated, perfused snake renal tubules. However, sodium reabsorption is apparently coupled to glucose and amino acid reabsorption in the gut (Schultz and Curran, 1970), and some studies (Vogel et al., 1965; 1966) on frogs (Rana ridibunda) have suggested that the reabsorption of filtered sodium by the proximal renal tubule is also responsible for regulating renal tubular organic acid secretion. In the present study, when all of the sodium was removed from the perfusion fluid, the net urate transport from bath to lumen still was unchanged. Since the removal of sodium from the perfusion would have essentially eliminated sodium reabsorption, there is no evidence for the coupling of sodium reabsorption to urate secretion in the present study. Thus, it is also not surprising that ethacrynic acid, which apparently blocks renal tubular sodium reabsorption (Giebisch, Boulpaep, and Whittembury, 1971), failed to influence net urate secretion even in a concentration as high as $10^{-3}$ M.
In summary, it appears that removal of potassium from the bathing medium eliminates the active transport of urate into the cells across the peritubular membrane and that under these circumstances cell urate enters the lumen by a passive process (Figure 14). It does not appear likely that the permeability of the tubule epithelium is altered under these conditions. The active transport of urate into the cells and subsequent secretion into the lumen can be restored by restoring potassium to the bath.


