Active Ion Transport in the Renal Proximal Tubule

I. Transport and Metabolic Studies

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ABSTRACT   Various aspects of the interrelationship between ion transport and cellular metabolism were investigated using a suspension of rabbit cortical tubules that were mainly proximal in nature. Using the intact tubules, the compartmentation of K within the renal cell was studied by performing ^{42}K uptake studies. The oxygen consumption (Q_{O_2}) of the tubules was measured under similar conditions, as well as when the Na pump was stimulated by increasing Na^+ entry with nystatin. In addition, the state 3 rate of respiration was measured when the mitochondria of digitonin-permeabilized tubules were stimulated by ADP. At 37 and 25°C, a single-compartmental uptake of ^{42}K was observed, which suggests that extracellular K^+ communicates with a single compartment within the renal cell. Between 37 and 15°C, the ouabain-sensitive Q_{O_2} and the initial ^{42}K uptake rate were parallel in an Arrhenius-type plot, which indicated that active ion transport and oxidative phosphorylation remain tightly coupled within this temperature range. At all temperatures between 37 and 15°C, nystatin stimulated the Q_{O_2}, which demonstrates that the entry of Na^+ into the renal cells was rate limiting for active Na^+ transport throughout this temperature range. Between 37 and 20°C, the nystatin-stimulated Q_{O_2} was nearly equal to the state 3 rate of respiration, which suggests that active ion transport may be limited by ATP availability under these conditions. At 15°C, nystatin addition stimulated the Q_{O_2} well below the state 3 respiratory rate.

INTRODUCTION

The basic model for transepithelial Na transport across the proximal tubule involves the movement of Na across the apical membrane down an electrochemical gradient and the subsequent active transport of this ion across the basolateral membrane by the Na,K-ATPase in exchange for K (Giebisch, 1961; Whittembury et al., 1961). Previous studies have demonstrated that under physiological

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conditions, the rate-limiting step for this process is the entry of Na across the luminal membrane (Stroup et al., 1974; Spring and Giebisch, 1977). Thus, under physiological conditions, the Na pump is unsaturated with respect to Na. The movement across the luminal membrane is complicated by the variety of ways in which Na may enter the cell, including cotransport with organic solutes (e.g., sugars, amino acids) or with anions (e.g., lactate, phosphate), exchange for cellular H^+, coupled NaCl entry, and other possibilities. Studies with proximal tubule brush border membranes have enabled these pathways to be identified and characterized (for a review, see Kinne and Schwartz, 1978).

Previous studies of isotopic Na and K fluxes in tissue slices and in suspensions of separated renal tubules have demonstrated multicompartmental uptakes of these ions (Kleinzeller et al., 1962; Mudge, 1951, 1953; Burg et al., 1964; Burg and Orloff, 1966). However, these studies may have been affected by diffusional limitations of ions, substrates, and oxygen because of the thickness and heterogeneity of the tissue slices and the closed lumens of the separated tubules. This is the first of three papers in which a suspension of separated rabbit proximal tubules with open lumens was used to investigate various aspects of renal transport and metabolism. In this paper, the compartmentation of cellular K was investigated by monitoring the uptake of ^{42}K. In addition, the interrelationship of the mitochondria, the Na,K-ATPase, and luminal Na entry were specifically investigated as a function of temperature. The tight coupling between Na pump (Na,K-ATPase) activity and mitochondrial oxidative phosphorylation enabled the rate of oxygen consumption (Q_{O_2}) of the tubule suspension to be used as a quantitative measurement of Na pump activity. For comparison, the Na,K-ATPase hydrolytic activity was measured using lysed proximal tubule membranes.

In the following articles (Soltoff and Mandel, 1984a, b), we further examine the function and activity of the Na,K-ATPase in intact rabbit proximal tubule suspensions. In the second paper (Soltoff and Mandel, 1984a), the response of active transport to alterations of the extracellular and intracellular Na and K is examined. In the third paper (Soltoff and Mandel, 1984b), the quantitative dependence of Na pump activity on intracellular ATP is investigated by altering the ATP content in a graded fashion by using rotenone, an inhibitor of oxidative phosphorylation. Taken together, these papers present a detailed investigation of the characteristics of Na pump activity in the intact proximal tubule and allow for a more complete understanding of its control by ionic and metabolic means.

MATERIALS AND METHODS

Renal Tubule Suspension

The protocol and solutions used to obtain the tubule preparation have been modified from the original report by Balaban et al. (1980). Here we describe the modified procedure. Female New Zealand White rabbits (Bunny Haven, Durham, NC), 3–4 kg, were injected with 2,000 U heparin (The Upjohn Co., Kalamazoo, MI) and anesthetized with ether 15–20 min later. A ventral incision was made, the descending aorta was clamped rostral to the kidney, and a cannula was inserted in the aorta near the junction

of the renal arteries. The kidneys were perfused in situ at $37\,°C$ at a rate of 45–50 ml/ min with hypertonic solution A (Table I) equilibrated with 95% O_2/5% CO_2. The renal veins were cut after the initiation of perfusion. After 5–10 min, the perfusion was continued at a rate of ~30 ml/min for an additional 10 min with an identical solution containing ~0.8 mg/ml collagenase. The kidneys were subsequently reperfused with the original medium for 5–10 min to wash out the collagenase. The kidneys were rapidly excised and placed in ice-cold isotonic solution B, the renal capsules were removed, and the cortex was dissected and minced with fine scissors. The cortical tissue was then placed in a beaker containing solution B and was magnetically stirred at $4\,°C$ for 30 min to disperse the tubules. This suspension was filtered through three layers of gauze to remove unseparated tubules and washed twice by centrifugation ($\approx$50 g for 2 min) and resuspension of the tubule pellet. To remove nonvital single cells and cellular debris (see Balaban et al., 1980), 20-ml aliquots of the suspension were centrifuged ($\approx$50 g) for 20 min on a 5-ml cushion of solution C, which contained 25% (wt/vol) Ficoll (400,000 mol wt). The layer of tubules on top of the Ficoll cushion was removed by suction and resuspended three additional times by centrifugation and replacement of the supernatant with cold (0– $4\,°C$) fresh solution B. The final pellet was resuspended at the desired tubule concentration, generally 3–6 mg protein/ml.

The resulting tubule preparation was mainly proximal in origin. In a morphological study of five different preparations of tubules, in which several microscopic fields were examined from each preparation, 85.7 ± 1.3% of the tubules were proximal tubules. The remaining tubules consisted mostly of distal and collecting tubules. Visual inspection showed that 87.3 ± 3.1% of the proximal lumens were open and therefore appeared to be capable of transepithelial transport. In experiments in which the extracellular medium was varied by alterations in Na and/or K (Soltoff and Mandel, 1984a, b), the appropriate

TABLE I

Experimental Solutions

Solution	A	B	C	D
mosmol	*mM*	*mM*	*mM*	*mM*
Osmolarity	325	295	295	295
NaCl	115	115	136	—
NaHCO$_3$	25	25	—	—
NaH$_2$PO$_4$	2	2	2	—
CaCl$_2$	1	1	1	—
KCl	5	5	5	120
KH$_2$PO$_4$	—	—	—	5
MgSO$_4$	1	1	1	—
Glucose	5	5	5	—
Lactate	4	4	4	—
Alanine	1	1	1	—
Glutamate	—	—	—	5
Malate	—	—	—	5
Butyrate	—	1*	—	1
HEPES	—	—	4	10
Dextran	0.6%	0.6%	0.6%	—
Ficoll	—	—	25%	—
EGTA	—	—	—	2
Mannitol	25	—	—	—
pH	7.4	7.4	7.4	7.4

* Butyrate was not present during preparation of the tubule suspension.

solution was substituted beginning with the tubule resuspensions after the centrifugation on Ficoll. Unless indicated otherwise, the tubules were incubated at 37°C for 15–20 min and gassed with a 95% O_2/5% CO_2 mixture before all measurements. The suspension was then transferred to thermostatted chambers that were magnetically stirred, and the respiratory rates were measured using a Clark-type oxygen electrode.

Digitonin-treated Tubule Suspension

The state 3 respiratory response, defined by Chance and Williams (1956) using mitochondria exposed to exogenous ADP, was measured in digitonin-treated tubules as described by Harris et al. (1981). Digitonin permeabilizes the plasma membrane and enables the mitochondria to be exposed to exogenously added ADP. In brief, the tubules were obtained as mentioned above and were maintained in the normal suspension medium (solution B) at 0–4°C. Immediately before each measurement of oxygen consumption, a portion of the tubule suspension was centrifuged at 50 g for 2 min, and the supernatant was drawn off and replaced with the mitochondrial assay medium (solution D, Table I). After resuspension in this medium, the tubules were again centrifuged, and the supernatant was replaced with fresh mitochondrial assay medium. The tubules were then resuspended, gassed with 100% O_2, and incubated for several minutes at 37°C before exposure to digitonin and the addition of 0.38 mM ADP, which was previously determined to give the maximum response (Harris et al., 1981). Digitonin was added in an amount (0.11– 0.19 mg/mg protein) sufficient to achieve a maximal stimulation of respiration by ADP. All measurements were made in the presence of 10^{-4} M ouabain, which was added immediately before digitonin.

^{42}K Uptake

Tubule suspensions (~10 ml) were incubated at the appropriate temperature in solution B for ~30 min. The suspension was mixed well with a magnetic stirrer and was gassed with a 95% O_2/5% CO_2 mixture at a rate that provided adequate oxygenation of the tubules. Sufficient ^{42}KCl was added at time zero to produce a final isotope activity of ~1 μCi/ml of suspension. $^{42}K_2 CO_3$ was obtained from North Carolina State University (Department of Nuclear Engineering, Raleigh, NC), and neutralized with HCl. Samples of the suspension were removed at various time intervals after isotope addition (time zero). Aliquots of 0.3–0.5 ml of suspension were layered onto 0.4 ml of phthalate in a 1.5-ml microcentrifuge tube, and each tube was centrifuged for at least 10 s using either a Brinkmann (Westbury, NY) 3200 or an Eppendorf (Westbury, NY) 5412 centrifuge. All tubes were kept on ice before adding the samples. The phthalate was a 2:1 mixture of dibutyl/dioctyl phthalate. The end of the uptake period for each sample was the instant at which the centrifuge was started, which was 2–4 s after the sample had been removed from the suspension. The results of this centrifugation produced a pellet of tissue below and a volume of extracellular medium above the phthalate layer. The upper medium was transferred to a separate tube, and care was taken to remove all remaining isotope activity above the phthalate by rinsing that portion of the tube several times with distilled water. The gamma activity of the extracellular medium and the pellet was counted in an Intertechnique (Dover, NJ) CG 30 gamma counter. The isotope activity of the pellet was at least 10 times larger than that of the background. The K concentration of the extracellular medium was later measured using atomic absorption spectrophotometry to determine the specific activity. In several experiments, the chemical Na and K contents in the tissue pellet were also measured (see *Tissue Electrolyte Content*).

For each sample, the K^+ uptake (nmol/mg protein) was calculated as

$$K^+ \text{ uptake} = \frac{A_T}{P_T \cdot SA_m},$$

where A_T is the radioactivity in the tissue pellet, P_T is the amount (mg) of pellet protein, and SA_m is the specific activity in the medium ($cpm \cdot nmol^{-1}$). The initial uptake rate (nmol/ mg protein·min) was calculated from the slope of the initial portion of the cation uptake as a function of time. This function was linear during the interval of time used to calculate the slope, and a regression line through these points had a correlation coefficient of 0.98– 0.99. For the 37°C experiments, this rate was calculated from 4 samples that were taken within a 0.8–1.1-min interval after the isotope addition; for the 25°C experiments, there were 4–7 samples within a 1.0–1.8-min interval; for the 15°C experiments, there were 8–11 samples within a 5.4–6.3-min interval. The uptake of ^{42}K was also measured in the presence of 10^{-4} M ouabain. Based on these results, the ouabain-sensitive uptake rates were calculated. It should be noted that the kinetics of the K uptake were minimally affected by the presence of extracellular K trapped in the uptake samples. If the extracellular space is ~50% of the total pellet volume (see Table II), and the extracellular and intracellular K concentrations are 5 and 125 mM, respectively, the trapped K will be only 4% (5/125) of the total. Since each sample pellet contained approximately the same amount of protein (and thus the same extracellular space), and the stirring mechanics of the tubule suspension promoted the rapid equilibration of ^{42}K, it was assumed that the extracellular contribution was similar in all of the samples. Since the uptake rate is effectively calculated as the incremental uptake of ^{42}K over time, a constant extracellular (trapped) component will contribute little, if any, to the calculated rate.

The rate constant k (min^{-1}) of the K uptake was calculated from the following equation:

$$k = \frac{\ln[1 - K_{uptake(t)}/K_{uptake}(\infty)]}{t}$$

where $K_{uptake(t)}$ is the measured uptake at time t (minutes), and $K_{uptake}(\infty)$ is the maximum value that was reached. Each uptake experiment was separately normalized to the maximum value, and the data were fit by computer using a nonlinear technique without derivatives (Brent, 1973).

Several assumptions were made in this analysis. The tubules were presumed to be at a steady state during the course of the ^{42}K exposure. Also, in order to determine the ouabain-sensitive component of ^{42}K uptake, it was assumed that measurements made in the presence of ouabain inhibited only the contribution of the Na,K-ATPase and that the remaining (e.g., passive) properties of the membrane were not affected.

Oxygen Consumption

The rate of respiration of the tubule suspension was measured using a Clark-type oxygen electrode and an oxymeter (model 53 Oxygen Monitor; Yellow Springs Instrument Co., Yellow Springs, OH) to monitor the disappearance of oxygen from closed, thermostatted 1.6-ml chambers. The oxygen tension was calibrated by measuring the amount of room air oxygen that was dissolved in 150 mM NaCl at the temperature at which the experiment was performed.

Tissue Electrolyte Content

To determine the tissue Na and K contents, an aliquot of the tubule suspension was layered on a 0.4-ml phthalate cushion in a 1.5-ml microcentrifuge tube and centrifuged

in an identical manner as was done for the radiolabeled K experiments. A portion of the volume above the phthalate was used to measure the extracellular ion concentration. The remainder of this layer was removed, and the tube was rinsed several times with distilled water to remove all traces of chemical Na and K. The phthalate layer was completely removed, and the tissue pellet was exposed to a volume (usually 1.3 ml) of extraction solution (6% perchloric acid, 1 mM EDTA, 4 mM CsCl) for at least 24 h. This solution and the extracellular medium were measured at appropriate dilutions against known standards using an atomic absorption spectrophotometer (model 460; Perkin-Elmer Corp., Norwalk, CT).

Because the lumens of the tubules were open, the total water volume of the pellet included ~50% trapped extracellular medium (see below). This produces only a small error in the measurement of the tissue K content, but a much larger overestimation of the tissue Na content is made because of the high Na concentration in the extracellular medium. Where noted in the results, the estimated extracellular contamination was subtracted from the total extracted ion content using an extracellular volume of 2 μl/mg protein (see Table II) to obtain a better approximation of the true tissue ion content.

Extracellular Space and Cell Volume

Experiments were performed using radiolabeled water and extracellular markers to determine the extra- and intracellular volumes of the tubule pellets that were obtained after rapid centrifugation of the tubule suspension through phthalate. The intracellular volume was calculated as the difference between the total water content and the extracellular space of the pellet.

Polyethylene glycol compounds of two molecular weights, 900 (PEG 900) and 4,000 (PEG 4,000), were used to determine the extracellular space. The final concentrations of radioactivity in the tubule suspension were as follows (in μCi/ml suspension): [^{3}H]H$_2$O, 1–5; [^{3}H]PEG 900, 0.2–1.1; [^{3}H]PEG 4,000, 2; and [^{14}C]PEG 4,000, 0.5. In several experiments, the tubules were simultaneously exposed to [^{3}H]PEG 900 and [^{14}C]PEG 4,000. Otherwise, the isotopes were added separately to suspensions in parallel. The tubules were exposed to the isotopes at 37°C for 15–30 min, after which time duplicate aliquots of ~0.5 ml were taken from the suspension, added to 1.5-ml centrifuge tubes that contained 0.4 ml of a phthalate mixture (2 dibutyl:1 dioctyl), and rapidly centrifuged. An aliquot of the extracellular medium above the phthalate was removed for radioactive analysis, the remaining volume was drawn off, and the tube was rinsed several times with distilled water to remove all traces of radioactivity. The phthalate was removed and ~1.3 ml of extraction solution (6% perchloric acid, 1 mM EDTA, 4 mM CsCl) was added. The pellets were extracted for at least 12 h at room temperature, and aliquots of the extraction solution were measured for radioactive analysis.

In several experiments, the pellets were solubilized after the extraction period in order to measure any residual radioactivity that may have remained in the pellet. Less than 4% additional radioactivity was recovered. Radioactivity was measured by scintillation counting in a Beckman (Fullerton, CA) LS-250 Liquid Scintillation System. Aqueous samples (1 ml) were added to 10 ml of scintillation fluid (Bray's solution).

Na,K-ATPase Activity

The Na,K-ATPase enzyme activity was measured in lysed membranes of the normal tubule suspension. Tubules were first obtained by the method described above. After resuspension in the normal medium (solution B), they were centrifuged at low speed (≈50 g) for 2 min. The supernatant was drawn off and replaced with 5–10 vol of distilled water, and the resulting suspension was stirred at room temperature for ~10 min. This suspension

was frozen in liquid nitrogen, thawed at room temperature, and stored at $-20\,^\circ$C in several aliquots for future analysis. The effects on renal tubules of hypotonic exposure and freeze thawing to maximize the Na,K-ATPase activity have been described elsewhere (Doucet et al., 1979). Unless otherwise noted, the total ATPase activity was measured by the rate of liberation of inorganic phosphate when a dilution of this preparation was incubated in a medium that contained 140 mM NaCl, 10 mM KCl, 5.5 mM $MgCl_2$, 0.125 mM EGTA, 20 mM Tris (pH 7.4), and 5 mM ATP. The Na,K-ATPase activity was the component of total ATPase activity that was sensitive to 10^{-3} M ouabain. Inorganic phosphate was extracted into an organic medium (Seals et al., 1978) and measured by a colorimetric procedure (Martin and Doty, 1949). Appropriate controls were made for nonenzymatic phosphate appearance. All samples were assayed in duplicate or triplicate.

ATP Content

Aliquots (0.5 ml) of the proximal tubule suspension were added to an equal volume of ice-cold 6% perchloric acid/1 mM EDTA and mixed using a vortex mixer. These samples were then rapidly centrifuged. The supernatants were removed and the pellets were frozen for later protein determination. The supernatants were neutralized with 1.5 M KOH/0.4 M imidazole/0.3 M KCl and were centrifuged to precipitate $KClO_4$. The supernatants were removed and frozen at $-20\,^\circ$C until analysis. The ATP content was measured by a fluorometric assay that measured NADPH produced by a hexokinase/glucose-6-phosphate dehydrogenase enzyme system (Lowry and Passonneau, 1972).

Protein Content

Tissue pellets were solubilized in 0.1 N NaOH/5% deoxycholate, and the protein contents were determined by using either the biuret method (Gornall et al., 1949) or the procedure used by Lowry et al. (1951). Both techniques gave equivalent values, but for small amounts of proteins, it was necessary to use the latter procedure. Bovine serum albumin was used as the protein standard.

Chemicals and Drugs

Collagenase (Type 4) was obtained from Sigma Chemical Co. (St. Louis, MO) or Worthington Biochemical Corp. (Freehold, NJ). $[^3H]H_2O$, $[^3H]PEG$ 900, $[^3H]PEG$ 4,000, and $[^{14}C]$-PEG 4,000 were purchased from New England Nuclear (Boston, MA). Digitonin, nystatin (mycostatin), HEPES, ATP, and ADP were obtained from Calbiochem-Behring Corp. (San Diego, CA). Dextran T-40 and Ficoll were purchased from Pharmacia Fine Chemicals (Piscataway, NJ). Other chemicals and their sources including the following: dioctyl phthalate (Aldrich Chemical Co., Inc., Milwaukee, WI); dibutyl (n-butyl) phthalate and dimethyl sulfoxide (Fisher Scientific Co., Fair Lawn, NJ); ATP, ouabain, and Tris (Sigma Chemical Co.). All other chemicals were reagent grade. Nystatin was dissolved in dimethyl sulfoxide.

Statistics

Unless otherwise noted, values are reported as means $\pm$ SE.

RESULTS

Intracellular Volume and Extracellular Space

The results of the determinations of the intra- and extracellular spaces are shown in Table II. All values refer to measurements of the spaces in the tissue pellets

TABLE II

Total Water Space, Extracellular (PEG) Space, and Intracellular Space of the Proximal Tubule Suspension

		Intracellular space*
	µl/mg protein	*µl/mg protein*
Total water space	4.39 ±0.15 (12)	
PEG 900 space	2.39 ±0.16 (6)	2.00
PEG 4,000 space	1.96 ±0.08 (5)	2.43

* Intracellular space = total water space − PEG space.

that were obtained by rapid centrifugation of the tubule suspension. The total water space, 4.39 ± 0.15 µl/mg protein, represents the entire volume of intracellular and extracellular space occupied in the tissue pellet. PEG 900 occupied a larger space (2.39 ± 0.16 µl/mg protein) than did PEG 4,000 (1.96 ± 0.08 µl/mg protein). Using these values, the calculated intracellular volumes were 2.00 and 2.43 µl/mg protein, respectively. Preliminary studies using autoradiographic techniques indicated that PEG 900 entered the intracellular compartment of the proximal tubule (LeFurgey, A., S. P. Soltoff, and L. J. Mandel, unpublished results). This agrees with results obtained in rat kidney slices by McIver and Macknight (1974). Thus, the intracellular space calculated using the PEG 4,000 space appears to be a better indication of the true intracellular volume. In experiments performed without isotopic markers, the dry weight/wet weight and protein/dry weight ratios were determined to be 0.249 ± 0.002 (n = 8) and 0.710 ± 0.033 (n = 6), respectively. The wet weight included the entire mass of the tissue pellet, including the trapped extracellular space.

^{42}K Uptake

The results of a ^{42}K uptake study performed at 37°C are shown in Fig. 1. Five separate uptakes were measured at this temperature from one preparation of tubules: four control studies, and one in the presence of 10^{-4} M ouabain. The control uptakes were reproducible, and saturation was observed within ~11 min after isotope addition. The isotopic uptake was severely diminished in the presence of 10^{-4} M ouabain. Based on results such as those shown in Fig. 1, the ouabain-sensitive component of the ^{42}K uptake was calculated. The samples obtained from each preparation were normalized to the maximum uptake level, and the accumulation of data from all the experiments was plotted in Fig. 2 as a function of time after isotope addition. Using a nonlinear technique without derivatives (Brent, 1973), the best fit was obtained by the equation $Y = 1 - 1.0517\ e^{-0.403t}$, and the line shown in the figure was drawn from this equation. When the data were fitted by the sum of exponentials in the form $1 - Ae^{-at} - Be^{-bt} - Ce^{-ct} - \ldots$, the values of the rate constants a, b, and c were distinguishable only by differences in the one-thousandths place. Thus, the simplest fit of the
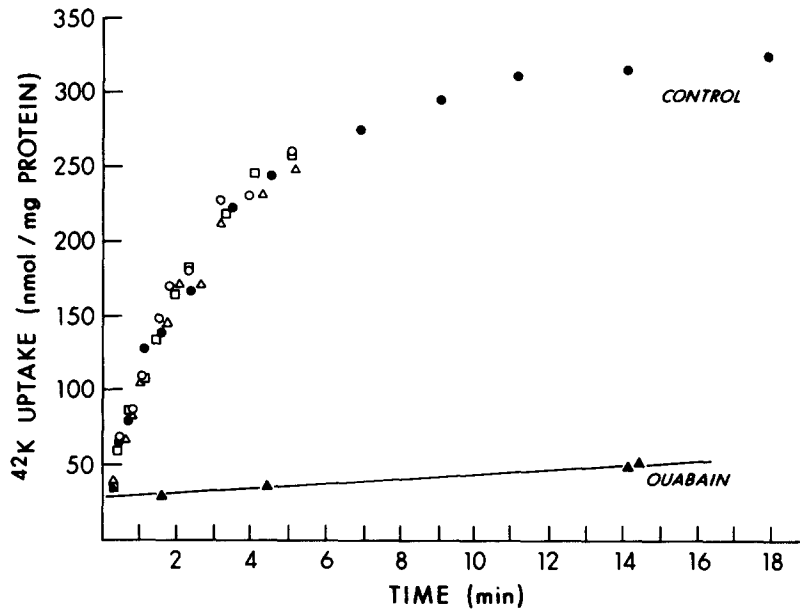
FIGURE 1. The uptake of ^{42}K by proximal tubules in suspension at 37°C in the presence and absence of 10^{-4} M ouabain plotted as a function of the time after the addition of isotope. The different symbols represent five separate uptake studies, one of which was conducted using tubules that were exposed to ouabain for 30 min prior to the addition of ^{42}K. Four uptakes were monitored in the absence of ouabain. All five studies shown here were conducted using tubules obtained from the same rabbit.

data was to a single exponential. This finding suggests that Fig. 2 depicts the Na,K-ATPase-mediated uptake of K into a single kinetic compartment. The computed rate constant ($k = 0.403$ min^{-1}) of this uptake corresponds to a $t_{1/2}$ value of 1.72 min. The mean values of these parameters that were measured in each individual uptake experiment were $k = 0.372 \pm 0.018$ (7) min^{-1} and $t_{1/2} = 1.89 \pm 0.09$ (7) min. The ouabain-sensitive component of the initial rate of ^{42}K uptake represents the steady state activity of the Na pump (Na,K-ATPase) of the proximal tubules in suspension. At 37°C, the ouabain-sensitive ^{42}K initial uptake rate was 88.9 ± 4.3 (9) nmol/mg protein·min.

Altering the incubation temperature had a marked effect upon the rate of ^{42}K uptake, as shown in Fig. 3 and Table III. At 37°C (Fig. 3A), the uptake is rapid and quickly reaches saturation. At 25°C (Fig. 3B), the total isotopic uptake saturated ~25 min after the isotope was added to the tubule suspension. The ouabain-sensitive ^{42}K uptake was best fitted by the equation $1 - 1.02e^{-0.174t}$, which describes a single compartment with a $t_{1/2}$ value of 3.98 min. At 15°C (Fig. 3C), the uptake had not yet saturated by 40 min, which was the longest time that the uptake was followed, and this precluded an accurate compartmental analysis at this temperature. The ouabain-sensitive initial ^{42}K uptake rates were 42.7 ± 0.8 (5) and 10.0 ± 2.2 (3) nmol/mg protein·min at 25 and 15°C,
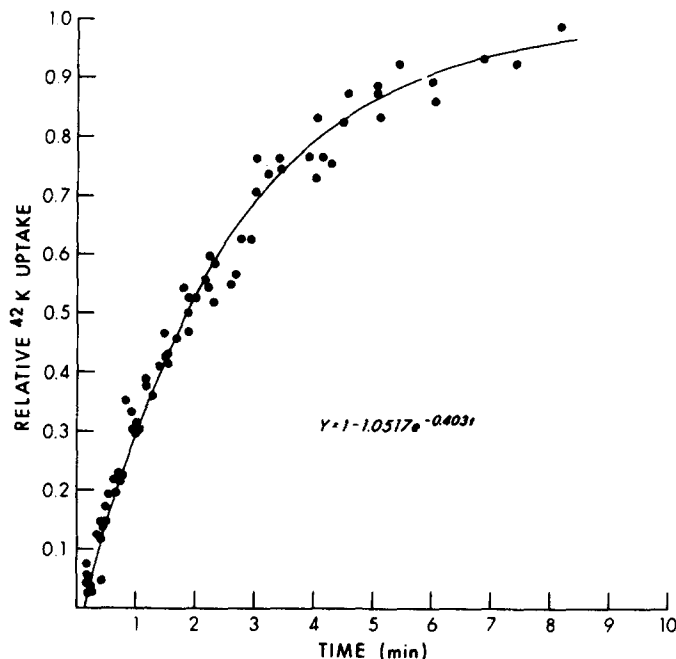
FIGURE 2. Composite of the ouabain-sensitive ^{42}K uptake by proximal tubules in suspension at 37°C. The values were normalized to the point at which the isotopic uptake reached saturation. The line represents a computer fit of the data to the equation shown having a single exponential. The data represent seven different uptake experiments, including the four control experiments shown in Fig. 1 and three additional experiments. The four uptakes from Fig. 1 were each normalized to the saturation value obtained in one of the experiments (closed circles in Fig. 1). In the other three experiments, each uptake was followed until saturation was achieved, and each uptake was normalized using the saturation value.

respectively. Representative ouabain-sensitive ^{42}K uptakes at these three temperatures are shown together in Fig. 4 so that they can be directly compared.

The level at which the isotopic uptake at 37°C became saturated, 303.8 ± 7.0 (4) nmol/mg protein, measured the amount of cellular K$^+$ that was rapidly exchangeable with the extracellular medium. However, when the tissue K contents were measured chemically in samples collected during the ^{42}K uptakes, the extracted content was ~40 nmol K$^+$/mg protein larger than the content calculated using the isotopic saturation value and the specific activity of the medium, as shown in Fig. 3. The ^{42}K uptake in the presence of ouabain at 37°C (Fig. 3A) was an exception in that the extracted K content was equivalent to the isotopic value. Although the isotope uptake showed single-compartmental kinetics, the difference between the extracted and the calculated contents may be due to a K pool that is inaccessible to or only slowly exchangeable with isotopic K (see Discussion).
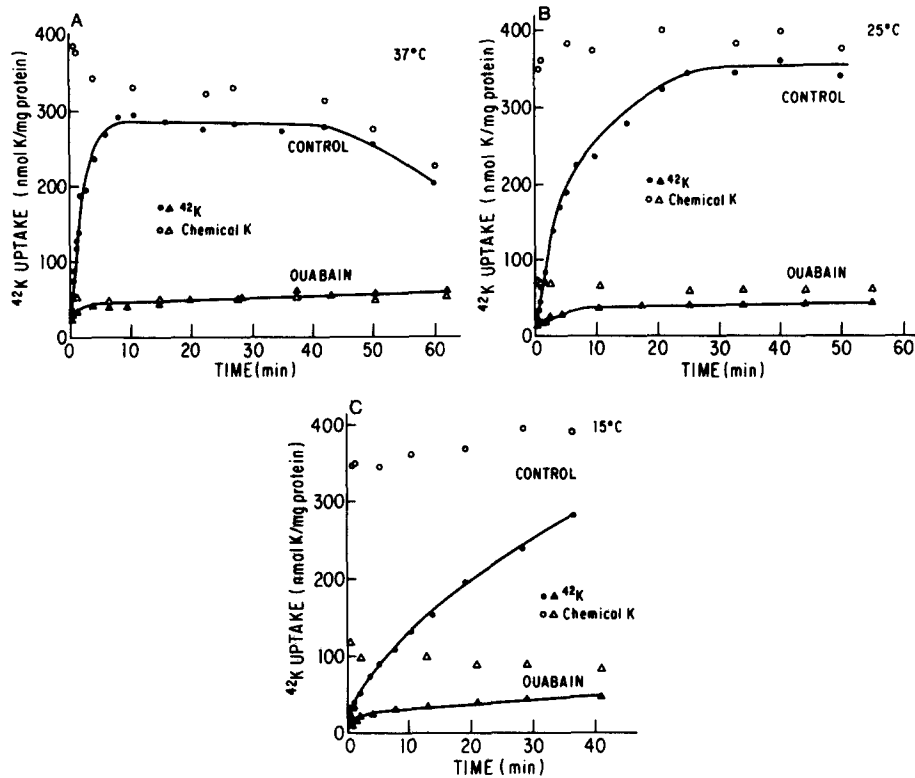
FIGURE 3.   Time course of the uptake of ^{42}K (closed figures) by proximal tubules in suspension at 37 (*A*), 25 (*B*), and 15°C (*C*) in the presence and absence of 10^{-4} M ouabain. Also shown (open figures) are the chemical K$^+$ contents that were measured by atomic absorption spectrophotometry in several samples after tissue extraction of the ions. Lines were drawn by eye.

Temperature Dependence of Active Transport and Metabolism

The temperature dependence of various transport and transport-related metabolic processes was examined, and the results were plotted in Arrhenius fashion as a function of reciprocal temperature to facilitate the examination of activation enthalpies.

The ouabain-sensitive steady state rate of oxygen consumption of the tubule suspension is a nonlinear function of reciprocal temperature that can be graphically portrayed as two straight lines that intersect at 25°C (Fig. 5). This relationship is parallel to that of the ouabain-sensitive initial uptake rates of ^{42}K, and thus both processes have similar activation enthalpies. The calculated enthalpies for the Q_{O_2} relationship are 14.1 and 24.1 kcal/mol for the 25–37°C and 15–25°C ranges, respectively. For this same range of temperatures, the calculated enthalpies for the initial rates of ^{42}K uptake were 11.2 and 24.8, respectively. The parallel nature of these different measurements is not surprising since the tight coupling between transport and oxidative metabolism has been well estab-

TABLE III

Effects of Temperature on Several Parameters of the Proximal Tubules

Temperature	Q_{O_2}			^{42}K uptake rate	Ion content		ATP content	Na,K-ATPase activity
	Ouabain-sensitive	Nystatin	ADP		K	Na		
°C	*nmol O_2/mg prot·min*			*nmol K/mg prot·min*	*nmol/mg prot*		*nmol/mg prot*	*nmol Pi/mg prot·min*
37	13.7	37.3	38.6	88.9	334.9	124.7	6.39	276.8 (2)
	±0.6 (7)	±1.2 (7)	±2.6 (3)	±4.3 (9)	±7.6 (4)	13.5 (4)	±0.44 (7)	
30	8.4	27.1	24.2				6.22	163.0 (2)
	±0.2 (8)	±0.8 (7)	±0.6 (3)				±0.15 (8)	
25	6.0	18.0	16.2	42.7	421.5	177.0	5.74	125.3 (2)
	±0.2 (9)	±0.7 (9)	±0.3 (3)	±0.8 (5)	±23.7 (3)	±23.4 (3)	±0.20	
21								67.4 (2)
20	3.4	10.4	12.2				6.41	
	±0.1 (9)	±0.6 (9)	±0.2 (3)				±0.27 (7)	
18								49.3 (1)
15	1.7	4.5	7.7	10.0	432.7	182.1	6.07	34.4 (1)
	±0.1 (7)	±0.3 (9)	±0.5 (3)	±2.2 (3)	±13.0 (4)	±19.5 (4)	±0.31 (8)	

The values represent the means ± SE with the number of observations in parentheses.

* Ion contents were measured after a 30-min incubation at the appropriate temperature. The values were corrected for contamination from the extracellular solution.

lished in this preparation at 37°C. The present results suggest that this tight coupling also persists at lower temperatures.

Since numerous investigators have found a linear relationship between the net transepithelial Na transport rate and the oxygen consumption rate, the Q_{O_2} data described above may be assumed to represent the activation enthalpies of the rate-determining step for the transepithelial Na transport. The identity of this step can be investigated with the aid of nystatin, a polyene antibiotic that increases the permeability of sterol-containing membranes to both Na and K (Cass and
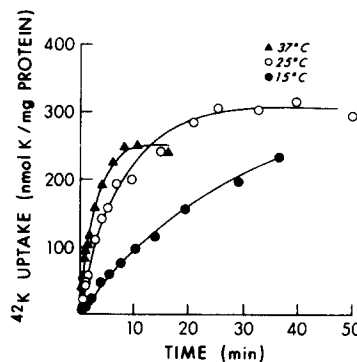

FIGURE 4.   Comparison of the ouabain-sensitive uptakes of ^{42}K by proximal tubules in suspension at 37, 25, and 15°C. For each temperature, the values were obtained by subtracting the ouabain-sensitive component of the uptake from the total ^{42}K uptake. Also shown are the ouabain-sensitive initial rates measured at each temperature.
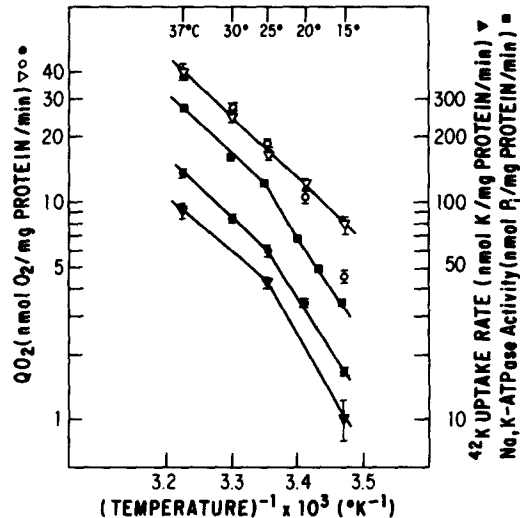
FIGURE 5. Arrhenius plot of several measurements of proximal tubules in suspension at temperatures between 15–37°C. Shown are the following: state 3 (∇), the rates of oxygen consumption of digitonin-treated tubules exposed to 0.38 mM ADP; nystatin (○), the nystatin-stimulated rates of oxygen consumption; Na,K-ATPase (■), the Na,K-ATPase hydrolytic activity of cortical tubule membranes; Q_{O_2} (●), the ouabain-sensitive rates of oxygen consumption; and ^{42}K (▼), the ouabain-sensitive initial rates of ^{42}K uptake. Lines were drawn by eye. The nystatin data are not connected by line. See Table III for numerical values and number of observations.

Dalmark, 1973). Added to a suspension of kidney tubules, it dissipates the normal Na^+ and K^+ gradients across the plasma membrane. This results in a stimulation of the Na pump activity by the increase in intracellular Na^+ concentration, which produces an increase in the oxygen consumption rate (Harris et al., 1981). As shown in Fig. 5, nystatin was found to stimulate the ouabain-sensitive rate of respiration at every temperature studied over the range of 15–37°C. Thus, in the absence of nystatin, at these temperatures the Na pump appears to be unsaturated with respect to intracellular Na. This suggests that the entry of Na into the kidney cell is normally the rate-limiting step of net Na transport. Based on these considerations, it may be concluded that the unstimulated ouabain-sensitive rates of oxygen consumption as a function of reciprocal temperature provide a measure for the activation enthalpies of the Na entry step in proximal tubules.

The nystatin-stimulated rate of respiration of tubules at 37°C (in the absence of ouabain) was previously found to be identical to the ADP-stimulated respiratory rate (state 3 rate) of digitonin-permeabilized cortical tubules (Harris et al., 1981). This finding demonstrated that nystatin promoted the maximal utilization of the respiratory capacity of the mitochondria. In the present study, shown in Fig. 5 and Table III, these rates were also equivalent at 37°C and were within ±15% between 20 and 30°C; only at 15°C was the nystatin-induced Q_{O_2} substantially (42%) lower than the state 3 rate. Since the Na entry step is no longer rate

limiting in the presence of nystatin, the observed dependence on temperature of the nystatin-stimulated Q_{O_2} (Fig. 5) could reflect the properties of either the mitochondrial respiratory chain or the Na,K-ATPase. To differentiate between these two possibilities, the temperature dependence of each of these processes was studied separately and, in addition, the total cellular ATP was measured at each temperature.

The mitochondrial state 3 respiratory rates were measured in proximal tubules after permeabilization with digitonin followed by ADP addition, as described by Harris et al. (1981). As depicted in Fig. 5, the state 3 rate displayed a single linear function of reciprocal temperature that rendered a calculated activation enthalpy of 12.9 kcal/mol. The temperature dependence of the Na,K-ATPase activity was measured using membranes obtained by hypotonic lysis plus freeze thawing of the normal tubule preparation (see Materials and Methods). Plotted as a function of reciprocal temperature (Fig. 5), it could be fitted to two linear components with a break around $25\,°C$, and each component was parallel to the ouabain-sensitive unstimulated Q_{O_2} and the initial ^{42}K uptake rates. The calculated activation enthalpies are 12.9 kcal/mol for the $37-25\,°C$ range and 21.9 kcal/mol for the $25-15\,°C$ range. The cellular ATP content was measured in the tubules to determine whether changes in ATP contributed to the observed temperature dependence of Q_{O_2}. As shown in Table III, the ATP contents of the kidney tubules were not significantly different at any of the temperatures studied.

Thus, the state 3 rate, the Na,K-ATPase activity, the ouabain-sensitive unstimulated Q_{O_2}, and the ouabain-sensitive initial rate of ^{42}K uptake all have similar enthalpies between 25 and $37\,°C$ and (except for the state 3 rate) also have similar enthalpies between 15 and $25\,°C$. The nystatin-stimulated Q_{O_2} is significantly different (lower) than the state 3 rate only at $15\,°C$. This suggests that between 20 and $37\,°C$ the ability of the mitochondria to produce ATP, which is measured by the state 3 response, is the factor that limits the increased Na pump activity in the presence of nystatin. A different limitation, perhaps the temperature dependence of the Na,K-ATPase activity, appears to restrict the response at $15\,°C$.

DISCUSSION

The role of the Na,K-ATPase in epithelial transport has been well documented, and the renal proximal tubule has been the focus of much attention. The general model that seems most appropriate relates the entry of Na across the luminal membrane to its transfer across the basolateral membrane by the Na,K-ATPase, with the former process being rate limiting for net transport. All of the data presented here support this general model and contribute further information on each of these transport steps and their relationship to cellular metabolism.

Using the value obtained in this study for the initial rate of ^{42}K uptake under steady state conditions at $37\,°C$, ~90 nmol/mg protein·min, the amount of Na^+ that is transported transepithelially by energy-dissipative pathways can be estimated. Assuming a 3 Na:2 K stoichiometry and isosmotic volume transport, a calculated value of 135 nmol Na^+/mg protein·min ($3/2 \times 90$) reflects a net

isosmotic volume transport of 900 nl H_2O/mg protein·min. This can be converted to conventional flux values for isolated perfused proximal tubules in terms of units of tubule length by using the conversion factor of Schmidt and Horster (1978), 33 μg dry wt/100 mm tubule length, along with values obtained in this study for the tubule suspension, 0.71 mg protein/mg dry weight. This gives a value of 0.21 nl/mm tubule length·min. In isolated perfused rabbit tubules, values for fluid reabsorption are ~1 nl/mm·min for proximal convoluted tubules (PCT) and 0.4 nl/mm·min for proximal straight tubules (PST). Since the tubule suspension is made up of both PCT and PST segments, this calculated value (0.21 nl/mm·min) is in good agreement with previous studies (Frömter et al., 1973; Kiil, 1977), which showed that about one-third of net Na^+ absorption was active and about two-thirds was due to passive movement or to solvent drag.

^{42}K Uptake

A unique finding of this study was the uptake of ^{42}K into a single compartment of the cortical tubules. Earlier studies of cortical slices (Kleinzeller et al., 1962; Mudge, 1951, 1953) and tubule suspensions (Burg et al., 1964; Burg and Orloff, 1966) found multicompartmental ^{42}K uptakes. Such studies were affected by diffusional limitations, nephron heterogeneity, and closed lumens, which caused the cells to be metabolically compromised and restricted full expression of transepithelial transport. Balaban et al. (1980) demonstrated that O_2 diffusional limitations were absent in the tubules used in the present studies, but were present in cortical slices. The single compartment found in this study probably reflects the relative homogeneity of the preparation, and the rate constant reflects the lack of diffusional and metabolic limitations experienced in other investigations.

The mean saturation level obtained in the 37°C isotopic ^{42}K uptakes was ~305 nmol/mg protein, as compared with ~350 nmol/mg protein measured by chemical extraction. Thus, ≅85% of cellular K^+ appears to be rapidly exchangeable. The rest of the K^+ may be in an intracellular compartment to which ^{42}K is inaccessible or only slowly accessible, and thus is excluded by the isotopic studies. Such a K pool might exist in the mitochondria, which make up ~25% of the intracellular volume in these tubules, and which are known to have a substantial concentration of K. In a study of the distribution of ^{42}K in isolated cardiac cells, the mitochondria were found to have 23% of the total cellular chemical K, but ^{42}K had only limited access to the mitochondria (Altschuld et al., 1981). The data in the present studies are in agreement with the existence of a single compartment that is in communication with the extracellular medium and for which the rate of turnover is related to the rate of transport in the proximal tubule. Using a value of 2.4 μl/mg protein for intracellular volume (Table III), the rapidly exchangeable K compartment would render a cytosolic K^+ concentration of ~125 mM. This value is similar to those measured in the kidney using chemical (Burg and Orloff, 1966; Kubota et al., 1980) or electron microprobe measurements (Beck et al., 1980; Thurau et al., 1981), but larger than those obtained with ion-selective microelectrodes (for a review, see Giebisch et al., 1981).

Temperature

Q_{O_2} AND K UPTAKE   The tight coupling between the rate of ion transport and the rate of oxygen consumption at 37°C has been previously demonstrated using this proximal tubule suspension (Harris et al., 1981) as well as other kidney preparations (Thurau, 1961; Torelli et al., 1966; Whittam and Willis, 1963). Although the ouabain-sensitive initial rates of ^{42}K uptake presented here were measured only at three temperatures, the parallel nature of this measurement with the ouabain-sensitive rate of oxygen consumption as a function of reciprocal temperature (Fig. 5) suggests that net transport and metabolism remain tightly coupled between 15 and 37°C. The K/O_2 ratio appears to be constant over this temperature range. A linear plot of the mean values of the ouabain-sensitive initial K uptake rates as a function of the ouabain-sensitive rate of oxygen consumption for 15, 25, and 37°C has a slope of ~6.5 and a correlation coefficient of 0.99, and intersects the origin. Admittedly, measurements made at additional temperatures within this range would be welcome. In a previous study (Harris et al., 1981) using the same substrate regimen as in these studies, the K/O_2 ratio was found to be ~9 upon transient stimulation by K of the Na,K-ATPase in tubules initially exposed to a nominally zero concentration of extracellular K. The differences in the ratios may reflect the different experimental conditions and/or techniques that were used (transient stimulation vs. steady state; extracellular K electrode vs. isotopic uptake) rather than true stoichiometric differences between steady state and dynamic conditions (see Soltoff and Mandel, 1984b).

Respiration and K^+ transport have also been measured in other mammalian species, particularly to examine the relative resistance to cold of the renal cortex in hibernators. Willis (1968) observed that the K/O_2 ratio was decreased at 5°C (compared with values obtained at 15–37°C) in kidney slices of guinea pigs and rats, but was undiminished in hamsters, ground squirrels, and rabbits. Thus, active transport (ouabain-sensitive K^+ uptake into K^+-depleted tissue) in nonhibernating rodents (rabbits being an exception) appeared to be more sensitive to temperature than was the transport-related respiration. As the temperature was decreased from 38 to 15°C in that study, an increase in the K/O_2 ratio was measured for rabbit because the cold caused a relatively greater reduction of transport-related Q_{O_2} than K uptake. This finding is in contrast to the constant K/O_2 ratio reported here over the 15–37°C range. These differences may be due to the use of cortical slices, which may be metabolically compromised, as discussed above.

Discontinuous Arrhenius Plots

Numerous investigators have studied the temperature dependence of the Na,K-ATPase activity using the isolated enzyme or broken membrane preparation. A partial listing of the results is presented in Table IV, along with several studies in which the activity of the Na pump was monitored in intact cells by measuring K^+ influx. Relatively few studies have been performed using intact cells. With few exceptions, discontinuities are found in Arrhenius plots, and studies over the temperature range of 15–37°C can most often be fitted to two straight lines

TABLE IV

Enthalpy Values for Na,K-ATPase (Na Pump) Activity in Various Tissues

Tissue	Preparation	Measurement*	Arrhenius plot discontinuity	Break point	Above break	Below break	Reference
				°C	kcal/mol		
Red blood cell							
Human	Intact	K influx	Yes	≈25[‡]			Stewart et al., 1980
LK sheep	Intact	K influx	No		(23.4)		Joiner and Lauf,
HK sheep	Intact	K influx	Yes	28.8	9.3	27.8	1979
Guinea pig {	Membrane	Na,K-ATPase	Yes	≈30[‡‖]	18.0	43.1	
{	Red cell ghost	K influx	Yes[‖]	—[‖]	10.8	29.5	Ellory and
Ground {	Membrane	Na,K-ATPase	Yes	≈30[‡‖]	0.05	37.4	Willis, 1976
squirrel {	Red cell ghost	K influx	Yes	≈30[‡‖]	6.9	20.2	
Tight epithelia							
Frog skin	Intact isolated	Short-circuit current	Yes	13.5[¶]			Takenaka, 1963
Brain							
Ox	Microsomes	Na,K-ATPase	Yes	20		1538	Taniguchi and Iida, 1972
Rat	Microsomes	Na,K-ATPase	Yes	20	7.8		Gruener and Avi-Dor, 1966
Kidney							
Hamster cortex	Membrane	Na-K-ATPase	Yes	16.4[‖]	18.8	43.5	Willis and Li, 1969
	Slice	K influx	Yes	17.7[‖]	9.3	20.7	Willis, 1968
Dog cortex	Microsomes	Na,K-ATPase	Yes	21	11.3	44	Le Grimellec et al., 1982
Rabbit cortex	Microsomes	Na,K-ATPase	Yes	20	13.5	28.5	Charnock et al., 1971
Lamb outer medulla	Purified	Na,K-ATPase	Yes	20	15.2	32.6	Grisham and Barnett, 1973
Guinea pig cortex	Homogenate	Na,K-ATPase	No**		(17)		Post et al., 1965
Rabbit proximal tubule	Membrane	Na,K-ATPase	Yes	25	12.9	21.9	This study
mal tubule	Intact	K influx	Yes	25	11.2	24.8	

* K influx is the ouabain-sensitive component; Na,K-ATPase activity is biochemical by determination.
[‡] Inflection point was calculated from data.
[‖] E_a values were calculated from raw data.
[‖] Curvilinear E_a values were measured at two extremes: 30–37°C, 15–20°C.
[¶] Examined for the 6–20°C range.
** Examined for the 18–44°C range.

that intersect at a breakpoint. This point seems to be around 16–21 °C for renal tissue and brain, and closer to 30 °C for red blood cells. It is difficult to determine which process is responsible for the break in the activation curve. Such a break has been attributed to a lipid transition (Taniguchi and Iida, 1972; Grisham and Barnett, 1973; but see Silvius and McElhaney, 1981) or to a change in a rate-limiting step. More conclusive mechanisms will have to await intimate knowledge regarding the molecular nature of the translocation steps. In this study, the temperature dependence of Na entry (i.e., the ouabain-sensitive Q_{O_2}) and the Na,K-ATPase enzyme activity are parallel, and both display a break at 25°C (Fig. 5). Assuming that the same relationship would be obtained for the Na,K-ATPase at subsaturating concentrations of Na^+, this is consistent with the observation that the K^+ contents of the tubules are similar (within 20%) over the 15–37°C temperature range (see Table III). Clearly, these dependencies must diverge at lower temperatures, since the renal cells accumulate Na^+ and release

K^+ at 0–5°C (Burg and Orloff, 1964; Willis, 1968). It may be speculated that the parallel temperature dependence reflects similar processes occurring at both the luminal and basolateral membranes, possibly emphasizing that Na entry may be a carrier-mediated process, as has been suggested by numerous investigators (see Sachs, 1977; Aronson, 1981). The activation energies for free diffusion of Na^+ and K^+ in water are 4.2 and 4.7 kcal/mol, respectively (Longsworth, 1955), values well below the calculated enthalpies for the measurements that represented the Na entry process. De Smedt and Kinne (1981) observed a linear Arrhenius plot in hog kidney brush border membrane vesicles for Na-independent D-glucose transport and Na-independent phosphate uptake, processes deemed to occur via simple diffusion, but discontinuities in the Arrhenius plot were observed for Na-glucose and Na-phosphate cotransport as well as for alkaline phosphatase activity. Other investigators have found differences between the temperature sensitivity of luminal and basolateral membranes transporters and enzymes. Le Grimellec et al. (1982) observed that the breakpoint of the Na,K-ATPase activity in dog kidney basolateral membranes was 21.4°C, but the breakpoint occurred at 26°C for the alkaline phosphatase activity in brush border membranes. Moreover, in red blood cells, the breakpoint ($\cong$25°C) of Na pump activity (ouabain-sensitive K^+ influx) was different from that ($\cong$20°C) of Na^+-K^+ cotransport (furosemide-sensitive K^+ influx) (data of Stewart et al., 1980, redrawn in Ellory and Willis, 1981).

ADP

The single activation enthalpy of the ADP-stimulated state 3 mitochondrial respiratory response over the full range of experimental temperatures (Fig. 5) contrasts sharply with the multiple activation enthalpies demonstrated by the ionic transport processes. The temperature dependence of mitochondria state 3 respiration is difficult to associate with an individual rate-limiting step in the complex process of oxidative phosphorylation. Discontinuities in Arrhenius plots of the activities of respiratory enzymes contained within the membrane have been found in mitochondria from other tissues, but were absent for the matrix enzyme malate dehydrogenase (Watson et al., 1975) or when the membranes were disrupted with detergents (Raison et al., 1971). Such changes were associated with alterations of the lipid components of the mitochondrial membrane. A single activation enthalpy was observed over the temperature range of 5–35°C in hamster liver mitochondria in the presence of some substrates but not others (Liu et al., 1969). The extramitochondrial ionic conditions may also be important, since a discontinuous Arrhenius plot was obtained for rat liver mitochondria suspended in a medium that lacked K (Lee and Geer, 1974).

Nystatin

The large respiratory capacity of the mitochondria and constancy of the cellular ATP content suggest that the proximal cell has sufficient energy at all tempera-

tures to sustain unstimulated levels of active transport. However, when the Na,K-ATPase activity of the tubule was stimulated with nystatin, the mitochondrial energy production may have become limiting over the 20–37°C range, as discussed earlier, since the nystatin-stimulated Q_{O_2} is nearly identical to the state 3 Q_{O_2} over this temperature range. It was previously observed that the ATP content drops slightly upon the addition of nystatin to the proximal tubule suspension at 37°C (Harris et al., 1981). Although nystatin promotes the utilization of the full respiratory capacity of the proximal cell between 20 and 37°C, the ATP production appears to be insufficient to fully meet the increased energetic demand that is produced by the higher levels of intracellular Na. Since the state 3 rate and the ATP content at 15°C indicate that there should not be an energetic limitation at this temperature, another process may be rate limiting. The 15–20°C portion of the nystatin response is parallel to the Na,K-ATPase activity over this temperature range, and so perhaps the rate limitation involves the Na,K-ATPase.

In summary, the results of the ^{42}K uptake studies suggest that extracellular K^+ communicates directly with a single compartment, presumably the cytosol, via the Na,K-ATPase in the proximal tubule cell. Na pump activity and mitochondrial oxidative phosphorylation remain tightly coupled between 15 and 37°C. When the Na pump is stimulated to maximal activity by exposure of the tubules to the cationophore nystatin between 20 and 37°C, the rate-limiting step for Na transport changes from the rate of Na^+ entry into the cell to the rate of ATP production.

REFERENCES

Altschuld, R., C. Hohl, A. Ansel, and G. P. Brierly. 1981. Compartmentalization of K^+ in isolated adult rat heart cells. *Arch. Biochem. Biophys.* 209:173–184.

Aronson, P. S. 1981. Identifying secondary active solute transport in epithelia. *Am. J. Physiol.* 240:F1–F11.

Balaban, R. S., S. P. Soltoff, J. M. Storey, and L. J. Mandel. 1980. Improved renal cortical tubule suspension: spectrophotometric study of O_2 delivery. *Am. J. Physiol.* 238:F50–F59.

Beck, F., R. Bauer, U. Bauer, J. Mason, A. Dörge, R. Rick, and K. Thurau. 1980. Electron microprobe analysis of intracellular elements in the rat kidney. *Kidney Int.* 17:753–763.

Brent, R. C. 1973. Algorithms for Minimizations without Derivatives. Prentice-Hall, Inc., Englewood Cliffs, NJ. 116–167.

Burg, M. B., E. F. Grollman, and J. Orloff. 1964. Sodium and potassium flux of separated renal tubules. *Am. J. Physiol.* 206:483–491.

Burg, M. B., and J. Orloff. 1964. Active cation transport by kidney tubules at 0°C. *Am. J. Physiol.* 207:983–988.

Burg, M. B., and J. Orloff. 1966. Effect of temperature and medium K on Na and K fluxes in separated renal tubules. *Am. J. Physiol.* 211:1005–1010.

Cass, A., and M. Dalmark. 1973. Equilibrium dialysis of ions in nystatin-treated red cells. *Nat. New Biol.* 244:47–49.

Chance, B., and G. R. Williams. 1956. The respiratory chain and oxidative phosphorylation. *Adv. Enzymol.* 17:65–134.

Charnock, J. S., D. M. Doty, and J. C. Russell. 1971. The effect of temperature on the activity of $(Na^+ + K^+)$-ATPase. *Arch. Biochem. Biophys.* 142:633–637.

De Smedt, H., and R. Kinne. 1981. Temperature dependence of solute transport and enzyme activities in hog renal brush border membrane vesicles. *Biochim. Biophys. Acta.* 648:247–253.

Doucet, A., A. I. Katz, and F. Morel. 1979. Determination of Na-K-ATPase activity in single segments of the mammalian nephron. *Am. J. Physiol.* 237:F105–F113.

Ellory, J. C., and J. S. Willis. 1976. Temperature dependence of membrane function. Disparity between active potassium transport and $(Na^+ + K^+)$ ATPase activity. *Biochim. Biophys. Acta.* 443:301–305.

Ellory, J. C., and J. S. Willis. 1981. Phasing out the sodium pump. *In* Effects of Low Temperature on Biological Membranes. G. J. Morris and A. Clark, editors. Academic Press, Inc., London. 107–119.

Frömter, E., G. Rumrich, and K. J. Ullrich. 1973. Phenomenologic description of Na^+, Cl^-, and HCO_3^- absorption from the proximal tubules of rat kidney. *Pflügers Arch. Eur. J. Physiol.* 343:189–220.

Giebisch, G. 1961. Measurements of electrical potential differences on single nephrons of the perfused *Necturus* kidney. *J. Gen. Physiol.* 44:659–678.

Giebisch, G., T. Kubota, and M. G. O'Regan. 1981. Ion-activity measurements in renal tubular epithelium. *In* The Application of Ion-selective Microelectrodes. T. Zeuthen, editor. Elsevier/North-Holland Biomedical Press, New York. 47–60.

Gornall, A. G., C. J. Bardawill, and M. M. David. 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* 177:751–766.

Grisham, C. M., and R. E. Barnett. 1973. The role of lipid-phase transitions in the regulation of the (sodium + potassium) adenosine to triphosphatase. *Biochemistry.* 12:2635–2637.

Gruener, N., and Y. Avi-Dor. 1966. Temperature-dependence of activation and inhibition of rat-brain adenosine triphosphatase activated by sodium and potassium ions. *Biochem. J.* 100:762–767.

Harris, S. I., R. S. Balaban, L. Barrett, and L. J. Mandel. 1981. Mitochondrial respiratory capacity and Na^+- and K^+-dependent adenosine triphosphatase-mediated ion transport in the intact renal cell. *J. Biol. Chem.* 256:10319–10328.

Joiner, C. H., and P. K. Lauf. 1979. Temperature dependence of active K transport in cation dimorphic sheep erythrocytes. *Biochim. Biophys. Acta.* 552:540–545.

Kiil, F. 1977. Renal energy metabolism and regulation of sodium reabsorption. *Kidney Int.* 11:153–160.

Kinne, R., and I. L. Schwartz. 1978. Isolated membrane vesicles in the evaluation of the nature, localization, and regulation of renal transport processes. *Kidney Int.* 14:547–556.

Kleinzeller, A., K. Janacek, and A. Knotkova. 1962. A simple method for measuring steady-state ion compartments and rate constants of ion fluxes in tissue slices. *Biochim. Biophys. Acta.* 59:239–241.

Kubota, T., B. Biagi, and G. Giebisch. 1980. Intracellular potassium activity measurements in single proximal tubules of *Necturus* kidney. *Curr. Top. Membr. Transp.* 13:63–72.

Lee, M. P., and A. R. L. Geer. 1974. The effect of temperature on mitochondrial membrane-linked reactions. *J. Biol. Chem.* 249:7541–7549.

Le Grimellec, C., M.-C. Giocondi, B. Carriere, S. Carriere, and J. Cardinal. 1982. Membrane fluidity and enzyme activities in brush border and basolateral membranes of the dog kidney. *Am. J. Physiol.* 242:F246–F253.

Liu, C.-C., J. L. Frehn, and A. D. La Porta. 1969. Liver and brown fat mitochondrial response to cold in hibernators and non-hibernators. *J. Appl. Physiol.* 27:83–89.

Longsworth, L. G. 1955. Diffusion in liquids and the Stokes-Einstein relation. *In* Electrochemistry in Biology and Medicine. T. Shadlovsky, editor. John Wiley & Sons, New York. 225–247.

Lowry, O. H., and J. V. Passonneau. 1972. A Flexible System of Enzymatic Analysis. Academic Press, Inc., New York and London.

Lowry, O. H., N. L. Rosenbrough, A. L. Farr, and R. J. Randell. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.

Martin, J. B., and D. M. Doty. 1949. Determination of inorganic phosphate. Modification of isobutyl alcohol procedure. *Anal. Chem.* 21:965–967.

McIver, D. J. L., and A. D. C. Macknight. 1974. Extracellular space in some isolated tissues. *J. Physiol. (Lond.).* 239:31–49.

Mudge, G. H. 1951. Studies on potassium accumulation by rabbit kidney slices: effect of metabolic activity. *Am. J. Physiol.* 165:113–127.

Mudge, G. H. 1953. Electrolyte metabolism of rabbit kidney slices: studies with radioactive potassium and sodium. *Am. J. Physiol.* 173:511–522.

Post, R. L., A. K. Sen, and A. S. Rosenthal. 1965. A phosphorylated intermediate in adenosine triphosphate-dependent sodium and potassium transport across kidney membranes. *J. Biol. Chem.* 240:1437–1445.

Raison, J. K., J. M. Lyons, and W. W. Thomson. 1971. The influence of membranes on the temperature-induced changes in the kinetics of some respiratory enzymes of mitochondria. *Arch. Biochem. Biophys.* 142:83–90.

Sachs, G. 1977. Ion pumps in the renal tubule. *Am. J. Physiol.* 233:F359–F365.

Schmidt, U., and M. Horster. 1978. Na-K stimulated adenosine triphosphatase: methodology for quantifications in microdissected renal tubule segments from freeze-dried and fresh tissue. *In* Methods in Pharmacology; Vol. 4B: *Renal Pharmacology.* M. Martinez-Maldonado, editor. Plenum Press, New York. 259–295.

Seals, J. R., J. M. McDonald, D. Bruns, and L. Jarett. 1978. A sensitive and precise isotopic assay of ATPase activity. *Anal. Biochem.* 90:785–795.

Silvius, J. R., and R. N. McElhaney. 1981. Non-linear Arrhenius plots and the analysis of reaction and motional rates in biological membranes. *J. Theor. Biol.* 88:135–152.

Soltoff, S. P., and L. J. Mandel. 1984a. Active ion transport in the renal proximal tubule. II. Ionic dependence of the Na pump. *J. Gen. Physiol.* 84:623–642.

Soltoff, S. P., and L. J. Mandel. 1984b. Active ion transport in the renal proximal tubule. III. The ATP dependence of the Na pump. *J. Gen. Physiol.* 84:643–662.

Spring, K. R., and G. Giebisch. 1977. Kinetics of Na$^+$ transport in *Necturus* proximal tubule. *J. Gen. Physiol.* 70:307–328.

Stewart, G. W., J. C. Ellory, and R. A. Klein. 1980. Increased human red cell cation passive permeability below 12°C. *Nature (Lond.).* 286:403–404.

Stroup, R. F., E. Weinman, J. P. Hayslett, and M. Kashgarian. 1974. Effect of luminal permeability on net transport across the amphibian proximal tubule. *Am. J. Physiol.* 226:1110–1116.

Takenaka, T. 1963. Effects of temperature and metabolic inhibitors on the active Na transport in frog skin. *Jpn. J. Physiol.* 13:208–218.

Taniguchi, K., and S. Iida. 1972. The effect of phospholipids on the apparent activation energy of (Na$^+$-K$^+$)-ATPase. *Biochim. Biophys. Acta.* 274:536–541.

Thurau, K. 1961. Renal Na reabsorption and O$_2$ uptake in dogs during hypoxia and hydro-chlorothiazide infusion. *Proc. Soc. Exp. Biol. Med.* 106:714–717.

Thurau, K., F. Beck, J. Mason, A. Dörge, and R. Rick. 1981. Inside the cell: an electron microprobe analysis of the renal tubular electrolyte concentration. *In* Epithelial Ion and Water Transport. A. D. C. Macknight and J. P. Leader, editors. Raven Press, New York. 137–145.

Torelli, G., E. Mella, A. Faelli, and S. Costantini. 1966. Energy requirements for sodium reabsorption in the *in vivo* rabbit kidney. *Am. J. Physiol.* 211:576–580.

Watson, K., E. Bertoli, and D. E. Griffiths. 1975. Phase transitions in yeast mitochondrial membranes. *Biochem. J.* 146:401–407.

Whittam, R., and J. S. Willis. 1963. Ion movements and oxygen consumption in kidney cortex slices. *J. Physiol. (Lond.).* 168:158–177.

Whittembury, G., N. Sugina, and A. K. Solomon. 1961. Ionic permeability and electrical potential differences in *Necturus* kidney cells. *J. Gen. Physiol.* 44:689–712.

Willis, J. S. 1968. Cold resistance of kidney cells of mammalian hibernators: cation transport vs. respiration. *Am. J. Physiol.* 214:923–928.

Willis, J. S., and N. M. Li. 1969. Cold resistance of Na-K-ATPase of renal cortex of the hamster, a hibernating animal. *Am. J. Physiol.* 217:321–326.