

## Inhibition of Renal Metabolism. Relative effects of arsenate on sodium, phosphate, and glucose transport by the rabbit proximal tubule.

P C Brazy, ... , L J Mandel, V W Dennis

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### Research Article

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# Inhibition of Renal Metabolism

## RELATIVE EFFECTS OF ARSENATE ON SODIUM, PHOSPHATE, AND GLUCOSE TRANSPORT BY THE RABBIT PROXIMAL TUBULE

P. C. BRAZY, R. S. BALABAN, S. R. GULLANS, L. J. MANDEL, and V. W. DENNIS,  
*Division of Nephrology and Department of Physiology, Duke University, and  
Veterans Administration Medical Centers, Durham, North Carolina 27710*

**ABSTRACT** These studies examine the inhibitory effects of arsenate on the transport of sodium, phosphate, glucose, and para-aminohippurate (PAH) as well as oxidative metabolism by proximal convoluted tubules from the rabbit kidney. Transport rates were measured with radioisotopes in isolated and perfused segments. Metabolic activity was monitored through oxygen-consumption rates and NADH fluorescence in parallel studies in suspensions of cortical tubules. The addition of 1 mM arsenate to the perfusate reduced fluid absorption rates from  $1.24 \pm 0.17$  to  $0.66 \pm 0.19$  nl/mm · min ( $P < 0.01$ ) and lumen-to-bath phosphate transport from  $9.93 \pm 3.47$  to  $4.25 \pm 1.08$  pmol/mm · min ( $P < 0.01$ ). Similar concentrations of arsenate reduced glucose transport only slightly from  $66.1 \pm 6.0$  to  $56.8 \pm 4.6$  pmol/mm · min ( $P < 0.05$ ) and had no effect of PAH secretion. Removing phosphate from the perfusate did not affect the net transport of sodium or glucose. In suspensions of tubules, arsenate increased oxygen consumption rates by  $20.5 \pm 2.9\%$  and decreased NADH fluorescence by  $10.8 \pm 1.5\%$ . These effects on metabolism were concentration dependent and magnified in the presence of ouabain. The data indicate that arsenate's main effect is to uncouple oxidative phosphorylation, and that graded uncoupling of oxidative metabolism causes graded reductions in the net transport of both sodium and phosphate. Glucose transport is inhibited only slightly and PAH secretion is not affected. Thus, partial as opposed to complete inhibition of metabolism reveals that different relationships exist between net

sodium transport and the transport of phosphate, glucose, and PAH by the proximal renal tubule.

### INTRODUCTION

Inhibitors such as acetazolamide, amiloride, ouabain, and phlorizin are useful in identifying biochemical sites involved in the movement of various solutes across specific membrane systems. The usefulness of inhibitors, however, is limited by the extent to which we know their mechanisms. We know that arsenate inhibits phosphate transport in a number of systems including the mammalian kidney (1), Ehrlich ascites tumor cells (2), yeast (3), bacteria (4), *Euglena* (5), and isolated membrane vesicles derived from renal brush border material (6), but the mechanisms of inhibition are uncertain. Arsenate may interfere with phosphate transport by direct competition at some transport site (2-6), or by inhibiting the activity of glycolytic enzymes such as glyceraldehyde 3-phosphate dehydrogenase (7), or by the uncoupling of oxidative phosphorylation at the mitochondrial level (8).

The objective of these studies is to gain further information on the biochemical sites where sodium transport interacts with the transport of phosphate and other solutes handled significantly by the proximal convoluted tubule. As with certain organic solutes (9) that may have metabolic as well as transport effects, the possible contributions of intraluminal phosphate to cotransport and intracellular metabolism must be separated conceptually and experimentally. In this regard, we reasoned that arsenate, which may substitute for phosphate in a number of biochemical (7, 8) and transport events, (2-6) would be a useful inhibitor for studying the various processes that involve phosphate and that affect solute transport. Accordingly, these studies describe the effects of arsenate on transport and metabolism by the proximal convoluted tubule, identify the dominant mechanism involved in the inhibitory effects, and

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describe for the first time the variability that appears to exist in the linkage of specific solute transport processes to oxidative metabolism. The data indicate that arsenate uncouples mitochondrial oxidative phosphorylation in a concentration-dependent fashion and that graded reductions in oxidative metabolism affect the transport of sodium and phosphate more than the transport of glucose or para-aminohippurate (PAH).<sup>1</sup> Phosphate absorption by the proximal convoluted tubule is therefore linked as closely as sodium absorption to oxidative metabolism.

## METHODS

**Isolated perfused tubules.** Segments of proximal convoluted tubules from rabbit kidney were isolated and perfused *in vitro* according to techniques described (10). Young female New Zealand White rabbits (1–3 kg) were maintained on rabbit chow (Ralston Purina Co., St. Louis, Mo.) and water before they were killed. Tubular segments from superficial and juxtamedullary nephrons were dissected from cortical slices. No attempt was made to select either superficial or juxtamedullary nephrons, or early or late proximal convoluted segments. We perfused the tubules *in vitro* with a continuously exchanging bathing medium consisting of commercial rabbit serum (Granite Diagnostics Inc., Burlington, N. C.) or a similar fluid maintained at 37°C, pH 7.35–7.45, and isosmotic with the perfusion fluid. The perfusate was a physiological fluid containing (mM): sodium chloride, 105; sodium bicarbonate, 25; potassium chloride, 5; magnesium sulfate, 1; monosodium phosphate, 2; calcium chloride, 1.5; sodium lactate, 10; L-alanine, 5; and D-glucose, 7. Bathing media other than rabbit serum, when used, were made directly from the corresponding perfusion fluids by adding either, 3 g/dl dialyzed dextran, 40,000 mol wt (Pharmacia Fine Chemicals AB, Uppsala, Sweden), or 6 g/dl defatted albumin (11), and enough calcium chloride for a final total calcium concentration of 3 mM. Before use, dextran was dialyzed exhaustively against doubly distilled water using a Spectrapor membrane (Spectrum Medical Industries Inc., Los Angeles, Calif.) with a nominal retention at 6–8,000 daltons. Dialysis removed lower molecular weight polymers and potential impurities. The concentration of the dialyzed dextran was estimated by weighing an air-dried aliquot. This indicated that ~10% of the original dextran was lost in the dialysis procedure. Aliquots were frozen and stored until the day used.

Transport rates for phosphate and glucose were determined using radioisotopes. We measured the lumen-to-bath fluxes of phosphate ( $J_{\text{po}_4}^{\text{b}}$ ) or glucose ( $J_{\text{glc}}^{\text{b}}$ ), as the difference between the amount of solute delivered and the amount collected according to the balance equation:

$$J_s = \frac{V_i C_i^* - V_o C_o^*}{L} \cdot \frac{[S]}{C_i^*}, \quad (1)$$

where  $J_s$  is the unidirectional solute flux (pmol/mm·min);  $V_i$  and  $V_o$  are the perfusion and collection rates, respectively;  $C_i^*$  and  $C_o^*$  (cpm/nl) represent the concentration of [<sup>14</sup>C]-

glucose or of [<sup>32</sup>P]O<sub>4</sub> in the perfusate and collected fluid, respectively; and [S] (pmol/nl) is the chemical concentration of the relevant solute in the perfusion fluid. Phosphate and glucose fluxes were measured in separate tubules. For studies of  $J_{\text{glc}}^{\text{b}}$  more glucose was added to the perfusate (final concentration ~14 mM) to achieve glucose delivery rates compatible with saturation of the transport mechanism (12, 13). Fluid absorption rates ( $J_v$ ) were measured for each tubule with either [<sup>125</sup>I]iothalamate (Abbott Diagnostics, Diagnostic Products, No. Chicago, Ill.) or dialyzed [*methoxy*-<sup>3</sup>H]inulin (New England Nuclear, Boston, Mass.).

Bath-to-lumen fluxes of phosphate and PAH were measured by the accumulation in the lumen of radioisotopic solute added to the bath. Thus (14)

$$J_s^{\text{bl}} = \frac{V_o C_o^*}{X_b L} \quad (2)$$

where  $J_s^{\text{bl}}$  is the bath-to-lumen flux of phosphate ( $J_{\text{po}_4}^{\text{bl}}$ ; pmol/mm·min) or of PAH ( $J_{\text{PAH}}^{\text{bl}}$ ; fmol/mm·min);  $V_o$ ,  $C_o^*$ , and  $L$  are the same as in Eq. 1 and  $X_b$  is the specific activity of the appropriate solute in the bath.

The basic protocol for the perfusion experiments was to allow a 30-min equilibration period after initiating perfusion, and then to collect three or four timed samples using a constant-volume pipette of ~100 nl. After these initial collections, we changed the perfusion fluid or bath and collected three or four additional samples after a second equilibration period of ~20 min.

Radioisotopic activity was measured by liquid-scintillation spectrophotometry in Aquasol (New England Nuclear) plus 0.5% water by volume. Inorganic phosphate was measured by the phosphomolybdate method (Hycel Phosphorus Test, Hycel Inc., Houston, Tex.); glucose by the glucose-oxidase method (Glucostat, Worthington Biochemical Corp., Freehold, N. J.); pH and PCO<sub>2</sub> by electrodes (Instrumentation Laboratory, Inc., Lexington, Mass.) and osmolality by freezing-point depression (Precision Systems, Inc., Sudbury, Mass.).

Data from each tubule are reported as the mean of at least three collections for each set of experimental conditions and are expressed as the mean ± SE for the number of tubules studied. Statistical comparisons were made using the paired *t* test.

**Suspensions of cortical tubules.** We prepared suspensions of separated renal tubules from the rabbit cortex according to methods of Balaban et al. (15). Briefly, kidneys from female New Zealand White rabbits were flushed *in vivo* with 50 mg/dl collagenase (Sigma type I, Sigma Chemical Co., St. Louis, Mo.), excised, and the cortex was dissected. Cortical tubules were dispersed, washed several times at 5°C, and centrifuged on a cushion of Ficoll (400,000 mol wt) to remove nonvital cells and cellular debris. The final pellet was resuspended to a concentration of 5–6 mg tubule protein/ml in a solution that contained (mM): sodium chloride, 115; sodium bicarbonate, 25; potassium chloride, 5; monosodium phosphate, 4; calcium chloride, 2.3; magnesium sulfate, 2.1; D-glucose, 5; sodium lactate, 4; L-alanine, 1; 3 g/dl dialyzed dextran (40,000 mol wt); 300 mosmol/kg·H<sub>2</sub>O; and was maintained at pH 7.4 (37°C). These tubules have open lumens and high oxygen consumption rates that are inhibited up to 70% by ouabain (15).

Studies using cortical tubule suspensions were performed in a specially designed, temperature controlled, sealed chamber (15) that permitted simultaneous measurements of oxygen consumption and microfluorometry. To initiate an experiment, the tubule suspension was pre-equilibrated for 30–60 min with 95% O<sub>2</sub>, 5% CO<sub>2</sub> gas mixture at 37°C. An 8-ml aliquot of this suspension was then placed into the chamber.

<sup>1</sup> *Abbreviations used in this paper:* CCCP, m-chlorocarbonyl cyanide phenylhydrazine;  $J_{\text{PAH}}^{\text{bl}}$ , bath-to-lumen flux of PAH;  $J_{\text{po}_4}^{\text{b}}$ , bath-to-lumen flux of phosphate;  $J_{\text{glc}}^{\text{b}}$ , lumen-to-bath flux of glucose;  $J_{\text{po}_4}^{\text{b}}$ , lumen-to-bath flux of phosphate;  $J_v$ , fluid absorption rate; PAH, para-aminohippurate.

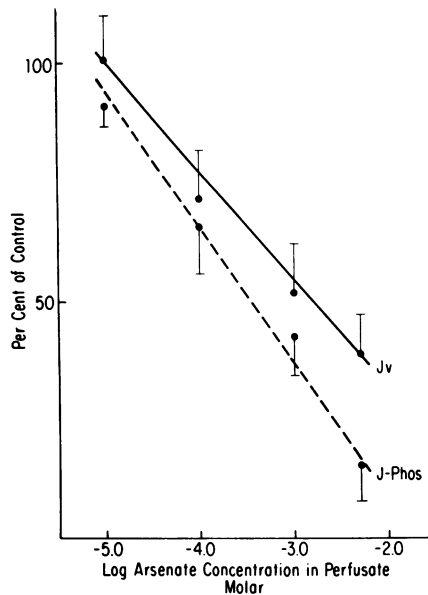


FIGURE 1 Effects of arsenate on fluid absorption rates ( $J_v$ ) and on the lumen-to-bath fluxes of phosphate ( $J_{\text{Phos}}$ ). Each point represents the mean  $\pm$  SE for three to five proximal convoluted tubules perfused initially with ultrafiltrate (control) and subsequently with ultrafiltrates plus the arsenate concentration indicated on the abscissa. 15 separate tubules were examined.

Oxygen consumption was monitored polarographically with a Clark oxygen electrode (Yellow Springs Instrument Co., Yellow Springs, Ohio). The oxygen tension in the suspension was recorded as a function of time and the slope reflected the oxygen consumption rate. Data are reported as the percent change in the oxygen consumption rate (the slope during the experimental period/the slope during control conditions  $\times$  100)  $\pm$  SE for a given number of suspensions.

Fluorometric measurements were performed on the tubule suspension as described (15). Briefly, the incident light on the chamber was obtained from a sharply filtered (366 nm peak) mercury arc lamp. Light was collected from the opposite side of the chamber by an objective lens focused near the center of the suspension. The collected light was focused on a beam splitter where 95% of the light was transmitted through a 450-nm filter to a photomultiplier tube. This photomultiplier tube recorded the fluorescent emission from NADH. The other 5% of the excitation and emitted light was directed through a 366-nm filter to a photomultiplier tube which recorded only the transmitted excitation light. This excitation light was subtracted electronically from the fluorescence signal to compensate for movement artifacts, for changes in the intensity of the arc lamp, and for scattering of light within the preparation (16). All results are expressed as the difference between the 450- and the 366-nm signals. An increase in 450 nm fluorescence indicates a net reduction of mitochondrial NAD (i.e., an increase in the reduced to oxidized NAD ratio).<sup>2</sup>

<sup>2</sup> Although NADH occurs in the cytoplasm as well as mitochondria, studies of skeletal muscle (17), cardiac muscle (18), and brain (19) indicate that >90% of the 450 nm fluorescence is attributable to bound mitochondrial rather than cytoplasmic NADH. Our own unpublished observations indicate that a similar situation exists for isolated proximal renal tubules.

A decrease in 450 nm fluorescence indicates a net oxidation of NAD (i.e., a decrease in the reduced to oxidized NAD ratio). The data were quantified by comparing the changes in emission of 450 nm light that occurred with each experimental condition to the intensity of emission that occurred during control conditions. This comparison is expressed as the percent change in fluorescence. All tubule suspensions were followed until anoxic conditions were achieved. The 0–100% range of the fluorescence signal was defined as the difference between the control and the anoxic state. The data are reported as the mean percent change in fluorescence  $\pm$  SE for a given number of suspensions. Protein content of each suspension was determined by the method of Lowry et al. (20).

As noted above, dextran replaced albumin or serum proteins in some studies because albumin interfered with fluorometry. Preliminary studies established that dialyzed dextran (3 g/dl in the bath) was isotonic to rabbit serum and had no effects on the transport of phosphate or glucose. That is, six proximal convoluted tubules were perfused with the standard artificial perfusate (see above), bathed initially with rabbit serum, and subsequently with an isotonic fluid that contained 3 g/dl dialyzed dextran. Fluid absorption rates averaged  $1.34 \pm 0.20$  nl/mm  $\cdot$  min with rabbit serum and  $1.34 \pm 0.16$  nl/mm  $\cdot$  min with dextran solution. Lumen-to-bath phosphate transport averaged  $6.35 \pm 1.88$  and  $6.32 \pm 2.00$  pmol/mm  $\cdot$  min, and glucose transport averaged  $69.3 \pm 4.3$  and  $70.8 \pm 5.1$  pmol/mm  $\cdot$  min during rabbit serum or dextran solution, respectively. These values were constant for at least 90 min after the change of fluid. Thus, dialyzed dextran at 3 g/dl in the bath supported volume, phosphate, and glucose transport to the same extent as serum proteins or albumin (10).

## RESULTS

**Transport studies.** Fig. 1 is a dose-response curve for the effects of intraluminal arsenate on fluid and phosphate absorption in isolated proximal convoluted tubules. 15 tubules were perfused initially with an ultrafiltrate of the rabbit serum used as the bath. Under these conditions, fluid absorption rates averaged  $1.08 \pm 0.10$  nl/mm  $\cdot$  min and lumen-to-bath phosphate transport averaged  $8.60 \pm 1.63$  pmol/mm  $\cdot$  min. Perfusion rates averaged  $12.06 \pm 0.81$  nl/min and the phosphate concentration in the perfusate was  $1.66 \pm 0.09$  mM. The addition of arsenate to the perfusate to final concentrations of 10  $\mu$ M–5 mM resulted in proportional reductions in both phosphate and fluid absorption rates. Arsenate in the perfusate had no effect on phosphate transport from bath to lumen which averaged  $0.69 \pm 0.07$  pmol/mm  $\cdot$  min for four tubules. If added to the bath only, arsenate to concentrations of 5 mM had no effect on either fluid or phosphate absorption.

We also examined the effects of arsenate on glucose and PAH transport. For these studies, the phosphate concentration in the perfusion fluid averaged  $1.67 \pm 0.08$  mM. As shown in Table I, 1 mM arsenate in the perfusate had no effect on PAH secretion which averaged  $319.9 \pm 67.0$  fmol/mm  $\cdot$  min for four proximal convoluted tubules. On the other hand, 1 mM arsenate in the perfusate reduced the lumen-to-bath flux of glucose from  $66.1 \pm 6.0$  to  $56.8 \pm 4.6$  pmol/mm  $\cdot$  min ( $P < 0.05$ ; Table II). For these studies, the glucose concentration in the

TABLE I  
Effect of Arsenate on PAH Secretion

Tubule	Length	$X_b$	$V_0$		$C_0$		$J_{PAH}^b$	
			C	E	C	E	C	E
	mm	cpm/fmol	nl/min		cpm/100 nl		fmol/mm·min	
1	0.9	0.418	8.37	12.80	573.2	462.4	129.1	157.3
2	1.3	0.341	6.99	9.38	2449.6	1808.1	386.1	382.5
3	1.4	0.431	13.48	14.45	1942.2	2029.0	434.1	485.6
4	0.8	0.295	11.12	25.04	700.9	280.9	330.3	298.0
Mean	1.1	0.371	10.01	15.41	1416.5	1145.1	319.9	330.8
SE	0.1	0.032	1.43	3.38	462.5	450.4	67.0	69.4
P			NS		NS		NS	

Four proximal convoluted tubules were perfused first with the control solution (C) and then with the same solution containing 1 mM sodium arsenate (E). The bath consisted of normal serum with 25  $\mu$ M PAH.  $V_0$  is the collection rate.  $X_b$  is the specific activity of [ $^3$ H]PAH in the bath and  $C_0$  is the concentration of isotope in the collected fluid.

perfusate averaged  $14.50 \pm 0.55$  mM and glucose delivery rates averaged  $156 \pm 14$  and  $176 \pm 9$  pmol/min during control and experimental periods, respectively. These values are consistent with maximal transport rates for glucose (12, 13).

Fig. 2 shows the relationship between the effects of intraluminal arsenate on phosphate transport from lumen to bath, as well as the effects on fluid absorption rates. Arsenate concentrations in the perfusate varied from 10  $\mu$ M to 10 mM. The data are plotted as fractional reductions over control values for each tubule. The equation for a linear regression is  $y = 0.85x + 0.01$  ( $r = 0.92$ ) and is very similar to the equation  $y = 0.77x$

+ 0.16 obtained previously for the fractional reductions in  $J_{PO_4}^b$ , and  $J_v$  that result from decreases in intraluminal sodium concentration (10).

Similarly, Fig. 3 shows the relationship between the fractional reduction in glucose transport from lumen to bath and the fractional reduction in fluid absorption rates. Arsenate concentrations in the perfusate varied from 0.1 mM to 10 mM. The degree of inhibition of glucose transport is less than that of phosphate (Fig. 2) but is similar to that observed for these degrees of reduction in fluid absorption that occur with reductions in intraluminal sodium concentration (10). Accordingly, the data in Figs. 2 and 3 show that arsenate inhibits

TABLE II  
Effect of Arsenate on Glucose Transport from Lumen to Bath

Tubule	$V_i$		$[G]_i$		$[G]_o$		$J_{Gc}^b$	
	C	E	C	E	C	E	C	E
	nl/min		mM		mM		pmol/mm·min	
1	8.10	12.64	13.87	13.65	8.62	9.81	52.3	56.4
2	9.11	12.04	15.37	15.50	1.70	8.02	83.9	60.0
3	12.91	10.37	15.21	15.13	8.23	8.08	85.6	75.7
4	9.68	10.95	15.29	15.30	11.47	12.91	58.6	45.6
5	13.04	13.95	15.25	15.13	9.86	11.79	56.6	45.1
6	11.59	13.17	11.99	11.87	2.39	4.35	59.5	58.0
Mean	10.74	12.19	14.50	14.43	7.04	9.16	66.1	56.8
SE	0.84	0.55	0.55	0.56	1.65	1.25	6.0	4.6
P	NS		NS		<0.05		<0.05	

Six proximal convoluted tubules (mean length  $1.22 \pm 0.19$  mm) were perfused first with a control solution (C), then with the same solution containing 1 mM arsenate (E). Normal rabbit serum was used as bath.  $V_i$  is the perfusion rate.  $[G]_i$  denotes the glucose concentration in the perfusate and  $[G]_o$  is the glucose concentration in the collected fluid as derived from changes in [ $^{14}$ C]glucose concentration.

the transport of sodium, phosphate, and glucose. The data cannot, however, distinguish between primary effects of arsenate on sodium transport with secondary inhibition of phosphate and glucose absorption, or primary effects of arsenate on phosphate transport with secondary effects on sodium and glucose absorption.

To separate these two major possibilities, we performed a similar series of studies in which phosphate absorption was eliminated by the removal of phosphate from the perfusion fluid. Phosphate in the perfusate was replaced by chloride but phosphate was present ( $2.25 \pm 0.06$  mM) in the rabbit serum used as the bath. For nine tubules,  $J_v$  averaged  $1.00 \pm 0.06$  nl/mm $\cdot$ min for the initial periods during perfusion with phosphate-containing ( $1.81 \pm 0.13$  mM) fluids and was unchanged at  $0.99 \pm 0.11$  nl/mm $\cdot$ min after removal of phosphate. The duration of exposure to phosphate-free perfusate was about 50 min. For these studies, we added radioisotopic phosphate to the bath to measure the intraluminal accumulation of phosphate from the bath. During perfusion with the phosphate-free perfusate, the concentration of phosphate at the collection end, as estimated by a rearrangement of Eq. 2, averaged  $0.04 \pm 0.01$  mM. We thus achieved a marked reduction in intraluminal availability of phosphate that had no discernible effect on  $J_v$ .

We used a similar procedure to evaluate the effects of phosphate-free perfusate on glucose transport. For these studies, the glucose concentration in the perfusate was  $12.66 \pm 0.20$  mM and glucose delivery rates averaged  $150.4 \pm 12.8$  and  $165.3 \pm 11.9$  pmol/min during control and experimental periods, respectively. For seven tubules,  $J_{\text{Gluc}}^b$  averaged  $80.4 \pm 7.0$  pmol/min $\cdot$ min during perfusion with control fluids and  $77.7 \pm 7.3$  pmol/min $\cdot$ min during perfusion with phosphate-free perfusate. Although intraluminal arsenate reduced fluid, phosphate, and glucose absorption, the marked reduction in avail-

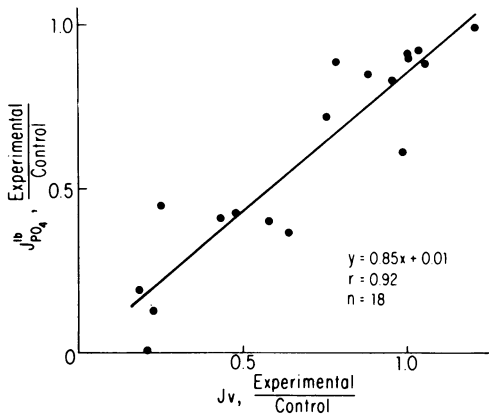


FIGURE 2 Relative effects of intraluminal arsenate on phosphate transport and on fluid absorption rates. Each point represents a single tubule.

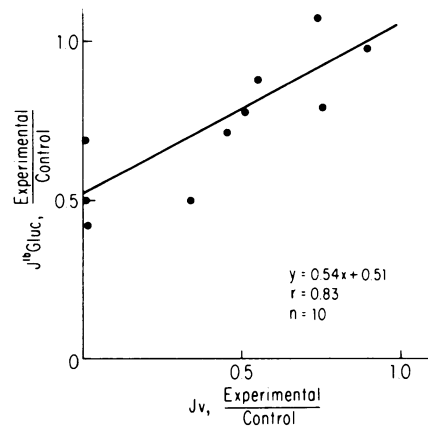


FIGURE 3 Relative effects of arsenate on glucose transport and on fluid absorption rates. Each point represents a single tubule.

ability of intraluminal phosphate did not affect fluid or glucose absorption. These data indicate that the effects of arsenate on sodium and glucose transport cannot be attributed primarily to arsenate-induced reductions in phosphate absorption.

We wanted to see if the effects of arsenate on the proximal convoluted tubule involved any interaction with phosphate. Accordingly, we examined the effects of changes in phosphate concentration on the inhibitory effects of arsenate. Table III lists the effects of 1 mM arsenate in the perfusate on  $J_v$  and  $J_{\text{PO}_4}^b$  during perfusion with 1.80, 0.85, or 0.0 mM phosphate in the perfusate. Two points are notable. First, arsenate reduced both  $J_v$  and  $J_{\text{PO}_4}^b$ , more markedly in the presence of the lower concentration of phosphate. Second, arsenate reduced  $J_v$  even in the absence of phosphate. Thus, the inhibitory effects of arsenate involve some interaction with phosphate but intraluminal phosphate is not required as would be expected if arsenate acted by direct inhibition of the luminal uptake of phosphate.

**Metabolic studies.** The inhibition of sodium, phosphate, and glucose transport by arsenate raised the possibility that arsenate may be acting as an inhibitor of cellular metabolism rather than as a specific inhibitor of transport. To determine any direct metabolic effects, we examined the effects of arsenate on oxygen consumption rates and on the redox state of NAD in suspensions of cortical tubules.

Fig. 4 shows the effects of arsenate on the oxygen consumption rate and on the change in 450 nm fluorescence for a typical study. For this particular study, arsenate was added to the bathing medium for a final concentration of 10 mM. Because these tubules have open lumens, arsenate presumably has direct access to the luminal surface. As noted in Fig. 4, this concentration of arsenate increased the oxygen consumption rate slightly (27%) and also decreased NADH fluores-

TABLE III  
Interaction between Phosphate and Arsenate

[PO <sub>4</sub> ] <sub>i</sub>	[PO <sub>4</sub> ] <sub>o</sub>		V <sub>i</sub>		J <sub>v</sub>		J <sub>B</sub>	
	C	E	C	E	C	E	C	E
mM	mM		nl/min		nl/mm <sup>2</sup> ·min		pmol/mm <sup>2</sup> ·min	
1.80±0.15	0.99±0.26	1.51±0.14	13.03±0.42	13.44±1.67	1.24±0.17	0.66±0.19*	9.93±3.47	4.25±1.08*
0.85±0.06	0.58±0.12	0.78±0.09	10.48±1.97	10.38±1.97	1.11±0.37	0.24±0.09*	3.35±0.21	0.46±0.48*
0.0	0.04±0.00	0.09±0.01	14.84±1.42	12.20±1.37	1.11±0.13	0.22±0.13*	—	—

Four tubules were studied at each of the three concentrations. Values are the mean±SE for each group. [PO<sub>4</sub>]<sub>i</sub> and [PO<sub>4</sub>]<sub>o</sub> denote the phosphate concentrations in the perfusate and collected fluid, respectively. V<sub>i</sub> is the perfusion rate. C denotes control periods during perfusion with artificial perfusate. E denotes experimental periods with 1 mM arsenate in the perfusate.

\* Asterisk indicates statistically significant differences between control and experimental periods by the paired *t* test.

cence (15%). The simultaneous increase in oxygen consumption and decrease in NADH fluorescence are consistent with the uncoupling of oxygen consumption from the phosphorylation of ADP to ATP (8, 21, 22) and with the stimulation of cellular ATPase activity (22).

To distinguish these two possibilities, we repeated the studies in the presence of 0.5 mM ouabain to inhibit sodium-potassium ATPase activity totally, and thereby decrease the respiratory rate of the tissue. These conditions would tend to magnify any uncoupling action related to arsenate. Fig. 5 shows a representative study from this series. The addition of ouabain decreased the rate of oxygen consumption and increased NADH fluorescence (22). The subsequent addition of arsenate increased oxygen consumption and decreased NADH fluorescence more dramatically than in Fig. 4. From these data we favor the interpretation that arsenate acts by uncoupling oxidative phosphorylation, rather than by stimulating ATPase activity, although the participation of an ATPase not sensitive to ouabain cannot be excluded.

Table IV lists the effects of different concentrations of arsenate on oxygen consumption rates and on changes in NADH fluorescence, as measured in four different preparations of tubules in the absence or presence of 0.5 mM ouabain. The preparations were exposed to each concentration of arsenate. Arsenate at concentrations >0.1 mM increased oxygen consumption and decreased NADH fluorescence progressively. As shown in Fig. 1, Tables II and III, these are the same concentrations of arsenate that inhibit proximal sodium, phosphate, and glucose transport.

The preceding data indicate that the dominant effect of arsenate was to uncouple oxidative metabolism. To test this further, we examined the effect of *m*-chlorocarbonyl cyanide phenylhydrazine (CCCP), a known uncoupler of oxidative metabolism (23). Because of apparent binding of CCCP to serum proteins, these studies were performed using dextran solution as the bath-

ing medium. As shown in Table V, CCCP was added to the bath for a final concentration of 1 μM decreased fluid and phosphate absorption to the same degree (64.0 ±9.2 and 72.1±8.1%, respectively) but decreased glucose transport significantly less (42.5±6.4%, *P* < 0.02).

In separate studies, 1 μM CCCP increased oxygen consumption rates by 51.2±2.3% in four suspensions of cortical tubules. CCCP at 10 μM increased oxygen consumption by 115.3±11.0%. Fluorometric measurements were not possible because CCCP fluoresces. Nonetheless, the striking increase in oxygen consumption with the decrease in J<sub>v</sub> indicate that CCCP acts under these conditions as a mitochondrial uncoupler similar to its action in other systems (23). Moreover, the pattern of inhibition of volume, phosphate, and glucose transport caused by CCCP is similar to that observed with arsenate.

## DISCUSSION

The effects of arsenate on sodium, phosphate, and glucose absorption, NADH fluorescence, and oxygen consumption indicate that arsenate may act in the proximal tubule as an uncoupler of ATP production from mitochondrial respiration. The simultaneous increase in respiration and decrease in the level of mitochondrial NADH associated with arsenate may be interpreted on the basis of known mitochondrial behavior. Two types of conditions would elicit these responses in isolated mitochondria; either a transition to a more active state that would stimulate respiration and increase the rate of ATP production, or an uncoupling action that would stimulate respiration but would inhibit ATP production (21, 22, 24). The first response might have occurred if arsenate increased the rate of ATP use but this seems unlikely because arsenate decreased net sodium transport, a major pathway for the use of ATP (25, 26). Moreover, the studies with ouabain (Table III) demonstrate that arsenate exerts

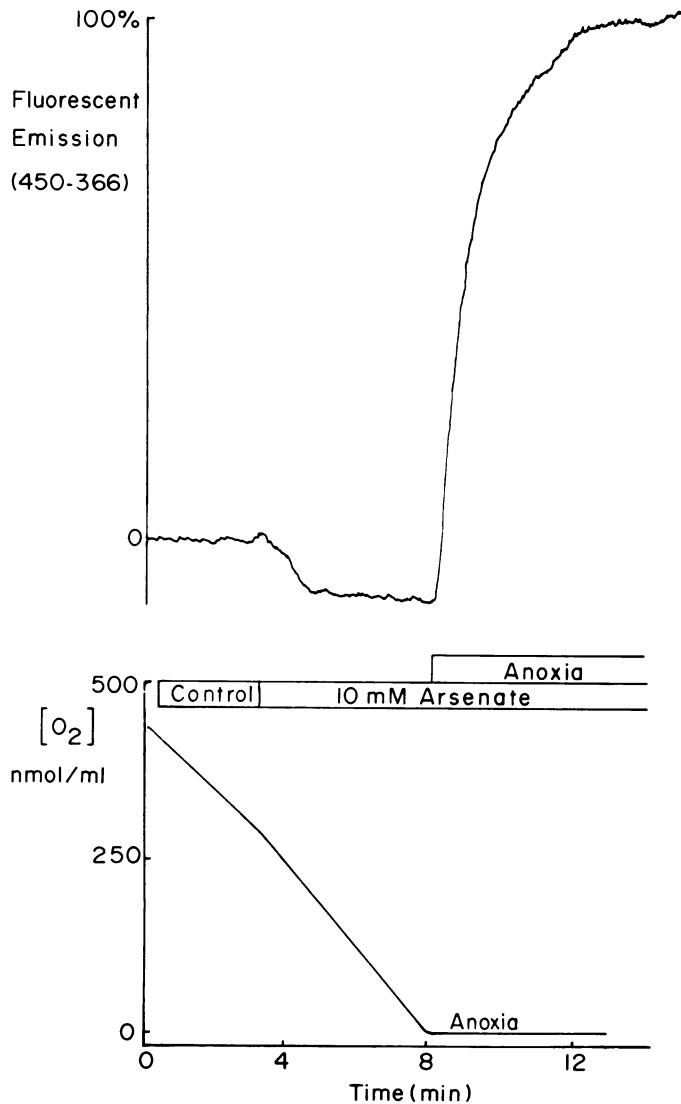


FIGURE 4 Representative study: simultaneous effects of 10 mM arsenate on oxygen consumption rate and on NADH fluorescence.

its metabolic effects even during complete inhibition of the sodium-potassium ATPase. Therefore, arsenate-induced increases in ATPase activity seem unlikely. Although we cannot exclude the possibility of activation of an ouabain-insensitive ATPase, the present data are most consistent with the interpretation that arsenate uncouples mitochondrial oxidative phosphorylation in intact proximal convoluted renal tubules as it does in the rat liver (8).

In addition to its action as an uncoupler of oxidative phosphorylation, arsenate competes with phosphate for binding sites on glycolytic enzymes (7) and may be converted to arsenite (27), a potent sulfhydryl group inhibitor that affects enzymes such as alpha-ketoglutarate and pyruvate dehydrogenases (28, 29). If the me-

tabolic effects of arsenate resulted from either of these two mechanisms, oxygen consumption would be expected to decrease because of fewer substrates available for the tricarboxylic acid cycle. Indeed, Crane and Lipmann (8) observed that arsenite decreased oxygen consumption by isolated mitochondria. In the present studies, the observed increase in oxygen consumption is not compatible with these mechanisms and suggests that these types of inhibition, if they do occur, are quantitatively unimportant. That is, arsenate's dominant mechanism appears to be inhibition of mitochondrial oxidative phosphorylation. In this regard, it should be noted that in the absence of ouabain, arsenate even at concentrations of 10 mM increased oxygen consumption by only 27%. This may be considered as



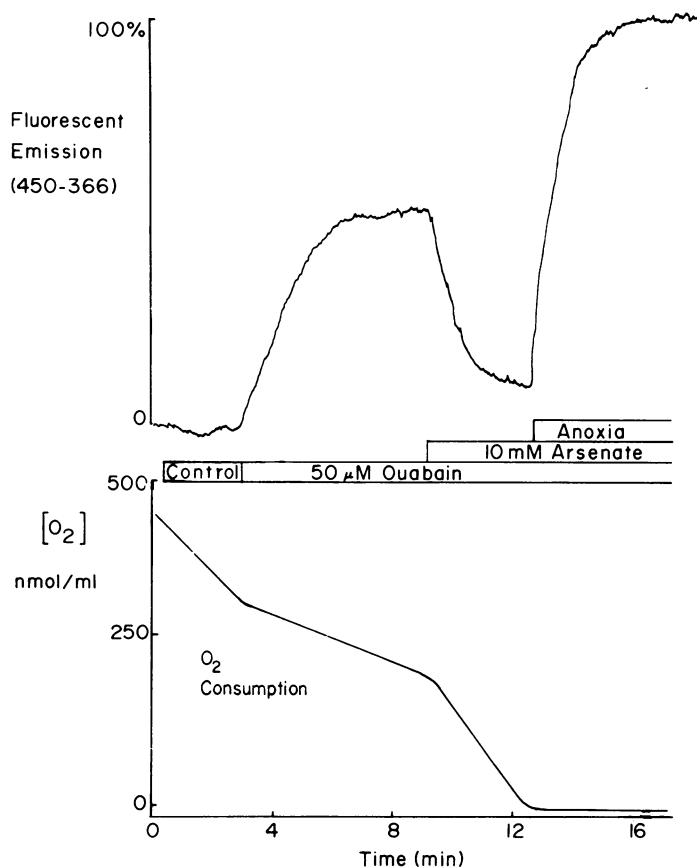


FIGURE 5 Representative study: simultaneous effects of 10 mM arsenate on oxygen consumption rate and on NADH fluorescence in the presence of ouabain 50  $\mu$ M.

a weak uncoupling action relative to the 115% increase in respiration observed under similar conditions in response to 10  $\mu$ M CCCP.

Studies of membrane vesicles from the proximal tubule of the rat demonstrate that arsenate inhibits the sodium-driven uptake of phosphate (6). We, therefore, considered that arsenate may inhibit transport and oxidative metabolism by blocking the luminal uptake of phosphate. For membrane vesicles, however, the  $K_m$  for phosphate transport is 0.08 mM and the inhibitory constant for arsenate ( $K_i$ ) is 1.1 mM. If one assumes that these constants apply to the rabbit proximal tubule in vitro, an arsenate concentration of about 25 mM would be required to inhibit phosphate transport by 50% at an ambient phosphate concentration of 1.8 mM. As shown in Fig. 1, 50% inhibition of  $J_{P_{i0}}$  occurred in the present studies at an arsenate concentration of about 1 mM. Moreover, the elimination of phosphate from the perfusate had no discernible effect on fluid or glucose absorption, and arsenate inhibited  $J_v$  even in the absence of phosphate in the perfusate. Thus, inhibition of the luminal entry of phosphate cannot account for the profound inhibitory effects of arsenate.

Although there were some necessary differences in the conditions that prevailed during the transport and the metabolic studies, none seems so major as to preclude a general comparison of the concentration dependencies of the transport and metabolic effects of arsenate. Thus, we note from Fig. 1 and from Table IV that the effects of arsenate on fluid absorption and oxidative metabolism had their onset at similar concentrations of about 0.1 mM and that further changes occurred as the arsenate concentration was increased to 1 mM. These data suggest that a very tight relationship exists between net sodium transport and energy availability in the isolated proximal tubule. That is, although previous studies have demonstrated profound reductions in proximal fluid absorption in response to potent uncouplers of oxidative phosphorylation (30, 31) and to metabolic inhibitors such as cyanide (31, 32), the present studies demonstrate graded reductions in transport in response to increasing concentrations of a weak uncoupler. To a first approximation, the graded reductions in fluid absorption (and phosphate absorption) correspond with the graded reductions in oxidative metabolism (Table IV). These partial, as opposed

**TABLE IV**  
*Effects of Arsenate on Oxygen Consumption and NADH Fluorescence in Nonperfused Isolated Tubules*

Arsenate mM	Oxygen consumption (percent change)		NADH fluorescence (percent change)	
	No ouabain	0.5 mM ouabain	No ouabain	0.5 mM ouabain
0.1	0	+10.4±2.5	0	-5.4±1.1
0.5	+13.3±2.7	+33.4±2.2	-7.0±1.2	-18.4±2.0
1.0	+20.5±2.9	+53.6±3.8	-10.8±1.5	-26.4±2.4
1.5	+26.9±3.6	+72.1±5.0	-14.1±2.1	-35.0±2.9

Data are mean±SE for four suspensions. Oxygen consumption averaged 32.0±4.3 nmol O<sub>2</sub>/mg protein·min in the absence of ouabain and 10.5±1.3 nmol O<sub>2</sub>/mg protein·min in the presence of ouabain.

to complete, reductions provide opportunities to examine the relationship between oxidative metabolism and transport rates for specific solutes.

If one accepts the conclusion from these studies and others (8) that arsenate inhibits cellular metabolism and thus net sodium transport, it is perhaps not surprising that arsenate also reduces the absorption of phosphate and glucose. There is now abundant evidence that the transport of these two solutes is linked closely to sodium transport. The elimination of net sodium transport by ouabain eliminates the net transport of phosphate (33) and glucose (12). The partial replacement of intraluminal sodium with choline causes proportional reductions

in phosphate, glucose, and net sodium fluxes (10, 34, 35). Moreover, specific symports for sodium and phosphate (6), and for sodium and glucose (36–38) have been described for the brush border of the proximal renal tubule. Thus, there seems to be little question that cotransport of sodium and phosphate, and sodium and glucose occur at some point, probably the brush border, in the series of events that results ultimately in the absorption of these solutes.

The present data emphasize, however, that despite their common dependency on sodium transport, glucose transport and phosphate transport are inhibited to different degrees by various conditions that partially inhibit net sodium transport. Whether caused by arsenate or CCCP as in the present studies (Figs. 1–3; Table V), or by substitution of choline for intraluminal sodium as in our previous studies (10), inhibition of net sodium transport affects the transport of phosphate more profoundly than the transport of glucose. The presence of this differential in the pattern of inhibition seems firm and various mechanisms may be considered.

First, inhibition of cellular metabolism by arsenate or CCCP may increase the intracellular concentration of inorganic phosphate by impaired ATP production. An increase in the intracellular concentration of inorganic phosphate would reduce the entry gradient for phosphate without necessarily affecting the gradient for glucose. In this regard, Wilson et al. (39) inhibited the metabolism of renal cells in culture and demonstrated an increase in intracellular phosphate concentration. It seems likely therefore that this mechanism could apply in the present studies. Its role is less certain, however, in our previous studies in which inhibi-

**TABLE V**  
*Effect of CCCP on Absorption of Fluid, Phosphate, and Glucose*

Tubule	V <sub>i</sub>		J <sub>s</sub>		J <sub>ph</sub>		J <sub>glc</sub>	
	C	E	C	E	C	E	C	E
	nl/min		nl/mm·min		pmol/mm·min		pmol/mm·min	
1	17.55	19.16	1.45	0.54	1.83	0.65	70.4	51.7
2	17.29	18.48	1.63	0.75	4.89	1.36	78.8	48.9
3	18.84	22.26	1.74	1.00	4.68	2.78	—	—
4	17.74	6.28	1.27	0.73	9.97	2.90	—	—
5	15.09	15.82	0.59	0.04	4.35	0.01	74.9	33.8
6	16.48	16.70	0.65	0.07	16.28	2.51	94.5	46.6
Mean	17.17	18.12	1.22	0.52	7.00	1.70	79.6	45.2
SE	0.52	0.98	0.20	0.18	2.15	0.50	5.2	3.9
P	NS		<0.001		<0.05		<0.02	

Six proximal convoluted tubules were perfused initially with the standard solution (C) containing 1.81±0.05 mM phosphate and extra D-glucose (9.82±0.21 mM). The tubules were bathed with the same solution plus 3 g/dl dextran. During the experimental period (E), 1 μM CCCP was present in the bath. The mean perfused length was 1.57±0.22 mm. Except in tubules 3 and 4, fluid, phosphate, and glucose transport were measured simultaneously using three isotopes.

tion occurred via replacement of intraluminal sodium with choline (10).

Second, although these methods of inhibition may reduce the electrochemical gradient for sodium that apparently mediates the cellular accumulation of phosphate and glucose, these two solutes may be coupled differently to this gradient. The result is that phosphate transport may be more sensitive to a partial reduction than glucose transport. For example, a greater portion of phosphate entry may depend on the chemical gradient for sodium, whereas a greater portion of glucose entry may depend on the transmembrane electrical potential. In this regard, Beck and Sacktor (37, 38) demonstrated that electrical potentials are sufficient to cause glucose uptake across membrane vesicles from the rabbit proximal tubule even in the absence of a chemical gradient for sodium. On the other hand, Hoffman et al. (6), and Barrett et al. (40) demonstrated that phosphate uptake into membrane vesicles is affected only slightly by electrical potential, and that much of the phosphate entry may occur via neutral complexes. Our own studies (10) show that the presumed depolarization of the proximal luminal membrane by the initiation of glucose transport also inhibits phosphate transport only slightly. Accordingly, if partial inhibition of cellular metabolism via arsenate or CCCP, or replacement of intraluminal sodium with choline reduces the chemical gradient for sodium more than the electrical potential, phosphate entry might be reduced more readily than glucose entry. Alternatively, a component of glucose entry may occur independently of sodium transport, although this seems unlikely in view of the observations that ouabain (12) or removal of sodium (35) eliminates glucose absorption.

There may be other explanations for the differential inhibition of phosphate and glucose transport that occurs with the partial inhibition of net sodium transport. Indeed, the various possibilities are not mutually exclusive and may combine. Further clarifications will require measurements of changes in intracellular electrical potentials and ionic concentrations.

The partial inhibition of metabolism achieved in these studies with arsenate did not cause any alteration in the rate of PAH secretion. In view of the present uncertainty regarding the existence of PAH coupling to sodium transport (41, 42), these results indicate only that PAH secretion is not critically dependent on a completely intact oxidative phosphorylation system.

Finally, we should comment further on the experiments in which phosphate was removed from the perfusate. These results demonstrated that elimination of phosphate transport by itself had no discernible effect on fluid absorption. This was expected because phosphate efflux from the lumen at 8 pmol/mm·min can account for, potentially, only about 5% of net sodium transport that averages about 150 pmol/mm·min (43).

In summary, partial as opposed to complete inhibition of net sodium transport by the proximal convoluted tubule reveals that phosphate transport is reduced much more profoundly than glucose or PAH transport. Thus, although the transport of these solutes may be sodium dependent, variability exists in the linkage of specific solute transport processes to net sodium transport.

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