

# Sodium, Phosphate, Glucose, Bicarbonate, and Alanine Interactions in the Isolated Proximal Convoluted Tubule of the Rabbit Kidney

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**ABSTRACT** Interactions among the transport systems involved with sodium, bicarbonate, glucose, phosphate, and alanine absorption in isolated segments of the rabbit proximal convoluted tubule were examined with radioisotopic techniques to measure glucose, phosphate, and fluid absorption rates. The composition of the perfusate and bath varied from normal, physiological fluids to fluids deficient in a single solute. The deletion of glucose from the perfusate increased the lumen-to-bath flux of phosphate from  $5.51 \pm 1.15$  to  $8.32 \pm 1.34$  pmol/mm·min ( $P < 0.01$ ). Similar changes occurred when glucose transport was inhibited by phlorizin  $10 \mu\text{M}$  in the perfusate. The deletion of alanine from the perfusate increased the lumen-to-bath flux of phosphate from  $6.55 \pm 1.08$  to  $9.00 \pm 1.30$  pmol/mm·min ( $P < 0.01$ ) but did not affect glucose transport significantly,  $80.1 \pm 10.1$  vs.  $72.5 \pm 5.4$  pmol/mm·min. Replacement of intraluminal sodium with choline, elimination of potassium from the bath, and removal of bicarbonate from the lumen and bath each reduced glucose, phosphate, and fluid absorption. These data indicate that the proximal absorptive processes for glucose and for phosphate include elements that are dependent upon some function of sodium transport. Additionally, the effects on phosphate transport of deleting glucose or alanine occur independent of any changes in net sodium transport and are opposite the effects of deleting bicarbonate. These differences may relate to the observations that the transport of glucose and alanine is electrogenic while that of bicarbonate is not. Regardless of possible mechanisms, the data demonstrate that important changes in the absorption rates of different

solutes handled significantly by the proximal convoluted tubule may occur in response to changes in specific components of proximal sodium transport.

## INTRODUCTION

Interactions among the various absorptive processes of the proximal renal tubule may be inferred from the occurrence of acquired and inherited disorders characterized by glucosuria, phosphaturia, and amino aciduria (1, 2) and from direct observations of the influence of specific solutes such as glucose on the renal handling of others such as phosphate (3–8). Although it is not clear how the transport of solutes as chemically different as glucose and phosphate or glucose and amino acids may interrelate, data obtained largely from intestinal transport systems have led to three basic theories. First, the various transport processes involved in the movement of these solutes may compete directly for limited available metabolic energy (9, 10). Second, the involved solutes may be transported by a single polyfunctional carrier having kinetic characteristics that are altered by interactions with different solutes (10). Third, the interacting transport systems may be mutually affected by some consequence of a dominant transport event such as sodium movement (11–14). Such consequences may include the maintenance of an extracellular to intracellular sodium gradient (11, 13, 14), the flow of sodium ions or sodium current (15), and the steady-state transmembrane electrical potentials related to cation transport (16).

With regard specifically to the kidney, relatively little attention has been directed toward the possibilities of direct interactions among the transport processes of solutes other than sodium. Emphasis instead has been placed on the regulatory roles of extrinsic humoral and physical factors. However, it seems reasonable that considerations intrinsic to the tubular epithelium itself may also significantly affect the renal handling of the

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major solutes. Accordingly, the purpose of the present studies was to look for relationships among the transport systems involved in the movement of glucose, phosphate, alanine, and bicarbonate across the proximal renal tubule and to consider potential mechanisms. Studies were performed *in vitro* to isolate transport events from systemic factors such as parathyroid hormone and insulin which may otherwise affect tubular function (7, 8). The results indicate that glucose, phosphate, and alanine compete for transport across the proximal convoluted tubule and this competition appears to be mediated through the mutual dependence of these solutes upon sodium transport. Primary reductions in sodium transport decrease the absorption of both phosphate and glucose while interference with the availability for transport of either glucose or alanine results in increases in phosphate absorption. Details of these interactions suggest that phosphate absorption may be inhibited by electrogenic sodium transport related to glucose and alanine while unaffected or enhanced by nonelectrogenic sodium transport related to bicarbonate absorption. Thus, electrical coupling of solute transfers may occur in the proximal renal tubule in a pattern similar to that described for other absorbing epithelia.

## METHODS

Segments of proximal convoluted tubules were dissected primarily from superficial nephrons of female New Zealand white rabbits maintained on rabbit chow (Ralston Purina Co., St. Louis, Mo.). Tubules were perfused *in vitro* with continuously exchanged bathing medium consisting of artificial serum or commercial rabbit serum (Microbiological Associates, Walkersville, Md.) maintained at 37°C, pH 7.35 to 7.45, and isosmotic with the perfusion fluid. Artificial perfusion fluids as described in Table I were used. Artificial bathing media, when used, were made directly from the corresponding perfusion fluids by adding 6 g/100 ml defatted albumin (17)

TABLE I  
Composition of Perfusion and Bathing Fluids

	A	B	C	D	Normal rabbit serum	Artificial serum
	mM					
Sodium	144.2	148.9	148.6	147.4	145.4	143.3
Potassium	5.1	5.4	5.2	4.8	5.1	5.3
Chloride	109.5	114.4	117.1	134.2	101.1	110.3
Bicarbonate	23.1	22.5	20.3	—	24.8	21.7
Glucose	6.4	—	5.8	6.5	6.5	7.4
Alanine	(5.0)	(5.0)	—	(5.0)	*	(5.0)
Phosphate	1.8	1.8	1.6	2.0	2.0	2.0

Values represent the means for all determinations. Values in parentheses represent gravimetric estimates. Dashes refer to omitted solutes. In addition to the above, all artificial perfusion fluids contained 1.5 mM CaCl<sub>2</sub>, 1.0 mM MgSO<sub>4</sub>, and 10 mM lactate. \* Total amino acid concentration in pooled rabbit sera averaged 5.4±0.1 mM of which glycine averaged 1.2 mM, alanine 0.7 mM, and other amino acids were distributed in lower concentrations. The amino acid profile in pooled sera was similar to the profile measured in the serum of individual rabbits (unpublished observations).

and sufficient calcium chloride to give a final total calcium concentration of 3 mM. All perfusion fluids contained either <sup>125</sup>I-iothalamate (Abbott Diagnostics, N. Chicago, Ill.) or dialyzed [*methoxy*-<sup>3</sup>H]inulin (New England Nuclear, Boston, Mass.) as markers of fluid absorption and perfusion rate.

Epithelial transport rates for phosphate and for glucose were determined with radioisotopes. The lumen-to-bath fluxes of phosphate ( $J_{Po}^b$ )<sup>1</sup> or of glucose ( $J_S^b$ ) were measured as the difference between the amount of solute delivered and the amount collected according to the balance equation (18):

$$J_S^b = \frac{V_1 C_1^* - V_0 C_0^*}{L} \cdot \frac{[S]_l}{C_1^*}, \quad (1)$$

where  $J_S$  is the unidirectional solute flux (picomoles per millimeter per minute),  $V_1$  and  $V_0$  (nanoliters per minute) are the perfusion and collection rates, respectively,  $L$  (millimeters) is the length of tubule perfused as measured by eyepiece of micrometer,  $C_1^*$  and  $C_0^*$  (counts per minute per nanoliter) represent the concentrations of [<sup>14</sup>C]glucose or of <sup>33</sup>PO<sub>4</sub> in the perfusate and collected fluid, respectively, and  $[S]_l$  (picomoles per liter) is the chemical concentration of the relevant solute in the perfusion fluid. This equation assumes that no significant changes occur in the specific activities of the radioisotopic solutes between the perfusate and the collected fluid. Support for this assumption is derived from demonstrations that movements of glucose (19) and of phosphate (18) are largely unidirectional<sup>2</sup> from the lumen to the bath when normal concentrations are present in the bath and also from the concordance between chemical and radioisotopic concentrations of glucose (21) and phosphate (22) along the rat proximal tubule perfused *in vivo*.

Bath-to-lumen fluxes of glucose and of phosphate were estimated from the accumulation in the lumen to radioisotopic solute added to the bath. Thus (23):

$$J_S^b = \frac{V_0 C_0^*}{X_b L}, \quad (2)$$

where  $J_S^b$  (picomoles per millimeter per minute) is the bath-to-lumen flux of glucose ( $J_S^b$ ) or of phosphate ( $J_{Po}^b$ );  $V_0$ ,  $C_0^*$ , and  $L$  are as above for Eq. 1 and  $X_b$  is the specific activity of the appropriate solute in the bath. As discussed by Schafer et al. (23), this equation neglects the backflux of transported solute which is considered to be insignificant.

In some instances, the lumen-to-bath and bath-to-lumen fluxes of phosphate were determined simultaneously with <sup>33</sup>PO<sub>4</sub> in the perfusate and <sup>32</sup>PO<sub>4</sub> in the bath (18). In all studies fluid absorption rates were measured with the standard equation (24).

Although the data to be presented constitute an admixture of glucose or phosphate fluxes measured from either bath to lumen or from lumen to bath, one basic protocol was followed. For each proximal convoluted tubule, a unidirectional flux

<sup>1</sup> Abbreviations used in this paper:  $J_S^b$  bath-to-lumen flux of glucose,  $J_{Po}^b$ , bath-to-lumen flux of phosphate;  $J_S^l$  bath-to-lumen flux of solute;  $J_S^b$  lumen-to-bath flux of glucose,  $J_{Po}^b$ , lumen-to-bath flux of phosphate;  $J_S^u$  unidirectional solute flux;  $J_v$ , fluid absorption rate.

<sup>2</sup> Tune and Burg (20) estimated that the bath-to-lumen flux of glucose in the isolated proximal convoluted tubule averaged 18.8 pmol/mm·min or approximately 20% of the lumen-to-bath flux. More recent estimates in our laboratory (19) with pipette sealants and measuring net fluid absorption simultaneously indicate bath-to-lumen values of 9 pmol/mm·min or approximately 10% of the lumen-to-bath flux. We consider this degree of backflux to be negligible.



(solution B) from zero to 0.5, 2.5, 6.0, or 12.0 mM and by changing the perfusion rate over the range from 5 to 20 nl/min. As illustrated in Fig. 2, inhibition of phosphate absorption occurred at glucose delivery rates as low as 5 pmol/min and inhibition appeared to be maximal at delivery rates greater than 30 pmol/min. According to Tune and Burg (20) and as confirmed by us (19), glucose absorption in this system is not saturated until delivery rates exceed approximately 150–200 pmol/min.

The effects of phlorizin, a well-characterized inhibitor of glucose transport (30), were examined both in the presence and in the absence of intraluminal glucose. Paired studies were done in seven proximal convoluted tubules, each perfused on a random basis with both glucose-free (solution B) and glucose-containing perfusate (solution A). As shown in Fig. 3, the lumen-to-bath flux of phosphate averaged  $4.77 \pm 0.82$  pmol/mm·min during perfusion with fluid containing 6.38 mM glucose and increased to  $9.11 \pm 1.81$  pmol/mm·min in the presence of phlorizin,  $10 \mu\text{M}$ , in the lumen ( $P < 0.02$ ). For these same tubules in the absence of glucose in the perfusate,  $J_{\text{PO}_4}^{\text{lb}}$  averaged  $9.70 \pm 1.72$  pmol/mm·min and was unchanged at  $9.70 \pm 1.52$  upon the addition of phlorizin. In six additional tubules perfused with solution A and bathed in rabbit serum,  $10 \mu\text{M}$  phlorizin in the lumen had no effect on the bath-to-lumen flux of phosphate which averaged  $0.36 \pm 0.10$  pmol/mm·min in the absence of phlorizin and  $0.21 \pm 0.06$  in the presence of phlorizin. Thus, in the presence of glucose, intraluminal phlorizin enhanced phosphate absorption to an extent similar to that observed upon the removal of glucose and yet in the absence of glucose phlorizin had no additional effect on phosphate transport. Accordingly, the effects of phlori-

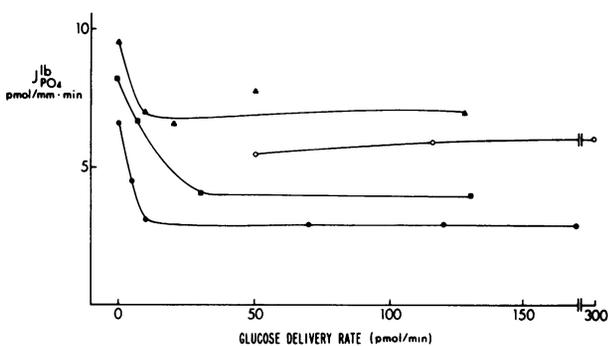


FIGURE 2 Relationship of phosphate absorption to glucose delivery rate. Four proximal convoluted tubules were perfused initially with artificial fluids having low glucose concentrations and subsequently with fluids having glucose concentrations ranging from 0.5 to 12 mM. Perfusion rates were also varied and glucose delivery was calculated as the product of the glucose concentration in the perfusate and the perfusion rate. Rabbit serum was used as bath.

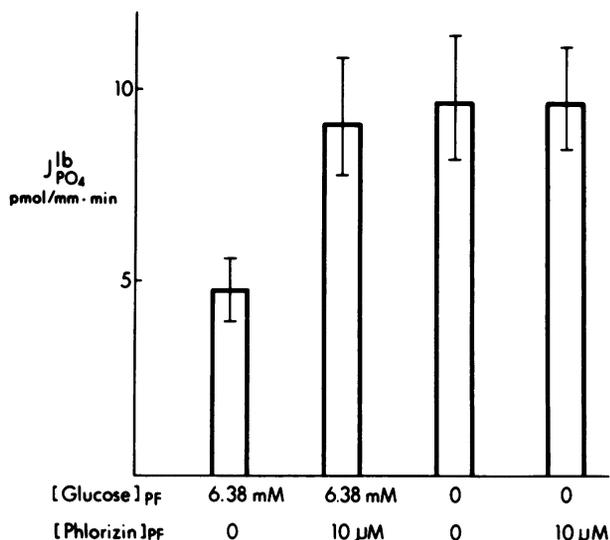


FIGURE 3 Glucose dependence of the effect of phlorizin on phosphate absorption. Seven proximal convoluted tubules were perfused initially with either glucose-containing or glucose-free fluid and subsequently with the same fluid containing  $10 \mu\text{M}$  phlorizin. Each tubule was then perfused with the alternate perfusate, again with and without phlorizin. Rabbit serum was used as bath.

zin on phosphate absorption appear to be related directly to the effects of phlorizin on glucose transport. As noted previously (19, 28), phlorizin caused a small but significant reduction in fluid absorption rate.

*Effects of alanine on phosphate transport.* The proximal handling of alanine, a representative neutral amino acid, and of glucose appear to be closely related. The presence of these two solutes is largely responsible for the lumen-negative transepithelial electrical potential difference associated with proximal sodium transport (28, 29, 31). Because, as shown above, glucose transport inhibited phosphate absorption, it seemed logical to examine also the effects of alanine. Seven convoluted segments (Table II) were perfused on a random basis with both solution A containing 5 mM alanine and with alanine-deleted solution A wherein sodium chloride replaced alanine. Rabbit serum was used as the bath. Under these conditions the presence of alanine decreased  $J_{\text{PO}_4}^{\text{lb}}$  from  $9.00 \pm 1.30$  pmol/mm·min to  $6.55 \pm 1.08$  pmol/mm·min ( $P < 0.01$ ). Fluid absorption rates were unchanged at  $0.78 \pm 0.06$  nl/mm·min. In three studies,  $J_{\text{PO}_4}^{\text{lb}}$  was also unchanged at  $0.77 \pm 0.26$  pmol/mm·min. Thus, alanine also inhibited phosphate absorption and did so without measurable effects on net sodium transport.

*Effects of alanine on glucose transport.* The possibility existed that these inhibitory effects of alanine on phosphate transport may have been mediated via alanine-induced increases in glucose transport. Therefore, the effects of alanine on glucose trans-

TABLE II  
Effect of Alanine on Phosphate Transport from Lumen-to-Bath

Tubule	$V_i$		$J_v$		$J_{po}^b$	
	+Ala	-Ala	+Ala	-Ala	+Ala	-Ala
	nl/min		nl/mm·min		pmol/mm·min	
1	18.00	14.31	0.80	1.06	10.08	11.84
2	18.16	18.73	0.70	0.64	8.25	12.18
3	12.35	12.47	0.58	0.59	6.05	7.34
4	12.70	16.20	1.01	1.06	8.56	10.10
5	13.32	13.89	0.88	0.92	7.40	12.12
6	12.73	9.40	0.55	0.52	2.46	3.57
7	12.88	15.43	0.78	0.70	3.06	5.82
Mean	14.31	14.35	0.76	0.78	6.55	9.00
Mean paired difference	-0.04±1.02		-0.03±0.04		-2.44±0.53	
P	NS		NS		<0.01	

Seven proximal convoluted segments were perfused in random order with both solution A containing alanine 5 mM (+Ala) and with solution C wherein alanine was replaced with sodium chloride (-Ala). Normal rabbit serum was used as the bath.  $V_i$  is the perfusion rate,  $J_v$  is the fluid absorption rate and  $J_{po}^b$  is the lumen-to-bath flux of phosphate.

port were also examined. As listed in Table III, in eight proximal convoluted segments perfused under conditions consistent with saturated glucose transport, the addition of alanine to the perfusate tended to reduce rather than increase glucose transport which averaged  $80.1 \pm 10.1$  pmol/mm·min in the absence of alanine and

$72.5 \pm 5.4$  in the presence of alanine ( $P > 0.05$ ). Although these data indicate that the effects of alanine on phosphate transport are not likely to be mediated through increases in glucose transport, it is perhaps surprising that alanine had no demonstrable effect on glucose absorption. This may reflect inadequate sensitivity of the methods to detect relatively small changes in large fluxes.

*Relationship of phosphate absorption to sodium transport.* We demonstrated previously that phosphate absorption is completely inhibited when fluid absorption is eliminated by  $10 \mu\text{M}$  ouabain in the bath but phosphate is not affected when  $J_v$  is eliminated with raffinose (18). This suggested that phosphate transport was related to sodium transport rather than to volume absorption per se as might occur with significant entrainment or solvent drag. Additional studies were performed, however, to extend these observations and to examine further the possibility that phosphate transport in the proximal convoluted tubule is dependent on sodium transport (18, 32). Control conditions for these studies consisted in perfusion with solution A with artificial serum derived from solution A as the bath. During the experimental periods, sodium transport was reduced by either the removal of potassium from the bath and replacement with sodium (33) or by reductions in intraluminal sodium concentration by substitution with choline (33). As shown in Fig. 4, the removal of potassium from the bath decreased  $J_{po}^b$  from  $8.72 \pm 1.46$  pmol/mm·min to  $1.20 \pm 0.16$  pmol/mm·min ( $P < 0.001$ ). Fluid absorption rates decreased from  $1.10 \pm 0.08$  to

TABLE III  
Effect of Alanine on Glucose Transport from Lumen to Bath

Tubule	$V_i$		[G]i		[G]o		$J_e^b$	
	A	C	A	C	A	C	A	C
	nl/min		mM		mM		pmol/mm·min	
1	14.09	13.30	8.99	9.60	4.92	4.51	64.3	73.1
2	16.15	15.97	10.00	11.43	4.98	3.74	93.7	125.9
3	12.46	13.59	12.21	11.93	3.19	4.92	67.2	65.8
4	15.62	13.69	9.99	10.10	7.50	7.96	63.6	52.1
5	14.76	14.68	14.15	14.43	8.00	6.58	97.7	121.8
6	24.13	20.36	14.71	13.15	12.32	10.30	58.1	58.7
7	12.88	15.43	14.71	13.15	9.09	7.43	75.0	84.0
8	24.05	28.54	12.60	12.76	9.00	9.54	60.7	59.7
Mean	16.77	16.95	12.17	12.07	7.38	6.87	72.5	80.1
SE	1.66	1.84	0.81	0.58	1.03	0.84	5.4	10.1
P	NS		NS		NS		NS	

Eight proximal convoluted tubules were perfused in random sequence with solution A containing 5 mM alanine (column A) and with solution C wherein sodium chloride replaced alanine (column C). Normal rabbit serum was used as bath. [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 [ $^{14}\text{C}$ ]glucose concentration.

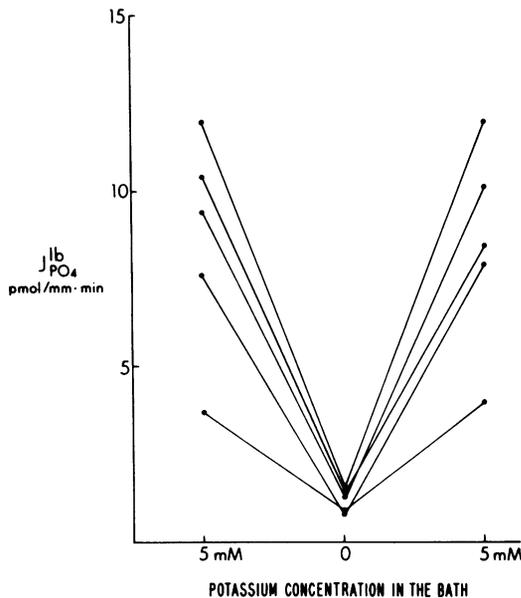


FIGURE 4 Influence of potassium availability on phosphate absorption. Five proximal convoluted tubules were perfused throughout with artificial perfusate (solution A). The bath was either artificial serum containing 5 mM potassium or artificial serum without potassium and having an equimolar substitution with sodium.

$0.06 \pm 0.04$  nl/mm·min. Both effects were reversible. In the second group, reducing the sodium concentration in the perfusate from  $147.7 \pm 0.9$  to  $73.5 \pm 0.5$  mM or to  $39.4 \pm 0.6$  mM resulted in progressive decreases in both  $J_v$  and in  $J_{PO_4}^{lb}$ . Because the artificial serum used as the bath had a sodium concentration averaging  $143.3 \pm 0.7$  mM and because sodium transport in the proximal tubule is bidirectional (34), the sodium concentration in the collected fluid may be expected to be increased over the reduced value in the perfusate. The sodium concentration in the collected fluid was not measured directly and thus the data are expressed as reductions in phosphate transport over control values as compared to reductions in  $J_v$  over control values. As shown in Fig. 5, for 16 studies in 13 tubules there was a positive linear correlation between the reduction in phosphate transport and the reduction in  $J_v$ . These data together with our previous observations (18) indicate clearly that the rate of phosphate absorption in this system is directly related to the rate of sodium transport. In addition, although the dispersion of the data from these types of studies precludes a precise determination, the estimated  $y$ -intercept in Fig. 5 is 0.16. This may indicate that only a small fraction of phosphate transport is independent of sodium and this presumably includes the sodium-independent diffusional component of  $J_{PO_4}^{lb}$ , which averages about 8% of the total flux (18).

*Relationship of glucose absorption to sodium transport.* Similar studies were performed while measur-

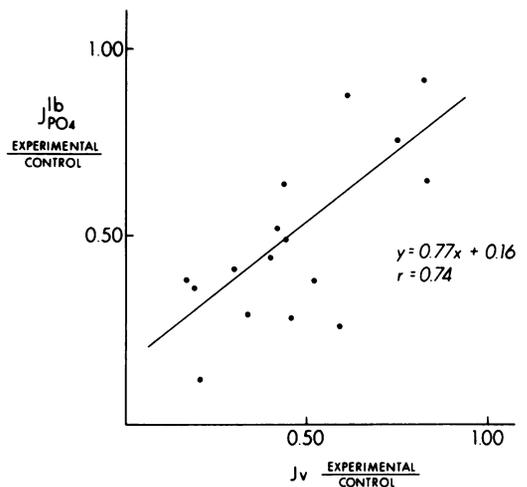


FIGURE 5 Influence of intraluminal sodium on phosphate transport. Each tubule was perfused during the control period with solution containing normal sodium concentration and during the experimental period with perfusate having partial replacement of sodium chloride with choline chloride. Artificial serum was used as bath. The ratio of the experimental to control values for phosphate transport is plotted along with the ratio of the experiment to control values for fluid absorption rate.

ing glucose transport from lumen to bath. For three tubules, the removal of potassium from the bath reduced  $J_{PO_4}^{lb}$  from  $62.8 \pm 8.4$  to  $30.4 \pm 12.0$  pmol/mm·min ( $P < 0.01$ ). Glucose delivery rates averaged  $246.8 \pm 28.2$  pmol/min in the presence of potassium and  $298.8 \pm 41.2$  pmol/min in the absence of potassium. As before, the deletion of potassium from the bath reduced  $J_v$  from  $1.20 \pm 0.18$  to  $0.18 \pm 0.12$  nl/mm·min.

Reductions in intraluminal sodium concentration by replacement with choline also reduced glucose transport (Table IV). When solution A, having a sodium concentration of  $144.9 \pm 3.1$  mM, was used as perfusate and artificial serum was used as the bath,  $J_{PO_4}^{lb}$  averaged  $87.4 \pm 3.4$  pmol/mm·min and was reduced to  $50.5 \pm 7.1$  pmol/mm·min ( $P < 0.01$ ) when the sodium concentration in the perfusate was  $46.8 \pm 4.2$  mM. The degree of reduction in glucose transport was notably less complete than the reduction observed for phosphate transport. This suggests that a significant component of glucose absorption in the proximal tubule may be independent of sodium transport.

*Effects of bicarbonate on the transport of glucose and of phosphate.* Thus far, the data indicate definite relationships among glucose, phosphate and sodium transport, but it must be acknowledged that sodium transport in the proximal convoluted tubule involves a series of transport processes including not only an electrogenic<sup>3</sup> process related to glucose and alanine

<sup>3</sup> "Electrogenic" in this context refers simply to the generation of a transepithelial electrical potential difference.

TABLE IV  
Effect of Intraluminal Sodium on Glucose Transport

Tubule	[Na]i		Glucose delivery		J <sub>v</sub>		J <sub>g</sub> <sup>b</sup>	
	C	E	C	E	C	E	C	E
	mM		pmol/min		nl/mm·min		pmol/mm·min	
1	137.5	38.5	145.6	209.0	0.90	-0.16	85.9	55.4
2	142.0	41.0	168.4	240.8	0.94	0.39	92.2	67.4
3	150.0	53.0	215.8	104.5	0.68	0.40	92.8	34.2
4	150.0	55.0	222.7	164.9	0.42	-0.22	78.5	45.1
Mean	144.9	46.8	188.1	179.8	0.74	0.10	87.4	50.5
SE	3.1	4.2	18.6	28.8	0.12	0.17	3.4	7.1
P			NS		<0.01		<0.01	

Four proximal convoluted tubules were perfused initially with solution A (C, control) and subsequently with perfusate having partial replacement of sodium chloride with choline chloride (E, experimental). Artificial serum derived from solution A was used as bath throughout. [Na]i denotes sodium concentration in the perfusate.

(28, 29, 31) but also a nonelectrogenic component related to bicarbonate absorption (31, 33, 35). The preceding data demonstrate that the total elimination of net sodium transport or the global reduction associated with reduced intraluminal sodium availability are both associated with reductions in phosphate and glucose transport. In contrast, however, the elimination of glucose and of alanine transport and presumably an associated component of sodium flux (29) resulted in increases in phosphate transport.

The next series of studies was designed to observe the effects of reducing the bicarbonate-mediated component of sodium transport (25, 31, 33, 35). For these studies individual segments were perfused with solu-

tion A as well as with solution D in which sodium bicarbonate was replaced with sodium chloride. The artificial bathing media were made directly from these fluids and were maintained at pH 7.4 by appropriate adjustments in the partial pressure of carbon dioxide. As demonstrated in Table V, the removal of bicarbonate from the perfusate and the bath of five tubules decreased J<sub>g</sub><sup>b from 11.71±2.34 pmol/mm·min to 1.00±0.44 (P < 0.01) while J<sub>v</sub> was reduced from 1.26±0.08 to 0.38±0.06 nl/mm·min (P < 0.001). Conversely, the addition of bicarbonate to the bath alone or to the perfusate and bath each increased sodium and phosphate transport. Attempts to examine bicarbonate in the perfusate alone resulted in tissue disruption</sup>

TABLE V  
Effect of Bicarbonate on Phosphate Transport

	J <sub>v</sub>				J <sub>g</sub> <sup>b</sup>			
	A	B	C	D	A	B	C	D
	nl/mm·min				pmol/mm·min			
Bicarbonate in perfusate	+	-	-	+	+	-	-	+
Bicarbonate in bath	+	-	+	+	+	-	+	+
	1.14	0.60	0.81	0.70	4.40	0.31	3.52	2.00
	1.28	0.45	0.81	0.94	8.87	1.16	7.30	9.04
	1.58	0.32	1.20	1.42	17.47	0.90	16.24	14.31
	1.20	0.30	0.58	0.97	12.16	0.05	7.99	12.12
	1.10	0.25	0.43		15.63	2.59	7.97	
Mean	1.26	0.38	0.77	1.01	11.71	1.00	8.60	9.37
SE	0.08	0.06	0.13	0.15	2.34	0.44	2.08	2.69
P		<0.001	<0.01	<0.05		<0.01	NS	NS

Five proximal convoluted tubules were perfused in random sequence with solution A containing bicarbonate and with solution D wherein bicarbonate was replaced with chloride. The bath was artificial serum at pH 7.4 and contained bicarbonate or not as indicated. P values relate changes to the initial control values listed in column A.

which progressed from the perfusion end presumably because of the high intraluminal pH resulting from the low ambient PCO<sub>2</sub> required to maintain the bicarbonate-free bath at pH 7.4. It is clear, however, that in contradistinction to the addition of glucose or of alanine, the addition of bicarbonate increased rather than decreased phosphate transport. As cited above, this effect may be assumed to relate to the effect of bicarbonate on sodium transport rather than volume absorption per se.

Similar studies were performed in five additional convoluted tubules during measurements of glucose transport. These tubules were perfused with both solution A containing bicarbonate and also with bicarbonate-free solution D. Artificial serum made from these fluids was used as the bathing medium. As shown in Table VI, J<sub>g</sub><sup>b</sup> was reduced from 68.1±7.3 pmol/mm·min in the presence of bicarbonate to 54.1±5.5 pmol/mm·min in the absence of bicarbonate (*P* < 0.02). Fluid absorption rates decreased from 0.91±0.19 to 0.54±0.16 nl/mm·min (*P* < 0.01). Thus, in contradistinction to the effects of deleting alanine, the removal of bicarbonate from the system and the associated decrease in net sodium flux reduced glucose transport in a qualitative pattern similar to that observed above for phosphate absorption. The fractional reduction in glucose transport is less substantial than the reduction in phosphate absorption, again suggesting that significant glucose transport may occur independent of sodium transport.

## DISCUSSION

This description of the complex interactions occurring among solute transfers in the proximal convoluted

tubule serves two purposes. First, it provides detailed descriptions of three major transport phenomena occurring in the proximal tubule of the rabbit kidney; the direct inhibition of phosphate absorption by glucose and alanine, the sodium dependency of both glucose and phosphate transport, and the inhibition of phosphate and glucose transport by the elimination of the bicarbonate-mediated component of sodium transport. Each of these major observations is useful in understanding certain previously described phenomena regarding the renal handling of phosphate and to a lesser extent glucose. More importantly, the combined observations furnish a basis for considering various mechanisms whereby the proximal transport of one solute may affect the absorption of others.

The first major observation of these studies is that glucose and phosphate compete for transport across the epithelium of the proximal convoluted tubule (3–8) and that this competition occurs independent of any systemic factors such as volume expansion, insulin, or parathyroid hormone activity. Whether replaced by urea or by sodium chloride, the deletion of glucose from the perfusion fluid resulted in consistent increases in phosphate absorption averaging approximately 60%. Moreover, similar increases in phosphate transport occurred when glucose was present in the perfusate but when its transport was inhibited by phlorizin. In the absence of glucose, phlorizin itself had no direct effect on phosphate handling and so the data indicate that glucose-induced reductions in phosphate absorption are related to the actual transport of glucose and not merely to its presence in the lumen. These observations would seem to explain in large part why glucose infusions sufficient to cause glucosuria result in increased phosphate excretion in man (4) and in the dog (3, 5, 8) while phosphaturia does not occur with phlorizin-induced (3, 5) or with renal glucosuria (36), conditions associated with glucosuria but without increases in glucose transport. Furthermore, because most data indicate that phlorizin interacts with glucose transport at the luminal brush border (19, 37–39) and perhaps at the glucose receptor site specifically (39), the present data suggest that glucose and phosphate enter their transport pathways through separate initial membrane events or receptors and that competition for transport occurs beyond this step. That is, these data do not favor the concept of a single polyfunctional carrier mechanism involving both glucose and phosphate. Pitts and Alexander (3) reached a similar conclusion on the basis of earlier clearance studies in the dog.

Although it seems clear that glucose and phosphate are not competing for a common initial transport event or receptor site, a number of other possible interactions between glucose transport and phosphate need to be considered. First, in an *in vitro* system such as this, metabolically derived energy may be marginal and as

TABLE VI  
Effect of Bicarbonate on Glucose Transport

Tubule	J <sub>v</sub>		Glucose delivery		J <sub>g</sub> <sup>b</sup>	
	C	E	C	E	C	E
	nl/mm·min		pmol/min		pmol/mm·min	
1	0.99	0.61	154.4	153.9	65.3	53.7
2	0.89	0.68	165.3	169.9	79.7	55.2
3	0.54	0.25	117.3	115.1	59.0	40.9
4	1.59	1.05	167.2	164.8	47.8	47.4
5	0.58	0.10	181.5	207.9	88.9	73.5
Mean	0.91	0.54	157.1	162.3	68.1	54.1
SE	0.19	0.17	10.8	14.9	7.3	5.5
<i>P</i>	<0.01		NS		<0.02	

Five proximal convoluted tubules were perfused during control periods (C) with solution A containing normal bicarbonate and bathed in artificial serum derived from solution A. During experimental periods (E), the same tubules were perfused with solution D containing no bicarbonate and bathed in artificial serum derived from solution D.

the transport of one solute is decreased, more energy may become available for the transport of another. This seems unlikely for at least three reasons. First, the present data derived from an *in vitro* system are in accord with similar data obtained *in vivo* in man (4) and in the dog (3, 5, 8) demonstrating that saturation of renal glucose absorption inhibits phosphate transport. Second, in the present studies, there was no suggestion of any proportionality between the reduction in phosphate transport and the expected rate of glucose transport. That is, Tune and Burg (20) demonstrated previously in this system that glucose absorption increases as glucose delivery rates are increased toward saturating levels of about 200 pmol/min. However, for the studies depicted in Fig. 2, the inhibition of phosphate transport occurred and appeared to remain constant after glucose delivery rates of less than 30 pmol/min were achieved. The degree of reduction in phosphate absorption was thus not proportional to the rate of glucose transport which may be expected to increase with further increases in glucose delivery. Imai et al. (29) described apparently similar relationships for glucose delivery and sodium transport. Third, comparable increases in phosphate transport occurred when alanine was deleted from the perfusate even though amino acid transport rates, and probably energy requirements, may be expected to be considerably less than the rate of glucose absorption (40).

It also seems unlikely that phosphate absorption is linked directly to phosphorylation of transported glucose since phosphate transport decreased as glucose absorption increased (Fig. 1 and 2) and because phlorizin enhanced phosphate transport while reducing glucose absorption. Similarly, the effects of glucose and alanine on transepithelial electrical potential difference are not likely to be responsible, *per se*, because the presence of these two solutes in the perfusate is associated with lumen negativity (28, 29, 31) and this effect, if anything, would be expected to increase rather than decrease the driving forces for the movement of phosphate from the lumen to the bath.

More likely mechanisms relating phosphate transport to that of glucose and alanine may reside in the mutual dependence of these absorptive processes upon sodium transport. The present studies demonstrate clearly that the transport processes involved in the movement of glucose and of phosphate across the epithelium of proximal convoluted tubules are dependent upon some interaction with sodium. With regard to phosphate, the elimination of potassium from the bath, the reduction in intraluminal sodium concentration by replacement with choline, and the elimination of bicarbonate from the ambient fluids each resulted in marked decreases in net sodium and in phosphate transport. For glucose, elimination of potassium from the bath, reduction in intraluminal sodium concentra-

tion, and ouabain (19) each reduced glucose absorption rates. These observations extend previous microperfusion studies performed *in vivo*, demonstrating that the severe condition of replacement of sodium with choline in both the luminal and peritubular capillary fluids of the proximal tubule of the rat eliminates the active transport of glucose (37), phosphate (32), and certain amino acids (41). Collectively, the data seem sufficient to conclude that the absorption of glucose, phosphate, and probably amino acids by the proximal renal tubule occur via transport processes that are dependent, at least in part, upon some function of sodium transport. In this regard, however, a significant portion of glucose absorption may occur in the proximal tubule independent of sodium transport (Table IV).

Models of epithelial transport based on sodium dependence emphasized originally the important regulatory role of the chemical gradient for sodium maintained between the extracellular and intracellular microenvironment by the operation of a metabolically dependent, ouabain-sensitive sodium extrusion system located at the antiluminal or serosal surfaces of absorbing epithelia (11, 42). The maintenance of a low intracellular sodium concentration relative to the external medium is thought to serve as the major transducer between cellular metabolism and solute transport at the brush border. Any interference with factors maintaining this gradient would be expected to reduce the driving force and therefore the absorption rate of any solute dependent upon its intensity. Such interferences may be regarded as primary inhibitors of sodium transport and would include maneuvers such as elimination of potassium from the bath, ouabain, reduced availability of sodium in the lumen, and, presumably, the complete removal of bicarbonate, each of which has been shown in the present (Tables IV-VI; Fig. 3-5) or in related studies (18, 19) to inhibit sharply the proximal absorption of both phosphate and glucose.

In addition to the chemical gradient for sodium, however, more recent considerations emphasize that electrical potentials across the mucosal surface are also important determinants of the rate and direction of sodium-coupled transfers. Evidence has been accumulated (43, 44) and summarized by Schultz (45) indicating that for various absorbing epithelia, including the proximal tubule of the newt (44), the carrier-mediated, sodium-dependent entry processes for glucose and alanine result in the translocation of charge and are thus rheogenic or current generating. Such a situation is also likely to obtain in the proximal convoluted tubule of the rabbit, although definitive information in the form of transmembrane electrical potentials is lacking at this time for obvious technical reasons. Rheogenic entry processes not only contribute to the transmembrane electrical potential difference but are themselves affected by any changes in its intensity. Accordingly, any

change in the rheogenic entry of a solute such as glucose or alanine may enhance or deter the entry of any other solute such as phosphate which may be influenced by this electrical potential. Such interactions are referred to as electrical coupling as distinguished from chemical coupling such as occurs, presumably, in co-transport. Electrical coupling has recently been demonstrated between sodium-dependent glucose and alanine transfers across isolated membrane vesicles derived from brush-border material of the rat small intestine (15).

With regard to the present studies, electrical coupling between the sodium-dependent transport of glucose and of phosphate could explain the lack of direct proportionality between the extent of glucose transport and the degree of inhibition in phosphate transport (Fig. 2). Moreover, such a model would explain why electrogenic transport of glucose and alanine may inhibit phosphate transport whereas nonelectrogenic bicarbonate-dependent sodium transport does not. In essence, since the bicarbonate-dependent component probably represents sodium and hydrogen ion exchange (46), there is no opportunity for electrical coupling. Along these lines, it seems likely that the inhibition of both glucose and phosphate transport by the complete removal of bicarbonate reflects the degree of reduction in sodium transport rather than any specific effect of bicarbonate although it must be acknowledged that the mechanism whereby bicarbonate affects sodium transport in the proximal tubule remains unclear (46). The major point to be made is that electrical as well as chemical consequences of sodium transport appear to be involved in these various interactions.

It should be emphasized that the present studies are inadequate to define precisely whether these or other mechanisms may underlie the inhibition of phosphate transport by glucose and alanine. However, the present studies do demonstrate unequivocally that the transport of glucose and of phosphate by the isolated proximal convoluted renal tubule occurs, at least in part, via sodium-dependent processes. Primary and marked inhibition of sodium transport reduces the transport of both glucose and phosphate, and presumably other solutes, while the primary inhibition of specific sodium-dependent solutes may result in increases in the absorption of others by mechanisms that are suggested but still basically undefined.

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