Effect of Acute and Chronic Vanadate Administration on Sugar Transport in Rat Jejunum (42827)

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Abstract. **Vanadate is known to have an insulin-like action which stimulates sugar transport in some systems like adipocytes and muscle cells, but in other systems it inhibits sugar transport by decreasing the activity of (Na^+ +K^+)-ATPase. To evaluate whether these two opposing actions may influence sugar transport across the intestine, we studied the effects of acute and chronic vanadate administration on the uptake of glucose, galactose, and 3-O-methylglucose in isolated rat intestinal cells. The sugar uptake measurements were also coupled by determinations of rubidium-86 uptake as a measure of the activity of the Na-K pump. Both acute and chronic vanadate administration reduced rubidium uptake by the cells but the reduction did not uniformly influence the uptakes of the three sugars in question which were stimulated by the acute exposure of the cells to vanadate. Glucose uptake was also stimulated by chronic vanadate administration, but the uptakes of galactose and 3-O-methylglucose were respectively unaffected or inhibited by chronic vanadate. The findings suggest that the effect of vanadate on sugar transport is dependent on the net difference between two actions of vanadate: (i) stimulation of a receptor site (possibly an insulin receptor site) in the intestinal cell membrane and (ii) inhibition of the Na-K pump. During acute vanadate exposure, the stimulation of the receptor site was very likely a dominant feature which overwhelms the inhibition of the pump. Chronic exposure to vanadate led, on the other hand, to only a limited degree of stimulation of the receptor site and the inhibition of the Na-K pump became evident in the uptake measurements of galactose and 3-O-methylglucose. Glucose uptake, however, was stimulated by chronic vanadate ingestion due, very likely, to an increase in the metabolism of this sugar which occurred only with prolonged exposure of the rat intestine to vanadate.** [P.S.E.B.M. 1989, Vol 190]

There is an increasing interest in the trace metal vanadium because of its suggested role in the regulation of cell functions (1–3) through its varied actions on cell enzymes including the (Na^+ +K^+)-ATPases (1, 4–6). Vanadium is present in most mammalian tissues (1, 7) and its environmental concentration is reported to be increasing (8, 9). We have previously tested the effects of vanadate (pentavalent vanadium) on water and electrolyte transport across the rat jejunum (10, 11) and found evidence to suggest the presence of a dual action of vanadate on the rat intestine. At low concentrations, vanadate stimulated water and electrolyte secretion by increasing the activity of adenylate cyclase, but at higher concentrations it decreased absorption by inhibiting the intestinal membrane (Na^+ +K^+)-ATPase. To further evaluate the effects of vanadate on the intestine, we tested, in the present study, the effects of acute and chronic vanadate administration on sugar absorption in the rat. Vanadate has been reported to have varied effects on sugar transport. It acts like insulin in stimulating the transport of glucose (12–15) and it can, like ouabain (16) inhibit sugar transport by its inhibitory action on the activity of (Na^+ +K^+)-ATPase (1, 5, 6, 10, 11). These effects, however, were observed when vanadate was administered acutely and in relatively large concentrations. It is unclear at present what effects vanadate may have on the intestine when it is administered over a prolonged period of time and with concentrations that could resemble those that occur with pollution.

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Materials and Methods

Animal Feeding. Weanling male Sprague-Dawley rats were housed under controlled conditions with a

constant temperature (24°C) and a 12:12-hr light-dark schedule. They were fed Ralston Purina rat chow and water *ad libitum* until their weights were about 200 g when they were randomized into groups: a control and four vanadate-fed groups. The control group was given the chow diet and water *ad libitum* and the other four groups were given vanadate (orthovanadate; Pflatz and Bauer Inc., Stamford, CT) through drinking water in 38, 75, 150, and 300 ppm concentrations. To prevent polymerization, the pH of the administered solution was adjusted to 7. The rate of growth of the rats and their food and fluid intakes were monitored during 30 days of this regimen. At the end of this period the rats were tested in the experiments that are described below. The acute effect of vanadate was also tested on control rats that weighed between 250 and 300 g, about the same weight as that of the rats that were chronically given vanadate (75 ppm) for a period of 1 month.

Isolation of Intestinal Cells and Uptake Studies. Intestinal villus cells were isolated using a modification of the methods of Weiser (17) and Wahawisan *et al.* (18). The rats were killed by cervical dislocation and segments of jejunum (30-cm long) were resected and flushed free of their intestinal contents by rinsing them in isotonic saline solution. The segments were everted and filled to distention with saline and incubated in a solution containing 96 m*M* NaCl, 1.5 m*M* KCl, 27 m*M* Na citrate, 8 m*M* KH_2PO_4, and 5.6 m*M* Na^2HPO_4 (pH 7.4, t 25°C). After 15 min of incubation the segments were removed and placed in an EDTA buffer containing 149.5 m*M* NaCl, 3 m*M* K_2HPO_4, 0.6 m*M* NaH_2PO_4, 1.5 m*M* EDTA, and 1 m*M* dithiothreitol (pH 7.4, t 25°C). Incubation in this buffer was continued for 4 min during which the segments were shaken gently to dislodge their cells. The segments were then removed and placed in a similar but fresh solution for another 4-min period. Three successive incubations were done and their collections of mainly villus cells (18) were pooled together and passed through a 130-μm nylon mesh (TETKO, HC3-10). The filtrate was then centrifuged at 900*g* for 5 min and the pellet resuspended and washed twice with Tris-buffered saline solution containing 110 m*M* NaCl, 5 m*M* KCl, 20 m*M* Tris base, 10 m*M* mannitol, and 5 m*M* L-glutamine (pH adjusted to 7.4 with 1 *N* HCl, t 35°C). A villus cell preparation was obtained with a 75–80% viability as determined by trypan blue exclusion. The effect of the acute exposure of the cells to vanadate was tested by preincubating the cells for 30 min in the Tris buffer solution containing vanadate.

Uptake was started by adding aliquots of the cell suspension to test solutions that contained the Tris buffer, a labeled sugar, and Ca^{2+} and Mg^{2+} to make the concentration of these divalent cations 1.2 m*M* in the final solution. The usual cell count of the final mixture was about 10^6 cells/ml about equivalent to 1.5 mg protein/ml. The cells were mixed thoroughly with the test solution during a period of 1 min, after which 10 volumes of cold nonlabeled buffer were added to end the uptake process by diluting the isotope and cooling the cells. The cell mixture was then filtered through Whatman GF/B filter papers using a Hoeffer filtration system. The filters were washed three times with the buffer and their isotopic label (mostly intracellular ^{14}C-sugar) was determined by liquid scintillation. In preliminary experiments, [^{14}C]inulin was added to the test solution and the extracellular space of the washed cells was found to be less than 1% of the cell counts, a value that was considered negligible and no extracellular markers were therefore used in all of the subsequent experiments. Three labeled dextro sugars were examined: glucose, 3-*O*-methylglucose (3-*O*-MeGlc), and galactose (New England Nuclear Research Products-Dupont, Boston, MA). Fluxes were determined per protein content of the cell mixture as determined by the Lowry method (19).

Rubidium Uptake. The activity of the intestinal Na-K pump in the control and vanadate-fed rats was compared by measuring the cellular uptake of rubidium, a K substituent. Cells isolated as described above were preincubated for 10 min in the presence of one of the three sugars and then tested by exposing them for 60 sec to a solution containing 1 m*M* rubidium chloride and tracer quantities of ^{86}Rb. In preliminary experiments, the uptake of the labeled rubidium was linear within the first 90 sec of exposure to the isotope. The amount of isotope reaching the cells during the initial 60-sec period determined the initial rate of uptake of ^{86}Rb which is considered an adequate estimate of the pump activity of intact cells.

Determination of Glucose Metabolism. The rates of glucose metabolism by the vanadate-fed and the control rat intestines were determined according to the method of Mallet *et al.* (20). Mucosal sheets of intestinal cells were obtained as described previously (21) by scraping the intestinal mucosal surface with a glass slide. The sheets were then incubated in Tris-buffered isotonic saline solution (37°C, pH 7.4) that was equilibrated under 100% oxygen. Aliquots of the mucosal cell suspensions were placed in Erlenmeyer flasks that contained central wells containing 0.5 ml of Hyamin. The flasks were incubated in a Dubnoff shaker and stirred at a rate of 1 oscillation/sec. After a period of equilibration of 10 min, [^{14}C]glucose (uniformly labeled) was added to the cell suspension and the preparation was incubated for 30 min, after which the flasks were cooled in ice for a period of 2 hr before the contents of the wells were collected and counted by liquid scintillation. The cell suspension was centrifuged at 27,000*g* and the isotope in the supernatant fluid was determined. The pellet was dried in an oven at 90°C for 12 hr and its weight was determined. The counts (cpm) were corrected to disintegrations per min (dpm) using a quench correction curve. The $^{14}CO_2$ liberated

during a 30-min period was considered as a measure of glucose metabolism. In preliminary experiments carbon-labeled CO_2 liberation as a function of time of incubation was linear up to 60 min. This agrees with what was observed by Mallet *et al.* (20) and the 30-min period of incubation chosen for the present studies was representative of the catabolic rate of glucose.

Statistical Analyses. The data presented are expressed as mean ± SE. Comparisons between various experimental and control groups were made using Student's *t* test.

Results

Growth Pattern and Intestinal Weights. As shown in Table I the food intake and growth rates of the rats tested during 30 days of vanadate ingestion were about equal in the control rats and in the rats given 38 or 75 ppm vanadate concentrations. This however, was not the case in the rats that were fed the higher concentration of vanadate. Their food intake was relatively poor and they failed to grow as much as the controls. Twenty per cent of the rats fed the 300 ppm vanadate concentration died before the completion of the feeding period and all rats of this group were therefore excluded from the study. The group fed the 150 ppm vanadate concentration was also excluded because of their nutritional deficiency and their failure to gain weight during the feeding period. Differences in intestinal weights were also noted between the control and the vanadate-fed groups. The wet weights of three of the vanadate-fed groups were statistically increased in comparison to the controls but the dry weights of the intestine were about equal in all five groups. The ratio of the wet to dry weights, which is representative of the volume of the intestinal cells, was increased in all of the animals that were fed the higher concentration of vanadate (75, 150, and 300 ppm) but not in the animals given the 38 ppm concentration. The latter group was excluded because the low dose of vanadate ingested produced no changes in intestinal cell water as compared with controls. All of the subsequent studies were done on animals that were given the 75 ppm concentration because their nutrition was comparable to that of the controls and they exhibited changes in their intestinal weights.

Uptake of Sugars by Intestinal Cells. The uptake of glucose, galactose, and 30-MeGlc was tested in cells that were acutely or chronically exposed to vanadate. In preliminary experiments uptake of labeled 3-*O*-MeGlc was measured as a function of time. During the initial 60 sec, the uptake was linear, suggesting that the loss of tracer from the cells was negligible and the uptake rate was very likely a true measure of influx into the cell. In all of the subsequent studies, uptake was therefore measured after a period of 60 sec of exposure to a labeled sugar. The limitation of this period to only 60 sec was also intended to limit the metabolism of glucose and galactose and to reduce the dissociation of the isotopic label from the sugars. The acute effect of vanadate on glucose uptake was also tested in preliminary studies to determine the concentration of vanadate needed to produce a significant response in sugar transport. These studies, which are shown in Table II, point out that a 10^{-3} *M* vanadate concentration produces the most significant increase in glucose uptake and this concentration was therefore used in all of the studies performed. As shown in Figure 1, acute and chronic vanadate administration caused different effects on glucose, galactose, and 3-*O*-MeGlc uptakes. As compared with the control untreated rats, acute vanadate administration enhances the uptake of all three sugars tested whereas chronic vanadate administration causes an increase in glucose uptake but no changes or a small decrease in galactose uptake and a decrease in 3-*O*-MeGLc uptake. Vanadate, acutely or chronically administered, enhanced sugar uptake by increasing the

Table II. The *In Vitro* Acute Effect of Vanadate on Glucose Uptake by Intestinal Cells

Vanadate concentration (*M*)	Glucose uptake (μmoles/min/mg protein)
0	0.31 ± 0.03[a]
10^{-5}	0.34 ± 0.03
10^{-4}	0.51 ± 0.05[b]
10^{-3}	0.75 ± 0.08[c]

[a] Results are mean ± SE of 12 observations in six rats.
[b] $P < 0.01$.
[c] $P < 0.001$ compared with the control in the absence of vanadate.

Table I. Characteristics of the Rat Groups Studied

	Control (0)	Vanadate fed (ppm)			
		38	75	150	300
Vanadate intake (mg/day)	0	2.2 ± 0.2[a]	5.0 ± 0.6	9.2 ± 1.2	14.6 ± 3.7
Caloric intake (cal/day)	54.3 ± 3.7	56.6 ± 4.7	53.6 ± 4.7	37.1 ± 4.9	32.4 ± 3.8
Body weight gain (g/day)	2.90 ± 0.22	2.93 ± 0.24	2.32 ± 0.31	1.61 ± 0.24[b]	0.74 ± 0.10[c]
Wet weight of intestine (g)	4.58 ± 0.17	4.64 ± 0.19	6.87 ± 0.61[b]	6.67 ± 0.58[b]	5.32 ± 0.31[b]
Dry weight of intestine (g)	1.31 ± 0.18	1.28 ± 0.17	1.26 ± 0.06	1.11 ± 0.10	1.07 ± 0.09
Intestinal wet:dry weight ratio	3.54 ± 0.33	3.63 ± 0.34	5.43 ± 0.44[b]	6.01 ± 0.45[b]	4.96 ± 0.19[b]

[a] Results are means ± SE of eight rats.
[b] $P < 0.01$ as compared with the respective controls.
[c] $P < 0.001$ as compared with the control.

maximal velocity of transport (J_{max}) without changing the half-maximal saturation constant (K_t). The inhibition of 3-*O*-MeGlc and possibly of galactose that occurred with chronic vanadate ingestion was due to an increase in both K_t and J_{max}. This inhibition was, furthermore, more evident with 3-*O*-MeGlc than with galactose and the percentage of increase in K_t was respectively higher.

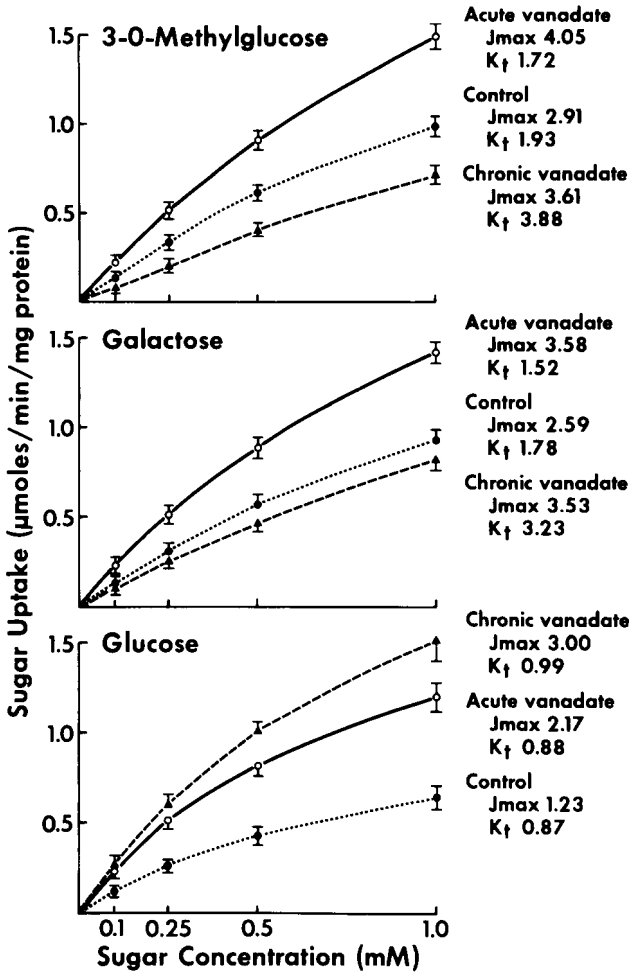

Figure 1. Comparison of the effects of acute and chronic vanadate administration on glucose, galactose, and 3-*O*-methylglucose uptake by intestinal cells. Results are means ± SE of determinations in eight rats. Lineweaver-Burk plots were used to derive the kinetic constants. The curves were drawn using the indicated constants.

To compare the significance of the difference in the kinetic parameters of the sugar uptake studies, the kinetic characteristics of the data shown in Figure 1 were calculated using the logarithmic transformation method of Barber *et al.* (22). This method allows for a determination of the variance of the kinetic constants and of the maximal velocities. The result of these studies are shown in Table III and they clearly indicate that acute vanadate increases the uptakes of the three sugars studied by significantly increasing J_{max} without changing K_t. Chronic vanadate on the other hand produces no significant changes in the transport characteristics of 3-*O*-MG or galactose but it increased the J_{max} of glucose uptake.

Rubidium Uptake. Table IV summarizes the results of the uptake of rubidium by intestinal cells. Under control conditions rubidium uptake was not different in cells that were preincubated with any of the three sugars tested and was not different between cells preincubated in the presence or absence of the sugars. Both acute and chronic exposure to vanadate caused a decrease in rubidium uptake, the decrease being of about the same magnitude in both the acute and chronic conditions. The presence of the sugars glucose, galactose, or 3-*O*-MeGlc did not statistically alter the rate of uptake of ^{86}Rb, although the inhibition seemed to be of a lesser degree when the cells were preincubated in glucose than in any of the other two sugars (the difference though was not statistically significant).

Glucose Metabolism. The metabolism of glucose was compared between the intestinal tissues that were exposed to vanadate and in tissues of the control animals. As shown in Table V $^{14}CO_2$ production from [^{14}C]glucose was not affected by the acute exposure of the intestinal cells to vanadate but chronic administration of vanadate caused a significant increase in the

Table III. The Kinetic Characteristics of Sugar Uptake in Intestinal Cells[a]

	K_t	J_{max}
3-*O*-Methylglucose		
Control	1.87 ± 0.39	2.82 ± 0.27
Acute	1.67 ± 0.33 (NS)[b]	3.93 ± 0.37 ($P < 0.01$)
Chronic	3.76 ± 0.62 (NS)	3.50 ± 0.39 (NS)
Galactose		
Control	1.73 ± 0.35	2.51 ± 0.30
Acute	1.47 ± 0.28 (NS)	3.47 ± 0.36 ($P < 0.05$)
Chronic	3.13 ± 0.60 (NS)	3.42 ± 0.39 (NS)
Glucose		
Control	0.84 ± 0.18	1.19 ± 0.09
Acute	0.85 ± 0.17 (NS)	2.10 ± 0.17 ($P < 0.01$)
Chronic	0.96 ± 0.21 (NS)	2.91 ± 0.23 ($P < 0.001$)

[a] The kinetic parameters of data in Figure 1 calculated with the logarithmic transformation method of Barber *et al.* (22).
[b] NS, not significant.

Table IV. Effect of Acute and Chronic Vanadate Administration on the Uptake of ^{86}Rb by Intestinal Cells[a]

Preincubation with	Uptake (μmoles/min/mg protein)			
	Glucose	Galactose	3-*O*-MeGlc	No sugar
Control	1.39 ± 0.12	1.42 ± 0.11	1.58 ± 0.13	1.53 ± 0.12
Acute vanadate	1.03 ± 0.08[b]	0.83 ± 0.06[b]	0.86 ± 0.06[b]	0.81 ± 0.05[b]
Chronic vanadate	0.91 ± 0.07[b]	0.81 ± 0.04[b]	0.86 ± 0.05[b]	0.83 ± 0.04[b]

[a] Results are mean ± SE of 12 determinations in six rats.
[b] Differs from the respective control values, $P < 0.01$. There were no significant differences between the uptake values in the presence of the various sugars.

Table V. The Effect of Vanadate on $^{14}CO_2$ Production from [^{14}C]Glucose Metabolism

	dpm/mg dry weight	Statistical significance
Control	178 ± 18[a]	
Acute vanadate		
10^{-4} *M*	167 ± 18	NS[b]
5×10^{-4} *M*	150 ± 17	NS
10^{-3} *M*	211 ± 23	NS
Chronic vanadate (75 ppm)	368 ± 41	$P < 0.001$

[a] Values are mean ± SE of eight determinations. Data are normalized for incubation of mucosal strips with uniformly labeled 1 μCi [^{14}C] glucose for 30 min.
[b] NS, not significant.

rate of glucose metabolism as evidenced by the prominent rise of $^{14}CO_2$ liberation under this condition.

Discussion

The results presented suggest that vanadate may have two effects on sugar transport in the intestinal cell: an inhibitory effect that is due to a reduced activity of the membrane (Na^+ +K^+)-ATPase and a stimulatory, insulin-like effect that activates a sugar transport site in the cell membrane. The degree of inhibition or stimulation by vanadate seems however, to vary with the sugar that is being transported and whether vanadate is administered acutely or chronically. Vanadate is recognized as a potent inhibitor of (Na^+ +K^+)-ATPase in many tissues (1, 4–6), including the intestine (10, 11). In the present studies, there were several findings to suggest that intestinal (Na^+ +K^+)-ATPase was inhibited by both acute and chronic vanadate administration. We have shown previously that the rat intestinal (Na^+ +K^+)-ATPase is inhibited by acute exposure to a 10^{-3} *M* solution of vanadate (11). A similar inhibition of the Na-K pump must occur with chronic exposure to vanadate. In favor of this conclusion is the fact that rubidium influx decreased equally after treatment with acute and chronic vanadate. Moreover, chronic vanadate administration to rats caused a significant swelling of the intestinal cells which is similar to that observed when the intestine is exposed to ouabain (21). Chronic vanadate also decreased galactose and possibly 3-*O*-MeGlc uptake by a mechanism similar to that observed with other (Na^+ +K^+)-ATPase inhibitors such as ouabain, cyanide, dinitrophenol, and iodoacetate (16).

The effects of vanadate on glucose, galactose, and 3-*O*-MeGlc transport reflect to some degree the influence of the oxyanion on the Na-K pump but they also reveal another effect of the anion on sugar transport. In the rat gut sac preparation, Tolman *et al.* (23) found that vanadate causes a decrease in glucose uptake from the mucosal side of the intestine and a decrease in the transfer of glucose across the serosal side of this tissue. These findings, which are explainable on the basis of inhibition of (Na^+ +K^+)-ATPase, do seem to be at variance with the present results which revealed a stimulatory effect of acute vanadate administration on glucose influx into the intestinal cells. The gut sac preparation experiments that are described by Tolman *et al.* (23) measured, however, the effect of vanadate on the net movement of glucose across the gut wall. These measurements are the resultant of at least two unidirectional fluxes across the epithelium and they could be unaffected by the influx of glucose into the intestinal epithelial cell. Furthermore, in the gut sac experiments, incubation of the intestine with glucose is carried out for a longer period of time (60 min) than in the present experiments (60 sec). A difference in the degrees of metabolism of glucose must exist between these two types of experiments and that could be responsible, at least in part, for the observed difference in the effect of vanadate on glucose transport.

The stimulatory effect of vanadate on sugar transport is revealed in the results of the experiments that tested the acute effect of vanadate on sugar uptake. Vanadate enhanced the uptakes of glucose, galactose, and 3-*O*-MeGlc, an effect that is similar to the effect of insulin on sugar transport (12–15). Chronic exposure of the intestine to vanadate increased only the uptake of glucose, but not of galactose or 3-*O*-MeGlc. Two possible explanations can be considered in explaining these results. One explanation assumes that the difference in the metabolism of the sugars in question may have an influence on the response of the cell to vanadate. Glucose, which is known to be well metabolized by the intestine, is readily stimulated by vanadate, whereas galactose which is only minimally metabolized, and 3-*O*-MeGlc which is not metabolized at all are not

as well stimulated. Our studies with $^{14}CO_2$ liberation from labeled glucose support this explanation.

The observed action of vanadate on sugar entry into the cells could also be explained on the basis of the dependence of the uptake process on two factors: the Na gradient across the cell membrane which is controlled by $(Na^+ + K^+)$-ATPase and the degree of binding of the sugar to the carrier site of the membrane. The stimulatory effect of vanadate on sugar uptake by the intestinal cells is similar to what has been observed in other tissue cells such as fat (15, 23–26), muscle (26), and liver (23) and it appears to be of a nature similar to that of insulin. Insulin has been reported to have no effects on sugar absorption in man (27) but in the everted intestinal sac preparation it causes an increase in glucose adsorption (28). Similarly, in studies of the isolated rabbit intestine in an Ussing chamber, insulin increased the net absorption of 3-*O*-MeGlc (29). Vanadate, like insulin, may stimulate the phosphorylation of the insulin receptor of cell membranes (12–15). In favor of this theory is the finding, in the present studies, of a vanadate-induced increase in J_{max} with a minimal change in K_t. These kinetic changes are similar to what was observed by many investigators (30–33) who found that insulin increases the J_{max} of glucose uptake without changing the K_t. Such changes indicate that vanadate, like insulin, increases the number of carrier sites in the membrane. This could account for the observed increase in sugar uptake that was observed with acute exposure of the intestine to vanadate. The chronic effect of vanadate may also be related, as suggested by Clark *et al.* (33), to differences in the phosphorylation of the insulin receptor. Glucose may enhance the phosphorylation more than galactose or 3-*O*-MeGlc, particularly since its metabolism is increased. The possible relationship, however, between the degree of phosphorylation and the catabolism of glucose remains to be verified further.

Finally, the results of the rubidium uptake studies suggest that vanadate decreases in all instances the activity of the $(Na^+ + K^+)$-ATPase. This indicates that what determines the nature of the effect of vanadate on sugar transport into the cell is the resultant of two opposing actions: a decrease in the pump activity and an increase in stimulation of the insulin receptor site. In the present study the net effect of acute vanadate on these two opposing actions produced a stimulation of the uptake of the three sugars into the intestinal cell. Chronic vanadate, on the other hand, caused an increase in glucose uptake and a minimal to moderate decrease in galactose and 3-*O*-MeGlc uptakes.

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