Metabolic Requirements for Sodium Transport in the Rat's Ileum[1] (36147)

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It is well known that sodium (Na) is necessary for the absorption of actively transported hexoses in the intestine (1), and since the latter enhances Na absorption (2) this phenomenon appears to be mutually dependent. Most investigators believe that glucose stimulates net Na transport (Q_{Na}) because it either is metabolized (3) or actively transported regardless of whether or not it is metabolized (4). Many reports have appeared which indicate that the energy for Q_{Na} is produced by glycolysis (5, 6), Krebs cycle activity (7) and/or oxidative phosphorylation (8). Many of these conclusions, however, were derived from studies using metabolic inhibitors which are known to have nonspecific effects. The present *in vitro* study was designed to determine the metabolic requirements for Q_{Na} in the rat's ileum. This was accomplished by studying Q_{Na} in the presence of substances which may or may not be metabolized by the ileum.

Methods. Everted ileal sacs were prepared from nonfasted male Holtzman rats, weighing 280–300 g, and mounted on lucite cannulae according to a previously described procedure (9). The sacs were suspended in tubes containing 50 ml of Krebs–Ringer bicarbonate solution (mucosal solution) and incubated at 37° (10). Aerobic conditions were maintained by aerating the solutions with 95% O_2 and 5% CO_2, while 95% N_2 and 5% CO_2 were used for anaerobic conditions. The bubbling of CO_2 through the solutions maintained the pH at 7.4. This procedure, which utilized a cannulated sac, permitted the study of transport in the same segment over several sequential periods of 1 hr. One ml of this solution containing 175 mg inulin/100 ml as a water marker was placed in the serosal sac (serosal solution). Both mucosal and serosal solutions were changed at the end of each hourly period. During the hour of preincubation and in all subsequent hours, unless otherwise noted, 10 m*M* D-glucose was present in both solutions. When galactose, pyruvate, mannitol and glycerol (all 10 m*M*) were used, they replaced glucose. The sodium salt of pyruvate replaced sodium chloride and the ionic concentration was not altered except for chloride. Mannitol was used to balance the solutions osmotically. Since it was shown in a previous report (11) that steady-state conditions were not attained until the end of 1 hr of incubation, the present investigation used the succeeding 2 hours to study Q_{Na}.

Some question may arise whether some of these substances can enter the cells and be metabolized. Therefore, in a series of cannulated everted sacs different radioisotopes were added to the mucosal solution at the beginning of the second hour, and total activity of the initial and final mucosal and serosal solutions was ascertained. Concentrations of 0.05 μCi/ml of D-glucose-U-^{14}C, D-galactose-1-^{14}C, glycerol-U-^{14}C or Na pyruvate-U-^{14}C were used. In another series, ileal segments were everted but not cannulated and then preincubated in Krebs–Ringer phosphate solution aerated with 100% O_2 at 37° for 1 hr. They were then placed in a 50 ml Erlenmeyer flask containing 10 ml of the Krebs phosphate solution with 0.05 μCi of D-glucose-U-^{14}C, glycerol-U-^{14}C, Na pyruvate-U-^{14}C, D-glucose-1-^{14}C or D-glucose-6-^{14}C. After aerating the contents of the flask with 100% O_2, a CO_2 trap was attached. This consisted of a wide mouth scintillation vial containing filter pa-

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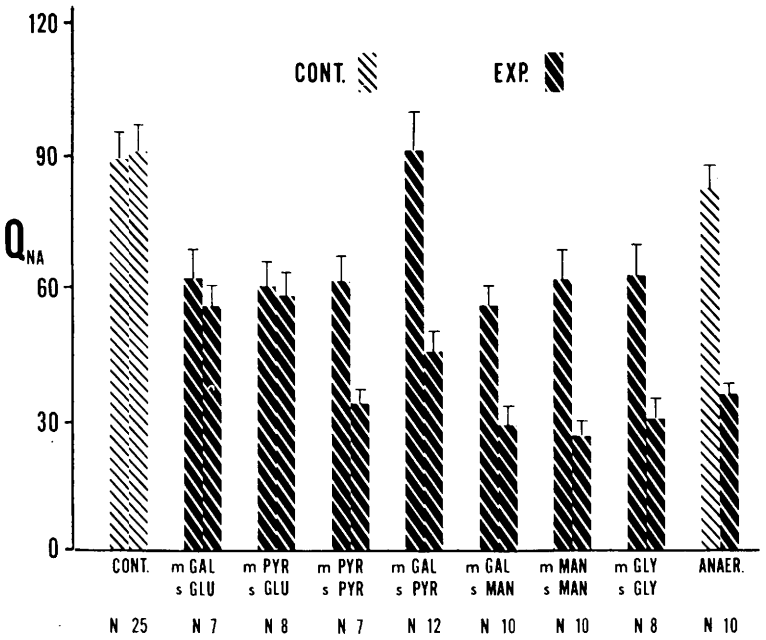

FIG. 1. Comparison of the effects of different substrates on the net transport of sodium (Q_{Na} = μEq/hr/sac), m =mucosal solution, s = serosal solution, CONT. = control, EXP. = experimental, GLU = glucose, PYR = pyruvate, GAL = galactose, MAN = mannitol, GLY = glycerol, ANAER. = anaerobic, N = no. of animals. $p < .001$ for all hours of all experimental groups except the first hour of gal–pyr ($p > .80$) when compared to the control group. First bar of pair represents the first hour and the second bar represents the second hour.

per wetted with 0.6 ml of 1 *N* NaOH and attached to the flask with a 3.5 cm section of 2 cm diam latex tubing. The flasks were incubated at 37° in a Dubnoff shaker for 1 hr. Scintillation fluid (0.1% PPO and 0.005% POPOP in 1:1 toluene:2-methoxyethanol) was added directly to the vial serving as the CO_2 trap and the radioactivity was determined. The total activity of aliquots of the initial and final media also were determined. Corrections for quenching were made with the external standard ratio and activity was counted to an error of 1% with a Beckman LS100 liquid scintillation counter.

Na was determined by flame photometry (IL model 143) and inulin by a colorimetric method (12). Glucose was determined with the Glucostat method on protein-free filtrates (13). Inulin, which is neither absorbed nor metabolized by the rat's ileum, was used to assay the net transport of fluid. The use of inulin as a marker in this method has been reported by Clarkson and Rothstein (9) and the calculations are as follows:

$$Q_{Na} = \left[\frac{I_i}{I_f} \times Na_f\right] - Na_i = \mu E_q/hr/sac.$$

I_i = initial (zero time) inulin conc (mg%) in 1 ml of serosal solution placed in sac; I_f = final (60 min) inulin conc (mg%) in volume of serosal solution after 1 hr; Na_f = μEq of Na/ml of serosal solution after 60 min; Na_i = μEq of Na/ml of zero time serosal solution.

All values are expressed as mean ± standard error and statistical significance was computed according to the Student *t* test.

Results. The mean net transport of Na (Q_{Na}) observed in preparations from 25 animals during the 2 hr period following the preincubation period is illustrated in Fig. 1. Glucose was present in both bathing solutions during both hours. In this control group the 3% difference between the 2 hourly periods was not significant ($p > .90$) and hence was

TABLE I. Development of Sodium Concentration Differences (D_{Na}) Between Final Serosal and Mucosal Solutions.

Substrate[a]		mEq Na/liter		P values[c]	
M	S	First hr	Second hr	First hr	Second hr
glu	glu	9.0 ± 1.1[b]	6.8 ± 1.0	–	–
gal	glu	7.9 ± 0.8	6.2 ± 0.6	$> .05$	$> .05$
pyr	glu	11.4 ± 1.0	12.4 ± 1.1	$< .005$	$< .005$
pyr	pyr	4.5 ± 0.7	2.5 ± 0.3	$< .001$	$< .001$
gal	pyr	3.0 ± 0.9	0.3 ± 0.7	$< .001$	$< .001$
gal	man	1.5 ± 0.9	0.0 ± 0.7	$< .001$	$< .001$
man	man	4.9 ± 1.2	1.0 ± 1.0	$< .001$	$< .001$
gly	gly	2.7 ± 1.1	0.6 ± 1.0	$< .001$	$< .001$
anaer		10.0 ± 0.8	0.6 ± 0.4	$> .05$	$< .001$

[a] M = mucosal solution, S = serosal solution, gal = galactose, glu = glucose, pry = pyruvate, man = mannitol, gly = glycerol, anaer = anaerobic.

[b] = Mean ± SE.

[c] = Significance of difference from controls (glu-glu).

considered the control for the other 7 groups in which glucose was replaced by various substrates in both mucosal and serosal solutions. The replacement of glucose with other substrates in the mucosal solutions in the first hour significantly decreased Q_{Na} ($p < .001$) in all groups except when galactose was placed on the mucosal side and pyruvate on the serosal side. The Q_{Na} of these 6 groups was depressed from control levels by approximately 35% ($p < .001$) in the first hour and there were no significant differences among the various groups.

When everted sacs with substrates other than glucose, in both mucosal and serosal solutions, were continued through the second hour further significant decreases ($p < .001$) in Q_{Na} occurred in 4 groups (Fig. 1). This occurred in groups with the same substrate on both sides (pyruvate, mannitol and glycerol) and in the group with galactose and mannitol. In 2 groups with glucose on the serosal side and with either pyruvate or galactose on the mucosal side, Q_{Na} was the same in both the first and second hour. There was no significant difference between these 2 groups. The Q_{Na} of the galactose (mucosal)–pyruvate (serosal) group decreased 50% in the second hour; however, the Q_{Na} during the second hour was not significantly different from the 2 groups with only glucose on the serosal side, but was markedly different ($p < .001$) from those without glucose. Anaerobic conditions depressed Q_{Na} 65% in the second hour and this decrease was not different from the group with mannitol on both sides.

In the control sacs a difference in Na concentration (D_{Na}) developed between the solutions bathing the 2 sides of the tissue. The serosal concentration of Na was 6.8–9.0 mEq Na/liter greater than the mucosal concentration after 1 hour incubation (Table I). This difference increased further during the second hour ($p < .005$) when pyruvate replaced glucose on the mucosal side. On the other hand, it decreased significantly in all groups ($p < .05$) or was abolished when glucose was absent from both solutions. It was also abolished by anaerobic conditions.

The movement of labelled substrates and their metabolites into the everted sacs is summarized in Table II. Galactose movement was greater with pyruvate than with glucose

TABLE II. Transport of Substrates and Their Metabolites into Serosal Sacs During the First Hour.

Substrate[a]			Transport rate (μmoles/g wet wt/hr)	
M	S	L	into sac	from mucosal side
glu	glu	glu	9.1 ± 2.3[b]	
gly	gly	gly	26.8 ± 2.3	
pyr	pyr	pyr	30.4 ± 4.3	
gal	pyr	gal	2.0 ± 0.25	22.4 ± 0.30
gal	glu	gal	1.6 ± 0.16[c]	11.6 ± 0.06[d]

[a] M = mucosal solution, S = serosal solution, L = substrate with label on mucosal side. Other symbols same as Table I.

[b] Mean ± S.E. for 6 animals in each group.

[c] Not significantly different from preceding group (gal–pyr).

[d] Significantly ($P < .05$) different from preceding group (gal–pyr).

on the serosal side. The appearance of more galactose in the serosal sac with pyruvate as the substrate was not significantly different from that observed when glucose was present, but the disappearance of galactose from the mucosal solution was significantly ($p < .05$) greater with pyruvate. Glycerol and pyruvate movement into the sacs with their metabolites was greater than glucose and its metabolites. The CO_2 production from labelled substrates (Table III) was greatest with pyruvate; however, more glycerol was metabolized to CO_2 than glucose and the C-1/C-6 ratio was 2.91.

The final serosal glucose concentration in control sacs was 57.2 ± 6.2 mg/100 ml, and 0.92 ± .12 mg glucose remained of the initial 2.0 mg in 1 ml (10 m*M*) placed in 13 sacs.

Discussion. The Q_{Na} by these everted sacs was similar in both the first and second hour of the control experiments, that is when glucose was present on both serosal and mucosal sides. The ability of these preparations to transport Na indicated that functional deterioration had not occurred during this time. The preincubation hour was not used since it had been previously shown that, insofar as the Q_{Na} is concerned, the preparation had not yet attained a steady state (11). When glucose was replaced on the mucosal side only, Q_{Na} decreased in the first hour and remained at this depressed level during the second hour. Whenever glucose was replaced on both sides, the Q_{Na} during the first hour was similar to that seen in groups when glucose was replaced on only the mucosal side; however, in the second hour Q_{Na} decreased even further. This undoubtedly is due to the accumulation of glucose in the tissue during the preincubation period and its subsequent metabolism after removal of this hexose from the bathing medium (8). This suggests that intracellular stores of glucose sustain the level of Q_{Na} in the first hour when glucose is removed from the bathing solutions and comparisons of Q_{Na} must be made on second hour data when tissue stores of glucose would be largely depleted.

If Krebs cycle activity provided the energy for this increment of Q_{Na}, pyruvate would supply it, providing that it can enter the cell and be metabolized. Pyruvate did not support Q_{Na} any better than mannitol which is not metabolized by the ileum. The pyruvate-U-^{14}C study showed that it can enter the cell and be metabolized. Although this tissue consists of a heterogenous cell population, others have shown that the contribution to metabolism by other than epithelial cells is minimal (14). It is possible that the decreased Q_{Na} may be due to an increased serosal to mucosal flux of Na. This could occur following tissue deterioration in the ab-

TABLE III. Metabolism by Ileal Tissue of Substrates to Carbon Dioxide.

Substrate	CO_2 Production
Glucose-U-^{14}C	.179 ± .02[a]
Glucose-1-^{14}C	.224 ± .01
Glucose-6-^{14}C	.077 ± .01[b]
Glycerol-U-^{14}C	.278 ± .04[b]
Pyruvate-U-^{14}C	.521 ± .02[b]

[a] Mean ± SE μmoles of substrate metabolized/g wet wt/hr.

[b] Significantly different ($P < .001$) from Glucose-U-^{14}C.

sence of utilizable substrate. Others, however, have shown that without substrates the absence of a net flux of Na was the result of a decrease in the mucosal to serosal flux, and there were no significant differences in the serosal to mucosal fluxes whether or not glucose was present in the rat's ileum (4). Glycerol, which is shown here to readily enter the cell and be metabolized, also did not sustain Q_{Na} when compared with the mannitol group. Glycerol kinase is present in this tissue (15) and it is suggested that after glycerol is phosphorylated it enters the glycolytic pathway and is oxidized producing one half of the adenosine triphosphate as an equimolar amount of glucose. This evidence, that glycolytic activity does not provide the energy for the increment of Q_{Na} dependent upon exogenous substrates, is supported by other findings. Glycolytic activity with the formation and transfer of lactate is not depressed by anaerobic conditions (16, 17), yet the Q_{Na} under anaerobic conditions was reduced to levels similar to those when exogenous substrates were removed for 2 hr (mannitol–mannitol group in hour 2). Again, this decrease in net flux cannot be attributed to an increase in the passive serosal to mucosal flux (increased tissue permeability) since it has been shown that anaerobic conditions do not increase this passive flux (8). All of these findings suggest that glycolysis, Krebs cycle activity, and oxidative phosphorylation do not provide the energy for glucose dependent Q_{Na} in the rat's ileum. In this study the glucose dependent increment is at least 70% of the total. The Q_{Na} requirements of a glucose dependent and oxygen dependent metabolic pathway could be satisfied with hexose monophosphate shunt activity. The C-1/C-6 ratio of 2.91, which is similar to that reported by others (18), indicates that more than half of the glucose entering the cell may be metabolized in the hexose monophosphate shunt. However, it has been shown that approximately one-third of the glucose entering the cell ends up as lactic acid (19). The remaining two-thirds which can be metabolized to CO_2 still allows for an active shunt pathway to be present.

The reciprocal dependence of Q_{Na} and actively transported hexoses was investigated with glucose and galactose, both of which are actively transported but only glucose is metabolized. In these 2 groups, glucose on the serosal side was the only exogenous source of energy for Q_{Na}. One group had galactose on the mucosal side while the other had pyruvate, which as stated earlier did not support the net transport of Na. The Q_{Na} was the same in both groups suggesting that any requirement of an actively transported hexose for Q_{Na} is minimal since pyruvate cannot serve this purpose. In these 2 groups Q_{Na} is supported equally in both the second and first hour in contrast to the other experimental groups. Glucose placed in the serosal sac was available for metabolism since it decreased over 50% in the control group. This suggests that glucose availability is the factor limiting Q_{Na}. It is considered unlikely that glucose moves from the serosal to the mucosal side and then is transported into the cell to facilitate Q_{Na} in the group with pyruvate (mucosal) and glucose (serosal) present.

During the first hour in the glucose laden cell, with pyruvate on the serosal side and galactose on the mucosal side, Q_{Na} equaled that of the control group. Neither pyruvate nor galactose alone produced this increment of Q_{Na}. It is suggested that the latter is produced by an increased transport of galactose which is stimulated by pyruvate. This is consistent with the isotope study, which demonstrated that pyruvate was better than glucose on the serosal side in stimulating galactose transport. This increment of Q_{Na} could be the result of the Na requirement for galactose transport or possibly an isosmotic fluid transfer accompanying the hexose transport. The Na concentration difference (D_{Na}) was significantly smaller in this group although the Q_{Na} was the same as in control experiments. This indicated that fluid transfer was closer to isosmosity. In addition, when glucose was present as an energy source and there was no need to actively transport a hexose (pyruvate–glucose group), the D_{Na} was significantly greater. This also suggests that an increment of Q_{Na} stimulated by an actively transported hexose is in isosmotic fluid. The basal levels of Q_{Na} that were observed

in the second hour under all conditions may result from utilizing endogenous substrates. The increment that remains under anaerobic conditions cannot be due to active hexose transfer since glucose transport ceases under these conditions (16).

Summary. The glucose dependent stimulation of Na transport in the ileum of the rat could not be duplicated with glycerol or pyruvate in an *in vitro* everted sac, although the latter 2 substrates were metabolized by the ileum to CO_2 at a faster rate than glucose. Galactose stimulated Na transport, provided that an energy source was available for the movement of this hexose. This glucose dependent increment of Q_{Na} resulted primarily from the metabolism of glucose which did not occur by glycolysis, Krebs cycle activity or oxidative phosphorylation, and secondarily from the active transport of a hexose.

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