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The Non-Essentiality of Sodium Ions for Intestinal Calcium Transport.* (28708)

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In studies on the transfer of calcium across the intestinal wall, it has been established that an active transport system exists(1,2). Although the importance of the calcium transport mechanism in the overall calcium economy of the organism is unknown, it is generally accepted that a metabolically mediated system is available for control and modification of calcium absorption. Evidence further suggests that, at high concentrations of intestinal calcium, intestinal transfer is facilitated(3) but not necessarily by active transport as usually defined, *i.e.*, net movement against an electrochemical gradient by an energy dependent mechanism.

Sodium ions have been found essential for intestinal transport of glucose(4,5), amino acids(6) and other organic substances by both *in vitro* and *in vivo* techniques. Specifically in regard to glucose transport, Crane *et al*(4) suggested that sodium acts as an integral part of the glucose-carrier complex whereas Csaky(6) is of the opinion that sodium is necessary for the energy-dependent phase of the transport mechanism. The sodium ion is specifically required for the glucose system and cannot be replaced by lithium, potassium or choline(5,7).

Although calcium has been shown to be necessary for optimal functioning of some membrane transport systems(8-10), the essentiality of sodium ion for calcium transport has not been established. Schachter *et al*(11) observed that one-half of the Na^+ in the Wilson-Wiseman solution could be replaced by choline with no change in S/M ratios† for Ca^{45}; this was interpreted as showing that Na^+ does not compete with Ca^{++} for binding sites in the transport system.

The present studies were undertaken to investigate whether or not sodium is required for calcium transport by rat intestine *in vitro*; this should indicate whether there is a possible relationship between calcium transfer and transport of other substances by the intestine.

Methods. The *in vitro* procedures used herein were described previously(12) and were based upon the techniques of Schachter and Rosen(1) and Wilson and Wiseman(13). Duodenal segments were taken from young male rats of the Carworth strain, everted, washed in cold physiological saline, and blotted. The segment lengths were standardized at 8 cm. One end of the segment was tied with suture and 0.6 ml of incubating solution labeled with Ca^{45} was injected into the other end which was then tied, forming the closed sac. The sac was placed in a 25 ml Erlenmeyer flask containing 5 ml of the Ca^{45} labeled incubating solution, gassed for 1 minute with O_2 and incubated at 37°C for 1 or 2 hours. At the end of the incubation period, the solution within the sac was re-

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† Refer to *Methods* section for definition of S/M ratio.

TABLE I. Ionic Composition of Solutions Used in *in vitro* Gut Sac Experiments.

	I	II	III	IV
	(Molar concentration in solution)			
NaCl	.135	.0135	—	—
KCl	.011	.011	.011	.011
$CaCl_2$	4×10^{-5}	4×10^{-5}	4×10^{-5}	4×10^{-5}
Na_2HPO_4	.008	.008	.008	.008
LiCl	—	—	.135	—
Choline Cl	—	—	—	.135

moved, the volume determined, and an aliquot (0.2 ml) dried on a planchet. A similar volume of the external solution was also pipetted into a planchet and dried. The Ca^{45} activities in the internal and external solutions were measured by usual counting procedures with a Geiger-Muller system; self-absorption corrections were found to be unnecessary. The results are presented as the ratio of the Ca^{45} in the serosal solution (internal) to the mucosal solution (external), *i.e.*, the S/M ratio. Four sacs were employed in each group for a given experiment although duplicate experiments were usually run in order to establish that the reported data were reproducible.

The different solutions were based on the Schacter-Rosen incubating medium(1). The compositions are given in Table I. All were iso-osmotic with the basal medium except solution II which was deliberately made hypo-osmotic, and all contained Ca^{45} at the level of about .04 μc per ml.

Oxygen uptake by intestinal mucosal tissue was determined by conventional Warburg manometric procedures(14). The mucosa was obtained by stripping everted rat duodenum with a pair of blunt forceps. The manometric data are expressed as microliters (μl) of O_2 consumed per mg of wet mucosal tissue.

Results and discussion. The transfer of calcium by an active process *in vitro* was again shown in the present studies since calcium was moved against a concentration gradient (S/M>1). This also means that calcium was transferred against an electrochemical gradient, because it has been repeatedly found that the serosal surface is electrically positive in respect to the mucosal surface(15). From Table II, it may be seen that Ca^{++} transport was inhibited when solution II (hypo-osmotic) was used (Exp. A). When Li^+ replaced Na^+ (Exp. B), the S/M ratio was greater than unity but less than that for the gut sacs incubated in the sodium solution (I). In the next experiment (C), choline$^+$ was found to substitute entirely for sodium in respect to calcium transport and the S/M ratio achieved was about the same as for the control series. Further, in an additional experiment, the .008 M Na_2HPO_4 buffer was replaced with .008 M K_2HPO_4; the Ca^{45} S/M ratios in control solution (I) and the solution completely devoid of Na^+ were $3.6 \pm .6$ and $4.6 \pm .9$, respectively, and the S/M ratios for 3-0-methyl glucose were $2.2 \pm .4$ and $0.8 \pm .01$, respectively. From this, it became apparent that sodium ion was not required as such by the calcium transport system and that the effect of Li^+ was to depress Ca^{++} transport rather than partially meet the Na^+ requirement. The effect of the hypo-osmotic solution (II) was undoubtedly due to general debilitation. This was borne out by studies on the influence of the various solutions on O_2 uptake by the mucosal tissue. The rate of oxygen consumption was not

TABLE II. Effect of the Major Cation in the Incubating Solution on Calcium Transport *in vitro*.

Exp	Major cation	Conc (M)	Solution composition*	S/M ratios†
A	Na^+	.135	I	3.4 (3.6 , 3.1 , 2.6 , 4.2)
	Na^+	.0135	II	.7 (.78, .64, .64, .65)
B	Na^+	.135	I	5.0 (4.9 , 3.5 , 4.6 , 6.8)
	Li^+	.135	III	1.8 (1.5 , 2.0 , 1.9 , 1.8)
C	Na^+	.135	I	3.3 (4.4 , 4.0 , 2.1 , 2.5)
	Choline Cl	.135	IV	3.8 (5.6 , 3.3 , 3.8 , 2.5)

* *Cf* Table I for composition of media.
† Serosal/mucosal ratios of Ca^{45} given as the mean with individual values in parentheses.

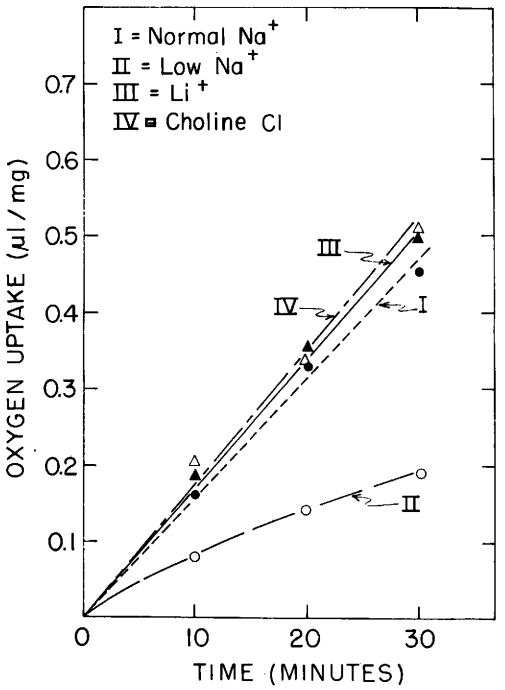

FIG. 1. Effect of the various incubating solutions on O_2 uptake by mucosal tissue. Each point represents the mean of 4 determinations.

greatly affected by replacing Na^+ with Li^+ or choline$^+$ (Fig. 1). However, the low Na^+ solution (II) significantly depressed oxidative metabolism by the mucosal tissue.

In the next series of experiments, the effect of the various solutions on the transport of 3-0-methyl glucose C^{14} was determined (Table III). As reported by others(6), sodium ion was found to be essential for active transport of 3-0-methyl glucose and, in this system, sodium ion could not be replaced by Li^+ or choline$^+$. This indicated that the transport systems for glucose and calcium are not interdependent, and that one can function under conditions in which the other is completely inhibited.

Dowdle *et al*(16) showed that deoxyglucose was a competitive inhibitor of calcium transport, suggesting that calcium and glucose shared a common step in the transport mechanism. The present results would tend to contradict this contention if sodium ion, as proposed by Crane(1), is a necessary part of the carrier complex. Again, if Na^+ is necessary for the transfer of energy to the glucose transport system as suggested by Csaky(17), the present results would suggest that the manner of energy transfer to the calcium system is different from that for glucose.

An additional study was undertaken to determine if the inhibitory effect of Li^+ could be reversed when replaced by Na^+. As shown in Table IV, Li^+ apparently caused permanent damage to the calcium transport system and the subsequent incubation in the Na^+ solution could not reverse the lithium effect. This is further evidence of differences between the calcium and glucose transport mechanisms because the lithium effect on the glucose transfer mechanism was shown to be reversible by Csaky and Zollicoffer(4).

Summary. Using an *in vitro* everted duodenal preparation from the rat, an assessment was made of the essentiality of the sodium ion in calcium transport. Choline chloride completely replaced sodium chloride whereas LiCl was partially inhibitory to this system. In confirmation of other work, sodium ion was shown to be essential for glucose transport. These data would indicate

TABLE III. Effect of the Major Cation in the Incubating Solution on Glucose (3-0-Methyl Glucose-C^{14}) Transport *in vitro*.

Exp	Major cation	Conc (M)	Solution composition*	S/M ratios†
A	Na^+	.135	I	1.31 (1.23, 1.19, 1.39, 1.41)
	Na^+	.0135	II	.87 (.87, .88, .88, .85)
	Li^+	.135	III	.86 (.86, .86, .85, .88)
B	Na^+	.135	I	1.37 (1.29, 1.39, 1.23, 1.57)
	Choline Cl	.135	IV	.84 (.84, .85, .80, .87)

* *Cf* Table I for composition of media.

† Serosal/mucosal ratios of 3-0-methyl glucose-C^{14} given as the mean with individual values in parentheses.

TABLE IV. Non-reversibility of Li^+ Inhibition on Calcium Transport *in vitro* by Na^+.

Group	Solution bathing mucosal surface* 0-30 min	30-90 min	Solution bathing serosal surface* 0-90 min	Ca^{45} S/M ratios†
1	I (Na^+)	—	I (Na^+)	3.8 (3.2 , 4.2 , 5.1 , 2.7)
2	I (Na^+)	I (Na^+)	I (Na^+)	3.2 (3.3 , 3.0 , 3.4 , 3.0)
3	III (Li^+)	—	I (Na^+)	1.7 (1.5 , 1.7 , 1.7 , 1.8)
4	III (Li^+)	I (Na^+)	I (Na^+)	1.0 (.95, 1.22, 1.00, .84)

* *Cf* Table I for composition of incubating solution.
† Serosal/mucosal ratios of Ca^{45} given as the mean with individual values in parentheses.

that sodium ion is not an essential requirement for active calcium transport, and would tend to separate the calcium transport system from several other transport systems.

Note added in proof: After this manuscript was submitted, a report by H. E. Harrison and H. C. Harrison *(Am. J. Physiol.,* 1963, v205, 107) has appeared in which similar conclusions were reached.

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Immunological Studies in Familial β 2-Macroglobulinaemias.* (28709)

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The recent finding of familial β 2-macroglobulinaemia(1,2) has led us to initiate investigations in relatives of patients with Waldenström macroglobulinaemia (W.M.)(3), multiple myeloma and "paraproteins" of unknown aetiology. Up to now 91 relatives of 30 patients with W.M. have been studied and, in 4 of these families, a familial incidence of β 2-macroglobulinaemia has been found. Certain characteristics of the macroglobulins (M.G.) in these 4 families, with special reference to their immunological structure, are reported here.

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Materials and methods. Sera were sub-