# THE REQUIREMENTS OF ANIONS FOR TRANSPORT OF CALCIUM IN RAT DUODENUM

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## 1. Introduction

Although there is controversy about the mechanism of intestinal transport of calcium, the transport process is an energy-dependent, cation-oriented, saturable process that moves calcium against concentration and electropotential gradients [1]. *In vitro* studies using the everted-gut-sac technique showed that efficient calcium transport can take place in a medium containing in addition to calcium only two other physiologically important ions i.e. sodium and chloride [2]. Although the stimulatory effect of sodium ion on calcium transport seems to be well documented [2, 3] no data concerning the role of chloride anion are available. On the other hand it has been shown in our laboratory that phosphate anion which seems to play an important role in calcium movement in rat distal ileum is not essential in duodenum [4].

In this communication we present evidence that there is an anion requirement for active transport of calcium. This requirement can be met by chloride, bromide, iodide, nitrate and acetate, but not by phosphate.

## 2. Experimental

Calcium transport was studied with the use of everted 5 cm duodenal gut sacs prepared from Wistar rats (5–6 weeks old) by the method described in [2]. The animals were maintained on standard laboratory diet and fasted for 12 hr before they were killed. The basal incubation medium contained 50 mM sodium-phosphate buffer pH 7.4, 20 mM glucose, 0.4 mM $CaCl_2$ with addition of $^{45}Ca$ (20 $\mu$Ci/100 ml) and 240 mM mannitol. Depending on the experiment mannitol was replaced by other components in proportion allowing the same osmotic pressure of the incubation medium to be maintained. The everted sacs were filled with 0.5 ml of medium and placed in 25 ml Erlenmeyer flasks containing 5 ml of medium. The duodenal preparations were incubated for 90 min at 37° under continous oxygen flow. Following incubation, the inside fluids were collected quantitatively. $^{45}Ca$ radioactivity was measured in liquid scintillation counter (Nuclear Chicago Mark I) using a scintillation fluid containing 100 mg POPOP and 4 PPO per 1 ℓ of toluene:methanol, 7:3, v/v. Calcium transport was calculated from the difference between $^{45}Ca$ amount in the total final sac content and this value in the initial sac content and expressed as nmoles/sac/90 min.

## 3. Results and discussion

As shown in table 1 calcium transport by everted sacs of rat dudenum depends on the presence of sodium chloride in the incubation medium. Lowest concentration of NaCl needed for fully efficient calcium transport was 50 mM. These results are in accordance with previous experiments in which different incubation media were used [2, 5]. In order to determine whether the requirement of sodium chloride was due only to sodium-dependency of calcium transport [2, 3] experiments using sodium phosphate medium were performed. Under such conditions the only chloride anions present (in concentration 0.8 mEq/ℓ) were these added as $CaCl_2$, and concentrations of $Na^+$ and phosphate could vary. As reported in table 2 negligible calcium transport was observed over 63–126 mEq/ℓ concentration range of

Table 1
Effect of sodium chloride on calcium transport by everted sacs incubated in high-phosphate medium.

| Concentration of NaCl in the medium [mM] | Calcium transport (nmoles/sac/90 min) |
|---|---|
| 0 | 0 |
| 10 | 0 |
| 20 | 38 |
| 35 | 168 |
| 50 | 240 |
| 70 | 250 |
| 120 | 258 |

Incubation conditions as described in Experimental except that 50 mM phosphate-Tris buffer pH 7.4 instead of 50 mM sodium-phosphate buffer was used. Results are the means of 3 sacs.

$Na^+$ when phosphate was the major anion of the incubation system. However, fully efficient calcium transport was restored by the addition of sodium chloride. These results can be interpreted as evidence that chloride but not phosphate is required for the process of active transport of calcium. This view is further supported by the observations (table 3) that calcium transport by everted sacs in the presence of 50 mM sodium-phosphate buffer increased with the increasing concentration of sodium chloride, choline chloride and tetramethyl-ammonium chloride in the

Table 2
Effect of phosphate and chloride anions on calcium transport by everted sacs.

| Incubation conditions | | | Calcium transport (nmoles/sac/90 min) |
|---|---|---|---|
| Concentration of sodium-phosphate buffer [mM] | 50 mM NaCl added | Concentration of $Na^+$ [mEq/ℓ] | |
| 35 | – | 63 | 50 |
| 35 | + | 113 | 280 |
| 50 | – | 91 | 60 |
| 50 | + | 141 | 247 |
| 70 | – | 127 | 50 |
| 70 | + | 177 | 240 |

Incubation conditions as described in Experimental. Results are the means of 3 sacs.

Table 3
Effect of sodium chloride, choline and tetramethylammonium chloride on calcium transport by everted sacs incubated in medium containing 50 mM sodium-phosphate buffer.

| Additions | | Calcium transport (nmoles/sac/90 min) |
|---|---|---|
| None | | 60 |
| NaCl | 5 mM | 100 |
| | 10 mM | 138 |
| | 20 mM | 180 |
| | 35 mM | 265 |
| | 50 mM | 290 |
| | 70 mM | 290 |
| | 120 mM | 220 |
| Choline chloride | 10 mM | 133 |
| | 20 mM | 166 |
| | 35 mM | 219 |
| | 50 mM | 203 |
| | 70 mM | 232 |
| | 120 mM | 236 |
| Tetra-methyl-ammo-nium chloride | 10 mM | 78 |
| | 20 mM | 220 |
| | 35 mM | 223 |
| | 50 mM | 295 |
| | 70 mM | 255 |
| | 120 mM | 290 |

Incubation conditions as described in Experimental. Results are the means of 3 sacs.

incubation system. Fully effective transport was observed over the same concentration range (35–120 mM) of all the compounds investigated. In order to study the specificity of chloride effect the influence of sodium bromide, sodium iodide, sodium nitrate and sodium acetate was investigated. As shown in table 4 all these compounds stimulated transport of calcium by everted sacs incubated in the presence of 50 mM sodium-phosphate buffer. However, sodium nitrate and sodium acetate inhibited calcium transport at concentration above 50 mM and 70 mM, respectively.

The results presented suggest strongly that the process of active transport of calcium is anion-dependent. However, from these data it is not possible to speculate on the role of anions in the mechanism of calcium transport.

Table 4
Effect of sodium bromide, sodium iodide, sodium nitrate and sodium acetate on calcium transport by everted sacs incubated in medium containing 50 mM sodium-phosphate buffer.

| Additions | | Calcium transport (nmoles/sac/90 min) |
|---|---|---|
| None | | 50 |
| NaBr | 5 mM | 71 |
| | 10 mM | 200 |
| | 20 mM | 216 |
| | 35 mM | 290 |
| | 50 mM | 220 |
| | 70 mM | 270 |
| NaI | 5 mM | 109 |
| | 10 mM | 244 |
| | 20 mM | 250 |
| | 35 mM | 275 |
| | 50 mM | 263 |
| | 70 mM | 230 |
| $NaNO_3$ | 5 mM | 120 |
| | 10 mM | 140 |
| | 20 mM | 235 |
| | 35 mM | 243 |
| | 50 mM | 190 |
| | 70 mM | 71 |
| | 120 mM | 0 |
| $CH_3COONa$ | 10 mM | 65 |
| | 20 mM | 163 |
| | 35 mM | 237 |
| | 50 mM | 180 |
| | 70 mM | 180 |
| | 120 mM | 0 |

Incubation conditions as described in Experimental. Results are the means of 2 sacs.

## References


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