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Distribution of Ca^{2+}-ATPase, ATP-dependent Ca^{2+}-transport, calmodulin and vitamin D-dependent Ca^{2+}-binding protein along the villus-crypt axis in rat duodenum

E.J.J.M. van Corven [a], C. Roche [b] and C.H. van Os [a]

[a] *Department of Physiology, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen (The Netherlands) and* [b] *INSERM 445, Hôpital E. Herriot, 69374 Lyon Cedex 02 (France)*

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The migration of intestinal epithelial cells from the crypts to the tips of villi is associated with progressive cell differentiation. The changes in Ca^{2+}-ATPase activity and ATP-dependent Ca^{2+}-transport rates in basolateral membranes from rat duodenum were measured during migration along the crypt-villus axis. In addition, vitamin D-dependent calcium-binding protein and calmodulin content were measured in homogenates of six cell populations which were sequentially derived from villus tip to crypt base. Alkaline phosphatase activity was highest at the tip of the villus (fraction I) and decreased more than 20-fold towards the crypt base (fraction VI). ($Na^+ + K^+$)-ATPase activity also decreased along the villus-crypt axis but in a less pronounced manner than alkaline phosphatase. ATP-dependent Ca^{2+}-transport in basolateral membranes was highest in fraction II (8.2 ± 0.3 nmol Ca^{2+}/min per mg protein) and decreased slightly towards the villus tip and base (fraction V). The youngest cells in the crypt had the lowest Ca^{2+}-transport activity (0.9 ± 0.1 nmol Ca^{2+}/min per mg protein). The distribution of high-affinity Ca^{2+}-ATPase activity in basolateral membranes correlated with the distribution of ATP-dependent Ca^{2+}-transport. The activity of Na^+/Ca^{2+} exchange was equal in villus and crypt basolateral membranes. Compared to the ATP-dependent Ca^{2+}-transport system, the Na^+/Ca^{2+} exchanger is of minor importance in villus cells but may play a more significant role in crypt cells. Calcium-binding protein decreased from mid-villus towards the villus base and was undetectable in crypt cells. Calmodulin levels were equal along the villus-crypt axis. It is concluded that vitamin D-dependent calcium absorption takes primarily place in villus cells of rat duodenum.

Introduction

The biochemical and morphological properties of enterocytes change during maturation and migration along the crypt-villus axis. Undifferentiated crypt cells display a high mitotic activity [1,2], and during maturation the villus cells acquire their characteristic enzyme composition, transport systems and morphology [3–7]. Villus cells have an absorptive function, while crypt cells may be responsible for electrolyte secretion [8,9]. The duodenum is the main site of active Ca^{2+} absorption and this process is regulated by the vitamin D metabolite $1\alpha,25(OH)_2D_3$ [10–12]. It is not known at present which epithelial cells on the villus-crypt axis absorb Ca^{2+} most avidly in the duodenum.

Abbreviations: PMSF, phenylmethanesulphonyl fluoride; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; HEDTA, *N*-(2-hydroxyethyl)ethylenediamine-*N*,*N*′,*N*′-triacetic acid; Hepes, 4(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

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Since there is no technique available which permits in situ analysis of transcellular Ca^{2+}-transport at different levels of the villus, we decided to isolate enterocytes from different positions along the villus-crypt axis. In these cell populations various parameters were investigated which are thought to be related to transcellular Ca^{2+}-transport; e.g. Ca^{2+}-ATPase, ATP-dependent Ca^{2+}-transport in basolateral membranes and Ca^{2+}-binding protein in whole cells. Intracellular Ca^{2+} may play a crucial role in regulating absorptive and secretory activities of enterocytes [9,13,14]. Since Ca^{2+}-ATPase and ATP-dependent Ca^{2+}-transport in basolateral membranes also have an important role in the Ca^{2+}-homeostasis of enterocytes it is from this point of view of interest to know the distribution along the villus-crypt axis.

Materials

Tris-ATP, oligomycin, theophylline, valinomycin, EGTA, HEDTA and dithiothreitol were from Sigma (St. Louis). Monensin was from Calbiochemicals (La Jolla). $^{45}CaCl_2$ (20 mCi/mg) was purchased from New England Nuclear (Dreieich, F.R.G.). All other chemicals were of the purest grade.

Methods

Isolation of enterocytes and basolateral plasmamembranes

Different populations of villus and crypt cells were isolated according to Harrison and Webster [15]. Eight male wistar rats between 180 and 210 g, fed ad libitum, were killed by a blow on the head. The first 20 cm of small intestine were removed and rinsed with ice-cold 150 mM NaCl, containing 1 mM dithiothreitol. Everted pieces were tied onto metal rods and vibrated at 4°C in 150 mM NaCl with 1 mM dithiothreitol with low amplitude (2 mm) and high frequency (60 Hz). After the first minute, shedded debris was discarded. The first population of cells was collected after a 10 min vibration period in the absence of EDTA (fraction I). Subsequent periods of vibration in the presence of 2.5 mM EDTA gave the following fractions: 10 min (fraction II); 10 min (fraction III); 15 min (fraction IV); 20 to 25 min (fraction V). After a total of 1 h vibration, crypt cells were harvested by scraping. Cells collected in the presence of EDTA were pelleted ($800 \times g$ for 10 min) and washed with ice-cold 150 mM NaCl. Homogenization and isolation of basolateral membrane vesicles were carried out as previously described [16].

Enzyme assays

Protein was determined with a commercial Coomassie blue kit (Bio-Rad) using γ-globulin as standard. $(Na^+ + K^+)$-ATPase and Ca^{2+}-ATPase assays and Ca^{2+}-uptake studies were done on the day of isolation. Ca^{2+}-ATPase activity was measured at 37°C in a medium containing (mM): KCl, 150; NaN_3, 1; ouabain, 4; theophylline, 5; Tris-ATP, 3; Mg^{2+}, 5; Tris-Hepes, 20 (pH 7.4). ATP-hydrolysis stimulated by 1 μM free Ca^{2+} was considered to be the result of high-affinity Ca^{2+}-ATPase activity. Ca^{2+}-ATPase was assayed only in basolateral membrane fractions [17]. $(Na^+ + K^+)$-ATPase, alkaline phosphatase, sucrase, succinic acid dehydrogenase, NADPH-dependent cytochrome *c* reductase and thiamine pyrophosphatase were determined as before [16,17]. Latency in $(Na^+ + K^+)$-ATPase activity was studied by incubating homogenates and basolateral membranes with Triton X-100 at 37°C for 15 min. Triton concentrations for optimal activation of $(Na^+ + K^+)$-ATPase were 0.05 mg Triton/mg protein for homogenates and 0.5 mg Triton/mg protein for basolateral membranes.

Ca^{2+}-uptake studies

ATP-dependent Ca^{2+}-uptake studies were done at 37°C in an uptake medium containing (mM): KCl, 150; $MgCl_2$, 5; Tris-Hepes, 20 (pH 7.4) and 1 μM free Ca^{2+} (0.47 mM total Ca^{2+}) buffered with EGTA, 0.5, and HEDTA, 0.5. The free Ca^{2+} concentration was calculated as described in detail by Van Heeswijk et al. [18]. ATP-dependent Ca^{2+}-uptake rates are defined as the differences between Ca^{2+}-uptake rates in the presence and in the absence of 3 mM Tris-ATP. The uptake medium contained 2 μCi/ml ^{45}Ca. The reaction was started by adding membranes to the uptake medium without ATP. After 1 min an aliquot was taken and quenched in 1 ml ice-cold stop solution (uptake medium + 0.5 mM $LaCl_3$). Membranes were collected on Schleicher and Schüll filters

(ME25, 0.45 μm). Filters with samples were washed twice with 2 ml ice-cold stop solution. 2 min after starting the reaction, 3 mM Tris-ATP was added to the uptake mixture and 1 min later another aliquot was taken and filtered as above. 1 min of incubation in the presence of ATP gives initial-rate values, while in the absence of ATP there is no further increase in Ca^{2+}-uptake after 1 min of incubation [16,18]. Ca^{2+}-uptake studies were run in triplicates.

Na^+/Ca^{2+} exchange activity was measured as previously reported [19]. In the absence of ATP, Ca^{2+}-uptake via the Na^+/Ca^{2+} exchanger is studied with an outwardly directed Na^+ gradient. For this, basolateral membrane vesicles were loaded with 150 mM NaCl and diluted 20-fold into an uptake medium containing 150 mM KCl, 5 μM free Ca^{2+} (0.55 mM total Ca^{2+}), 75 μCi ^{45}Ca/ml and 10 μg/ml valinomycin. Short incubations are carried out by mixing a droplet of membrane vesicles pipetted against the wall of the test tube, with the uptake medium. By activating the vortex for mixing, an automatic pipettor is triggered which quenches the uptake reaction with a stop solution after a preset time (1–15 s) [18].

Assay of Ca^{2+}-binding protein and calmodulin

Ca^{2+}-binding protein content of the six cell populations was determined by an equilibrated chromatographic column procedure [20,21]. The cells were homogenized and centrifuged at 100 000 $\times g$ for 1 h. 8 mg of the lyophylized redissolved supernatant were chromatographed on a Sephadex G-50 (fine) column (100 × 0.6 cm) which was previously equilibrated with 0.02 M ammonium acetate (pH 7.2) containing 10 μM $CaCl_2$ and 5000 cpm ^{45}Ca/nmol Ca. The area of the second peak is proportional to the amount of Ca^{2+}-binding protein loaded onto the column [21] (2 nmol Ca_{bound} = 1 nmol Ca^{2+}-binding protein).

Calmodulin content of the six cell populations was determined as follows: homogenates of the six cell populations were centrifuged at 100 000 × g for 1 h. The supernatant was purified after Teo et al. [22]. The pellet was dialyzed and treated as described by Teo et al. [22]. The purified cytosolic fraction and the membrane fraction were used for gel electrophoresis and in a phosphodiesterase assay. SDS-polyacrylamide gel electrophoresis was performed in 12% polyacrylamide slab gels [23] that were silver-stained [24]. Samples were run in the presence of 1 mM Ca^{2+} or 1 mM EGTA, after Gitelman and Witman [25]. Stimulation of cAMP-dependent phosphodiesterase was determined by a modification of the one-step procedure of Teo et al. [22], described in detail by Flik et al. [26]. Both methods, SDS-polyacrylamide gel electrophoresis and the phosphodiesterase assay, yielded similar calmodulin contents.

Results

The specific activities of alkaline phosphatase, sucrase and $(Na^+ + K^+)$-ATPase in the six cell fractions obtained from rat duodenum are shown in Figs. 1, 2 and 3. The protein yield during the six vibration periods is plotted cumulatively on the x-axis. Despite longer vibration times the yield of protein decreases after the third period, which indicates that it becomes increasingly difficult to shed cells further down the villus towards the crypt area. The specific activity of alkaline phosphatase in homogenates decreases more than 20-fold from fractions I to VI. A similar pattern of alkaline phosphatase activity was reported to correlate with a gradient of cell removal from villus tip to crypt base [5,27]. Sucrase activity peaks in the second fraction and then decreases as sharply as alkaline phosphatase. An identical distribution of sucrase along the villus crypt axis has been reported before for rabbit small intestine [28] and further supports our claim that fractions I to VI represent cells that are sequentially derived from villus tip to crypt base. It is of interest to note that alkaline phosphatase is enriched 2-fold while sucrase activity is decreased 2-fold in basolateral membrane fractions. This result demonstrates that alkaline phosphatase is native to basolateral membranes in rat small intestine, as reported before by Hanna et al. [29] and Ghijsen et al. [17].

In Fig. 3 specific activities of $(Na^+ + K^+)$-ATPase in homogenates and basolateral membranes are shown. Although less pronounced, there is a gradient of $(Na^+ + K^+)$-ATPase activity along the villus-crypt axis, which is of similar magnitude to that reported by Rowling and Sepulveda [28] in rabbit small intestine.

In Table I, purification data for four enzymes

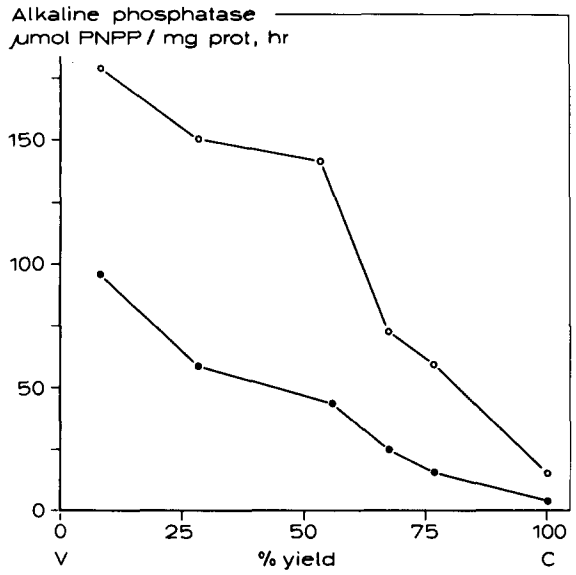

Fig. 1. Specific activities of alkaline phosphatase in homogenates (●) and basolateral membrane fractions (○) along the villus (V)-crypt (C) axis. The fractions are expressed as percentage of the total yield in cell protein. Mean values are given of eight experiments. S.E. values are within 10% of the mean for all points.

measured in the six basolateral membrane fractions are summarized. For $(Na^+ + K^+)$-ATPase the purification factors are not significantly different from each other (tested with Student's t-test, $P > 0.2$). The purification of $(Na^+ + K^+)$-ATPase is somewhat lower than previously published [16]. However, in the present study $(Na^+ + K^+)$-ATPase assays were run in the presence of Triton X-100 and it has been demonstrated that $(Na^+ + K^+)$-ATPase in basolateral membranes has a somewhat lower latency than in homogenates. Therefore, earlier purification factors tend to be somewhat overestimated [18]. The basolateral membranes from crypt cells are significantly more contaminated with mitochondrial membranes than the villus fractions. For the smooth endoplasmic reticulum marker, the opposite is observed. The Golgi marker, thiamine pyrophosphatase, seems to be present in equal amounts in villus and crypt basolateral membrane fractions.

In basolateral membrane vesicles the initial rates of ATP-dependent Ca^{2+}-uptake were determined and the results are shown in Fig. 4. Fraction II has the highest transport capacity for Ca^{2+} and the activity decreases towards the crypt area. Fraction

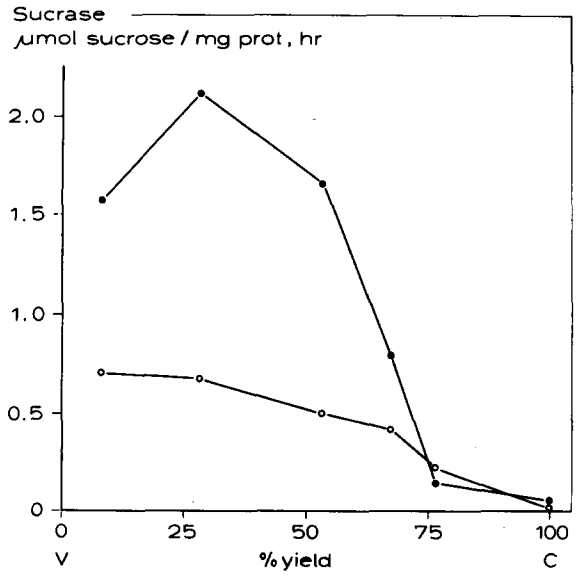

Fig. 2. Specific activities of sucrase in homogenates (●) and basolateral membrane fractions (○) along the villus-crypt axis. Mean values of four experiments.

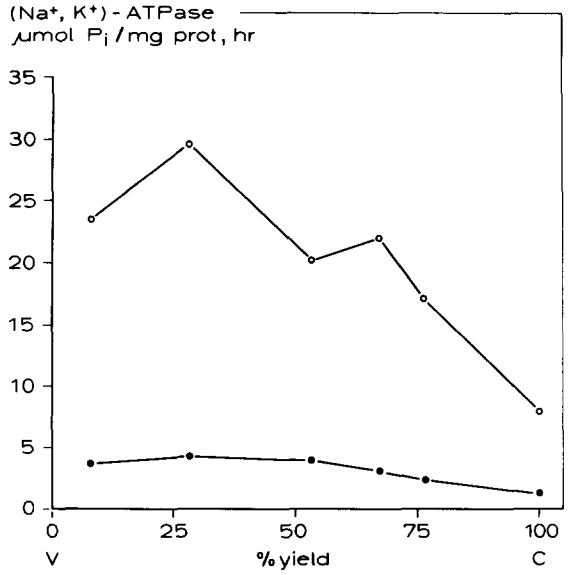

Fig. 3. Specific activities of $(Na^+ + K^+)$-ATPase in homogenates (●) and basolateral membrane fractions (○). Mean values of eight experiments, measured in the presence of Triton X-100. S.E. values are within 15% of the mean for all points. Latency studies on $(Na^+ + K^+)$-ATPase activity revealed that the percentage resealed vesicles in fractions I–VI is 50, 51, 59, 62, 41 and 49%, respectively ($n = 4$). These values are not significantly different (2-sided Student's t-test, $P > 0.2$).

TABLE I

PURIFICATION FACTORS OF FOUR MARKER ENZYMES IN BASOLATERAL MEMBRANE PREPARATIONS OF ENTEROCYTES FROM VILLUS TIP TO CRYPT BASE

Purification factors are calculated as specific activities in BLM fractions divided by those in homogenates. n.d., not determined due to lack of sufficient material.

Fraction	$(Na^+ + K^+)$-ATPase ($n = 8$)	Succinic dehydrogenase ($n = 8$)	NADPH-dep. cyt. *c* reductase ($n = 4$)	Thiamine pyrophosphatase ($n = 3$)
I	6.2	0.37	1.4	n.d.
II	7.5	0.37	3.2	1.4
III	5.8	0.35	3.2	1.6
IV	8.3	0.60	3.5	n.d.
V	9.5	0.40	2.3	n.d.
VI	6.4	0.82 [a]	0.9 [b]	2.0

[a] Significantly different from values I–V ($P < 0.01$) (Student's *t*-test).
[b] Significantly different from values I–V ($P < 0.005$).

I, the oldest villus cells which are shedded in the absenceo f EDTA, has a significantly lower transport capacity than fraction II. The high-affinity Ca^{2+}-ATPase activity in BLM preparations is also shown in Fig. 4. Again, highest activity is observed in fraction II, but the differences among fractions I to V are not statistically significant.

In crypt basolateral membranes a significant ATP-dependent Ca^{2+}-uptake activity could be measured despite the fact that no significant Ca^{2+}-ATPase activity was present. The ATP-dependent Ca^{2+}-uptake process in crypt preparations was therefore studied in more detail and the result is shown in Fig. 5. In the presence of ATP there is a continuous uptake of Ca^{2+} with time but the transported Ca^{2+} cannot be released with the Ca^{2+}-ionophore A23187. This is in striking contrast with the ATP-dependent Ca^{2+}-transport in villus basolateral membrane vesicles where transported Ca^{2+} is readily released by A23187 (Fig. 6). The data suggest that in crypt basolateral membranes ATP induces Ca^{2+} transport and Ca^{2+} is not accumulated in the intravesicular space but probably bound, hence A23187 cannot release transported Ca^{2+}. The difference in Ca^{2+}-content of villus and crypt basolateral membranes after 30 min of incubation in the presence of A 23187 also suggests that there are more Ca^{2+} binding sites on crypt than on villus basolateral membranes.

Comparison of ATP-dependent Ca^{2+} transport rates in different membrane populations is only justified when these membranes have the same percentage of resealed inside-out oriented vesicles. The degree of resealing, judged on the basis of latency in $(Na^+ + K^+)$-ATPase activity, is similar

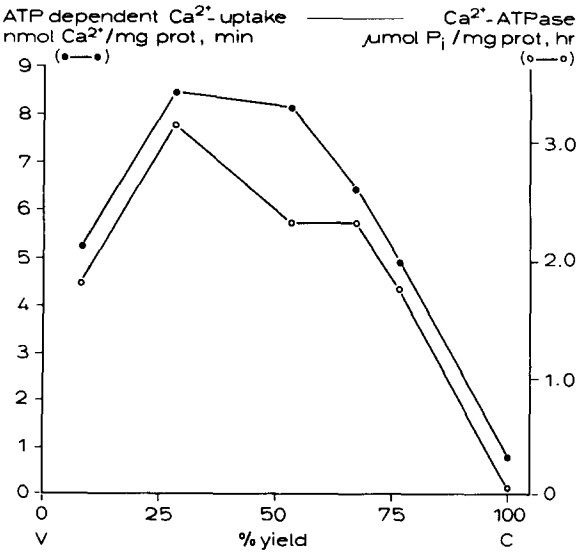

Fig. 4. Specific activities of ATP-dependent Ca^{2+}-transport (●) and Ca^{2+}-ATPase (○) in basolateral membrane fractions along the villus-crypt axis. ATP-dependent Ca^{2+}-transport is corrected for aspecific Ca^{2+}-binding observed in the absence of ATP. Mean values of eight experiments. S.E. values are within 10% of the mean for ATP-dependent Ca^{2+}-transport and within 20% of the mean for Ca^{2+}-ATPase.

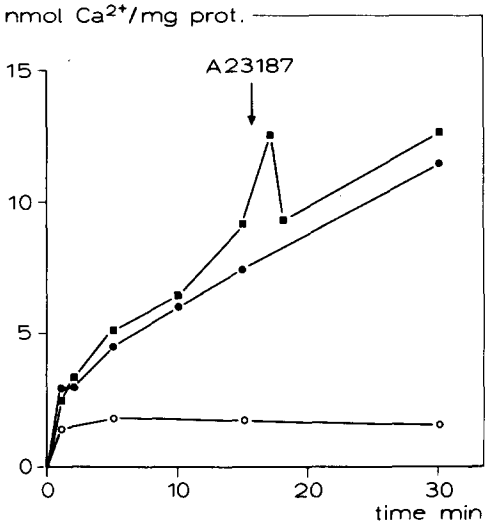

Fig. 5. Effect of ATP on Ca^{2+}-uptake in basolateral membranes from crypt cells (fraction VI). ■, Ca^{2+}-uptake in the presence of ATP. Arrow indicates addition of ionophore A23187; ●, Ca^{2+}-uptake in the presence of ATP and A23187; ○, Ca^{2+}-uptake in the absence of ATP. Free Ca^{2+} concentration is 1 μM. A23187 concentration is 10 μg/ml.

for the six populations (legend of Fig. 3). Another test for leakiness of the vesicles is the rate at which, for example, mannitol equilibrates across the membrane. Mannitol-uptake studies were done in pooled villus fractions I to V and in the crypt fraction VI. The results are shown in Fig. 7. Although the vesicular space of crypt basolaterals is smaller than that of villus material (1.5 vs. 2.2 μl/protein), it is clear that this small difference in size cannot explain the 10-fold difference in Ca^{2+}-transport rates observed between villus fraction II and crypt fraction VI.

The basolateral preparations I to VI are contaminated with membrane fragments from endoplasmic reticulum (Table I). To test whether this contamination contributes to ATP-dependent Ca^{2+}-uptake, the effect of oxalate on Ca^{2+}-transport was studied. In a combined villus fraction (I–V), the ATP-dependent Ca^{2+}-uptake in the presence and absence of 15 mM oxalate was 23.4 $\pm$ 4.2 and 26.2 $\pm$ 3.8 ($n = 3$) nmol Ca^{2+}/mg protein, respectively, after 30 min of incubation with 10 mM ATP (free Ca^{2+} was 1 μM). In crypt basolateral membranes these values were 12.5 $\pm$ 2.9 and 10.5 $\pm$ 3.0 ($n = 3$) nmol Ca^{2+}/mg protein, respectively. We conclude that oxalate does not increase ATP-driven Ca^{2+}-uptake in villus or in crypt membranes, which makes a contribution of endoplasmic reticulum unlikely [32–35]. Oligomycin (10

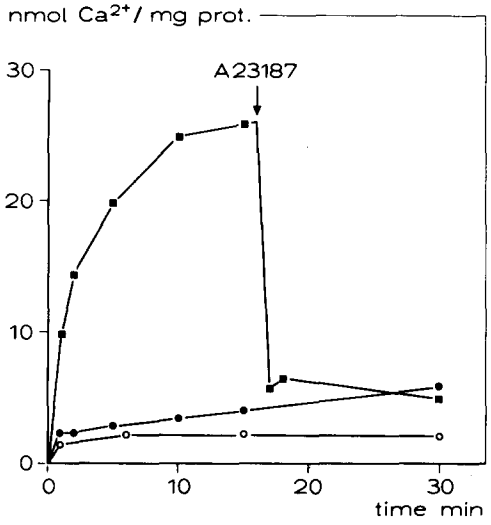

Fig. 6. Effect of ATP on Ca^{2+}-uptake in basolateral membranes from villus cells (pooled fractions I–V). ■, Ca^{2+}-uptake in the presence of ATP. Arrow indicates addition of ionophore A23187; ●, Ca^{2+}-uptake in the presence of ATP and A23187; ○, Ca^{2+}-uptake in the absence of ATP. Ca^{2+} and A23187 concentrations as for Fig. 5.

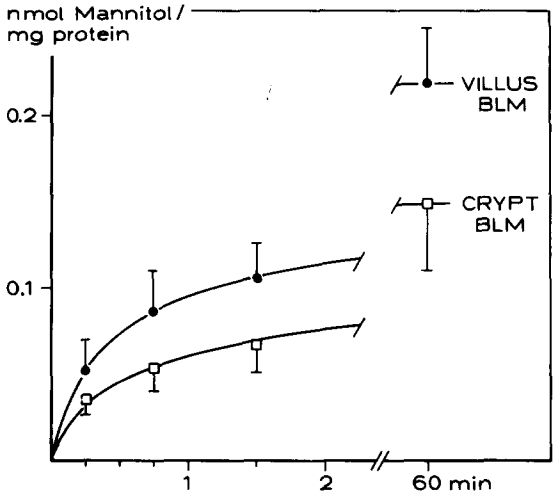

Fig. 7. Time course of mannitol uptake in basolateral membrane vesicles from villus (pooled fractions I–V) and crypt cells (fraction VI). Mannitol concentration is 0.1 mM. Data points are mean values (S.E. presented as bars) of 4 and 3 experiments with villus and crypt material, respectively. Uptake experiments were done as published previously [30,31].

TABLE II

Na^+/Ca^{2+} EXCHANGE ACTIVITIES IN BASOLATERAL MEMBRANES FROM VILLUS AND CRYPT CELLS

Villus is the combined fractions II–V, crypt is fraction VI. Membrane vesicles were preloaded with 150 mM NaCl (1 h on ice) and diluted 20-fold in equimolar NaCl (Na_i^+/Na_0^+) or KCl medium (Na_i^+/K_0^+). Membranes were preincubated with 10 μg/ml valinomycin (Val) or with 10 μg/ml valinomycin and 2 μM monensin (Val + Mon). One experiment consists of triplicate incubations of 15 s at 5 μM free Ca^{2+}. This represents initial rates of uptake [18,19]. Mean values plus S.E. are given with numbers of experiments in parentheses.

	(Ca^{2+}-uptake in nmol/mg protein per 15 s)		
	Na_i^+/Na_0^+	Na_i^+/K_0^+ + Val	Na_i^+/K_0^+ + Val + Mon
Villus	0.77 ± 0.05	0.96 ± 0.08	0.79 ± 0.06 ($n = 8$)
Crypt	1.14 ± 0.14	1.36 ± 0.19	1.18 ± 0.13 ($n = 8$)

μg/ml) was tested on ATP-dependent Ca^{2+}-uptake in fractions I–VI but had no effect, ruling out a mitochondrial contribution [16].

In basolateral membranes of crypt cells the ATP-dependent Ca^{2+}-transport rate is low and it is therefore of interest to measure the activity of the Na^+/Ca^{2+} exchange system which has previously been shown to be present in villus cells [19]. In Table II, Na^+-gradient-dependent Ca^{2+}-uptake in the absence of ATP is given for basolateral membranes of villus and crypt cells. About half of the vesicles are leaky (legend Fig. 3) and inside-out as well as rightside-out vesicles contribute to Na^+-gradient-dependent Ca^{2+}-uptake [18,37]. Therefore, the capacity of Na^+/Ca^{2+} exchange amounts to 1.5 and 1.8 nmol Ca^{2+}/min per mg protein in villus and in crypt basolateral membranes, respectively. Compared to the ATP-dependent Ca^{2+}-transport system the Na^+/Ca^{2+} exchanger is of minor importance in villus cells but may play a more significant role in the Ca^{2+}-homeostasis of crypt cells.

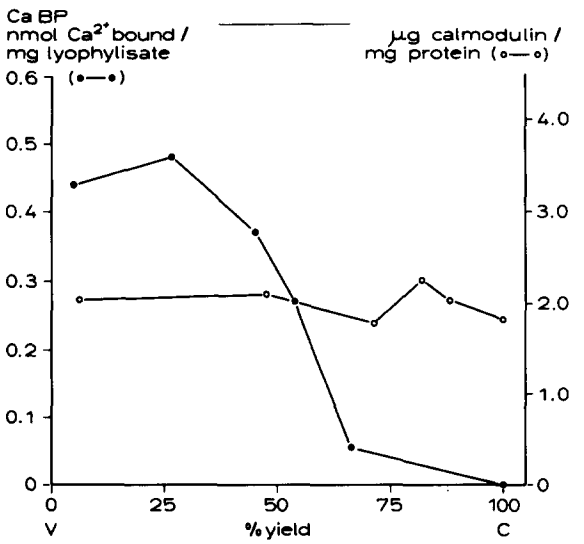

Fig. 8. Distribution of vitamin D-dependent calcium-binding protein (CaBP) and calmodulin along the villus-crypt axis. Calmodulin was 95% membrane-bound in all fractions. Mean values of two experiments are given.

The distribution of vitamin D-dependent Ca^{2+}-binding protein and of calmodulin along the villus crypt axis is given in Fig. 8. Ca^{2+}-binding protein content decreases toward the crypt area but calmodulin levels are constant. A similar dissociation between Ca^{2+}-binding protein and calmodulin has been reported to occur longitudinally in the small intestine [38].

Discussion

The distribution of alkaline phosphatase, sucrase and ($Na^+ + K^+$)-ATPase among the six fractions obtained in this study by a slightly modified method of Harrison and Webster [15] is similar to that reported by other investigators [5,27,28]. This particular result strongly suggests that fractions I–VI represent cells that are sequentially released from the villus tip to the crypt base. The purification factors for the basolateral membrane marker, ($Na^+ + K^+$)-ATPase, are not significantly different for the six cell fractions. The rate of ATP-dependent Ca^{2+}-uptake in basolateral membrane vesicles did not correlate with alkaline phosphatase and ($Na^+ + K^+$)-ATPase activities in

these fractions, since the decrease in alkaline phosphatase was more pronounced and the decrease in $(Na^+ + K^+)$-ATPase was less pronounced towards the crypt area. Active Ca^{2+}-transport was also dissociated from mitochondrial and Golgi membrane markers because crypt basolateral membranes were relatively more contaminated with succinic dehydrogenase than villus fractions and thiamine pyrophosphatase activity was equal in all fractions. Contamination with smooth endoplasmic reticulum deserves attention in view of the evidence that in a variety of cells endoplasmic reticulum is involved in ATP-dependent Ca^{2+}-sequestration, as for example in exocrine cells [32,33,35], hepatocytes [34,39], brain tissue [40], platelets [41], neutrophils [42] and insuloma cells [43]. Ca^{2+}-transport in endoplasmic reticulum is stimulated by oxalate [32–35,39]. In the present basolateral membrane preparations of small intestinal cells oxalate had no effect on ATP-dependent Ca^{2+}-transport. This result suggests that fragments derived from endoplasmic reticulum do not contribute to Ca^{2+}-uptake in this crude basolateral membrane preparation. At the moment there is no information about possible Ca^{2+}-pumps in endoplasmic reticulum of enterocytes but this point deserves further investigation. It may be that Ca^{2+}-uptake in endoplasmic reticulum is more sensitive to mechanical damage during centrifugation than the plasma membrane Ca^{2+}-transport system. Oxalate-stimulated Ca^{2+}-transport in endoplasmic reticulum from smooth muscle was reduced dramatically by centrifugation, while the oxalate-independent Ca^{2+}-uptake in plasma membranes was hardly reduced [44]. On density gradients no further separation between endoplasmic reticulum and basolateral membranes can be obtained, since their marker enzymes have an overlap in the same density regions [45]. Other more elaborate methods such as partitioning in aqueous polymer two-phase systems give a good resolution of marker enzymes but destroy the ATP-dependent Ca^{2+}-transport (Ref. 45; and unpublished observation).

Vitamin D-dependent Ca^{2+}-binding protein is perhaps the best-defined molecular expression of vitamin D action [47]. Ca^{2+}-binding protein levels in the intestine correlate directly with the efficiency of vitamin D-dependent calcium absorption [47]. In the rat, Ca^{2+}-binding protein is abundantly present in the duodenum and hardly detectable in the ileum, which has been shown independently by radioimmunoassay and by the chromatographic procedure used in the present study [38,48]. Our study indicates that Ca^{2+}-binding protein is more or less confined to the absorbing cells in the villus region. A recent immunocytochemical study on Ca^{2+}-binding protein in rat duodenum also reveals a gradient of Ca^{2+}-binding protein from villus tip to the upper crypt region, in agreement with our findings [49]. The fact that Ca^{2+}-binding protein and ATP-dependent Ca^{2+}-transport are most prominent in absorbing villus cells justifies the conclusion that vitamin D-dependent calcium absorption takes place primarily in villus cells. Apparently, calmodulin is of minor importance, since its distribution along the villus-crypt axis and along the small intestine is dissociated from Ca^{2+}-binding protein and Ca^{2+}-ATPase [38,50].

Although we have measured the Ca^{2+}-ATPase activity along the villus crypt axis, it remains entirely possible that this activity does not reflect the transcellular absorptive Ca^{2+}-transport rate. The entry step into the cell across the brush-border membrane might be rate-limiting in transcellular Ca^{2+}-transport. We have not attempted to measure the Ca^{2+}-permeability of the brush-border membrane for several reasons. Firstly, the luminal membrane of crypt cells lacks a brush border and methods for isolation of this membrane have not yet been described. Secondly, information on the real Ca^{2+}-permeability of brush-border membrane vesicles is difficult to obtain, since Ca^{2+}-binding to membrane sites obscures the transmembrane flux (Ref. 51; and our unpublished observations). Nevertheless, in view of the close correlation between the Ca^{2+}-binding protein content and the rate of transcellular Ca^{2+}-transport [48], we have confidence in our conclusion that Ca^{2+}-absorption takes place primarily in the mid- to upper villus region.

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References

1 Leblond, C.P. and Messier, B. (1958) Anat. Rec. 132, 247–255
2 Bertalanffy, F.D. (1960) Acta Anat. 40, 130–148
3 Webster, H.L. and Harrison, D.D. (1969) Exp. Cell Res. 56, 245–253
4 Fortin-Magana, R., Hurwitz, R., Herbst, J.J. and Kretchmer, N. (1970) Science 167, 1627–1628
5 Weiser, M. (1973) J. Biol. Chem. 248, 2536–2541
6 Chen, H. and Leblond, C.P. (1974) Am. J. Anat. 141, 461–480
7 King, I.S., Sepulveda, F.V. and Smith, M.W. (1981) J. Physiol. 319, 355–368
8 Welsh, M.J., Smith, P.L., Fromm, M. and Frizzel, R.A. (1982) Science 218, 1219–1221
9 Donowitz, M. (1983) Am. J. Physiol. 245, G165–G177
10 Walling, M.W. (1977) Am. J. Physiol. 233, F488–F494
11 Wasserman, R.H. and Fullmer, C.S. (1983) Annu. Rev. Physiol. 45, 375–390
12 Favus, M.J. (1985) Am. J. Physiol. 248, G147–G157
13 Frizzell, R.A. (1977) J. Membrane Biol. 35, 175–187
14 Donowitz, M. and Asarkoff, N. (1982) Am. J. Physiol. 243, G628–G635
15 Harrison, D.D. and Webster, H.L. (1969) Exp. Cell Res. 55, 257–260
16 Ghijsen, W.E.J.M., De Jong, M.D. and Van Os, C.H. (1982) Biochim. Biophys. Acta 689, 327–336
17 Ghijsen, W.E.J.M., De Jong, M.D. and Van Os, C.H. (1980) Biochim. Biophys. Acta 599, 538–551
18 Van Heeswijk, M.P.E., Geertsen, J.A.M. and Van Os, C.H. (1984) J. Membrane Biol. 79, 19–32
19 Ghijsen, W.E.J.M., De Jong, M.D. and Van Os, C.H. (1983) Biochim. Biophys. Acta 730, 85–94
20 Buckley, M. and Bronner, F. (1980) Arch. Biochem. Biophys. 202, 235–241
21 Ueng, T.H., Golub, E.E. and Bronner, F. (1979) Arch. Biochem. Biophys. 197, 205–217
22 Teo, T.S., Wang, T.H. and Wang, J.H. (1973) J. Biol. Chem. 248, 588–595
23 Laemmli, U.K. (1970) Nature 227, 680–685
24 Morrissey, J.H. (1981) Anal. Biochem. 117, 307–310
25 Gitelman, S.E. and Witman, G.B. (1980) J. Cell Biol. 98, 764–770
26 Flik, G., Van Rijs, J.H. and Wendelaar Bonga, S.E. (1984) Eur. J. Biochem. 138, 651–654
27 Lawson, A.J., Smit, R.A., Jeffers, N.A. and Osborne, J.W. (1982) Cell Tissue Kinet. 15, 69–80
28 Rowling, P.J.E. and Sepulveda, F.V. (1984) Biochim. Biophys. Acta 771, 35–41
29 Hanna, S.D., Mircheff, A.K. and Wright, E.M. (1979) J. Supramol. Struct. 11, 451–466
30 Mircheff, A.K., Van Os, C.H. and Wright, E.M. (1980) J. Membrane Biol. 52, 83–92
31 Wright, E.M., Van Os, C.H. and Mircheff, A.K. (1980) Biochim. Biophys. Acta 597, 112–124
32 Ponnappa, B.C., Dormer, R.L. and Williams, J.A. (1981) Am. J. Physiol. 240, G122–G129
33 Bonis, D. and Rossignol, B. (1982) FEBS Lett. 137, 63–66
34 Famulski, K. and Carafoli, E. (1982) Cell Calcium 3, 263–281
35 Kribben, A., Tyrakowski, T. and Schulz, I. (1983) Am. J. Physiol. 244, G480–G490
36 Hildmann, B., Schmidt, A. and Murer, H. (1982) J. Membrane Biol. 65, 55–62
37 Philipson, K.D. and Nishimoto, A.Y. (1982) J. Biol. Chem. 257, 5111–5117
38 Thomasset, M., Molla, A., Parkes, O. and Demaille, J.G. (1981) FEBS Lett. 127, 13–16
39 Burgess, G.M., McKinney, J.S., Fabiato, A., Leslie, B.A. and Putney, J.W. (1983) J. Biol. Chem. 258, 15336–15345
40 Blaustein, M.P., Ratzlaff, R.W. and Schweitzer, E.S. (1980) Fed. Proc. 39, 2790–2795
41 Lapetina, E.G., Watson, S.P. and Cuatrecasas, P. (1984) Proc. Natl. Acad. Sci. USA 81, 7431–7435
42 Prentki, M., Wolheim, C.B. and Lew, P.D. (1984) J. Biol. Chem. 259, 13777–13782
43 Prentki, M., Janjic, D. and Wolheim, C.B. (1984) J. Biol. Chem. 259, 14054–14058
44 Raeymakers, L. and Casteels, R. (1984) Cell Calcium 5, 205–210
45 Mircheff, A.K., Sachs, G., Hanna, S.D., Labiner, C.S., Rabon, E., Douglas, A.P., Walling, M.W. and Wright, E.M. (1979) J. Membrane Biol. 50, 543–563
46 Mircheff, A.K., Ahnen, D.J., Islam, A., Santiago, N.A. and Gray, G.M. (1985) J. Membrane Biol. 83, 95–107
47 Wasserman, R.H. and Fullmer, C.S. (1983) Annu. Rev. Physiol. 45, 375–390
48 Pansu, D., Bellaton, C., Roche, C. and Bronner, F. (1983) Am. J. Physiol. 244, G695–G700
49 Taylor, A.N., Gleason, W.A. and Lankford, G.L. (1984) J. Histochem. Cytochem. 32, 153–158
50 Ghijsen, W.E.J.M., and Van Os, C.H. (1982) Biochim. Biophys. Acta 689, 170–172
51 Braun, H.J., Birkenhäger, J.C. and De Jonge, H.R. (1984) Biochim. Biophys. Acta 774, 81–90