Proc. Natl. Acad. Sci. USA
Vol. 74, No. 8, pp. 3612–3616, August 1977
Physiological Sciences

Calcium translocation by Golgi and lateral-basal membrane vesicles from rat intestine: Decrease in vitamin D-deficient rats

(microvilli/secretion)

ROGER A. FREEDMAN*†, MILTON M. WEISER*‡§, AND KURT J. ISSELBACHER*‡

* Gastrointestinal Unit, Massachusetts General Hospital, Boston, Massachusetts 02114; † Harvard–Massachusetts Institute of Technology Program in Health Sciences and Technology, Boston, Massachusetts 02114; the ‡ Harvard Medical School; and § Howard Hughes Medical Institute, Miami, Florida 33152

Contributed by Kurt J. Isselbacher, June 3, 1977

ABSTRACT **Intestinal Ca^{2+} transport was studied in membrane vesicles isolated from microvillus, Golgi, and lateral-basal membrane preparations. Ca^{2+} uptake by these vesicles was measured by determination of $^{45}Ca^{2+}$ associated with these membranes after collection by micropore filtration. Golgi membranes showed the highest initial rate and equilibration level of Ca^{2+} uptake. Approximately 90% of this Ca^{2+} uptake was into an osmotically responsive space, suggesting that what was measured was predominantly Ca^{2+} translocation. Vitamin D-deficient rats showed a markedly diminished rate of uptake and level of equilibration. These data indicate that a Ca^{2+}-translocating process was associated with Golgi membranes to a greater extent than with surface membranes and that this process was markedly decreased in vitamin D-deficient rats. The results suggest that the Golgi apparatus participates in intestinal Ca^{2+} absorption.**

A vitamin D-dependent Ca^{2+} transport process is an important property of the small intestine epithelium, but the molecular mechanisms of Ca^{2+} transport and vitamin D-dependent steps are not well-defined (1, 2). Some studies have suggested that the microvillus membrane is the locus of the active transport and/or vitamin D-dependent step (3–7), whereas others have implicated the lateral-basal membrane (8, 9). Still other investigators have emphasized intracellular factors in intestinal Ca^{2+} transport, particularly a vitamin D-dependent Ca^{2+}-binding protein (CaBP) (10, 11) or mitochondrial Ca^{2+} sequestration (12). The Golgi apparatus is a cellular organelle known to sequester various substances in preparation for secretion (13) or transcellular transport, as in intestinal fat absorption (14). Warner and Coleman (15) studied Ca^{2+} localization within the intestine with an electron probe x-ray microanalyzer and found discrete Ca^{2+} sequestrations in the supranuclear and lateral-basal membrane areas of the cell, which correspond to the distribution of Golgi membranes.

The present study analyzes the Ca^{2+}-translocation properties of Golgi, lateral-basal, and microvillus membranes, all of which were isolated from intestinal epithelium as vesicle preparations. Vesicles derived from Golgi membranes were found to be more active in Ca^{2+} translocation than surface membranes and this Ca^{2+} translocation was markedly decreased in vitamin D-deficient animals.

MATERIALS AND METHODS

Animals. Weanling male albino rats were obtained from Holtzman Co. (Madison, WI). To make rats vitamin D deficient, they were fed a purified low-calcium diet containing 0.02% calcium and 0.30% phosphorus with no vitamin D and housed under incandescent light. The animals were sacrificed after 2–3 weeks (weight, 50–60 g) on this regimen.

Tissue Preparation. The animals were stunned and then killed by cervical dislocation. The small intestine was removed, washed with 0.154 M NaCl, and slit open longitudinally and the mucosa was obtained by scraping. A purified microvillus membrane was prepared by the method of Hopfer *et al.* (16). Lateral-basal membranes were prepared by a modification of methods described by Mircheff and Wright (17). The intestinal scrapings were diluted in 5 mM NaEDTA (pH 7.0), 15 ml per rat at 0°, and homogenized with 10 strokes of a loose-fitting-pestle Dounce homogenizer. All subsequent procedures were done at 0–4°. The homogenate was then centrifuged for 20 min at 55,000 × *g*, and the pellet was resuspended in buffer A (0.25 M sorbitol/12.5 mM NaCl/0.5 mM NaEDTA/5 mM histidine–imidazole buffer, pH 7.5), 15 ml per rat, and further homogenized with 75 strokes in the Dounce homogenizer. This homogenate was diluted with buffer A (100 ml per rat) and centrifuged at 1400 × *g* for 15 min in a Beckman 30 rotor. The supernatant was then centrifuged at 55,000 × *g* for 45 min. The pellet was resuspended in sorbitol (60 g/100 ml) in 5 mM histidine–imidazole buffer (pH 7.5), 15 ml per rat, by 10 strokes with a motor-driven Potter–Elvehjem homogenizer. The suspension (2.4 ml) was put into an SW-41 tube (Beckman no. 31411) and overlayed with 3, 4, and 3 ml each of sorbitol (40, 30, and 20 g/100 ml, respectively) in 5 mM histidine–imidazole buffer, pH 7.5. The discontinuous density gradients were placed in a Beckman SW-41 rotor and centrifuged at 200,000 × *g* for 2 hr. Fractions were collected beginning from the top of the gradient. Each fraction was diluted with 100 mM sodium *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonate (Na·Hepes) buffer, pH 7.0, and centrifuged at 78,000 × *g* for 2 hr, and the resultant pellet was resuspended in 0.5–1.0 ml of 100 mM NaCl/20 mM Na·Hepes, pH 7.0/1 mM $MgCl_2$.

Enzyme Assays. Sucrase (β-fructofuranoside, β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) was determined by the method of Messer and Dahlqvist (18). Alkaline phosphatase [orthophosphoric-monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1] was determined as described by Weiser (19). Na^+,K^+-ATPase (ATP phosphohydrolase, EC 3.6.1.3) was determined according to Quigley and Gotterer (20). NADPH-cytochrome *c* reductase (NADPH–cytochrome reductase, NADPH:ferricytochrome oxidoreductase, EC 1.6.2.4) was assayed by the method of De Duve *et al.* (21) and cytochrome oxidase (cytochrome *c* oxidase, ferrocytochrome *c*: oxygen oxidoreductase, EC 1.9.3.1) was assayed according to Cooperstein and Lazarow (22). Galactosyltransferase activity was measured as described (23). The exogenous glycoprotein

The costs of publication of this article were defrayed in part by the payment of page charges from funds made available to support the research which is the subject of the article. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: CaBP, Ca^{2+}-binding protein; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

Table 1. Marker enzyme specific activities of sorbitol density-gradient fractions from normal and vitamin D-deficient rats

Fraction	Galactosyltransferase activity, cpm × 10^{-3}/mg protein per 60 min		Na^+,K^+-ATPase activity, μmol/mg protein per 15 min		Sucrase activity, nmol/mg protein per 15 min	
	Control	D-deficient	Control	D-deficient	Control	D-deficient
20/30	52	53	<0.01	0.06	<0.01	<0.01
30/40	18	18	1.80	1.85	<0.01	<0.01
40	8	8	1.18	1.13	<0.01	0.40
40/50	6	5	0.31	0.09	4.03	4.90
50	2	2	0.29	0.10	5.13	5.65

acceptor for galactosyltransferase was fetuin from which the terminal sialic acid and penultimate galactose had been removed (24).

Ca^{2+}-Uptake Assay. Ca^{2+} uptake was usually measured within 24 hr of completion of the membrane preparation. Membranes (final protein concentration, 0.03–0.10 mg/ml) were incubated at 25° in 4.2 ml of buffer B (20 mM Na·Hepes buffer, pH 8.0/2.5 mM $MgCl_2$/100 mM NaCl) and, unless otherwise noted, 0.5 mM $^{45}CaCl_2$ (0.88 μCi/mol). At various time intervals, 1.0-ml aliquots were removed and immediately filtered through nitrocellulose filters (Schleicher and Schuell no. BA85, 0.45 μm) that had been presoaked in 10 mM $CaCl_2$. The filters were dissolved in 10 ml of a Triton X-100 scintillation solution (Triton X-100, 1000 ml; toluene, 1834 ml; Liquifluor; 166 ml) and assayed in a liquid scintillation counter. Radioactivity nonspecifically bound to the nitrocellulose filters was determined by filtering incubation medium free of membranes and this value was subtracted from all experimental values.

All radioactive materials and Liquifluor were purchased from New England Nuclear Corp. Unlabeled UDP-galactose was obtained from Sigma Chemical Co. Protein was determined by the method of Lowry *et al.* (25).

RESULTS

Membranes isolated by density-gradient centrifugation by methods similar to those used here are known to form vesicles spontaneously, as are purified brush border membranes (16). Electron micrographs of the density-gradient membrane fractions confirmed their vesicular conformation (Fig. 1).

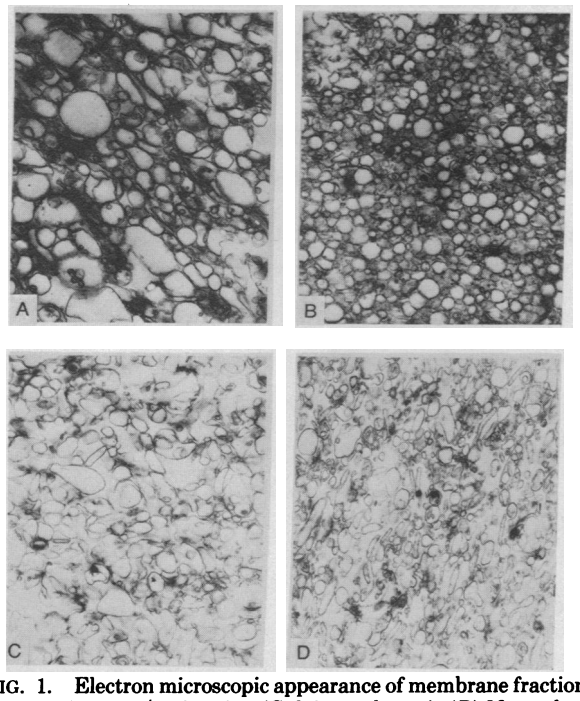

FIG. 1. Electron microscopic appearance of membrane fractions. (*A*) Normal rat, 20/30 fraction (Golgi membrane). (*B*) Normal rat, 40 fraction (lateral-basal membrane). (*C*) Vitamin D-deficient rat, 20/30 fraction (Golgi membrane). (*D*) Vitamin D-deficient rat, 40 fraction (lateral-basal membrane). (×10,000.)

Galactosyltransferase, Na^+,K^+-ATPase, and sucrase were used as marker enzymes for Golgi, lateral-basal, and microvillus membranes, respectively. Specific activities of these enzymes were measured in the density-gradient fractions and in the purified brush border membranes from both normal and vitamin D-deficient rats. The marker enzyme distribution of the preparations used in this study are summarized in Table 1 and are similar to that found with isolated cells and a sucrose gradient by M. M. Weiser, M. Neumeier, A. Quaroni, and K. Kirsch (unpublished data). There was no difference in enzyme distributions between membranes isolated from normal and vitamin D-deficient rats. The relative enrichment of galactosyltransferase and the very low Na^+,K^+-ATPase in the 20/30 fraction identified this fraction as being rich in membranes derived from the Golgi apparatus. Similarly, the 40 fraction was identified as lateral-basal membranes on the basis of Na^+,K^+-ATPase enrichment; the microvillus fraction was enriched in sucrase activity. Contamination by endoplasmic reticulum and mitochondria was insignificant.

When the membrane fractions obtained from the sorbitol density gradient were tested for Ca^{2+} uptake, the Golgi-rich 20/30 membrane fraction showed the greatest initial rate of uptake as well as the greatest equilibrium level (Fig. 2). Microvillus membrane vesicles prepared separately by the method of Hopfer *et al.* (16) showed low initial rates and equilibrium levels of Ca^{2+} uptake.

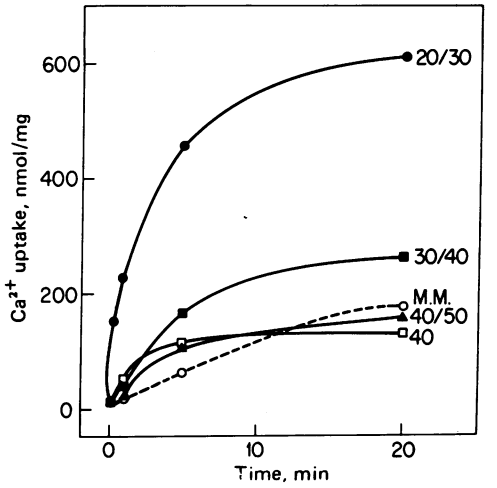

FIG. 2. Ca^{2+} uptake by various membrane fractions from the intestinal epithelial cell. ○, Microvillus membranes (M.M.) obtained by the method of Hopfer *et al.* (16). The other membrane fractions were obtained from sorbitol density-gradient centrifugation (see *Materials and Methods*). ●, Golgi-rich 20/30 fraction; ■, lateral-basal 30/40 fraction; □, lateral-basal 40 fraction; and ▲, 40/50 fraction. Membrane protein concentration in all incubations was 0.03 mg/ml.

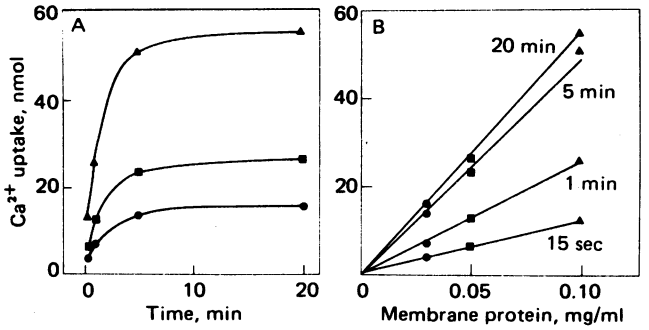

FIG. 3. Ca^{2+} uptake by the Golgi-rich 20/30 fraction at several membrane protein concentrations. (*A*) Time course of uptake. Membrane protein concentration: ●, 0.03 mg/ml; ■, 0.05 mg/ml; ▲, 0.10 mg/ml. (*B*) Same data plotted as a function of membrane protein concentration which shows that the uptake is directly proportional to protein concentration at all time points.

Ca^{2+} uptake by the Golgi-rich 20/30 fraction was studied with three different concentrations of membrane protein in the incubation medium. Fig. 3*A* indicates that equilibration of uptake was reached by 20 min at all three concentrations. Because the rate of uptake was greatest in the first 15 sec, this was taken as an approximation of the initial rate of uptake. Fig. 3*B* shows that the amount of uptake at all time points was proportional to the membrane protein concentration in the incubation medium. Ca^{2+} uptake was also measured over a range of Ca^{2+} concentrations (Fig. 4). The initial rate of Ca^{2+} uptake by membrane vesicles from the Golgi-rich 20/30 fraction showed saturation kinetics with half-maximal activity at a Ca^{2+} concentration of approximately 0.5 mM.

Uptake by membrane vesicles as determined by this micropore filtration technique measures both transport into the intravesicular space and binding to the membrane (26). The method of Hopfer *et al.* (16) was used to distinguish transport from binding by measuring Ca^{2+} uptake in the presence of increasing amounts of impermeable osmotically active cellobiose at concentrations that shrink the available intravesicular space. When Ca^{2+} uptake was plotted against the reciprocal of the total external osmolarity (Fig. 5), the slope was indicative of translocation into an osmotically responsive space. Extrapolation to infinite medium osmolarity (Y-intercept) yielded an approximation of the amount of membrane-bound Ca^{2+}, which was only 10% of the 20-min uptake.

When Ca^{2+} uptake by membrane vesicles obtained from vitamin D-deficient rats was measured, significant decreases in uptake were observed. This is shown in Fig. 6*A* in which the time course of Ca^{2+} uptake by Golgi-rich 20/30 vesicles from vitamin D-deficient rats is compared to that from normal rats. Decreases in both the initial rate (94% decrease) and the equilibrium level (81% decrease) of uptake were observed in vesicles from vitamin D-deficient rats. Lateral-basal membrane vesicles from vitamin D-deficient rats showed similar but less marked decreases in initial rate and equilibrium level of uptake (Fig. 6*B*). Lineweaver-Burk plots showed that half-maximal velocities were reached at similar Ca^{2+} concentrations for normal and vitamin D-deficient animals; however, the maximal velocity was decreased in vesicles from vitamin D-deficient animals.

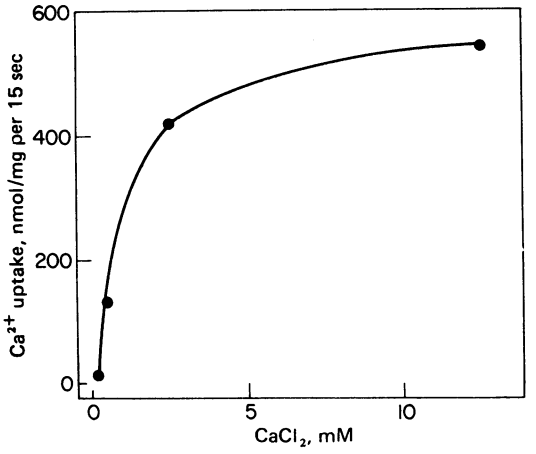

FIG. 4. The effect of Ca^{2+} concentration on the initial rate of Ca^{2+} uptake by Golgi-rich 20/30 membrane vesicles. Membrane protein concentration was 0.03 mg/ml.

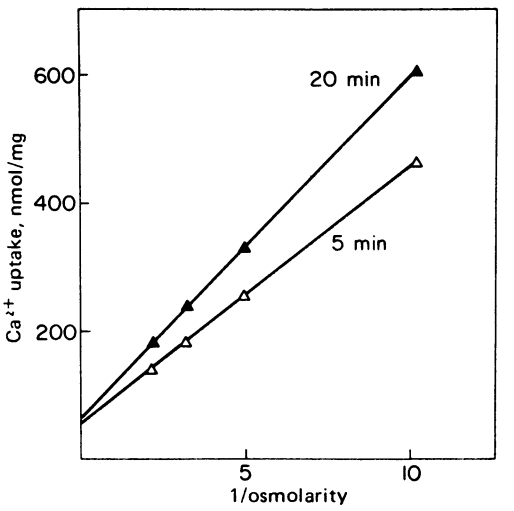

FIG. 5. Effect of medium osmolarity on Ca^{2+} uptake. Uptake by Golgi-rich 20/30 membrane fractions was measured at 5 min (△) and 20 min (▲) from media containing 25 mM NaCl, 20 mM Na·Hepes (pH 8.0), 2.5 mM $MgCl_2$, 0.5 mM $^{45}CaCl_2$, and enough cellobiose to give the indicated osmolarity (shown as inverse osmolarity). The osmolarity was calculated as the sum of contributions from all solutes, assuming ideal behavior. Membrane protein concentration in the incubation media was 0.03 mg/ml.

DISCUSSION

Transport of Ca^{2+} across the intestinal epithelial cell requires entry into the cell through the microvillus membrane, passage through the cell, and exit across the lateral-basal membrane.

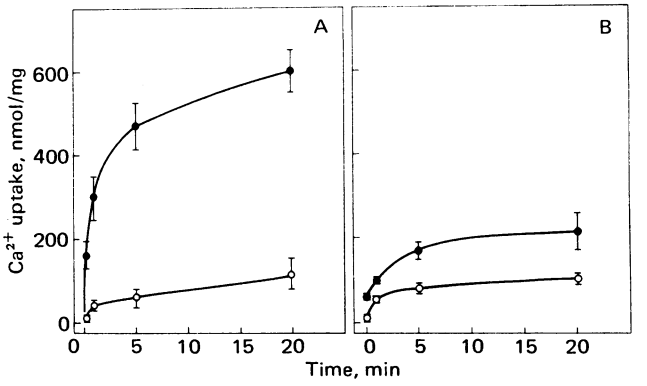

FIG. 6. Effect of vitamin D deficiency on Ca^{2+} uptake by intestinal membrane vesicles. (*A*) Calcium uptake by Golgi-rich 20/30 fractions from normal (●) and vitamin D-deficient (○) rats. (*B*) Ca^{2+} uptake by lateral-basal membrane 40 fractions from normal (●) and vitamin D-deficient (○) rats.

Vitamin D deficiency specifically reduces the ability of the intestinal epithelial cell to transport Ca^{2+}. A metabolite of vitamin D, 1,25-dihydroxycholecalciferol, has been shown to be the most active calciferol in enhancing Ca^{2+} transport through the epithelial cell, but the molecular mechanisms for Ca^{2+} transport and the vitamin D-affected steps are still unclear (1, 2).

In an attempt to define the molecular mechanism involved in intestinal Ca^{2+} transport and the vitamin D-dependent steps, we studied Ca^{2+} translocation into vesicles formed by various subcellular membranes isolated from intestinal mucosa of both normal and vitamin D-deficient rats. The uptake measured represents Ca^{2+} bound to the membrane as well as Ca^{2+} translocated into the intravesicular space (26). In the studies presented here, the membrane vesicles derived from intestinal Golgi membrane appeared to have the greatest rate of Ca^{2+} uptake as well as the greatest level at equilibrium (Fig. 2). When exposed to hyperosmolar media, these Golgi membrane vesicles showed a decrease in their ability to translocate Ca^{2+} that was inversely proportional to the medium osmolarity (Fig. 5). The data suggest that approximately 90% of the observed uptake at 20 min represented translocation into an osmotically responsive intravesicular space.

The apparent initial rate of uptake would be affected by the number of membranes forming closed vesicles and by the thickness of the membranes. Electron microscopic examination of Golgi, lateral-basal (Fig. 1), and microvillus (16) membrane vesicles revealed no detectable difference in either the thickness or the fraction of membranes forming closed vesicles. Therefore, the increased rate of Ca^{2+} uptake by Golgi as compared to lateral-basal and microvillus membrane vesicles suggests that the intestinal Golgi membranes have a greater intrinsic capacity to translocate Ca^{2+}. Unfortunately, we have no data on the "sidedness" of the vesicles—i.e., percent right-side-out (for Golgi, the cytoplasmic side of the membrane facing outward) or inside-out.

Golgi membrane vesicles prepared from vitamin D-deficient rats showed an initial rate of Ca^{2+} uptake that was only 6% of that observed with Golgi membranes from normal rats (Fig. 6*A*). The level of uptake at equilibrium was also decreased in vitamin D-deficient Golgi vesicles (Fig. 6*A*). Lateral-basal membrane vesicles from vitamin D-deficient rats showed similar but less marked decreases in initial rate and equilibrium level of uptake (Fig. 6*B*). It should be stressed that membrane vesicles from vitamin D-deficient animals showed no differences compared to normal rats in marker enzyme distribution along the sorbitol gradient (Table 1) and in their electron microscopic appearances (Fig. 1). Preliminary kinetic data suggest that there are fewer sites for Ca^{2+} translocation in the vitamin D-deficient membrane vesicles.

CaBP has been suggested as a vitamin D-dependent intracellular carrier. Imunofluorescent localization of CaBP however, indicates that the protein is a component of mucus and is not present within the cytoplasm of the absorptive cell (27) although it is isolated from the 38,000 × *g* supernatant of mucosal homogenates (11). The role of CaBP as a vitamin D-dependent intracellular carrier has been challenged by Spencer *et al.* (28) who have shown that correction of Ca^{2+} transport in vitamin D-deficient animals after injection of 1,25-dihydroxycholecalciferol preceded the detection of CaBP mRNA and that high levels of CaBP persisted after Ca^{2+} transport had returned to prerepletion levels.

It has also been proposed that mitochondria may sequester Ca^{2+} and be involved in transcellular Ca^{2+} transport (12). In vitamin D-deficient animals, electron microscope studies have shown $Ca(PO_3)_2$ crystals to be largely restricted to the microvillus region of cells; after vitamin D administration, many granules were observed in mitochondria and fewer in the microvilli (29). However, electron probe microanalyses have shown no Ca^{2+} localization within mitochondria, and a phosphorus signal was not associated with any Ca^{2+} localizations (15). These electron probe microanalyses also showed the Ca^{2+} tended to be located in a supranuclear position and near the lateral membrane. The Golgi apparatus of intestinal absorptive cells tends to be supranuclear as well as in areas near the lateral membrane.

The present results suggest that the Golgi apparatus may be involved in the process of intracellular Ca^{2+} accumulation; this Ca^{2+} uptake by the Golgi apparatus is affected by the vitamin D status of the animal. It is possible that the Golgi apparatus serves to transport Ca^{2+} vectorially out of the cell by an interaction with the lateral-basal membrane. In addition, Ca^{2+} uptake by lateral-basal membrane vesicles may represent the exit step of Ca^{2+} from the cell.

The association of the Golgi apparatus with secretion and Ca^{2+} transport is consistent with present concepts of cellular secretory mechanisms in a number of tissues. The Golgi apparatus functions to sequester and then secrete a variety of substances including endocrine and exocrine secretions (13, 30), immunoglobulins (31), and surface glycoproteins (32). Indeed, the Golgi apparatus is thought of as a universal "organelle of secretion," serving several functions in the secretory process: glycosylation of secretory glycoproteins, intracellular transport, concentration, storage, and secretion by exocytosis (13). Ca^{2+} has been shown to be required for the last step of this process—i.e., the release of secretory products by exocytosis (33). This Ca^{2+} requirement for secretion has been shown for the endocrine and exocrine parts of the pancreas, neurosecretory cells, granulocytes, and mast cells (33). There is evidence that in the pancreas (34) and in the adrenal medulla (33) Ca^{2+} is not only necessary for secretion but that relatively high concentrations of Ca^{2+} are released along with the secretory product. In invertebrates, intracellular Ca^{2+} granules are associated with the Golgi system in a variety of tissues and these granules have been shown to be secreted into intercellular spaces (35).

Intracellular membrane structures, in some cases shown to be Golgi membranes, are involved in the intestinal absorption of substances such as fats (14), vitamin B_{12} (36), immunoglobulins (37), and horseradish peroxidase (38). These substances traverse the intestinal cell from microvillus to lateral membrane and at some point become enclosed within intracellular membranes before their exit into the intercellular space. The present studies support the concept that during its absorption Ca^{2+} traverses the intestinal cell and is secreted from it with the help of the Golgi apparatus in a manner similar to that described for the above substances. The precise role of Ca^{2+} translocation by Golgi membranes and, in particular, its relationship to events in the microvillus and lateral-basal membranes require further investigation.

We thank Ms. Julia MacLaughlin for invaluable technical assistance and suggestions. This work was supported by Grants CA-16703, AM-04501, and AM-01392 from the National Institutes of Health.

1. DeLuca, H. F. & Schnoes, H. K. (1976) *Annu. Rev. Biochem.* **45**, 631–666.
2. Omdahl, J. L. & DeLuca, H. F. (1973) *Physiol. Rev.* **53**, 327–372.
3. Martin, D. L., Melancon, M. J., Jr. & DeLuca, H. F. (1969) *Biochem. Biophys. Res. Commun.* **35**, 819–823.
4. Melancon, M. J., Jr. & DeLuca, H. F. (1970) *Biochemistry* **9**, 1658–1664.

5. Kowarski, S. & Schachter, D. (1973) *J. Clin. Invest.* **52,** 2765–2773.
6. Adams, T. H., Wong, R. G. & Norman, A. W. (1970) *J. Biol. Chem.* **245,** 4432–4442.
7. Wong, R. G. & Norman, A. W. (1975) *J. Biol. Chem.* **250,** 2411–2419.
8. Schachter, D., Kowarski, S., Finkelstein, J. D. & Ma, R. W. (1966) *Am. J. Physiol.* **211,** 1131–1136.
9. Birge, S. J. & Gilbert, H. R. (1974) *J. Clin. Invest.* **54,** 710–717.
10. Wasserman, R. H. & Taylor, A. N. (1966) *Science* **152,** 791–793.
11. Wasserman, R. H., Corradino, R. A. & Taylor, A. N. (1968) *J. Biol. Chem.* **243,** 3978–3986.
12. Borle, A. B. (1973) *Fed. Proc.* **32,** 1944–1950.
13. Palade, G. (1975) *Science* **189,** 347–358.
14. Friedman, H. I. & Cardell, R. R., Jr. (1972) *J. Cell. Biol.* **52,** 15–40.
15. Warner, R. R. & Coleman, J. R. (1975) *J. Cell Biol.* **64,** 54–74.
16. Hopfer, U., Nelson, K., Perrotto, J. & Isselbacher, K. J. (1973) *J. Biol. Chem.* **248,** 25–32.
17. Mircheff, A. K. & Wright, E. M. (1976) *J. Membr. Biol.* **28,** 309–333.
18. Messer, M. & Dahlqvist, A. (1966) *Anal. Biochem.* **14,** 376–392.
19. Weiser, M. M. (1973) *J. Biol. Chem.* **248,** 2536–2541.
20. Quigley, J. R. & Gotterer, G. S. (1969) *Biochim. Biophys. Acta* **173,** 456–468.
21. De Duve, C., Pressman, B. C., Gianetto, K., Wattiaux, R. & Appleman, R. (1955) *Biochem. J.* **60,** 604–617.
22. Cooperstein, S. J. & Lazarow, A. (1951) *J. Biol. Chem.* **189,** 665–670.
23. Podolsky, D. K. & Weiser, M. M. (1975) *Biochem. Biophys. Res. Commun.* **65,** 545–551.
24. Podolsky, D. K. & Weiser, M. M. (1975) *Biochem. J.* **146,** 213–221.
25. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
26. Reed, K. C. & Bygrave, F. L. (1975) *Anal. Biochem.* **67,** 44–54.
27. Taylor, A. N. & Wasserman, R. H. (1970) *J. Histochem. Cytochem.* **18,** 107–115.
28. Spencer, R., Charman, M., Wilson, P. & Lawson, E. (1976) *Nature* **263,** 161–163.
29. Sampson, H. W., Matthews, J. L., Martin, J. H. & Kunin, A. S. (1970) *Calcif. Tissue Res.* **5,** 305–316.
30. Whur, P., Herscovics, A. & LeBlond, C. P. (1969) *J. Cell Biol.* **43,** 289–311.
31. Zagury, D., Uhr, J. W., Jamieson, J. D. & Palade, G. E. (1970) *J. Cell Biol.* **46,** 52–63.
32. Whaley, W. G., Dauwalder, M. & Kephart, J. E. (1972) *Science* **175,** 596–599.
33. Rubin, R. P. (1970) *Pharmacol. Rev.* **22,** 389–428.
34. Case, R. M. & Clausen, T. (1973) *J. Physiol. (London)* **235,** 75–102.
35. Simkiss, K. (1976) "Calcium in biological systems," in *Symposium of the Society for Experimental Biology XXX* (Cambridge University Press, Cambridge, England), pp. 423–444.
36. Weisberg, H., Rhodin, J. & Glass, G. B. J. (1968) *Lab. Invest.* **19,** 516–525.
37. Rodewald, R. (1971) *J. Cell Biol.* **45,** 635–640.
38. Cornell, R., Walker, W. A. & Isselbacher, K. J. (1971) *Lab. Invest.* **25,** 42–48.