Membrane-bound and Fluid-phase Macromolecules Enter Separate Prelysosomal Compartments in Absorptive Cells of Suckling Rat Ileum

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ABSTRACT The absorptive cell of the suckling rat ileum is specialized for the uptake and digestion of milk macromolecules from the intestinal lumen. The apical cytoplasm contains an extensive tubulocisternal system, a variety of vesicles and multivesicular bodies (MVB), and a giant phagolysosomal vacuole where digestion is completed. To determine if sorting of membrane-bound and fluid-phase macromolecules occurs in this elaborate endocytic system, we infused adsorptive and soluble tracers into ligated intestinal loops in vivo and examined their fates. Lysosomal compartments were identified by acid phosphatase histochemistry. Native ferritin and two ferritin-lectin conjugates that do not bind to ileal membranes (Con A, UEA_I) served as soluble tracers. Horseradish peroxidase binds to ileal membranes and thus was not useful as a fluid-phase tracer in this system. Cationized ferritin and a lectin that binds to terminal B-D-galactosyl sites on ileal membranes (Ricinus communis agglutinin[RCA_I]-ferritin) were used as tracer ligands. All tracers entered the wide apical invaginations of the luminal cell surface and were transported intracellularly. Membrane-bound tracers were found in coated pits and vesicles, and throughout the tubulocisternal system (where cationized ferritin is released from the membrane) and later, in large clear vesicles and MVB. In contrast, fluid-phase tracers appeared within 5 min in vesicles of various sizes and were not transported through the tubulocisternae; rather, they were concentrated in a separate population of vesicles of increasing size that contained amorphous dense material. Large clear vesicles, large dense vesicles, and MVB eventually fused with the giant supranuclear vacuole. Acid phosphatase activity was present in MVB and in the giant vacuole but was not present in most large vesicles or in the tubulocisternae. These results demonstrate that membrane-bound and soluble protein are transported to a common lysosomal destination via separate intracellular routes involving several distinct prelysosomal compartments.

Eucaryotic cells can incorporate extracellular macromolecules, both nonselectively (by fluid-phase endocytosis or pinocytosis) and selectively (by receptor-mediated or adsorptive endocytosis). In some cell types fluid-phase endocytosis seems to involve the formation of smooth membrane vesicles (32, 36, 41), whereas adsorptive endocytosis is invariably accomplished by the formation of vesicles from clathrin-coated membrane regions (2, 3, 11, 22, 37). When the two processes are conducted by separate membrane microdomains on the cell surface, solutes and ligands are already sorted to a large degree before entering internal membrane systems. In many cell types, however, fluid-phase and adsorbed macromolecules may be taken up together and sorted in endosomal compartments (1, 2, 16, 22, 26, 27, 30, 37).

Epithelial cells throughout the small intestine of neonatal rodents are highly specialized for endocytosis of luminal macromolecules. The membrane region involved in uptake is localized at the bases of the microvilli on the luminal cell surface, and the effective surface area of this region is amplified by deep membrane invaginations or pits between microvilli. Ultrastructural tracer studies of neonatal rat jejunum have demonstrated that soluble proteins (horseradish peroxidase [HRP])[1] and specific ligands (maternal immunoglobulin G) enter this sequestered extracellular space together and are

[1] *Abbreviations used in this paper:* CF, cationized ferritin; HRP, horseradish peroxidase; NF, native ferritin; RCA_I, *Ricinus communis* agglutinin.

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taken up together into a subjacent intracellular smooth membrane compartment (1, 30). Here they are sorted: ligand-receptor complexes appear to be withdrawn in coated vesicles and transported across the epithelium, whereas soluble tracers are transported exclusively to lysosomes (1, 29, 30).

The ileum of the neonatal rat is functionally different from the jejunum in that its absorptive epithelial cells have no receptors for maternal IgG; instead, they are specialized for uptake and intracellular digestion of milk macromolecules (5, 6). Although incorporation of luminal macromolecules in the ileum is thought to be a nonselective, fluid-phase process (6, 15, 20), the apical cytoplasm of ileal cells contains an elaborate array of membrane compartments, arranged in an orderly topographic sequence between the brush border and the nucleus: at the apical surface between microvilli are deep, wide invaginations of the apical plasma membrane; within and below the terminal web region is a labyrinthine system of narrow tubules and cisternae; a variety of clear vesicles, dense vesicles, and multivesicular bodies are postioned below the tubulocisternae; and finally, a giant supranuclear vacuole dominates the central cytoplasm (6, 15, 20, 42, 43). In this enormous lysosomal compartment, the degradation of milk macromolecules appears to be completed.

Previous investigators described the uptake of soluble tracers from the lumen and their delivery to the giant supranuclear vacuole by light and electron microscopy (5, 15, 20, 21, 35). These studies reported that lanthanum, HRP, and native ferritin (NF) follow a common pathway, percolating freely through the apical invaginations and the tubulocisternae (that were believed to be a continuous system, open to the lumen), and then being actively shuttled into the large vesicles that finally fuse with the giant supranuclear vacuole. The fate of adsorbed macromolecules such as experimental ligands, however, was not tested.

The elaborate morphologic organization of these membrane systems suggested to us that the ileal cell, like the jejunal cell, may be capable of sorting soluble and membrane-bound macromolecules. To test this hypothesis, we have applied a variety of ultrastructural tracers, including soluble proteins and experimental ligands, to the luminal surface of the ileal epithelium in suckling rats. We have defined in detail the intracellular routes followed by these tracers and have found that fluid-phase and membrane-bound proteins are transported through separate prelysosomal membrane compartments to a common lysosomal destination. The apical tubulocisternal system is demonstrated to be an intracellular endosomal compartment reserved for macromolecules that adsorb to ileal cell membranes, whereas fluid-phase tracers are transported in separate endosomal vesicles that appear to fuse with each other and concentrate their content. Our data suggest that the sorting process occurs rapidly, within the first few minutes of uptake and before entry into the major endosomal compartments.

MATERIALS AND METHODS

Experimental Animals: Sprague-Dawley rats (8–12-d old) were allowed to nurse up to the time of experimentation to maintain the differentiated function of the endocytic complex. Animals were anesthetized by an intraperitoneal injection of 0.1–0.2 ml of 25% urethane solution in PBS.

Ligated Loops In Vivo: Segments of distal ileum (3–4 cm long) were surgically ligated, leaving a loose ligature at the proximal end through which tracer protein solutions were injected. The ligature was tightened around the needle as it was withdrawn, providing a leak-free luminal compartment. For rinsing, a small cut at one end provided drainage while buffer solution was infused from the other end. Neonatal rats were maintained in a 37°C incubator under urethane anesthesia for up to 2 h before fixation of the ileal segment by infusion of fixative solution. Mesenteric circulation was maintained throughout the experiment.

Fixation and Tissue Processing: Mucosal samples were surgically removed after luminal infusion of a solution consisting of 2% fresh formaldehyde and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.4 at 22°C. Samples were then immersed in fixative and sliced at 0.5 mm. After 2–4 h of fixation, slices were rinsed in 0.1 M cacodylate buffer at 4°C, postfixed in 1% OsO_4 (in the same buffer), rinsed in cacodylate buffer, dehydrated, and embedded in Epon-Araldite. Thin sections were stained for 2–3 min with lead citrate and were examined with a JEOL 100 CX electron microscope.

Ultrastructural Tracers: Native ferritin (Polysciences, Inc., Warrington, PA) obtained as a purified cadmium-free solution in 0.01 M NaCl, was diluted in PBS and used at concentrations of 10, 25, and 50 mg/ml. Cationized ferritin ([CF]; Miles Laboratories, Inc., Elkhart, IN), supplied as an 11 mg/ml solution in 0.15 M NaCl, was diluted to 5.5 mg/ml with PBS. Affinity-purified lectin-ferritin conjugates (EY Laboratories, San Mateo, CA) were dialyzed against PBS to remove sodium azide and were used, with the appropriate cations, at a concentration of 1 mg/ml. To remove aggregates, all ferritin tracers were centrifuged at 12,000 *g* for 10 min prior to use.

Lyophilized horseradish peroxidase (Sigma type VI, Sigma Chemical Co., St. Louis, MO) was diluted in PBS to a concentration of 10 mg/ml. For HRP histochemistry, tissues were fixed for 2–4 h, sliced at ~100 μm, and rinsed in 0.1 M Na cacodylate buffer pH 7.0, and then in 0.1 M Tris buffer, pH 7.0. Mucosal slices were preincubated for 15 min (at 4°C in the dark) in a 0.05% solution of 3-3′-diaminobenzidine tetrahydrochloride (Polysciences Inc.) in Tris buffer pH 7.0, and then incubated in the same solution containing 0.1% H_2O_2, rinsed, postfixed in osmium tetroxide, and routinely embedded for thin sectioning.

Acid Phosphatase Histochemistry: For ultrastructural localization of acid phophatase, mucosal samples were fixed for 30–60 min in standard aldehyde solution and washed overnight in 0.1 M cacodylate buffer, pH 7.4 with 4% sucrose added. Tissue samples were sliced perpendicular to the mucosal surface at 50 μm on a Smith-Farquhar tissue chopper, and incubated for 60 min at 23°C in 0.05 M Tris-maleate buffer pH 5.2 containing sodium β-glycerophosphate and lead nitrate. Slices were then rinsed, postfixed in OsO_4, and routinely embedded for electron microscopy.

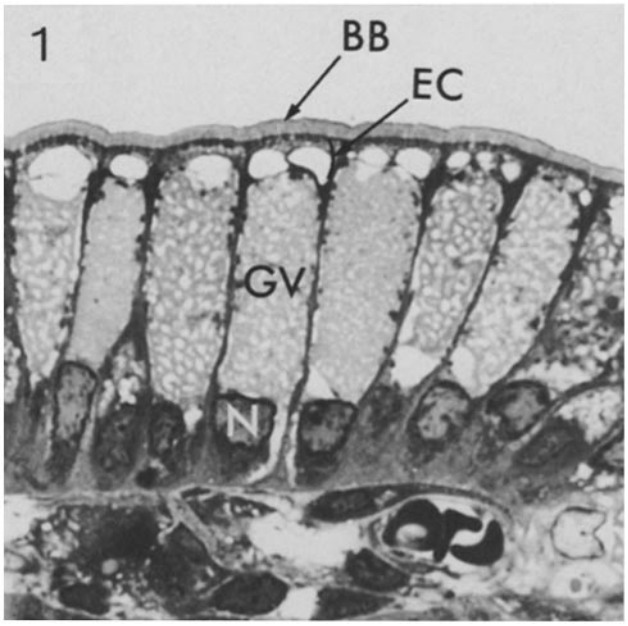

FIGURE 1 Light micrograph of absorptive cells on an ileal villus of a sucking rat. The cells are dominated by a giant supranuclear vacuole (*GV*). The narrow cytoplasmic region between the brush border (*BB*) and the giant vacuole is occupied by tubulocisternae and vesicles of the endocytic complex (*EC*). Only the large vesicles of this complex are visible by light microscopy. The nucleus (*N*) lies in the basal cytoplasm. × 780.

RESULTS

The major morphological features of the endocytic complex of suckling rat ileum, as described by previous authors (6, 15, 20, 21, 28, 35, 42, 43) are confirmed and extended here. The luminal surface of the absorptive cell displays an array of microvilli typical of intestinal absorptive cells. The central supranuclear region is dominated by a large vacuole (Fig. 1). Between the microvilli, the luminal surface is amplified by deep, wide invaginations whose membranes display a regular

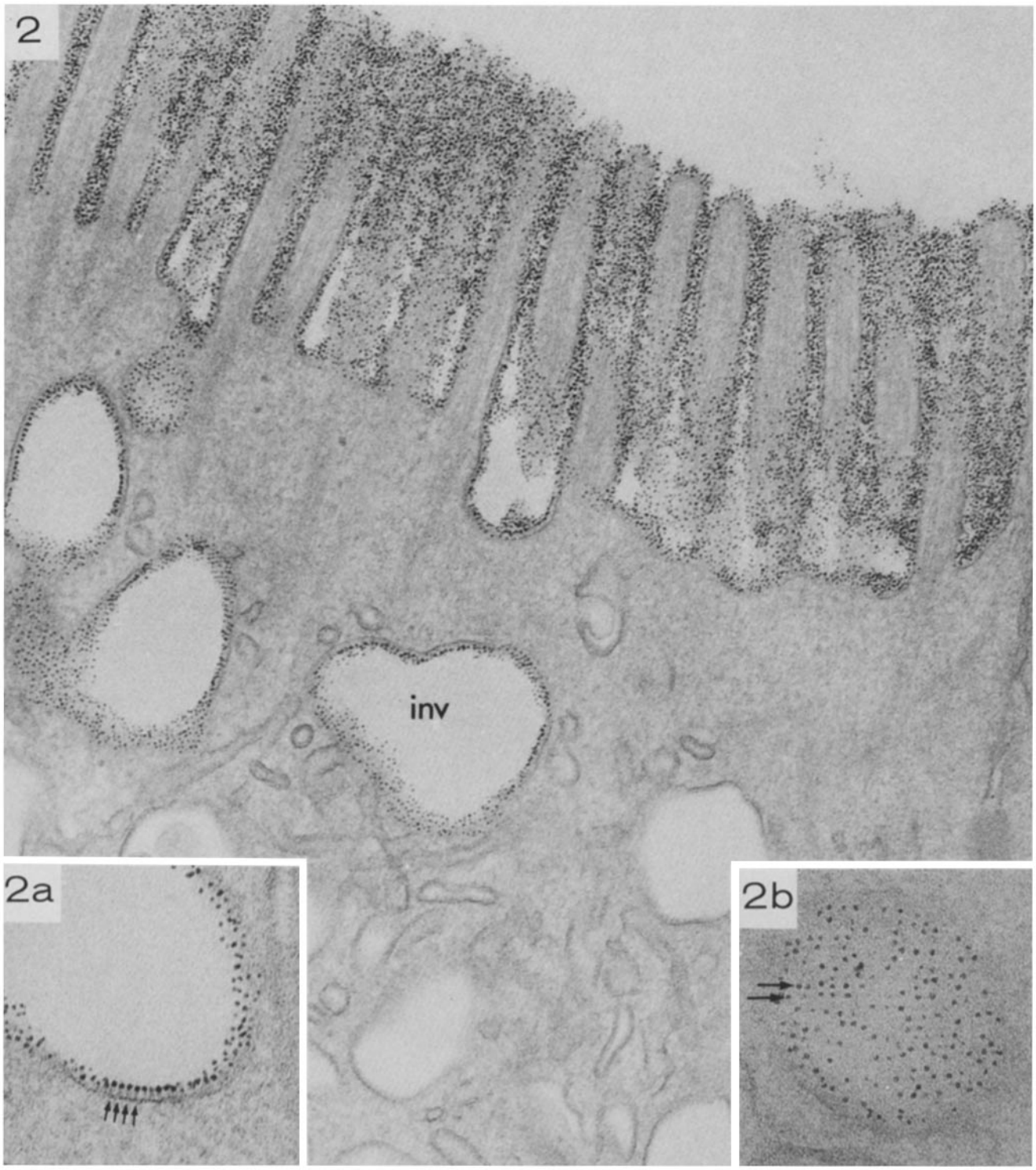

FIGURE 2 Prefixed ileum, exposed at 4°C to luminal cationized ferritin. CF binds to anionic cell surface sites on membranes in direct continuity with the luminal membrane. The microvilli and wide invaginations (*inv*) of apical membrane are labeled, but the narrow anastomosing tubules and cisternae of the apical cytoplasm are unlabeled and thus are not structurally connected to the apical invaginations. (*inset a*) Apical invaginations display an array of surface projections (arrows), each of which binds one molecule of CF. (*inset b*) Tangential section grazing the surface of an apical invagination. CF is bound to membrane projections in orderly linear arrays (arrows). Molecules of CF are spaced along the rows. × 74,000; × 126,000 (*inset a*); × 132,000 (*inset b*).

array of surface projections that have been previously reported and characterized in detail (18, 20, 28, 42). These surface arrays are also present on membranes of the extensive network of tubules and cisternae in the apical cytoplasm but are not present on the membranes of the various vesicles that lie above the giant vacuole or in the giant vacuole itself. We find that at least three morphologically-distinct populations of vesicles are interposed between the tubulocisternae and the giant vacuole: large, clear vesicles are usually located centrally, just below the tubulocisternae; vesicles with homogeneous, dense content tend to be positioned more laterally, and multivesicular bodies are congregated along the upper edge of the giant vacuole.

Acid phosphatase (APase) cytochemistry indicated that the giant vacuole and the adjacent multivesicular bodies are consistently APase positive, confirming previous light microscopic histochemical studies (6). A few of the largest dense-content vesicles and an occasional clear vesicle also show AcPase activity, but the vast majority of these vesicles, as well as the entire tubulocisternal system, are devoid of AcPase.

To visualize the entire extent of the apical membrane invaginations, tracers were applied to the luminal surfaces of cells whose membranes had been immobilized by aldehyde fixation. Both CF (Fig. 2) and HRP adhered to the membranes of microvilli and apical invaginations. Neither tracer entered the tubulocisternal system indicating that the tubulocisternae

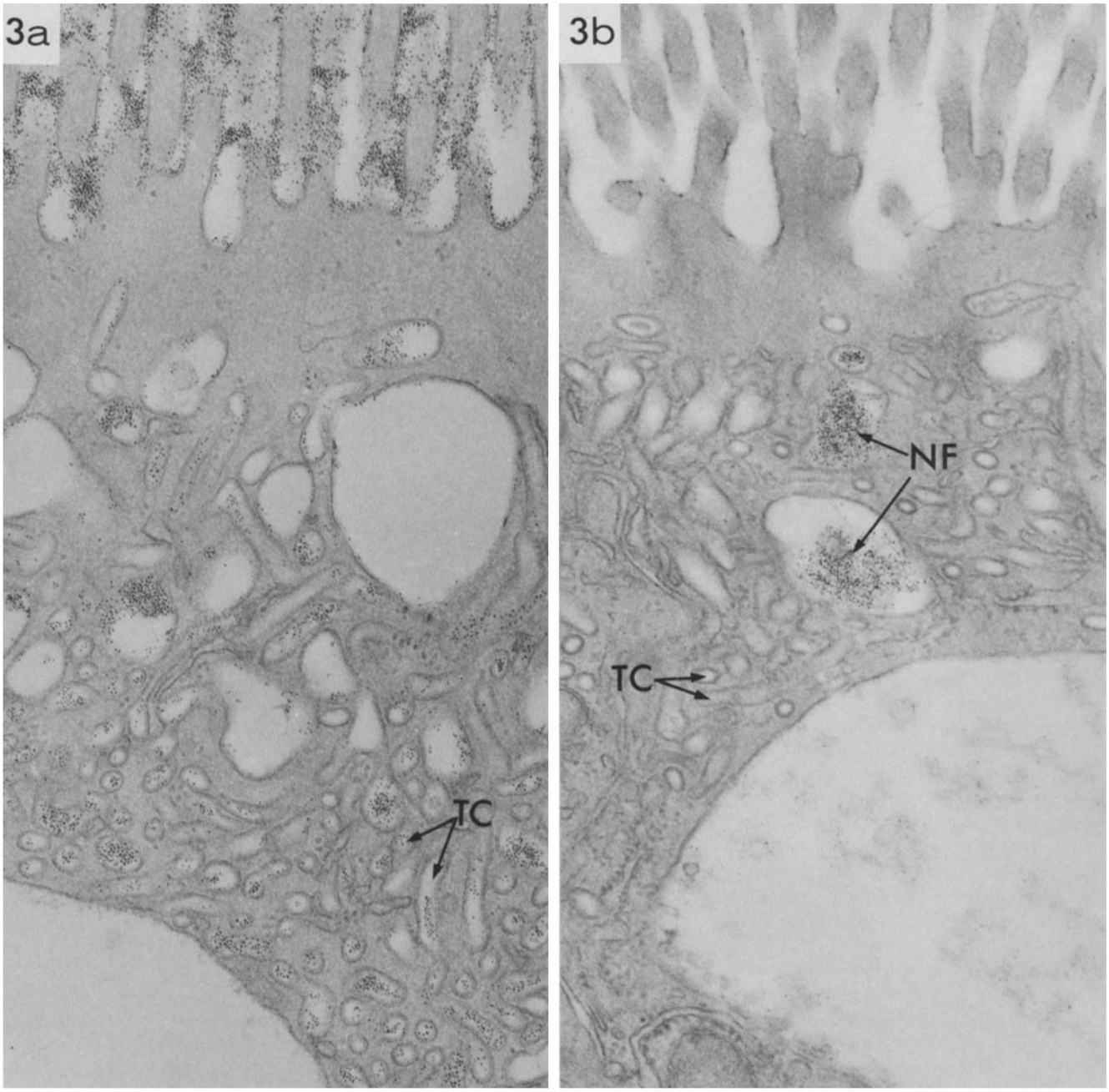

FIGURE 3 (*a*) Absorptive cell from a ligated loop, infused in vivo with CF for 10 min before fixation. CF has entered the tubulocisternal system (*TC*) where it is mostly membrane-bound. (*b*) Absorptive cell as in a but infused in vivo with native ferritin (*NF*) for 10 min before fixation. NF is present in vesicles but not in the tubules and cisternae. Some NF has entered a large vesicle. × 54,000.

are not continuous with the cell surface.

CF adhered in a random fashion to the plasma membrane or the glycocalyx on microvilli of fixed cells. On the apical invaginations, however, CF binding displayed a regular pattern (Fig. 2, *insets*), reflecting the arrangement of the surface arrays previously described (20, 28, 31). A single CF particle was present on each of the surface projections, so that CF was separated by a uniform distance from the membrane (Fig. 2*a*). In tangential sections, CF particles were seen spaced along the rows formed by the surface array (Fig. 2*b*). Since HRP adhered to components of the apical plasma membrane, it was not useful as a soluble tracer for this cell type. NF did not adhere to ileal cell membranes and was not retained on the luminal surfaces or in the apical invaginations of prefixed cells.

After luminal infusion in vivo, both CF and NF were rapidly endocytosed by ileal absorptive cells, but even at the earliest time intervals observed they were present in different intracellular compartments (Fig. 3). After 3, 5, and 10 min, CF appeared within the tubulocisternal system (Fig. 3*a*). NF, in contrast, was confined to vesicles in the apical cytoplasm and was generally not observed in tubulocisternae (Fig. 3*b*). After infusion of high concentrations (50 mg/ml) of NF, a few NF particles were seen in tubulocisternae; this small amount of NF is consistent with uptake of small volumes of fluid during adsorptive endocytosis. Since NF was not retained in pits or in forming vesicles that had not yet completely closed and sequestered their soluble content, the exact origin of the NF-containing vesicles could not be visualized. CF, attached to membrane-associated arrays, was seen in clathrin-coated pits along the apical invaginations and at the bases of microvilli (Fig. 4) as well as in coated vesicles in the apical cytoplasm. These observations suggest either that membrane-bound and fluid-phase macromolecules are taken up separately from the apical invaginations, or more probably, that they are sorted very rapidly after uptake in coated pits and vesicles.

At later times (20 and 30 min) NF and CF were collected in separate, larger prelysosomal vesicles (Fig. 5). Membrane-bound CF was still present in the tubulocisternae, but clusters of free CF appeared both in tubulocisternae and in large, clear vesicles (Fig. 5*a*). Very rarely, small amounts of CF were associated with the membrane of a clear vesicle. Free CF also appeared in multivesicular bodies that were AcPase positive (Fig. 6). From 30 to 60 min after luminal infusion of CF, clear vesicles and multivesicular bodies containing unbound tracer were observed in the process of fusing with the giant vacuole. NF, in contrast, was segregated in vesicles that contained dense, amorphous material (Fig. 5*b*). These vesicles became progressively larger and their content increasingly concentrated during 20 to 60 min of continuous NF uptake. Vesicles containing concentrated NF appeared to fuse with, and release their contents into the giant vacuole (Fig. 7), beginning at ~30 min after uptake.

Of the ferritin-conjugated lectins tested in these studies, only *Ricinus communis* agglutinin (RCA_I; specific for terminal β-D-galactose residues, reference 25) was bound to components of the plasma membranes of both microvilli and apical invaginations in vivo. This lectin tracer, like CF, was rapidly transported into the tubulocisternal system, then into large clear vesicles and multivesicular bodies, and finally into the giant vacuole (Fig. 8).

Other lectin-ferritin conjugates did not bind to ileal cell surfaces in vivo, including concanavalin A (specificity α-D-mannose and α-D-glucose), *Ulex europeaus* agglutinin (specificity L-fucose), and *Dolichos biflorus* agglutinin (specificity *N*-acetyl-D-galactosamine). These tracers were apparently taken up in the fluid phase since they did not enter tubulocisternae and were transported to the giant vacuole in the same types of vesicles that had carried NF.

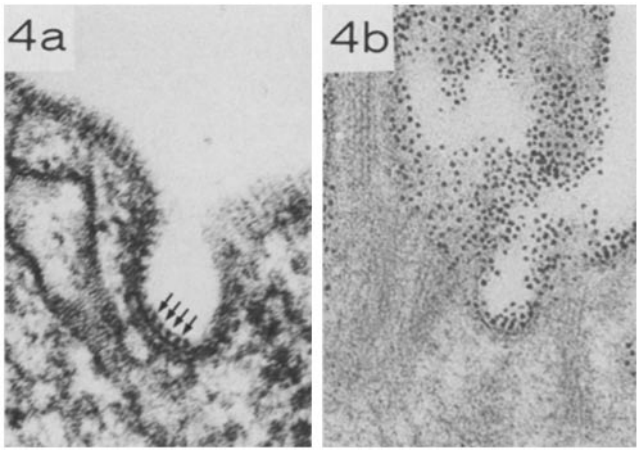

FIGURE 4 (*a*) Formation of coated vesicles on apical membranes. A coated pit along an apical invagination contains membrane projections in orderly linear arrays. (*b*) Coated pits also form at the bases of microvilli. After intraluminal infusion of cationized ferritin, membrane-bound CF is seen in coated pits. × 130,000 (*a*); × 86,000 (*b*).

Neither membrane-bound tracers (CF, RCA_I-ferritin) nor fluid phase tracers (NF, other lectin-ferritin complexes) were observed in the cisternae of the Golgi complex or in Golgi-associated vesicles at any time during the 90-min course of this study.

DISCUSSION

The process of adsorptive endocytosis, whose features have been summarized in recent reviews (2, 11, 37), has been elucidated principally through application of physiologic ligands to cells in vitro. In addition, ultrastructural tracer studies have demonstrated that nonphysiologic foreign macromolecules such as plant lectins and cationized ferritin, that can bind "opportunistically" to cell surface saccharides, may be internalized and delivered to lysosomes in the endocytic systems intended for physiologic ligands. Normally, however, most cell types would encounter such "opportunistic" ligands only in laboratory experiments.

The intestinal absorptive cell, unlike most cell types, is routinely exposed in vivo to a complex mixture of foreign macromolecules. Its apical plasma membrane faces the contents of the intestinal lumen that may include plant lectins, lectin-like surface proteins of microorganisms, cationic antigens, and toxins. Its situation is particularly challenging in the neonatal period when the epithelium of the entire small intestine is highly endocytic. Although infectious and allergic disorders of the intestinal mucosa are more common during this vulnerable period than in the adult (39), it is remarkable that this single cell layer is normally successful in preventing transepithelial passage of foreign proteins and opportunists, while at the same time it is conducting efficient transepithelial transport of maternal IgG in proximal regions and massive intracellular digestion of milk in distal regions.

Selective transport of maternal IgG in the duodenum-jejunum has been shown to occur by receptor-mediated endocytosis coupled with a transepithelial vesicular shuttle that bypasses the lysosomal system (1, 29, 30). In these studies, soluble tracer proteins that entered the initial endocytic compartment along with IgG were segregated from ligand-receptor complexes and transported to apical lysosomes for degradation (1, 30). In contrast, CF, that bound nonselectively to anionic groups on the endocytic membranes, was partially transported to lysosomes but was also carried across the jejunal epithelium in the vesicular shuttle intended for maternal IgG (30). This indicated that experimental tracer proteins that bind to intestinal cell surface saccharides can be used to trace the various intracellular paths that adsorbed luminal proteins may follow in vivo.

The endocytic membrane systems in absorptive cells of the suckling rat ileum are much more elaborate that one would predict for a single, lysosome-directed endocytic pathway for

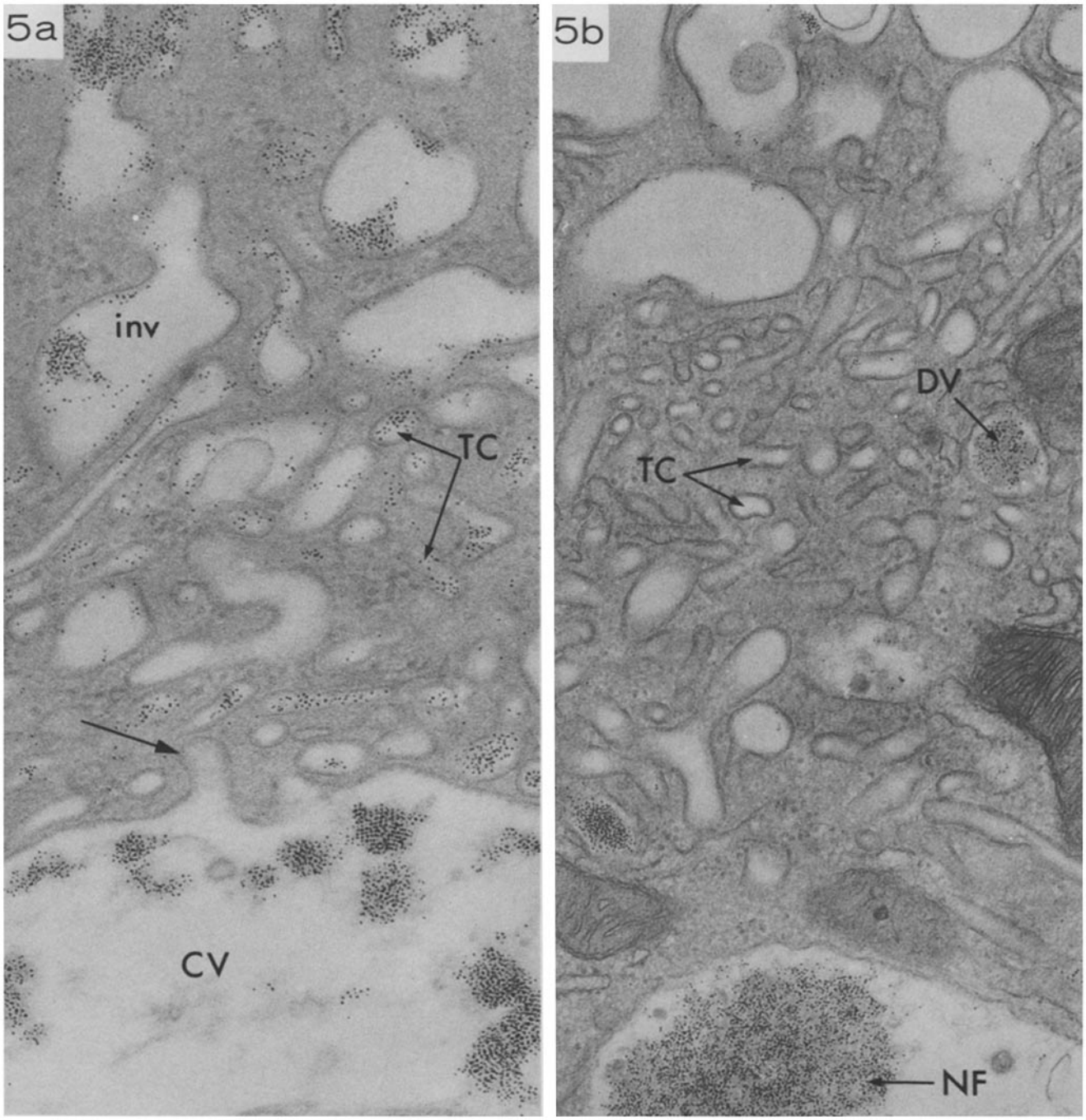

FIGURE 5 (a) Absorptive cell exposed in vivo to CF for 30 min before fixation. Membrane-bound CF is present on microvilli and apical invaginations, and throughout the tubulocisternae (*TC*). In addition, clusters of free CF are present in dilated cisternae, and in large clear vesicles (*CV*). A direct connection between the tubulocisternal system and the clear vesicle is indicated by the arrow. The tracer has not yet entered the grant supranuclear vacuole. *inv*, as in Fig. 5. (*b*) Absorptive cell from an ileal loop filled with native ferritin (*NF*) for 10 min, rinsed, and maintained for an additional 20 min prior to fixation. NF is present in small vesicles with dense content (DV), in multivesicular bodies, and is concentrated in large vesicles. × 58,000 (a); × 54,000 (*b*).

soluble milk macromolecules. Nevertheless, previous in vivo tracer studies reported that all proteins enter an open tubulocisternal system before uptake into vesicles and delivery to the giant vacuole (5, 6, 15, 20, 35). By applying tracers to prefixed tissue and at 4°C in vitro, we have shown that the tubulocisternae represent an internal compartment, and this implies that entry of luminal protein requires transient membrane fusion events, or a vesicular membrane shuttle. By following the transport routes of soluble and adsorbed tracers at short time intervals at 37°C, we have shown that soluble tracer proteins are not transported through the tubulocisternal system; rather, the tubulocisternae represent an extensive prelysosomal compartment that is reserved for macromolecules that can bind or adsorb to the invaginated membranes of the luminal cell surface.

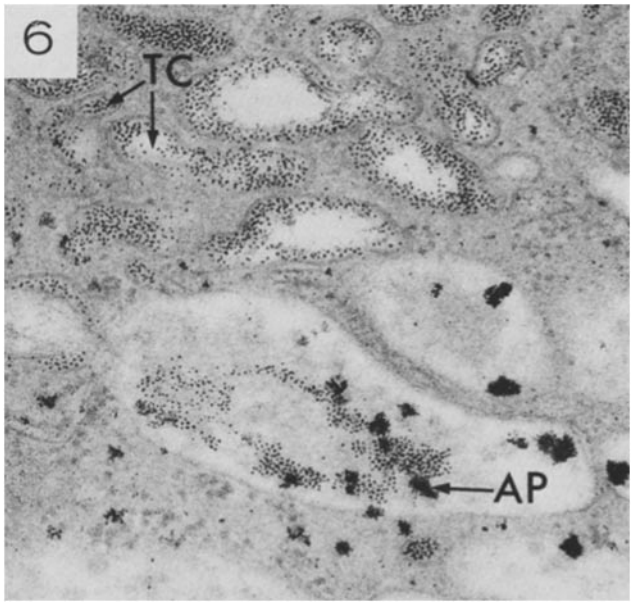

FIGURE 6 Apical cytoplasm of an absorptive cell exposed to CF for 45 min before fixation, and then processed for histological demonstration of acid phosphatase (*AP*) activity. AP activity is not present in the tubulocisternae (*TC*) or in vesicles containing membrane-bound CF. AP activity is present in larger vesicles whose lumens contain free CF. × 57,600.

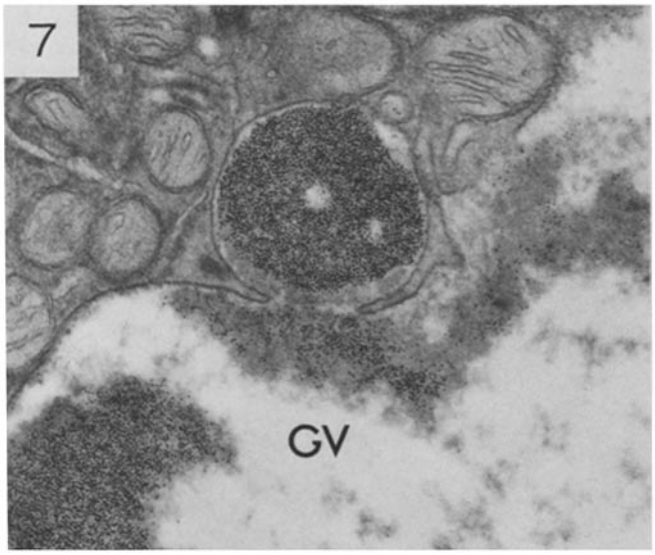

FIGURE 7 Absorptive cell from a ligated loop that was filled with NF for 10 min, rinsed, and maintained for 80 min prior to fixation. A vesicle containing concentrated NF appears to fuse with the giant vacuole (*GV*). × 54,000.

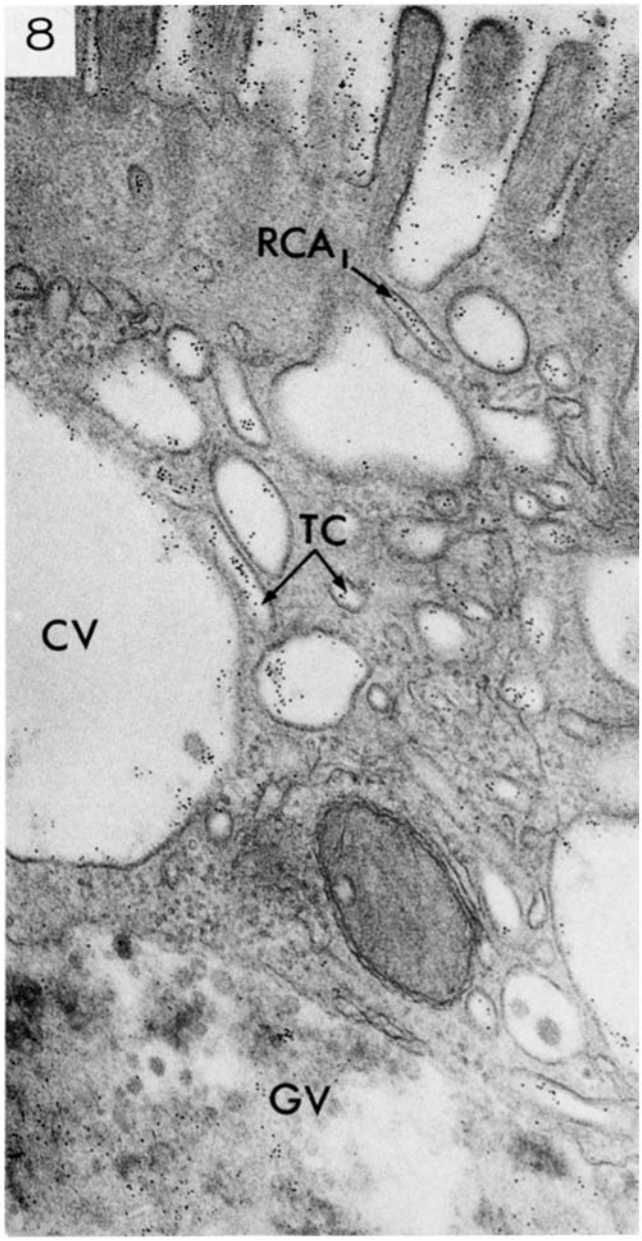

FIGURE 8 Apical cytoplasm of an absorptive cell from an ileal loop filled with ferritin-conjugated RCA$_I$ (*RCA$_I$*) for 30 min before fixation. RCA$_I$ binds to the membranes of microvilli and apical invaginations, and is transported through the tubulocisternal system following the same route as cationized ferritin. *TC*, tubulocisternae; *CV*, clear vesicles; *GV*, giant vacuole. × 54,000.

The apical invaginations and tubulocisternal membranes both display unique two-dimensional surface arrays that appear in sections as regular projections spaced at 15-nm intervals, previously shown to contain *N*-acetylhexosaminidase activity (18). We have decorated these projections with CF and have demonstrated the presence of CF-decorated projections in coated pits, suggesting that the membrane arrays are endocytosed and are transported to the tubulocisternae. Coated pits are also abundant along the tubulocisternal membranes (43), perhaps reflecting the retrieval and recycling of

membrane and enzyme arrays back to the apical invaginations, or indicating withdrawal of membrane for another destination. Tubulocisternal systems are known to play a role in endocytosis in other cell types, but little is known about the origin, composition, or movements of these membranes. In kidney proximal tubule cells, for example, an extensive system of apical tubules is involved in the uptake of protein from glomerular filtrate (14). Although these tubules may accomplish the recycling of endocytosed membrane (4), the tubular membranes differ antigenically from the intermicrovillar, endocytic microdomains of the apical plasma membrane (19). In hepatocytes (44) and hepatoma cells (10, 24), peripheral tubulocisternae function as an intermediate prelysosomal compartment through which ligands pass during receptor-mediated endocytosis. In the ileal cell, direct connections between tubulocisternae and large, clear, nonlysosomal vesicles were observed (see Fig. 5*a*) suggesting that the tubulocisternae of the ileal cell could also be analogous to the endosome-associated tubules (CURL) shown to be involved in uncoupling of asialoglycoprotein ligands from their receptors in liver cells (9).

The fate of the arrays or of the tubulocisternal membranes could not be followed with the tracers used in this study, because CF dissociated from the membrane arrays before leaving the tubulocisternal system. The release of CF from the membranes occurs prior to its arrival in a lysosomal compartment; release begins in the tubulocisternae and with few exceptions, is complete in the large, clear nonlysosomal vesicles where tight, semicrystalline CF clusters are free in the lumens. This large, polycationic tracer presumably cross-links cell surface anions (7), and it is possible that anionic macromolecules are released and participate in the formation of large clusters of free tracer. Clustering and release of CF has been observed during transepithelial transport across vascular endothelial cells (33). We assume that the tubulocisternae and large clear vesicles may be acidic compartments comparable with endosomal compartments in other cell types (23, 38), but whether acidification promotes release of CF from anionic sites has not been directly tested.

The prelysosomal compartments and the multivesicular lysosomes of the ileal absorptive cell are arranged in clear topographical sequence above the giant supranuclear vacuole, but the Golgi complex is invariably positioned several microns away, at the lateral borders of the cell near the base of the giant vacuole. Neither CF nor RCA_I-ferritin were observed in Golgi-associated vesicles or Golgi cisternae at any time interval (up to 1.5 h). These findings differ from those of previous studies on cultured cells. Gonatas et al. (12, 13) applied HRP-conjugated lectins and soluble HRP to neurons and neuroblastoma cells in culture, and showed that after endocytosis, adsorbed tracers were localized in cisternae and vesicles associated with the *trans* face of the Golgi complex (GERL) and in lysosomes or residual bodies whereas fluid-phase HRP was directed only to lysosomes. Similarly, ultrastructural studies of receptor-mediated endocytosis of HRP-conjugated α_2-macroglobulin in cultured fibroblasts indicated that physiologic ligands pass through a Golgi-associated membrane compartment before being delivered to lysosomes (40). In nonpolarized, cultured cells the Golgi complex is often located close to endocytic vesicles and tubules, and the intracellular positions of these compartments are not strictly defined; indeed, their positions may rapidly change during endocytic activity (17). In epithelial cells of the neonatal ileum, in contrast, the Golgi complex is consistently and clearly separated from endosomal compartments. We conclude that in these highly polarized cells, passage of ligand through Golgi-associated compartments is not a prerequisite for entry into lysosomes.

Fluid-phase endocytosis is a ubiquitous activity of cells that may result in the uptake and degradation of significant quantities of soluble proteins (32, 36, 37). The degree to which soluble proteins are "sorted" from membrane-bound ligands, and the rapidity of the sorting process, seems to vary widely among cell types. Even within the intestine of the neonatal rat, regional differences in absorptive functions can be correlated with differences in the patterns of macromolecular uptake, transport, and sorting. In the jejunum, Abrahamson and Rodewald (1, 30) found that fluid phase tracer (HRP) and receptor-bound IgG were present together in the same intracellular compartment (the apical tubules and vesicles) 30 min after uptake, and were subsequently sorted. In the ileum, in contrast, membrane-bound CF is segregated in the tubulocisternal system and fluid-phase NF is diverted to small vesicles within 3 to 10 min after uptake. Despite the abundance of fluid-phase vesicles, the process of their formation could not be visualized because "open" vesicles do not sequester NF, and prior to 10 min of uptake, very little NF could be observed in cells. In some cell types, fluid-phase vesicles seem to be derived from noncoated regions of the plasma membrane. The apical invaginations of ileal cells are only partially clathrin-coated on the cytoplasmic side, but their luminal surfaces are completely covered by periodic arrays (26). Adsorption of CF to these arrays is uniform over the entire invaginated membrane, and the arrays are taken up in coated pits. Since no smooth undecorated membrane microdomain seems to be available for purely fluid-phase uptake, it is possible that both CF and NF were taken up in coated vesicles and that very rapid sorting occurred in the first few minutes after uptake (16, 22). In any event, significant amounts of soluble tracers were never observed in the tubulocisternae, indicating that fluid-phase macromolecules must be segregated from adsorbed macromolecules very early and not after incorporation into this endosomal compartment.

During the first 20 min of NF uptake, the tracer appeared in vesicles that became progressively larger as they approached the giant vacuole. At the same time, NF became increasingly concentrated until the largest vesicles were packed with ferritin, along with the dense amorphous material that probably represents milk macromolecules that were taken up along with the tracer. These observations suggest that the membranes of these vesicles fuse with each other and actively remove water before fusing with lysosomal compartments. Similar concentrating activity, observed in fluid-phase vesicles of other cell types (36) has been taken as evidence for the presence of ion-transporting enzymes in pinosome membranes and a recent report indicates that fluid-phase endocytic compartments may also be acidified (8).

Sorting of fluid-phase and membrane-bound protein into separate endocytic pathways may facilitate a variety of functions in specialized cell types. In the suckling rat jejunum, it serves to direct macromolecules to different destinations: IgG-receptor complexes are addressed to the basolateral cell surface whereas soluble protein is targeted to lysosomes (1, 30). In macrophages, where the ultimate lysosomal destination of ligands and fluid-phase macromolecules is the same (32, 37), separation of adsorptive and fluid-phase endocytic pathways may facilitate efficient prelysosomal retrieval of mannose

receptors (34). In the absorptive cell of suckling ileum, both fluid phase and membrane-bound macromolecules seem to be destined for lysosomal digestion, yet they are sorted and transported separately. The purpose of this sorting capacity, or of the elaborate adsorptive endocytic pathway that features enzyme arrays on prelysosomal membranes, is not yet clear. It is possible that sorting allows for retrieval and recycling of luminal membrane enzymes and/or receptors, or for selective transport of milk hormones to a nonlysosomal destination. These possibilities cannot be readily tested using multivalent experimental ligands such as CF or lectins since these tracers cross-link membrane binding sites and this may "address" them to lysosomes. Nevertheless, we have recently observed the transepithelial transport of small amounts of CF in this cell (unpublished observation). Further studies testing specific milk components as candidate ligands are needed to elucidate the physiological importance of the separate endocytic pathways in the neonatal ileum.

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