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Study on the origin of apical tubules in ileal absorptive cells of suckling rats using concanavalin-A as a membrane-bound tracer

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Summary. The ileal absorptive cells of suckling rats exhibit high levels of endocytic activity being engaged in nonselective uptake of macromolecules from the intestinal lumen. The apical cytoplasm usually contains an extensive network of small, membrane-limited tubules (apical tubules: AT), in addition to newly formed endocytic vesicles and large endocytic vacuoles. To determine whether the AT are directly involved in the endocytic process by carrying the tracer into the cell, we have analysed movements of the apical cell membrane of the ileal absorptive cells by using a membrane-bound tracer (horseradish peroxidase-labelled cancanavalin-A: Con-A HRP). The ileal absorptive cells were exposed in vitro to Con-A HRP for 10 min at 4° C, incubated for different times in Con-A free medium at 37° C, and prepared for electron microscopy. After 1 min incubation at 37° C, invaginations of the apical cell membrane, including coated pits, and endocytic vesicles were labelled with HRP-reaction product, whereas the AT and large endocytic vacuoles were negative. After 2.5 min, almost all the large endocytic vacuoles were labelled with reaction product, which was seen in their vacuolar lumen and along the luminal surface of their limiting membrane. A few AT with reaction product were seen in the apical cytoplasm; they were in frequent connection with the reaction-positive large endocytic vacuoles. With increasing incubation time, the number of the labelled AT increased. Thus, after 15 min at 37° C, the apical cytoplasm was fully occupied by the reaction-positive AT. The ends of these AT were often continuous with small spherical coated vesicles. No reaction product was detected in the Golgi complex at any time after incubation. These observations indicate that the AT located in the apical cytoplasm probably originate by budding off from the large endocytic vacuoles, rather than being involved in the process of endocytosis.

Key words: Endocytosis – Absorptive cells – Ileum – Intestine, small – Apical tubules – Membrane recycling – Rat

The absorptive cells throughout the small intestine of neonatal rodents are developmentally specialized to take up luminal macromolecules by active endocytosis (for reviews, see Walker 1981; Bainter 1986). Recent studies have indicated that the absorptive cells of the jejunum of suckling rats selectively transport maternal antibodies into the circulation without lysosomal degradation (Rodewald 1980; Abrahamson and Rodewald 1981). This specific transport contrasts with the pathway of nonspecific luminal macromolecules that are taken up by the absorptive cells of the ileum and transported to lysosomes where they are degraded (Rodewald 1973). Both of these cells are replaced abruptly at three weeks of age by absorptive cells of apparently adult form and function (Ono 1975; Hirano and Kataoka 1986).

Electron-microscopic observations of the ileal absorptive cells of suckling rats have shown a well-developed "endocytic complex" (Wissig and Graney 1868; Knutton et al. 1974), including deep invaginations of the apical cell membrane between the bases of the microvilli, newly formed endocytic vesicles, large prelysosomal endocytic vacuoles, and, finally, a giant supranuclear, lysosomal vacuole. Besides these structures, a labyrinthine system of small membrane-limited tubules, referred to as "apical tubules" (AT) (Graney 1968), "apical tubular system" (Staley et al. 1972; Worthington and Graney 1973; Chase and Munn 1980) or "apical canalicular system" (Bainter 1986), has always been observed in the apical cytoplasm. It has been proposed that the AT are derived from intermicrovillous, tubular invaginations of the apical cell membrane and are related to the mechanism of endocytosis.

The AT have occurred in the ileal absorptive cells of all the neonatal mammals so far examined (Clark 1959; Graney 1968; Wissig and Graney 1968; Cornell and Padykula 1969; Staley et al. 1972; Walker et al. 1972; Knutton et al. 1974; Chase and Munn 1980; Gonella and Neutra 1984; Staley and Bush 1985), and also represent a feature common to other types of absorbing epithelia, which take up protein macromolecules by endocytosis (Hatae et al. 1984). We have previously demonstrated a specific helical structure in the AT of several absorbing epithelia (kidney proximal tubule, visceral yolk sac and ductuli efferentes) by use of a fixative containing formaldehyde, glutaraldehyde and osmium tetroxide, and indicated in kidney proximal tubule cells that the AT are formed by budding off from the large endocytic vacuoles during the process of endocytosis (Hatae et al. 1986a, b).

The present study was undertaken to determine whether a similar helical structure is present in the AT of ileal absorptive cells, and to elucidate the origin of the AT during endocytosis. For this purpose segments of the ileal tissues from neonatal suckling rats were fixed by the same fixative

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as in the previous study (Hatae et al. 1986a), and the ultrastructure of the AT was examined by electron microscopy. The other segments were incubated for different times, after labelling the apical surface of the ileal absorptive cells with horseradish peroxidase-conjugated concanavalin-A (Con-A HRP), and the intracellular transport of the tracer was analysed with special reference to the AT.

Materials and methods

Suckling Wistar rats between 9 and 11 days of age were allowed to nurse up to time of experimentation. Under light ether anesthesia, pieces of the distal ileum, 2–3 cm in length, were removed 1–2 cm proximal to the caecum, slit longitudinally and cut into small segments (about 3–4 mm square).

Ultrastructure

For ultrastructural examination of ileal absorptive cells, segments of the ileum were fixed for 1 h at 4° C in a mixture of 1% formaldehyde, 1.5% glutaraldehyde and 1% osmium tetroxide diluted with 0.07 M Sörensen phosphate buffer (pH 7.2) containing 3% sucrose (Hatae et al. 1986a). After rinsing with distilled water, the specimens were stained en bloc for 1 h in 1% uranyl acetate in acetate-Veronal buffer (pH 5.0) (Farquhar and Palade 1965), and embedded in Epon 812 after dehydration in graded alcohols. Thin sections were cut with diamond knives on a Porter-Blum MT-2 microtome, double-stained with uranyl acetate (saturated in 50% ethanol) and lead citrate, and examined under a JEOL 200CX electron microscope operating at 100 kV.

Con-A HRP labelling

For exposure of the mucosa to the tracer in vitro, segments of the ileum were rinsed for 5 min at 4° C in Eagle's minimal essential medium (MEM) (Nissui Seiyaku Co., Japan) which was gassed with O_2-CO_2 (95% – 5%) and adjusted to pH 7.0, then incubated for 10 min at 4° C in MEM containing 2.5 mg/ml Con-A HRP conjugates (Sigma Chem. Co.). The samples were three times rinsed to eliminate unbound tracer in MEM at 4° C and then immediately fixed or incubated again for 1, 2.5, 5, 15 min at 37° C in Con-A HRP-free MEM. The specimens were fixed for 2 h at 4° C in a mixture of 1% formaldehyde and 1.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) containing 3% sucrose. After washing overnight in the same buffer at 4° C, the ileum segments were stained for peroxidase activity for 1 h at room temperature with a medium containing 0.05% 3, 3′-diaminobenzidine and 0.01% hydrogen peroxide in 0.1 M cacodylate buffer (pH 7.0) (Graham and Karnovsky 1966). They were then rinsed for 1 h at 4° C in the same buffer and postfixed for 1 h at room temperature in an aqueous solution of 1% osmium tetroxide and 1.5% potassium ferrocyanide (Karnovsky 1971). After rinsing in distilled water, the samples were stained en bloc for 1 h in 0.5% uranyl acetate in 0.1 M acetate-Veronal buffer (pH 5.0) and embedded in Epon 812 after dehydration in graded alcohols. Thin sections were examined under a JEOL 100S electron microscope operating at 80 kV without staining.

Control

After rinsing in MEM for 5 min at 4° C, segments of the ileum were incubated for 10 min at 4° C in MEM containing 0.75 mg/ml HRP (type VI, Sigma Chem. Co.) (corresponding to the concentration of HRP in 2.5 mg/ml Con-A HRP) rinsed three times in MEM at 4° C and then immediately fixed or incubated again for 1, 2.5, 5, 15 min at 37° C in HRP-free MEM. Fixation, diaminobenzidine reaction, post-fixation and embedding were carried out as already described.

Results

Ultrastructure of the endocytic complex

The ultrastructural appearance of the ileal absorptive cells of suckling rats has already been presented in previous publications (Clark 1959; Graney 1968; Wissig and Graney 1968; Cornell and Padykula 1969; Knutton et al. 1974), thus our description will be mainly focused on the endocytic complex of these cells.

The apical cytoplasm appeared to be honeycombed with a well-developed endocytic complex under a brush border of microvilli (Fig. 1). The central cytoplasm was dominated by a giant lysosomal vacuole and the nucleus was compressed toward the base of the cell. The lysosomal vacuole contained either a fine flocculent material or clumps of dense material aggregated about the periphery. At high magnification, the apical cell membrane at the base of the microvilli formed numerous wide, vesicular and saccular invaginations extending deeply into the apical cytoplasm (Fig. 2). These invaginations often bifurcated as they penetrated the cytoplasm. The outer surface of the microvilli was associated with a glycocalyx coat, but most of the invaginations displayed a regular array of surface projections that have been previously identified as digestive enzyme protein, *n*-acetylglucosaminidase, which is presumed to be engaged in excising sugar moieties in the maternal milk (Jakoi et al. 1976; Robertson et al. 1976). Numerous coated vesicles (~160 nm in diam.) were formed by pinching off from coated pits between the bases of the microvilli or along the invaginations of the apical cell membrane (Fig. 2, left inset). Under the apical cell surface, were found numerous, round or elongated saccular, endocytic vesicles (150–500 nm in diam.) whose luminal membrane was covered with fine projections. The cytoplasmic side of the vesicles showed either a clathrin coat or smooth contour. The morphological characteristics of these vesicles were very similar to those of the invaginations of the apical cell membrane. Thus, it was very difficult to determine whether these vesicles had become already detached from the apical cell membrane, or whether they were invaginations opening toward the apical surface that was out of the plane of sectioning. In the deeper levels of the apical cytoplasm, spherical or irregularly shaped, large endocytic vacuoles (1000–3000 nm in diam.) were found (Fig. 2). The luminal content of these vacuoles was electron-lucent, but their luminal membrane was covered with fine projections.

Besides these endocytic vesicles and vacuoles, nemerous AT were identified in the apical cytoplasm (Fig. 2). The AT were cylindrical with fairly constant diameter (70–90 nm) and oriented in various directions throughout the apical cytoplasm. They often branched and anastomosed each other, so that they formed a labyrinthine system of interconnecting channels. Unlike the case observed in other types of absorbing epithelia, the AT of the ileal absorptive cells showed clear, electron-lucent content without

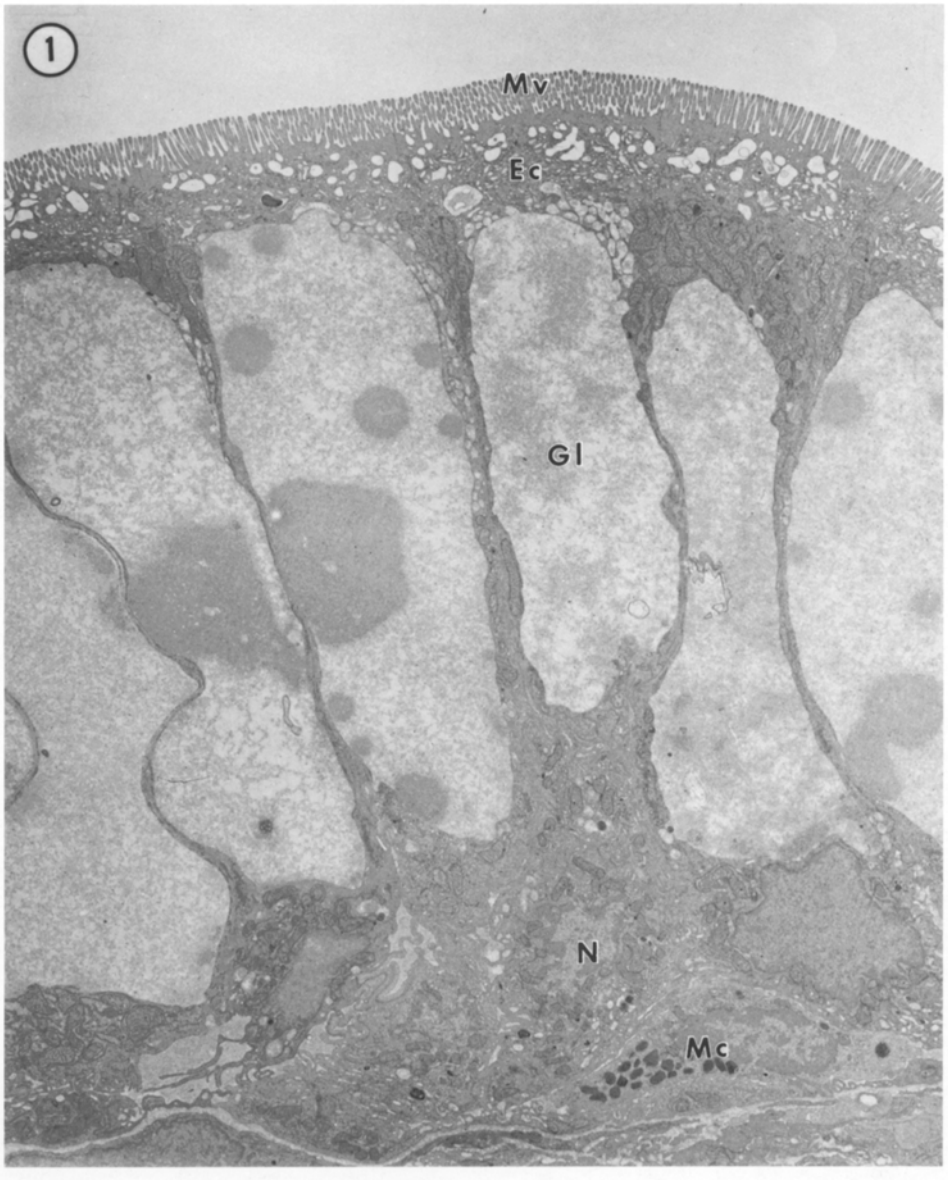
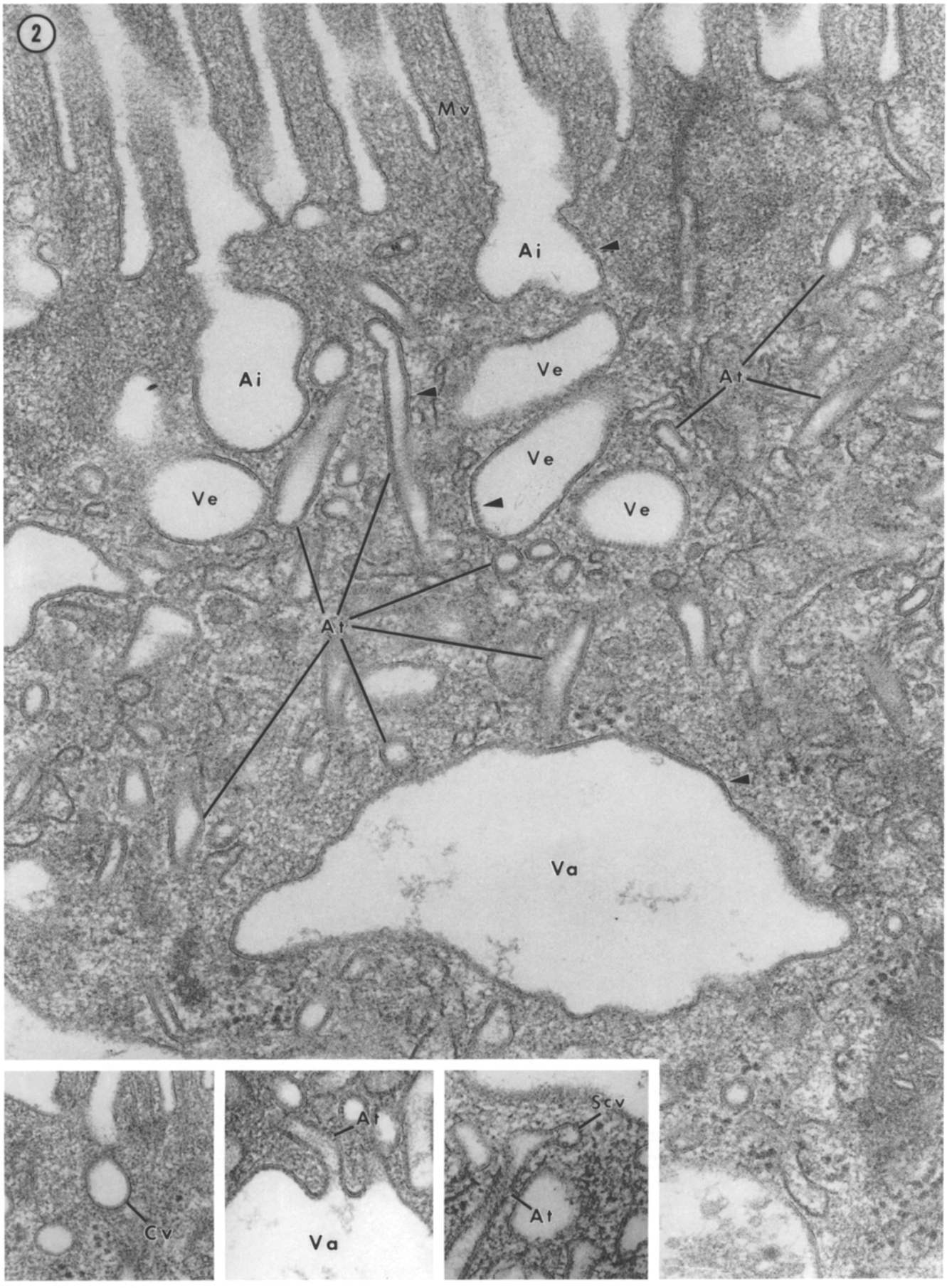


Fig. 1. Survey electron micrograph of absorptive cells from a suckling rat ileum fixed with a mixture of formaldehyde, glutaraldehyde and osmium tetroxide. The apical surface of the cells is provided with closely packed microvilli (*Mv*), and the subjacent zone is characterized by a well-developed endocytic complex (*Ec*). The central cytoplasm is occupied by a giant lysosomal vacuole (*Gl*) which compressed the nucleus (*N*) toward the base of the cell. The giant lysosomal vacuole is filled with either a flocculent material or clumps of dense material. A migratory cell (*Mc*) is present in the basal extracellular space. ×3900

Fig. 2. Ultrastructure of the endocytic complex of ileal absorptive cells fixed with a mixture of formaldehyde, glutaraldehyde and osmium tetroxide. Saccular invaginations (*Ai*) of the apical cell membrane are seen between the bases of the microvilli (*Mv*). The area beneath the microvilli contains numerous apical tubules (*At*), endocytic vesicles (*Ve*) and large endocytic vacuoles (*Va*), whose luminal surface is covered with fine projections of digestive enzymes (*arrowheads*). ×75800. *Inset: Left.* Coated vesicle (~160 nm in diam.) (*Cv*) formed by pinching off from a coated pit at the base of the microvilli. ×55600. *Middle.* Large endocytic vacuole (*Va*) to which an apical tubule (*At*) is connected. ×83000. *Right.* Apical tubule (*At*) to the end of which a small, spherical coated vesicle (~90 nm in diam.) (*Scv*) is connected.

Fig. 3. Apical cytoplasm of ileal absorptive cells labelled with Con-A HRP for 10 min at 4° C. Con-A binding sites are revealed by the presence of HRP-reaction product with oxidized diaminobenzidine. Reaction product is found on the luminal surface of the microvilli (*Mv*), invaginated intermicrovillous cell membrane (*Ai*), including coated pits (*Cp*), and some intracytoplasmic vesicular structures (*arrowheads*) which open to the intestinal lumen, but not within apical tubules (*At*), endocytic vesicles (*Ve*) and large endocytic vacuoles (*Va*). ×23500. *Inset:* Coated pit labelled with HRP-reaction product, which is more electron-dense than that on the surface of the microvilli. ×69600

Fig. 4. Apical cytoplasm of ileal absorptive cells at 1 min incubation after labelling with Con-A HRP. Reaction product is mainly found on the luminal surface of the microvilli (*Mv*), invaginated cell membrane, including coated pits (*Cp*), and within endocytic vesicles (*Ve*). No reaction is found in apical tubules (*At*) and large endocytic vacuoles (*Va*). ×23500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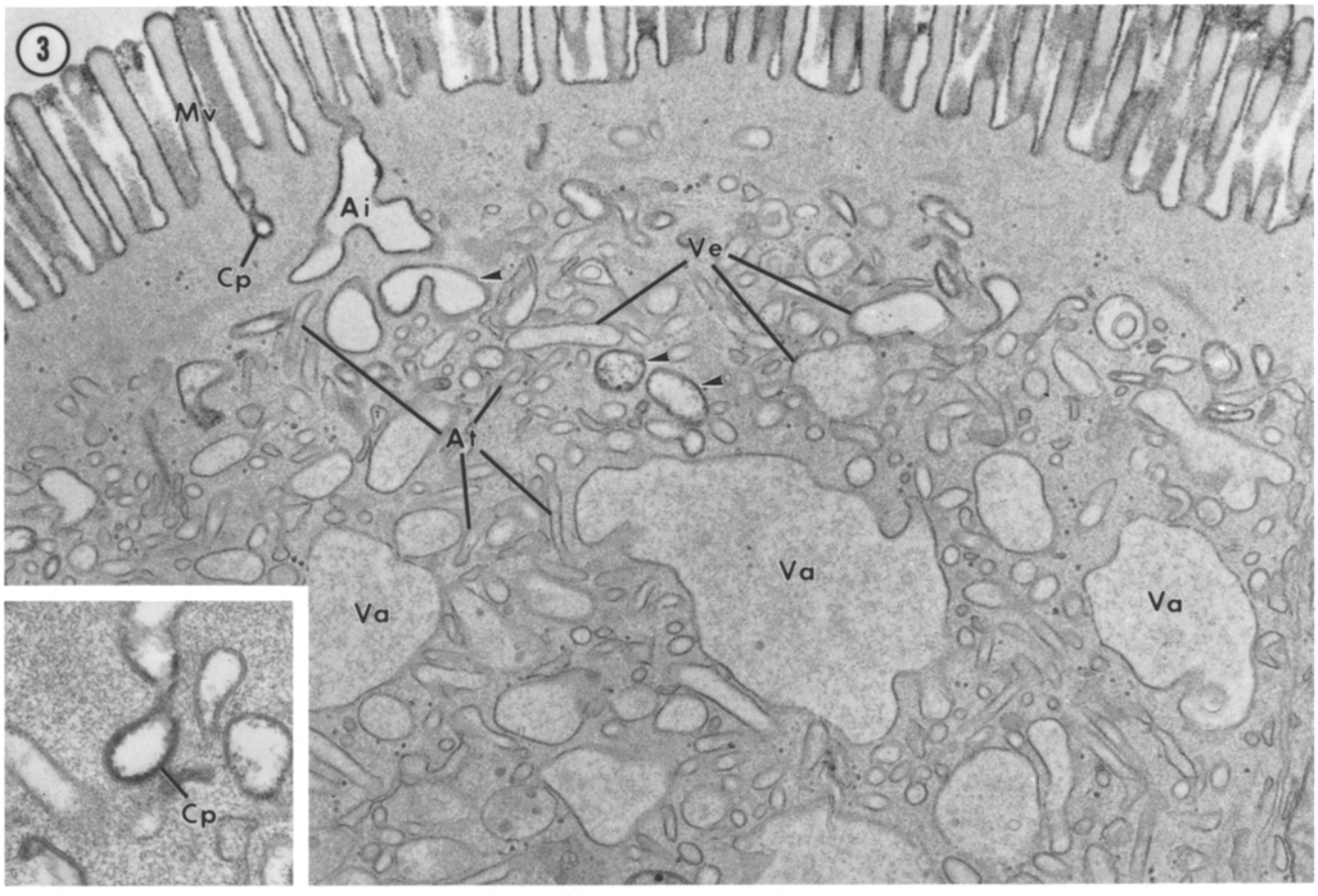
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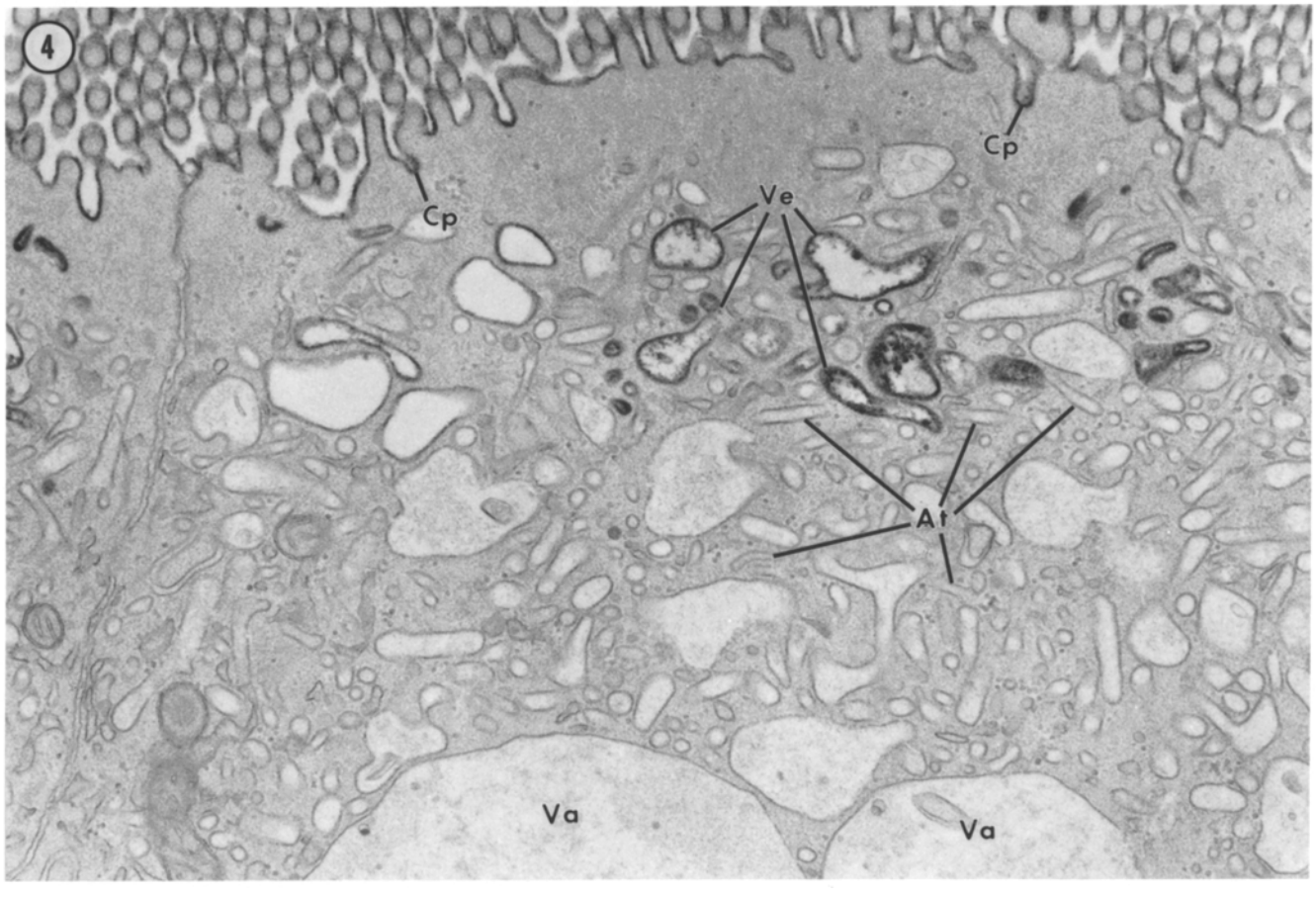
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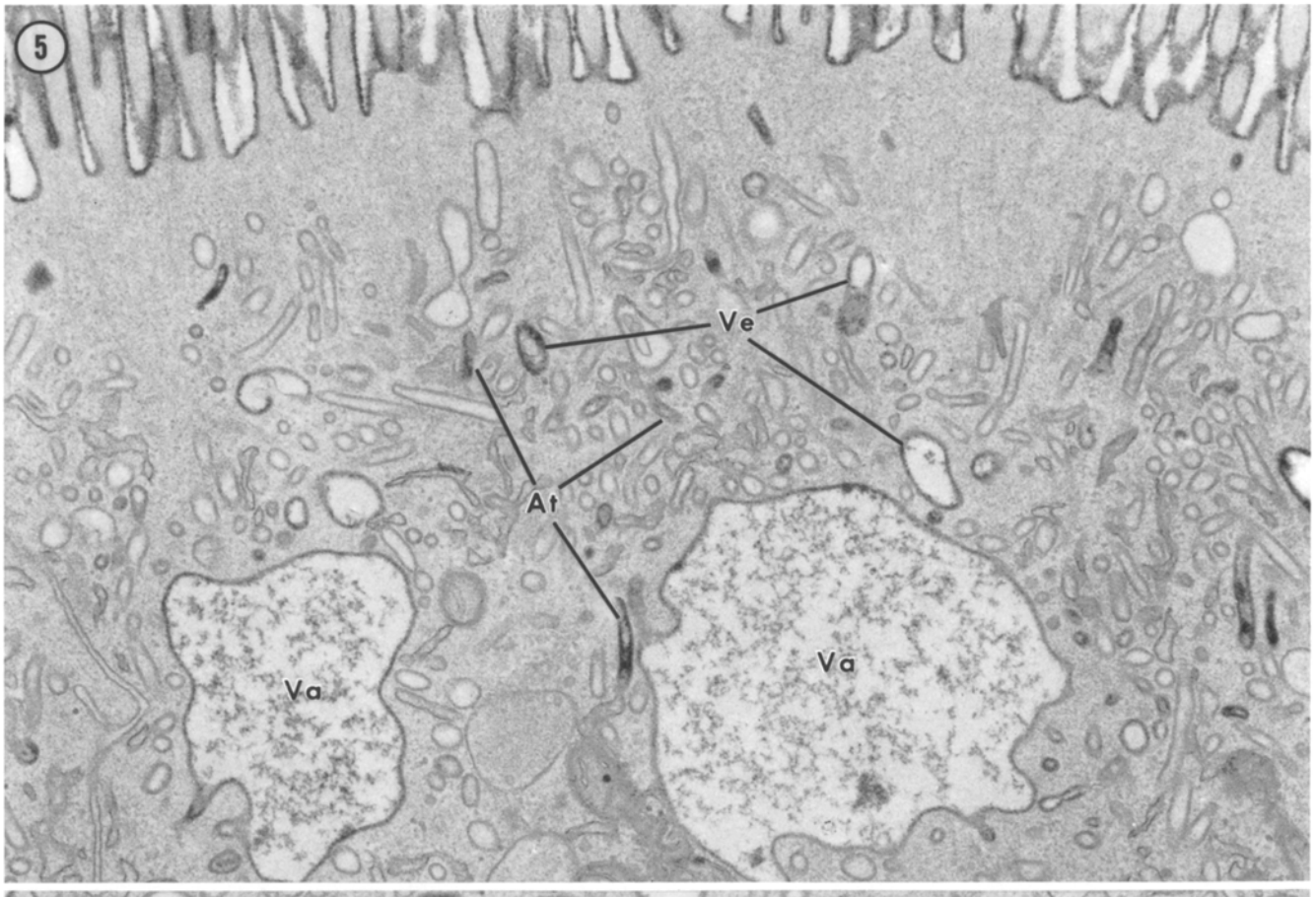


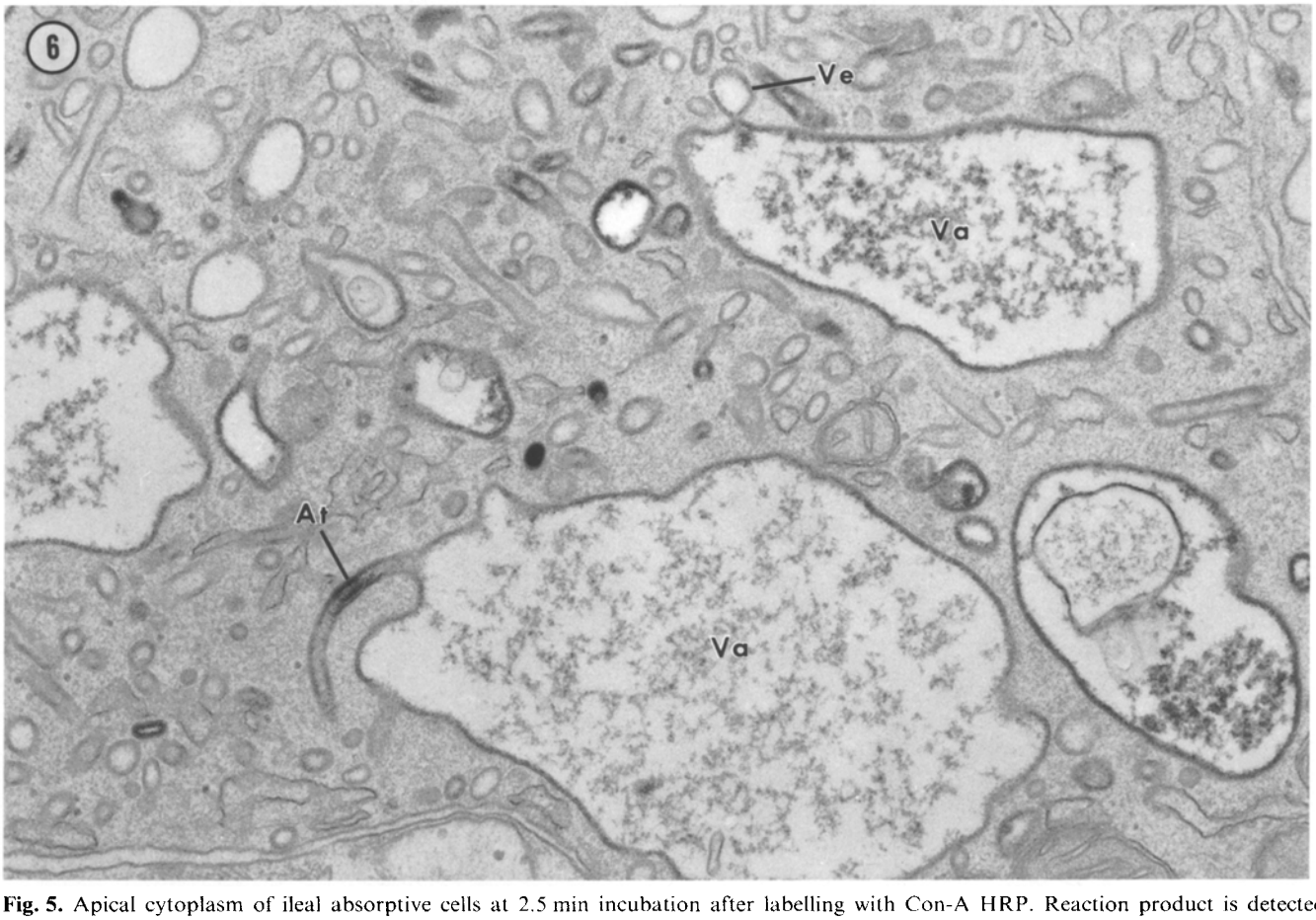
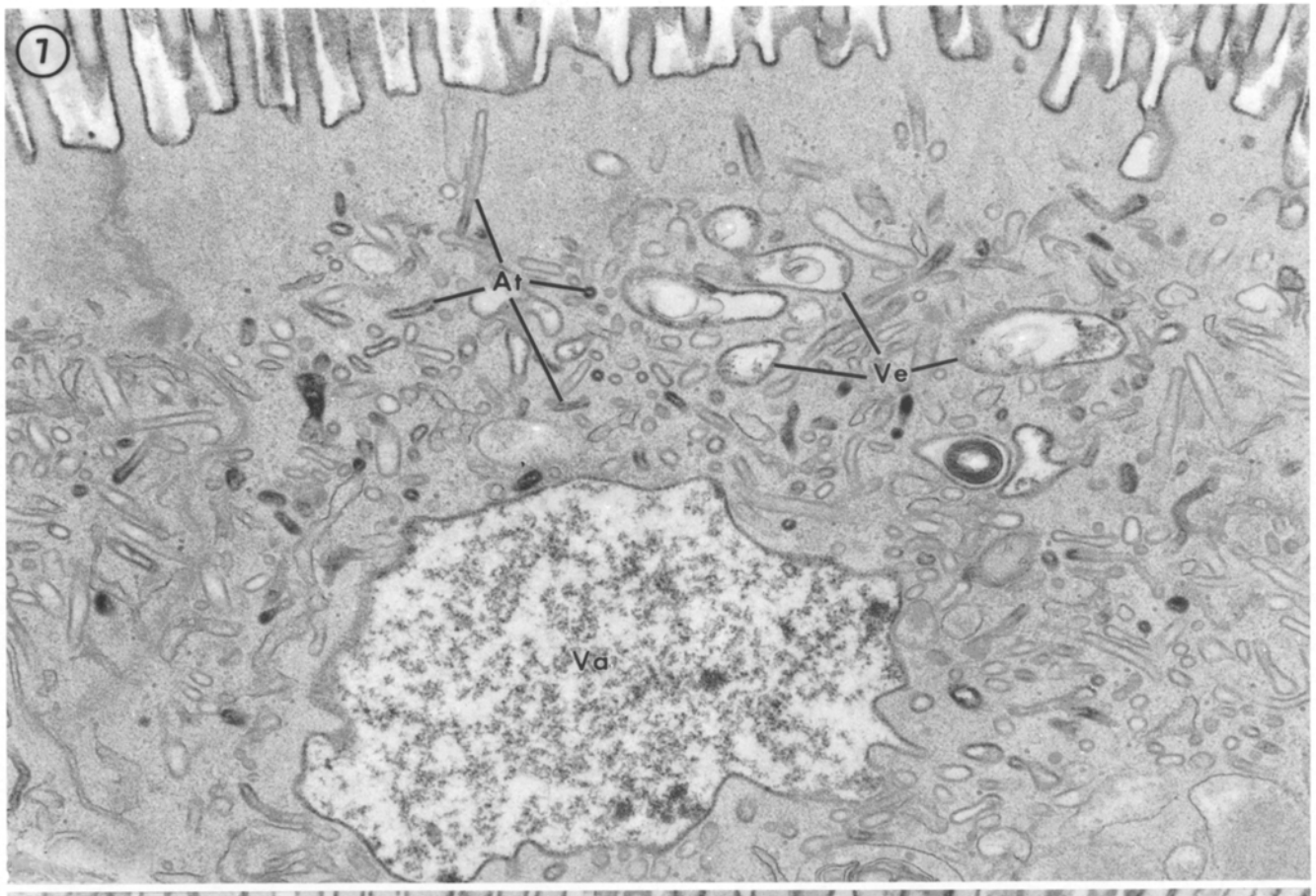


Fig. 5. Apical cytoplasm of ileal absorptive cells at 2.5 min incubation after labelling with Con-A HRP. Reaction product is detected in endocytic vesicles (Ve) and large endocytic vacuoles (*Va*), whereas only a few apical tubules (*At*) are reaction-positive. × 23 500

Fig. 6. Con-A HRP-positive large endocytic vacuoles (*Va*) at 2.5 min incubation after labelling with Con-A HRP. Reaction product is seen in the vacuolar lumen and along the inner surface of the limiting membrane of the large endocytic vacuoles to which labelled endocytic vesicles (*Ve*) and apical tubules (*At*) are connected. × 45 600



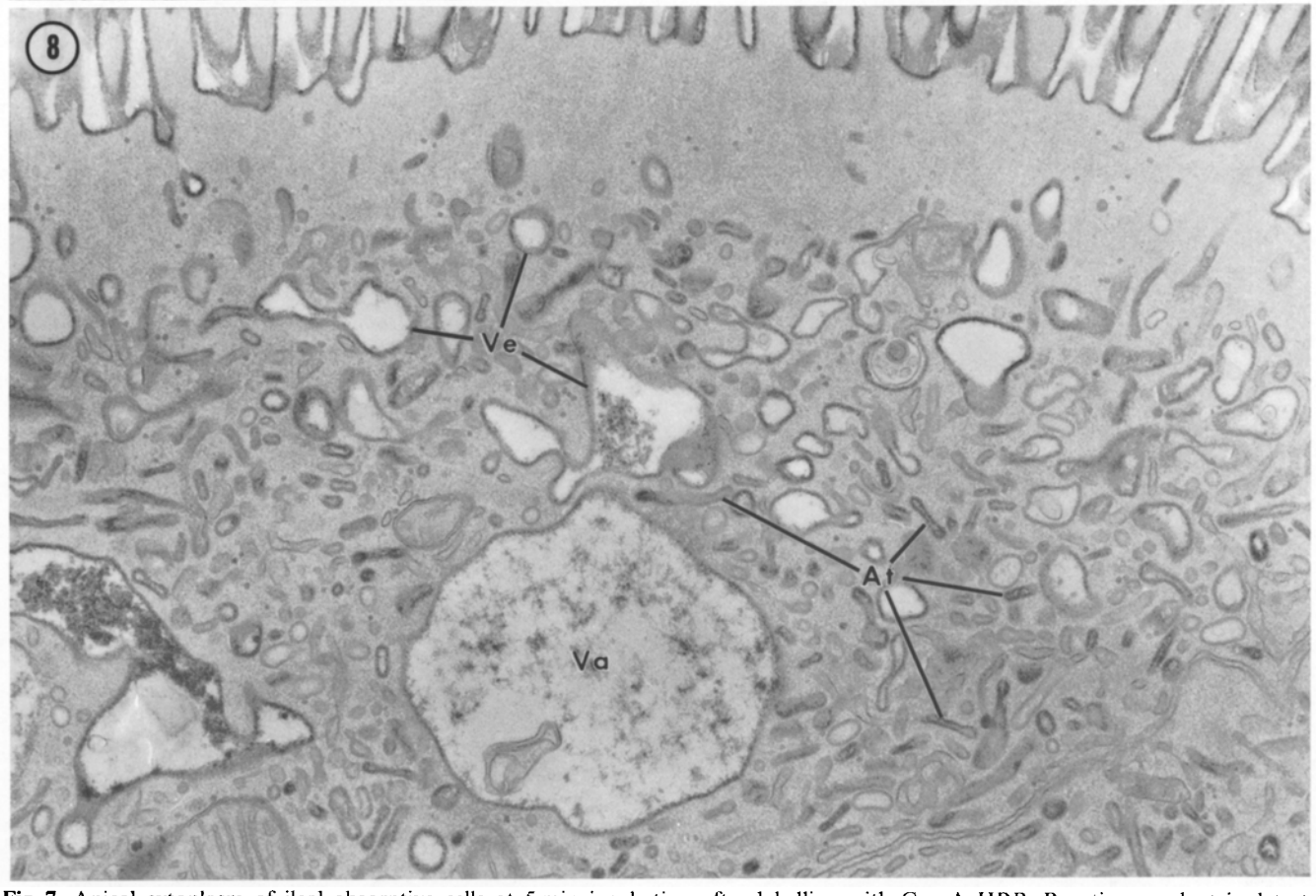


Fig. 7. Apical cytoplasm of ileal absorptive cells at 5 min incubation after labelling with Con-A HRP. Reaction product is detected in endocytic vesicles (*Ve*), large endocytic vacuoles (*Va*) and several apical tubules (*At*). The reaction-positive apical tubules are distributed not only close to the large endocytic vacuoles but beneath the apical cell surface. × 25000

Fig. 8. Apical cytoplasm of ileal absorptive cells at 15 min incubation after labelling with Con-A HRP. The apical cytoplasm is fully occupied by abundant Con-A HRP-positive apical tubules (*At*), endocytic vesicles (*Ve*) and large endocytic vacuoles (*Va*). × 25000

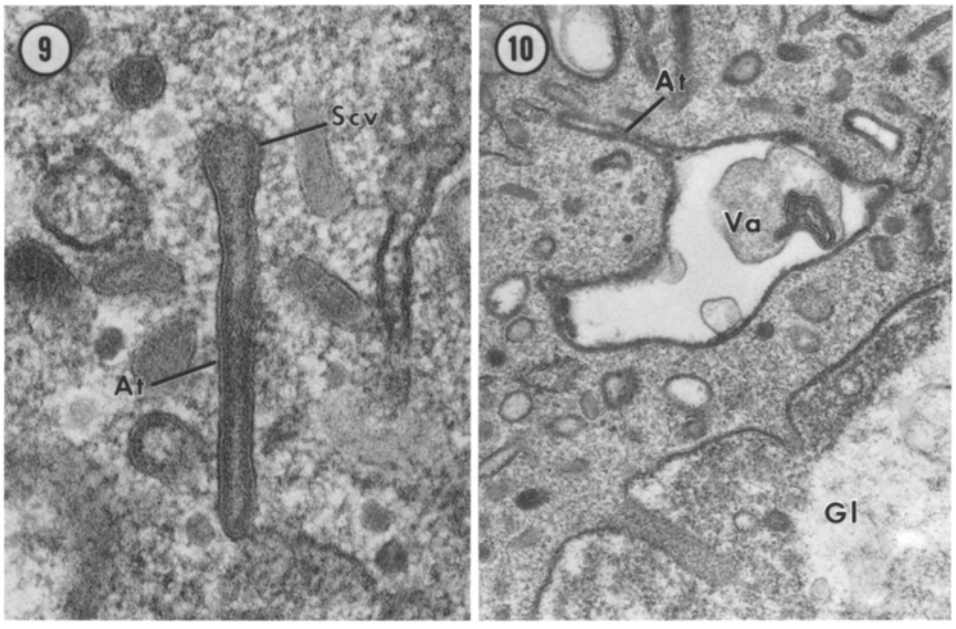


Fig. 9. Apical tubule (*At*) connected with a small, spherical coated vesicle (~90 nm in diam.) (*Scv*) at 15 min incubation after labelling with Con-A HRP. Intense reaction product is seen within the apical tubule and coated vesicle. ×111500

Fig. 10. Giant lysosomal vacuole (*Gl*) and large endocytic vacuole (*Va*) at 15 min incubation after labelling with Con-A HRP. Reaction product is seen in the giant lysosomal vacuole and large endocytic vacuole. A labelled apical tubule (*At*) is connected with the large endocytic vacuole. ×34600

any structural differentiation, but their luminal membrane beared fine projections similar to those found on the surface of the invaginations of the apical cell membrane, the endocytic vesicles and the large endocytic vacuoles. Although the AT with the surface projections were occasionally seen close to the apical cell surface, no direct contact was observed between them; in contrast, the end of the AT was frequently connected with the large endocytic vacuoles (Fig. 2, middle inset) or small-spherical coated vesicles (Fig. 2, right inset). In general, the size of these coated vesicles was smaller (~90 nm in diam.) than that of the coated vesicles seen along the apical cell surface.

Localization of Con-A HRP on the endocytic complex

When the ileal absorptive cells were exposed to Con-A HRP for 10 min at 4° C, labelling was only found along the apical cell surface of these cells (Fig. 3). Intense HRP-reaction product was found along the outer surface of the microvilli and the intermicrovillous invaginations of the apical cell membrane, including coated pits. In general, the reaction product in the coated pits was more electron-dense than that seen on the surface of the microvilli (Fig. 3, inset). In the vicinity of the apical cell surface, several intracytoplasmic vesicles were also labelled, indicating an open connection to the cell surface, but the AT, endocytic vesicles and large endocytic vacuoles were not.

When Con-A HRP-labelled ileal absorptive cells were incubated for 1–15 min at 37° C in lectin-conjugate-free medium, the lectin conjugate was internalized and transferred to various intracellular membrane compartments. After 1 min, many of the coated pits and endocytic vesicles were labelled with an intense or a moderate reaction, particularly along the luminal surface of their limiting membrane (Fig. 4). However, the AT and the large endocytic vacuoles showed no reaction product. After 2.5 min, almost all the large endocytic vacuoles had become labelled with intense reaction product, which was seen in their vacuolar lumen and along the inner surface of their limiting membrane (Fig. 5). Occasionally, fusion of the positively reacting endocytic vesicles with the large endocytic vacuoles was seen (Fig. 6). A few AT showing an moderate reaction were observed in the apical cytoplasm, and they were frequently connected with the reaction-positive endocytic vacuoles. After 5 min, the AT showing an intense or a moderate reaction were observed more frequently than at 2.5 min, and they were distributed not only close to the large endocytic vacuoles but beneath the apical cell surface (Fig. 7). Connection of the reaction-positive AT with the large endocytic vacuoles was common. After 15 min, almost all the AT and the large endocytic vacuoles were labelled with reaction product (Fig. 8). Although the labelled AT fully occupied the apical cytoplasm, they were never continuous with the apical cell surface; in contrast, the small, spherical coated vesicles (~90 nm in diam.) showing an intense reaction were often connected with the end of the AT (Fig. 9). At this time, some of the giant lysosomal vacuoles had become labelled with weak reaction product (Fig. 10). At no time did the Golgi complex, which was located basolaterally to the nucleus, or the basolateral cell membrane show any reaction product.

Control

No reaction product was found in any ileal absorptive cells after the exposure of the ileum segments to HRP for 10 min at 4° C either with or without subsequent incubation for 1, 2.5, 5, 15 min at 37° C in HRP-free medium.

Discussion

Previous electron-microscopic studies on the process of macromolecular absorption by the ileal absorptive cells of neonatal mammals have revealed that different tracers (ferritin, saccharated iron oxide, colloidal gold and HRP) were taken up from the intestinal lumen into the absorptive cells by the same initial pathway; they were taken into the endocytic vesicles formed by the invaginations of the apical cell membrane, and then actively transferred into the large en-

docytic vacuoles that finally fuse with the giant lysosomal vacuoles (Clark 1959; Graney 1968; Staley et al. 1972; Walker et al. 1972; Ono 1975; Chase and Munn 1980).

The origin and role of the AT in the endocytic process has been a subject of much debate. In early studies, the AT were believed to originate from the tubular invaginations of the apical cell membrane between the bases of the microvilli at the initial stage of endocytosis and deliver their load to the large endocytic vacuoles. On the other hand, data accumulated indicating that the AT cannot be originated as simple pinocytic invaginations of the apical cell membrane. Graney (1968) and Bainter (1986) suggested that the AT are preformed structures open to the intestinal lumen with intermicrovillous pores and represent an organelle specialized for nonselective uptake of macromolecules. Chase and Munn (1980), from their study on the uptake of polycationic ferritin by the newborn piglet ileum, concluded that nonspecific interaction of polycationic ferrithin with the invaginated cell membrane, as a function of charge-density, leads to opening of the sub-apical tubular system, allowing entry of the tracer into the transport system of the cell. Recently, Gonnella and Neutra (1984), using tracers for adsorptive and fluid-phase endocytosis in a study of suckling rat ileum, suggested that the AT are intracellular compartments which are only involved in adsorptive endocytosis by acting as an intermediary between the apical cell membrane invaginations and the large prelysosomal endocytic vacuoles.

By using Con-A HRP as a membrane-bound tracer, our present study indicates that the AT in ileal absorptive cells of suckling rats represent intracellular compartments which do not directly participate in the endocytic processes, but are originated from the large endocytic vacuoles.

Con-A conjugates, which bind noncovalently to mannosyl and glycosyl moieties of cell-surface components (Nicolson 1974; Brown and Hunt 1978), have been extensively used to label an external cell surface and to demonstrate adsorptive endocytosis (Willingham et al. 1981; Hermo and Morales 1984; Morales et al. 1984; Kugler and Miki 1985). After labelling of the ileal absorptive cells at 4° C, Con-A binding sites were detected by the presence of HRP-reaction product in the apical cell membrane with a well-developed glycocalyx coat, i.e., the outer surface of the microvilli and the deep intermicrovilous invaginations of the cell membrane. In the vicinity of the apical cell surface, some intracytoplasmic vesicles of varying diameter were also labelled with reaction product, indicating an open connection to the intestinal lumen. On the other hand, the AT and the large endocytic vacuoles were not labelled with reaction product. This indicates that these structures are intracellular compartments which are not continuous with the apical cell surface.

When Con-A HRP-labelled ileal absorptive cells were incubated for 1–15 min at 37° C in lectin-free culture medium, the lectin conjugates were rapidly internalized and revealed movements of the apical cell membrane during endocytosis. The present study has demonstrated that, after 1 min, many of the coated pits and endocytic vesicles were labelled with reaction product, but the AT and large endocytic vacuoles were negative. After 2.5 min, the large endocytic vacuoles were densely labelled presumably by fusion with reaction-positive endocytic vesicles and, for the first time, a few labelled AT were seen. These AT were in frequent connection with the reaction-positive endocytic vacuoles, and the reaction in these structures was seen in their vacuolar lumen and along the luminal surface of their limiting membrane. These findings indicate that these AT are *not* directly involved in the endocytic process, but are probably formed by budding off from the large endocytic vacuoles. The increase in Con-A HRP-labelled AT with increase time of incubation presumably reflects an increased number of AT being derived from the densely labelled vacuoles.

The AT in the apical cytoplasm is characteristic not only of ileal absorptive cells in neonatal mammals, but of other types of absorbing epithelia which take up protein macromolecules by active endocytosis, i.e., kidney proximal tubule (Christensen 1986), visceral yolk sac (Miki and Kugler 1986), ductuli efferentes (Hermo and Morales 1984), posterior intestine of stomachless fishes (Iida and Yamamoto 1986), trophotaenial placenta of viviparous fishes (Schindler and de Vries 1987) and pericardial cells and nephrocytes of some insects (Crossley 1983). We have previously demonstrated the presence of a specific helical structure in the lumen of the AT of several absorbing epithelia (Hatae et al. 1986a). In the present observations, the AT in suckling rat ileal cells bear digestive enzyme arrays on the luminal surface of their limiting membrane, whereas no ordered structures could be detected in the tubule lumen. This indicates the presence of structural variation in the AT, which probably reflects the functional diversity in different absorbing epithelia.

Although uptake of luminal macromolecules in the ileum is thought to be nonselective, fluid-phase, the tubule-vacuole complex in the ileal cells is reminiscent of CURL (compartment of uncoupling of receptor and ligand) in other cell types (Geuze et al. 1983; Dautry-Varsat and Lodish 1984; Harding et al. 1985) engaged in receptor-mediated endocytosis. It has been proposed that small tubules detach from the CURL and carry receptors to the apical cell membrane. Recently, Christensen (1982), Kugler and Miki (1985), using a marker of adsorptive endocytosis in their studies of rat kidney proximal tubules and rat yolk sacs respectively, have suggested that the AT are not involved in the absorptive process, but have a role in the mechanism by which some membrane components are recycled from large endocytic vacuoles back to the apical cell membrane. In fact, in the present and other previous studies (Shibata et al. 1983; Gonnella and Neutra 1984), digestive enzyme arrays were present on the membrane of the apical invaginations, the large endocytic vacuoles and the AT, but the giant lysosomal vacuoles lacked these enzymes on their limiting-membrane. These findings suggest that the enzyme arrays on the apical invaginations are transferred to the large endocytic vacuoles by the membrane movements during endocytosis and recycled back from these vacuoles to the apical cell surface via the AT, thereby allowing retrieval of these enzymes for extracellular digestion of sugar moieties in the maternal milk. Iida and Yamamoto (1985), however, have not observed direct contact between the AT and the apical cell surface in their study on HRP-uptake by the posterior intestine of stomachless fishes. In the present and other previous study on the rat ileum (Worthington and Graney 1970; Hirano and Kataoka 1986), the AT were not seen in direct contact with the apical cell surface; in contrast, they were frequently connected with small-spherical coated vesicles (~90 nm in diam.). This indicates that these vesicles probably detach from the AT and serve for

the retrieval and recycling of membrane material back to the apical cell surface.

In the present study, the Golgi complex showed no reaction product at any time after incubation. This result differs from that obtained in cultured cells (Gonatas et al. 1977; Willingham and Pastan 1980; Willingham et al. 1981) and secretory epithelia (Herzog and Farquhar 1977; Herzog and Reggio 1980). In these cells, Golgi-associated vesicles or Golgi cisternae were labelled by tracers for adsorptive or receptor-mediated endocytosis, and they were thought to be involved in membrane recycling. Recent studies, however, indicated that the Golgi complex in absorbing epithelia was not labelled during endocytic process (van Deurs et al. 1981; Christensen 1982; Gonnella and Neutra 1984; Hermo and Molares 1984; Iida and Yamamoto 1985; Kugler and Miki 1985; Hatae et al. 1986b). This suggests that direct membrane recycling via the Golgi complex is probably of minor importance in the absorbing epithelia.

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