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Intracellular Transport Pathways in Endodermal Cells of the Rat Visceral Yolk Sac

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Using horse radish peroxidase (HRP) and HRP-labeled concanavalin A, we examined intracellular transport pathways in endodermal cells of the rat visceral yolk sac. Morphologically, membrane-bound structures in these cells were regarded as coated vesicles, apical vacuoles, lysosome and apical canaliculi. The coated vesicles were further classified into three groups; large, medium-sized and small coated vesicles. Those of large size were found only in the supranuclear cytoplasm, while those of the medium-size, only in the basolateral cytoplasm. Small vesicles were found in the supranuclear and basolateral cytoplasm and in the Golgi areas. It is well known that the large coated vesicles, apical vacuoles and lysosmes are directly involved in endocytosis which occurs at the apical surface, and the apical canaliculi, in membrane recyling from the apical vacuoles and lysosmes to the apical cell membrane. Histochemically, by 30 min, the membrane-bound structures labeled with these tracers were found only in the supranuclear cytoplasm, but at 60 min, some small labeled vesicles were noted also in the basolateral cytoplasm and in the Golgi areas. These findings suggest that though most substances and membrames endocytosing at the apical surface are transported to the lysosmes, some might be transported to the basolateral cytoplasm and Golgi areas in the form of the small coated vesicles. The medium-sized coated vesicles were not labeled with the histochemical tracers, suggesting that they might be endocytosed at the basolateral cell membrane.

Key Words

Intracellular transport,
Visceral yolk sac endoderm,
Endocytosis,
Concanavalin A,
Horse radish peroxidase.

INTRODUCTION

Visceral yolk-sac endoderm of the rat embryo is a very active absorptive epithelium which plays an important role in mediation of embryonic nutrition, particularly prior to the establishment of a cholioallantoic placenta.[1] Biochemical studies revealed that proteins taken up by the endodermal cells are intracellularly degraded to amino acids, and transferred to the embryo,[2–4] Morphologically, it has been shown that exogenous proteins are rapidly taken up into the apical clathrin-coated invaginations (coated pits) and transported to lysosomes[5] which contain various kinds of hydrolytic enzymes.[6,7] On the other hand, it was reported that a number of molecules such as albumin, transferrin and immunogloburin G are also transferred from mother to embryo across the visceral yolk-sac endoderm.[8–17] These molecules

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might be transported via intracellular transport pathways other than the lysosomal pathway, because their activity and molecular form should be kept intact. To date, however, morphological evidence which suggests the presence of these intracellular transport pathways in the endodermal cells has not yet been reported.

Concanavalin A (Con A) is useful for labeling apical cell membranes, because it binds to mannosyl and glycosyl moieties in the cell-surface components, and therefore makes it possible to study the intracellular movements of labelled apical cell membranes[23]. In the present study, therefore, using horseradish peroxidase (HRP) and HRP-labeled Con A as makers of fluid-phase and adsorptive (receptor-mediated) endocytosis, we examined the distribution of the endocytosed materials and apical cell membranes in the endodermal cells of the rat visceral yolk-sac at the electron microscopic level. Special reference was made to the intracellular transport pathways other than the main route, i.e., from the apical surface to the lysosomes.

METERIALS AND METHODS

Animals

Abult Wistar rats (at least 3 months old) were mated overnight, and the following morning, pregnancy was confirmed by the presence of sperm in a vaginal smear. It was assumed that rats with a positive smear had mated within 2 hr of midnight (12:00 pm), and the conceptuses were thus presumed to be 0.5 days old at noon on the following day.[18] At 10.5 days of pregnancy, the rats were decapitated under light ether anesthesia, and the uterus was removed and immediately transferred to Hank's balanced salt solution for dissection.

Whole embryo culture

The uterus was carefully opened, and the decidual masses containing the embryo and its yolk sac along with the adherent trophoblast and ectoplacental cone were dissected from it under a dissecting microscope. The decidual masses and the parietal yolk sac were separated, but the visceral yolk sac and the ectoplacental cone were left intact (whole embryo). These whole embryos were then cultured for 24 hr at 37°C in 100% homologous heat-inactivated serum according to the method of New et al.[19]

Ultrastructure

The 24-hr-cultured whole embryos were fixed for 1 hr at 4°C in a mixture of 2% glutaraldehyde and 2% paraformaldehyde dissolved in 0.1 M cacodylate buffer (pH 7.4), or in the same fixative containing 0.1% saponine. After brief rinsing in the same buffer, the visceral yolk sacs were cut into small pieces with a razor blade and postfixed for 1 hr at 4°C in 1% osmium tetroxide dissolved in the cacodylate buffer. They were then dehydrated in a series of graded alcohols and embedded in an epoxy resin mixture (Quetol 812, Nacharai tesc, Japan). Ultrathin sections were cut with a diamond knife, contrasted with uranyl acetate and lead citrate, and examined in an electron microscope, JEM 100-SX (JEOL, Tokyo).

Experiment using HRP-labeled concana-

valin A

The 24-hr-cultured whole embryos were rinsed for 10 min at 4°C in phosphate buffered saline (PBS) and then incubated for 10 min at 4°C in PBS containing 100 μg/ml HRP-labeled concanavalin A (Con A-HRP) (Sigma, USA). These whole embryos were washed three times in PBS at 4°C to remove the excess lectin conjugate, and cultured again for 5, 10, 20, 30 and 60 min at 37°C in Con A-HRP-free rat serum.

Experiment using HRP

The 24-hr-cultured whole embryos were further cultured for 5, 10, 20, 30 and 60 min at 37°C in the rat serum containing 30 μg/ml HRP (corresponding to the concentration of HRP in 100 μg/ml Con A-HRP).

Histochemical procedures

These whole embryos treated with Con A-HRP and HRP were fixed for 1 hr at 4°C in a mixture of 0.5% glutaraldehyde and 1% paraformaldehyde dissolved in 0.1 M cacodylate buffer (pH 7.4) containing 6% sucrose. During the fixation, the visceral yolk sacs were removed from the embryos, and cut into small pieces with a razor blade. After rinsing for 1 hr at 4°C in the same buffer containing 7% sucrose, the yolk sacs were incubated for 1 hr at room temperature in a medium containing 0.05% 3, 3'—diaminobenzidine tetrahydrochloride and 0.05% H_2O_2 diluted with 0.1 M cacodylate buffer (pH 7.3). They were then rinsed for 1 hr at 4°C in the same buffer and postfixed for 1 hr at 4°C in 2% osmium tetroxide dissolved in 0.1 M cacodylate buffer (pH 7.4). After washing in the same buffer, these materials were dehydrated in a series of graded alcohols and embedded in the epoxy resin mixture. Ultrathin sections were examined in the electron microscope without any contrast staining.

RESULTS

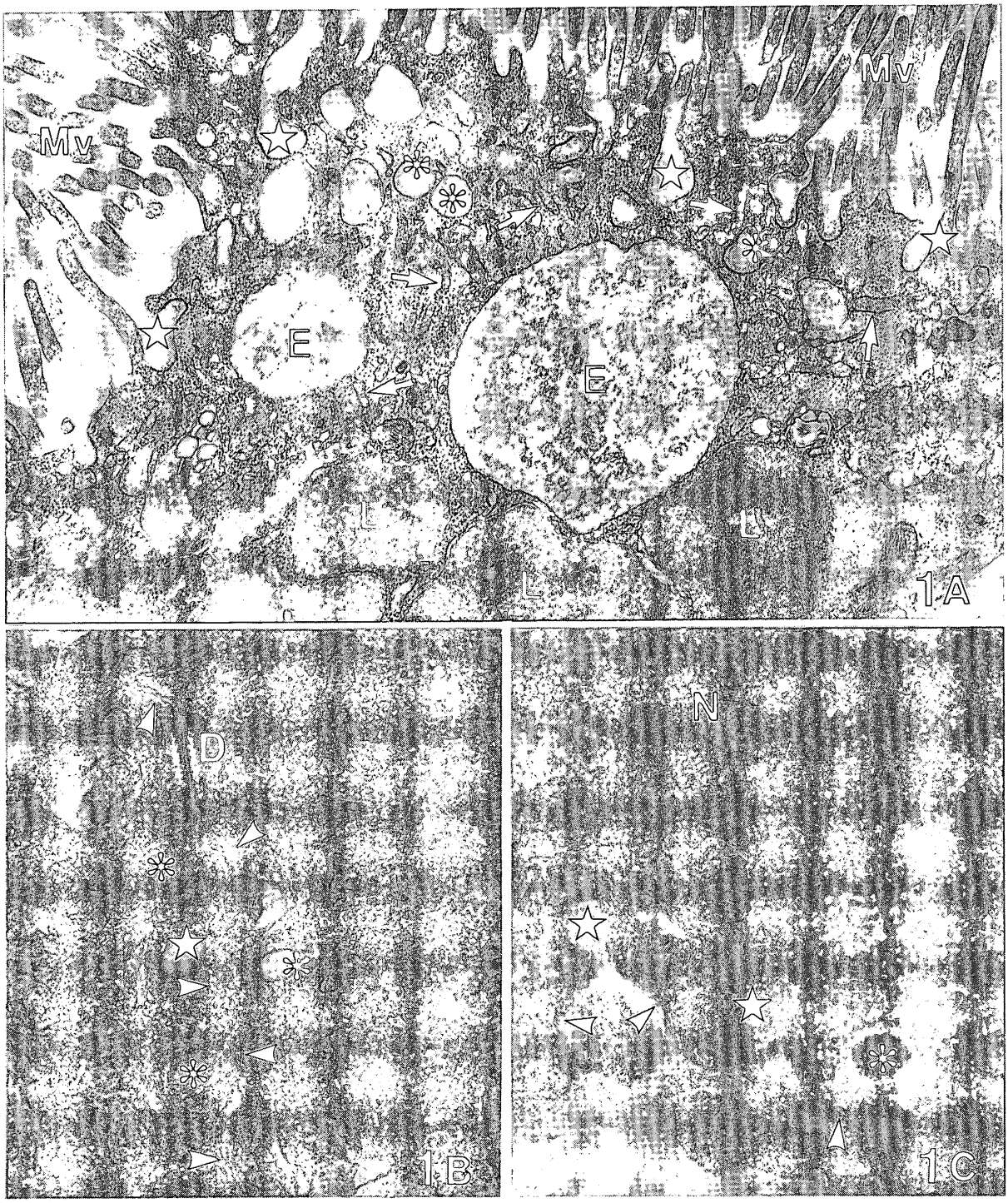
General appearance of the endodermal cells

Ultrastructure of the visceral yolk-sac endoderm of the 24-hr-cultured whole embryos (corresponding to the 11.5-day-old rat embryo in vivo) was essentially the same as that of the endodermal cells of the 10.5- and 12.5-day old rat embryo *in vivo*.[20,21]

Between the basis of microvilli lining along the apical surface, several spherical and saccular clathrin-coated pits[22] were observed (Fig. 1A). The most apical layer of the cytoplasm contained several round clathrin-coated vesicles. It was suggested that some of them had already detached from the apical cell membrane, while the others were still continuous with the apical cell membrane at different planes.[23] In the next layer of the cytoplasm, some large, round or irregularly-shaped vacuoles were noted (endosomes). They were always devoid of the clathrin coat along the outer surface of the limiting membrane. In the next layer, while still apical to the nucleus, some large electron-dense lysosomes were observed. Besides these structures, numerous membrane-bound round or tubular structures (apical canaliculi) were distributed mainly in the supranuclear cytoplasm.

In the cytoplasm lateral and basal to the nucleus, some coated pits and

coated vesicles were encountered mainly in the vicinity of the cell membrane (Fig. 1B, C). Golgi complexes were usually found in the cytoplasm basal or lateral to the nucleus. Mitochondria were often found in the cytoplasm apical to the nucleus, while sections of rough-surfaced endoplasmic reticulum (RER) were randomly distributed throughout the cytoplasm.



Fgiure 1. Electron micrograph of the endodermal cell of a 24-hr-cultured rat embryo (corresponding to the 11-day-old rat embryo *in vivo*). Picture A: Between the basis of microvilli (*MV*) lining along the apical surface of the cell, several coated pits (*stars*) are seen. The most apical layer of the cytoplasm contains several large coated vesicles (*asterisks*), and the next layer, some endosomes (*E*). Lysosomes (*L*) occupy the cytoplasm just apical to the nucleus. In the supranuclear cytoplasm, numerous apical canaliculi (*arrows*) are distributed among these membrane-bound structures. Picture B: In the cytoplasm lateral to the nucleus, some coated pits (*star*) and coated vesicles (*asterisks*) are observed mainly in the vicinity of the lateral cell membrane (*arrowheads*). *D:* desmosome. Picture C: In the cytoplasm basal to the nucleus (*N*), some medium-sized coated pits (*stars*) and coated vesicles (*asterisk*) are found. Picture A: × 28,000, Picture B: × 48,000, Picture C: × 64,000.

Distribution of the coated vesicles in the endodermal cells

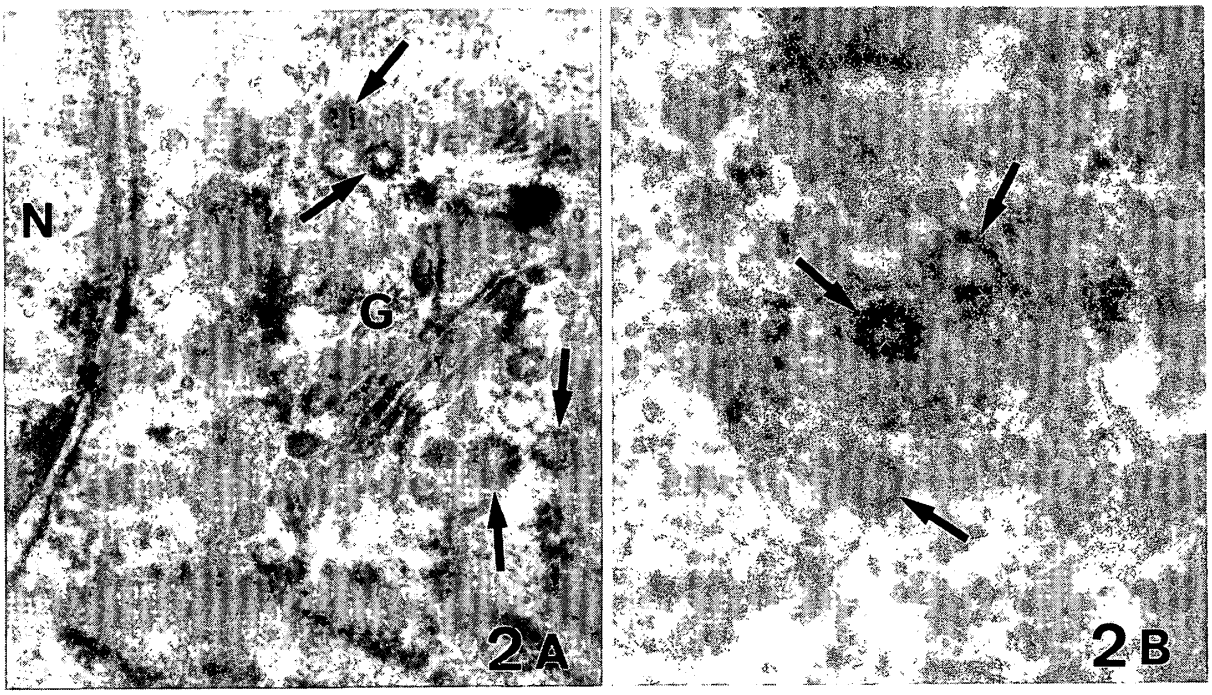
Treatment of the visceral yolk-sac endoderm with saponin during fixation allowed us to examine in detail the morphological characteristics and distribution of the vesicles in the endodermal cells. The coated vesicles were roughly classified into three groups according to their diameter: Large coated vesicles measured 200-500 nm; medium-sized coated vesicles 120-200 nm; and small coated vesicles, 80-105 nm in diameter, respectively (Table 1). The large ones were noted only in the supranuclear cytoplasm, those of medium-size, only in the lateral and basal cytoplasm (Table 2). While the small ones were distributed in various portions of the cytoplasm (Fig. 2A, B). About 40% of them were found in the supranuclear cytoplasm, about 10% in the basolateral cytoplasm, and about 50% in the Golgi areas.

Histochemical experiments

In the endodermal cells treated with Con A-HRP for 10 min at 4°C, distinct labeling was detected only between the basis of microvili and along the luminal surface of the coated pits (Fig. 3A). In the most apical layer of the cytoplasm, some labeled vesicular structures were observed. But they should be regarded as the coated pits which were still open to the apical surface at different planes, because at 4°C, endocytosis does not occur. In the cytoplasm lateral or basal to the nucleus, or in the Golgi areas, labeling with this tracer was not observed anywhere (Fig. 3B).

The large coated vesicles in the supranuclear cytoplasm began to be labeled with HRP and HRP-labeled Con A within 10 min after the start of the whole embryo culture with these histochemical tracers, and at 20 min, most of the large coated vesicles were labeled (Fig. 4A). The endosomes began to be labeled at 20 min, and the lysosomes and apical canaliculi at 30 min (Fig. 4B). By this time, the labeled membrane-bound structures were detected only in the supranuclear cytoplasm. In the lateral or basal cytoplasm or in the Golgi areas, distinct labeling with these traces was not detected.

At 60 min, almost all lysosomes and most of the apical canaliculi were labeled with these tracers. In the lateral and basal cytoplasm, some



Fgiure 2. Electron micrograph of the endodermal cell of a 24-hr-cultured rat embryo treated with saponin. Picture A: Small coated vesicles (*arrows*) seen in the Golgi area (*G*). Picture B: Small coated vesicles (*arrows*) seen in the lateral cytoplasm. Picture A: × 64.000, Picture B: × 75,000.

Table 1. Coated visocles in the rat visceral yolk-sac endoderm.

Coated vesicles	Diameter
Large CV (n=100)	200-500 nm (377 ± 72 nm)
Medium-sized CV (n=100)	120-200 nm (166 ± 19 nm)
Small CV (n=100)	80-105 nm (96 ± 9 nm)

small labeled vesicles were noted mainly in the vicinity of the lateral and basal cell membrane (Fig. 5A, B). Small labeled vesicles were noted also in the Golgi areas (Fig. 5C). The medium-sized coated vesicles seen in the lateral and basal cytoplasm were not labeled at any time examined in the present study.

DISCUSSION

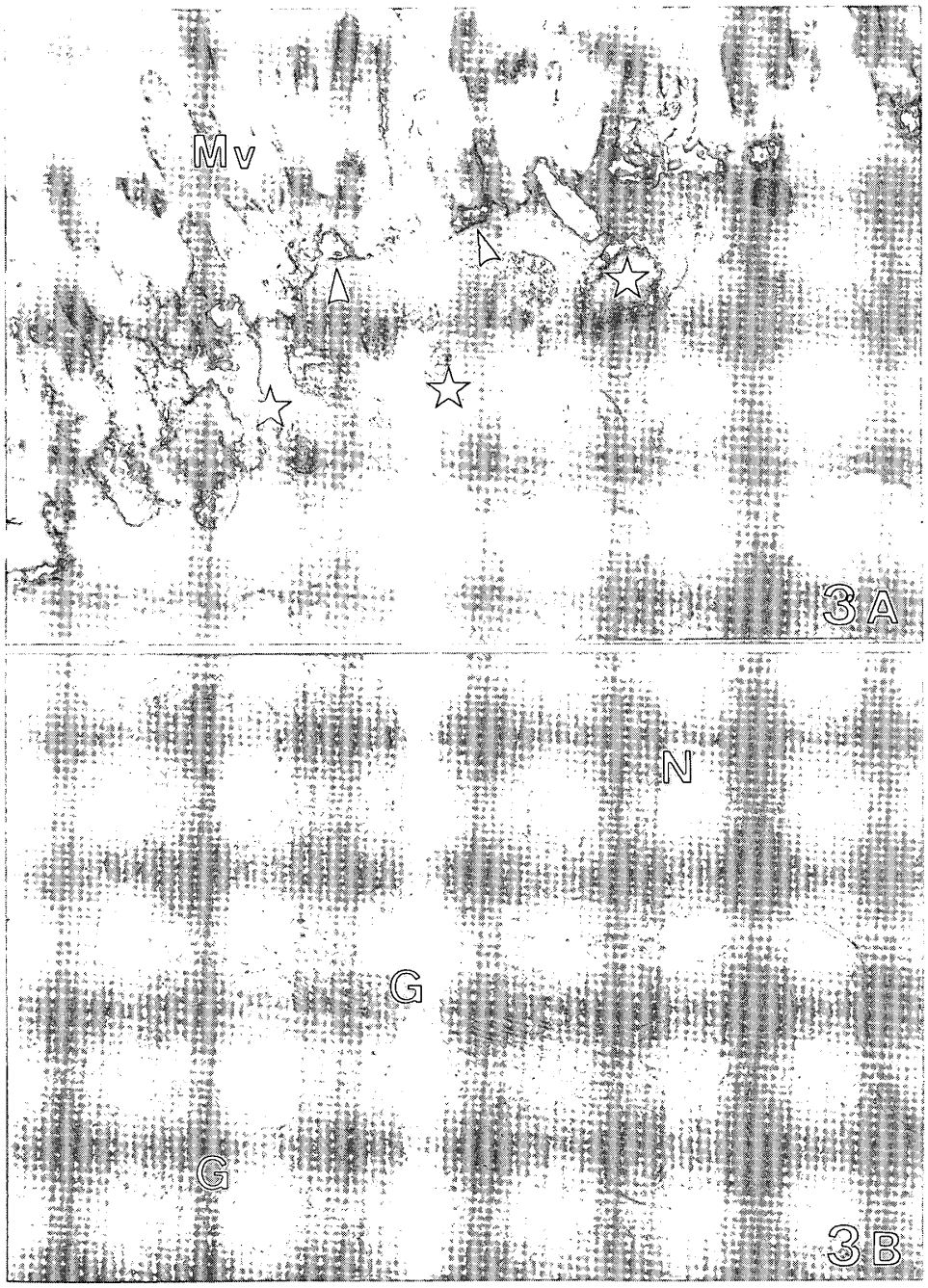
In absorptive cells such as endodermal cells of the rodent visceral yolk-

Table 2. Distribution of coated vesicles in the rat isceral yolk sac endoderm

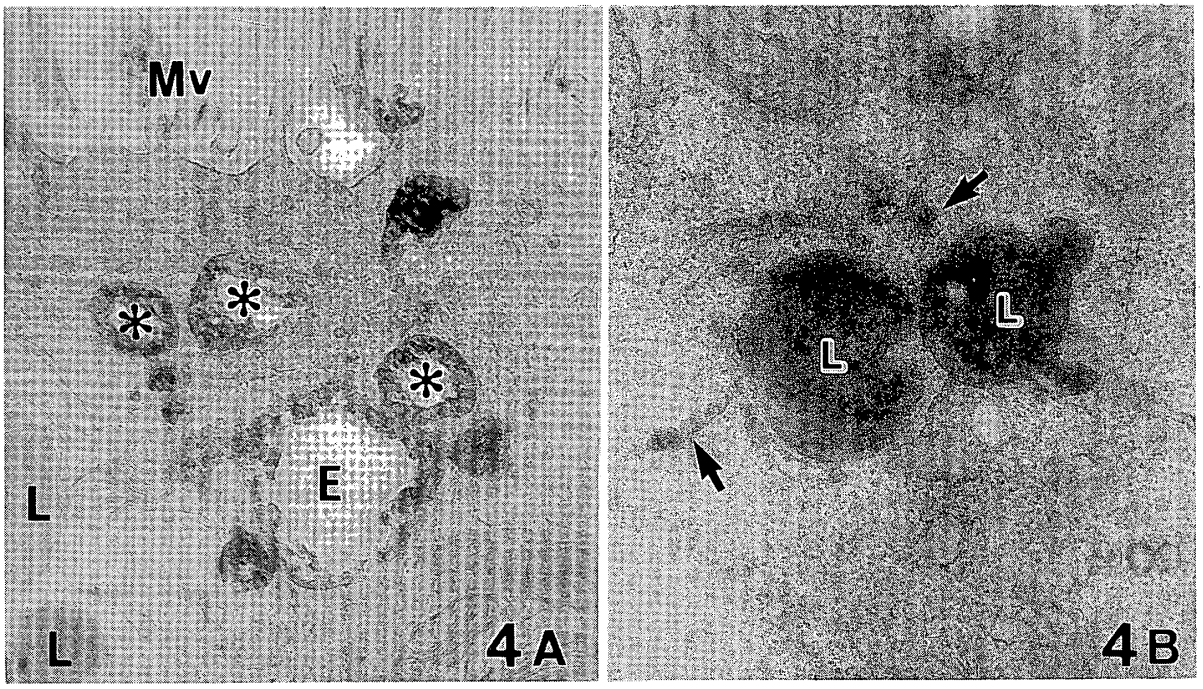
Coated vesicles	Distribution
Large CV	Supranuclear cytoplasm 100%
Medium-sized CV	Basolateral cytoplasm 100%
Small CV	Supranuclear cytoplasm 40% Basolateral cytoplasm 10% Golgi areas 50%

sac, epithelial cells of the small intestine and renal proximal tubule cells, numerous membrane-bound structures are absorved, and are generally known as coated vesicles, apical vacuoles (endosomes), lysosomes and apical canaliculi (apical tubuli). In the present study, we found that there were three types of coated vesicles (large, medium-sized and small coated vesicles) in the endodermal cells, and each type exhibited specific intracellular localization. It is well known that the large coated vesicles seen only in the apical cytoplasm, apical vacuoles and lysosomes are directly involved in endocytosis which occurs at the apical surface. The apical canaliculi which form an anastomosing network in the apical cytoplasm[24] are consider to serve as a vehicle for membrane recycling from the apical vacuoles and lysosomes to the apical cell membrame.[23,25,26] The medium-sized coated vesicles were found only in the basolateral cytoplasm. Along the basolateral cell membrame, clathrin-coated pits were also found. The clathrin coat is thought to provide a structural scaffold for invagination of the membrane.[27] Thus, these medium-sized coated vesicles can regarded as endocytosed vesicles, rather than those for exocytosis at the basolateral cell membrace. These vesicles were not labeled with the histochemical tracers which were exposed to the apical surface of the endodermal cells. The small coated vesicles were distributed in the supranuclear and basolateral cytoplasm and in the Golgi areas. To date, however, the significance of the small coated vesicles seen in the apical and basolateral cytoplasm of the endodermal cells has not yet been fully discussed.

The present study showed that by 30 min, membrane-bound structures labeled with the histochemical tracers were detected only in the apical cytoplasm. At 60 min, almost all membrane-bound structures in the supranuclear cytoplasm were labeled, and some small labeled vesicles were found also in the basolateral cytoplasm, mainly in the vicinity of the basolateral cell membrane. This suggests that these labeled vesicles were transported from the supranuclear cytoplasm to the basolateral cyto-

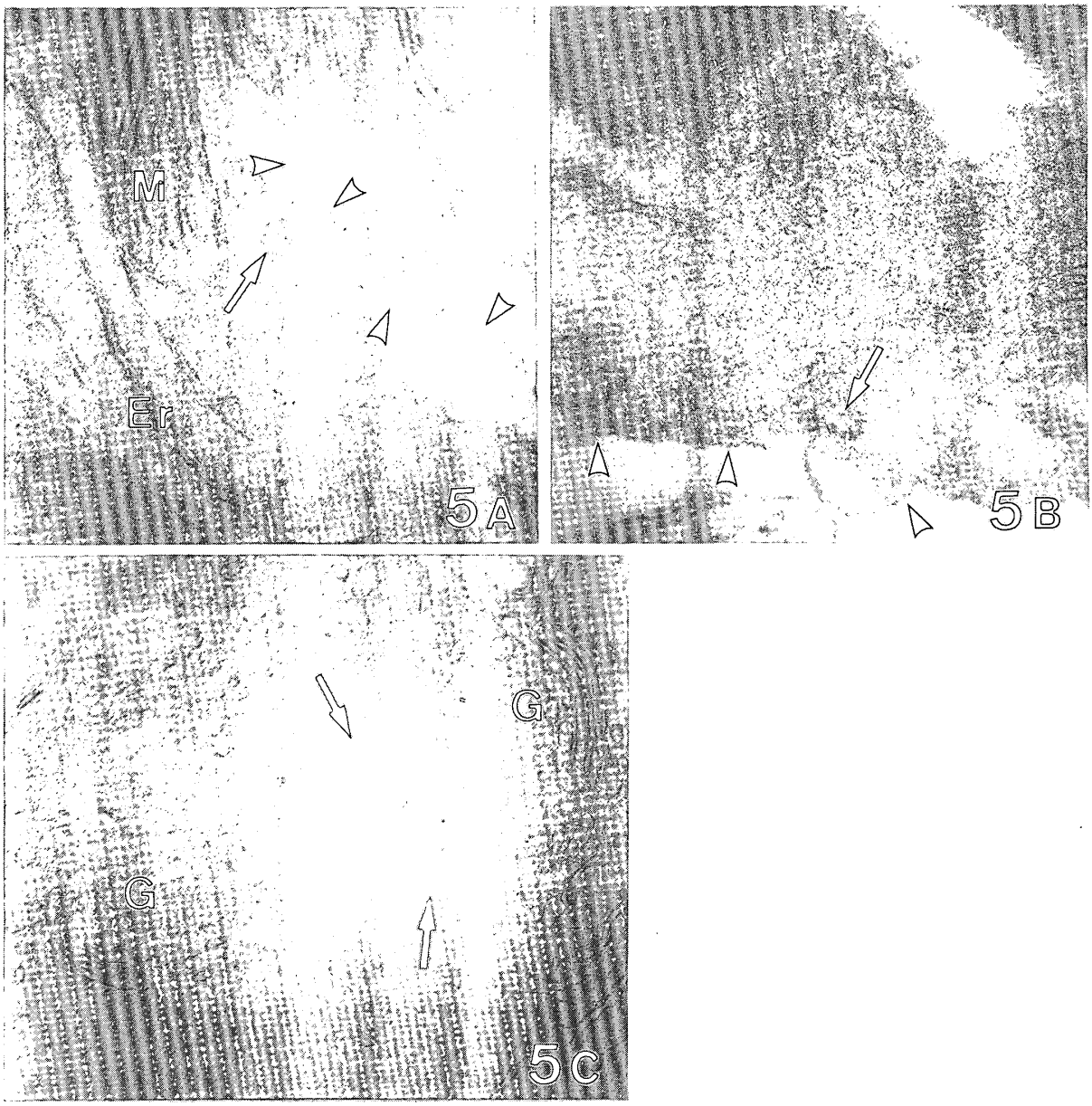


Fgiure 3. Endodermal cells of a 24-hr-cultured rat embryo labeled with Con A-HRP at 4°C for 10 min. Picture A: Distinct labeling is demonstrated between the base (*arrowheads*) of microvilli (*MV*) and in the coated pits (*stars*). The labeled membrane-bound structures in the most apical layer of the cytoplasm are considered to be coated pits which might be continuous with the apical surface at the different planes, because endocytosis does not occur at such low temperature. Picture B: In the Golgi area (*G*) located in the cytoplasm lateral to the nucleus (*N*), no distinct labeling is observed anywhere. Picture A: × 33,000, Picture B: × 57,000



Fgiure 4. Picture A: The endodermal cell of a 24-hr-cultured rat embryo which was treated with Con A-HRP for 10 min at 4°C and further cultured for 20 min at 37°C. Distinct labeling is noted along the luminal surface of coated vesicles (*asterisks*) and endosome (*E*). Picture B: The endodermal cell of a 24-hr-cultured rat embryo cultured with HRP for 30 min at 37°C. Almost all lysosomes (*L*) and some apical canaliculi (*arrows*) are strongly labeled with this tracer. Picture A: × 31,000, Picture B: × 50,000

plasm. Morphological observations in the present study revealed that most of the small vesicles in the basolateral cytoplasm were coated with clathrin. Hatae et al.[28] reported that small coated vesicles detached from the apical canaliculi were often observed in the ileal absorptive cells of suckling rats. This suggests that also in the endodermal cells, the small coated vesicles in the supranuclear cytoplasm might form from the apical canaliculi. Roberts et al.[29] reported that in the rat endodermal cells, IgG receptors were localized in the apical canaliculi, and suggested that IgG molecules were non-selectively endocytosed at the luminal surface, bound to IgG receptors in the apical canaliculi, and released to the interstitium after delivery to the basolateral membrane. It was also reported that albumin and transferrin were transported from the maternal blood serum to the embryo through the visceral yolk sac endoderm.[8–17,30] These substances are not likely to be degraded in the lysosomes because they should keep their molecular from and biological activity intact. Alstiel and Branton[31] stated that clathrin coat prevents the intimate contact required for fusion of the membrane bilayers. Pearse and Bretscher[27] and Klinger and Klüter[32] suggested that coated vesicles are the apparatus by which



Fgiure 5. The endodermal cell of a 24-hr-cultured rat embryo treated with Con A-HRP for 10 min at 4°C and further cultured for 60 min at 37°C. Picture A: A small labeled vesicle (*arrow*) is found in lateral cytoplasm in the vicinity of the lateral cell membrane (*arrowheads*). *Er:* endoplasmic reticulum, *M:* mitochondria. Picture B: A small labeled vesicle (*arrow*) seen in the basal cytoplasm very near from the basal cell membrane (*arrowheads*). Picture C: Small labeled vesicles (*arrow*) seen in the Golgi area (*G*). Picture A: × 88,000, Picture B: × 88,000, Picture C: × 52,000

membrane proteins are sorted out to their different destinations. These findings taken together suggest that these small coated vesicles might be important for intracellular transport of some specific endocytosed molecules.

Some labeled small vesicles were found also in the Golgi areas at 60 min. Since these areas exhibited no labeled vesicles by 30 min, they might have been transported from the supranuclear cytoplasm to the Golgi areas. It was said that in absorptive cells such as choroid plexus epithelium[33], suckling rat ileum[28,34], nonciliated cells of the ductuli efferentes[35], and renal proximal tubules[36], endocytosed tracers were not detected in the Golgi areas. On the other hand, in the secretory cells such as anterior pituitary cells, thyroid follicles and exocrine pancreas [37–40], endocytosed tracers were detected in the Golgi areas. It is said that in the secretory cells, limiting membranes of secretory granules fused with the surface cell membrane are endocytosed and recycled back to the Gogli areas. It was reported that the rat visceral yolk-sac endoderm actively synthesizes and secretes a variety of proteins including α-fetoprotein, retinol-binding protein, ceruloplasmin and transferrin.[41–46] Several kinds of digestive enzymes are also synthesized and transferred to the lysosome and apical cell membranes.[7,47] The findings of the present study suggest that in the endodermal cells, some membranous elements might be recycled back to the Golgi areas from the apical cell membrane and/or the membrane-bound structures in the supranuclear cytoplasm.

The results of the present study suggest that in the endodermal cells of the rat visceral yolk sac, though most of the substances and membranes endocytosed at the apical surface are transported to the lysosomes, some might be transported to the basolateral cytoplasm and Golgi areas as small coated vesicles. These small coated vesicles might play important roles in intracellular transport of some specific substances and in membrane recycling to the Golgi areas.

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