Evidence for the Sorting of Endocytic Vesicle Contents during the Receptor-mediated Transport of IgG across the Newborn Rat Intestine

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ABSTRACT Fc receptors on the luminal membranes of intestinal epithelial cells in the neonatal rat mediate the vesicular transfer of functionally intact IgG from the intestinal lumen to the circulation. In addition, there is a low level of nonselective protein uptake, but in this case transfer does not occur. To determine whether a specialized class of endocytic vesicles could account for the selective transfer of IgG, mixtures of IgG conjugated to ferritin (IgG-Ft) and unconjugated horseradish peroxidase (HRP) were injected together into the proximal intestine of 10-d-old rats, and the cellular distribution of these two different tracers was determined by electron microscopy. Virtually all apical endocytic vesicles contained both tracers, indicating simultaneous uptake of both proteins within the same vesicle. However, only IgG-Ft bound to the apical plasma membrane, appeared within coated vesicles at the lateral cell surface, and was released from cells. HRP did not bind to the luminal membrane and was not transferred across cells but was confined to apical lysosomes as identified by acid phosphatase and aryl sulfatase activities. To test the possibility that the binding of IgG to its receptor stimulated endocytosis, HRP was used as a fluid volume tracer, and the amount of HRP taken up by cells in the presence and absence of IgG was measured morphologically and biochemically. The results demonstrate that endocytosis in these cells is constitutive and occurs at the same level in the absence of IgG. The evidence presented indicates that the principal selective mechanism for IgG transfer is the binding of IgG to its receptor during endocytosis. Continued binding to vesicle membranes appears to be required for successful transfer because unbound proteins are removed from the transport pathway before exocytosis. These results favor the proposal that IgG is transferred across cells as an IgG-receptor complex.

The cellular route of maternal IgG transport across the intestinal epithelium of the neonatal rat has been studied using IgG conjugated to tracers for electron microscopy (27–29). These previous studies and others that have employed physiological methods (5, 17, 21, 22) have shown that IgG first binds to Fc receptors on the brush-border membranes of epithelial cells that line the duodenum and proximal jejunum. The binding of IgG to receptors is pH dependent: IgG binds at pH 6.0–6.5 but not at pH 7.4 (17, 30, 31, 38). The bound IgG enters the cells within endocytic tubules that form at the bases of the microvilli and is then transferred to spherical coated vesicles and released at the lateral surface into the intercellular space (27–29). We have recognized, however, that a small yet significant amount of apparently nonselective protein uptake also occurs by endocytosis, but transfer across the cell and release of these proteins is not observed (1, 29).

The present investigation was undertaken to examine specifically the mechanism whereby intestinal epithelial cells of the newborn rat selectively segregate different proteins that enter the cells by endocytosis. For our experiments, we used horseradish peroxidase (HRP) as a fluid volume tracer and ferritin conjugated to IgG (IgG-Ft) as a specific label for receptor-

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mediated IgG transport. The cellular distribution of these proteins was then determined by electron microscopy. Our results demonstrate that HRP and receptor-bound IgG enter cells simultaneously within the same endocytic vesicles. In addition, we observe essentially a constant level of endocytic activity in these cells, irrespective of the presence of IgG. However, only IgG is released at the lateral surface of the cells. In contrast, HRP remains within cells and is transported into lysosomes in the apical cytoplasm. Therefore, the contents of endocytic vesicles appear to be sorted out during intracellular transport, and only membrane-bound protein is transferred, possibly complexed with receptor, across the cell.

MATERIALS AND METHODS

Animals

Female Sprague-Dawley random-bred rats with litters 10–12 d old were purchased from Flow Laboratories, Dublin, Va.

Proteins

Rat gamma-globulins (Cohn fraction II) and crystallized bovine serum albumin (BSA) were purchased from Miles Laboratories, Inc., Elkhart, Ind. HRP (type VI) was obtained from Sigma Chemical Co., St. Louis, Mo. Native horse spleen ferritin (EM grade) was purchased from Polysciences, Inc., Warrington, Pa. All proteins were dialyzed against normal saline before use.

Ferritin Conjugation

IgG was purified from rat gamma-globulins by chromatography on DEAE cellulose by methods previously described (28). Ferritin was dialyzed against 0.1 M EDTA, pH 7.4, to remove heavy metal contaminants (10) and was conjugated to the purified IgG (using 4,4′-difluoro-3,3′-dinitro-diphenyl sulfone) by the method of Sri Ram et al. (34). The crude conjugate was purified by Pevikon block electrophoresis (24) and was then determined to be free of unconjugated IgG and unbound ferritin by immunoelectrophoresis. The conjugate was concentrated to 7 mg/ml ferritin by ultrafiltration with an Immersible-CX Molecular Separator (Millipore Corp., Bedford, Mass.) and dialyzed into normal saline.

Transport Experiments

Young rats, 10–12 d old, were anesthetized with ether, and the proximal jejunum was exposed and cannulated *in situ* as described previously (31). A second cannula was inserted 3 cm distal to the first to serve as a drain. The segment was rinsed by gently injecting 3.0 ml of warm saline into the proximal cannula.

To trace the cellular fate of proteins that enter IgG-transporting cells by nonselective endocytosis, a solution of HRP (1.5 mg/ml) was injected into cannulated segments. Segments were then incubated *in situ* for periods of 10, 20, 30, 60, and 120 min. For double-tracer experiments, mixtures of IgG-Ft (1.5 or 5 mg/ml Ft) and HRP (1.5 mg/ml) were injected together and the segments were incubated *in situ* for 10, 30, and 60 min. After incubation, the segments were briefly rinsed with warm saline, and were excised, fixed, and processed histochemically for HRP as described below.

Morphometric and Biochemical Studies

To determine whether IgG stimulated endocytosis in IgG transporting cells, the relative level of endocytosis was compared in the presence and absence of IgG. For these studies, intestinal segments were cannulated, and mixtures of IgG or BSA (3.5–5.0 mg/ml) together with HRP (1.5 mg/ml) or unconjugated ferritin (25 mg/ml) were injected into the segments. The segments were incubated *in situ* for 10 min. The segments were then quickly rinsed with saline and excised. In morphometric studies, tissue exposed to the protein and tracer were fixed and processed for microscopy. For each experiment, sections of standard thickness (70 nm) were cut, and at least 21 electron micrographs were taken of equivalent fields of the brush-border region of cells that lined the upper half of villi. These fields were selected from areas where the microvilli were sectioned longitudinally, but, otherwise, the fields were randomly photographed. The number of endocytic vesicles that contained tracer was counted from photographic prints and was expressed as a function of positive vesicles per unit area of luminal cell surface. This surface area included the contribution of the microvilli, which were estimated to contribute 93% of the total surface area. Values were analyzed for statistical significance by use of a *t* test.

For biochemical studies, animals that had been separated from their mothers for a minimum of 14 h were used to minimize the possibility of the presence of residual, maternal IgG within the intestine. Jejunal segments were injected with IgG or BSA along with HRP, or HRP alone, and, after 10 min, epithelial cells from these segments were isolated by a modification of the method of Evans et al. (8) as described previously (31). The isolated cells from each individual (~0.1 ml of packed cells) were lysed in 1.0 ml of 0.1% Triton X-100, and each lysate was analyzed for peroxidase activity by use of the spectrophotometric assay of Steinman and Cohn (36). The peroxidase activity was then compared with standards of known HRP concentrations. The total cellular protein in the isolate was determined with a modified Lowry procedure (4) to reduce interference from EDTA and detergent. The amount of HRP uptake for each animal was expressed as nanograms HRP per milligram of isolated cellular protein. Values were analyzed for statistical significance by a one-way analysis of variance.

Enzyme Histochemistry

In segments that were injected with tracers for electron microscopy, tissue strips were fixed for 30 min in 2% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 6.0, and for an additional 90 min in fixative buffered to pH 7.4. Tissue was then washed overnight in several changes of 0.1 M buffer plus 3.5% sucrose. Tissue exposed to HRP was incubated for 60 min in a medium containing 0.05% diaminobenzidine and 0.01% H_2O_2 in 0.1 M sodium phosphate buffer, pH 6.0 (11).

In other experiments, uninjected intestinal segments were excised and processed to assess the possible presence and distribution of lysosomal enzymes. For acid phosphatase, tissue strips were fixed for 2 h in 2% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.3, plus 3.5% sucrose and washed overnight in cacodylate buffer. Tissue was then chopped to a thickness of 40 μm with a Smith and Farquhar tissue chopper (DuPont Co., Sorvall Biomedical Div., Newtown, Conn. [DuPont-Sorvall]) and incubated for 60 min in a modified Barka-Anderson medium containing sodium-β-glycerophosphate and lead nitrate in 0.05 M Tris-maleate buffer, pH 5.2 (3). For aryl sulfatase, 100-μm sections were incubated for 90 min with *p*-nitrocatechol sulfate and barium chloride in 0.05 M acetate buffer, pH 5.5 (16).

Electron Microscopy

After aldehyde fixation and substrate incubation, as required, tissue was postfixed in buffered 2% osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in Epon (19). Silver to pale gold thin sections were cut with diamond knives on a Porter-Blum MT-2 microtome (DuPont-Sorvall) and stained for 1–5 min in lead citrate (26). Sections were examined in a Philips EM 200 electron microscope microscope operated at 60 kv.

RESULTS

Transport of HRP

When HRP was injected into the lumen of the small intestine and the preparation was incubated *in situ*, HRP reaction product could be detected within numerous vesicles within the apical cytoplasm (Fig. 1). However, HRP was not transferred across the cells and into the intercellular space, as we have reported in other studies that used this tracer (1, 29). HRP was seldom visualized in more basally located vesicles and did not appear within the small coated vesicles associated with the lateral plasma membrane. Instead, much of the reaction product to HRP appeared to be concentrated within apical vacuoles and dense bodies. In addition, tracer was not evident within cells that lined the lower third of intestinal villi, indicating that these younger cells did not take up detectable amounts of HRP by endocytosis.

Distribution of Lysosomal Enzymes

To determine whether vesicles that appeared to contain high concentrations of HRP might also contain lysosomal enzymes, intestinal tissue from the jejunum of the newborn rat was processed to assess the distribution of the diagnostic lysosomal enzymes, acid phosphatase and aryl sulfatase. Acid phospha-

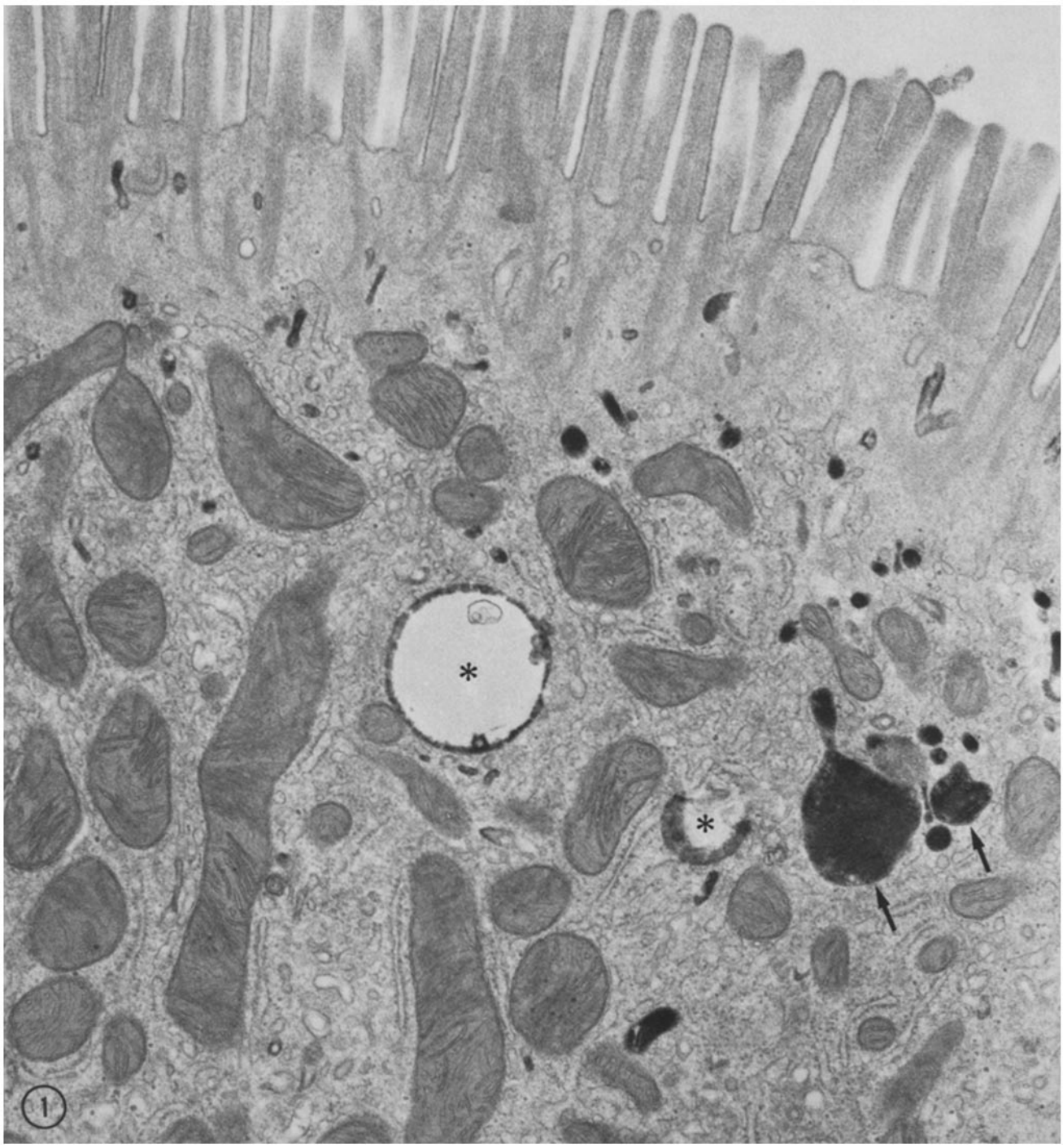

FIGURE 1 Electron micrograph of the apical region of intestinal epithelial cells incubated with HRP for 2 h. HRP is present within endocytic vesicles and small apical vacuoles II (*). High concentrations of reaction product are seen within several dense bodies (arrows). × 29,000.

tase activities were demonstrated within dense bodies that appeared similar in morphology and location to the large, dense vesicles described previously that contained high concentrations of HRP (Fig. 2*a*). Acid phosphatase activity was notably absent, however, within endocytic vesicles and within the apical vacuoles. The distribution of aryl sulfatase was similar to that described for acid phosphatase. These dense bodies that contained the lysosomal enzymes occurred predominantly within the apical cytoplasm. In some instances, Golgi vesicles occasionally contained acid phosphatase but not aryl sulfatase. In addition, acid phosphatase also appeared as a brush-border enzyme on some cells in the jejunum, but the amount of enzyme present on individual cells, as estimated by the intensity of staining, was highly variable (Fig. 2*b*).

Endocytosis in the Presence and Absence of IgG

Whether endocytosis was stimulated by the binding of IgG to its membrane receptor was examined by use of morphological and biochemical methods. For morphological analysis, Ft

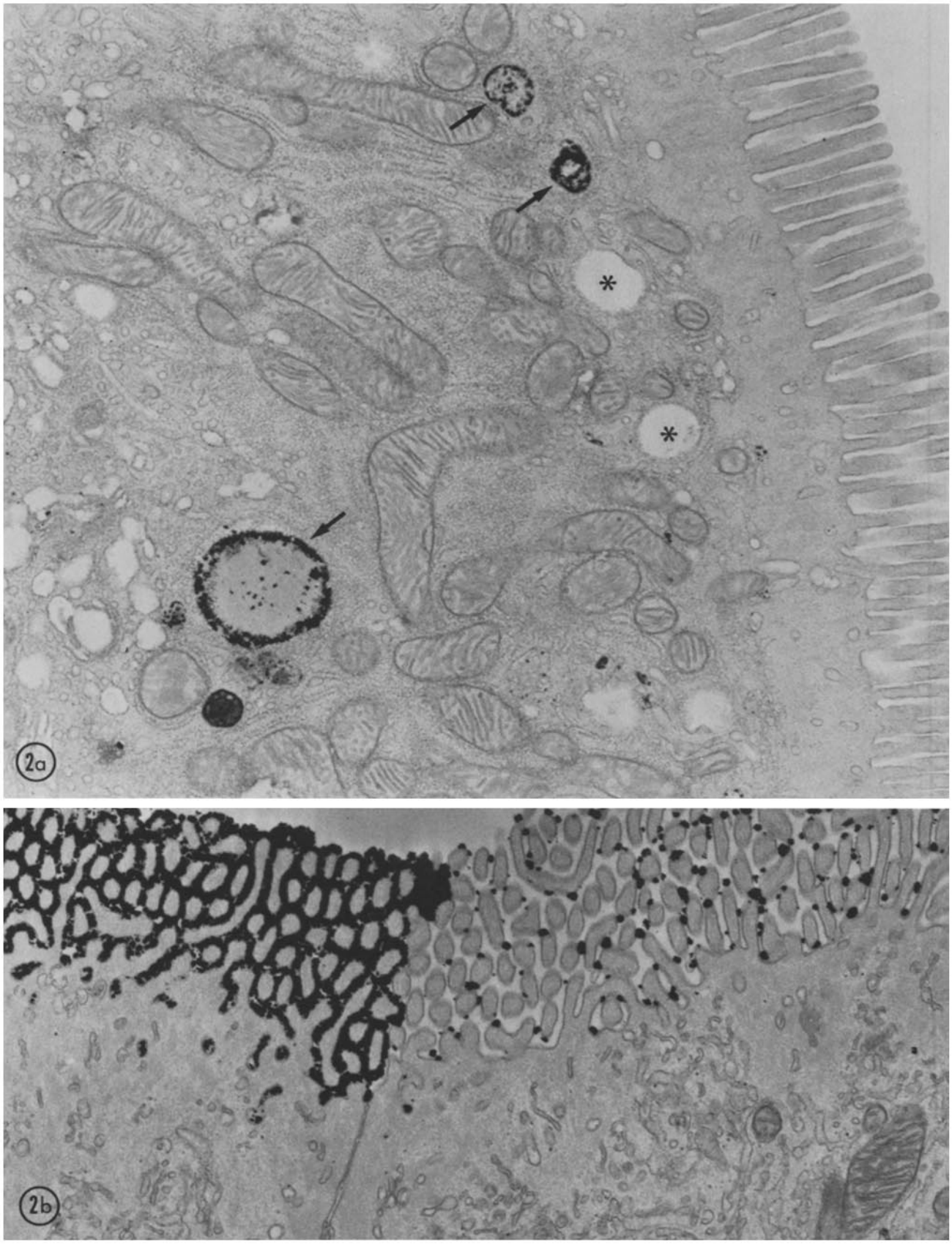

FIGURE 2 Micrographs from the apical portion of jejunal epithelial cells processed for acid phosphatase activity. × 25,000. (*a*) The lead phosphate precipitate appears confined primarily to vesicles (arrows) that correspond to the dense bodies seen in Fig. 1 that contained HRP. Acid phosphatase is not apparent in apical vacuoles (*). (*b*) Acid phosphatase is also present as a brush-border enzyme on some neonatal cells in the jejunum. The amount of enzyme present on individual cells, however, is highly variable, as shown in this micrograph.

or HRP was injected into intestinal segments in the presence or absence of IgG. After a 10-min incubation, the number of endocytic vesicles that contained tracer within fixed cells was determined in order to estimate the rate of endocytosis.

When unconjugated Ft was used as a space-filling tracer for endocytosis, relatively few profiles of endocytic vesicles appeared to contain Ft, even at the high concentration (25 mg/ml Ft) used in this experiment (Fig. 3 *a*; Table I). We presume these estimates for Ft-containing vesicles to be low estimates because there is a high probability that a given section profile will not contain Ft if the tracer is present at low density. HRP proved to be a more sensitive indicator of endocytosis in these cells, and vesicles that had taken up HRP within 10 min could be readily identified (Fig. 3 *b*). After a 10-min incubation period, nearly all of the tracer within the cells appeared within endocytic vesicles that were 1 μm or less from the bases of the microvilli (Fig. 3 *b*). For this reason, only positive vesicles within 1 μm of the cell surface were scored from prints. By

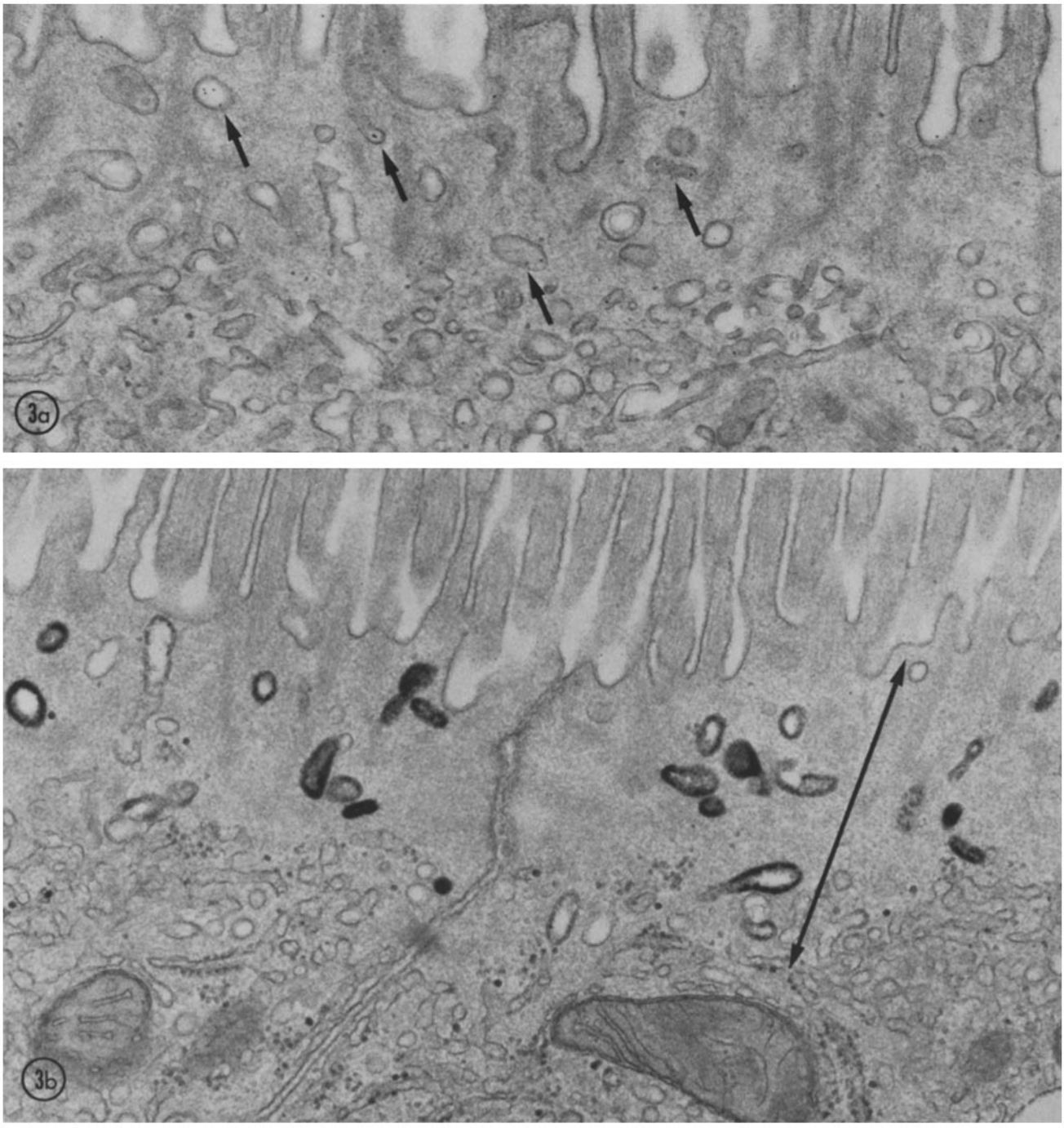

FIGURE 3 Mixtures of IgG or BSA and space-filling tracers for endocytosis were injected into jejunal segments, and the segments were incubated *in situ* for 10 min. The number of endocytic vesicles that contained tracer was then counted from prints. × 54,000. (*a*) Representative micrograph from the brush-border region of cells that were incubated with IgG and 25 mg/ml ferritin. A few vesicles within the terminal web contain small numbers of ferritin particles (arrows). (*b*) Micrograph from cells that were incubated with IgG and 1.5 mg/ml HRP for 10 min. Several vesicles that contain HRP reaction product can be readily distinguished. Most vesicles occur within 1 μm from the bases of the microvilli (arrow).

estimating the section thickness to be 70 nm, we calculated the number of vesicles that contained tracer per unit area of luminal membrane surface. The results from these experiments (Table I) show that although fewer vesicles contained Ft than HRP, the number of vesicles that contained either tracer did not change significantly in the presence of IgG ($P > 0.5$).

As an independent method, an enzymatic assay for HRP was also performed on epithelial cells that were isolated from intestinal segments that had been injected with HRP in the presence or absence of IgG. The results are summarized in Table II. Unexposed epithelial cells possessed no detectable endogenous peroxidase activity. As was the case within the morphological assay, there was no significant difference in the uptake of HRP in the presence or absence of IgG ($P > 0.05$).

These morphological and biochemical results together indicate that IgG binding to its receptor does not appear to stimulate or otherwise affect bulk endocytosis by the epithelial cells.

Sorting of Vesicle Contents

To evaluate the possibility that different endocytic vesicle populations are responsible for selective and nonselective protein uptake, intestinal sgements were incubated with a mixture of HRP and IgG-Ft over a period of 15–60 min. Both tracers appeared together within a majority of endocytic vesicles, indicating simultaneous uptake (Fig. 4). However, only the IgG-Ft conjugate appeared bound to the apical membrane at the bases of microvilli and within endocytic pits. In addition, a far greater number of Ft particles entered cells as compared with the extremely low level of uptake of the unconjugated Ft that was used at a much higher concentration in the endocytosis experiments noted previously. This markedly enhanced uptake of IgG-Ft was therefore interpreted to be attributable virtually entirely to the specific interaction of the conjugate with the IgG receptor.

When the two tracers were incubated within intestinal segments for 60 min, the overall distribution of HRP within the cells appeared essentially the same as that previously observed: HRP remained confined to endocytic and lysosomal vesicles within the apical cytoplasm (Fig. 5). IgG-Ft also appeared within these apical vesicles. However, only IgG-Ft was found in vesicles located in more basal regions of the cell surrounding the Golgi complex and lateral plasma membrane. Frequently, IgG-Ft particles were visualized within spherical coated vesicles (Fig. 6 *a*) and vesicles bordering and apparently fusing with the lateral membranes of cells (Fig. 6 *b*). IgG-Ft was also detected free within the intercellular space, indicating transport of IgG across the cell (Fig. 6 *c*). HRP was not observed in coated vesicles nor was it transferred to the intercellular spaces. This evidence indicates that the free HRP had been removed from the IgG transport pathway.

TABLE I

Morphological Assay of Endocytosis in the Presence and Absence of IgG

Stimulating protein	Tracer	No. of prints scored	No. of positive vesicles
			$\mu m^{-2}/10$ min ± SEM
IgG (5.0)*	Ft (25)*	23	1.3 ± 0.4
BSA (5.0)		22	1.4 ± 0.7
IgG (3.5)	HRP (1.5)	21	4.1 ± 0.2
BSA (3.5)		23	4.0 ± 0.1

Mixtures of IgG or BSA and tracers for electron microscopy were injected into the proximal jejunum of 10-d-old rats, and the preparation was incubated *in situ* for 10 min. Exposed tissue was then processed for microscopy. The number of endocytic vesicles that contained tracer was counted from photographic prints and expressed as a function of apical cell surface area as described in Materials and Methods. *SEM*, standard error of the mean.

* Concentrations in parentheses are in milligrams per milliliter.

TABLE II

Biochemical Assay of Endocytosis in the Presence and Absence of IgG

Stimulating protein	Tracer	No. of animals	HRP uptake in 10 min
			ng HRP/mg cell protein ± SEM
IgG (3.5)*	HRP (1.5)*	8	1.32 ± 0.18
BSA (3.5)	HRP (1.5)	7	2.1 ± 0.4
None	HRP (1.5)	3	1.93 ± 0.55

Mixtures of IgG or BSA and HRP were injected into jejunal segments of 10-d-old rats and incubated *in situ* for 10 min. Epithelial cells that were isolated from injected segments were then lysed in Triton X-100, and the lysate was assayed for HRP activity and total protein content as described in Materials and Methods. *SEM*, standard error of the mean.

* Concentrations in parentheses are in milligrams per milliliter.

DISCUSSION

We used mixtures of IgG conjugated to ferritin and unconjugated HRP to distinguish selective IgG transport from nonselective fluid endocytosis in IgG-transporting cells of the neonatal rat jejunum. Our results show that selective and nonselective uptake probably occur within the same endocytic vesicles but that only receptor-bound IgG is transferred across cells. In contrast, unbound proteins that enter cells have a different intracellular fate. These proteins appear to be segregated from IgG and concentratrated within lysosomes. In addition, evidence is presented that indicates that IgG binding does not significantly stimulate endocytosis in these cells. These results are consistent with the hypothesis that the selection of IgG for transfer across cells requires the binding of IgG to its receptor on the membranes of endocytic vesicles. Continued binding to vesicle membranes appears also to be required, because the fluid contents of endocytic vesicles are removed from the IgG transport pathway.

Transport of HRP

Our experiments with free HRP have shown that cells that line the uppermost one-half to two-thirds of intestinal villi take up significant amounts of HRP by endocytosis. However, HRP is not transferred across these cells but appears to be concentrated within apical lysosomes.

The localization of free HRP exclusively to apical endocytic vesicles and lysosomes is strikingly different from the cellular distribution of HRP that we have observed when HRP-IgG conjugates (29) or HRP-anti-HRP immune complexes (1) are incubated within intestinal segments. In these cases, reaction product of HRP appears bound to the apical plasma membrane, occurs within numerous tubules and coated vesicles throughout the cytoplasm, and can be readily detected within the intercellular spaces by 30 min. Therefore, the apical distribution of unconjugated HRP which we report here cannot be attributed simply to the enzymic degradation of HRP during the course of intracellular transport. Rather, the intracellular fate of HRP appears to depend upon whether or not HRP becomes linked to the IgG receptor. Thus, at least two different

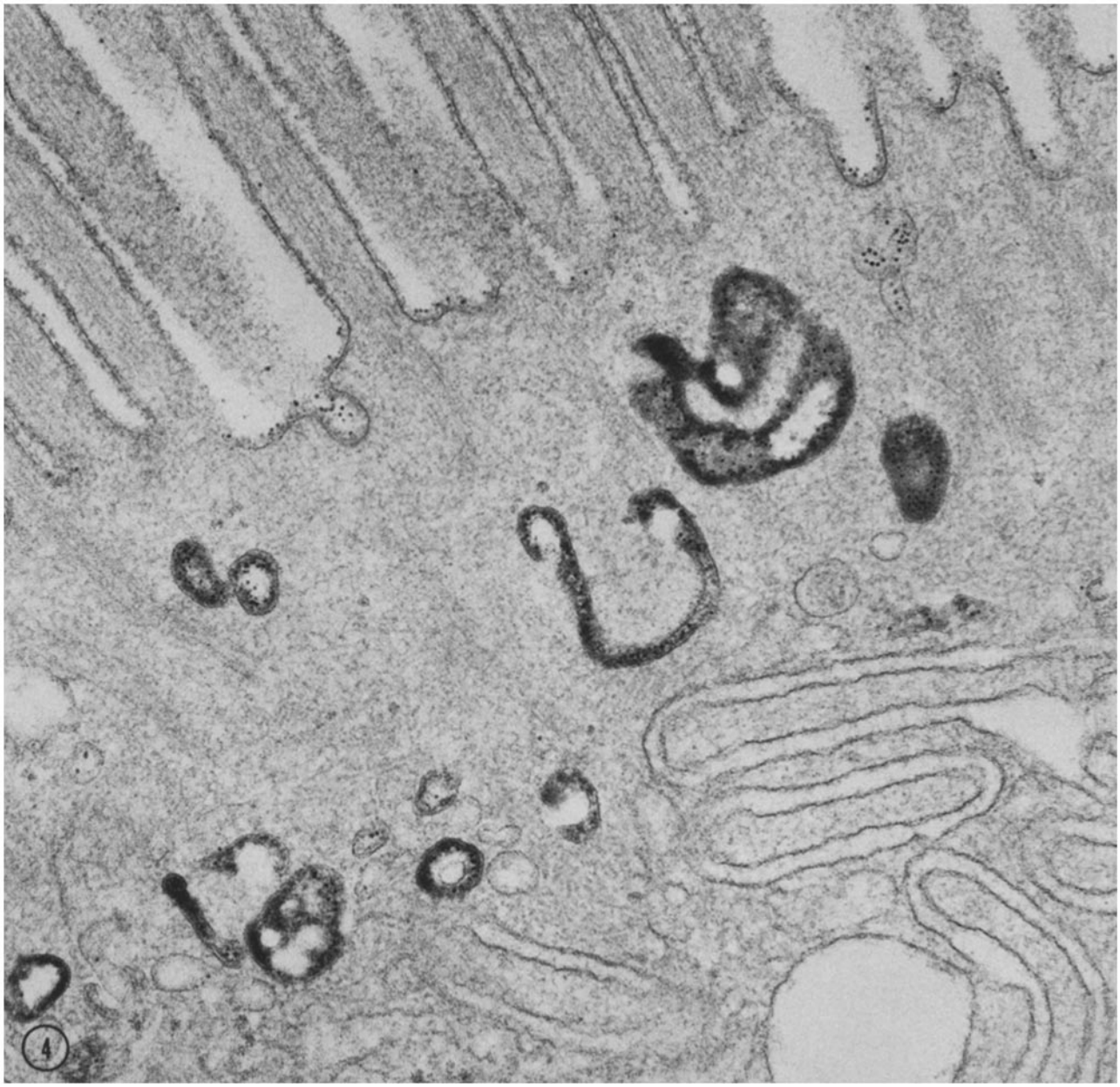

FIGURE 4 This micrograph depicts the brush-border region from jejunal cells that were exposed to mixtures of IgG-Ft (1.5 mg/ml) and HRP (1.5 mg/ml) for 30 min. Numerous IgG-Ft particles appear bound to the apical plasma membrane. HRP does not bind to the membrane but in most instances occurs together with IgG-Ft within the endocytic vesicles in the terminal web. × 82,000.

routes exist for proteins that enter IgG-transporting cells by endocytosis: transfer across cells, as is the case for IgG conjugates or HRP-anti-HRP immune complexes, and transport to lysosomes, as is the case for unconjugated HRP and presumably other proteins that fail to become linked with the IgG receptor. Under some conditions IgG may apparently take both routes within the cell. Morris and Morris (21, 22) found that when ^{125}I-IgG is injected into jejunal segments of the newborn rat ~40% of the administered dose is transmitted intact into the circulation. The rest is degraded, possibly by the lysosomes that we have identified here. This evidence also agrees with previous morphological experiments (28) that have localized some IgG-Ft particles within apical multivesicular bodies and that we observe here as well.

We did not detect HRP within epithelial cells on the lower third or at the bases of intestinal villi, and this indicates a negligible level of endocytic activity in these younger cells. Because these basal cells also do not display functional IgG receptors (31), the onset of endocytic ability by epithelial cells migrating out onto the villus may occur at the same developmental stage as the expression of IgG receptors.

Constitutive Endocytosis

One possible way to enhance the amount of selective protein uptake by IgG-transporting cells would be to have IgG stimulate endocytosis upon binding to its receptor. To test this possibility, the relative level of endocytosis was compared in the presence and absence of IgG by morphological and biochemical techniques. The results from these two different approaches indicate, however, that endocytosis is constitutive and is not stimulated by IgG.

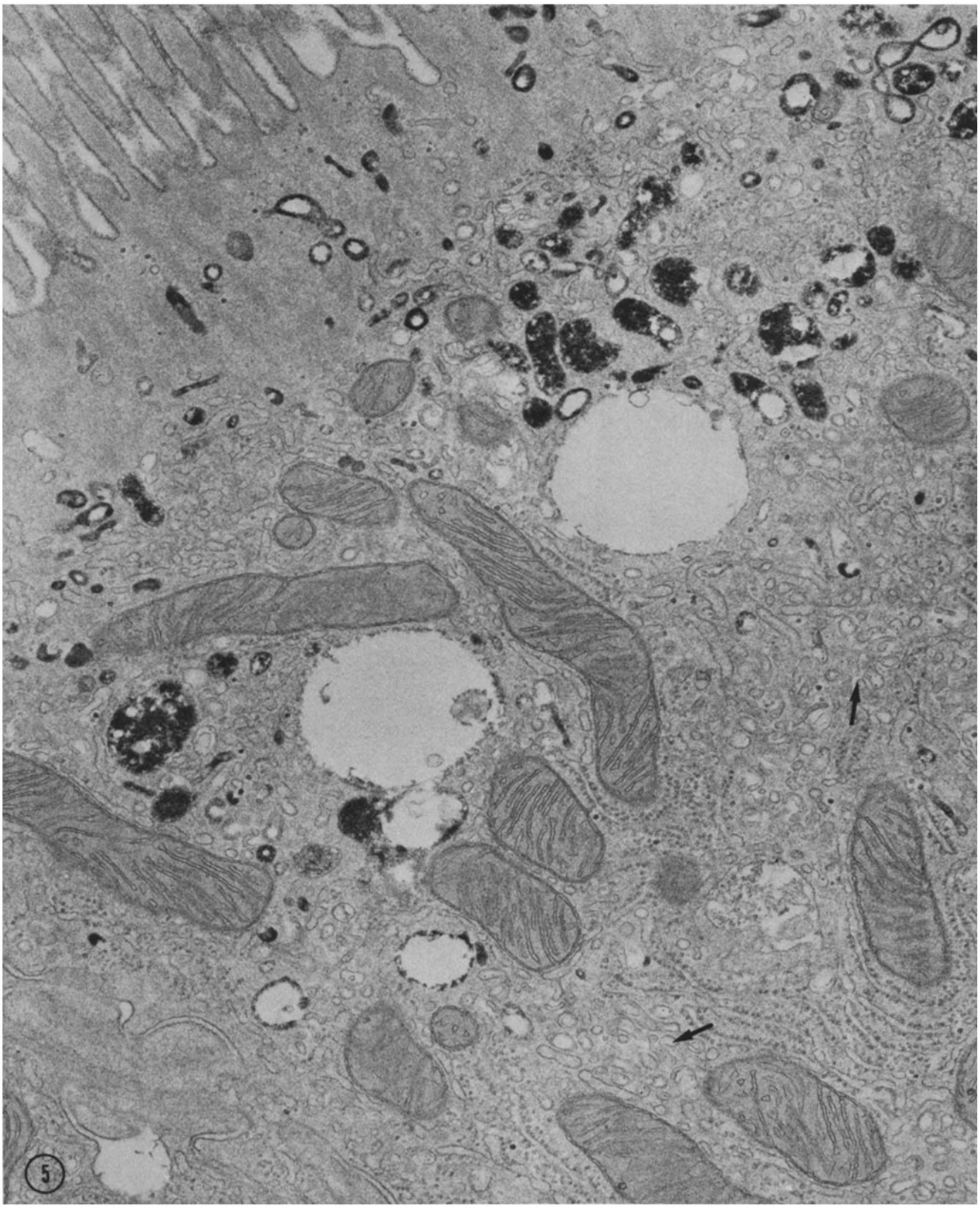

FIGURE 5 Low magnification view of cells incubated with IgG-Ft and HRP for 60 min. Notice that HRP remains confined to dense bodies and vesicles that occupy the apical cytoplasm. In contrast, most small vesicles within the more basal regions of the cell contain IgG-Ft exclusively (arrows). × 35,000.

The mechanisms that control endocytosis are poorly understood (33). Using soluble HRP as a fluid phase marker and mouse macrophages, Steinman and Cohn (36) measured the rate of constitutive endocytosis and subsequent lysosomal inactivation of HRP within these cells. In additional studies with immune complexes prepared with HRP and anti-HRP IgG (37), they discovered a 4,000-fold increase in the rate of endocytic HRP uptake and attributed this increase to the interaction

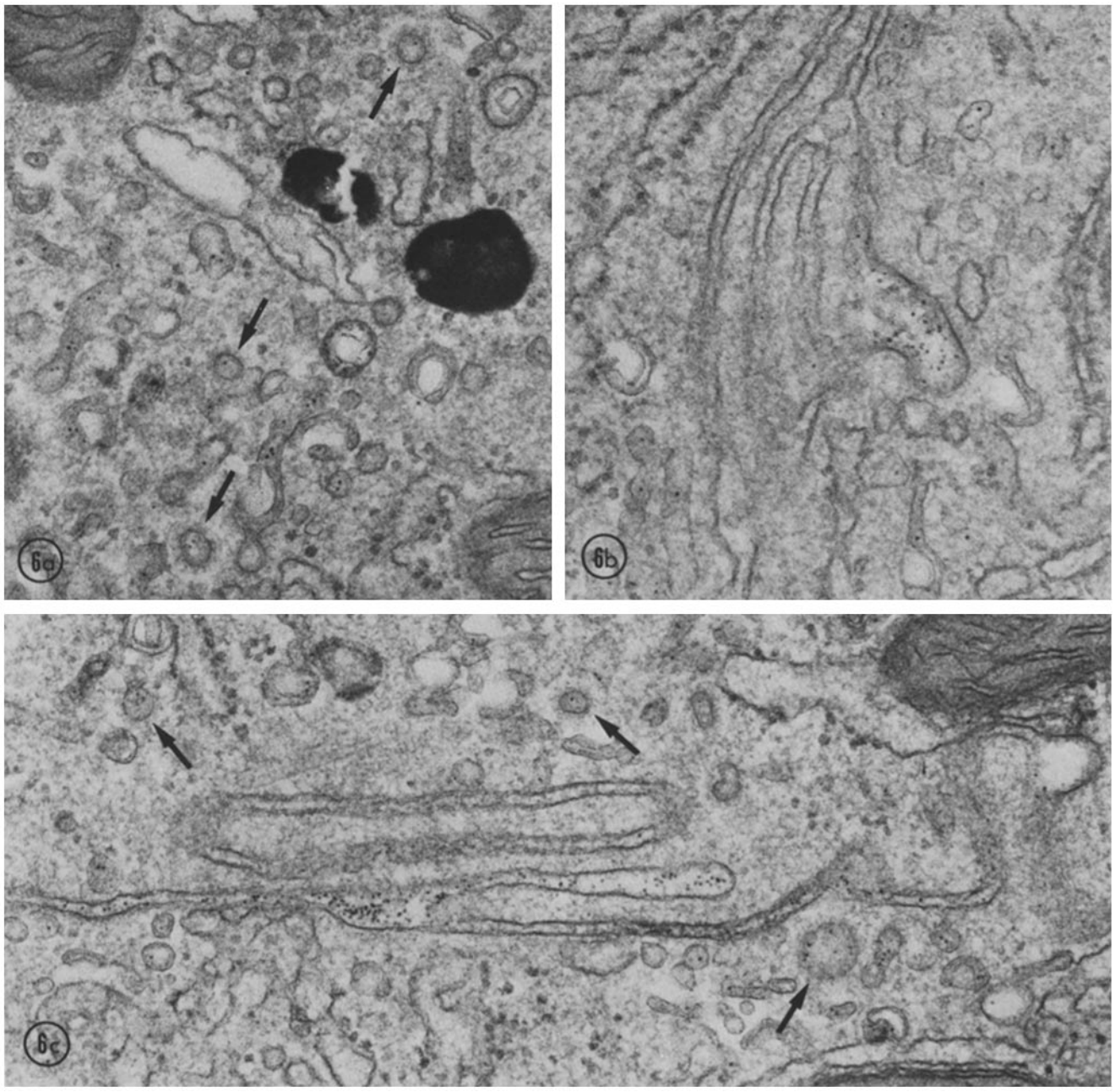

FIGURE 6 Cells incubated with IgG-Ft and HRP for 60 min. (*a*) Micrograph taken from the midregion of the cell. Notice that the two tracers appear in distinctly different vesicle types, unlike what is seen in Figs. 4 and 5. IgG-Ft is visualized in small tubules and coated vesicles (arrows), but HRP is seen only in larger, dense bodies. × 65,500. (*b*) Area near the lateral cell surface. A vesicle that contains IgG-Ft appears fused with the lateral membrane and is discharging its contents into the intercellular space. × 59,000. (*c*) Numerous IgG-Ft particles appear free within the intercellular space and within coated vesicles (arrows) that border the lateral membrane. HRP is not present in these compartments. × 65,000.

of the complexes with the macrophage Fc receptor. A dramatic increase in the rate of fluid uptake is similarly observed in other instances of receptor-mediated endocytosis. For example, when epithelial growth factor is added to human carcinoma A-431 cells, the rate of pinocytosis as measured by HRP uptake increases 10-fold over basal levels during the first 15 min of exposure (13). Endocytosis of HRP by toad bladder epithelium also increases significantly upon binding of antidiuretic hormone (20). In contrast, endocytosis in human fibroblasts appears to be constitutive and occurs continuously in the presence or absence of low-density lipoprotein (2, 7, 12).

Sorting of Vesicle Contents

Another possible mechanism that could explain the selective transfer of IgG in the presence of nonselective endocytosis would be the existence of different, specialized populations of endocytic vesicles, one for selective IgG uptake and transport, and a different class for the nonselective uptake of materials destined for intracellular digestion. To investigate this possibility, we injected IgG-Ft and unconjugated HRP simultaneously into intestinal segments and then compared the intracellular distribution of these two different tracers. Nearly every

endocytic vesicle that contained HRP also contained receptor-bound IgG-Ft. However, only IgG-Ft was transferred across cells within coated vesicles and released into the intercellular spaces. These results indicate that: (*a*) selective and nonselective protein uptake probably occur within the same endocytic vesicle, (*b*) the contents of endocytic vesicles are sorted during intracellular transport, and (*c*) only membrane-bound IgG is transferred across cells.

These results differ from what has been presented by other workers studying different examples of receptor-mediated protein transport. Moxon et al. (23) have proposed that different classes of endocytic vesicles in the fetal rabbit yolk sac account for the selective transfer of rabbit IgG across that tissue. Further, Haigler and co-workers (13), studying A-431 carcinoma cells, localized a ferritin conjugate of epithelial growth factor and unbound HRP within different vesicles when these tracers were administered to cells sequentially.

Although our experiments illustrate the intracellular sorting out of proteins, the precise site where this process takes place has not been firmly established. The apical cytoplasm of the neonatal jejunal cells contains a complex system of endocytic vesicles, as well as small vacuoles, multivesicular bodies, and lysosomes (28). Whether endocytic vesicles fuse with the apical vacuoles, which apparently lack lysosomal enzymes but are similar in morphology to organelles referred to by Willingham and Pastan (40) as receptosomes, is currently under investigation. Our identification of the site where sorting occurs is made more difficult because more than half of the internalized IgG may be digested intracellularly as indicated previously. Consequently, the simultaneous appearance of IgG-Ft and HRP within the lysosomal compartment should not be interpreted as evidence that sorting takes place after fusion of the endocytic vesicles with the lysosomes. Such a mechanism would require that the IgG to be transferred be somehow protected from digestion within the lysosomes, for example, by remaining complexed with receptors (6). The interpretation that we favor, instead, is that virtually all of the fluid within endocytic vesicles together with a portion of the IgG is removed from the IgG transport pathway and ultimately is digested within the lysosomes. The receptor-bound IgG that is destined for transfer, in contrast, is probably never exposed to the lysosomal hydrolases, ensuring that it is transferred intact across the cell.

Proposals have been made earlier (30, 31) that IgG is transferred across the neonatal rat intestine as an IgG-receptor complex. The observation that IgG binds to the brush-border membranes of intestinal epithelial cells at pH 6.0–6.5 but not at pH 7.4 (17, 29–31, 38) has led to the hypothesis that the pH dependency of binding could account for the vectorial transport of IgG across cells (30, 31). According to this model, receptors in the brush border bind IgG at pH 6.0–6.5, which is the ambient pH of the luminal fluid (30). IgG then enters the absorptive cell bound to the endocytic vesicle membrane. Subsequent to endocytosis, coated vesicles containing receptor-bound IgG appear to bud off from the tubular endocytic vesicles and ultimately fuse with the basolateral surface of the cell. The pH of the abluminal compartment, presumably near pH 7.4, would cause dissociation of IgG from the receptor and diffusion into the intercellular fluid compartment. Although the experiments described here do not prove that IgG is transferred complexed with receptor, the results are nevertheless consistent with such a mechanism. This implies that membrane-bound proteins and other membrane components, such as receptors, are transferred within the membrane from the apical to basolateral cell surface. The demonstration of pH-sensitive Fc receptors on the basolateral surface of isolated epithelial cells (31, 39) adds additional support to this concept.

The flow of receptors or other membrane components between different intracellular compartments has been either postulated or observed directly in other experimental systems. For example, to account for the large amount of vesicle membrane brought into macrophages and L cells during endocytosis, Steinman et al. (35) proposed that this membrane eventually reappears on the cell surface. Subsequent experiments by Schneider et al. (32), who labeled specifically the endocytic vesicle membrane of rat fibroblasts with anti–plasma membrane antibody, support the membrane recycling hypothesis. More recently, Louvard (18) has shown that fluorescent antibody to surface aminopeptidase in Madin-Darby canine kidney cells is entirely cleared from the apical cell surface within 30 min but that the enzyme reappears by 40 min. Several studies (9, 14, 15, 25) have traced the fate of surface membrane components labeled with cationic ferritin within a variety of secretory cell types. These studies have shown that tracer, initially membrane-bound, can enter several distinct subcellular compartments after endocytosis including the Golgi cisternae, lysosomes, and secretory vesicles. These experiments as well as ours illustrate that there may be a significant flow and exchange of membrane components between different regions of the plasma membrane and specific intracellular compartments.

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