

DISTRIBUTION OF IMMUNOGLOBULIN G RECEPTORS IN THE SMALL INTESTINE OF THE YOUNG RAT

RICHARD RODEWALD

From the Department of Biology, University of Virginia, Charlottesville, Virginia 22901

ABSTRACT

Conjugates of horseradish peroxidase (HRP) and immunoglobulin G (IgG) were used to map the distribution of cell surface receptors that can bind IgG at 0°C within the small intestine of 10–12-d-old rats. Luminal receptors are present only within the duodenum and proximal jejunum. In these locations, receptors are limited to absorptive cells that line the upper portion of individual villi. Near villus tips, receptors are relatively evenly distributed over the entire luminal plasmalemma. In the midregion of villi, receptors are unevenly distributed over the luminal surface. Receptors (*a*) specifically bind rat and rabbit IgG, (*b*) recognize the Fc portion of the immunoglobulins, and (*c*) bind at pH 6.0 but not pH 7.4. To determine whether IgG receptors are confined to the luminal portion of the plasmalemma, intact epithelial cells were isolated from the proximal intestine of 10–12-d-old rats and incubated with HRP conjugates at 0°C. The specific binding of rat IgG-HRP to cells at pH 6.0 indicates that IgG receptors, which are functionally similar to those found on the luminal surface, are also present over the entire abluminal surface of absorptive cells. These results are consistent with the transport of IgG to the abluminal plasma membrane in the form of IgG-receptor complexes on the surface of vesicles. Exposure of these complexes to the serosal plasma, which is presumably at pH 7.4, would cause release of IgG from the receptors. To assess possible inward movement of vesicles from the abluminal surface after discharge of IgG, intravenously injected HRP was used as a space-filling tracer in the serosal plasma. HRP could be visualized within the coated and tubular vesicles responsible for transport of IgG in the opposite direction. These vesicles may, therefore, provide a pathway whereby receptors shuttle between the luminal and abluminal surfaces of cells.

Several investigations have provided compelling evidence that maternal immunoglobulins that are selectively transported from the intestinal lumen into the circulation of young rats bind to specific receptors on epithelial cells. The receptor hypothesis was originally proposed by Brambell (for a review, see reference 5) and his colleagues to account for the selection, competition, and saturation exhibited during transport of specific immuno-

globulins (11–13). The hypothesis has been corroborated by histological evidence, which has shown that IgG immunoglobulins selectively bind to the luminal surface of absorptive cells in the proximal third of the small intestine as the first step in vesicular transport of the immunoglobulins across the cells (29, 30). Recent experiments have indicated that selective binding is pH dependent (17, 31, 32); it can occur at pH 6.0–6.5, which is

near the pH of the luminal contents, but not at pH 7.4–8.0. This suggests that IgG that binds to the luminal surface remains bound to the receptors during transport across the cells and then is released from the receptors at the abluminal cell surface when exposed to the interstitial plasma, which is presumably near pH 7.4 (32, 34).

This study utilized the selective binding that occurs at 0°C (32), a temperature at which the subsequent steps in transport apparently do not occur. IgG fractions conjugated to horseradish peroxidase were used, therefore, as tracers for both light and electron microscopy to map the tissue and cellular distribution of the receptors on the luminal surface of intestinal cells at this temperature. Other experiments, with isolated epithelial cells, indicate that receptors functionally identical to those on the luminal surface are also present on the abluminal plasmalemma, an observation consistent with the notion that IgG is transported to the abluminal compartment in the form of receptor-IgG complexes.

MATERIALS AND METHODS

Animals

Randomly bred female Sprague-Dawley rats with 9–10-d-old young were purchased from Flow Laboratories, Dublin, Va.

Proteins

Immunoglobulins (Cohn fraction II), crystallized bovine serum albumin (BSA), and anti-IgG antisera were purchased from Miles Laboratories, Inc., Elkhart, Ind. Mercuripapain and horseradish peroxidase (HRP), type VI, were obtained from Sigma Chemical Co., St. Louis, Mo.

IgG, Fc, and Fab Preparations

IgG was purified from fraction II powders by diethylaminoethyl cellulose (DEAE-cellulose) chromatography according to methods reported previously (30). Purified rat IgG was digested with papain by the technique used by Guyer et al. (10) to digest mouse IgG and was chromatographed on a DEAE-cellulose column (19). Purity of fragments was analyzed by immunoelectrophoresis. Each fragment yielded a single precipitin line when developed against anti-rat IgG antiserum.

Peroxidase Conjugations

Purified IgG, Fc, and Fab preparations and crystallized BSA were covalently conjugated to periodate-activated HRP, type VI, according to the method of Nakane and Kawaoi (25). Conjugations in each case were carried out at a 1:1 molar ratio of HRP to the second protein. Conjugates were tested for the presence of unconjugated HRP by chromatography on a Bio-Gel P-100 column (Bio-Rad Laboratories, Richmond, Calif.). The percent of HRP, monitored spectrophotometrically at 405 nm, which eluted with conjugates in the void volume indicated that >90% of the HRP was coupled in all preparations. Conjugates were

dialyzed against 0.1 M phosphate buffer, pH 6.0 or pH 7.4, and used at a concentration of 1.0–1.5 mg HRP per ml for all experiments.

Binding to Intestinal Segments

10–12-d-old rats were lightly anesthetized with ether, and, in each animal, an abdominal incision was made to expose the small intestine. A short cannula made from PE-50 polyethylene tubing (Clay-Adams Div., Becton, Dickinson & Co., Parsippany, N. J.) was inserted into the lumen through a small incision in the wall, 2–3 cm distal to the pylorus, and tied in place. A second incision was made 4–5 cm distal to this cannula, and the segment between the incisions was flushed with 3–5 ml of normal saline. A second cannula was then inserted through the distal incision and tied in place. In some experiments, a similar method was used to isolate a 4–5-cm segment of the distal ileum, ending at a point 2–3 cm from the caecum. In each case, the segment, cannulated at both ends, was quickly excised and immersed in saline at 0°C. The segment was cut in half, and each half was washed with 2–3 ml of cold 0.1 M phosphate buffers of appropriate pH. For direct binding studies, an HRP conjugate in buffer of the chosen pH was injected into the cannulated end of a segment until the light brown conjugate was observed flowing from the free end. The free end was then clamped, and additional conjugate was injected to balloon the segment slightly. After a 10-min incubation at 0°C, the segment was unclamped and rinsed with 2–3 ml of cold buffer at pH 6.0 or pH 7.4. Fixation was commenced by the injection of cold 2% glutaraldehyde in buffer at the same pH as the preceding rinse. The segment was then transferred to a pool of cold fixative, and a region of tissue in the middle of the segment was cut into small pieces for further processing. Tissue incubated at pH 6.0 was fixed for 30 min at this pH and then for an additional 90 min in glutaraldehyde buffered at pH 7.4. Tissue incubated at pH 7.4 was fixed for 2 h at this pH only.

In one experiment, the entire small intestine from 12-d-old rats was exposed to rat IgG-HRP at 0°C, pH 6.0 or pH 7.4. The procedure described above for short segments was followed except that the full length of the small intestine, from pylorus to ileal-caecal junction, was removed from the animals and treated as a single, intact segment.

Proximal segments were also used for competitive binding experiments. For these studies, segments were removed from animals and rinsed with pH 7.4 and then pH 6.0 buffers. Segments were then injected with unconjugated rat IgG (10 mg/ml) or with its Fc or Fab fragments (6–10 mg/ml) in buffer at pH 6.0. After incubation for 10 min at 0°C, the segments were rinsed with 3 ml of cold buffer at pH 6.0 or pH 7.4. Following this, segments were rinsed with an additional 1 ml of buffer at pH 6.0 and then incubated with rat IgG-HRP for 10 min in buffer at pH 6.0. The remaining steps were identical to those used in direct-binding experiments.

Binding to Isolated Cells

Intact epithelial cells were isolated from the proximal third of the small intestine by the method of Evans et al. (8), which utilizes 10 mM EDTA and gentle mechanical abrasion to release the epithelial cells. Released cell aggregates were drained into a cold, conical centrifuge tube but were not further disrupted. Cell suspensions were washed three times by centrifugation at 450 g and 4°C for 1 min and resuspended in 5-ml volumes of cold, phosphate-buffered saline at pH 6.0 or pH 7.4. After the third wash, samples of cells were resuspended in a 0.5-ml volume of

an appropriate HRP conjugate in buffer that contained 40 mg/ml BSA and that was at the same pH as the preceding washes. Cells were incubated in conjugates for 10 min at 0°C and then washed by centrifugation and resuspension, once in the same buffer with BSA and once in buffer alone. Finally, the cells were resuspended in 2% glutaraldehyde in 0.1 M phosphate buffer at the same pH used in previous steps, fixed, and further treated in suspension in a manner similar to that used for tissue blocks.

HRP Histochemistry

Fixed tissue blocks or cell suspensions were thoroughly rinsed in cold 0.1 M phosphate buffer, pH 7.4, that contained 0.1 M sucrose. To demonstrate the presence of HRP, samples were incubated in 0.05% diaminobenzidine-HCl (DAB) (Aldrich Chemical Co., Milwaukee, Wis.) and 0.01% H₂O₂ in 0.1 M phosphate buffer, pH 6.0, for 45 min at room temperature. Incubation was performed at pH 6.0 because HRP is several times more active at this pH than at pH 7.6, which is the pH originally recommended by Graham and Karnovsky (9, 35). After a brief rinse in cold buffer, samples were postfixated for 90 min in 2% OsO₄ in 0.1 M phosphate buffer, pH 7.4. Samples were then dehydrated in ethanol and embedded in Epon (22).

The whole intestine preparation, after fixation, was rinsed and reacted with DAB and H₂O₂ by infusion of the incubation medium through the lumen over a 45-min period. After the reaction, the lumen was thoroughly rinsed with cold buffer but was not further treated.

Abluminal Uptake of HRP

Lightly anesthetized 10–12-d-old rats were injected intravenously with 5 mg of HRP, type VI, per 25 g of body weight. HRP was administered at a concentration of 25 mg/ml in normal saline by way of the left femoral vein. After 5, 10, or 20 min, a short segment of the proximal small intestine, 3–4 cm distal to the pylorus, was removed and placed in 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, at room temperature. Small pieces

of tissue were cut from the segment and then processed by the same methods used to visualize HRP in conjugate experiments.

Microscopy

1–2- μ m thick sections were cut from polymerized Epon blocks with glass knives and a Porter-Blum MT2 microtome (DuPont Instruments-Sorvall, Newtown, Conn.). Unstained thick sections were examined with a Zeiss WL light microscope and photographed with a Nikon Microflex AFM camera attachment (Nippon Kogaku, Garden City, N. Y.). Silver to pale gold thin sections were cut with diamond knives and stained for 1 min in lead citrate (28). Thin sections were examined at 60 kV with a Philips 200 electron microscope.

RESULTS

Organ Distribution of IgG-HRP Binding

Rat IgG-HRP, when injected into the entire small intestine of a 12-d-old rat and allowed to bind at 0°C, pH 6.0, exhibited a gradient of binding along the length of the intestine (Fig. 1). As judged by the intensity of DAB staining, IgG-HRP bound strongly to the mucosal surface of the proximal half of the intestine, including the entire duodenum and the proximal portion of the jejunum. Staining gradually decreased in intensity over an ~4–6-cm region in the midportion of the small intestine. The distal region of the intestine, including the entire ileum, showed little or no binding. When the experiment was repeated with rat IgG at pH 7.4, no binding was observed on any region of the intestine.

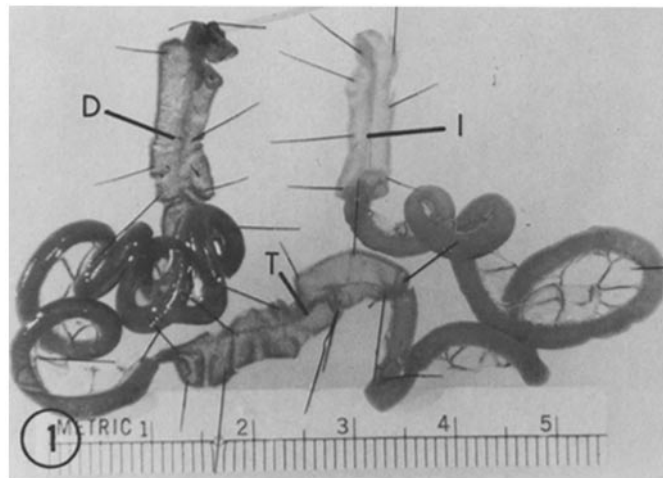


FIGURE 1 Whole mount of small intestine from a 10-d-old rat. The wall of the intestine has been cut to expose the mucosal surface in the duodenum (*D*), distal ileum (*I*), and transition region (*T*). Dense reaction product from bound rat IgG-HRP extends from the duodenum to the transition region, where a gradual decrease in staining is evident. $\times 1.3$.

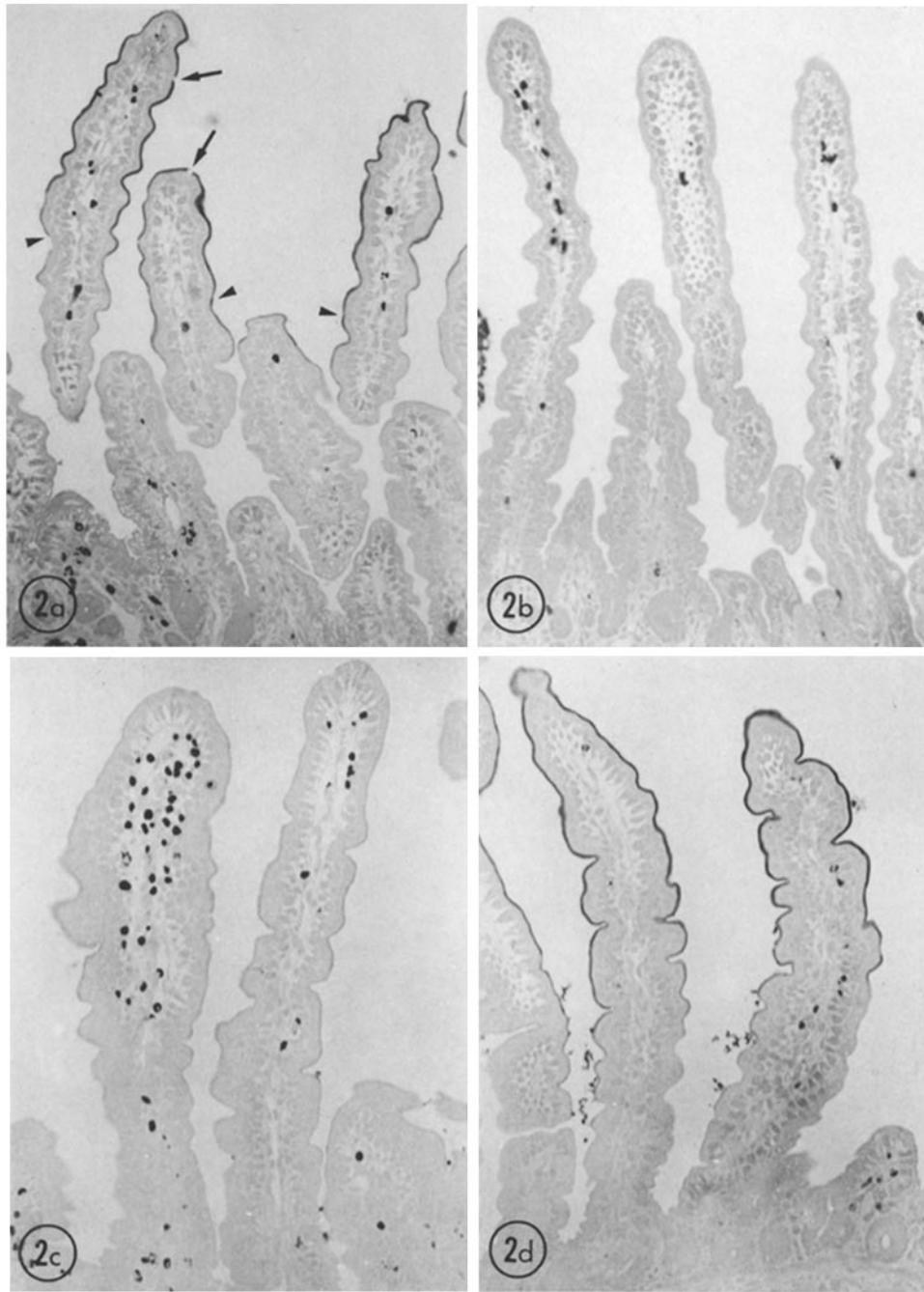


FIGURE 2 Thick sections of villi from the proximal jejunum of 10-d-old rats incubated with rat IgG-HRP under various conditions. Stained only for HRP. (a) Tissue incubated with rat IgG-HRP at pH 6.0. Conjugate staining is apparent as a dense band on the luminal surface of villus tips. Interruptions in staining occur over globet cells (arrows). Overall staining decreases in the midregion of villi, where discontinuities in the intensity of staining are often evident (arrowheads). (b) Tissue incubated with rat IgG-HRP at pH 7.4. No staining is evident on the villus surfaces. (c) Tissue pretreated with unconjugated rat IgG, rinsed with pH 6.0 buffer, and incubated with rat IgG-HRP at pH 6.0. Only a faint band of staining appears on the villus surfaces. (d) Tissue pretreated with conjugated rat IgG but rinsed with pH 7.4 buffer before incubation with rat IgG-HRP. Intense staining on the villus surfaces appears similar to that on tissue not pretreated with unconjugated IgG. $\times 190$.

Tissue Distribution of IgG-HRP Binding

The intensity of rat IgG-HRP staining in the proximal intestine at pH 6.0 varied over the length of the villi. As light microscopy revealed, binding was strongest on the outer third of individual villi and decreased gradually over the middle third (Fig. 2*a*). No binding was evident on the mucosal surface in the lowermost regions of villi or within crypts. Staining was limited to the striated border of absorptive cells and appeared as a dark-brown band, 1–2 μm thick. No binding occurred on the surface of goblet cells, and, where cells of this type were found, interruptions in the band of staining on the villus surface were evident. The decrease in staining on absorptive cells in the midregion of the villi was usually gradual and continuous. However, in some cases, isolated regions stained more intensely than neighboring regions (Fig. 2*a*). Over the outer third of the villus, staining on the surface of absorptive cells appeared uniform. When extruded cells were seen near villus tips, the entire surface of these cells was stained. Staining within the interior of the epithelial cells was not seen.

Specificity of Binding

Isolated segments were utilized to determine the specificity of binding to the mucosal surface of the proximal intestine of 10–12-d-old rats. The results of these experiments are summarized in Table I. Binding was specific for HRP conjugates of rat and rabbit IgG and occurred at pH 6.0 but not at pH 7.4 (Fig. 2*b*). The HRP conjugate made from the Fc portion of rat IgG bound but the Fab conjugate did not. To rule out the possibility that conjugation to HRP might influence the binding of IgG, competitive binding experiments with unconjugated proteins were performed. When proximal segments were allowed to bind unconjugated rat IgG at pH 6.0 and were then rinsed at the same pH and incubated with rat IgG-HRP, very little conjugate staining could be detected on the epithelium (Fig. 2*c*). However, when a segment was rinsed at pH 7.4 before IgG-HRP application at pH 6.0, mucosal staining appeared identical to that seen without pretreatment with unconjugated rat IgG (Fig. 2*d*). IgG-HRP binding could also be blocked by pretreatment with unconjugated rat Fc but not by pretreatment with Fab.

Additional direct binding experiments were undertaken to assess possible binding of rat IgG-HRP to the epithelium in (*a*) ileal segments from 10–12-d-old rats and (*b*) proximal segments from

rats at 22 d, an age by which selective transport of IgG has ceased (11). In neither case, could conjugate staining be detected anywhere on the villus surface at either pH 6.0 or pH 7.4.

IgG-HRP Binding to Cells in Intact Tissue

The pattern of binding of HRP conjugates to villi as observed by light microscopy was also apparent by electron microscopy. However, at the higher resolution, additional features of binding to individual absorptive cells were revealed. In the case of all conjugates that bound, staining was limited to the outer aspect of the luminal plasmalemma and was seen both on microvilli and on the surface of the frequent membrane invaginations at the bases of microvilli (Figs. 3 and 4*a*). The amount of reaction product was frequently greater within these invaginations than on the adjacent microvillar membranes (Fig. 4*b*). No staining was observed within the tight junctions or on other regions of the lateral plasmalemma. In mid-villus regions, where variations in intensity of staining were apparent by light microscopy, the amount of reaction product on the luminal surface changed abruptly at tight junctions (Fig. 4*c*). However, the amount of staining on individual absorptive cells remained relatively constant over their surface. Electron microscopy revealed no reaction product on the plasmalemma of goblet cells, and, again, an abrupt discontinuity in staining could be clearly noted at the junctions with adjacent absorptive cells (Fig. 4*d*). As was observed by light microscopy, there was no evidence for any uptake of conjugates into epithelial cells at 0°C.

IgG-HRP Binding to Isolated Cells

Because it was likely that junctional complexes prevented conjugates from reaching the abluminal

TABLE I
Conjugate Binding to Proximal Segments at 0°C

Conjugate	Incubation pH	Staining on villus surface
Rat IgG-HRP	6.0	+
Rat IgG-HRP	7.4	–
Rabbit IgG-HRP	6.0	+
Rabbit IgG-HRP	7.4	–
Chicken IgG-HRP	6.0	–
Sheep IgG-HRP	6.0	–
BSA-HRP	6.0	–
Rat Fc-HRP	6.0	+
Rat Fab-HRP	6.0	–

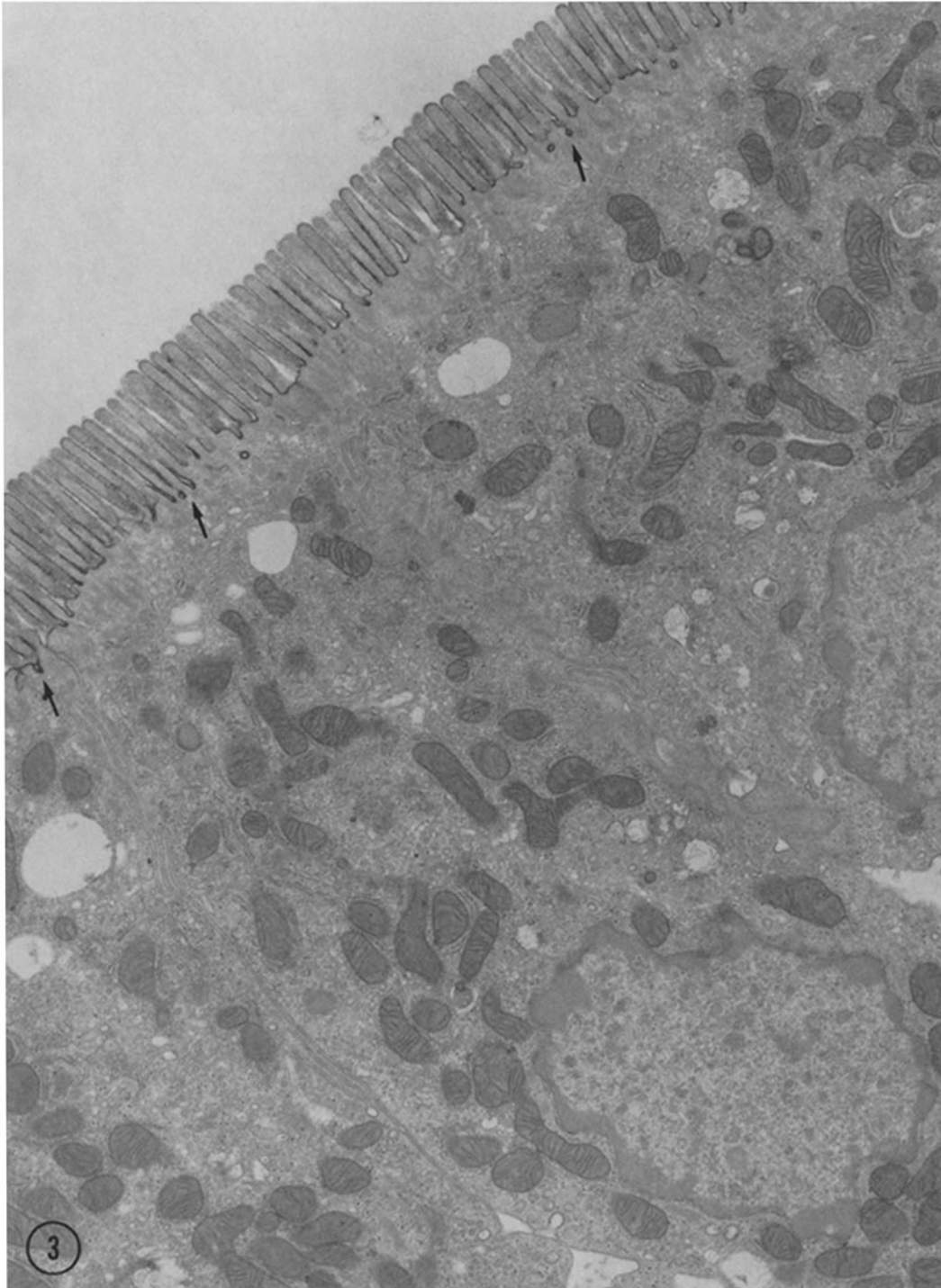


FIGURE 3 Electron micrograph of absorptive cells from the proximal jejunum of a 10-d-old rat that were incubated with rat IgG-HRP at pH 6.0. Reaction product is limited to the microvillus membranes and invaginations at the bases of microvilli (arrows). Stained for 1 min with lead citrate. $\times 1,200$.

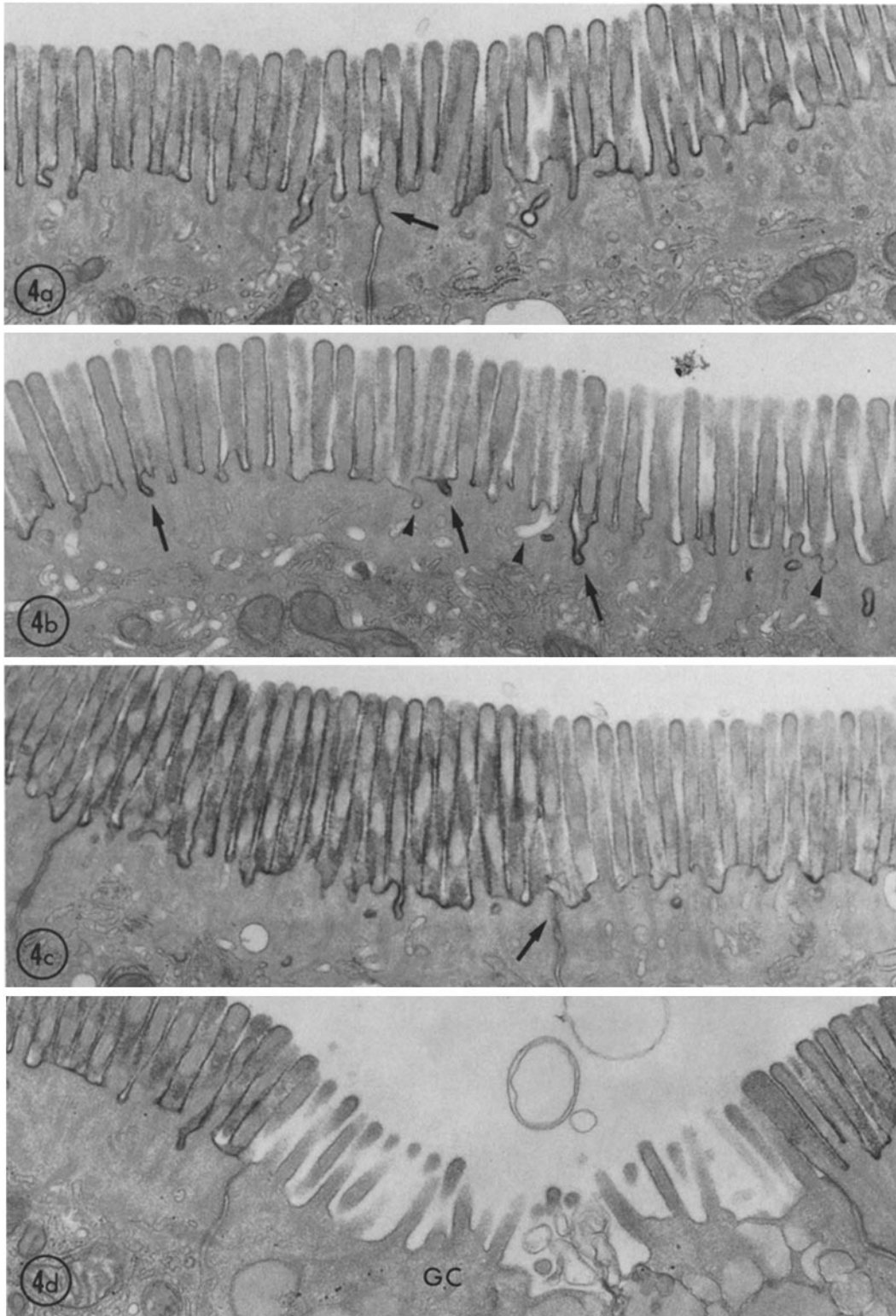


FIGURE 4 Luminal surfaces of epithelial cells in the proximal jejunum exposed to rat IgG-HRP, pH 6.0. (a) Epithelial cells near a villus tip. Reaction product is relatively evenly distributed over all of the luminal plasmalemma. No reaction product appears within or below the tight junction (arrow). (b) Epithelial cell in midregion of villus. Most reaction product is confined to invaginations near villus bases (arrows), although several of the invaginations show little staining (arrowheads). (c) Epithelial cells in midregion of villus. The overall intensity of surface staining varies on two adjacent cells, with an abrupt transition at the tight junction (arrow). (d) Epithelial cells near villus tip. The luminal surface of the goblet cell (GC) exhibits no reaction product. $\times 19,000$.

surface of cells in intact tissue, isolated epithelial cells were also prepared that could be suspended in conjugates under various pH conditions. Epithelial cells were isolated predominantly in the form of large sheets and clusters. Most epithelial cells retained the same basic morphological features seen in intact tissue. However, the epithelial basement membrane and most cells of the lamina propria were absent. A small but variable number of damaged cells, usually less than 10%, were present in each preparation. Typically, these cells appeared swollen and exhibited discontinuities in their plasma membranes. Damaged cells bound conjugates nonspecifically throughout their cell interior under all incubation conditions.

When isolated cells were incubated in the cold with rat IgG-HRP at pH 6.0 in the presence of BSA, binding could be detected by light microscopy on both the luminal and abluminal surfaces of large numbers of intact absorptive cells (Fig. 5a). The intensity of staining varied to some degree among absorptive cells, with some cell clusters showing little or no staining. On occasion, cell sheets were seen that exhibited a gradient of staining along their length, particularly on their luminal

surface. Cells that were positive, however, usually showed staining on their luminal and abluminal surfaces. In contrast to absorptive cells, goblet cells exhibited no staining on either region of the plasma membrane.

When isolated cells were incubated with rat IgG-HRP at pH 7.4 with BSA, virtually all staining on both the luminal and abluminal surfaces of absorptive cells was absent (Fig. 5b). Moreover, no staining was observed when cells were incubated with chicken IgG-HRP, sheep IgG-HRP, or BSA-HRP at pH 6.0 or pH 7.4. No experiments were performed with rabbit IgG-HRP or conjugates with IgG fragments. The specificity of binding for rat IgG-HRP at pH 6.0 on the abluminal plasmalemma was apparent only when cells were incubated with conjugate in the presence of unconjugated BSA. When BSA was removed from the incubation medium, rat IgG-HRP was found to bind to the abluminal surface of cells at both pH 6.0 and pH 7.4, although luminal binding was apparent only at pH 6.0.

Electron microscopy revealed that the surface binding of rat IgG-HRP at pH 6.0 in the presence of albumin occurred over almost the entire surface

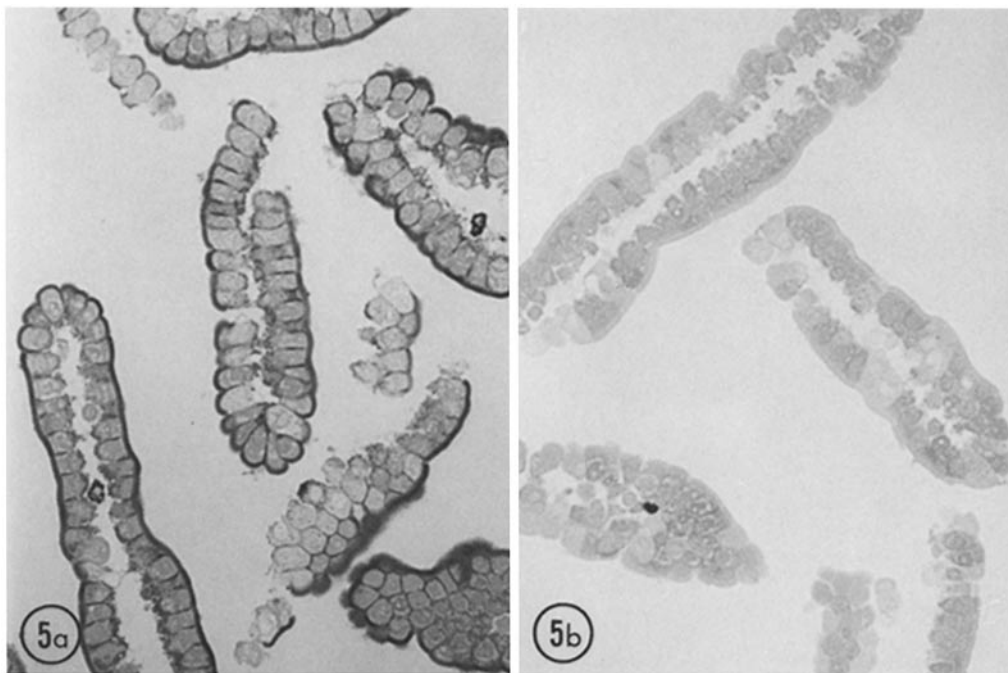


FIGURE 5 Thick sections of isolated epithelial cells incubated in HRP conjugates under different conditions. Stained only for HRP. (a) Cells incubated in rat IgG-HRP at pH 6.0. Reaction product is readily apparent over entire outer surface of cells. (b) Cells incubated in rat IgG-HRP at pH 7.4. Staining is absent from both luminal and abluminal surfaces of cells. $\times 350$.

of positively stained cells (Fig. 6). Only within the region of intact tight junctions of cell clusters did the plasmalemma fail to stain. Goblet cells ex-

hibited virtually no staining on their luminal and abluminal surfaces, although the membranes of adjacent absorptive cells usually showed strong

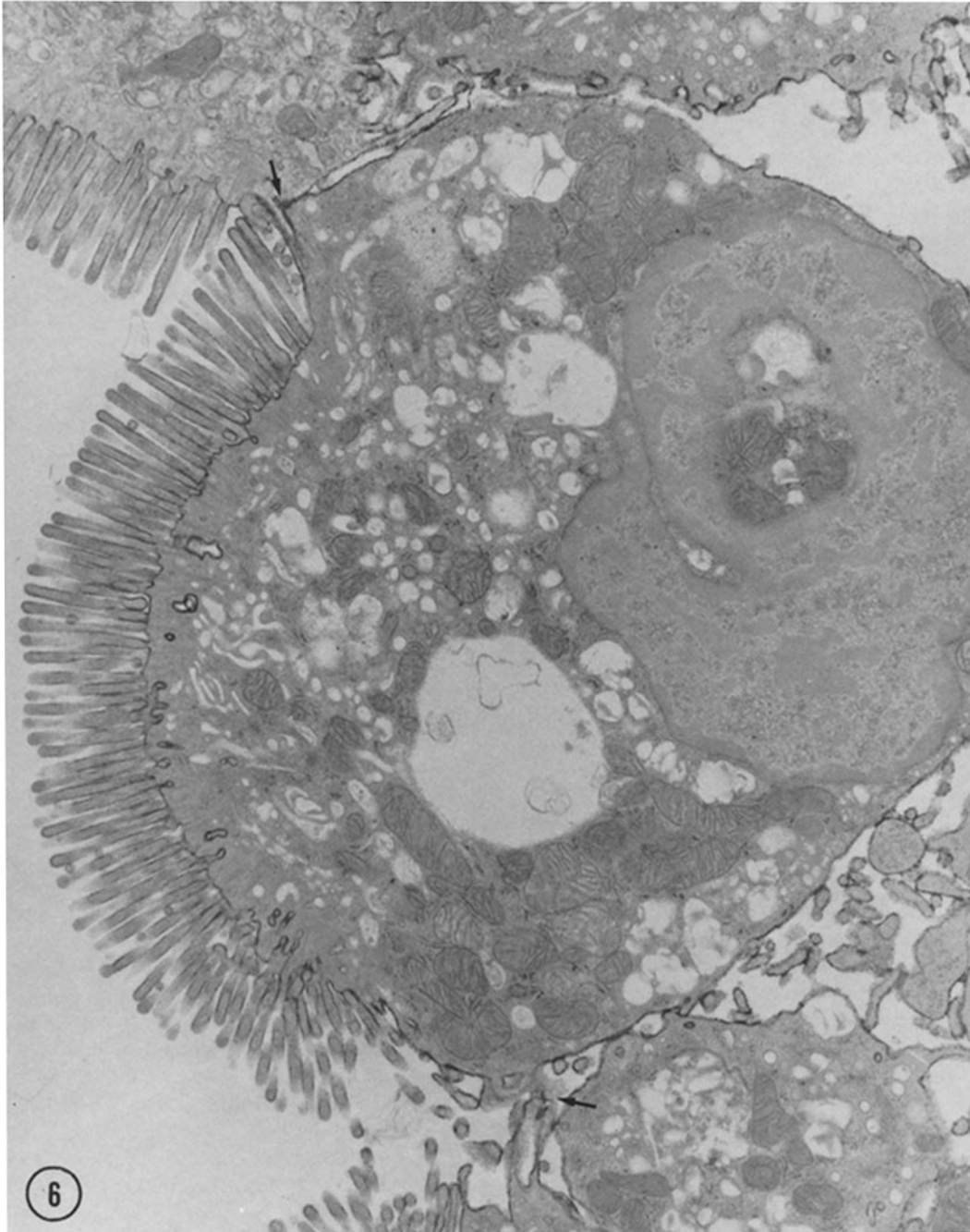


FIGURE 6 Electron micrograph of an isolated absorptive cell incubated in rat IgG-HRP at pH 6.0. Reaction product covers the entire luminal and abluminal surfaces. The regions of the junctional complexes with adjacent cells are denoted by arrows. $\times 1500$.

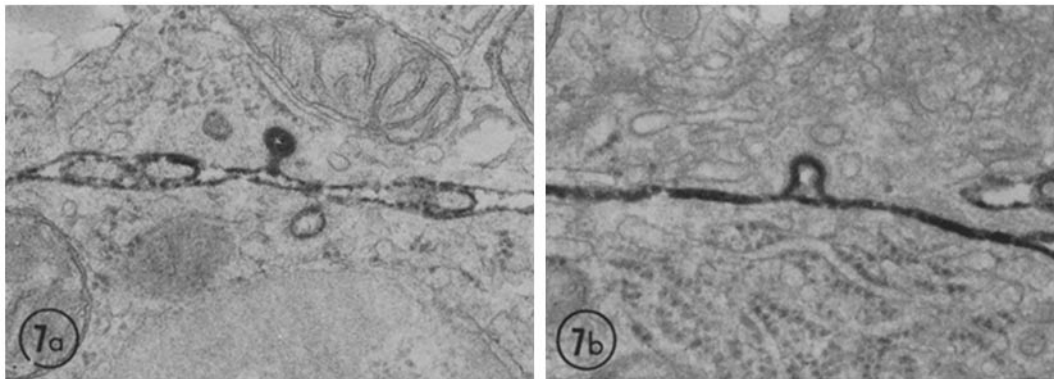


FIGURE 7 (a and b) Details of the lateral cell membranes of absorptive cells incubated in rat IgG-HRP at pH 6.0. Reaction product is evident both within coated pits and on adjacent membrane surfaces. $\times 47,000$.

staining.

The pattern of conjugate binding to the luminal plasmalemma of absorptive cells was similar to that observed in binding experiments with intestinal segments. Both microvillar membranes and surface pits exhibited staining (Fig. 6). Binding of IgG-HRP to the abluminal plasmalemma also extended over all membrane regions. Frequently seen on this surface were small invaginations, 0.1–0.2 μm in diameter, which, having a fibrillar coat on the cytoplasmic aspect of their membrane, resembled the coated pits that have been implicated in the discharge of immunoglobulins from the transporting cell (30, 31). Invariably, these invaginations exhibited strong staining on their exterior surfaces (Fig. 7a and b).

Abluminal Uptake of Unconjugated HRP

The possible inward movement from the abluminal surface of vesicles that might contain IgG receptors was evaluated indirectly by use of unconjugated HRP administered intravenously to intact 10–12-d-old rats at a sufficiently high dose to label completely the interstitial spaces surrounding the absorptive cells. After 5, 10, and 20 min, tissue from the proximal jejunum was fixed and examined for bulk endocytosis of the HRP into the interior of vesicles formed at the abluminal surface.

HRP reaction product filled the extracellular spaces between epithelial cells after even the shortest time period and penetrated up to, but not past, the tight junction (Fig. 8). Reaction product filled the coated pits found on the abluminal surfaces of absorptive cells. In addition, many vesicles within the cell interior showed staining (Figs. 8 and 9),

the numbers increasing with incubation time. These vesicles were particularly plentiful near the lateral cell surfaces at the level of the supranuclear Golgi complex (Fig. 9) and included both coated vesicles and small tubular vesicles indistinguishable morphologically from the coated and tubular vesicles that have previously been shown to be involved in IgG transport from lumen to abluminal surface (30). However, few of the tubular vesicles in the terminal web region were found to contain HRP, even after 20 min. On the other hand, vacuoles present in the apical cytoplasm often contained reaction product (Fig. 8), the amount appearing to increase with time. Rarely was any product visible in the lumen or on the luminal surface of cells. No staining appeared within elements of the rough or smooth reticulum or within cisternae of the Golgi complex.

DISCUSSION

Distribution of IgG Receptors

IgG-HRP conjugates were used in these studies to determine the qualitative distribution of membrane receptors for IgG on the surfaces of the intestinal epithelial cells. Two criteria were used to identify the receptor sites with the conjugates: (a) binding had to be specific for conjugates of immunoglobulins known to be transported by the neonatal rat intestine (11) and (b) binding had to be pH dependent, occurring at pH 6.0 but not at pH 7.4, as has been previously reported (17, 32). Conjugate binding to the luminal surface of proximal segments met these criteria, as has been briefly reported previously (32), and, in addition, was specific for the Fc portion of the IgG molecule.

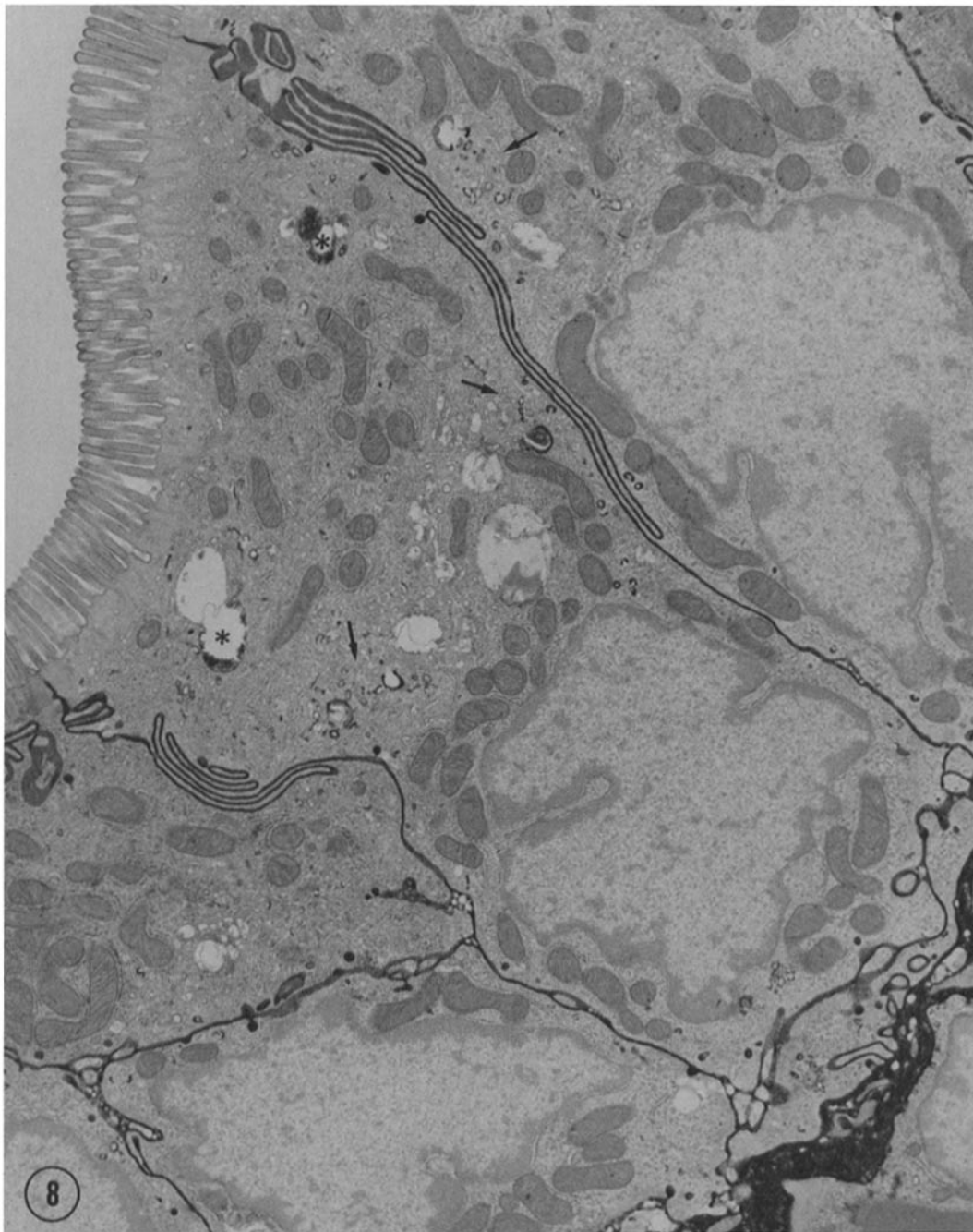


FIGURE 8 Absorptive cells from the proximal jejunum of an animal intravenously injected with unconjugated HRP. 10-min circulation time. Reaction product completely fills the extracellular spaces that constitute the abluminal compartment. Reaction product is also evident within several cytoplasmic vesicles, which are particularly numerous in the Golgi region (arrows). Staining is also seen within two apical vacuoles (*), although most apical vesicles and the surface of the luminal plasmalemma lack reaction product. $\times 8,500$.

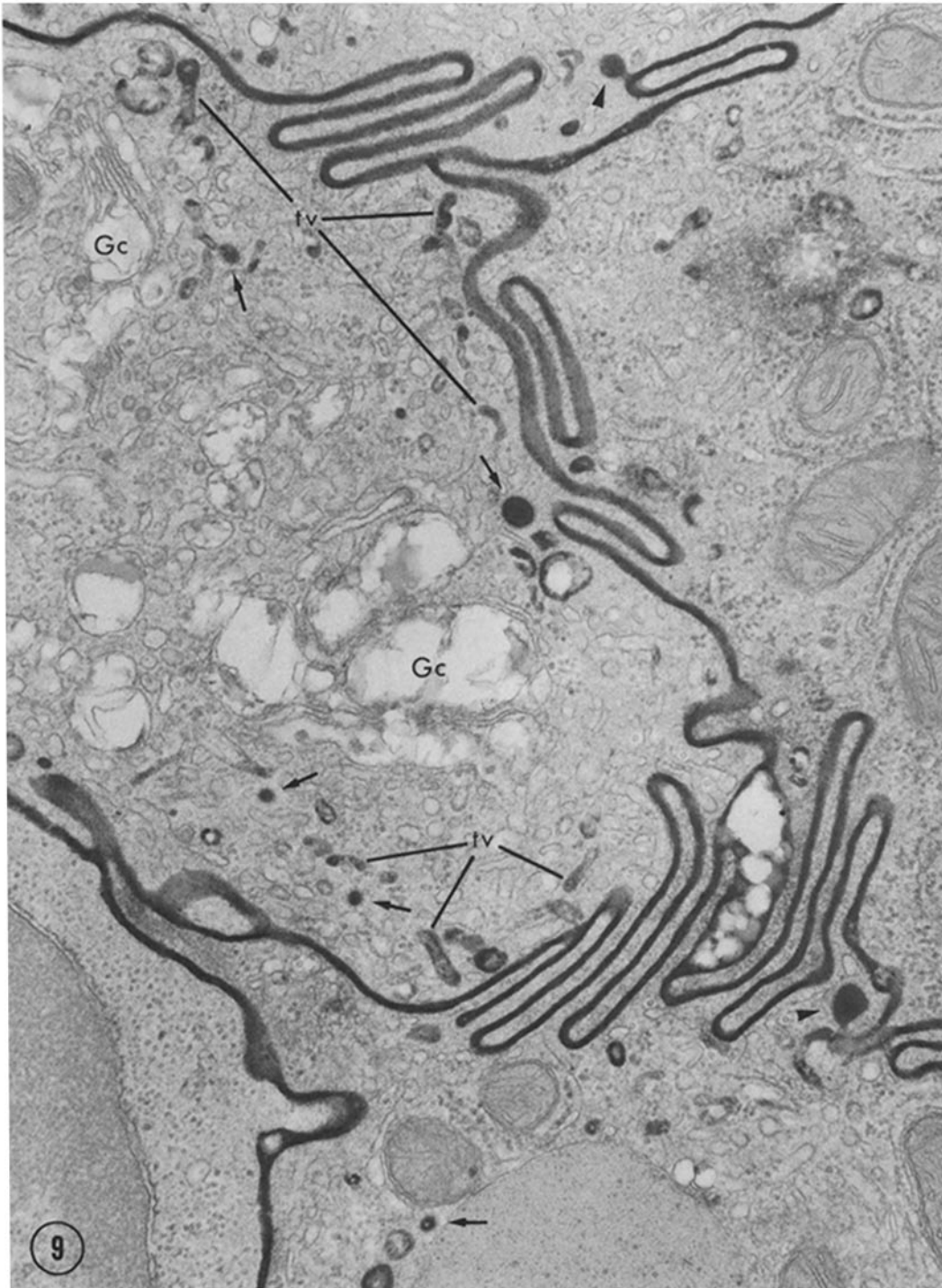


FIGURE 9 Detail of the Golgi region of absorptive cells from an animal intravenously injected with HRP. 10-min circulation time. Reaction product is evident within coated pits attached to the lateral cell membrane (arrowheads) and within coated vesicles apparently free in the cytoplasm (arrows). Several tubular vesicles (*tv*) also contain reaction product. However, the cisternae of the Golgi complex (*Gc*) show no staining. $\times 35,000$.

The latter observation is consistent with evidence that the Fc fragment, but not the Fab portion, can be transported across the intact small intestine (17). Furthermore, inhibition of conjugate binding with unlabeled IgG provides compelling evidence that the observed specificity and pH dependence of conjugate binding reflects the normal interaction of native IgG with receptors and is not the result of the alteration of IgG by conjugation to HRP.

Earlier studies with ferritin and HRP conjugates of IgG (29-31) have provided evidence that binding of IgG to the luminal surface of proximal absorptive cells is the primary selective event in transport across these cells. Tracers that are unable to bind to the luminal membrane enter these cells in very limited quantities and are not transmitted to the abluminal surface, implying that the distribution of receptors on luminal surfaces, as visualized by the use of conjugate binding at 0°C, should provide an estimate of the relative transport capabilities of various regions of the intestine and of various cells in a given region. The results of binding experiments lead to several important conclusions based on this interpretation.

Absorptive cells in the distal intestine do not bear IgG receptors on their luminal membrane and likely do not transport IgG to the circulation. This is apparent from the absence of conjugate binding in experiments both with ileal segments and with the whole small intestine. Results obtained from the whole intestine suggest that transport capability is high in both the duodenum and proximal jejunum and diminishes rapidly over a short region approximately midway down the small intestine; the entire ileum apparently lacks transport capacity. This conclusion agrees with previous unsuccessful attempts to detect transport of specific IgG antibodies and IgG conjugates in ligated segments from the distal third of the small intestine (23, 29, 30, 34). The method used in the present studies to visualize surface receptors, however, would not detect cells that selectively bind IgG exclusively on their internal membranes after, for instance, nonselective uptake of proteins from the lumen. Such a mechanism has been postulated as part of a scheme for selective IgG transport within distal cells (5, 15), although the lack of detectable transport in distal segments does not support this scheme.

Observations of IgG-HRP binding to the luminal surface in proximal segments indicate that

absorptive cells near the villus tip have a greater transport capability than cells near the villus base. In the adult intestine, absorptive cells arise by division of relatively undifferentiated cells in the crypt; the cells migrate toward the villus tip, where, after ~32-38 h, they are extruded into the lumen (21). Presumably, a similar turnover of cells occurs in the neonate. As might be expected, binding of IgG-HRP indicates that epithelial cells, when they originate in the crypt, lack receptors and hence transport capacity. Several observations of the cells in the midregion, where receptors are first evident, are particularly noteworthy. Firstly, absorptive cells were found that lacked surface receptors but appeared otherwise to have a fully differentiated microvillar surface, with frequent membrane invaginations at the bases of microvilli and numerous tubular vesicles in the nearby apical cytoplasm. Other nearby cells with limited overall staining were found, but they had occasional surface invaginations with high concentrations of bound conjugate. One possible interpretation of these observations is that differentiating absorptive cells first synthesize the basic membrane framework of the transport vesicles and only later synthesize or insert the specific receptors into these membranes. Newly synthesized receptors may move to the luminal surface within vesicles and then diffuse over the microvillar surface. Localized concentration of conjugate within some surface pits might also reflect movement of receptors to these regions as a means of concentrating IgG in the interior of a forming vesicle during initiation of transport. Secondly, in the midregion of villi, differences in overall intensity of surface staining of adjacent cells, are frequently observed suggesting that there is some variability among individual cells as to when receptors are first synthesized or as to how quickly they are displayed on the luminal surface.

It is extremely important that all of these characteristics of receptor binding in the proximal intestine of 10-12-d-old rats are lost in 22-d-old rats. A similar loss of binding has recently been reported by Borthistle et al. (4), who used radioiodinated IgG. Thus, the documented loss of transport capacity at 18-21 d of age (24, 30) almost certainly must result from the loss of functional receptors from the cell surface. However, the results do not differentiate between the possibilities that the epithelium is repopulated at 18-21 d, with absorptive cells no longer able to synthesize recep-

tors, or that receptors are, in fact, displayed by the cells but are degraded by luminal proteases that are activated at this age (2, 3, 16, 24).

Abluminal Receptors—Evidence for Receptor Transport

A model for IgG transport across absorptive cells in the proximal intestine has previously been postulated on the basis of the observed transport of several IgG tracers (30, 31). After the initial step, in which IgG binds to receptors on the luminal plasmalemma, the immunoglobulin is carried into the cell by endocytosis within tubular vesicles, which form at the bases of microvilli. Within the apical cytoplasm, the IgG is then transferred from the tubular vesicles to smaller, coated vesicles, which, in turn, migrate to the abluminal plasmalemma to discharge the IgG. The differences in binding of IgG at pH 6.0 and pH 7.4 (17, 32, 34) have led to the hypothesis that the IgG molecules, after binding to the receptors at the lower pH within the lumen, move across the cell as IgG-receptor complexes on vesicle membranes. Fusion of the coated vesicles with the abluminal surface and exposure of the complexes to the interstitial plasma, which is presumably near pH 7.4, would finally cause release of the IgG to complete the transport process.

Implicit in this model is that receptors are able to move from one structurally distinct membrane to another. These membranes would include the luminal plasmalemma, the membranes of the two vesicle types, and the abluminal plasmalemma. Receptor movement is plausible if these membranes behave as fluid-mosaic structures (33), at least during periods of membrane and vesicle fusion. Neither the binding experiments nor previous transport studies (29–31) provide evidence for the distribution of receptors on internal vesicle membranes. However, the studies with isolated cells do reveal the presence of receptors on the abluminal surface that are functionally very similar to the luminal receptors, as would be the case if these receptors are transported across the cells. Furthermore, the receptors on both luminal and abluminal membranes are not restricted to surface invaginations that might represent vesicles fused with the plasmalemma. These observations suggest some degree of lateral movement of receptors within, at least, the plasma membranes, as has also been proposed for surface receptors involved in endocytosis of low density lipoproteins in fibroblasts

(1). Although one might argue that the observed distribution of receptors on isolated epithelial cells may have resulted, in part, from the experimental procedures used for isolation, the maintenance of the cell suspension at 0°C, a temperature at which fluidity of the membranes of other cells is negligible (6, 7, 18), would minimize any artifactual redistribution of receptors. In this regard, abluminal receptors appeared even on large clusters of cells in which the zonula adherens appeared intact, which presumably would have restricted the lateral movement of receptors from the luminal to abluminal surface, as would be the case in intact tissue.

Receptor Reutilization

The fate of receptors at the abluminal surface after release of bound IgG is also an important consideration in the proposed transport scheme. Estimates of the rate and efficiency of transport indicate that luminal IgG can reach the circulation within 30 min (11, 20, 29). The rapidity of transport suggests that receptors may be reutilized for many transport cycles during the lifespan of the absorptive cell. Several mechanisms can be postulated by which the cell might reutilize the receptors. A likely possibility is a vesicular system (14, 26, 27) in which receptors, possibly with other membrane components, are transported back toward the luminal surface within the membranes of vesicles that form at the abluminal membrane.

To assess possible inward movement of vesicles from the abluminal plasmalemma, unconjugated HRP was injected intravenously into intact animals at a high concentration to act as a contents marker for any vesicles forming at the abluminal surface. In these experiments, an IgG conjugate could not be used to label the receptors directly because the pH at the abluminal surface presumably would prohibit binding of the conjugate to the receptors. The results of these experiments indicate that vesicles do form in this region of the plasmalemma and that many are morphologically similar to the coated and the tubular vesicles responsible for IgG transport in the reverse direction. Conversely, several membrane compartments that are not directly involved in IgG transport, including the Golgi cisternae and the rough and smooth endoplasmic reticulum (30, 31), did not contain HRP. These observations lead to a working hypothesis for receptor transport in which the coated and the tubular vesicles shuttle among each

other and their respective regions of the cell surface, the tubular vesicles fusing specifically with the luminal surface, and the coated vesicles fusing with the abluminal surface. Thus, the vesicles may represent a membrane pathway, discontinuous in time, through which receptors can move back and forth between the two regions of the cell surface. The receptors themselves need not display net movement because the pH dependence of IgG binding to the receptors is sufficient to allow vectorial transport of IgG from the lumen to the circulation (32).

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