INTESTINAL TRANSPORT OF ANTIBODIES
IN THE NEWBORN RAT

RICHARD RODEWALD

From the Department of Biochemistry, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104. Dr. Rodewald's present address is the Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115.

ABSTRACT

Evidence has been reported that the proximal small intestine of the neonatal rat selectively transports antibodies into the circulation. This study describes the morphology of the absorptive epithelial cells in this region of the intestine and their transport of several immunoglobulin tracers: ferritin-conjugated immunoglobulins (IgG-Ft) and antiperoxidase antibodies. Cells exposed to rat IgG-Ft bound the tracer on the membrane of tubular invaginations of the apical cell surface. Tubular and coated vesicles within the cell also contained the tracer, as did the intercellular spaces. Uptake of tracer was highly selective and occurred only with rat or cow IgG-Ft; when cells were exposed to chicken IgG-Ft, ferritin-conjugated bovine serum albumin, or free ferritin, tracer did not enter the cell or appear in the intercellular spaces. Experiments with rat and chicken antiperoxidase showed a similar selective uptake and transport of only the homologous antibody. When cells from the distal small intestine were exposed to the tracers, all tracers were absorbed nonselectively but none were released from the cells. Cells from the proximal small intestine of the 22-day-old rat failed to absorb even rat IgG-Ft. A model is presented for selective antibody transport in proximal cells of the neonatal rat in which antibodies are selectively absorbed at the apical cell surface by pinocytosis within tubular vesicles. The antibodies are then transferred to the intercellular space within coated vesicles. Distal cells function only to digest proteins nonselectively.

INTRODUCTION

The neonatal rat relies, in part, on passive immunity derived from its mother for protection against infection during early life. Antibodies present in the colostrum and milk of the mother are absorbed from the small intestine in the suckling rat and transported functionally intact into the circulation. Several features of this transport (see reviews: Brambell, 1958, 1966, 1970; Morris, 1968), most notably the high degree of selection for homologous IgG immunoglobulins (Halliday, 1955a, 1958; Bangham and Terry, 1957; Halliday and Kekwick, 1960; Morris, 1955a; Jordan and Morgan, 1968), have led to the hypothesis first proposed by Brambell et al. (1958) that transport is mediated by the selective binding of immunoglobulins to receptor sites present in the absorptive cells of the small intestine.

Quite naturally, several attempts have been made to demonstrate a morphological basis for selective binding and transport within the cells. Clark (1959) described a unique epithelial cell type in the lower jejunum and ileum of suckling rats which disappeared at an age when antibody transport ceased. Although a number of investigators (Clark, 1959; Kraehenbuhl et al., 1967; Graney, 1965, 1968; Kraehenbuhl and Campiche, 1969)
have shown that these cells can concentrate a variety of tracers including antibodies, no clear evidence has been presented for selective binding or release of immunoglobulins from the cells. Nevertheless, it has been generally believed that these cells are responsible for transport of antibodies to the circulation (Clark, 1959; Anderson, 1963, 1965; Graney, 1965, 1968; Bamford, 1966; Krachenbuhl and Campiche, 1969).

In a preliminary paper (Rodewald, 1970) this author reported evidence that, in fact, little if any selective transport occurs in the distal region of the intestine in comparison to a high level of transport in the proximal one-third of the intestine. Gross morphological differences have been recognized between absorptive cells in the proximal and distal small intestine (Clark, 1959; Graney, 1968), but proximal cells in the past have not been fully described or examined. This study, therefore, presents the fine structure of absorptive cells from the proximal jejunum of the 10-day-old rat and reports the vesicular transport across these cells of two homologous immunoglobulin tracers: rat immunoglobulins conjugated to horse spleen ferritin, and rat antibodies to horseradish peroxidase. Numerous control experiments with several heterologous tracers indicate that transport by proximal cells is highly selective. Other control experiments show that (a) the ability of these cells to transport homologous tracer is lost at 22 days of age, and (b) ileal cells are unable to transport any of the tracers intact to the circulation.

MATERIALS AND METHODS

Animals

Pregnant female Sprague-Dawley rats were obtained from Charles River Laboratories, Wilmington, Mass. Litters were born and raised in the laboratory. Mothers, after use of their litters, were used as a source of homologous immunoglobulins. White leghorn chickens used for the production of antiperoxidase antibody were purchased from Don Miller Farms, San Diego, Calif.

Tracer Preparation

PURIFICATION OF IgG IMMUNOGLOBULINS: IgG immunoglobulins were isolated from rat antisera by a one-step ammonium sulfate precipitation (Kendall, 1938) followed by DEAE-cellulose chromatography (Fahey, 1967) with 0.02 M phosphate buffer, pH 7.2. The IgG peak was concentrated by ultrafiltration (Craig, 1968). IgG immunoglobulins were also isolated from globulin powders, fractions II, commercially prepared from bovine, chicken, and rat sera (Pentex Biochemical, Kankakee, Ill.). Each globulin was dissolved in a small amount of phosphate buffer and chromatographed on DEAE-cellulose without prior salt precipitation. Since chicken globulins precipitate in dilute buffers (Benedict, 1967), 0.1 M phosphate buffer, pH 7.2, was used for preparing IgG immunoglobulin from this species.

FERRITIN CONJUGATION: The method of Sri Ram et al. (1963) was employed to conjugate covalently horse spleen ferritin to rat, chicken, and bovine IgG immunoglobulins by means of 4,4'-difluoro-3,3'-dinitrodiphenyl sulfone (FNPS) (K & K Laboratories, Inc., Plainview, N. Y.). The ferritin used for conjugation, though purchased as a X6 recrystallized cadmium-free preparation (Pentex), was dialyzed against EDTA according to the method of Farquhar and Palade (1961) to remove possible residual contaminants of heavy metals. After extensive dialysis of the crude conjugate mixture against normal saline, the ferritin-antibody conjugates were purified from free ferritin and IgG by preparative Pevikon-block electrophoresis as described by Osterland (1968). The spot corresponding to the conjugate was cut from the block, eluted from the Pevikon with cold normal saline, concentrated by ultrafiltration, and dialyzed exhaustively against normal saline. Immunoelectrophoresis (Borek and Silverstein, 1961) revealed the conjugate to be free of unconjugated ferritin and IgG. Ferritin was conjugated to bovine serum albumin (BSA) under conditions identical to those used for preparing IgG conjugates. Because of the high electrophoretic mobility of BSA, however, Pevikon-block electrophoresis did not allow total separation of the BSA conjugate from the unconjugated proteins. Immunoelectrophoretic analysis of the eluted conjugate indicated the presence of a small amount of free ferritin. No further purification, however, was attempted.

A sample of ferritin was also reacted with FNPS under conditions identical to those used for preparing IgG conjugates. Because of the high electrophoretic mobility of BSA, however, Pevikon-block electrophoresis did not allow total separation of the BSA conjugate from the unconjugated proteins. Immunoelectrophoretic analysis of the eluted conjugate indicated the presence of a small amount of free ferritin. No further purification, however, was attempted.

ANTIPEROXIDASE ANTIBODIES: Adult rats were immunized against horseradish peroxidase, type II (Sigma Chemical Co., St. Louis) by repeated subcutaneous injection of the protein in incomplete Freund's adjuvant (Freund and McDermott, 1942). Chickens were immunized against peroxidase by repeated intravenous injections of the protein in normal saline. 10 days after the last injection, animals were bled by heart puncture. Specific antiperoxidase antibodies were purified by passing the antisera through a column containing an insolubilized immunoadsorbent of peroxidase, type II (Avrameas and
Ternyck, 1969). Specific antibody, after elution from the column with 0.1 M glycine-HCl buffer, pH 2.8, was dialyzed against normal saline and concentrated by ultrafiltration.

Tissue Preparation

Morphological Studies: Young rats were lightly etherized without prior fasting. An abdominal incision was made to expose the small intestine, and a small segment of the upper jejunum (4-5 cm from the pylorus) or ileum (4-5 cm from the caecum) was quickly excised, placed in a pool of fixative, and cut into small pieces with razor blades. Tissue was fixed for 2-4 h at room temperature in 2% glutaraldehyde buffered at pH 7.3 with one of three buffers: 0.1 M cacodylate, 0.1 M phosphate, or 0.05 M phosphate with 0.05 M NaCl. 1-5 mM CaCl₂ was added to each buffer. Tissue was then rinsed at 2-4°C in four 30-min changes of buffer containing 0.1 M sucrose and postfixed in cold buffered 2% OsO₄. Tissue was finally dehydrated in a graded series of ethanol and embedded in Epon (Luft, 1961).

Ferritin Conjugate Studies: 10- or 22-day-old rats were isolated for 2 h, then lightly etherized. An abdominal incision was made in each rat to expose the intestine. Either the proximal or distal 1/3 of the small intestine was isolated in situ by tying silk thread ligatures around the intestine at points measured from the pylorus or caecum. Approximately 0.1 ml of one of the ferritin tracers (10-12 mg/ml in ferritin) was injected into the proximal end of the ligated portion, and a third ligature was tied to isolate the point of injection to prevent leakage of the tracer into the body cavity. The incision was then closed, and the animal was allowed to recover. After a 1-h transport period, tissue from the ligated region was fixed and embedded in Epon in the manner already described.

Antiperoxidase Studies: In experiments on transport of antiperoxidase, intestinal segments from 10-day-old rats were ligated as above and injected with either 0.1 ml of antiperoxidase antiserum or 0.1 ml of purified antiperoxidase antibody, 2 mg/ml in saline. After 1 h a small piece of the ligated segment was removed, cut into small pieces, and treated to reveal antiperoxidase according to the method of Leduc et al. (1968). Tissue was first fixed for 1 h at 4°C with 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, and washed overnight in buffer with 0.1 M sucrose in the cold. Tissue was then incubated for 1 h or overnight in 0.5 mg/ml peroxidase (type II) in wash buffer. After three 10-min rinses, tissue was refixed in buffered 2% glutaraldehyde for 15 min, again rinsed in wash buffer, then stained for peroxidase using the procedure of Graham and Karnovsky (1966). Tissue was incubated at room temperature for 30-60 min in 0.5 mg/ml 3,3′-diaminobenzidine hydrochloride (DAB) (Sigma Chemical Co.) in 0.05 M Tris-HCl buffer, pH 7.5, containing 0.01% H₂O₂. After incubation, tissue was postfixed in OsO₄, dehydrated, and embedded in Epon.

The specificity of staining for antiperoxidase antibodies was assessed in control experiments on tissue which had not been exposed to these antibodies. In one, tissue was subjected to the same staining procedures with peroxidase and DAB as done for experimental tissue. In the second, tissue was exposed to DAB alone after the first glutaraldehyde fixation. Neither control revealed peroxidase activity in absorptive cells.

Electron Microscopy

Silver-to-gray thin sections were cut with diamond knives in a Porter-Blum MT-2 microtome (Ivan Sorvall, Inc., Norwalk, Conn.) and mounted on bare copper grids. For morphological studies, sections were stained sequentially 5 min in a saturated solution of uranyl acetate and 5 min in lead citrate (Reynolds, 1963). For identification of ferritin and peroxidase reaction product, sections were stained only in lead citrate for 15-60 s. Sections were examined in an EM-6B electron microscope (Associated Electrical Industries, London) or a Philips 200 microscope (Philips Electronic Instruments, Mount Vernon, N. Y.).

RESULTS

Morphological Studies

Proximal Cells, 10-Day-Old Rat

The absorptive cell in the proximal jejunum (Fig. 1) is a low columnar cell with a striated border of closely packed microvilli. The nucleus is situated in the basal half of the cell with a well-developed Golgi complex immediately supranuclear. The lateral extracellular spaces are often dilated, especially toward the basal pole of the cell, but are sealed from the intestinal lumen by a zonula occludens at the luminal surface. The cell rests on a thin, often discontinuous basal lamina bordering the underlying lamina propria.

The most conspicuous characteristic of the cell (Figs. 1, 2) is the presence of numerous intracellular lipid droplets. These are confined primarily to the apical cytoplasm and are particularly abundant in the region of the Golgi complex. Lipid droplets are also observed in the lateral intercellular spaces as well as in most of the extracellular spaces in the lamina propria. With respect to these features, the neonatal cells appeared similar to the
FIGURE 1 Absorptive cells from the proximal jejunum, 10-day-old rat. The apical cell surface is covered with numerous microvilli (mv) projecting from the terminal web (tw). Many lipid droplets, partially extracted during fixation, can be seen in the apical cytoplasm (arrows), Golgi complex (Gc), and intercellular spaces (*). L, intestinal lumen; bl, basal lamina; end, capillary endothelial cell; rbc, red blood cell. X5,000.
FIGURE 2 Apical cytoplasm of proximal cells, 10-day-old rat. Several small pits (*) are shown extending into the terminal web from the luminal cell surface. Tubular vesicles (tv) and coated vesicles (cv) are plentiful in the subjacent cytoplasm where they are often found joined (arrows). Lipid does not appear in either of these vesicle types and is confined to rough and smooth elements of the endoplasmic reticulum (ER). av, apical vacuole; mvb, multivesicular body; zo, zonular occludens. X40,000.
lipid absorptive cells in the jejunum of the adult (Clark, 1959; Palay and Karlin, 1959 a, b; Cardell et al., 1967). A detailed examination of cell fine structure, however, reveals several important features of the cell which differentiate it from the cell in the adult. One such feature is seen at the base of the microvilli where there occur numerous invaginations of the plasma membrane (Figs. 2, 3), greatly exceeding in both size and number the apical pits observed in the adult jejunal cell (Palay and Karlin, 1959 a, b; Cardell et al., 1967). The invaginations range from slight depressions in the membrane to short tubular channels about 100 nm in diameter which penetrate the terminal web to a depth of over 0.5 µm. Of particular interest is the distinctive membrane structure of these pits (Fig. 3). On the luminal surface of the membrane can be seen a thin coating of dense, generally amorphous material 100-150 Å thick; a similar coating can less frequently be distinguished on the cytoplasmic surface.

A second distinctive characteristic of the cell is the appearance in the apical cytoplasm of two systems of vesicles. One consists of the closely associated rough and smooth elements of the endoplasmic reticulum (RER, SER), the organization of which appears identical to the organization of these structures in the cell of the adult (Palay et al., 1967) where they play a central role in the transport of lipid. For example, one can observe numerous small lipid droplets segregated within dilated cisternae of the highly anastomosing SER as well as points of continuity between elements of the SER and RER.

The remaining system of vesicles is composed in part of small tubular elements of varying length, which appear similar in structure to and are apparently derived from the surface pits of the terminal web region (Fig. 2). The membranes of these vesicles exhibit the distinctive luminal surface coat, though in contrast to the more apical pits, usually lack any obvious specialization on the cytoplasmic surface. Like the endoplasmic reticulum, the tubular vesicles extend throughout the apical cytoplasm but evidently do not participate in lipid transport. They show no structural continuity with the SER or RER, from which they are easily distinguished, and are entirely devoid of lipid droplets.

In close association with the tubular vesicles in the apical cytoplasm are seen numerous small coated vesicles. These vesicles are especially plentiful near the lateral cell margins and among the more basal tubular elements (Figs. 2, 4). The coated vesicles are spherical, range in size from 100 to 200 nm overall diameter, and possess luminal and cytoplasmic surface coats typical of these vesicles in other tissues (Roth and Porter, 1963, 1964). In addition, small coated pits of a size and membrane appearance very similar to the coated vesicles are commonly observed fused with the surfaces of the tubular vesicles as well as on the lateral plasma membrane (Figs. 2, 4). Coated vesicles do not seem to make contact with other organelles and, in particular, Golgi sacules, though such contacts have been reported for jejunal cells of the adult (Cardell et al., 1967).

Two other vesicle types are noteworthy in the apical cytoplasm. The first includes irregularly shaped multivesicular bodies, 0.3-0.5 µm diameter, containing a flocculent material of intermediate density and occasional small membranous inclusions (Fig. 2). The vesicles stain less densely but otherwise appear similar to the apical lysosomes described in cells of the adult intestine (Cardell et al., 1967). The second type of vesicle consists of infrequent small vacuoles mostly free of visible contents (Fig. 2).

**DISTAL CELLS, 10-DAY-OLD RAT**

Observations on epithelial cells in the ileum were essentially identical to those of Graney (1965, 1968), Wissig and Graney (1968), and Cornell and Padykula (1969). However, because some authors (Kraehenbuhl and Campiche, 1969; Orlic and Lev, 1973) have not clearly distinguished between proximal and distal cells, the identifying characteristics of distal cells should be briefly noted.

Distal cells are most easily recognized by their characteristic supranuclear vacuole, a giant spherical or ovoid vesicle up to 30 µm in length and occupying a central position in the cell (Fig. 5). Apical to the supranuclear vacuole and extending to the microvilli of the luminal cell surface is a zone of cytoplasm filled with an interconnecting system of pleomorphic pits, vesicles, and small vacuoles. These vesicles are considerably larger and more numerous than the small tubular vesicles of the proximal cells. A unique feature of the vesicles, described in detail by Wissig and Graney (1968), is the highly ordered array of small dense beads found on the luminal surface of their limiting membranes (Fig. 6). This beaded structure is never
FIGURE 3  Microvillus surface of a proximal cell, 10-day-old rat. Several surface invaginations can be seen, the membranes of which often exhibit amorphous coats on their luminal and cytoplasmic surfaces (arrowheads). ×60,000.

FIGURE 4  Lateral cell surface of proximal cells, 10-day-old rat. Figure 4 a, b. Coated vesicles are found free, attached to tubular vesicles (arrowhead), and open to the intercellular space (is). ×50,000.
Absorptive cell from the distal ileum, 10-day-old rat. In addition to the supranuclear vacuole (snv), the many apical vesicles and small vacuoles are clearly seen. Several vesicles are attached to the microvillus surface and are open to the intestinal lumen (arrows). Two vacuoles (*) can be seen apparently fusing with each other. Gc, Golgi complex. ×5,000.
Microvillus surface of a distal cell, 10-day-old rat. A large apical vesicle is seen open to the intestinal lumen. The luminal surface of the vesicle membranes has a characteristic beaded coat (arrowhead) not found on vesicles in proximal cells. $\times 54,000$

found on the membranes of vesicles in proximal cells. Both the beaded vesicles and large vacuoles contain varied concentrations of a finely fibrillar material interpreted as substances absorbed from the gut lumen (Clark, 1959; Graney, 1965, 1968). However, although beaded vesicles often are found forming long convoluted channels open to the lumen, neither the beaded vesicles nor the apical vacuoles ever show continuity with the lateral cell membrane. In the Golgi region, between the nucleus and supranuclear vacuole, are observed small numbers of coated vesicles. The coated vesicles appear only infrequently on the lateral cell membrane and are never found fused to the surface of the supranuclear vacuole.

**Proximal Cells, 22-Day-Old Rat**

Absorptive cells from the proximal jejunum of the 22-day-old rat appeared identical to jejunal cells as described for the adult rat (Palay and Karlin, 1959a, b; Cardell et al., 1967). These cells exhibit notable differences from the proximal cells of the 10-day-old rat. While the SER and RER retain their highly developed pattern in the apical cytoplasm, the surface pits of the microvillus cell membrane as well as the underlying tubular vesicles are substantially decreased in size and number by this age. The few tubular vesicles that do remain are limited to within and immediately below the terminal web. In the place of additional tubular vesicles are found much more numerous multivesicular dense bodies which range in size from 0.2 to 0.5 $\mu$m in diameter and are indistinguishable from the apical lysosomes of adult cells (Cardell et al., 1967). Finally, the number of coated vesicles in the cells is also noticeably reduced from the number at the earlier age. The remaining coated vesicles usually are found in the Golgi region of the cell where, in addition, coated pits are at times found on the lateral cell surface. No contact is evident between coated vesicles and the few tubular vesicles in the region of the terminal web.
Tracer Studies

Location of Tracers in Proximal Cells of the 10-Day-Old Rat

Absorptive cells of the proximal intestine which were subjected to the ligation procedure and 1-h incubation period showed no morphological change from normal cells, except for a marked decrease of intracellular lipid droplets in the SER, RER, and Golgi cisternae. This most probably resulted from the depletion of intraluminal lipid by cellular absorption during the incubation. Nevertheless, these and all other organelles appeared well preserved in the cells.

Rat IgG-Ft: When cells were exposed to rat IgG immunoglobulin conjugated to ferritin (IgG-Ft), numerous ferritin particles were found within the small pits of the apical cell surface (Fig. 7). This ferritin invariably adhered to the amorphous surface coat of the pit membranes (Fig. 7b). The localization of the tracer was quite specific for the membrane invaginations: even in areas where large numbers of ferritin particles filled the membrane pits, few ferritin molecules were found on the adjacent surfaces of the microvilli.

Within the apical cell cytoplasm, numerous ferritin molecules were present in the many tubular and coated vesicles (Figs. 7a, 8). Several of the coated pits attached to the tubular vesicles could be seen containing small numbers of tracer molecules (Fig. 8). Much smaller quantities of tracer were observed in the small vacuoles and multivesicular bodies. However, no ferritin ever appeared in the RER, SER, Golgi vesicles, other organelles, or free in the cytoplasmic matrix.

The most critical of these observations was that a portion of the tracer had traversed the cells and could be identified within the intercellular spaces (Fig. 9). Though the ferritin in these locations usually appeared highly dispersed, localized clusters of ferritin were frequently found in diluted regions of the spaces as if the tracer had been recently discharged from one of the adjacent cells (Fig. 9c). Ferritin was also frequently found in the coated pits attached to the lateral plasma membrane (Fig. 9a, b).

Unconjugated ferritin, ferritin conjugated to heterologous proteins: Control experiments with other ferritin tracers, with the exception of bovine IgG, gave distinctly different results from those for rat IgG-Ft. When proximal cells were exposed to untreated ferritin, ferritin treated with FNPS in the absence of a second protein, or ferritin conjugated to BSA or chicken IgG, no ferritin was found in the pits at the base of microvilli or elsewhere on the cell surface. Furthermore, no ferritin could be identified within any of the cytoplasmic vesicles or the lateral extracellular spaces.

Bovine IgG-Ft: Unlike other control conjugates, a considerable amount of bovine IgG-Ft entered and traversed the proximal cells. The localization of this conjugate on the apical cell surface, within the tubular and coated vesicles, and in the intercellular spaces closely paralleled that of rat IgG-Ft. However, in contrast to the results with rat IgG-Ft, much larger quantities of ferritin were apparent in the multivesicular bodies and small apical vacuoles (Fig. 10). In many of the vacuoles the predominant proportion of tracer closely adhered to the inner surface of the vacuole membrane.

Antibodies to peroxidase: The location in proximal cells of rat antiperoxidase antibodies, identified by reaction product to antibody-bound peroxidase, also closely paralleled the results with rat IgG-Ft. However, compared with the observations with the ferritin tracer, fewer of the tubular and coated vesicles within the cells were positive for the antiperoxidase antibodies. Nevertheless, high concentrations of specific reaction product in several tubular vesicles, the intercellular spaces, and the lamina propria indicated that antibody had crossed the cells (Figs. 11-13). When chicken antiperoxidase was substituted for the specific rat antibody in this experiment, all reaction product within the cells and extracellular spaces was abolished.

The results of experiments with ferritin and antiperoxidase tracers on proximal cells are summarized in Table I.

Location of Tracers in Distal Cells of the 10-Day-Old Rat

Ferritin tracers: No morphological changes were observed in the absorptive cells from distal ligated segments after being exposed to ferritin tracers. The location of ferritin within the cells was similar whether cells were exposed to rat IgG-Ft or untreated ferritin; other ferritin tracers were not tested. Ferritin particles were localized in small quantities within the apical beaded vesicles characteristic of these cells (Fig. 14) and in increasing concentrations in the small vacuoles and suprapi-
Figure 7: Apical surface of proximal cells exposed to rat IgG-Ft. Fig. 7 a: Ferritin can be identified in several pits at the base of the microvilli and within many of the underlying tubular vesicles. Little or no ferritin is observed in the multivesicular bodies (mvb) or vacuoles in this region. $\times 52,000$. Fig. 7 b: Ferritin in the surface pits appears to adhere to the surface of the membrane. $\times 60,000$. 

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FIGURE 8. Golgi region of a proximal cell exposed to rat IgG Ft. Ferritin is localized exclusively within tubular and coated vesicles (arrowheads). Golgi cisternae (Gc) are always devoid of tracer. ×52,000. Inset: A coated vesicle containing ferritin is seen attached to a tubular vesicle. ×67,000.

nuclear vacuole. Nevertheless, no tracer was ever identified in the lateral extracellular spaces. It is of interest that in tissue exposed to rat IgG-Ft, tracer was never found preferentially bound to the membranes of either the vesicles open at the apical cell surface or the other vesicles and vacuoles deeper in the cytoplasm. Golgi vesicles and coated vesicles were always free of tracer. These results were, therefore, identical to the results of Graney (1965, 1968) on distal cells in young rats fed free ferritin.

ANTIBODIES TO PEROXIDASE: Location of rat antiperoxidase antibodies was limited in distal cells to within the small apical vacuoles and to a lesser extent within the beaded vesicles and supranuclear vacuoles (Fig. 15). Poor penetration of peroxidase into the cells during staining may have

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been partially responsible for the lack of reaction product in the latter locations. As with ferritin tracers, no reaction product was found within the lateral extracellular spaces or lamina propria to indicate transport through the cell.

**Proximal Cells of the 22-Day-Old Rat**

The morphology of absorptive cells from the ligated proximal intestine of the 22-day-old rat was identical to that of untreated cells except for a decrease in the intracellular lipid. When a ligated

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**Figure 9.** Lateral cytoplasm of proximal cells exposed to rat IgG-Ft. Fig. 9 a: Tracer is seen in coated vesicles attached to the lateral plasma membrane as well as free in the intercellular space. ×32,000. Fig. 9 b: Same. ×65,000. Fig. 9 c. Localized clusters of ferritin in the intercellular space suggest recent release from adjacent coated vesicles similar to those shown (arrowheads). ×65,000.
segment was exposed to rat IgG-Ft for 1 h, tracer could rarely be identified within any cytoplasmic vesicles of the absorptive cells. Apical pits, small tubular vesicles, and coated vesicles were always devoid of tracer. Only the dense lysosomal bodies could, on occasion, be found to contain clusters of molecules resembling ferritin.

Experiments with antiperoxidase antibodies were not performed on animals of this age.

DISCUSSION

Site of Transport in the Intestine

The morphological observations with immunoglobulin tracers substantiate the preliminary observations (Rodewald, 1970) that the proximal small intestine is the primary site of transport of immunoglobulins to the circulation of the neonatal rat. With both rat immunoglobulin conjugated to ferritin and rat antiperoxidase antibody, evidence was found for transport across the absorptive epithelium in the proximal intestine. On the other hand, neither tracer could be found crossing the epithelium of the distal intestine. It is particularly important that in these experiments ligated segments of intestine were used which, while not visibly altering the integrity of the epithelial cells, ruled out circumvential transport of tracer across regions of the intestine not under observation. Although certain studies have used the detection of specific antibodies in the lamina propria of the distal intestine to justify a transport role for distal cells (Clark, 1959; Kraehenbuhl and Campiche, 1969), it is significant that in these studies the distal intestine was not tied off from more proximal regions. In these experiments the proximal intestine, though not studied, was also exposed to the immunoglobulins and could have accounted for the observed transport. The detection by Bamford (1966) of transport across everted ileal sacs in vitro is perhaps more significant but can be questioned on several grounds: (a) the observed transport was small, (b) transport in proximal segments was not measured, and (c) transport could also be detected in ileal sacs from 84- and 99-day-old rats (Bamford, cited by Morris, 1968) when selective transport in the intact rat was lacking.

Route of Transport across Proximal Absorptive Cells

The epithelial cells of the jejunum were found to contain a system of small tubular vesicles which could be easily distinguished from the lipid-conn-
taining endoplasmic reticulum and the larger beaded vesicles of distal cells. Observations with immunoglobulin tracers indicate that the tubular vesicles, together with coated vesicles, are responsible for antibody transport across the proximal cells.

Of the tracers used to demonstrate antibody transport, the ferritin conjugates proved most useful, owing to their ease of identification at low concentration in normally fixed tissue. Although chemical modification of immunoglobulin by conjugation to ferritin would be expected to decrease transport across the cells (Slade, 1970), it was not so drastic as to inhibit this transport completely. As shown by controls with unconjugated ferritin and BSA-Ft, transport could not be ascribed to the presence of ferritin alone, nor to the conjugating...
reagent (FNPS) on the proteins. Furthermore, the lack of transport of these control tracers showed that leakage between cells was not responsible for transport of the other antibody tracers.

The interpretation of the ferritin conjugate results relies on the assumption that, during transport, the ferritin did not become detached from the immunoglobulin and follow a separate route through the cells. This possibility seems remote since the same vesicle types in which ferritin was identified could also be shown to contain rat antiperoxidase antibody, a tracer which needed no modification before transport by the cells. Although intracellular localization of peroxidase antibodies was more limited, owing most likely to less than optimal penetration of peroxidase and substrate into fixed cells, the results with this tracer were essentially the same as those with the ferritin tracer.

Several features of the transport mechanism, briefly noted elsewhere (Rodewald, 1970), are apparent from the tracer studies:

(a) Antibodies are selected from other proteins at the apical cell surface within the small pits.
which form at the base of the microvilli. Ferritin when conjugated to rat immunoglobulin was found bound exclusively to these areas of the cell membrane. Unconjugated ferritin and ferritin conjugated to chicken immunoglobulin or bovine serum albumin were not bound. Whereas ferritin conjugated to bovine immunoglobulin appears to adhere to these membrane pits, previous work (Brambell et al., 1961) indicates that this immunoglobulin is selectively transported in the same way as the homologous immunoglobulin.

It seems likely that the antibody receptor sites proposed by Brambell et al. (1958) are localized on the pit membranes and may be represented by the amorphous material covering the luminal surface of those membranes. Selection, therefore, does not occur on an internal membrane of the cell as required in transport models for distal cells (Leisring and Anderson, 1961; Anderson, 1965; Brambell, 1966; Morris, 1968) in which pinocytosis of material from the intestinal lumen is completely nonselective. In contrast to this, selective binding of antibody on the proximal cell surface allows the initial step of pinocytosis to be the selective event.

(b) After pinocytosis, antibodies are segregated within the small tubular vesicles of the apical cytoplasm. During this stage of transport, as in later stages, the antibodies are always enclosed within membrane-limited vesicles and apparently do not pass across membranes or through the cytoplasmic matrix. It is also of interest that no evidence is found for immunoglobulins within the endoplasmic reticulum or Golgi cisternae, which, in these cells, are usually involved in the segregated transport of lipid into the circulation as in the adult rat (Strauss, 1966; Cardell et al., 1967).

(c) Immunoglobulins are transferred to coated vesicles which form on the surface of the tubular vesicles. Coated vesicles have in the past been associated with selective transport of proteins in other cells (see Kanaekei and Kadota, 1969). The present case provides a particularly clear example where these vesicles participate in such a process. However, the exact role the vesicles play in actual selection of antibodies is not fully evident. Although it has been suggested that one or the other surface coats of coated vesicles might function to bind proteins selectively (Roth and Porter, 1964; Bowers, 1964; Kanaekei and Kadota, 1969), it appears that in proximal cells the luminal surface coat of tubular vesicles is the primary site of antibody selection. It may well be that the formation of tubular vesicles at the luminal plasma membrane is not fully selective and allows the nonspecific uptake of some unbound protein; the coated vesicles could then provide a second opportunity for selection of the membrane-bound antibodies. The observation that small amounts of ferritin conjugate and antiperoxidase antibody are observed within vesicles resembling lysosomes suggests that not all proteins entering the cell are transported into the circulation and that some further selection might occur.

(d) Coated vesicles discharge antibodies at the lateral cell surface into the extracellular space. This is apparently accomplished by a process of reverse pinocytosis in which the membrane of the coated vesicle fuses with the plasma membrane of the cell. It seems unlikely that the appearance of tracer in the coated pits represents back diffusion into these pits from the extracellular space after transport of the tracer by some other route. Frequently, high local concentrations of tracer are seen in the intercellular spaces in close proximity to areas of cells containing large numbers of coated vesicles and pits. After release from the cells, antibody is free to diffuse into the lamina propria.

**Selection**

An important test for this mechanism of selective transport in proximal cells was to determine whether it showed the same degree of specificity as has been demonstrated for antibody transport in the intact rat. In this respect, the complete failure of proximal cells to transport BSA-Ft and unconjugated ferritin indicated a strong selection of immunoglobulins by this mechanism in accord

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**Table I**

| Protein administered | On apical cell surface | In cytoplasmic vesicles | In lateral extracellular spaces |
|----------------------|-----------------------|------------------------|--------------------------------|
| Rat IgG-Ft           | +                     | +                      | +                              |
| Bovine IgG-Ft        | +                     | +                      | +                              |
| Ft                   | -                     | -                      | -                              |
| Ft*                  | -                     | -                      | -                              |
| BSA-Ft               | -                     | -                      | -                              |
| Chicken IgG-Ft       | -                     | -                      | -                              |
| Rat antiperoxidase   | +                     | +                      | +                              |
| Chicken antiperoxidase| -                    | -                      | -                              |

Ft = horse spleen ferritin; Ft* = horse spleen ferritin treated with 4,4'-difluoro-3,3'-dinitrodiphenyl sulfone; BSA = bovine serum albumin; IgG = IgG serum immunoglobulin.
FIGURE 14 Apical surface of a distal cell, 10-day-old rat, exposed to rat IgG-Ft. Ferritin is found within the apical vesicles but is not preferentially bound to the membrane surfaces. Results with free ferritin were identical. X52,000.

with the physiological studies of Bangham and Terry (1957), Brambell et al. (1958), and Jordan and Morgan (1968). Furthermore, the tracer experiments showed selection in proximal cells among immunoglobulins produced in different animal species. Ferritin conjugates of rat and bovine IgG and rat antiperoxidase antibody all were transported, but the ferritin conjugate of chicken IgG and chicken antiperoxidase were not.

The results with bovine immunoglobulin disagree with experiments of Halliday (1955 a), who found no evidence for transport in the rat of functionally intact bovine antibodies. The reason for this discrepancy is apparent from later evidence (Brambell et al., 1961) indicating that bovine immunoglobulins are selectively transported to the circulation but lose their antibody activity in the process. These investigators inferred that transport of bovine immunoglobulins is carried out by the same mechanism which transports other immunoglobulins, since, as shown by Brambell et al. (1958), bovine immunoglobulin interferes strongly with the transport of homologous antibodies in the rat. The present study gives no indication at what point the bovine immunoglobulins might lose their antibody activity, but is otherwise consistent with these data.

Disappearance of Transport in Proximal Cells of the 22-Day-Old Rat

Another important criterion for the validity of a mechanism for antibody transport is that the
mechanism ceases to function at the age when transport ceases in the intact rat (Halliday, 1955b). The disappearance of the large supra-nuclear vacuoles in cells of the distal intestine by the end of the third week after birth has thus been used to justify the choice of this region as the site of transport (Clark, 1959; Graney, 1968). However, morphological changes alone do

**Figure 15** Apical cytoplasm of a distal cell exposed to rat antiperoxidase antibodies. Reaction product to antibody-bound peroxidase shows a distribution within apical vesicles similar to that for rat IgG-Ft and free ferritin. Reaction product is never found in the intercellular spaces (is). ×26,000.
not allow one to determine which cell type is responsible for transport. The proximal cells in the 22-day-old rat also exhibit important changes which, while perhaps less striking than changes in distal cells, are probably more directly related to the cessation of selective transport. The vesicles forming from the apical cell membrane markedly decrease in number and, more importantly, show no selective binding or uptake of rat IgG-Ft on their membrane surfaces. The fact that the only ferritin tracer found within these cells was present within the small dense lysosomal bodies suggests that any material that does enter these cells is degraded rather than transported to the circulation. This small amount of tracer seen in the dense bodies probably reflects a nonselective uptake similar to the uptake observed in cells of the adult (Cardell et al., 1967; Casley-Smith, 1967; Cornell et al., 1971). Quantitative estimates (Warshaw et al., 1971; Walker et al., 1972) indicate that transport of intact protein to the circulation is extremely small in the adult compared to the amount of antibody transport in the neonate (Halliday, 1955 b; Rodewald, 1970).

**Role of Distal Epithelial Cells**

Although distal cells were able to absorb and concentrate rat IgG-Ft and antibodies to horse-

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**FIGURE 16** Diagram of antibody transport across the proximal absorptive cells in the small intestine of the neonatal rat. (1) Antibodies selectively bind to the surface of specialized pits located on the apical plasma membrane. (2) Bound antibodies are absorbed by pinocytosis and become segregated within small tubular vesicles. (3) Antibodies are transferred from the tubular vesicles to spherical coated vesicles. (4) The coated vesicles discharge the antibodies at the lateral cell membrane by reverse pinocytosis.
radish peroxidase within their large vacuoles, no release of either tracer into the circulation was observed as was the case in proximal cells. A comparison of results with rat IgG-Ft and those with free ferritin failed to reveal any process within the cells selective for the immunoglobulin conjugate. The conjugate was not found preferentially bound to any membrane surfaces or segregated within any vesicle class which was not also accessible to free ferritin. It is most logical to conclude that uptake by these cells is entirely a nonselective process and is not associated with selective transport of antibodies. It should be emphasized that these results are by no means inconsistent with the observations in previous studies which well document the nonselective uptake of proteins by distal cells (Clark, 1959; Graney, 1963, 1968; Krachenbuhl et al., 1967).

Considerable evidence for the lysosomal nature of the vacuoles in distal cells has suggested that this nonselective uptake reflects primarily a digestive function (Vacek, 1964; Cornell and Padykula, 1969). This interpretation is consistent with evidence that intraluminal digestion is suppressed in the gut at this age (Hill, 1956; Mosinger et al., 1959; Clark, 1959). These observations can be incorporated into a simple model for the function of distal cells which takes into account the high level of antibody transport in the proximal intestine (Rodewald, 1970). It is suggested that distal cells do not transport antibodies at all and act only to degrade ingested material for the nutrition of the animal. Antibodies which will be transported are selected earlier from the luminal contents by proximal cells and are transported to the lamina propria. Other proteins, including those antibodies which the proximal cells fail to transport, then pass down the intestine to be nonselectively degraded in distal cells.

**SUMMARY**

The model proposed for the selective transport port across proximal cells is shown schematically in Fig. 16.

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