SELECTIVE ANTIBODY TRANSPORT IN THE PROXIMAL
SMALL INTESTINE OF THE NEONATAL RAT

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INTRODUCTION

Numerous physiological studies have demonstrated the highly selective nature of antibody transport in the small intestine of the neonatal rat (Halliday, 1955, 1958; Bangham and Terry, 1957; Halliday and Kekwick, 1960; Jordan and Morgan, 1968). γ G antibodies of the rat can be transferred functionally unaltered in large quantities from the lumen of the intestine into the circulation, whereas other milk and serum proteins are transported in much smaller quantities or not at all.

Recently investigators have proposed that selective antibody transport occurs across the epithelial cells in the distal region of the small intestine. However, to our knowledge no experiments have been reported in the literature which compare antibody transport in the proximal and distal portions of the intestine, even though many studies have emphasized marked morphological (Clark, 1959; Graney, 1968) and physiological differences (Clark, 1959; Koldovský and Chytil, 1965; Heringová et al., 1965; Noack et al., 1966) between these regions. We decided, therefore, to determine whether a difference similarly exists between these regions in their ability to transport antibodies. A simple ligation experiment reported in this paper indicates that the bulk of selective antibody transport occurs in the proximal one-third of the intestine. In a second experiment, tissue in the proximal region was exposed to immunoglobulin conjugated to ferritin which could be followed with the electron microscope to study the morphological basis of selection and transport of antibodies across the epithelial cells.

MATERIALS AND METHODS

Preparation of Immunoglobulins and Conjugates

Adult white rats were immunized to either hemocyanin from *Limulus polyphemus* (courtesy Dr. Fred Karush, University of Pennsylvania) or horsespleen ferritin (cadmium-free, crystallized six times; Mann Research Labs, Inc., New York) by repeated subcutaneous injection in Freund's adjuvant. γG immunoglobulins were purified from collected sera by ammonium sulfate fractionation (Kendall, 1938) followed by DEAE cellulose chromatography (Fahey, 1967). γG immunoglobulin to hemocyanin was conjugated to ferritin by the method of Ram et al. (1963). The antibody-ferritin conjugate was separated from unconjugated immunoglobulin and ferritin by preparative Pevikon block electrophoresis (Osterland, 1968).

Ligation Experiment

10-day-old rats from litters raised in the laboratory were isolated for 2 hr then lightly etherized. An abdominal incision was made in each rat to expose the intestine. Either the proximal or distal one-third of the small intestine was isolated *in situ* by tying silk thread ligatures around the intestine at points measured from the pyloris or caecum. 0.1 ml of the γG anti-ferritin immunoglobulin (40 mg/ml in saline) was injected into the proximal end of the ligated portion, and a third ligature was tied to isolate the point of injection. The intestine was replaced in the abdominal cavity, the incision was clipped closed, and the animal was allowed to recover. 1 hr after the injection a blood sample was taken by cardiac puncture, and the rat was sacrificed. This time is sufficient for considerable transport of antibodies (Halliday, 1955) without causing any noticeable deterioration of the ligated tissue. The amounts

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of anti-ferritin in the blood serum and purified immunoglobulin used for the injection were estimated by a passive hemagglutination assay (Boyden, 1951) in which ferritin was employed as sensitizing antigen. Anti-ferritin titers were expressed as the maximum dilution of the sample which gave positive agglutination of the ferritin-coated red blood cells.

Electron Microscopy

The ligation procedure was repeated on the proximal one-third of the intestine. Approximately 0.1 ml of immunoglobulin-ferritin conjugate was injected into the ligated portion. In control experiments to demonstrate specificity of transport, unconjugated ferritin was used in place of the conjugated ferritin. The concentration of ferritin in all experiments was adjusted to 12 mg/ml in saline as measured spectrophotometrically at 350 mμ (ϵ = 5.3 ml/mg·cm). After 1 hr the rat was reanesthetized, and a small piece of ligated tissue 4–5 cm from the pyloris was excised and immediately placed in fixative. Fixation consisted of 2½ hr in 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3, followed by exhaustive rinsing in the same buffer with 0.1 M sucrose, and postfixation in 2% osmium tetroxide. The tissue was dehydrated in ethanol and embedded in Epon (Luft, 1961). Silver to gray thin sections were cut on a Porter-Blum MT-2 microtome (Ivan Sorvall Inc., Norwalk, Conn.), stained for 1 min in lead citrate (Reynolds, 1963), and examined in an AEI EM6B electron microscope.

RESULTS

Comparison of Transport in Proximal and Distal Ligated Segments

The results of the ligation experiment as described above are presented in Fig. 1. The anti-ferritin concentration in the blood is expressed as a concentration quotient (C. Q.), the ratio of antibody titer in the serum divided by the titer of the injected immunoglobulin. In all cases the concentration quotient of the proximal ligated segment was higher than the value for the distal ligated segment. In five of the eight animals in which the distal segment was ligated, no transport could be detected at all within the limits of sensitivity of the hemagglutination assay. On the average, proximal transport was over 30 times greater than distal transport.

Location of Antibody-Ferritin Conjugate in Epithelial Cells

The general appearance of the epithelial cells in the proximal segment of the intestine was similar
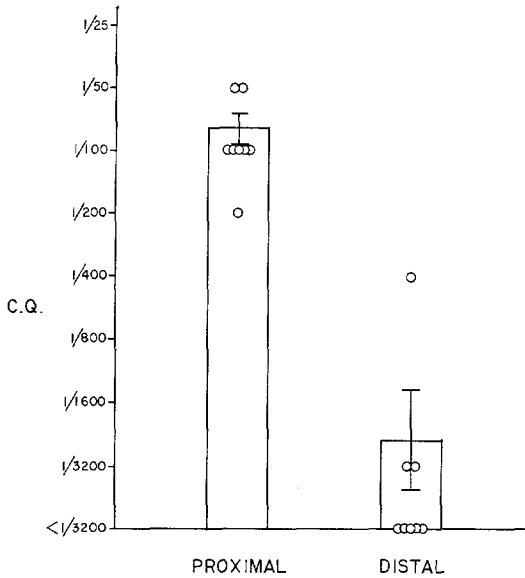

FIGURE 1   Comparison of anti-ferritin titers present in the circulation 1 hr after injection of anti-ferritin immunoglobulin into the proximal or distal ligated segment of the small intestine. C.Q. equals anti-ferritin titer in the serum divided by anti-ferritin titer in the injected immunoglobulin. Circles show values for individual animals. Bars and vertical lines show means and standard errors, respectively.

to that described by Clark (1959) and Graney (1968) for cells of the duodenum and upper jejunum in the rat at this age. The cells contained numerous small lipid droplets but lacked the large supranuclear vacuoles characteristic of more distal cells of the intestine at the same age (Clark, 1959; Graney, 1968).

1 hr after injection of the proximal ligated segment with ferritin-conjugated immunoglobulin, the epithelial cells showed numerous ferritin particles localized on the outer surface of the apical plasma membrane between microvilli (Figs. 2 a, b). The membrane in this region often formed small, irregularly shaped invaginations 0.2–0.5 μ in length which extended into the subjacent terminal web region. The surfaces of these invaginations were invariably covered with large numbers of ferritin particles. Rarely could ferritin be identified on the surface of microvilli or free in the lumen. Presumably, any ferritin conjugate in these locations was washed away during fixation.

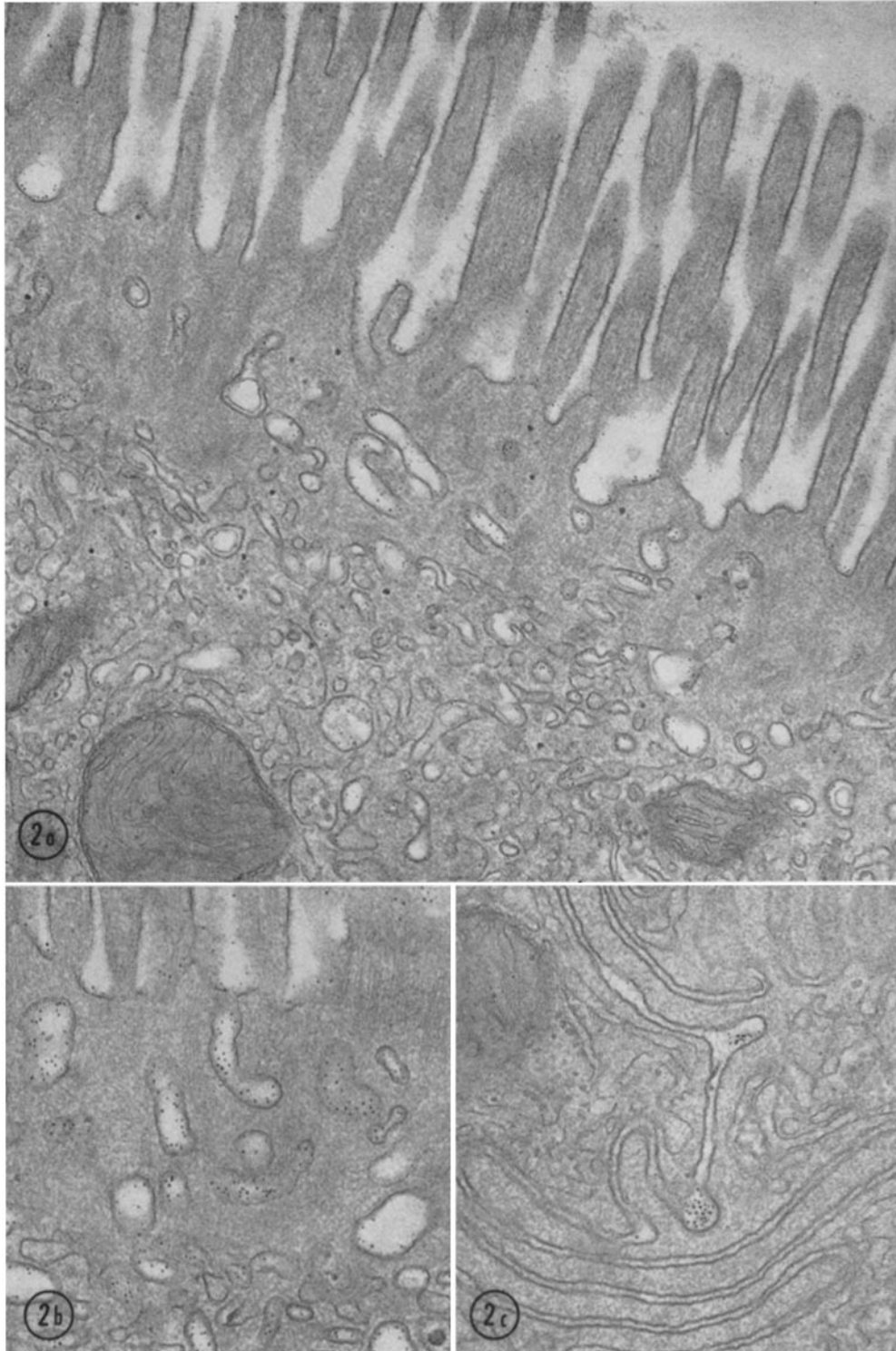Ferritin was also present within the epithelial

FIGURE 2   Epithelial cells from proximal ligated segment injected with ferritin-conjugated immuno-globulin.

FIGURE 2 *a*   Apical region of the cell showing ferritin on apical plasma membrane and within cytoplasmic vesicles. × 35,000.

FIGURE 2 *b*   Same. × 50,000.

FIGURE 2 *c*   Lateral extracellular space between two adjacent epithelial cells. Ferritin appears in clusters as if having been recently discharged by vesicles. × 50,000.
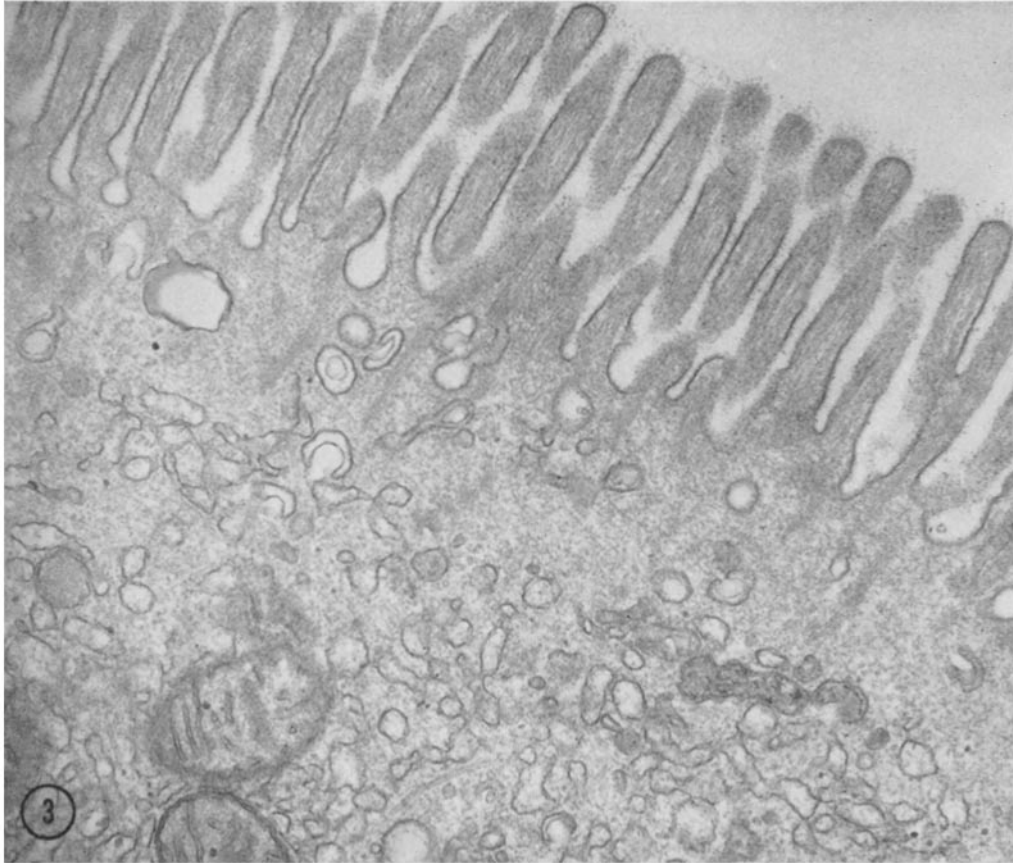
FIGURE 3   Control. Apical region of an epithelial cell from a proximal segment exposed to unconjugated ferritin. × 35,000.

cells where it was localized within small vesicles of the apical cytoplasm. Many of these vesicles appeared similar in size and shape to the surface invaginations between microvilli. At no time was any ferritin present free in the cytoplasm or within other cell organelles. Ferritin particles were also found within the extracellular spaces between adjacent epithelial cells. This ferritin usually appeared dispersed within these lateral spaces but was often encountered in more concentrated clusters as if having been recently discharged from the cell (Fig. 2 c).

In control experiments (Fig. 3) in which unconjugated ferritin was used for injection, no tracer could be detected on the apical cell membrane, within any cytoplasmic vesicles, or within the intercellular spaces.

DISCUSSION

Region of Maximal Antibody Transport

The results of the ligation experiment clearly indicate that the majority of selective antibody transport occurs in the proximal portion of the small intestine. The mean concentration quotient of 1.2×10^{-2} calculated for proximal transport, in fact, is of the same order of magnitude as the value of $1/32$ expected for maximal transport by the entire intestine as determined by Halliday (1958) using 12-day-old rats. The amount of antibody that reached the circulation nonselectively in our experiments as a result of the ligation procedure must have been very small and certainly no more than the mean C.Q. calculated for the distal segment. This small amount of antibody

detected in the serum after distal ligation may have resulted from unavoidable leakage of material through the site of injection or through other areas of tissue injury caused by the ligation procedure. Alternatively, it may represent a small but real amount of selective antibody transport by the distal intestine as reported by Bamford (1966) for inverted ileal sacs from 18-day-old rats. Nevertheless, we feel that the distal intestine does not contribute significantly to total transport of antibodies, contrary to what has been proposed by other investigators (Clark, 1959; Bamford, 1966; Brambell, 1966; Morris, 1968; Graney, 1968).

Intracellular Route of Transport— Preliminary Conclusions

The results of the ligation experiment led us to follow the morphological route of transport across the epithelial cells in the proximal region with a suitable tracer for which we chose ferritin-conjugated antibodies for the electron microscope. We would like to emphasize that the cells in this region, lacking the large supranuclear vacuole, are of a different morpnological cell type than the more distal cells studied by other authors and assumed by them to be responsible for selective transport (Clark, 1959; Bamford, 1966; Graney, 1968; Anderson, 1969; Kraehenbuhl and Campiche, 1969). Though both cell types had been recognized previously (Clark, 1959; Graney, 1968), the role of the proximal cells in antibody transport has until now been nearly completely ignored.

The observations on proximal cells exposed to antibody-ferritin conjugate support the conclusion that these cells are indeed responsible for antibody transport. Ferritin when coupled to antibody enters the epithelial cells in the proximal intestine and is transferred in vesicles to the intercellular space; unconjugated ferritin does not even enter the cell. We believe, therefore, that conjugation of the antibody does not destroy the ability to be transported and, in fact, the route of transport can be accurately followed with this conjugate as tracer.

We wish to report in this preliminary note two conclusions from our conjugate studies concerning the mechanism of antibody transport. First, selection of antibodies from other proteins appears to take place on the outer surface of the apical plasma membrane of the epithelial cell. This conclusion is based on the observation that ferritin when coupled to antibody adheres to localized

areas of the membrane at the base of microvilli, often within pinocytotic membrane invaginations. The presence of ferritin on the membrane does not appear to be a nonspecific effect of fixation since tracer does not appear on the immediately adjacent microvillus membranes or other cell surfaces. Furthermore, unconjugated ferritin does not adhere even to membranes at the bases of microvilli. The location of selective binding sites for antibody within the epithelial cells was first hypothesized by Brambell et al. (1958) from physiological evidence concerning the nature of antibody selection. Several authors (Leissring and Anderson, 1961; Anderson, 1965; Brambell, 1966; Morris, 1968) have since extended this hypothesis and suggested that the selective site is located on an intracellular membrane surface of distal intestinal cells and that selection occurs following nonselective pinocytosis of proteins at the apical surface. We suggest, instead, that antibody selection occurs prior to or at the same time as pinocytosis at the cell surface of the proximal cells and that other proteins are excluded from entering the cells. It is possible that binding of antibodies to specific sites on the membrane surface initiates the process of pinocytosis.

Second, the transfer of antibody occurs exclusively within membrane-limited vesicles. In our experiments ferritin is never observed free in the cytoplasm of the cells. Antibodies enter the cells segregated within the small tubular vesicles formed by pinocytosis at the apical cell surface. Other observations that we have made indicate that the antibodies are then transferred from the tubular vesicles to smaller, coated vesicles similar to those first described by Roth and Porter (1964). These vesicles then discharge the antibodies into the lateral extracellular space by reverse pinocytosis. A more detailed description of this mechanism for intracellular transport of antibodies will be the subject of a future communication.

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