

MACROMOLECULAR ABSORPTION

Mechanism of Horseradish Peroxidase Uptake and Transport in Adult and Neonatal Rat Intestine

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ABSTRACT

The immature small intestine of neonatal mammals is permeable to gamma globulins as a source of passive immunity. Allegedly, macromolecular absorption ceases when the epithelial cell membrane matures. However, some evidence exists that adult animals retain a limited capacity to transport antigenic and biologically active quantities of large molecules. In this study, the mechanism of absorption of the tracer protein, horseradish peroxidase (HRP), was tested in neonatal and adult rat gut sacs. Transport into serosal fluid was quantitated by enzymatic assay and monitored morphologically by histochemical techniques. A greater transport of HRP was noted in the adult jejunum compared to adult ileum and neonatal intestine. Morphologically, the uptake mechanism in adult intestine was similar to the endocytosis previously reported in neonatal animals. Like other endocytotic processes, HRP uptake in adult rats is an energy-dependent process as determined by metabolic inhibitors and temperature-controlled studies. An understanding of the mechanism whereby macromolecules are bound to intestinal membranes and engulfed by them is necessary before the action of physiologic macromolecules such as enterotoxins can be appreciated.

INTRODUCTION

The small intestine of certain neonatal mammalian species can absorb large quantities of macromolecules as a transient phenomenon for acquiring passive immunity (27). Immature intestinal epithelial cells engulf gamma globulins and other milk proteins forming small vesicles which coalesce as phagosomes. The proteins then traverse the cell either to be deposited in the intercellular space by a reverse endocytosis (exocytosis) or to combine with lysosomes for intracellular digestion (6, 12). Uptake specificity and the period of enhanced absorption vary with the species (14, 16, 21, 27), and certain factors in the diet (22) as

well as exogenous hormones (15) can cause a premature closure of the epithelial barrier to macromolecules.

Allegedly, macromolecular absorption ceases when the epithelial cell membrane matures. However, there is evidence to suggest that adult animals can continue to absorb large molecules in antigenic and biologically active quantities (1, 2, 4, 33). Danforth and Moore (10) noted that insulin injected into isolated loops of adult rat small intestine caused a hypoglycemic response. Furthermore, Bernstein and Ovary (3) demonstrated that haptens and larger antigens were absorbed

from guinea pig small intestine in sufficient quantities to evoke a passive cutaneous anaphylaxis response, and Korenblat et al (20) showed that a considerable number (15–30%) of normal adults responded to a physiologic oral milk protein load by developing milk precipitins. In addition, earlier studies from this laboratory (9, 34) presented evidence for the *in vivo* absorption of horseradish peroxidase from adult rat small intestine.

The present study was designed to define the mechanism of macromolecular absorption in the adult rat intestine. Using a tracer protein, horseradish peroxidase (HRP, mol wt 40,000), that could be visualized ultrastructurally and easily quantitated enzymatically, *in vitro* absorption from adult small intestine was compared to the neonatal transport pattern. Since pinocytosis is an energy-dependent process in neonatal intestine (23), as well as in other cellular systems (7, 18, 26), the energetics of HRP uptake by the adult epithelial cell were also investigated.

METHODS AND MATERIALS

Experimental Procedures

Female albino Charles River strain rats (Charles River Breeding Laboratories, Inc., Wilmington, Mass.), ranging in age from 15 to 80 days, were used. Since intestinal permeability to gamma globulins ceases at 20 days (6, 12), neonatal rats at ages 15 and 18 days were initially studied to assure an adequate representation of immature small intestine. However, the magnitude of absorption was virtually identical in both groups, and thereafter only the larger, 18-day old animals were used.

Animals fasted overnight were subjected to a laparotomy under ether anesthesia, and the small intestine was exposed. Two segments of gut were used as representative portions of jejunum and ileum, one 15 cm distal to the terminal portion of the duodenum and another the last 15 cm of ileum. After everting the intestine over a glass rod using the Wilson and Wiseman technique (35), 5-cm long sacs were made and incubated in flasks containing oxygenated Krebs-Ringer bicarbonate containing $10 \mu\text{M}$ HRP. The sacs were slowly oscillated in a Dubnoff incubator at 37°C for 60 min while a continuous 95% O_2 -5% CO_2 atmosphere was maintained. After incubation, serosal fluid was drained by syringe and the volume was determined. Representative sacs were saved for morphologic studies. Two methods for monitoring gut sac viability during the period of incubation were used. (a) sacs were examined by both light microscopy and electron microscopy for morphologic integrity, and (b) sacs were monitored for the active transport

of L-histidine- ^{14}C during the entire test period. Gut sac volumes were measured before and after incubation. Any sac with less than 90% of the original volume after incubation was considered to have leaked and was discarded.

Absorption Studies

Horseradish peroxidase (Sigma Chemical Co., St. Louis, Mo., 250 units/mg protein) was measured by a modification of the Worthington method (Worthington Biochemical Corp., Freehold, N. J.) (25). A 0.1 ml sample of test solution was mixed with 2.9 ml of a reaction mixture containing 0.003% H_2O_2 in phosphate buffer (0.1 M, pH 6.0) and 0.025 ml of an aqueous solution of O-dianisidine, di-HCl (10 mg/ml). Using a Gilford recording spectrophotometer (Gilford Instrument Laboratories, Oberlin, Ohio), the rate of increase in optical density at $460 \text{ m}\mu$ was determined. Protein concentration of HRP was measured by the method of Lowry et al. (24), using bovine serum albumin as a standard. At dilutions of HRP standard solutions below $10 \mu\text{M}$, there was no linear relationship between enzymatic activity and enzyme protein concentration. However, when serial dilutions of standard solutions were assayed in a buffer containing 1% bovine serum albumin and 0.1 M phosphate, pH 6.0, a linear relationship was noted between enzymatic activity and protein concentration. Enzyme protein concentration in sac fluid was subsequently determined from a standard curve relating enzyme activity to enzyme protein.

To determine the appropriate molecular size of the peroxidase in serosal fluid, a $\text{K}25 \times 40$ column with G-75 Sephadex gel in 0.1 M phosphate buffer, pH 6.0, was used (Pharmacia Fine Chemicals, Inc., Piscataway, N. J.). The column was calibrated with exogenous HRP and egg white ovalbumin (mol wt 45,000). The elution profile of standard HRP and comparable amounts of serosal fluid peroxidase were compared by assaying for peroxidase activity in the usual way.

Inhibition Studies

Representative inhibitors of cellular glycolysis and oxidative phosphorylation were used to determine the energy requirements for HRP absorption in adult small intestine. Concentrations of inhibitor that decreased the active transport of L-histidine- ^{14}C were used in subsequent absorption experiments.

Sodium fluoride (5×10^{-3} M) was used as an inhibitor of glycolysis, and 2,4-dinitrophenol (2×10^{-5} M), CF-CCP (1×10^{-6} M), a derivative of carbonyl cyanide phenylhydrazone, and S-13 (5×10^{-6} M), a salicylanilide analogue, were used as inhibitors of oxidative phosphorylation.

The effect of temperature (20°C and 4°C) on HRP absorption was also determined.

Morphologic Studies

Gut sacs were processed for morphologic studies as follows: after incubation with a mucosal medium containing HRP, single sacs from adult jejunum and ileum were examined by light and electron microscopy at 5, 15, 30, 60, and 90 min after incubation *in vitro*. Individual jejunal and ileal sacs from mature rats were also examined after 15 min of incubation with a mucosal medium containing either 1% bovine serum albumin in Krebs-Ringer buffer or 0.1 M phosphate buffer, pH 6, alone.

In addition, gut sacs from neonatal jejunum and ileum were examined by light and electron microscopy at 15, 30, and 60 min after incubation in HRP-containing media. Six gut sacs from adult animals were examined after 15 min and 30 min of incubation in HRP solution containing S-13 at inhibitory (5×10^{-6} M) or toxic levels (1×10^{-5} M), and six from adult animals incubated in HRP and 2,4-dinitrophenol at inhibitory (2×10^{-5} M) and toxic (1×10^{-4} M) concentrations.

As a control for the presence of endogenous peroxidase activity, gut sacs from both adult and neonatal jejunum and ileum were incubated in mucosal media containing 10 μ M bovine serum albumin (mol wt 68,000) and then examined at 15, 30, and 60 min.

After incubation *in vitro*, sacs were immersed in a cold solution of 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4. While immersed in this fixative, the tissues were diced into cubes and placed in fresh fixative for 1-2 hr. The small cubes of tissue were then rinsed in 0.1 M cacodylate buffer, pH 7.4, for about 18 hr. Those tissues which had been exposed to HRP were incubated for about 30 min at room temperature in 0.1 M Tris-HCl buffer, pH 7.6, containing 5 mg of diaminobenzidine solution (11) to which 0.1 ml of 1% H_2O_2 had been added; the incubation was continued for 15 min. After the cytochemical procedure, specimens were washed three times in cacodylate buffer. All specimens were fixed for 1 hr in Dalton's chrome osmium, and the tissues were stained en bloc with uranyl acetate for 1.5 hr, dehydrated in a graded series of ethanol and propylene oxide, and embedded in Epon-Araldite. Thin sections were cut on an LKB ultramicrotome (LKB Instruments, Inc., Rockville, Md.) with a diamond knife and were examined in a Philips EM 200 electron microscope. Only those specimens which had not been incubated in the cytochemical medium were then stained with lead and uranyl ions.

RESULTS

Absorption Studies

To minimize the difference in absorptive surface area between the jejunum and ileum and between the immature and adult small intestine, absorp-

tion rates were expressed as micromoles of enzyme in gut sac serosal fluid per milligram of mucosal homogenate per hour.

The results of HRP absorption in adult and 18-day old rats are shown in Fig. 1. The average absorption \pm SE was 2.89 ± 0.33 μ M/mg per hr in adult jejunum and 1.85 ± 0.22 μ M/mg per hr in adult ileum. The absorption pattern for the upper and lower small intestine of immature animals was 1.30 ± 0.25 μ M/mg per hr and 1.10 ± 0.22 μ M/mg per hr, respectively. Thus, the average absorption rate was significantly higher in the adult jejunum than in the adult ileum ($P < 0.005$) and the absorption in both sections of adult small intestine was significantly greater than in the corresponding portions of neonatal intestine ($P < 0.001$). No difference in absorption was noted between neonatal jejunum and ileum.

When pooled samples of serosal fluid containing absorbed HRP were concentrated by negative pressure dialysis and passed through a calibrated Sephadex G-75 column under conditions previously mentioned, HRP enzymatic activity was present in the same elution fraction as when the original enzyme solution was tested. This observa-

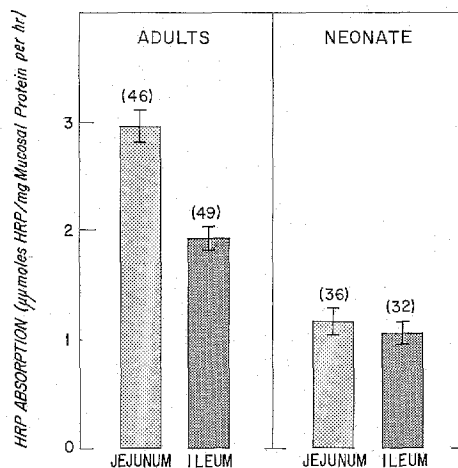


FIGURE 1 Average HRP absorption into jejunal and ileal gut sac serosal fluid from 12 adult and 16 neonatal rats. The SEM is represented by brackets, and the number of observations is enclosed in parentheses. Adult jejunal absorption is significantly greater than adult ileal absorption ($P < 0.005$). Adult jejunal and ileal absorption is also greater than in corresponding portions of neonatal intestine ($P < 0.001$). No significant difference is noted in transport from neonatal jejunum and ileum.

tion, similar to that shown in *in vivo* studies (34), suggests that no breakdown occurred in the measurable HRP as a result of absorption. To be certain that enzymatic activity in serosal fluid represented exogenous protein absorption, two additional experiments were done. HRP was labeled with Na-¹²⁵I (13) and absorption was measured by counting serosal fluid radioactivity after dialysis in a Beckman gamma counter (Beckman Instrument Inc., Fullerton, Calif.). Although the presence of iodinas in the intestinal mucosal epithelium (28) prevented a quantitative measurement of HRP absorption, the absorption pattern after dialysis in jejunal sac fluid ($1.01 \pm 0.09 \mu\text{M}/\text{mg}$ per hr.) and in ileal fluid ($1.34 \pm 0.13 \mu\text{M}/\text{mg}$ per hr) was similar to that measured by enzymatic assay. Gut sacs were incubated in a $10 \mu\text{M}$ bovine serum albumin solution, and the endogenous peroxidase activity was determined. No peroxidase activity was present in the serosal fluid, thus eliminating any contribution of endogenous peroxidase to the over-all enzyme measured.

Inhibition Studies

The effect on HRP absorption in the presence of S-13 ($5 \times 10^{-6} \text{ M}$) is shown in Fig. 2. A significant difference in both jejunal ($P < 0.01$) and ileal transport ($P < 0.005$) was noted compared to adult controls.

Table I summarizes the effect of temperature and other metabolic inhibitors on HRP absorption. A striking decrease in jejunal (33% of normal) and ileal (37% of normal) transport occurred at 4°C compared to 20°C. A decrease in absorption was also noted when CF-CCP (jejunum 59%, ileum 54%) and 2,4-dinitrophenol (49% and 50%) were used as inhibitors of oxida-

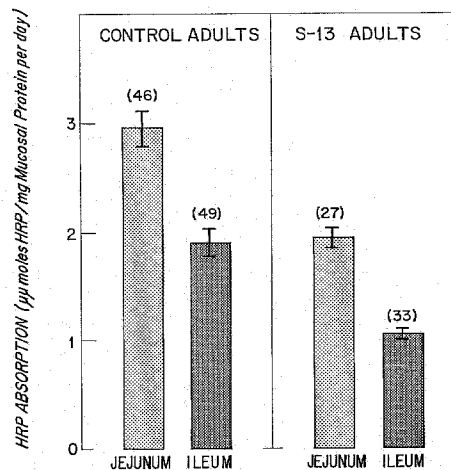


FIGURE 2 Average HRP absorption into serosal fluid of gut sacs from 8 animals incubated in the presence of inhibitor, S-13, ($5 \times 10^{-6} \text{ M}$), and from gut sacs of 12 control animals. A significant difference in absorption was noted when jejunum sacs ($P < 0.01$) and ileum sacs ($P < 0.005$) from both groups were compared.

TABLE I
Energetics of HRP Absorption

Condition	Absorption			
	Jejunum		Ileum	
	% of normal			
Temperature				
4°C	33	(22/22)	37	(21/24)
20°C	100	(8/8)	100	(7/8)
Metabolic inhibitors				
S-13	40	(19/27)	45	(27/33)
($5 \times 10^{-6} \text{ M}$)				
CF-CCP	59	(14/14)	54	(14/17)
($1 \times 10^{-6} \text{ M}$)				
2,4-DNP	49	(24/36)	50	(24/37)
($2 \times 10^{-5} \text{ M}$)				
Sodium fluoride	39	(12/20)	42	(16/19)
($5 \times 10^{-3} \text{ M}$)				

Numbers in parentheses refer to the number of observations of the total showing a significant decrease in absorption.

tive phosphorylation. In addition, NaF, an inhibitor of glycolysis, showed a decrease in jejunal (39%) and ileal (42%) absorption.

Because of the variation in total transport in different rats when exposed to metabolic inhibitors, sections of small intestine from a single animal were incubated in HRP media alone and with HRP in the presence of metabolic inhibitors. In a series of three animals, HRP absorption was greater in control segments than in those exposed to metabolic inhibitors.

Individual gut sacs were highly sensitive to slight changes in inhibitor concentration. A "toxic" response (Fig. 3) was frequently noted when inhibitor levels were increased by two- to fourfold. This toxic response was characterized by enzyme activity in serosal fluid 30-50 times the control values, by a reversal of differences between ileal and jejunal absorption, and by extensive cytolysis of intestinal epithelium.

Light and Electron Microscopy

Except in experiments with high concentrations of inhibitors, the epithelial lining of the gut sacs was largely preserved; only rare areas of epithelial denudation were observed, usually at the very tips of villi. The major changes at the light micro-

scopic level involved extensive vacuolation of the supranuclear cytoplasm of the absorptive cells and dilatation of the intercellular spaces between adjacent absorptive villus cells (Fig. 4). The cells lining the crypts retained their normal light and electron microscopic appearance. In some instances, even at the light microscopic level, one could see a pronounced accumulation of peroxidase reaction product along the basal lamina of the villus epithelium (Fig. 4) and in the intercellular spaces between adjoining absorptive cells. By electron microscopy, the microvilli were normal in appearance, and in general, the intercellular junctional zones retained their structural integrity. Reaction product was often present right up to the junctional zone (Figs. 5 and 6), but ordinarily could not be detected as passing through the tight junctions of the absorptive cells. Transmission of HRP into and within absorptive cells was very comparable to what we previously observed *in vivo* (9, 34) and followed the general form expected of an endocytic process. The major departure from the *in vivo* situation was the presence of HRP within large numbers of extensively developed cytoplasmic vacuoles (Fig. 5). Although our *in vivo* studies had shown HRP to be present in small numbers of similar structures (9, 34), they

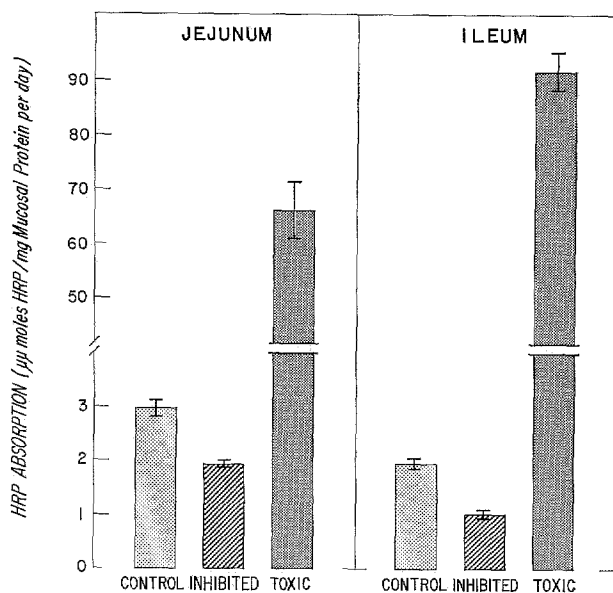


FIGURE 3 Toxic absorption response of rat intestinal epithelium to metabolic inhibitors. Adult rat gut sacs showed a 30- to 50-fold diffuse increase in HRP transport when concentrations of 2,4-dinitrophenol were increased from a 2×10^{-5} M (inhibitory levels) to a 1×10^{-4} M concentration, suggesting a cytotoxic effect.

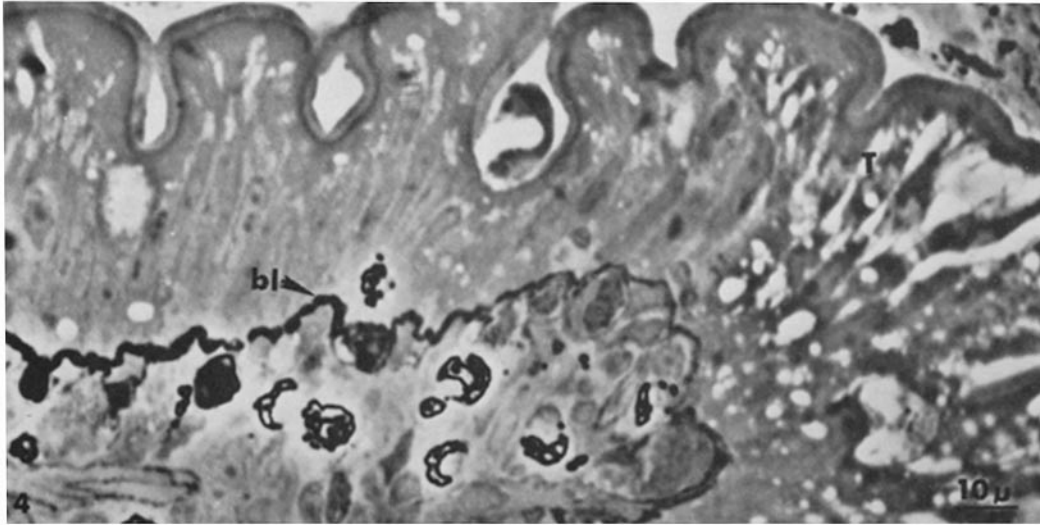


FIGURE 4 Intestinal villus in gut sac of adult rat incubated for 15 min in HRP medium. Note the extensive vacuolation at the tip of the villus (T) and reaction product along the basal lamina (bl) $\times 850$.

were much more abundant in the gut sacs. These large cytoplasmic vacuoles, seen by both light and electron microscopy, were broad structures often a couple of microns in diameter; they usually contained an electron-lucent center, a smooth-surfaced, trilaminar unit membrane, and most often showed the HRP reaction product limited to their periphery, closely adherent to the inner surface of the limiting membrane (Fig. 7). The development of an abundance of such large vacuoles appeared to be secondary to the conditions of the *in vitro* incubation rather than to HRP absorption *per se*, such structures were also abundant in gut sacs incubated *in vitro* with Krebs-Ringer buffer alone or in buffer containing 1% bovine serum albumin.

Transport of HRP in neonatal sacs was similar to that in adult animals (Fig. 8). Reaction product was present in vesicles within the tubulo-vacuolar system. Large vacuoles were present but the epithelium was intact at both the light and electron microscopic levels.

In control experiments in which bovine serum albumin instead of HRP was used in the mucosal incubation media, no endogenous peroxidase activity was apparent in ultrastructural sections of jejunal and ileal epithelial cells from neonatal and adult animals. Peroxidase activity was present, however, in blood vessels within the lamina propria.

The appearances of gut sacs incubated in non-toxic but inhibitory concentrations of metabolic inhibitors were essentially the same as in control animals (Fig. 9). No difference in the uptake pattern was noted. The cell structure was intact, tight junctions were preserved, and no significant cytolysis was noted at ultrastructural levels. When tissues were incubated with toxic concentrations of metabolic inhibitors, however, there was a stripping away of the epithelium from the lamina propria (Fig. 10), marked cytolysis, and loss of tight junction integrity. In addition, evidence of cellular degeneration was observed ultrastructurally.

DISCUSSION

HRP can cross the adult rat intestinal epithelial barrier as evidenced by these physiological and morphologic studies. This observation refutes the notion that rat intestine becomes impermeable to macromolecules 20 days after parturition (12, 14, 27). Furthermore, the transport mechanism appears to be an endocytotic process similar to that observed in neonatal animals (6, 8, 21).

Macromolecular uptake by immature intestinal epithelial cells has been extensively studied morphologically. Clark (6), using 10-day old mice and rats fed bovine gamma globulin and ovalbumin, noted vesicles containing these macromolecules in thin sections of ileal epithelial cells. He concluded

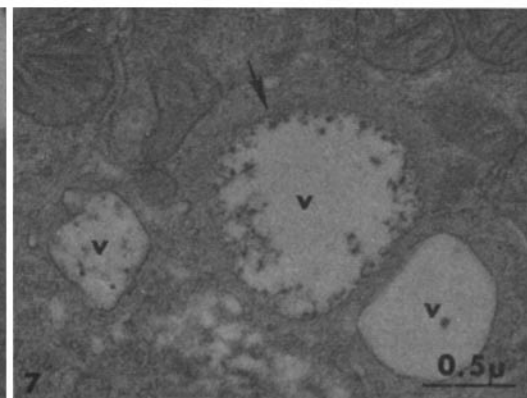
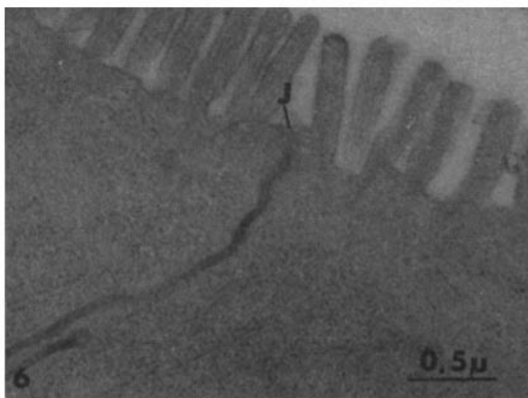
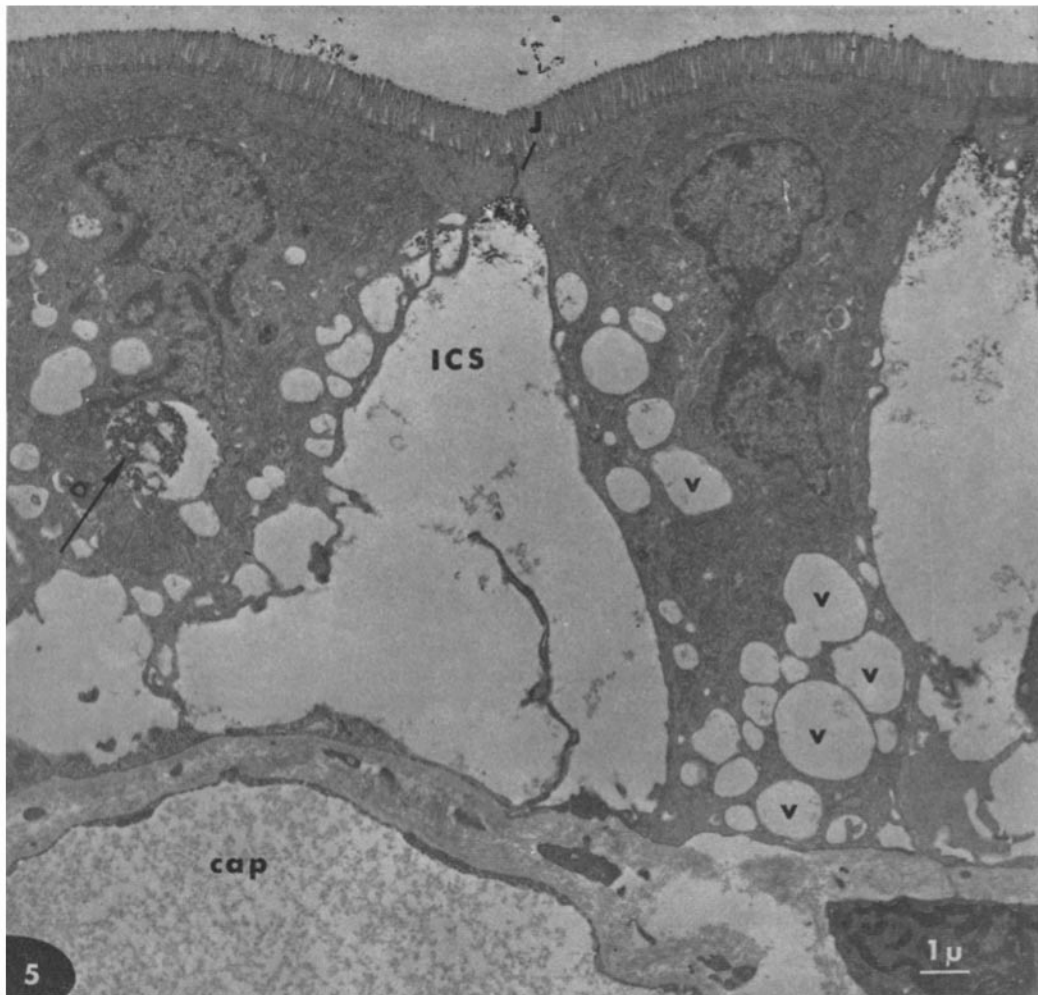


FIGURE 5 Low power electron micrograph of epithelial absorptive cells in gut sac of adult ileum incubated for 30 min in HRP medium. Note the intact intercellular junction (J) with reaction product in the dilated intercellular space (ICS). HRP is present in large vacuoles within the absorptive cell (→); cap, capillary. $\times 6500$.

FIGURE 6 Higher power view of intercellular junction between two absorptive cells. Note the intact junction (J). $\times 25,000$.

FIGURE 7 High power view of large intracellular vacuoles (V) with reaction product noted to be most concentrated at the periphery (→). $\times 25,000$.

that the immature gut absorbed proteins by pinocytosis. Cornell and Padykula (8), working with suckling rats, concluded that uptake might be nonspecific and that protein and lipids could be transported by a similar intracellular pathway. Kraehenbuhl and Campiche (21) showed that various species of newborns could engulf macromolecules, even if the macromolecules are not transmitted across the mucosa, suggesting that endocytosis was a more universal phenomenon than heretofore appreciated. They noted that species difference in adsorption was not dependent on uptake but on the extent of intracellular digestion by lysosomal enzymes.

In the present study, the morphologic appearance of HRP reaction product in the immature and adult small intestinal epithelial cells suggests that the mechanism of uptake in both groups of animals is very similar. HRP was first found to be adsorbed to the microvillus membrane, then contained within membrane-enclosed droplets of the apical microtubular region, and finally contained in large vacuoles above the nucleus. The presence of HRP in the extracellular space at the level of the nucleus and the failure to demonstrate reaction product in the basal area of the cell suggested that exocytosis occurred through the lateral cell boundary. This morphologic pattern corresponds to previously reported *in vivo* studies in adult rats (9).

Experiments designed to quantitate macromolecular absorption have largely dealt with the transport of immunoglobulins in newborn animals (2, 14, 27), using antibody titers and the specific activity of radioactively labeled gamma globulins. Uptake decreases markedly at 20 days, the time when an active immune system develops in the newborn. Little is known about the quantitative neonatal absorption of other macromolecules (12, 17). However, Clarke and Hardy (5) administered polyvinylpyrrolidone-¹²⁵I to young

rats and noted a decrease in radioactivity in the intestinal wall after 20 days. They concluded, as have others (6, 12), that intestinal closure to all macromolecules occurs at the same time as for gamma globulins. This was not the case in the present study, where adult jejunal and ileal absorption was found to exceed that of neonatal animals. The discrepancy between these observations undoubtedly relates to the sensitivity and the techniques used to measure transport. Uptake into the intestinal cell wall reflects both absorption and adsorption and is inaccurate as a measure of transport. In addition, iodineases in the intestinal cell wall (28) can release the unbound radioactive label, making quantitation of protein absorption exceedingly difficult. The decrease in radioactivity in mucosal homogenates noted after 20 days by these authors could also reflect decreased binding capacity as well as decreased absorption.

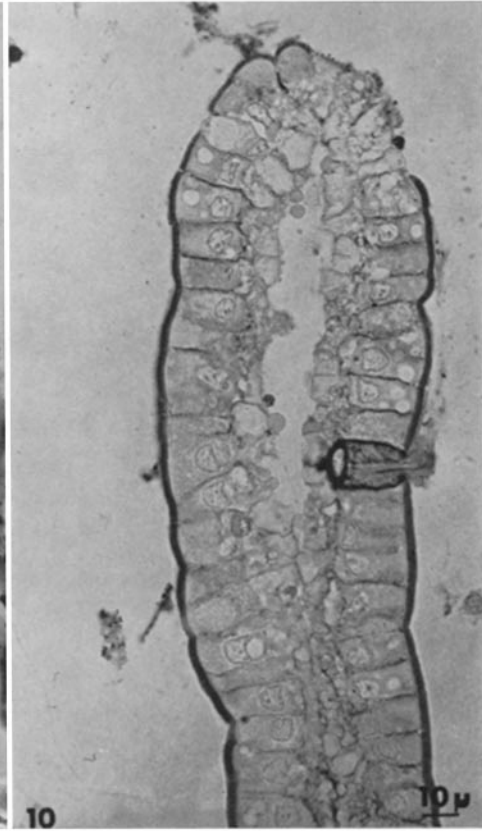
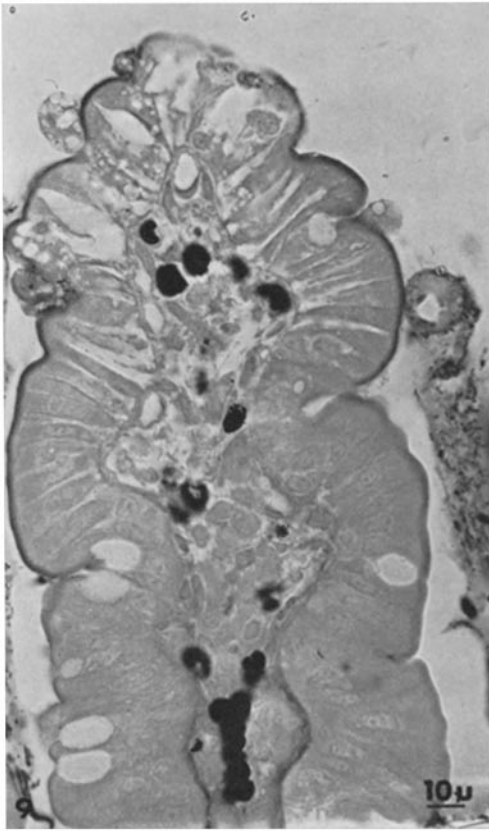
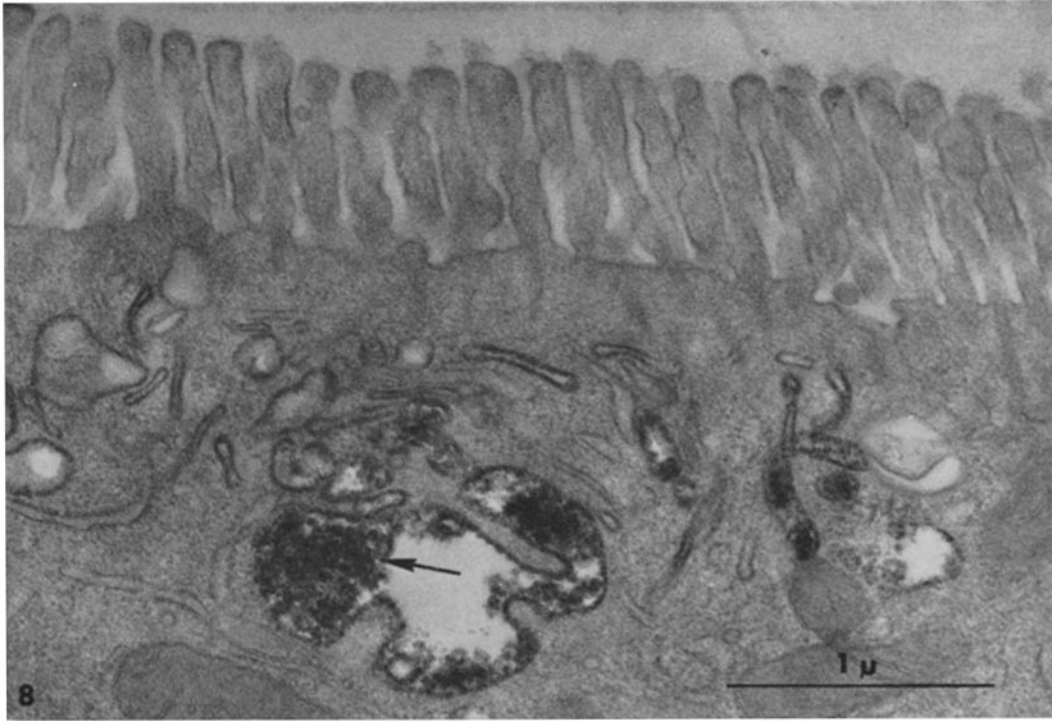
The assay used to quantitate HRP in serosal fluid was sensitive enough to distinguish between absorption in adult jejunum and that in ileum as well as that in neonatal intestine. The significantly greater HRP absorption in adult jejunum compared to ileum and compared to the upper and lower portions of neonatal intestine suggests that a selective process exists in the adult jejunum which accounts for the greater transport of this glycoprotein. Although data were expressed as absorption per mg mucosal protein in order to minimize the differences in surface area between portions of the adult and neonatal gut, a more accurate measurement of surface area is needed before this observation can be substantiated.

Rhodes and Karnovsky (29) recently reported that surgical trauma to guinea pig intestine caused an increase in HRP absorption near the site of injury. This same explanation conceivably pertains to *in vitro* absorption using the gut sac technique. However, they demonstrated a loss of

FIGURE 8 Absorptive cell from neonatal ileal gut sac incubated for 30 min in HRP reaction product. Note that the large vacuole containing the HRP is most concentrated in the periphery (→). $\times 35,000$.

FIGURE 9 Intestinal villus from an adult rat jejunal gut sac incubated in *inhibitory* concentrations of S-13 (5×10^{-6} M) and HRP medium for 30 min. Note that the cells have retained their normal integrity and are adherent to the underlying lamina propria. $\times 500$.

FIGURE 10 Intestinal villus from an adult rat jejunum incubated in *toxic* concentrations of S-13 (1×10^{-5} M) and HRP medium for 30 min. Note the loss of cell integrity, the stripping away of the epithelium from the lamina propria, and the marked permeation of HRP into the intercellular space. $\times 500$.



intercellular integrity as the primary cause of enhanced permeability. We noted no such alteration in the terminal epithelial junction. Furthermore, during the period of incubation, representative gut sacs retained viable morphologic features and continued to actively transport L-histidine-¹⁴C. The persistent difference in HRP transport in the adult jejunum and ileum also suggests a specific absorption process rather than non-specific alteration in permeability.

In various cell lines, Cohn (7) and others (18, 26, 32) have reported endocytosis to be an energy-dependent transport process. Using mouse macrophages, Cohn demonstrated a decrease in vesicle formation when the preparations were incubated with inhibitors of glycolysis, respiration, and oxidative phosphorylation (7). Lecce (23) showed a decrease in the uptake of fluorescent-labeled immunoglobulins in piglet intestinal cells when the incubation temperature was lowered to 0°C and when metabolic inhibitors were used. HRP uptake in the proximal tubular cells of adult rat kidney is also an energy-dependent process (26) requiring oxidative phosphorylation, glycolysis, and a specific sodium concentration. In our study, HRP absorption was significantly decreased in the majority of gut sacs incubated with appropriate concentrations of metabolic inhibitors and at 4°C. This observation, combined with the morphological appearance, suggests that macromolecules are absorbed by an energy-dependent pinocytotic process in adult rat intestinal epithelial cells.

The variable response of individual animals to slight changes in concentrations of metabolic inhibitors made the interpretation of data very difficult. Three patterns were noted: (a) a normal uninhibited response, (b) inhibition of absorption, and (c) sufficient cytotoxicity to cause markedly increased transmission across the small intestine. Levels of inhibitors sufficient to enhance absorption resulted in cell death, whereas when metabolism was inhibited at levels permitting continued cell viability a decrease in absorption was seen. This same observation was made by Ryser (30), measuring uptake of albumin-¹³¹I by sarcoma cells; when cytotoxicity occurred, a marked increase in uptake was noted.

This study underscores the potential role of the small intestine in the uptake of macromolecules under physiologic conditions. The process of HRP transport by the rat small intestine may

apply to the absorption of a wide variety of macromolecules, including bacterial enterotoxins such as those produced by *Vibrio cholerae* and enteropathic *Escherichia coli*, as well as to the absorption of antigenic quantities of sensitizing proteins implicated in hypersensitivity states. Evidence exists that these macromolecules can be absorbed by the normal adult intestine (19, 20, 31). Therefore, by understanding the mechanism of macromolecular transport, particularly those factors affecting membrane binding and induction of membrane invagination, we can develop insight into the pathophysiologic processes causing these diseases and begin to cope with preventing them.

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