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Observations on the Ultrastructure and Function of the So-Called "Microfold" or "Membraneous" Cells (M Cells) by Means of Peroxidase as a Tracer*

An Experimental Study with Special Attention to the Physiological Parameters of Resorption

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Summary. 79 NMRI mice and Wistar rats were used for ultrastructural investigations of the sequential uptake of horseradish peroxidase (HRP) by M cells. In addition the ultrastructure of the so-called tuft-cells was reported.

HRP, a foreign protein antigen, was applied either by injection (Owen 1977), or by stomach tube. After variable exposure times (5 min to 3 h) segments of the distal small intestine, containing Peyer's patches, mesenteric lymph nodes and liver tissue were removed. After fixation, they were reacted with H_2O_2-3,3′-diaminobenzidine tetrachloride and were examined by light and electron microscopy for HRP reaction products. The uptake of HRP mainly occurs through the M cells in the dome epithelium of Peyer's patches with a continual transport of the antigenic material into lymphoid cells, macrophages, and dendritic reticulum cells. In the 3 h specimens a few single HRP-positive lymphoid cells can be observed within the efferent lymphatics of Peyer's patches. In addition, a continual uptake of HRP by necrobiotic enterocytes was observed. It has also been shown that after 3 h HRP is located inside the Kupffer cells of the liver. These findings also support the presumption that antigenic material can be transmitted via the portal circulation. However, definite, quantitatively and permanently recorded uptake of HRP by brush border cells was not be observed.

To exclude a toxic effect of the applied HRP on the enterocytic epithelium additional resorptive-physiological investigations were performed using the in vivo-perfusion-recirculation method and in vitro-accumulation of L-phenylalanine.

Key words: Microfold (membraneous) cells – Tuft cells – Electron microscopy – Horseradish peroxidase – Sequential uptake.

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Introduction

The intestinal tract is a continually exposed immunobiological organ with an inner surface estimated to be approximately 200–300 m^2 in area. In view of the enormous amounts of microorganisms and alimentary antigens to which it is exposed it is an important surface in immunological homeostasis (Walker 1976). It has been estimated that about 90% of all immunoglobulins produced in the organism are directed against intestinal antigens (Ruchti et al. 1976, 1979).

A morphological correlate of the immunobiological marginal surface function is the "gut associated lymphoid tissue" (GALT). The development and functional maturation of the GALT are closely related to the course of bacterial settlement in the intestinal tract. Thus GALT belongs to secondary level lymphatic tissues, which only develop during the process of antigenic exposure (stimulation). The morphological and functional differentiations of the GALT are evidenced by the postulate that a continual but limited (controlled) antigen absorption takes place via the wall of the intestinal epithelium. Experimental inquires and clinical observations have shown that the sites of physiological antigen resorption are in the areas of specific lympho-epithelial differentiations of the mucosa (Walker et al. 1972; Walker and Isselbacher 1974, 1977; Bockman and Stevens 1977; LeFevre et al. 1978; Abe and Ito 1978; Joel et al. 1978; Ruchti et al. 1980; see also: Kraehenbuhl et al. 1979).

Overall, the studies by Owen (Owen and Jones 1974a, b; Owen 1977) have shown that specialized mucosal cells, referred to as "microfold" or "membraneous" cells (M cells) are responsible for direct antigen transport to the GALT.

The present study investigates the ultrastructure of M cells and the resorption pathway of horseradish peroxidase (HRP) applied either by stomach tube or by injection into the distal ileum. To exclude a toxic effect of the HRP on the mucosal epithelium, resorptive-physiological investigations were performed by the methods of in vivo-perfusion-recirculation and in vitro-accumulation of L-phenylalanine.

Table 1. Number ($n=79$) of animals (NMRI mice and Wistar rats) at different exposure intervals (1 min to 3 h)

Exposure times min	HRP (0.5%)		Saline (0.9%)	
	Per injectionem	Per stomach tube	Per injectionem	Per stomach tube
1	5		2	
5	3		2	
10	3	2	3	
15	3		2	
20	3			
30	3	2	2	
60	3	1	2	
90	3	2	2	
120	3	1	2	
150	3	1	2	
180	3	7	2	7
	35	16	21	7

Methods

Experiments concerning HRP[1] resorption were carried out with a total of 79 NMRI mice and Wistar rats. HRP was applied according to the injection method described by Owen (1977). In addition, a slight deviation from his method was by the use of a stomach tube (Table 1).

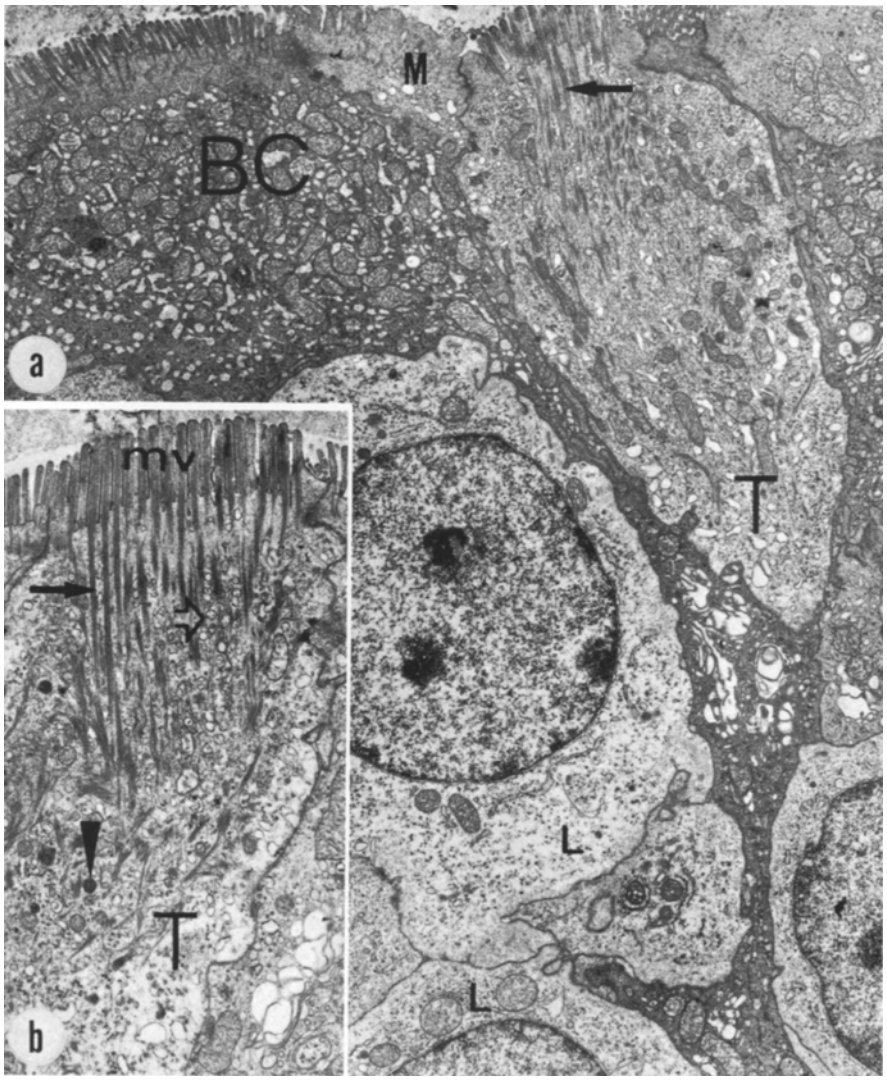


Fig. 1a, b. Epithelium over the dome of a lymphoid follicle from a control specimen ileal Peyer's patch of a mouse. Tuft cells (*T*): well developed microvillus (*mv*) with a deep-seated network of core-filaments (→). Between these core-filaments, a well developed cytocavitary system is visible (⇢). Multivesicular bodies (▼). Brush border cells (*BC*) and M cell (*M*) are seen in association with tuft cells. Intraepithelial lymphocytes (*L*). ×7,000

1 HRP, type VI, Sigma Chemical Co., St. Louis, Mo., USA

Before application of HRP the animals were starved for 12–24 h. During this time they received water ad libitum. The animals were laparotomised under ether and nembutal narcosis (0.06 mg/g body weight). 0.1 ml of a 0.5% HRP solution (HRP in 0.9% saline) were injected into the intestinal lumen proximal to the ileo-caecal valve in the area of the first Peyer's patches. Normal controls received 0.1 ml 0.9% saline without HRP. An intraluminal fixation was begun after variable exposure times (Table 1). Fixative: 2.4% glutaraldehyde and 0.8% paraformaldehyde in 0.08 M phosphate buffer (pH 7.4).

Segments of the ileum containing Peyer's patches, regional lymph nodes and segments of liver tissue were removed and placed into the fixative. Segments of the gut were cut longitudinally and pinned flat in a Petri dish. After 1 h, the Peyer's patches were cut into tissue strips and were again placed in fresh fixative. After 3.5 h of fixation at room temperature, the specimens were washed twice in 0.1 M phosphate buffer (pH 7.4). Tissue strips were then incubated for 15 min in 0.02% 3,3′-diaminobenzidine tetrachloride (DAB)[2] in 0.05 M tris-HCl buffer, pH 7.6. After that specimens were again incubated in fresh DAB solution containing, in addition, 0.01% H_2O_2. The incubation period lasted 3–6 min at 37° C, excluding all illumination (Graham and Karnovsky 1966; Malmgren and Olsson 1977). Following that specimens were washed twice in 0.1 M phosphate buffer (pH 7.4) and postfixed for 1 h in Caulfield's OsO_4 (Caulfield 1957). The tissue strips were then dehydrated through graded alcohols at room temperature and embedded in Epon 812. The cutting of 1 μ-thin sections stained with toluidine blue for light microscopy, made it possible to select representative slices for electron microscopy. Thin-sections were cut with a diamond knife on a Reichert OM U2 ultramicrotome. All sections were investigated with

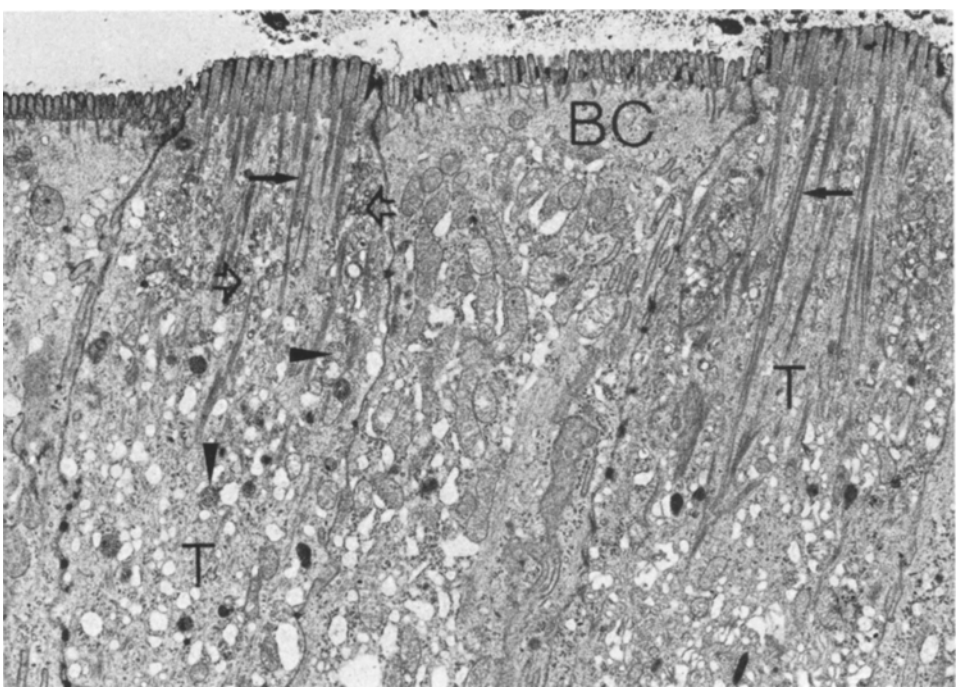


Fig. 2. Two tuft cells (*T*) with microvillous tuft, core-filaments (→), cytocavitary system (⇢) and multivesicular bodies (▼). Between tuft cells ordinary brush border cells (*BC*). HRP within the gut lumen, 10 min after injection. HRP resorption is not seen. ×7,750

2 DAB, Polysciences, Inc., Warrington, Pa., USA

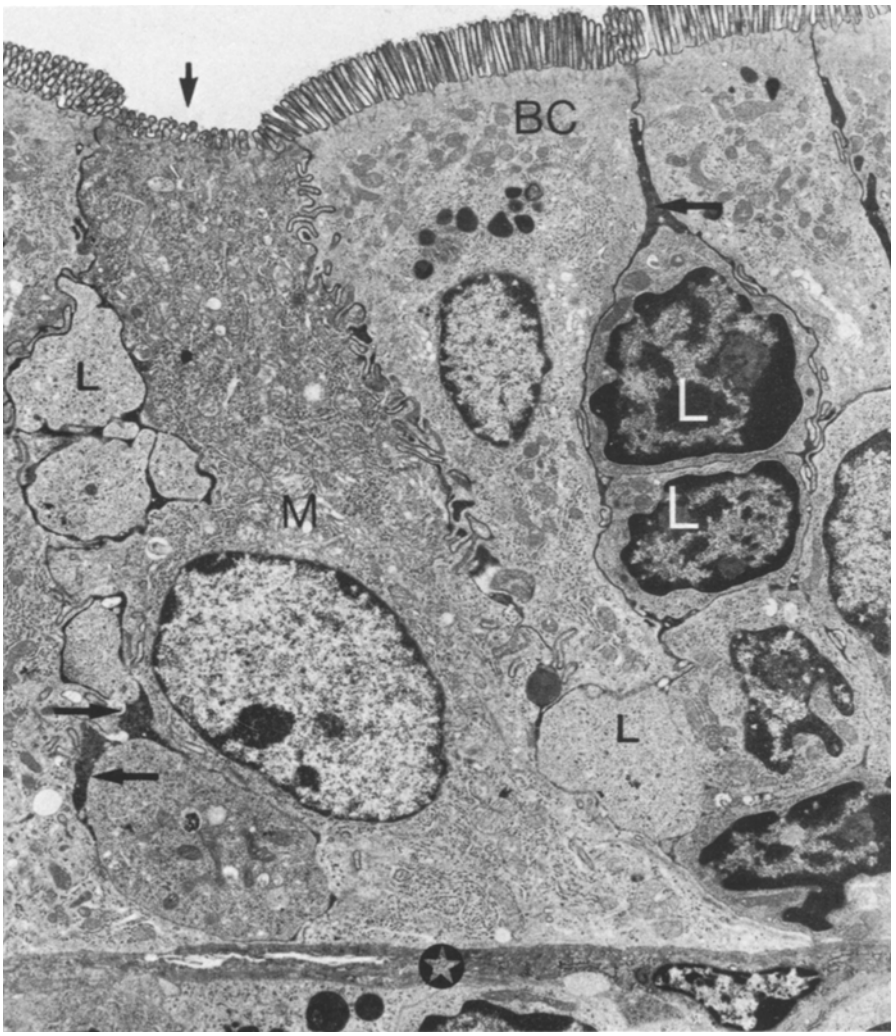


Fig. 3. Epithelium over the dome of a lymphoid follicle from a mouse ileal Peyer's patch. M cell (*M*) with irregular apical membraneous folds (↓) and basally located nucleus is situated between brush border cells (*BC*). Numerous intraepithelial lymphocytes (*L*). HRP located within the interstice (→). M cells, like brush border cells, rest on the basement membrane (✪). HRP specimen, 1 h. ×6,000

a Zeiss EM 9A electron microscope, either without additional staining or, in some cases, after staining with uranyl acetate and lead citrate.

To exclude HRP diffusion through the enterocytic epithelium caused by a toxic effect of the tracer, 27 Wistar rats (14 experimental animals, 13 controls) were used for resorptive-physiological experiments by the in vivo-perfusion-recirculation method (Menge et al. 1970) and the in vitro-accumulation of L-phenylalanine (Robinson and Felber 1965).

Parallel tissue blocks from the same material were stained with ruthenium red and inspected by electron microscopy as well. The ruthenium red dye was prepared according to the method described by Luft (1971) and by Gebbers and Otto (1974).

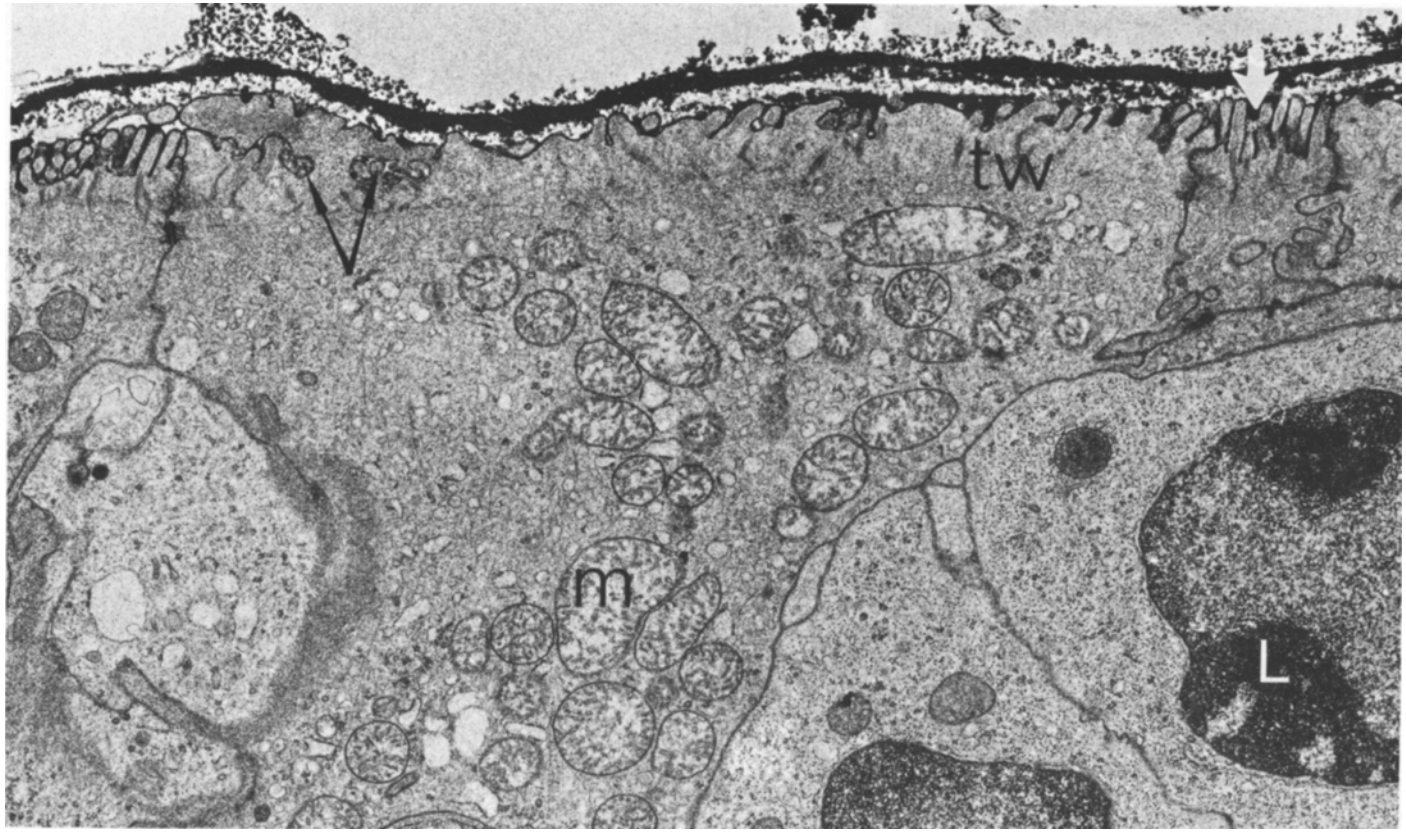


Fig. 4. Apical sections of M cells with irregular membraneous folds at their surface. Some microvilli are nearly fully developed (↓). Structures of the terminal web (*tw*) are only rudimentary. Mitochondria (*m*), intraepithelial lymphocytes (*L*). HRP specimen: Reaction product coasts the short irregular microvilli and extends down into pits in the M cell apex (→). × 9,400

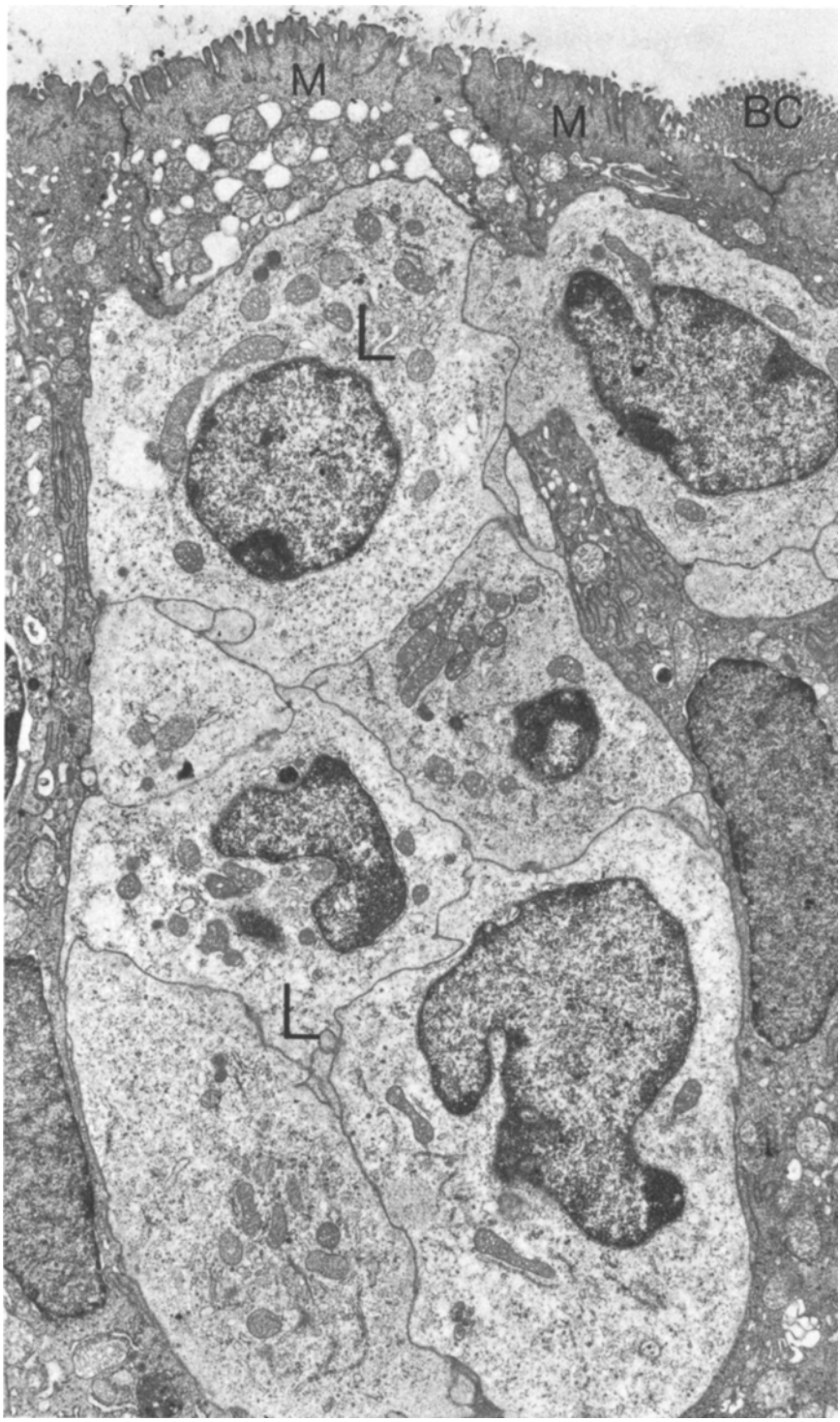


Fig. 5. M cells (*M*) with many intraepithelial lymphocytes (*L*). Control specimen. ×7,230

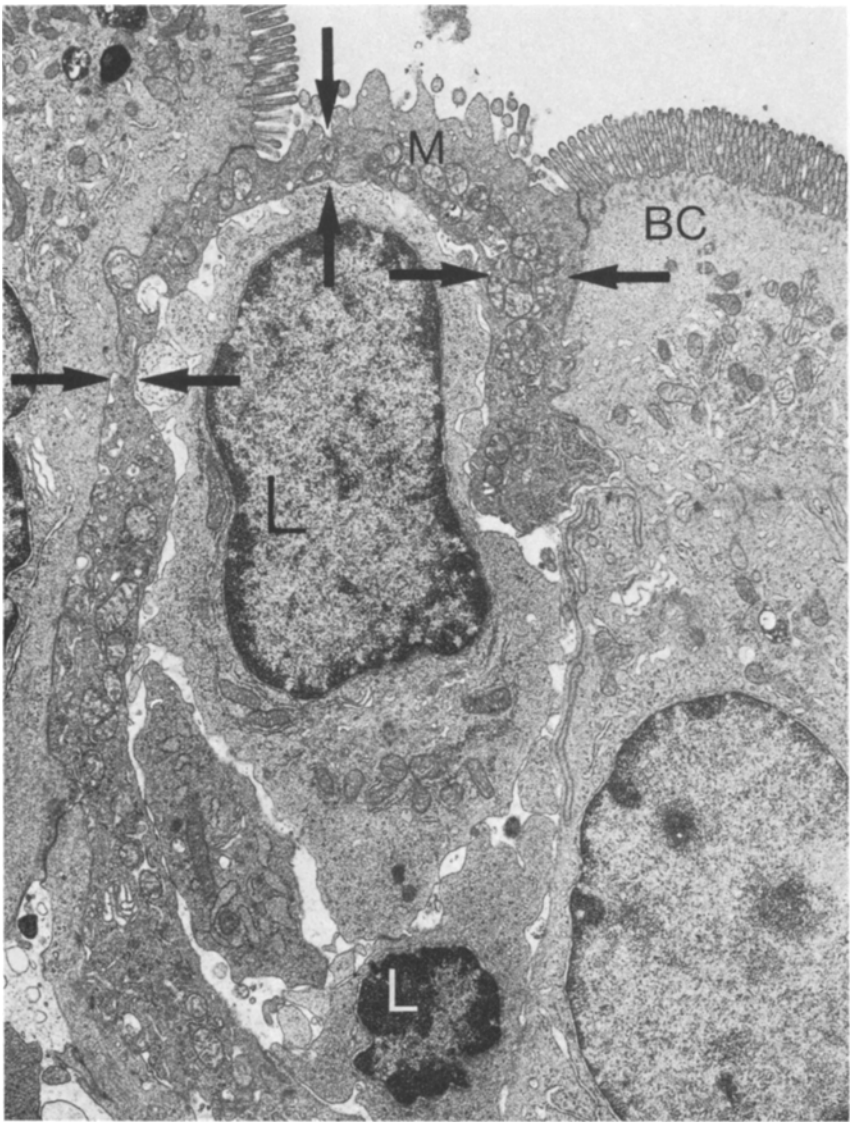


Fig. 6. Apical part of M cell (*M*) with associated lymphoid cells (*L*). Note the attenuated cytoplasm of the M cells (between arrows) which bridges the surface between brush border cells (*BC*), forming tight junctions together with them and producing a barrier between the lymphoid cells and the intestinal lumen. Control specimen. ×8,050

Results

The epithelial layer of the mucosa consists of several structurally and functionally dissimilar cells or cell systems (Otto 1978). Peyer's patches and solitary follicles of the appendix and the small intestine are covered by the so-called dome epithelium which contains numerous "membraneous" cells (M cells) as well as cells referred to as "tuft" cells.

Tuft cells are enterocytic cells with very well developed microvilli which project far into the gut lumen (Figs. 1, and 2). The surface of the microvilli is covered with the glycocalyx which

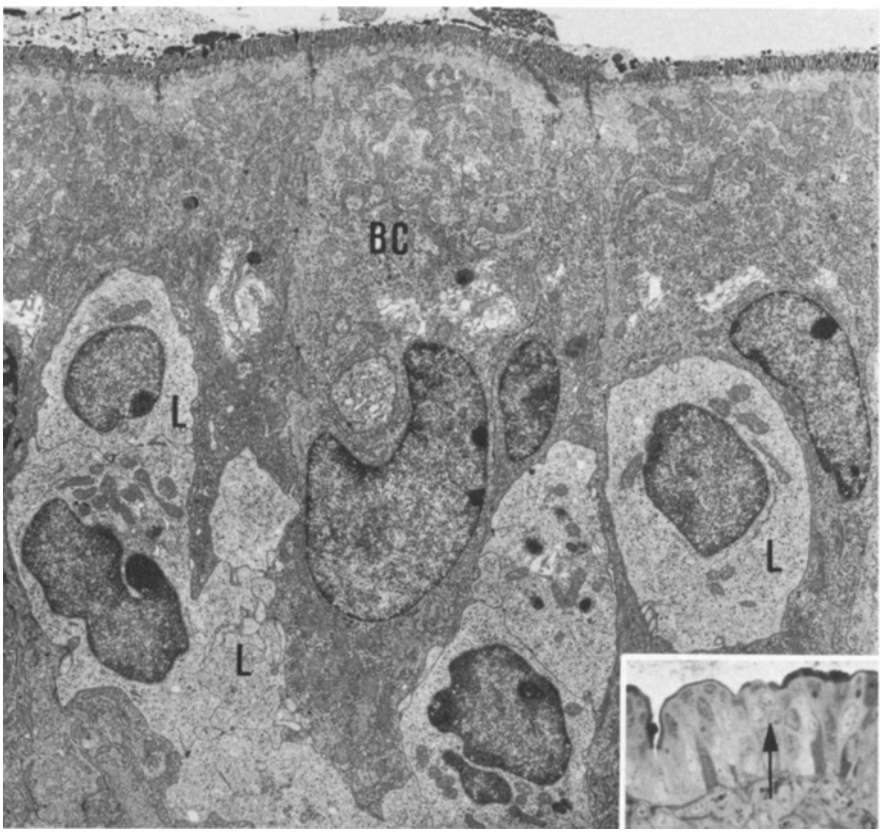


Fig. 7. Brush border cells (BC) over the dome of a lymphoid follicle from a mouse ileal Peyer's patch with numerous intraepithelial lymphocytes (*L*). HRP specimen, 30 min. × 5,600. Inset. Epithelial layer, so-called dome epithelium with numerous intraepithelial lymphocytes (↑). Semithin section, toluidine blue. × 380

can be stained with HRP and ruthenium red. Inside the microvilli so-called "core" filaments are developed, extending far into the basal cell areas down to the level of the nucleus. Between these filaments there is a system of caveolae and tubules (Fig. 2). Tuft cells contain comparatively few mitochondria. They contain so-called multivesicular bodies. Tuft cells are connected to bordering enterocytes by tight junctions as well as desmosomes. They can also be detected in the vicinity of M cells. Characteristic HRP resorption (as seen in the M cells) is not observed in tuft cells (Fig. 2).

M cells have baso-apical differentiation (Fig. 3). They rest on the enterocytic basement membrane which is often perforated. Instead of the usual brush border they have irregular surface projections and vesiculations with heavy endocytotic activity. Normal microvilli as well as core filaments and terminal web structures are seldom seen (Fig. 4). A glycocalyx is not often visible. M cells contain numerous mitochondria and a relatively well developed endoplasmic reticulum. Most of their nuclei are comparatively large and located in the basal part of the cell. They appear to be euchromatic, although chromatin condensations

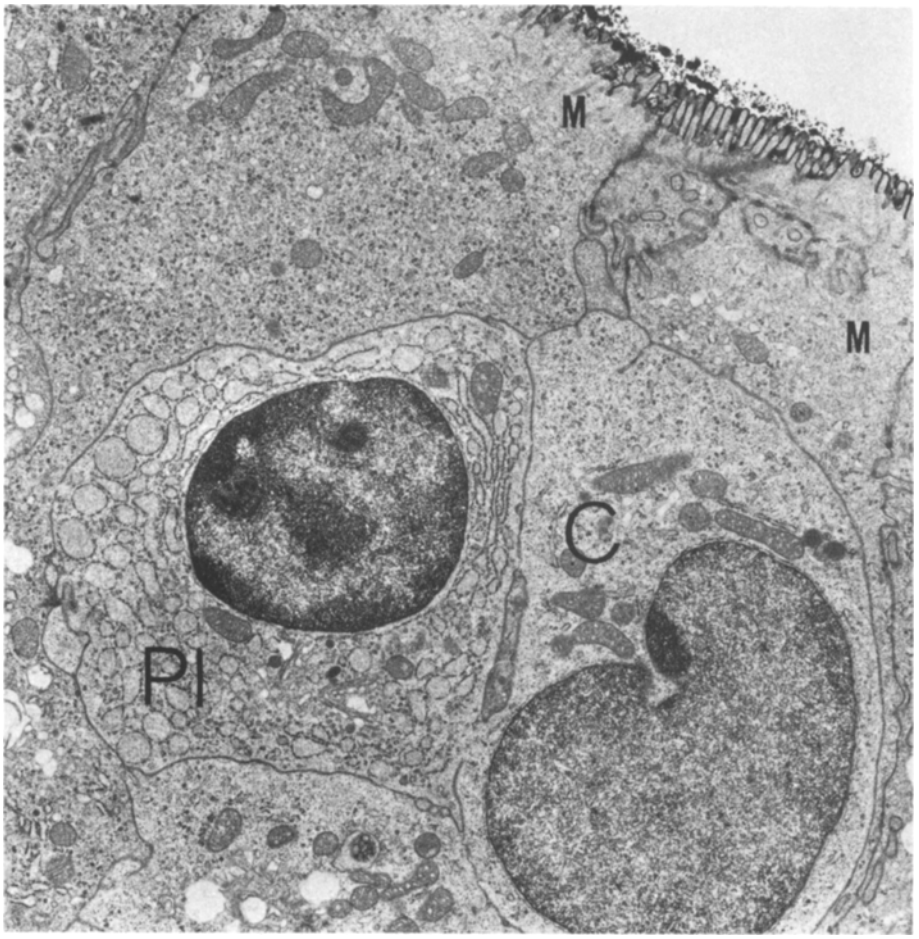


Fig. 8. Apical part of M cells (*M*) with intraepithelial lymphoid cells of distinctive differentiation. Plasmacytic cell (*PI*) and lymphoid cell with cerebriform nucleus (*C*). HRP specimen, 10 min. ×8,050

and larger nucleoli can often be found at the margin of these nuclei. Another characteristic of M cells is that of forming a disordered network which closely surrounds numerous (interepithelial) lymphocytes (Figs. 5 and 6). Lymphoid cells which are situated within the dome area of the epithelial layer (Fig. 7) are distinctly heteromorphic in their structure. Small lymphocytes are found together with lymphoid round-cells with cerebriform nuclei, lymphoblastic transformed cells and plasmacytoid cells (Fig. 8). Within this dome epithelium interepithelial lymphocytes approach the intestinal lumen up to a distance of approximately 0.3 μ but, remain separated from it by the cytoplasmic projections of M cells (Fig. 6).

After an intraluminal HRP (or ruthenium red) application, either by injection into the distal ileum or by use of a stomach tube, marked HRP (or ruthenium red) resorption took place.

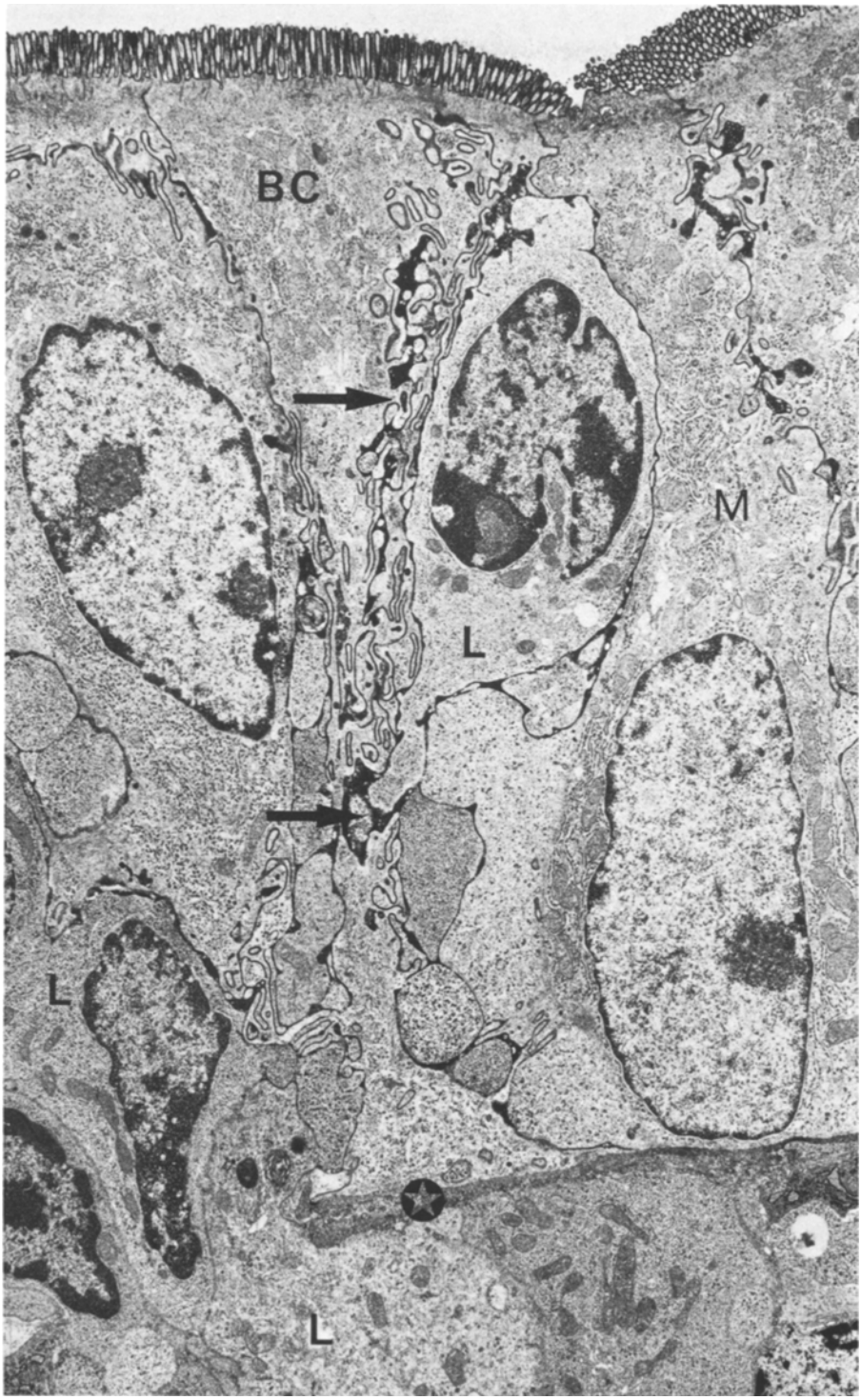


Fig. 9. Lymphoid follicle epithelium one h after application of HRP into the intestinal lumen. M cells (*M*) and associated lymphocytes (*L*) with well developed interdigitations. Brush border cells (*BC*). HRP reaction products are found within the intercellular space (→). Basement membrane (✪) HRP specimen, 1 h. ×6,200

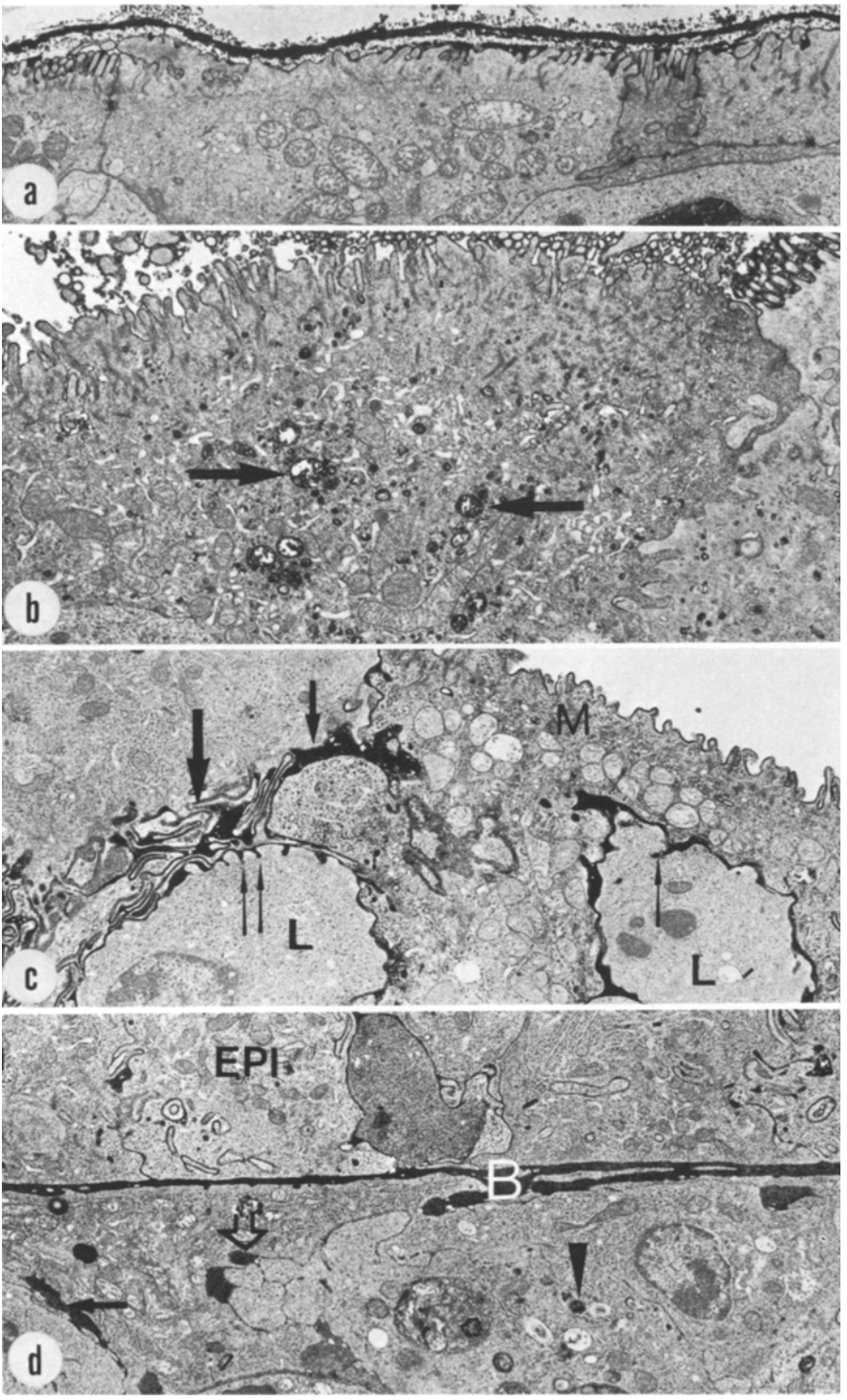
Five- and 10-min HRP Specimens. The applied HRP can be found in the area of the glycocalyx of enterocytic brush border cells and also adhering to the apical membrane folds of M cells and tuft cells (Figs. 4 and 10a). In some M cells apical membrane invaginations and a few single HRP-positive vesicles in the apical cytoplasm are observable. The lateral intercellular space does not show any HRP reaction products.

20- and 30-min Specimens. These specimens showed numerous HRP-positive vesicles within the apical cytoplasm of M cells (Fig. 10b). A peculiar membrane-associated HRP activity could be observed. Homogeneous and granular stained HRP-positive vesicles were comparatively rare. A significant reaction of the intercellular space with the tracer could not be recorded. Even in close relation to M cells, interepithelial lymphocytes remained free of HRP-positive reaction products. Brush border cells and tuft cells did not show any HRP uptake. In the deep-seated lymphatic tissue of solitary follicles and Peyer's patches HRP was also lacking.

One-Hour Specimens. Above all, HRP reaction products were detectable in the M cell-lymphocyte intercellular space (Figs. 9 and 10c). HRP could often be clearly detected within the intercellular space between brush border cells which are situated in the vicinity of M cells. However, HRP-labelled endocytotoic activity of these enterocytes was not recoreded. Between M cells and M cell associated lymphocytes well developed membrane interdigitations exist. The exterior cell membrane of the lymphocytes is clearly stained with HRP. Occasionally lymphocytes show membrane invaginations and HRP-positive submembraneous vesicles (Figs. 10c and 14). The marginal area of perforations along the basement membrane is often focally stained with HRP. Around these areas lymphocytes and macrophages can frequently be observed. They are partially located inside the epithelial layer as well as in the subepithelial layer near the basement membrane. Some of the macrophages in one-hour specimens already showed HRP-positive vesicles in their intraepithelial projections. Continual staining of the basement membrane or the subepithelial space together with staining of lympho-reticular cells was not seen.

120-, 150- and 180-min Specimens. When compared with the HRP reaction in the one-hour specimens these animals showed a nearly continuous reaction,

Fig. 10a–d. Sequential uptake of HRP after intestinal application. **a** Apical part of M cells, 10 min after HRP application. Reaction products coat the short irregular microvilli. ×5,600. **b** Apical part of M cells, 30 min after HRP application. Reaction products fill many vesicles (→) within the cytoplasm of the M cells. ×8,250. **c** M cells (*M*) and associated lymphocytes (*L*) after 1 h exposure to HRP. HRP has been transported into the M cell-lymphocyte intercellular space (→) as well as into the intercellular space to bordering columnar cells. Lymphocytes with HRP-positive membrane invaginations (↑↑). ×10,800. **d** Basal epithelium sector (*EPI*) with basement membrane (*B*) and subepithelial lymphoid tissue. Reaction products are found in the basal intercellular space of the epithelium, in the area of the basement membrane (*B*), as well as within the subepithelial lymphoid tissue. They are located partially in the extracellular space (←), adhering to membranes (⇓) or within subepithelial cells (▼). 120 min after introduction of HRP. ×8,400


a
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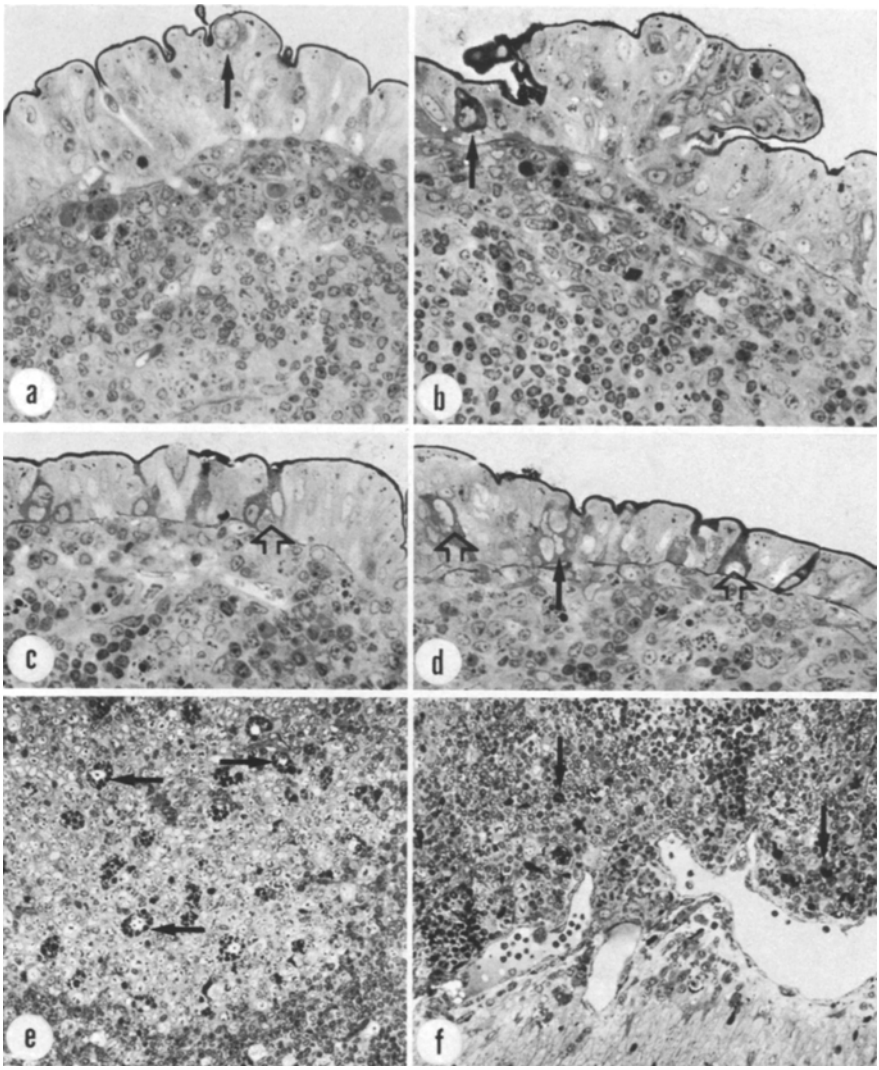

Fig. 11a–f. Light microscopy: Observations in Peyer's patches after 2 and 3 h HRP exposure. **a–d** Positive HRP reaction along the brush border, within the M cell-lymphocyte intercellular space (↑), in the area of the so-called "dark cells" (⇑), at the basement membrane and within some subepithelial macrophages. ×380. **e** Germinal center with HRP-positive macrophages (→). ×380. **f** Efferent lymphatics, the marginal area of a Peyer's patch with vessel-associated HRP-positive mononuclear cells (↓). ×380. Semithin sections, toluidine blue

around the basement membrane, within the subepithelial extracellular space, adhering to the surface of dendritic reticulum cells and inside macrophagocytic cells of Peyer's patches (Figs. 10d, 11 and 12). After 3-h exposure HRP-positive macrophages were already very abundant within the germinal centers (Fig. 11e). They were found directly around or within efferent lymphatics (Fig. 11f). Fur-

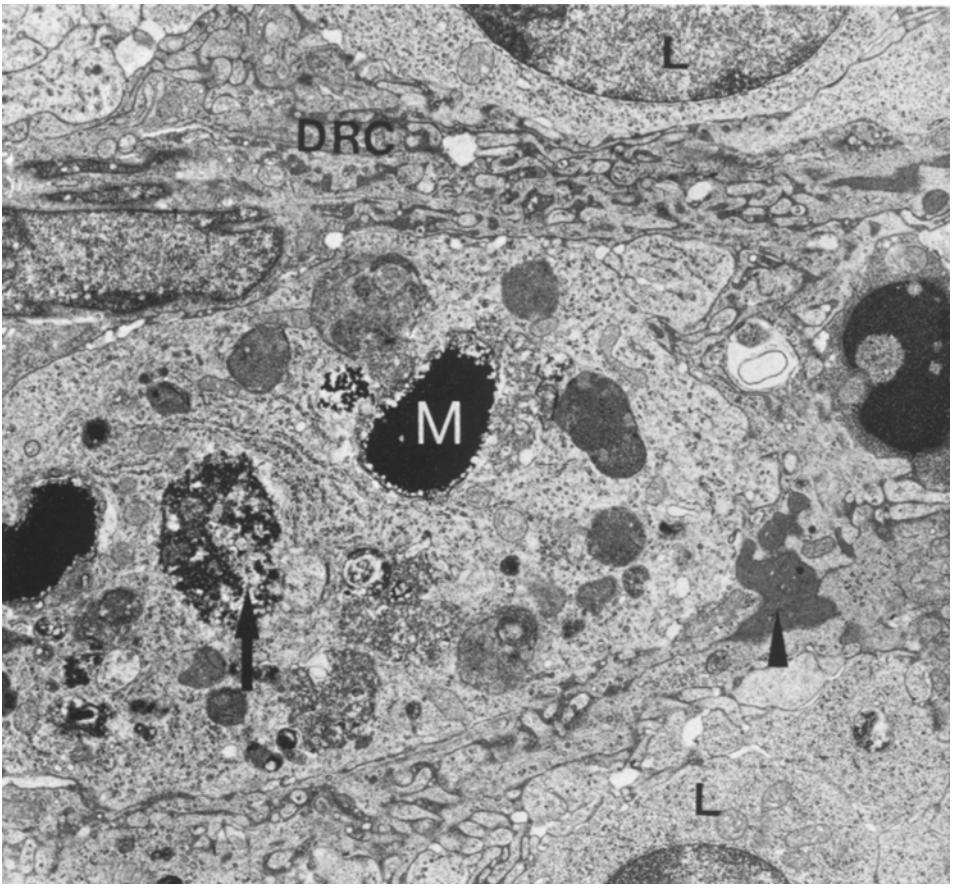

Fig. 12. Section of a lymphoid follicle from a mouse ileal Peyer's patch, 2 h after HRP application. Dissimilar electron-dense reaction products are found within macrophages (*M*, ↑), sometimes also extracellularily (▲). Lymphocytes (*L*). Projections of dendritic reticulum cells (*DRC*). × 5,350

thermore, HRP-positive reaction products in these specimens are seen inside the venous endothelium of intestinal vessels as well as in the Kupffer cells of the liver (Fig. 13).

Control Specimens. Controls received saline solution without HRP. The controls were sacrificed according to the corresponding HRP exposure times. At 5, 10, 20, 30, 60, 120, 150, and 180 min after application of saline solution the control specimens showed reaction products from endogenous peroxidase in erythrocytes and leucocytes, but not in the glycocalyx or the epithelial cells. Control specimens, fixed at variable intervals, differed only by a remarkable dilatation of the extracellular spaces.

The experiments have shown that an undetermined amount of HRP passes through the intestinal wall in the area of necrobiotic enterocytes. The cytoplasm of these necrobiotic cells showed a diffuse staining. Remarkably, the basal part of these necrobiotic cells was nearly always demarcated by interepithelial

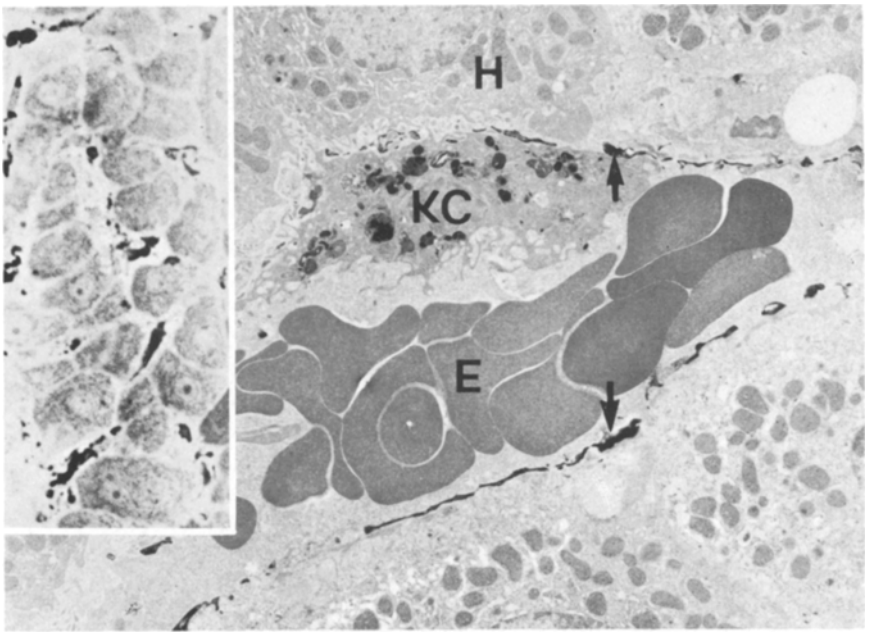

Fig. 13. HRP-positive Kupffer cells after 3 h HRP exposure. HRP reaction products are found within the cytoplasm of the Kupffer cells (*KC*) and membrane-associated of sinus endothelium (↑). Erythrocytes (*E*). Hepatocytes (*H*). ×4,000. Inset. HRP-positive Kupffer cells. Semithin section toluidine blue. ×380

lymphocytes (Fig. 14). These lymphocytes showed some small membrane invaginations containing HRP-positive reaction products. While brush border cells generally showed no HRP uptake, small HRP-positive vesicles are observable within brush border cells adjacent to necrobiotic cells. All of these HRP related observations and the deep invaginations between microvilli exemplify enterocytic resorption of this tracer (Fig. 15).

Discussion

The available ultrastructural observations concerning HRP uptake by the intestine show that the intestinal tract in the area of lymphofollicular aggregations contains certain specialized enterocytes (Reviews: Parrott 1976a, b; Thiery 1978; Asquith 1979). These are appearantly capable of selective resorption regarding larger molecular and antigenic substances (Bockman and Cooper 1973; Owen and Jones 1974a, b; Owen 1977; Walker and Isselbacher 1977; Ruchti et al. 1979, 1980). These cells enclose numerous lymphoid cells within their membranes. Their luminal surface is folded irregularly and demonstrates a highly developed endocytotic activity. Due to these special features they are referred to as "microfold" or "membraneous" (M cells) (Owen and Jones 1974a, b; Owen 1977). The origin of M cells remains undetermined, as well as the question of whether their lysosomes participate in the digestion of resorbed antigenic

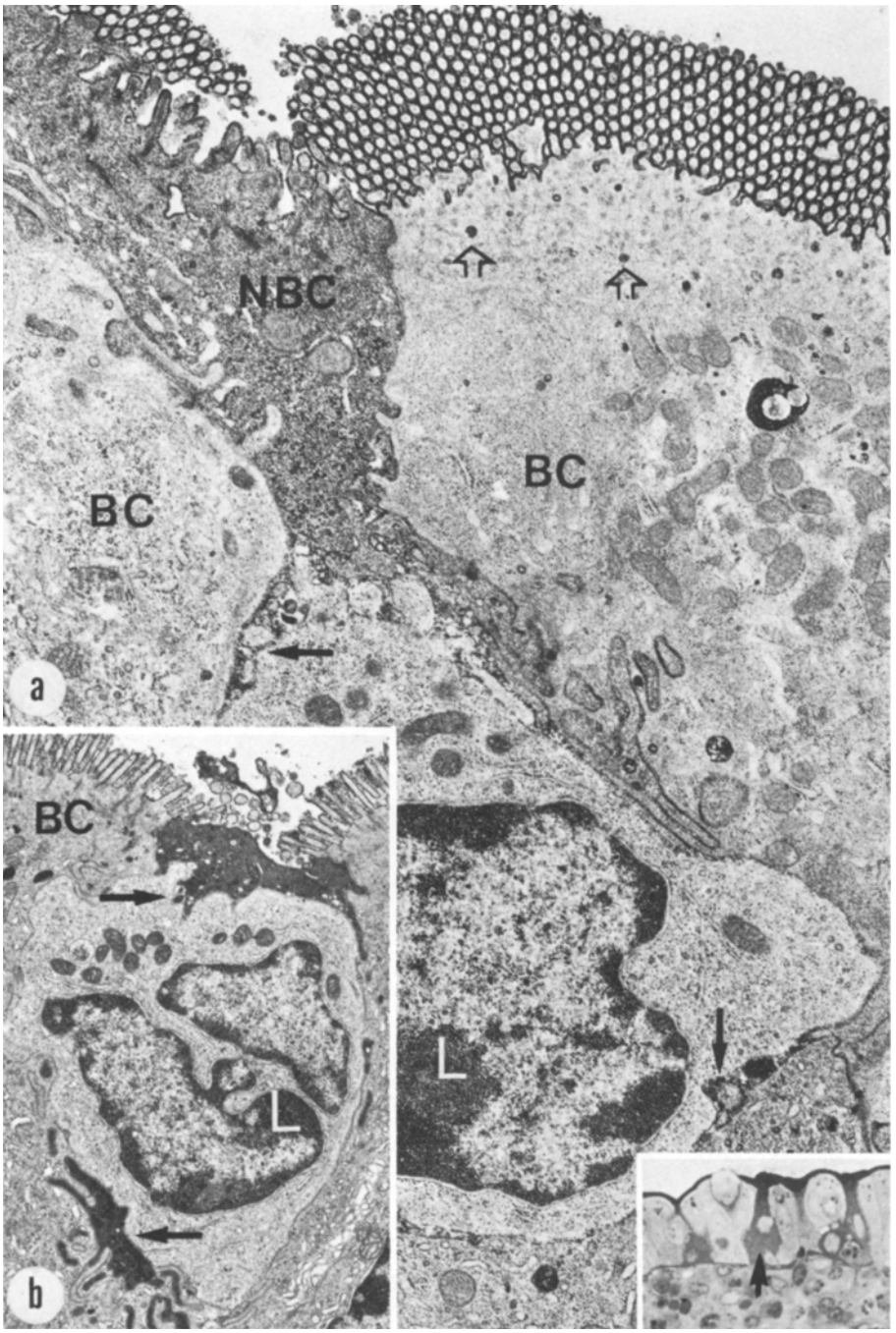

Fig. 14a, b. Necrobiotic brush border cells (*NBC*) 10 min (**a**, ×14,000) and 20 min (**b**, ×7,200) after application of HRP. The cytoplasm is diffusely stained with HRP reaction products. Within the depth of necrobiotic brush border cells, lymphoid cells are located with partially HRP-positive membrane invaginations (→). The bordering brush border cells (*BC*) are still intact. Reaction products coat the microvilli and extend down into pits in the cell apex. Within the apical cytoplasm of these brush border cells are some few HRP-positive vesicles (⇑). . Inset. Dome epithelium with necrobiotic "dark cells" (↑). The basement membrane which is associated with "dark cells" shows a positive HRP reaction. Semithin section, toluidine blue. ×380

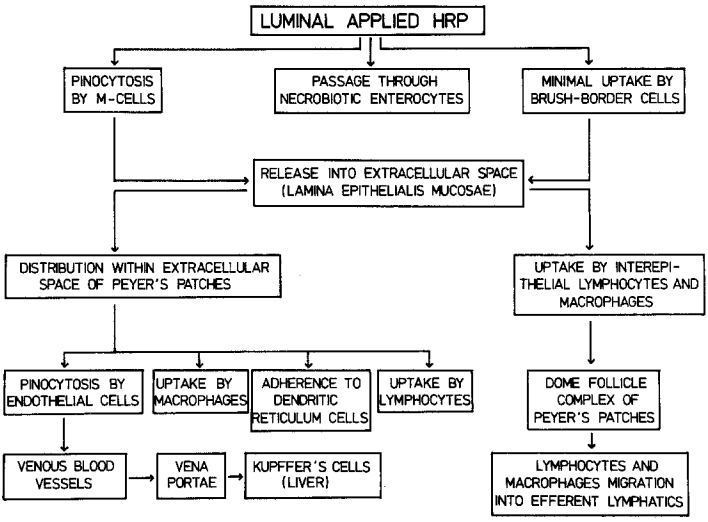

Fig. 15. Schematic synopsis of potential resorption paths of HRP across the intestinal epithelium. Further transport of resorbed HRP via blood and lymph vessels, and processing of HRP within the dome follicle epithelium of Peyer's patches. Complied from our observations and following literature: Cornell et al. (1971), Walker and Isselbacher (1974), Bockman and Stevens (1977), Owen (1977), and Joel et al. (1978)

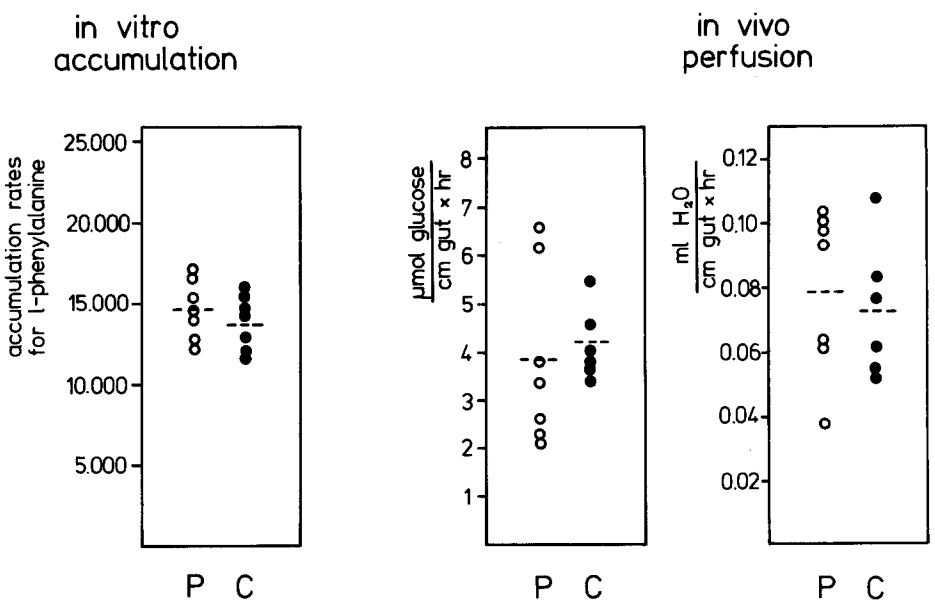

Fig. 16. List of the results concerning the examination of a potential toxicity of intraluminal applied HRP. *P* peroxidase exposed animals ($n=14$); *C* controls (saline exposed animals) ($n=13$). Left: Accumulation rates for L-phenylalanine (in vitro-method by Robinson and Felber (1965)). Right: Glucose and H_2O resorption (in vivo-perfusion-recirculation-method by Menge et al. (1970)). Both methods show that no statistically significant difference (*t*-test) between HRP exposed animals and controls exist regarding the resoprtion activity

material. In ultrastructural observations M cells do not appear to be a separate cell type. M cells show few subcellular structures which are poorly developed and are also characteristic of brush border cells (Owen 1977). Therefore, microvillous differentiations, core filaments and structures of the terminal web are found as exceptions (see Fig. 4). These observations demonstrate functional differentiation but do not demonstrate a separate histogenetic cell type (Leblond and Cheng 1976). The absence of the glycocalyx over the M cell surface may be related to their immaturity (Rao et al. 1972). It appears likely that M cells are both structurally differentiated and quantitatively variant, depending on the kind and duration of antigenic exposure. However, oral or parenteral application of HRP, bovine serum albumin (Walker et al. 1972, 1973, 1974) or ferritin (Bockman and Winborn 1966; Bockman and Cooper 1973) lead to a significant decrease in the resorption of these tracers. This phenomenon indicates a locally triggered reaction of the mucosa in respect to an active immunisation (Ruchti et al. 1980). An energy-dependent uptake mechanism of macromolecules observed by Lecce (1965) and Walker et al. (1972), and our ultrastructural findings, confirm the theory that M cells are functionally differentiated brush border cells.

Newborn mice and rats possess the ability to resorb intraluminally applied macromolecules by pinocytosis in all brush border cells [proteins and colloidal material: Clark (1959); ferritin: Graney (1968), Rodewald (1970), Shervey and Gardner (1973); adenovirus: Worthinton and Graney (1973); lipid: Cornell and Padykula (1969); carbon particles: Joel et al. (1978); HRP: Kraehenbuhl and Campiche (1969), Lev and Orlic (1973), Orlic and Lev (1973), Rodewald (1976), Kraehenbuhl et al. (1979)]. With termination of the suckling period the brush border cells loose their absorptive capacity. The controlled antigen uptake maintaining the immunological homeostasis of the organism appears to occur in adults only in the area of the GALT; mainly in solitary follicles and Peyer's patches. These are bordered by M cells at the luminal surface (Bockman and Cooper 1973; Owen 1977). In accordance with the observations of Owen (1977) and Joel et al. (1978) our results show that in adult mice and rats intraluminal applied HRP is resorbed nearly only by M cells.

The transepithelial passage of macromolecular particles takes place in a sequential manner. In the beginning, HRP is resorbed by means of pinocytosis in M cells and is then transported by vesicles. From the lateral cell membrane these vesicles extrude HRP into the extracellular space. M cell associated interepithelial lymphocytes as well as macrophages are responsible for a further uptake of HRP. In macrophages phagocytosed material (HRP, carbon particles) may persist for an extended period of time (Joel et al. 1978). Our results indicate an additional extracellular transport of HRP from the M cell associated intercellular space into the dome follicle area, with a possible adherence to dendritic reticulum cells or potential phagocytosis of the tracer by subepithelial macrophages.

After a 3 h exposure time, HRP could be detected also within the Kupffer cells of the liver. From these observations it can be postulated that the reticulohistiocytic compartement of the liver may also play a yet underestimated role in the processing of intestinal absorbed substances.

Table 2. In vitro-accumulation rates of L-phenylalanine

n	HRP specimens ($n=7$)		Control specimens ($n=7$)	
1.	14.320		15.450	
	17.550		13.800	
	17.200		13.170	
	17.120	$\bar{x}_{HRP_1}=16.730$	14.390	$\bar{x}_{C_1}=14.344$
	17.460		14.910	
2.	13.190		10.940	
	12.320		12.050	
	13.330		11.180	
	12.460	$\bar{x}_{HRP_2}=12.810$	12.000	$\bar{x}_{C_2}=11.758$
	12.750		12.620	
3.	16.600		14.150	
	18.690		14.370	
	16.840		14.430	
	16.830	$\bar{x}_{HRP_3}=16.940$	14.940	$\bar{x}_{C_3}=14.374$
	15.740		13.980	
4.	11.790		15.790	
	13.020		15.590	
	12.950		15.080	
	12.640	$\bar{x}_{HRP_4}=12.766$	15.000	$\bar{x}_{C_4}=15.530$
	13.430		16.190	
5.	15.670		15.920	
	15.120		16.670	
	15.000		16.200	
	15.210	$\bar{x}_{HRP_5}=15.282$	16.440	$\bar{x}_{C_5}=15.888$
	15.410		14.210	
6.	13.060		11.530	
	13.590		12.480	
	13.900		11.160	
	14.300	$\bar{x}_{HRP_6}=14.074$	11.760	$\bar{x}_{C_6}=11.778$
	15.520		11.960	
7.	12.820		14.070	
	15.460		12.030	
	14.860		12.180	
	15.150	$\bar{x}_{HRP_7}=14.616$	12.450	$\bar{x}_{C_7}=12.766$
	14.790		13.100	
		$\bar{\bar{x}}_{HRP}=14.745$		$\bar{\bar{x}}_{C}=13.777$
		$s_{HRP}=1.691$		$s_C=1.698$

t-test: test statistic ($\hat{t}$) = 1.06905 < fractile ($t_{12;\,0.975}$) = 2.18 (statistically not significant)
Level of significance $\alpha = 5\%$

In addition, our experiments show that HRP also passes through necrobiotic enterocytes, and through areas of discontinuous epithelium (epithelial desquamations). Necrobiotic enterocytes show a diffuse cytoplasmic HRP reaction ("dark cells"). Similar dark staining of necrobiotic cells was noted by Cornell et al. (1971) and by Owen (1977) with HRP, and by Boom et al. (1974) with ruthenium

Table 3. Glucose and H_2O resorption, in vivo-perfusion-recirculation-method

Glucose resorption		Volume (H_2O) resorption	
HRP specimens ($n=7$) (μmol/cm gut × h)	Control specimens ($n=6$) (μmol/cm gut × h)	HRP specimens ($n=7$) (ml/cm gut × h)	Control specimens ($n=6$) (ml/cm gut × h)
2.52	3.46	0.037	0.055
3.35	3.93	0.062	0.109
6.51	3.85	0.102	0.084
2.14	5.39	0.064	0.077
3.81	4.52	0.102	0.053
2.38	3.94 $\bar{x}_C=4.18$	0.094	0.061 $\bar{x}_C=0.073$
6.23 $\bar{x}_{HRP}=3.85$		0.098 $\bar{x}_{HRP}=0.080$	
t-test: test statistic ($\hat{t}$) = 0.60855 < fractile ($t_{11;\,0.975}$) = 2.20 (statistically not significant) Level of significance $\alpha=5\%$		t-test: test statistic ($\hat{t}$) = 0.50582 < fractile ($t_{11;\,0.975}$) = 2.20 (statistically not significant) Level of significance $\alpha=5\%$	

red. The basal part of these cells was nearly always demarcated by interepithelial lymphoid cells. The possible resorption pathways for the transport of macromolecular particles through the epithelial layer are listed in Fig. 15.

The intestinal uptake of HRP has been studied under various experimental conditions. The methods are much improved by a more physiological application of HRP. The advantage of a free HRP distribution along the intestinal wall is evident in comparison with earlier experiments using ligated loops of bowel (Cornell et al. 1971; Rhodes and Karnovsky 1971; Bockman and Cooper 1973). For this reason the intestinal injection method described by Owen (1977), was expanded to include an application method using a stomach tube. Although the injection method permits an exact recording schedule of the sequence of HRP uptake, it leads to several traumata, such as narcosis, laparotomy and mechanical manipulation of the gut with peristaltic irritation. In contrast, HRP application with a stomach tube, a substantially more physiological method, eliminates a potential artificial "resorption path" (HRP diffusion via discontinuities of traumatised epithelium). Nevertheless, an exact temporal correlation of the sequential HRP uptake is impossible. In addition, potential retrograde accumulation of HRP within the epithelia of the GALT can not always been excluded (Straus 1958; Hampton and Rosario 1967; Nopajaroonsri et al. 1974; Bockman and Stevens 1976).

HRP catalyses the reaction: $2H_2O_2 \rightarrow 2H_2O+O_2$. Therefore, HRP could potentially affect the energy metabolism of the intestinal mucosa. In high concentrations it may even cause structural damage to the enterocytes (see Owen 1977). To exclude HRP diffusion through the intestinal epithelium conditioned by a toxic effect on the enterocytes, resorptive-physiological investigations were performed: in vivo-perfusion-recriculation (Menge et al. 1970) and in vitro-accumulation of L-phenylalanine (Robinson and Felber 1965). The results are summarized in Table 2 and 3. Experiments using 0.5% HRP solution have

shown that a statistically significant difference concerning resorbtive capacity does not exist between saline-exposed and HRP-exposed animals (Fig. 16). Therefore, an HRP diffusion through toxically altered enterocytic epithelium can be excluded.

In the area of the dome epithelium a further cell type was recorded, observed frequently in close association with M cells. This differs structurally from brush border cells and from M cells (Figs. 1 and 2). The characteristic structural component of these cells is a very well developed microvillous tuft ("tuft cells") on the apical surface, extending far into the gut lumen (Isomäki 1973; Nabeyama and Leblond 1974). In contrast to M cells a close association with lymphocytes does not exist. In spite of a distinctive cytocavitary system, no resorption of the applied HRP could be observed at any time. The functional meaning of "tuft cells" remains unsettled.

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References

Abe K, Ito T (1978) Qualitative and quantitative morphologic study of Peyer's patches of the mouse after neonatal thymectomy and hydrocortisone injection. Am J Anat 151:227–234

Asquith P (ed) (1979) Immunology of the gastrointestinal tract. Churchill Livingstone, Edinburgh, London, New York

Bockman DE, Cooper MD (1973) Pinocytosis by epithelium associated with lymphoid follicles in the bursa of Fabricius, appendix, and Peyer's patches. An electron microscopic study. Am J Anat 136:455–478

Bockman DE, Stevens W (1977) Gut-associated lymphoepithelial tissue: Bidirectional transport of tracer by specialized epithelial cells associated with lymphoid follicles. J Reticuloendothel Soc 21:245–254

Bockman DE, Winborn WB (1966) Light and electron microscopy of intestinal ferritin absorption. Observations in sensitized and non-sensitized hamsters (Mesocricetus auratus). Anat Rec 155:603–622

Boom A, Daems WT, Luft JN (1974) On the fixation of intestinal absorptive cells. J Ultrastruct Res 48:350–360

Caulfield JB (1957) Effects of varying the vehicle for OsO_4 in tissue fixation. J Biophys Biochem Cytol 3:827–830

Clark SL (1959) The ingestion of proteins and colloidal materials by columnar absorptive cells of the small intestine in suckling rats and mice. J Biophys Biochem Cytol 5:41–49

Cornell R, Padykula HA (1969) A cytological study of intestinal absorption in the suckling rat. Am J Anat 125:291–315

Cornell R, Walker WA, Isselbacher KJ (1971) Small intestinal absorption of horseradish peroxidase. A cytochemical study. Lab Invest 25:42–48

Gebbers J-O, Otto HF (1974) Electron microscope studies on the intestine using ruthenium red. Z Zelforsch 147:271–283

Graham RC, Karnovsky MJ (1966) The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: Ultrastructural cytochemistry by a new technique. J Histochem Histochem 14:291–302

Graney DO (1968) The uptake of ferritin by ileal absorptive cells in suckling rats. An electron microscope study. Am J Anat 123:227–253

Hampton JC, Rosario B (1967) The passage of exogenous peroxidase from blood capillaries into the intestinal epithelium. Anat Rec 159:159–170

Isomäki AM (1973) A new cell type (tuft cell) in the gastrointestinal mucosa of the rat. A transmission and scanning electron microscopic study. Acta Path Microbiol Scand, Sect A, Suppl-No 240

Joel DD, Laissue JA, LeFevre ME (1978) Distribution and fate of ingested carbon particles in mice. J Reticuloendothel Soc 24:477–487

Kraehenbuhl JP, Bron C, Sordat B (1979) Transfer of humoral secretory and cellular immunity from mother to offspring. Curr Top Pathol 66:105–157

Kraehenbuhl JP, Campiche MA (1969) Early stages of intestinal absorption of specific antibodies in the newborn: An ultrastructural, cytochemical and immunological study in the pig, rat and rabbit. J Cell Biol 42:345–365

Leblond CP, Cheng H (1976) Identification of stem cells in the small intestine of the mouse. In: Cairnie AB, Lala PK, Omond DG (eds) Stem cells of renewing cell populations. Academic Press, New York, San Francisco, London, p 7–31

Lecce JG (1966) In vitro absorption of γ-globulin by neonatal intestinal epithelium of the pig. J Physiol 184:594–604

LeFevre ME, Vanderhoff JW, Laissue JA, Joel DD (1978) Accumulation of 2 µm latex particles in mouse Peyer's patches during chronic latex feeding. Experientia 34:120–129

Lev R, Orlic D (1973) Uptake of protein in swallowed amniotic fluid by monkey fetal intestine in utero. Gastroenterology 65:60–68

Luft JH (1971) Ruthenium red and violet. I. Chemistry, purification, methods of use for electron microscopy and mechanisms of action. Anat Rec 171:347–368

Malmgren L, Olsson Y (1977) A sensitive histochemical method for light- and electronmicroscopic demonstration of horseradish peroxidase. J Histochem Cytochem 25:1280–1283

Menge H, Bloch R, Schaumlöffel E, Riecken EO (1970) Transportstudien, morphologische, morphometrische und histochemische Untersuchungen zum Verhalten der Dünndarmschleimhaut im operativ ausgeschalteten Jejunalabschnitt der Ratte. Z ges exp Med 153:74–90

Nabeyama A, Leblond CP (1974) "Caveolated cells" characterized by deep surface invaginations and abundant filaments in mouse gastro-intestinal epithelia. Am J Anat 140:147–165

Nopajaroonsri C, Luk SC, Simon GT (1974) The passage of intravenously injected colloidal carbon into lymph node parenchyma. Lab Invest 30:533–538

Orlic D, Lev R (1973) Fetal rat intestinal absorption of horseradish peroxidase from swallowed amniotic fluid. J Cell Biol 56:106–119

Otto HF (1978) Funktionelle Morphologie. In: Bartelheimer H, Classen M, Ossenberg FW (eds) Der kranke Dünndarm. III. Hamburger Medizinisches Symposium, Witzstrock, Baden-Baden, Köln, New York, p 3–23

Owen RL (1977) Sequential uptake of horseradish peroxidase by lymphoid follicle epithelium of Peyer's patches in the normal unobstructed mouse intestine: An ultrastructural study. Gastroenterology 72:440–451

Owen RL, Jones AL (1974a) Epithelial cell specialization within human Peyer's patches: An ultrastructural study of intestinal lymphoid follicles. Gastroenterology 66:189–203

Owen RL, Jones AL (1974b) Specialized lymphoid follicle epithelial cells in the human and nonhuman primate: A possible antigen uptake site. In: Scanning electron microscopy/1974 (Part III). Proceedings of the Workshop on Advances in Biomedical Applications of the SEM IIT Research Institute, Chicago, p 697–704

Parrott DMV (1976a) The gut as a lymphoid organ. Clinics in Gastroenterology 5:211–228

Parrott DMV (1976b) The gut-associated lymphoid tissues and gastrointestinal immunity. In: Ferguson A, MacSween RNM (eds) Immunological aspects of the liver and gastrointestinal tract. MTP Press, Lancaster

Rao SN, Mukherjee TM, Williams AW (1972) Quantitative variations in the disposition of the enteric surface coat in mouse jejunum. Gut 13:33–38

Rhodes RS, Karnovsky MJ (1971) Loss of macromolecular barrier function associated with surgical trauma to the intestine. Lab Invest 25:220–229

Robinson JWL, Felber J-P (1965) Compartments of the uptake of amino-acids by intestinal fragments during in vitro incubation. Gastroenterologia 104:335–342

Rodewald R (1970) Selective antibody transport in the proximal small intestine of the neonatal rat. J Cell Biol 45:635–640

Rodewald R (1976) Intestinal transport of peroxidase-conjugated IgG fragments in the neonatal rat. In: Hemmings WA (ed) Materno-foetal transmission of immunoglobulins, Cambridge University Press, New York, p 137–153

Ruchti C, Keller HU, Hess MW, Cottier H (1976) Die Epithelschranke des Darms. Bedeutung und Funktion in der Infektabwehr. Fortschr Med 94:531–534

Ruchti C, Luscieti P, Laissue J, Schaffner Th, Hess MW, Cottier H (1979) Funktionelle Morphologie des intestinalen Immunsystems. Z Gastroenterol Verh-Bd 14:16–24

Ruchti Ch, Gerber HA, Hess MW, Keller HU, Cottier H (1980) Zur Barrierefunktion des Menschlichen Darms: Mechanismen des Antigeneintritts und der antimikrobiellen Schutzfunktionen. Therapeutische Umschau 37:161–167

Shervey PD, Gardner PJ (1973) Intestinal absorption of ferritin in the suckling rat. Am J Anat 137:471–476

Straus W (1958) Colorimetric analysis with N,N-dimethyl-p-phenylenediamine of the uptake of intravenously injected horseradish peroxidase by various tissues of the rat. J Biophys Biochem Cytol 4:541–550

Thiery G (1978) L'appendice du lapin: Un modèle d'appareil immunitaire appliqué l'étude de l'immunité épithéliale. Ann Immunol (Inst Pasteur) 129C:503–522

Walker WA (1976) Host defence mechanisms in the gastrointestinal tract. Pediatrics 57:901–916

Walker WA, Isselbacher KJ (1974) Uptake and transport of macromolecules by the intestine: Possible role in clinical disorders. Gastroenterology 67:531–550

Walker WA, Isselbacher KJ (1977) Intestinal antibodies. N Engl J Med 297:767–773

Walker WA, Cornell R, Davenport LM, Isselbacher KJ (1972) Macromolecular absorption. Mechanism of horseradish peroxidase uptake and transport in adult and neonatal rat intestine. J Cell Biol 54:195–205

Walker WA, Isselbacher KJ, Bloch KJ (1972) Intestinal uptake of macromolecules: Effect of oral immunization. Science 177:608–610

Worthington BB, Graney DO (1973) Uptake of adenovirus by intestinal absorptive cells of the suckling rat. I. The neonatal ileum Anat Rec 175:37–61

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