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THE EPITHELIUM COVERING PEYER'S PATCHES IN YOUNG MILK-FED CALVES

AN ULTRASTRUCTURAL AND ENZYME HISTOCHEMICAL INVESTIGATION *

By

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LANDSVERK, T.: *The epithelium covering Peyer's patches in young milk-fed calves. An ultrastructural and enzyme histochemical investigation.* Acta vet. scand. 1981, *22*, 198—210. — Between the ordinary villi over Peyer's patches there are small domes or "pseudovilli" caused by bulges in the lymphoid tissue. These "pseudovilli" were studied in 5 healthy milk-fed, about 3-week-old, pre-ruminant calves. Scanning electron microscopy revealed that the "pseudovilli" were covered by a specialized follicle associated epithelium (FAE). The FAE had poorly developed microvilli and often extensive folding of the cell surface close to the cell borders. By transmission electron microscopy the tips of the marginal folds of the FAE seemed to fuse, probably in the process of enfolding bulk material from the lumen. The FAE apical cytoplasm contained numerous thick-walled and bristle-coated invaginations, tubules and vesicles indicative of micropinocytosis. Multivesicular bodies and large vacuoles were frequent. Indications of extracellular unloading of residual bodies were found. Intraepithelial lymphocytes tended to group together, and some were rich in rough endoplasmic reticulum. Enzyme histochemistry showed weak reactions of adenosine triphosphate splitting enzyme and aminopeptidase in the FAE luminal cell border. Cytoplasmic acid phosphatase showed a marked basal-apical decrease along the "pseudovillus" probably caused by the onset of endocytosis. The results of this study appear compatible with the concept that the FAE takes up macromolecules from the lumen by pinocytosis and sensitizes lymphocytes.

Peyer's patches; epithelial cells; microfolds; endocytosis; enzymes; calves.

Peyer's patches play a vital role in the establishment of the intestinal immune response since they generate precursors of IgA secreting plasma cells (*Gowans & Knight* 1964, *Craig &*

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Cebra 1971, *Müller-Schoop & Good* 1975, *Pierce & Gowans* 1975, *Husband & Gowans* 1978). It is believed that the lymphocytes of Peyer's patches get their information concerning antigens through a specialized epithelium overlying the Peyer's patches lymphoid tissue. Pinocytosis and evidence of heavy lymphocytic infiltration have been found in this epithelium (*Bockman & Cooper* 1973, *Owen & Jones* 1974, *Owen* 1977).

There has been an increasing interest in the gut associated lymphoid tissue in domestic animals. Studies concerned with morphology, developmental traits, and migration patterns of lymphocytes have been reported in pigs (*Chapman et al.* 1974, *Chu et al.* 1979), lambs (*Reynolds* 1980), and calves (*Waksman* 1973). In a previous study a brief description of the light and scanning electron microscopic appearance of the domes or "pseudovilli" overlying Peyer's patches in pre-ruminant milk-fed calves was given (*Landsverk* 1979). In the young calf, solitary follicles of lymphoid tissue are found at any level of the small intestine. However, accumulation of follicles into a continuous row forming Peyer's patches occurs only in the posterior 15 % of the small intestine.

The present study reports the enzyme histochemistry and fine structure of the epithelium covering the "pseudovilli" over Peyer's patches, with special reference to its possible function in antigen transport.

MATERIALS AND METHODS

Intestinal specimens were obtained from 5 healthy 17-, 20-, 21-, 22- and 23-day-old calves, which also had been used in previous studies (*Landsverk* 1979, 1980). The calves were fed whole cow's milk 110 ml/kg body weight/day. For the purpose of this study samples were taken from various sites in the posterior 15 % of the small intestine, in the anti-mesenteric portion thickened by Peyer's patches. Further details of sites and sampling procedures are given in the previous report (*Landsverk* 1979). The methods for light microscopy (LM), scanning electron microscopy (SEM), and enzyme histochemistry have likewise been described. The enzymes studied were: alkaline phosphatase (EC 3.1.3.1), adenosine triphosphate splitting enzyme, acid phosphatase (EC 3.1.3.2), β-D-galactosidase (EC 3.2.1.23), aminopeptidase (EC 3.4.11.2), non-specific esterase and succinate dehydrogenase (EC 1.3.99.1).

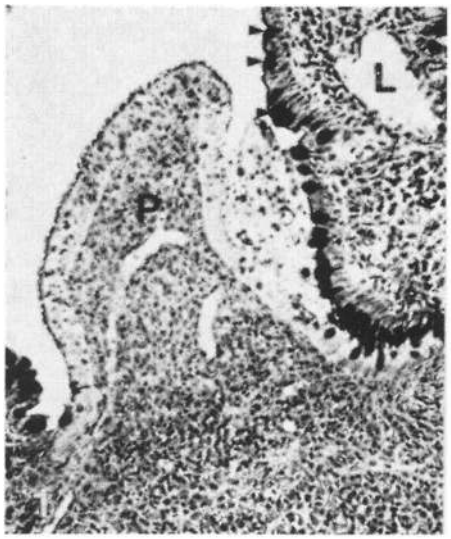
Additional methods included freeze-fracture of the intestinal tissue. The intestinal tissue was fixed in diluted Karnovsky's fixative (*Karnovsky* 1965) with 0.9 % glutaraldehyde and 0.7 % paraformaldehyde in 0.14 mol/l cacodylate buffer and dehydrated in ethanol. Freeze-fracture was carried out on specimens dehydrated in ethanol (*Humphreys et al.* 1978). The specimens were enclosed in gelatin capsules, which were submersed and fractured in liquid nitrogen. The specimens were thawed in ethanol, equilibrized in acetone, and critical point dried with carbon dioxide as the transitional fluid. The specimens were treated further for SEM as prevously described (*Landsverk* 1979).

For transmission electron microscopy (TEM) the same initial fixative as above was used. The tissue was post-fixed in 2 % cacodylate buffered osmium for 2 h, dehydrated in acetone, and embedded in Araldite. About 1 μm thick sections for light microscopy were stained with toluidine blue, and thin sections were cut with glass or diamond knives and stained with lead citrate and uranyl acetate. The sections were examined in a Siemens Elmiskop I A.

RESULTS

The histological characteristics of the "pseudovilli" covering Peyer's patches in the calf small intestine have been described (*Landsverk* 1979) and may be summarized as follows: the "pseudovilli" represent bulges of lymphoid tissue of Peyer's patches covered by a vacuolated columnar epithelium, devoid of goblet cells, with a thin brush border and heavily infiltrated by leukocytes (Figs. 1 and 2). When compared with the epithelium of the nearby crypts of Lieberkühn, the epithelium covering the "pseudovilli" was shown to lack the basophilia and the pyroninophilia of the crypt cells; in this respect the "pseudovillous" epithelium resembled the ordinary epithelium covering the nearby ordinary villi.

By enzyme histochemistry the luminal cell border of the epithelium covering the "pseudovilli" showed a weak reaction of adenosine triphosphate splitting enzyme (Fig. 3) and L-leucine and L-alanine aminopeptidase. These enzymes showed strong reaction in the brush border of nearby ordinary villi. Alkaline phosphatase gave no staining of the luminal cell border. However, intraepithelial cells, probably neutrophils, were positive (Fig. 4). Acid phosphatase was shown as a granular deposit in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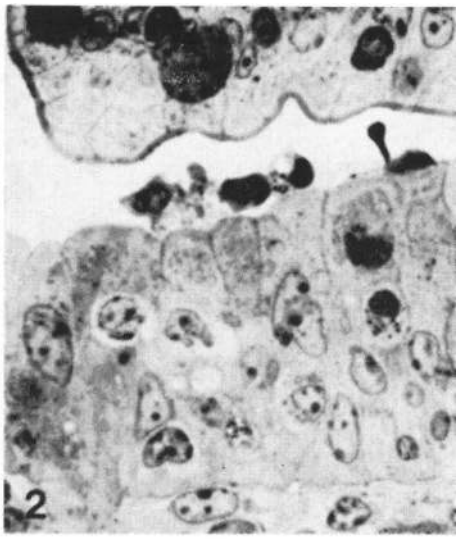
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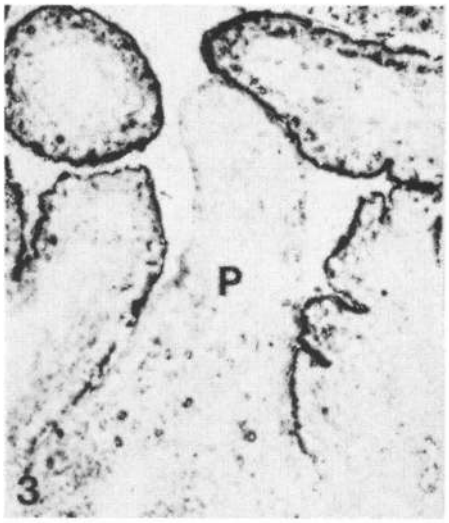
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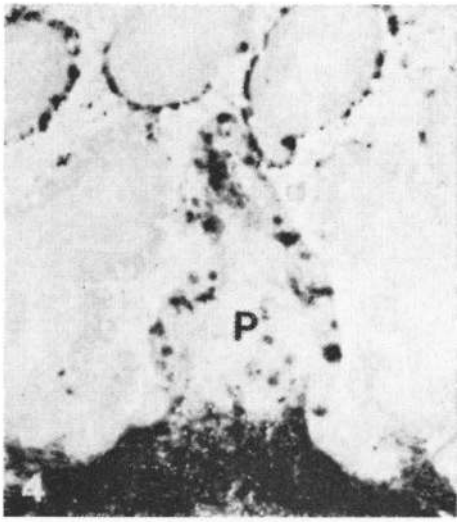
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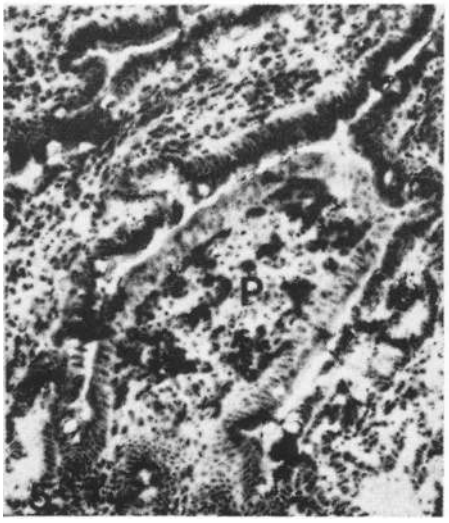
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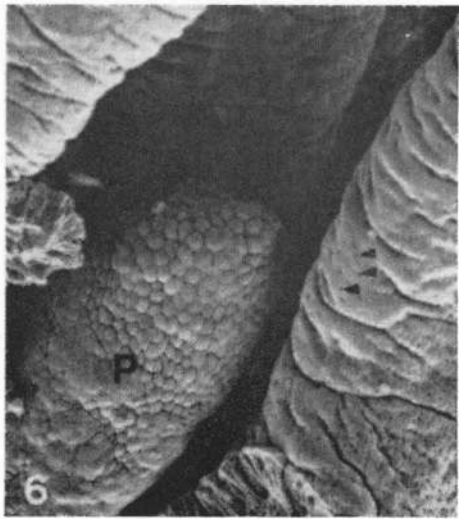
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Figure 1. The "pseudovillus" (P) consists of a dome of lymphoid tissue covered with a columnar epithelium. The epithelium lacks goblet cells and shows frequent leukocyte migration. The ordinary villus has a central lacteal (L) and many dark stained goblet cells (arrows). AB-PAS, × 150.

Figure 2. Columnar epithelium of the "pseudovillus" with a thin brush border. Note the thicker brush border of the ordinary epithelium in the upper portion of the micrograph. The "pseudovillous" epithelium is infiltrated by leukocytes. Leukocytes also cover the luminal plasma membrane. About 1 μm thick Araldite section, toluidine blue. × 750.

Figure 3. ATP splitting enzyme. The luminal surface of the epithelial cells along the "pseudovillus" (P) shows a weak reaction in contrast to the strong reaction in the brush border of ordinary villi. No counterstain. × 110.

Figure 4. Alkaline phosphatase. Discontinuous staining in the brush border of apical portions of ordinary villi. The "pseudovillus" (P) has positive focal staining of the epithelium probably due to migrating neutrophils, whereas the luminal cell surface appears negative. No counterstain. × 110.

Figure 5. Acid phosphatase. Most of the "pseudovillus" (P) has a relatively weak staining of the apical epithelial cell cytoplasm in contrast to the strong staining in the corresponding zone of ordinary epithelial cells. Note the relatively strong reaction in the supranuclear cytoplasm of the epithelial cells at the base of the "pseudovillus". This reaction decreases up along the „pseudovillus". Positive cells in the lamina propria of the "pseudovillus" are interpreted as macrophages. Lightly counterstained with hematoxylin. × 150.

Figure 6. Scanning electron micrograph of a "pseudovillus" (P) residing at the base of ordinary villi. Note the protruding epithelial cells of the "pseudovillus". The ordinary villi have a relatively smooth surface with transverse furrows. Arrows indicate openings of goblet cells in an ordinary villus. × 300.

Figure 7. Scanning electron micrograph, "pseudovillus". The epithelial cells have concentric folds encircling a central area with microvilli. × 3,000.

Figure 8. Scanning electron micrograph, "pseudovillus". Some cells show a tuft-like arrangement of microvilli (arrows) with a flattened cell periphery containing low ridges. Other cells show a variable development of low folds or small microvilli. Two leukocytes (L) are stuck to the surface. × 4,000.

Figure 9. Scanning electron micrograph. Apical area of a "pseudovillus". The plasma membrane contains low folds only. Note the pores in the center of the cells. A leukocyte (L) with pseudopods is seen at the surface. × 2,000.

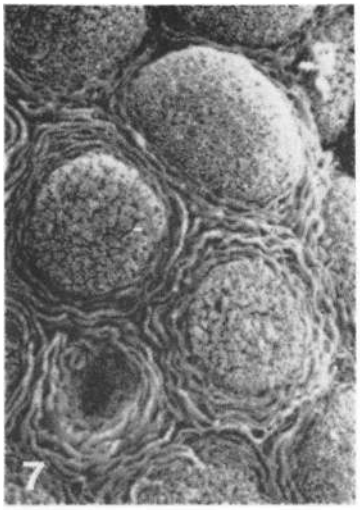
Figure 10. Scanning electron micrograph of a freeze-fractured specimen, "pseudovillus". The fractured surface contains vacuoles (arrows) of various sizes. In the vacuoles particulate material is seen. In the upper portion of the micrograph the luminal plasma membrane contains concentric folds and microvilli. × 3,000.

Figure 11. Scanning electron micrograph of a freeze-fractured specimen, "pseudovillus". The fractured surface in the lower portion of the micrograph contains vesicles and tubules, the latter especially prominent beneath the concentric folds. The folds are interconnected with bridge-like formations (arrows). × 10,000.

Figure 12. Scanning electron micrograph of a freeze-fractured specimen, ordinary villus. The epithelial cells have regular, rod-shaped tightly spaced microvilli. N = nucleus. × 3,000.

Figure 13. "Pseudovillous" epithelium with vacuoles (V), a lymphocyte (L), a globule leukocyte (G), and neutrophils (N), the latter also in the lumen. One of the epithelial cells (D) shows several autophagic vacuoles indicating cell degeneration. × 3,500.

Figure 14. "Pseudovillous" epithelium. Glycocalyx (black arrows) covers the trilaminar plasma membrane of a microvillus or microfold. In the center of the pyramidal structure several vesicles are seen (white arrows). × 52,000.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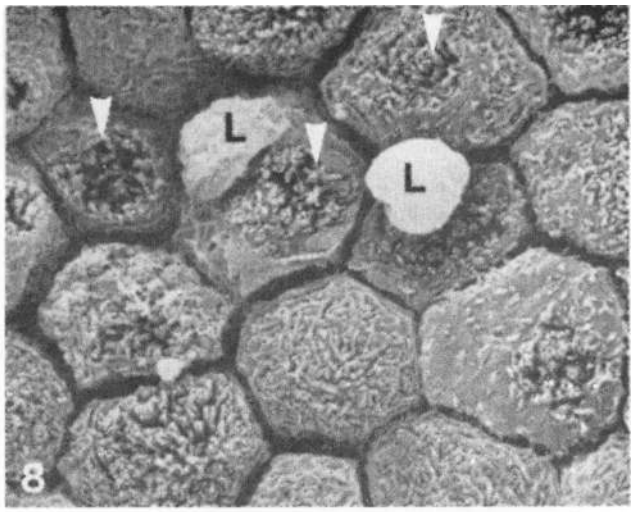
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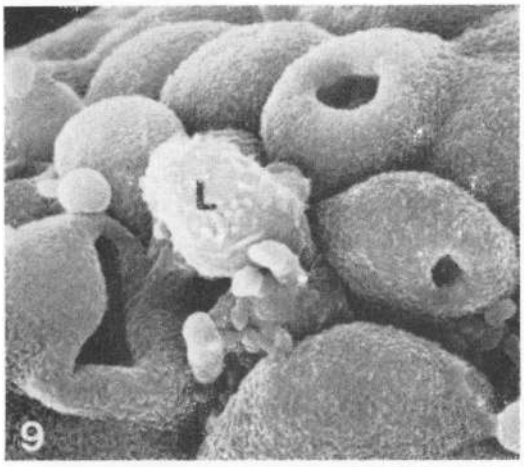
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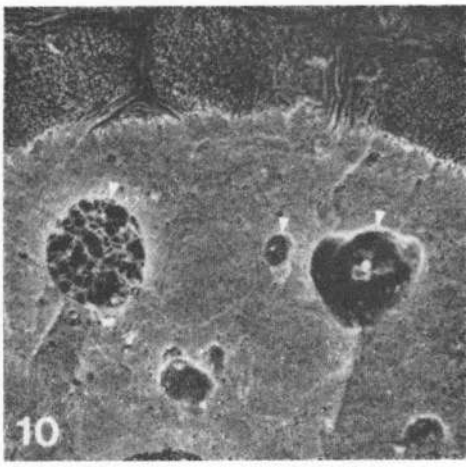
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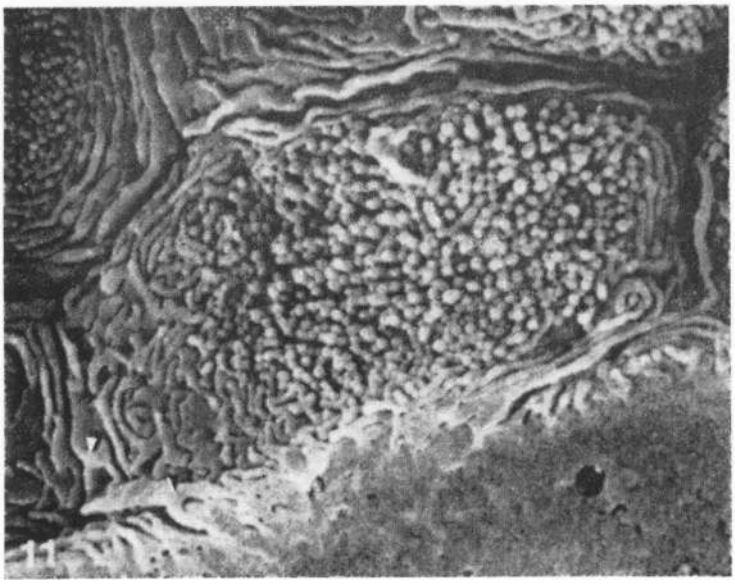
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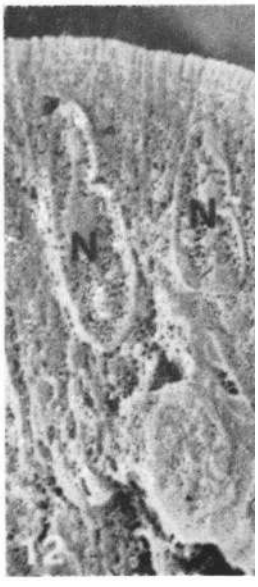
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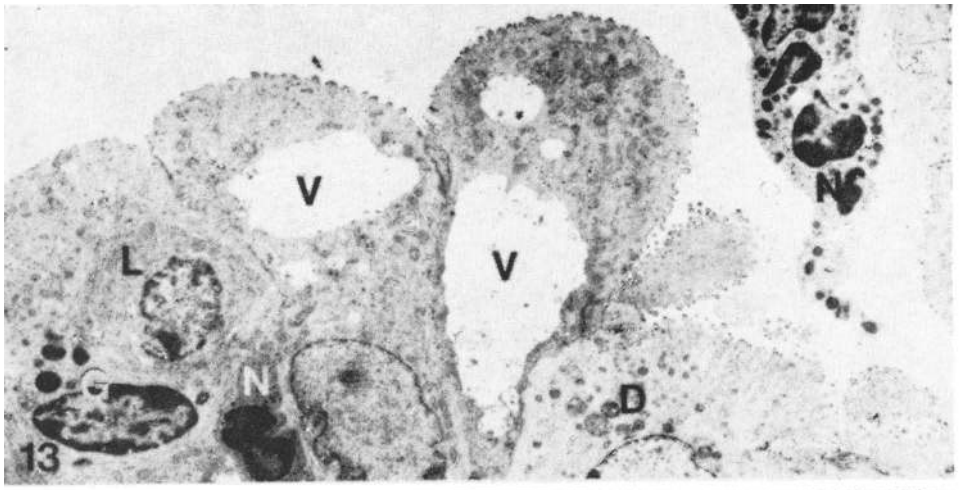
Figure 15. "Pseudovillous" epithelium. The microvilli are covered by a glycocalyx (black arrows) and show a sparse development of longitudinal filaments. Invaginations are seen at the base of the microvilli (white arrows). × 45,000.
Figure 16. "Pseudovillous" epithelium, transverse sections of folds close to the cell border. A fold is seen to bend back (arrow). Tubules extend from the apical plasma membrane deep into the cell cytoplasm. × 23,000.
Figure 17. "Pseudovillous" epithelium. Folds in proximity to the cell border seem to have fused (arrow). Cytoplasmic vesicles and invaginations (I) of the apical plasma membrane are seen. × 45,000.
Figure 18. "Pseudovillous" epithelium. Luminal cell debris (Cd) is probably being internalized by invagination (I) of the apical plasma membrane. Cytoplasmic vesicles and tubules show a bristle coat (arrows) on their cytoplasmic surface. × 45,000. Inset. A serial section from the same specimen showing electron opaque deposits, probably cell debris, contained within a vesicle of the cytoplasm. × 45,000.
Figure 19. "Pseudovillous" epithelium. A dark-stained necrotic cell (Nc) and a neighbour cell apparently engulfing necrotic cell debris. Cytoplasmic bodies (B) containing electron opaque material. × 12,000.
Figure 20. "Pseudovillous" epithelium. The cytoplasm contains multivesicular bodies (Mvb) and numerous vesicles and tubules. Continuity between a vesicle and a multivesicular body is shown (arrow). × 10,000.
Figure 21. "Pseudovillous" epithelium. A multivesicular body containing vesicular structures and more electron opaque membrane-bound particles. Arrow indicates a smaller dense body. × 45,000.
Figure 22. Scanning electron micrograph of a freeze-fractured specimen, "pseudovillous" epithelium. A cytoplasmic body, probably a multivesicular body, contains spherical bodies supposed to correspond to the vesicles seen by TEM. × 9,000.
Figure 23. Scanning electron micrograph of a freeze-fractured specimen, "pseudovillous" epithelium. Cytoplasmic vesicles contain fine particulate material. × 20,000.
Figure 24. "Pseudovillous" epithelium. A vacuolar structure (Vs) contains membrane fragments (arrows) and membraneous particles of various sizes. The appearance of the particles corresponds to those of the multivesicular or residual bodies. The vacuolar structure communicates with the intercellular space (In). Numerous cytoplasmic vesicles are seen in proximity to the vacuolar structure. × 32,000.
Figure 25. "Pseudovillous" epithelium. Cytoplasmic bodies (B) with a varying electron opaque content, interpreted as residual bodies, G = Golgi apparatus. × 34,000.
Figure 26. "Pseudovillous" epithelium. Large and small electron opaque bodies (arrows) in the dilated intercellular spaces (In). A large cytoplasmic electron opaque body (B) is seen close to the dilated intercellular space. × 11,000.
Figure 27. Enlarged area of Fig. 26 showing membrane bound dense particles (arrows) and small membraneous or vesicular structures in the intercellular space (In). × 20,000.
Figure 28. "Pseudovillous" epithelium. Two epithelial lymphocytes (L) in close apposition. Many multivesicular bodies (arrows) and bodies with electron opaque contents are seen in proximity to the lymphocytes. × 6,800.
Figure 29. "Pseudovillous" epithelium. A lymphocyte (L) in the intercellular space close to the base of the cell. The lymphocyte cytoplasm is rich in rough endoplasmic reticulum. B = basement lamina, E = epithelial cells. × 16,000.
Figure 30. "Pseudovillous" epithelium. Electron opaque, particulate and vesicular structures are seen in a "pocket" between an epithelial cell (E) and a lymphocyte (L). × 20,000.
Figure 31. "Pseudovillous" subepithelial lamina propria. B = basement lamina. Macrophages (M) contain necrotic cells. F = fibroblast. Arrows indicate dilated endoplasmic reticulum of subepithelial fibroblasts. × 3,500.



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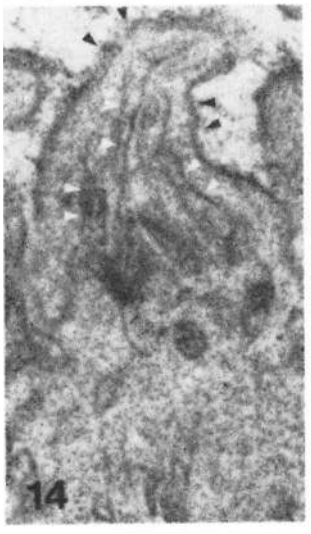
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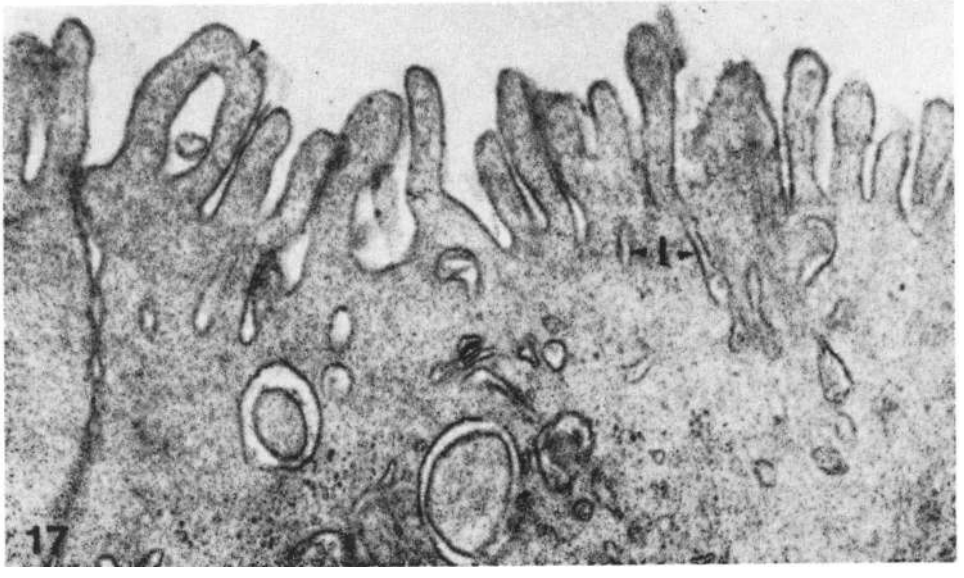
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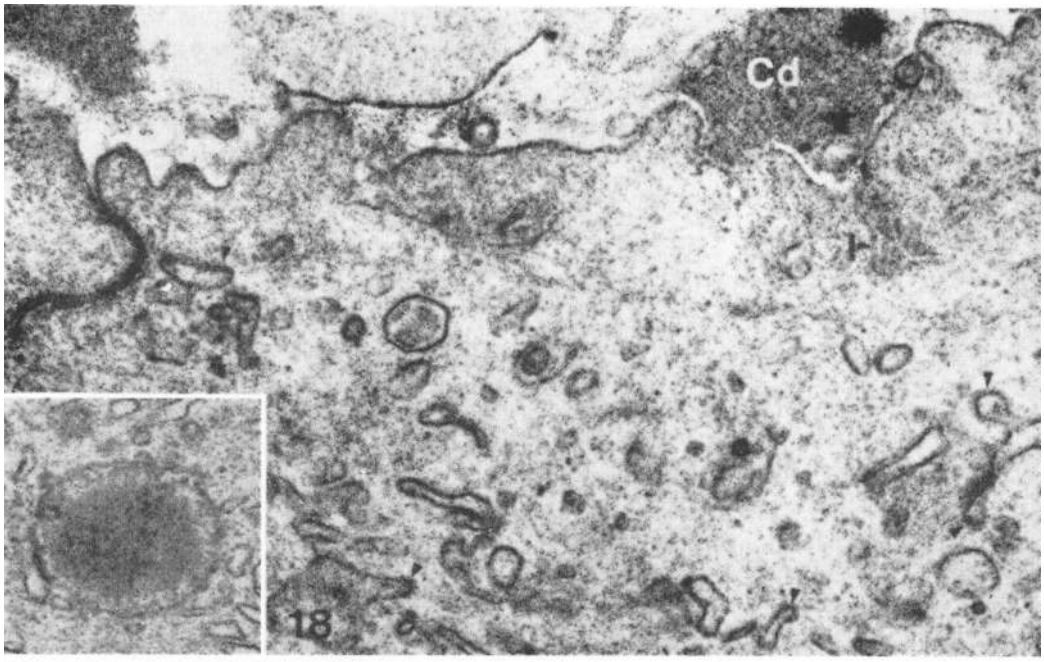
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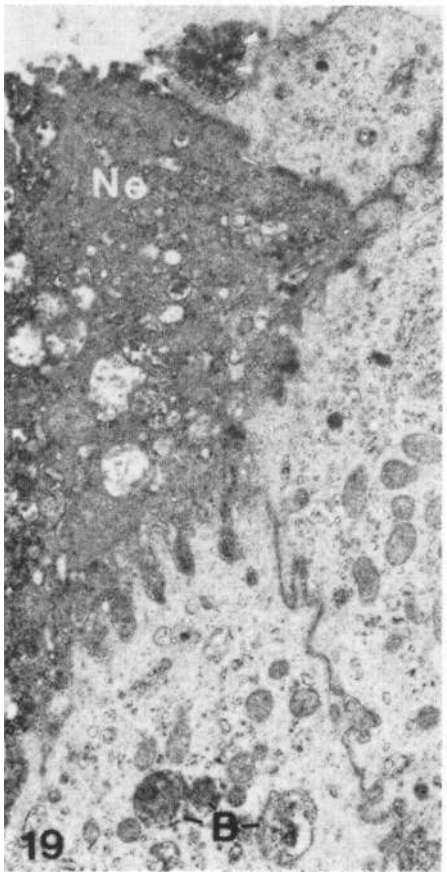
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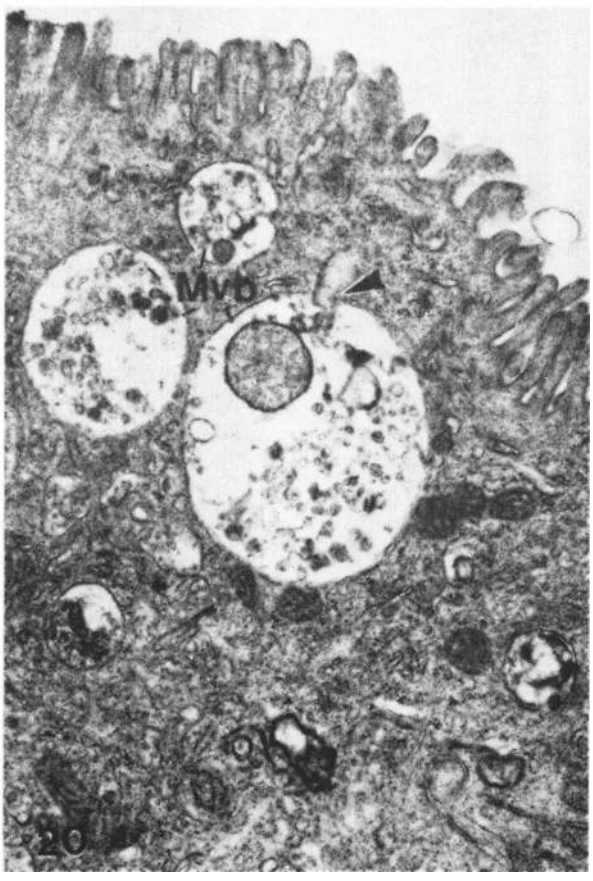
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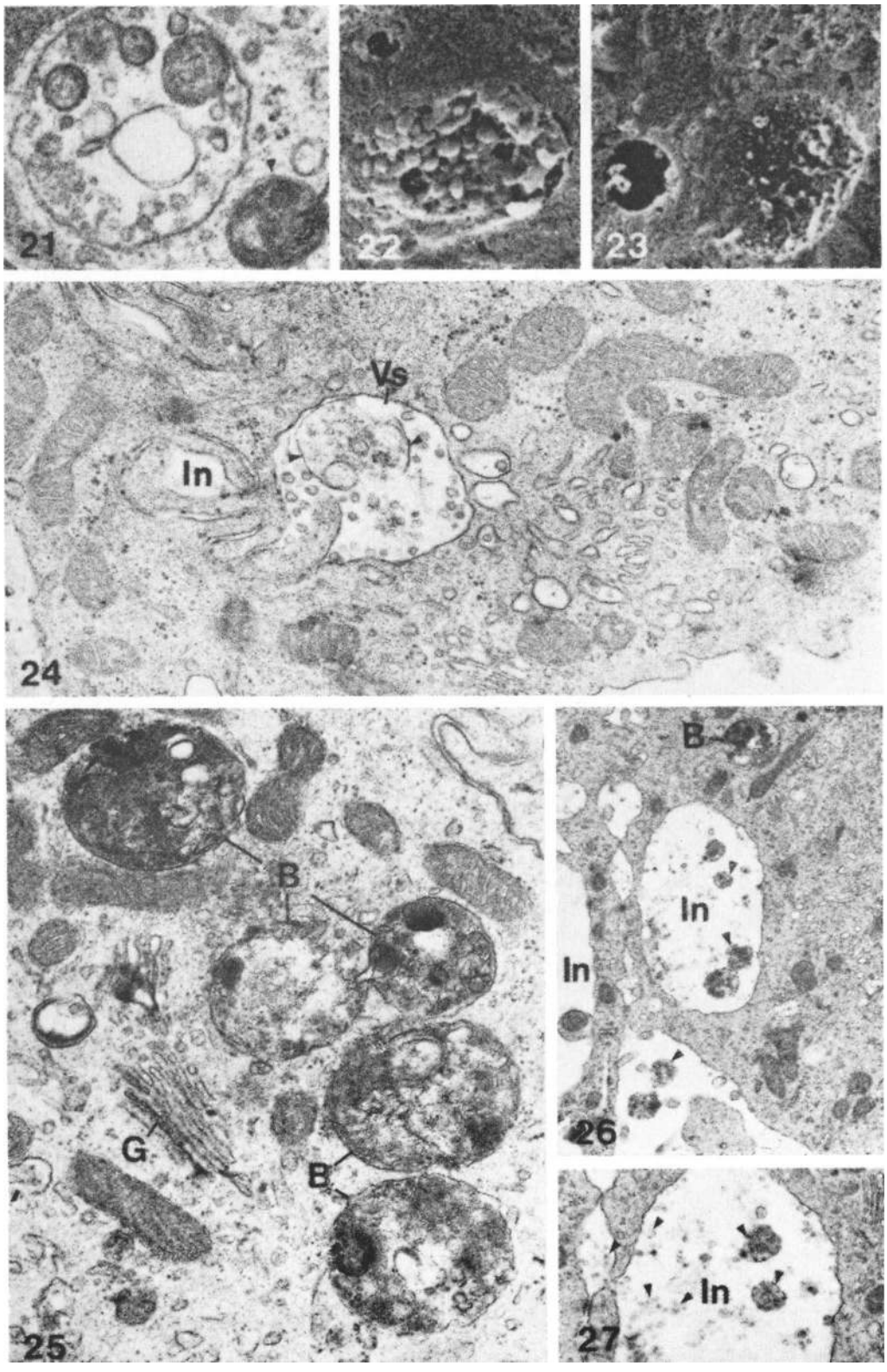
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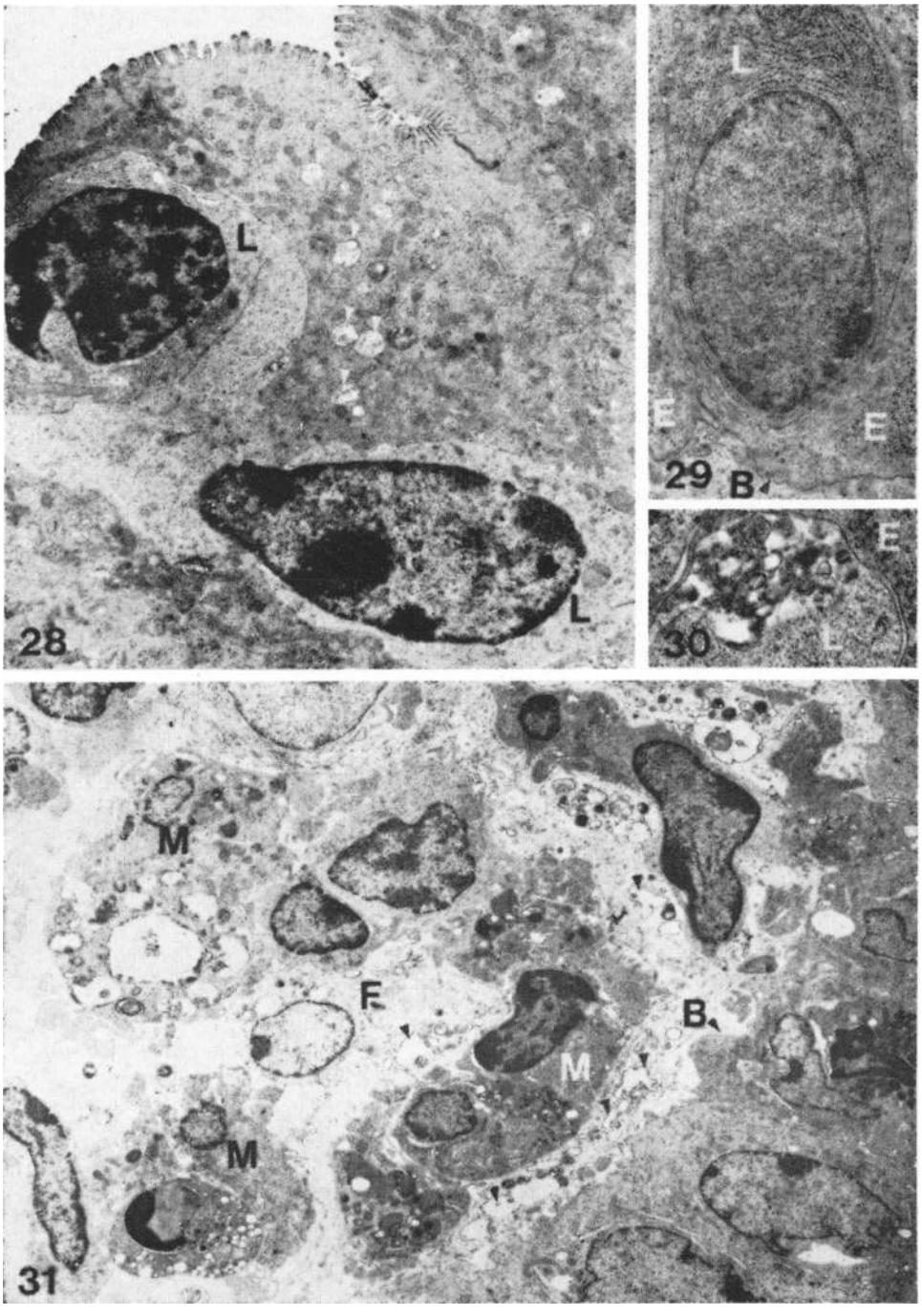
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the supranuclear cytoplasm of the epithelial cells at the base of the "pseudovilli", whereas the epithelium in the upper portion of the "pseudovilli" showed a weak reaction (Fig. 5). Succinate dehydrogenase showed a reaction similar to that seen in ordinary villi, while β-D-galactosidase and non-specific esterase reactions were not detected under the conditions used. In the lamina propria of the "pseudovilli" esterase and acid phosphatase positive cells, probably macrophages, had accumulated (Fig. 5).

The low power SEM appearance of the "pseudovilli" has been described (*Landsverk* 1979): between the ordinary villi over Peyer's patches there are small villi-like structures or "pseudovilli", covered with protruding epithelial cells (Fig. 6). With high power SEM the luminal plasma membrane of the protruding cells varied in appearance from one area to another (Figs. 7—11). In some areas especially close to the tip of the "pseudovilli" a nearly flattened cell surface was seen (Fig. 9). In most cells, however, a pattern for the general arrangement of the cell surface could be delineated. Towards the lateral cell borders there was extensive folding, the direction of the folds following the course of the cell borders (Figs. 7, 10 and 11). Microvilli, sometimes with a tuft-like appearance (Fig. 8), were developed in the center of the cell surface. In some cells situated near the apex of the "pseudovilli" conspicuous pores were seen in the plasma membrane (Fig. 9). Leukocytes were sometimes seen on the surface of the epithelial cells (Figs. 8 and 9). Freeze-fracture of the "pseudovillous" epithelium revealed intracytoplasmic vacuoles and multivesicular bodies (Figs. 10, 11, 22 and 23). The microvilli of the "pseudovillous" epithelial cells were short and irregular, in contrast to the long and slender microvilli of the ordinary villous epithelial cells (Figs. 11 and 12).

TEM demonstrated that the "pseudovillous" epithelium consisted of a population of columnar cells with nuclei mostly in near basal position. Almost all the epithelial cells showed a similar structure (Fig. 13). The luminal plasma membrane was coated by a glycocalyx as in epithelial cells covering ordinary villi (Figs. 14 and 15). Rootlets and central core of the microvilli showed variable development but were mostly sparse (Fig. 15). No distinct terminal web was seen. A marginal fold of the plasma membrane was sometimes seen to bend back so that its tip approached an adjacent fold (Fig. 16). Apparently the fold made contact and fused (Fig. 17). From the folded areas pro-

jections of tubules extended deep into the apical cytoplasm. Numerous invaginations of the plasma membrane were found at the base of the microvilli outside the folded areas (Fig. 15). The membrane of the tubules and vesicles appeared to have the same thickness as the luminal plasma membrane, and their cytoplasmic surface was often bristle-coated (Fig. 18). Some cells seemed to engulf cell debris (Figs. 18 and 19). The apical cytoplasm of the epithelial cells often contained large vacuoles and multivesicular bodies (Figs. 13, 20 and 21). The internal vesicles of the multivesicular bodies were apparently formed by infoldings of the limiting membrane, possibly corresponding to the spherical bodies seen by freeze-fracture (Fig. 22). Electron opaque bodies with a more indistinct particulate and membranous contents were interpreted as residual bodies (Fig. 25). Intermediary stages between multivesicular and residual bodies were frequent, suggestive of a progressive development from multivesicular into residual bodies. The "pseudovillous" epithelial cells contained an ovoid nucleus with a distribution of chromatin almost corresponding to that of ordinary villous epithelial cells. The cells had considerable amounts of rough endoplasmic reticulum. Unattached ribosomes varied but were mostly few, and Golgi complexes were prominent. The apical portion of the lateral cell membranes had tight junctions, desmosomes, and extensive interdigitations. The basal portion of the intercellular space was often vastly expanded and sometimes contained clumps of particulate or vesicular material (Figs. 24, 26 and 27). The appearance of the material resembled that contained within the multivesicular and residual bodies. Occasionally the contents of the cytoplasmic bodies seemed to be discharged into the intercellular space (Fig. 24).

In the epithelium migrating leukocytes were frequent and consisted of lymphocytes, neutrophils, and globule leukocytes (Figs. 13, 28 and 29). Neutrophils, globule leukocytes, and occasional lymphocytes were also seen covering the luminal surface of the "pseudovillous" epithelial cells. The lymphocytes contained within the epithelium tended to group together and showed a wide variation with respect to the occurrence of rough endoplasmic reticulum. In some lymphocytes the ribosomes were dispersed throughout the pale cytoplasm, and endoplasmic reticulum was not seen. In other lymphocytes quite extensive amounts of rough endoplasmic reticulum were found. The mi-

grating leukocytes were often contained within the intercellular spaces, but frequently the position of the leukocytes in relation to the epithelial cells could not be assessed with certainty. In the space between the plasma membranes of the migrating leukocytes and the epithelial cells, accumulations of the vesicular or particulate material were sometimes seen (Fig. 30). Sometimes such material was contained within phagosomes of the neutrophils. The basement lamina was interrupted by the migrating leukocytes. Single epithelial cells with the characteristics of ordinary villous epithelial cells were occasionally found among the "pseudovillous" enterocytes. Goblet cells and endocrine cells were not seen in the "pseudovilli". Sometimes, signs of cell degeneration with predominance of autophagic vacuoles or overt necrosis were observed (Figs. 13 and 19).

As in ordinary villi, a subepithelial layer of fibroblasts and collagen was formed. Noteworthy among the subepithelial cells were lymphocytes and the frequent occurrence of macrophages, some being rich in phagosomes. Necrotic cells were often contained within the phagosomes (Fig. 31).

DISCUSSION

In the present study the specialized epithelium covering the lymphoid tissue over Peyer's patches in the calf has been described. This follicle-associated epithelium (FAE) has poorly developed microvilli, with numerous cytoplasmic tubules, vesicles and vacuoles similar to those in the FAE described in chicken bursa of Fabricius, man, pig and mouse Peyer's patches and man and rabbit appendix (*Andrew & Shimuzu* 1966, *Shimuzu & Andrew* 1967, *Bockman & Cooper* 1973, 1975, *Owen & Jones* 1974, *Owen* 1977, *Chu et al.* 1979). In the present study a relatively homogenous population of FAE cells was found. In contrast, *Owen & Jones* described scattered "membraneous" epithelial cells or "M" cells in the epithelium over Peyer's patches in man and mouse. The designation "M" cells was given because of the cell's interaction with lymphocytes, i.e. the specialized cells formed a "membrane" between the lymphocytes and the lumen. In the present material no morphologic differences were found between epithelial cells interacting with lymphocytes and most of the other epithelial cells of the "pseudovilli". The term follicle-associated epithelium (FAE) is therefore used in accordance with *Bockman & Cooper* (1975).

The lack of pyroninophilia and the nuclear and cytoplasmic maturity as seen by TEM suggest a specialization and differentiation as compared with crypt cells. The FAE cells appear to be structurally related to the absorbing villous epithelium described in neonatal suckling animals (*Clark* 1959, *Kraehenbuhl & Campiche* 1969). This epithelium internalizes proteins by micropinocytosis, a capacity vital to the absorption of immunoglobulins. The FAE cells may possibly have escaped the postnatal redifferentiation that occurs in ordinary villous epithelial cells. The special surface folds of the "pseudovillous" epithelial cells have, however, not been described in neonatal calves. The FAE cells also resemble the caveolated cell, another cell type in the intestinal tract. These cells are interposed between ordinary absorbing cells and have extensive invagination of the apical plasma membrane (*Isomäki* 1973, *Nabeyama & Leblond* 1974, *Weyrauch* 1979); the typical abundant microfilaments present in the caveolated cells are, however, not seen in the FAE.

In other studies the FAE cells have been shown to be capable of pinocytosis of electron opaque tracers (*Bockman & Cooper, Owen*). The typical surface architecture found in the present FAE cells with prominent folds in peripheral parts of the luminal plasma membrane may be interpreted as a specialization related to pinocytosis. Although low and rather irregular surface folds have previously been reported in FAE of several species (*Owen & Nemanic* 1978) the concentric organization found in the present material has not been described. Based on the present observations it is presumed that luminal bulk material is enclosed by the fusion of these folds and transported into the cytoplasm by membrane flow. The sequence of such a process occurring in endothelial cells was delineated by *Fawcett* (1965). Invagination of the luminal plasma was, however, also found outside the folded areas in the present material and it is possible that different forms of pinocytosis existed. Larger invaginations appeared to be formed by pinocytosis of cell debris, and the pores seen with SEM might have been created in such a manner. The thick membranes of the apical vesicles and tubules of the FAE cells support the supposition that endocytosis rather than exocytosis is taking place. The formation of bristle coats on the cytoplasmic surface of the vesicles and tubules has generally been associated with endocytosis (*Friend & Farquhar* 1967, *Geuze & Kramer* 1974, *Moxon et al.* 1976). The occurrence of multi-

vesicular bodies may be related to the pinocytic process. Inclusion of electron opaque tracers into the multivesicular bodies has been shown to occur during endocytosis (*Friend & Farquhar, Nicander & Pløen* 1979). The possible function of the multivesicular bodies may be to absorb environmental macromolecules and/or to store redundant cell membrane (*Locke & Collins* 1968, *Abrahams & Holtzman* 1973).

It is thought that the lymphocytes of Peyer's patches are sensitized by contact with antigens communicated by the FAE (*Owen* 1977). Pinocytosis of antigens is probably essential in this connection. In experimental studies localization of macromolecule tracers in endocytic vesicles and at the surface of lymphocytes in their intraepithelial position has been described (*Bockman & Cooper, Owen*). The significance of the multivesicular bodies and residual bodies described in the present study relative to the function of the FAE cells is open to question. The particular and vesicular material contained within the intercellular spaces or "pockets" between the leukocytes and epithelial cells probably resulted from exocytosis of the contents of cytoplasmic bodies with similar contents, i.e. residual bodies. Exocytosis of residual bodies appears to be unusual in higher animals (*de Duve & Wattiaux* 1966) but has been suggested for liver cells (*Kerr* 1970, *Munnell & Cork* 1980) and kidney cells (*Maunsbach* 1969). It is not known whether the intercellular material of the FAE in the present material had any significance for maturation of epithelial lymphocytes. Activation of lymphocytes in their epithelial position was indicated by the occurrence of rough endoplasmic reticulum in some of the lymphocytes. Such a transformation of lymphocytes has also been suggested to occur in other FAE epithelia (*Owen & Jones, Chu et al.*).

In the present study a relatively sparse lymphocyte infiltration of the epithelium was found. *Shimuzu & Andrew* observed a moderate lymphocyte infiltration of the FAE epithelium in young rabbit appendix and a progressive increase of this infiltration with age. Hence, the moderate infiltration reported here may be age-dependent.

The weak reaction of aminopeptidase and ATP-splitting enzyme found in this study may be related to reduced intracellular synthesis of these enzymes or caused by the reduction in surface area of the luminal plasma membrane as compared with ordinary villous enterocytes. For the ATP-splitting enzyme the last expla-

nation is the most probable. There is a very distinct and steady increase in the reaction of this enzyme from bottom to top of the calf intestinal crypt which appears to be correlated to microvillous development (*Landsverk* 1979 unpublished). The weak acid phosphatase reaction of apical FAE cells in the present material coincides with observations in FAE of rabbit appendix (*Schmedtje* 1965). The marked decrease of the acid phosphatase reaction from the base of the "pseudovillus" and up along the "pseudovillus" suggests an effective mobilization and consumption of the enzyme, probably in connection with the onset of endocytosis.

The weak acid phosphatase of most of the FAE may indicate a sparse lysosomal equipment in these cells, and may have consequences for the defensive ability of the FAE cells. There are indications that the FAE is, in fact, deficient in defensive capacity. It has been shown that the Peyer's patches are a weak point for infection with certain bacteria (*La Brec & Formal* 1961, *Carter & Collins* 1974, *Hohmann et al.* 1978). In the FAE subepithelial tissue of rabbit appendix there are aggregates of bacteria which otherwise appear avirulent (*Friedenstein & Goncharenko* 1965, *Schmedtje, Shimuzu & Andrew*). The heavy epithelial infiltration by neutrophils and globule leukocytes demonstrated in the present report may be looked upon as a compensatory mechanism. Cells with features common to globule leukocytes are frequent in the calf intestinal mucosa (*Landsverk* 1979), and their migration to an epithelial position has been seen in certain parasitic infections in cattle (*Rahko* 1969). In the "pseudovillus" it is apparently a local stimulus that is responsible for the migration, since such an infiltration is slight in the nearby ordinary villi. The accumulation of macrophages in FAE subepithelial position are in accordance with another study of Peyer's patches in mice (*Sobhon* 1971) and may be an additional trait of a compensatory defense mechanism. However, there is also evidence that these macrophages may handle wornout migrating cells (*Sawicki et al.* 1977), and that phenomenon seemed to be a prominent feature in the present study too. In germinal centers of Peyer's patches an abundance of tingible body macrophages engulf dying lymphocytes (*Sobhon*). The interrelationship of pathogenic agents and the "pseudovilli" will be one of the subjects in a subsequent paper (*Landsverk* in prep.).

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SAMMENDRAG

Epitelet over Peyerplettene hos unge melke-fôrede kalver. En ultrastrukturell og enzymhistokjemisk undersøkelse.

Mellom de ordinære villi over Peyerplettene hos unge kalver er det små fremhvelvninger av lymfoid vev. Disse fremhvelvninger eller „pseudovilli" ble studert hos 5 friske, omkring 3 uker gamle, melkefôrede kalver. Skanning elektron mikroskopi viste at „pseudovilli" var utkledt av et spesialisert follikel-assosiert epitel (FAE) som hadde sparsom utvikling av mikrovilli og karakteristiske konsentriske folder i den luminale celleoverflate. Transmisjonselektronmikroskopisk undersøkelse tydet på at disse foldene kunne smelte sammen og inkorporere partikler i cytoplasmaet. Invaginasjoner av cellemembranen var hyppige, spesielt under foldene. Noen invaginasjoner viste tydelige „børster" på cytoplasmaoverflaten, et forhold som ble tatt til inntekt for en slik pinocyttose. Vakuoler, multivesikulære legemer og residuallegemer forekom ofte i FAE; noen ganger syntes innholdet i residuallegemene å bli avlevert til intercellulærspaltene ved eksocyttose. Intraepitheliale lymfocytter opptrådte ofte gruppevis og noen hadde rikelig med endoplasmatisk retikulum. Enzymhistokjemi ga svake reaksjoner av adenosin trifosfat-spaltende enzym og aminopeptidase i FAE's celleoverflate. Sur fosfatase i cytoplasma av FAE viste en markert reduksjon fra basis til apicale partier av „pseudovilli", antagelig i forbindelse med endocyttose. Resultatene fra dette studiet synes forenlige med den oppfatning at FAE pinocytterer makromolekyler og sensiterer lymfocytter.

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