Mem. Inst. Oswaldo Cruz, Rio de Janeiro, Vol. *85* (4): 435-443, oct./dec. 1990

HISTOLOGICAL AND ULTRASTRUCTURAL STUDIES OF THE MARSUPIAL *DEDELPHIS ALBIVENTRIS* PEYER'S PATCHES

V. B. COUTINHO; H. B. COUTINHO; T. I. ROBALINHO; E. S. O. SILVA; H. F. SEWELL* & A. D. McKINNON*

Centro de Pesquisas Aggeu Magalhães – FIOCRUZ, Caixa Postal 7472, 50730 Recife, PE, Brasil
*Department of Pathology, University of Aberdeen, AB9 2ZD, Scotland, UK

Differing from the studied Eutheria the white belly opossum Peyer's patches do not present a conspicuous dome. M cells are located in the inner layer of bilaminal invaginations formed at the bottom of the villi. A great variation in the morphology of M cells was observed. The enterocytes located at the epithelial inner layer may present endocytic vesicles, and the microvilli are shorter than the microvilli of enterocytes lining the small intestine. As these morphological aspects have been described to exist in the enterocytes of the lactent opossum small intestine it was surmised that the opossum Peyer's patches special epithelium could represent the persistence in adult animals of a cellular pattern established before the intestinal maturation had occurred.

Key words: M cells – Peyer's patch – *Didelphis albiventris*

In lactent mammals the small intestine is lined by an epithelium which is able to absorb macromolecules by endocytosis. In the duodenum coated vesicles transfer to the lamina propria macromolecules captured by specific receptors in the glycocalix; in the jejunum-ileum ingested milk is absorbed by endocytosis and digested intracellularly by lysosomal activity (Clark, 1959; Williams & Beck, 1969; Rodewald, 1970, 1973, 1980). Part of the nerve growth factor and epidermal growth factor absorved by the ileum epithelial cells of lactent rats may escape lysosomal degradation and are transported in vesicles to the basolateral cell surface for release by exocytosis (Siminosky et al., 1986; Gonnella et al., 1987). During weaning the epithelial cells are substituted by enterocytes unable to promote endocytosis of macromolecules. The substitution of a type of epithelium permeable to macromolecules by another one devoid of this capability has been considered to be the determinant of physiological intestinal closure (Rundell & Lecce, 1973).

The administration of glucocorticoids to lactent rodents was able to induce a precocious intestinal closure (Overton, 1965; Carlile & Beck, 1983). In the lactent opossum the administration of cortisol determined the abrupt substitution of the cells able to promote the endocytosis of macromolecules by enterocytes morphologically similar to the cells present in the adult small intestine lining (Coutinho et al., in press).

The presence of cells able to absorb macromolecules was reported to exist in the epithelial lining of the Peyer's patch domes of *adult* Eutheria, a fact that allowed the understanding of the mode of penetration of antigens, viral and bacterial, from the intestinal lumen to the interior of the organism (Owen & Jones, 1974; Owen, 1977; Wolf et al., 1981; Rosen et al., 1981; Kohbata et al., 1986; Owen et al., 1986).

As the study of the histophysiology of the Peyer's patches has been documented in Eutheria, we believed it to be necessary and important to study the histological and ultrastructural features of Peyer's patches in the Methatheria. We have studied *Didelphis albiventris* (a marsupial of easy capture in the urban area of Recife, Brazil) as our experimental model.

MATERIALS AND METHODS

Thirty-tree adult opossum after a 24 h fast

This work was supported by research grants from Agreement Conselho Nacional de Pesquisas/The British Council, SUDENE and Bank of Brasil Foundation.

Received 23 April 1990
Accepted 23 August 1990

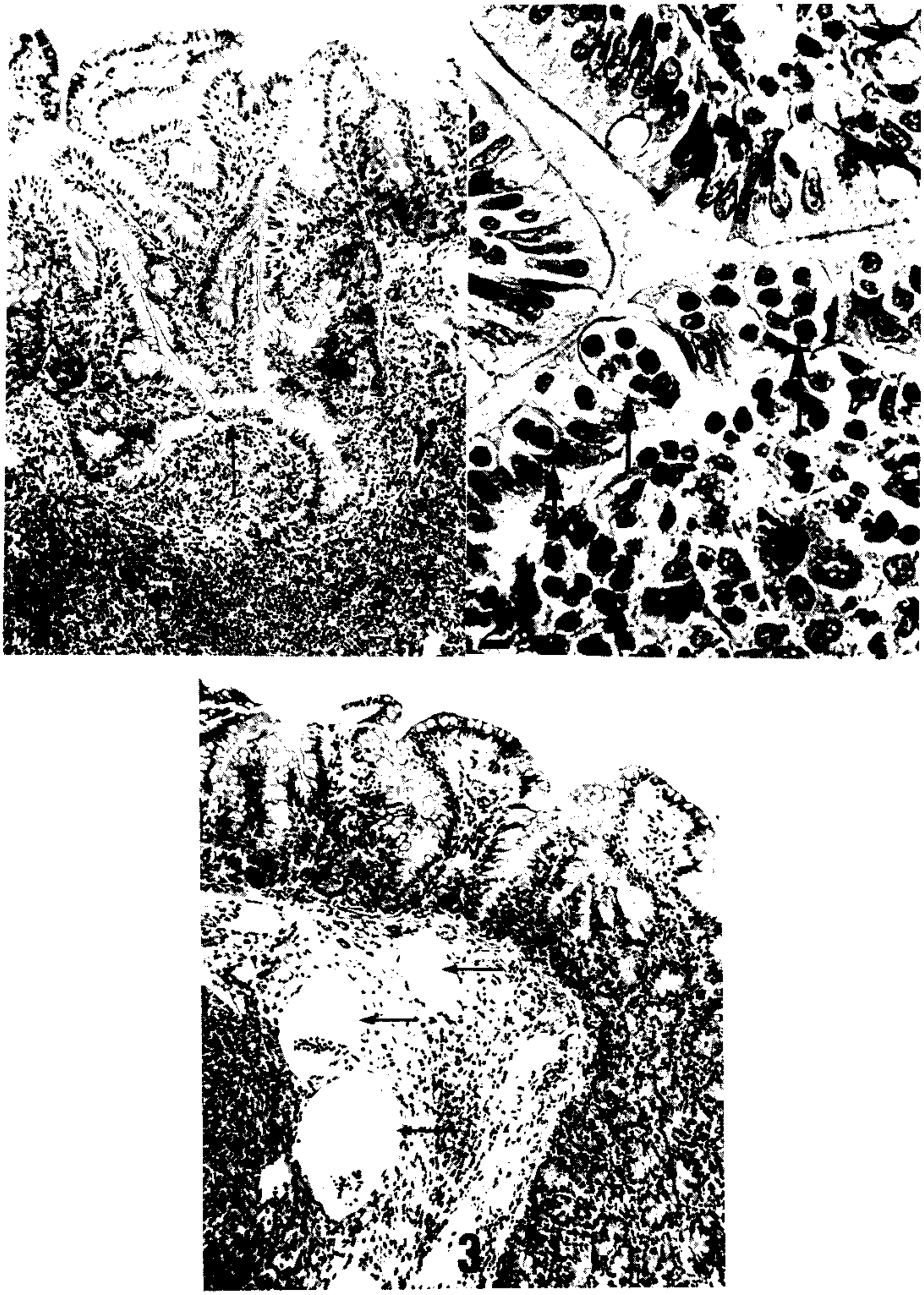

Fig. 1: opossum Peyer's patch. Arrow indicates the dome epithelial lining equivalent. H. E. x 50. Fig. 2: higher magnification for Fig. 1. Arrows indicate M cell. H. E. x 200. Fig. 3: arrows indicate laterally located lymphatic vessels containg lymphocytes. H. E. x 50.

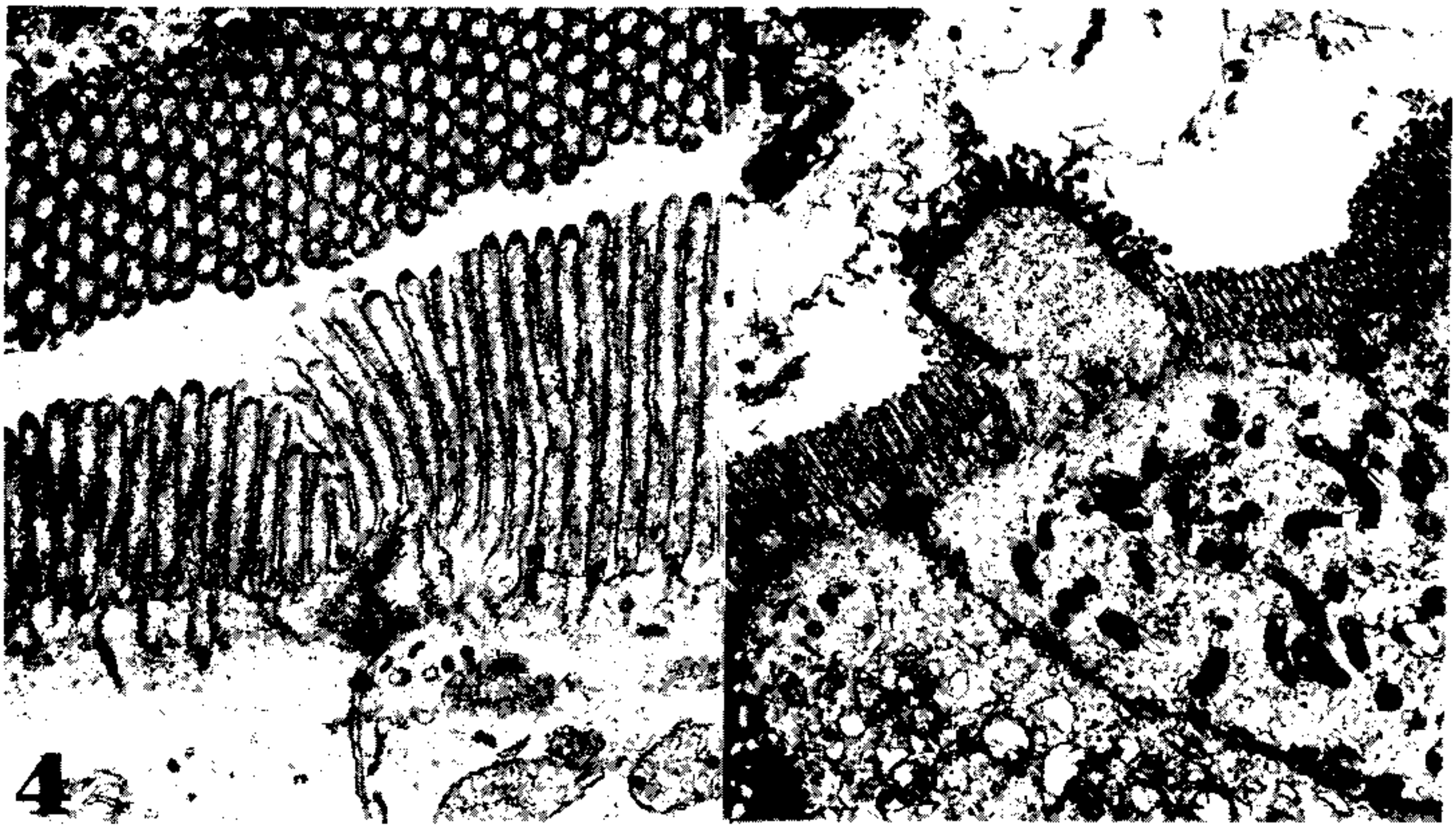

Fig. 4: electronmicrography of Peyer's patch enterocyte presenting shorter microvilli than adjacent enterocyte. x 16.000. Fig. 5: electronmicrography of an enterocyte presenting short microvilli on a cellular protusion. x 9.300.

were anesthetized by ether; the small intestine was removed and sectioned longitudinally; Peyer's patches were removed and fixed in Bouin's or Karnovsky's fixatives. In 7 animals, isolated loops of the jejunum-ileum were filled with saline and the animal sacrificed 30 min later; Peyer's patches were removed and fixed as reported.

Bouin fixed material was embedded in paraffin or JB-4 resin (Polyscience, Inc., Warrington, PA, USA). Sections of 5 μm were stained by haematoxylin and eosin.

For ultrastructural studies the pieces were fixed for 24 h in Karnovsky fixative, washed in 0.2 M cacodilate buffer and postfixed in 1% osmic acid for 2 h. After dehydration, embedding was done using Emix-Medium resin (EM Scope Laboratories, London, England). Thick sections were stained by toluidine blue. Thin sections (60-90 nm) were placed on grids, counterstained with 7% uranyl acetate and lead acetate. An EM Zeiss Model EM-109 and an EM Jeol Model JEM-100S were used for the ultrastructural studies.

RESULTS

Histological observations – The opossum Peyer's patches measure 5 to 8 mm diameter. They are disclike structures located at the opposite surface to the attachment of the mesentery to the small intestine. Most of them are located in the terminal ileum but one or two are occasionally found in the duodenum. In the region of the patches the intestinal villi are shorter than in the adjacent tissues. Lieberkuhn crypts are observed in the patches and communicate with the bottom of the villi. It was noted that in portions of the lamina propria devoid of *muscularis mucosae* there was ingrowth of the bottom of the villi to form bilaminal invaginations that extended parallel to the surface. The inner layer of this invagination is in contact with a lymphatic nodule and possesses large cells with clear cytoplasm and to which are attached several lymphocytes. At optical level, cells similar to the enterocytes are located among those clear cells. Goblet or Paneth cells are not present in the inner layer epithelium. However, they were noticed in the outer layer of the invagination and lining the tubelike structure that connects the invagination with the base of the villi (Figs 1, 2).

In the areas overlying the invaginations crypts of Lieberkuhn were observed. Several lymphatic nodules with germinal centers are present in the Peyer's patch. Conspicuous lymphatic vessels with lymphocytes in their lumens are present laterally or between the

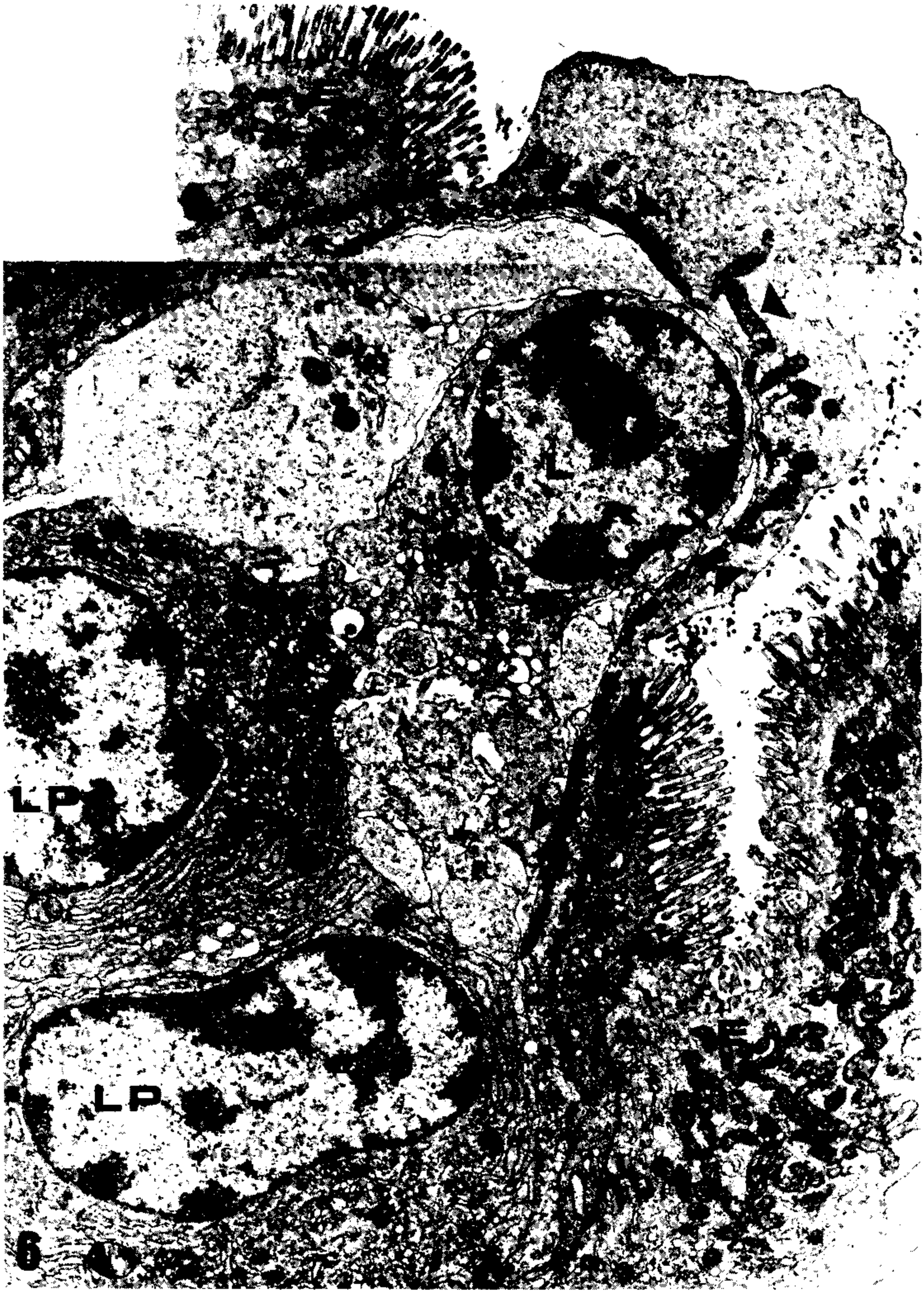

Fig. 6: electronmicrography of a M cell devoid of microvilli. The lymphocytes within it cytoplasmic niches are at different stages of plasmocytoid differenciation. Arrowheads indicate mitochondria; L: lymphocyte; LP: plasmocytoid lymphocytes; E: enterocytes. x 11.000.

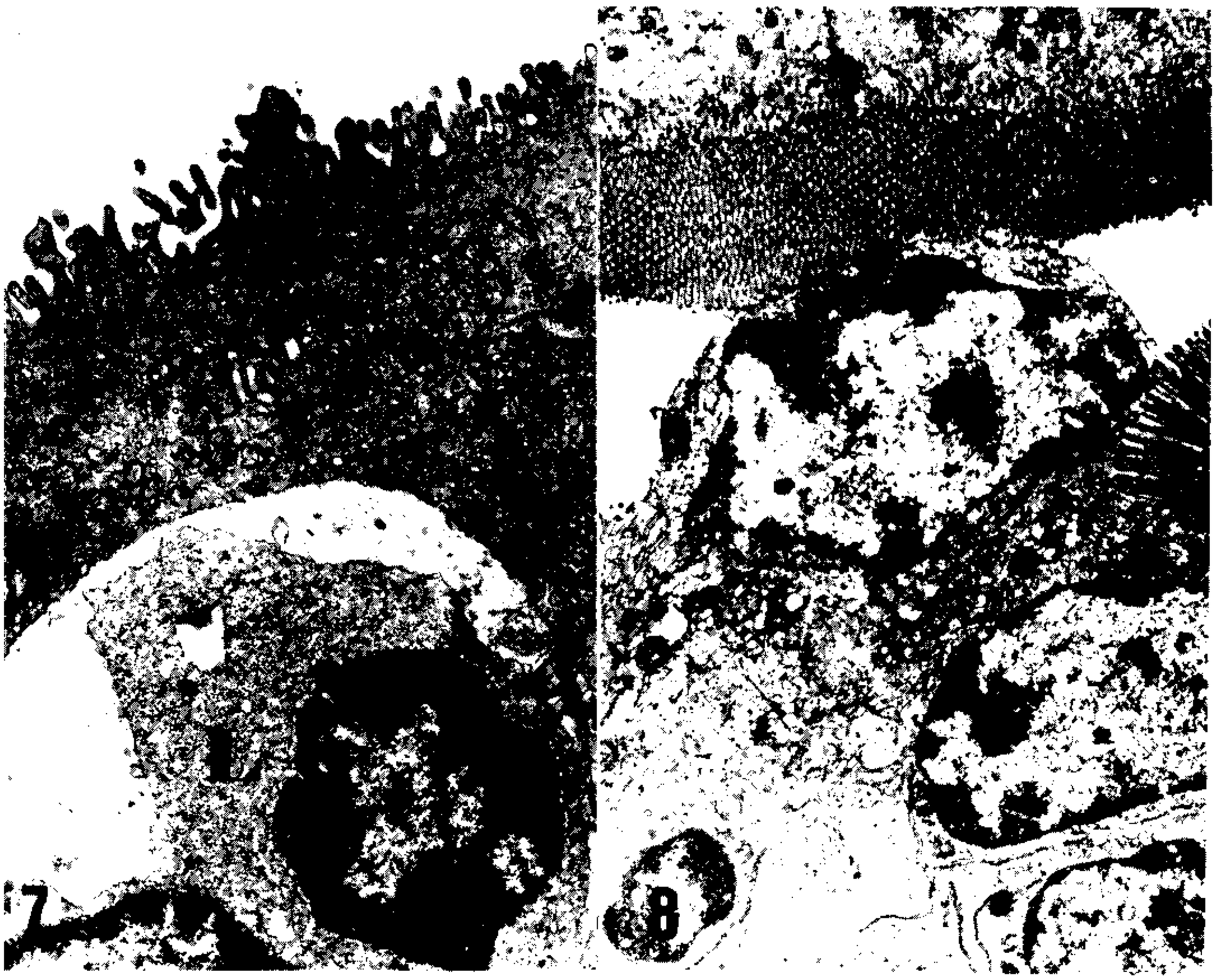

Fig. 7: electronmicrography of a M cell presenting microvilli of different shape and length. L: lymphocyte. x 9.300. Fig. 8: electronmicrography presenting a lymphocyte migrating into the intestinal lumen.

nodules and the muscular layer. The lining of the villi epithelium is formed by prismatic enterocytes devoid of cytoplasmic vesicles, and goblet cells (Fig. 3).

Ultrastructural observations – At EM level a considerable variation in the morphological aspects of the cellular population lining the inner layer of the epithelial invagination was observed. Laterally to the invagination the typical enterocytes that present long microvilli are abruptly substituted by cells with short microvilli and endocytic vesicles in their cytoplasm (Fig. 4). The number and length of the microvilli vary from cell to cell. Occasionally, on the surface of a single cell, areas of short microvilli alternate with areas of long microvilli (Fig. 5). Cells containing lymphocytes in cytoplasmic "niches" are usually devoid of or contain a few short microvilli. These cells that may be considered as equivalent of the M cells described in the Eutheria Peyer's patches, present a great variation in the morphology of their cellular apical poles. Whilst some M cells maintain the same superficial level of the neighbouring enterocytes, other M cells demonstrate cytoplasmic protusion into the intestinal lumen. In such cells several lymphocytes are noted within their cytoplasm. Elongated mitochondria are located in the apical pole and lateral areas of the M cells, the nuclei of which are always located at the basal pole. The lymphocytes within the M cell cytoplasm exhibit varied stages of plasmacytoid differentiation; whilst the lymphocytes located at the upper surface of the M cells do not possess a conspicuous rough endoplasmic reticulum, the lymphocytes in the middle or basal areas are rich in rough endoplasmic reticulum. Lymphocytes migrating into the lumen do not show plasmacytoid differentiation. Numerous plasmacytoid cells are present in Peyer's patches lamina propria. The space between the lymphocytes and the wall of the M cell cytoplasmic "niches" is occupied by a flakelike material of low electron density, comparable to the content of the endocytic vesicles (Figs 6, 7, 8).

Fig. 9: cytoplasmic vesicles are present in M cell and enterocyte of an animal treated by intraluminar injection of saline. M: M cell; E: enterocyte; P: unidentified Protozoan. x 15.000.

In fasting animals the enterocytes present in the opossum Peyer's patches are usually devoid of cytoplasmic vesicles, whilst a few small vesicles are present in the apical cytoplasm of the typical M cells. However, in the animals where segments of the small intestine were filled with saline, all cells lining the lower layer of the cellular invaginations possess apical vesicles of variable size containing a flakelike material (Fig. 9).

Junctional complexes are present in all cells lining the inner layer of the epithelial invagination.

DISCUSSION

In the opossum the localization of Peyer's patches in the duodenum and distal portion of the ileum is equivalent to that described in mice by Chin & Hudson (1971).

In Eutheria, Peyer's patches represent areas of the small intestine where lymphatic nodules are lined by a thin mucosa devoid of intestinal villi or crypts (Faulk et al., 1971). The mucosa lining the lymphatic nodule is in the form of a thin dome laterally delineated by sulci. The dome epithelium is composed of short cells containing intracytoplasmic lymphocytes; goblet cells are absent in the epithelial dome (Faulk et al., 1971; Bockman & Cooper, 1973). Three different cellular types were described in the epithelial dome: (1) cells morphologically equivalent to the enterocytes; (2) cells presenting long microvilli (tuft cells) and (3) a special type of cell denoted as the M cell which is able to acommodate lymphocytes within its cytoplasm, and to perform endocytic activity (Owen, 1977).

The demonstration of M cells and the comprehension of their function, identified in the intestinal epithelium of *adult* Eutheria a cell type able to promote the absorption of macromolecules. Such function had been previously stated to occur in lactent mammals only until the establishment of intestinal closure. Thus, the M cells were recognized as the site of entry for intestinal antigens, lectins, viruses and bacteria into the organism. The presence of B lymphocytes in the M cells is interpreted as allowing a rapid contact with the absorbed antigens. Following migration of the B cells into the submucosa and subsequent lymphatic drainage and blood circulation, expansion of lymphocyte clones has been demonstrated together with selective homing to the intestinal lamina propria of immunocytes able to secrete specific IgA antibodies.

Because of the presence of villi on the whole surface, the opossum Peyer's patches do not present a domelike structure as has been reported in the Eutheria. Only the lower of the epithelial invaginations formed at the bottom of villi are in contact with the lymphatic nodules and contain cells equivalent to the Eutheria dome lining.

In the special epithelium of opossum Peyer's patches only two cell types were identified: enterocytes and M cells. Cells with the morphologic features ascribed to the tuft cells were not seen in the opossum. When saline was injected into the small intestinal lumen both types of cells had demonstrable endocytic vesicles. Similar observations were reported by Calado et al. (1986) who showed increased endocytic vesicles in the enterocytes of the lactent cat when the intestinal lumen was filled with ingested milk. Coutinho et al. (in press) reported a precocious gut maturation in the cortisol treated lactent opossum; the newly formed enterocytes possessed longer microvilli than those observed in the non-treated lactent marsupial, a fact equivalent to the observation of Overton (1965) in the cortisone treated lactent rat. As the present of short microvilli and endocytic capability are both observed in the enterocytes of the lactent opossum and in the enterocytes lining the dome equivalent of Peyer's patches of adult marsupial, it is surmised that such a cell type represents the maintenance in adult animals of a histophysiological feature present in the lactent animals small intestine until weaning occurs.

Whilst Bye et al. (1984) have considered the M cells as a special cell type, Smith & Peacock (1980), and Owen (1983) suggested that M cells may represent a differentiated form of the enterocytes lining Peyer's patches. The existence of M cells in the opossum with or without microvilli, and the differences in length and number of microvilli in the observed enterocytes of the opossum Peyer's patches could suggest that all cells lining the lower layer of the opossum Peyer's patches constitute a single cell type. The shape variation of the apical pole of the cells may be surmised as a

result of the tension exerted by the intracytoplasmic lymphocytes when they become bound in the cellular membranes forming their "niches". The tension on the lateral membrane of the enterocyte could also be responsible for the reduction in length and number of microvilli.

In the opossum M cells, lymphocytes are lodged in "niches" at different cells levels; the lymphocytes located in the neighbourhood of the basal pole are noted to present plasmacytoid differentiation. Contrastingly the cells located close to the upper surface of the M cells do not have a conspicuous rough endoplasmic reticulum. The lymphocytes observed migrating into the lumen were also poor in rough endoplasmic reticulum. These interesting variations in lymphocyte morphology and differentiation may relate to different lymphocyte lineages. Equivalents of T and B lymphocytes may exist within the opossum, and in the region of the Peyer's patch they may mediate interactions with absorbed antigens, one result of which may be the variation in lymphocyte cytomorphology noted in this study.

ACKNOWLEDGEMENT

To Miss M. C. C. Lacerda for her typing.

REFERENCES

BYE, W. A.; ALLAN, C. H. & TRIER, J. S., 1984. Structure, distribution, and origin of M cells in Peyer's patches of mouse ileum. *Gastroenterology, 86:* 789-801.

BOCKMAN, D. E. & COOPER, M. D., 1973. Pinocytosis by epithelium associated with lymphoid follicles in the Bursa of Fabricius, Appendix, and Peyer's patches. An eletron microscopic study. *Am. J. Anat., 136:* 455-478.

CALADO, T. C.; COUTİNHO, V. B.; MELO, L. M.; MENDONÇA, M. A. & COUTINHO, H. B., 1986. Modificações citológicas observadas nos enterócitos de gato lactente decorrentes da endocitose do leite materno. III Jornada Científica do CCBi/UFAL.

CARLILE, A. E. & BECK, F., 1983. Maturation of ileal epithelium in the young rat. *J. Anat., 137:* 357-369.

CHIN, K. N. & HUDSON, G., 1971. Ultrastructure of Peyer's patches in the normal mouse. *Acta Anat., 78:* 306-318.

CLARK, S. L., 1959. The ingestion of proteins and coloidal materials by columnar absortive cells of the small intestine in suckling rats and mice. *J. Biophys. Biochem. Cytol., 5:* 41-51.

COUTINHO, V. B.; COUTINHO, H. B. & COUTINHO, E. M., 1989. Effects of hydrocortisone acetate treatment on the small intestine of the lactent marsupial *Didelphis albiventris. Anat. Anzeiger.* (in press).

FAULK, W. P.; McCORMICK, I. N.; GOODMAN, J. R.; YOFFEY, I. M. & FUDENBERG, H. H., 1971. Peyer's patches: morphologic studies. *Cell. Immunol., 1:* 500-520.

GONNELLA, P. A.; SIMINOSKI, K.; MURPHY, R. A. & NEUTRA, M. R., 1987. Transepithelial transport of epidermal growth factor, by absorptive cells of suckling rat ileum. *J. Clin. Invest., 80:* 22-32.

KOHBATA, S.; YOKOYAMA, H. & YABUUCHI, E., 1986. Cytopathogenic effect of *Salmonella typhi* GIFU 10007 on M cells of murine ileal Peyer's patches in ligated ileal loops: an ultrastructural study. *Microbiol. Immunol., 30:* 1225-1237.

OVERTON, J., 1965. Fine structure of the free cell surface in developing mouse intestinal mucosa. *J. Exp. Zool., 159:* 195-202.

OWEN, R. L., 1977. Sequencial 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, R. L., 1983. A new pathophysiology of M cells. Good news and bad news from Peyer's patches. *Gastroenterology, 85:* 468-469.

OWEN, R. L.; APPLE, R. T. & BHALLA, D. K., 1986. Morphometric and cytochemical analysis of lysosomes in rat Peyer's patch follicle epithelium: their reduction in volume fraction and acid phosphatase content in M cells compared to adjacent enterocytes. *Anat. Rec., 216:* 521-527.

OWEN, R. L. & JONES, L., 1974. Epithelial cell specialization within human Peyer's patches: an ultrastructural study of intestinal lymphoid follicles. *Gastroenterology, 66:* 189-203.

RODEWALD, R. B., 1970. Selective antibody transport in the proximal small intestine of neonatal rat. *J. Cell Biol., 45:* 635-670.

RODEWALD, R. B., 1973. Intestinal transport of antibodies in the newborn rat. *J. Cell Biol., 58:* 189-211.

RODEWALD, R. B., 1980. Immunoglobulin transmission in mammalian young and the involvement of coated vesicles. In C. D. Ockleford & A. White (eds). *Coated vesicles.* Cambridge University Press, England.

ROSEN, L. von; PODJASKI, B.; BETTMANN, I. & OTTO, H. F., 1981. Observations on the ultrastructure and function of the so-called "Microfold" or "Membraneus" cells (M cells) by means of peroxidase as a tracer. *Virchows Arch.* (Pathol. Anat.), *390:* 289-312.

RUNDELL, J. D. & LECCE, J. G., 1973. Independence of intestinal epithelial turnover from cessation of absorption of macromolecules (closure) in neonatal mouse, rabbit, hamster and guinea pig. *Biol. Neonate, 20:* 51-57.

SIMINOSKI, K.; GONNELLA, T.; BERNANKE, J.; OWEN, L.; NEUTRA, M. & MURPHY, R. A., 1986. Uptake and transepithelial transport of nerve growth factor in suckling rat ileum. *J. Cell Biol., 103:* 1979-1990.

SMITH, M. W. & PEACOCK, M. A., 1980. M cell distribution in follicle-associated epithelium of mouse Peyer's patch. *Am. J. Anat., 159:* 167-175.

WILLIAMS, R. M. & BECK, F., 1969. A histochemical study of gut maturation. *J. Anat., 105:* 487-501.

WOLF, J. L.; RUBIN, D. H.; FINBERG, R.; KAUFFMAN, R. S.; SHARP, A. H.; TRIER, J. S. & FIELDS, B. N., 1981. Intestinal M cells: a pathway for entry of reovirus into the host. *Science, 212:* 471-472.