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Morphological Study of Microfold Cells of Intestinal Lymphoid Follicles in Peyer's Patches

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ABSTRACT. A study was made of the morphology of microfold cells (M cells) of the human and rat Peyer's patch, and the morphological changes occurring due to aging and disease were examined. In the mature rat, the microfolds were classified into four types, and the microfolds were found to differ according to age. A microfold jutting out as a long, thin projection was noted in a patient with an intestinal infection. There was a close relationship between the enclosure of lymphoid cells by M cells and age in that with increasing age there were more M cells with enclosed lymphoid cells as well as more lymphoid cells within one M cell. It was also shown that pinocytosis as described by Fawcett occurs along with micropinocytosis in the incorporation of macromolecules.

Key words : Microfold cell — M cell — Lymphoid cell — Pinocytosis

There are numerous kinds of gut-associated lymphoid tissue such as Peyer's patches and lymphoid follicles which gradually increase in number and size from the tonsils and duodenum to the cecum in the mucosa of the mammalian gut. It is known that this gut-associated lymphoid tissue functions as a local immune system to cope with the constant introduction of antigenic macromolecules. As shown by the work of Owen *et al.*[1] and numerous other functional morphological studies, mostly on mice, cells having a unique morphology are found among follicle-associated epithelial cells. These cells have been described as microfold cells or membranous epithelial cells and are referred to as M cells. Studies concerning human M cells are few, and no study has been performed of the morphological changes accompanying aging. In a functional morphological study of M cells, the author examined the ultrastructure of human M cells and morphological changes of M cells due to aging, employing rats where human materials could not be used.

MATERIALS AND METHODS

The author examined portions of the ileum fixed immediately after surgical resection from a 42-year-old male with cancer of the ileocecum, 54-year-old male with cancer of the ascending colon, 57-year-old male with Crohn's disease and 34-year-old male with Yersinia enteritis, and the jejunum of a 14-month-old girl with congenital stenosis of the jejunum.

Peyer's patches from the ileum and jejunum of three each of 10-day-old, 20-day-old and one-year-old male SD rats were examined in the study.

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Materials were fixed in 2.5% glutaraldehyde, and the Peyer's patches and solitary lymphoid follicles were observed under the dissecting microscope, after which lymphoid follicles were removed en bloc. Light microscopic observation was followed by examination under the electron microscope. Transmission electron microscopic samples were fixed for 2 hours in 1% osmium tetroxide, dehydrated through an ethanol series, transferred to propylene oxide and embedded in epoxy resin. Ultrathin sections were cut with glass knives on a Porter-Blum MT2-B ultramicrotome, stained with uranyl acetate and lead citrate, and viewed under a Hitachi H-500 electron microscope. Materials for scanning electron microscopic observation underwent fixation in 1% osmium tetroxide and dehydration through an ethanol series, and after being transferred to isoamyl acetate, were critical-point dried, evaporationcoated with gold-palladium and viewed under a Hitachi HHS-2R electron microscope.

RESULTS

Human M cells. In small intestine tissue stained with toluidine blue, lymphoid follicles within Peyer's patches were observed under the dissecting microscope as hemispherically shaped domes of various sizes, from large ones of 900 μm to small ones of 300 μm. In mice, each Peyer's patch usually has 6 domes, but in the human tissue samples, no such regularity was observed.

Certain cells in HE and PAS stained specimens observed under the light microscope appeared to be M cells because of the difficulty of viewing any brush border, the cells being sunken into the surface of follicle-associated epithelium and the cells having mononuclear cells enclosed within them, but definite confirmation was not possible.

Large domes were observed under the scanning electron microscope as hemispherical protuberances with a diameter of 900 μm, and small domes as 300 μm-diameter protuberances (Fig. 1). With higher magnification, cells sunken into the mucosal epithelium were recognized (Fig. 2, arrow). At very high magnification, microfolds were revealed on the surface of these sunken cells. There was no surface coat covering these cells. Most of these M cells were distributed around domes. There were about 36 enterocytes to one M cell.

The basic structure of human M cells as observed under the transmission electron microscope was similar to that of mouse M cells.[2] There were two lymphoid cells enclosed in one of the M cells examined. The microfolds formed a sharp border, and there was no surface coat. The lymphoid cells enclosed in the M cells had complex cytoplasmic projections (Fig. 4). Enlargement of the luminal surface revealed a distinct difference between the microvilli of the columnar cells and the microfolds of the M cells. In the apical region of the cytoplasm of M cells there were numerous small vesicles (Fig. 5). M cells and columnar cells make contact with each other through desmosomes and junctional complexes in the same manner as adjacent columnar cells do (Fig. 6). Lymphoid cells enclosed within M cells send out complex cytoplasmic projections, while projections of the M cell push into the lymphoid cells, so that the two cells are intimately interlocked with each other. In one instance a projection of an M cell was seen to be incorporated into a lymphoid cell

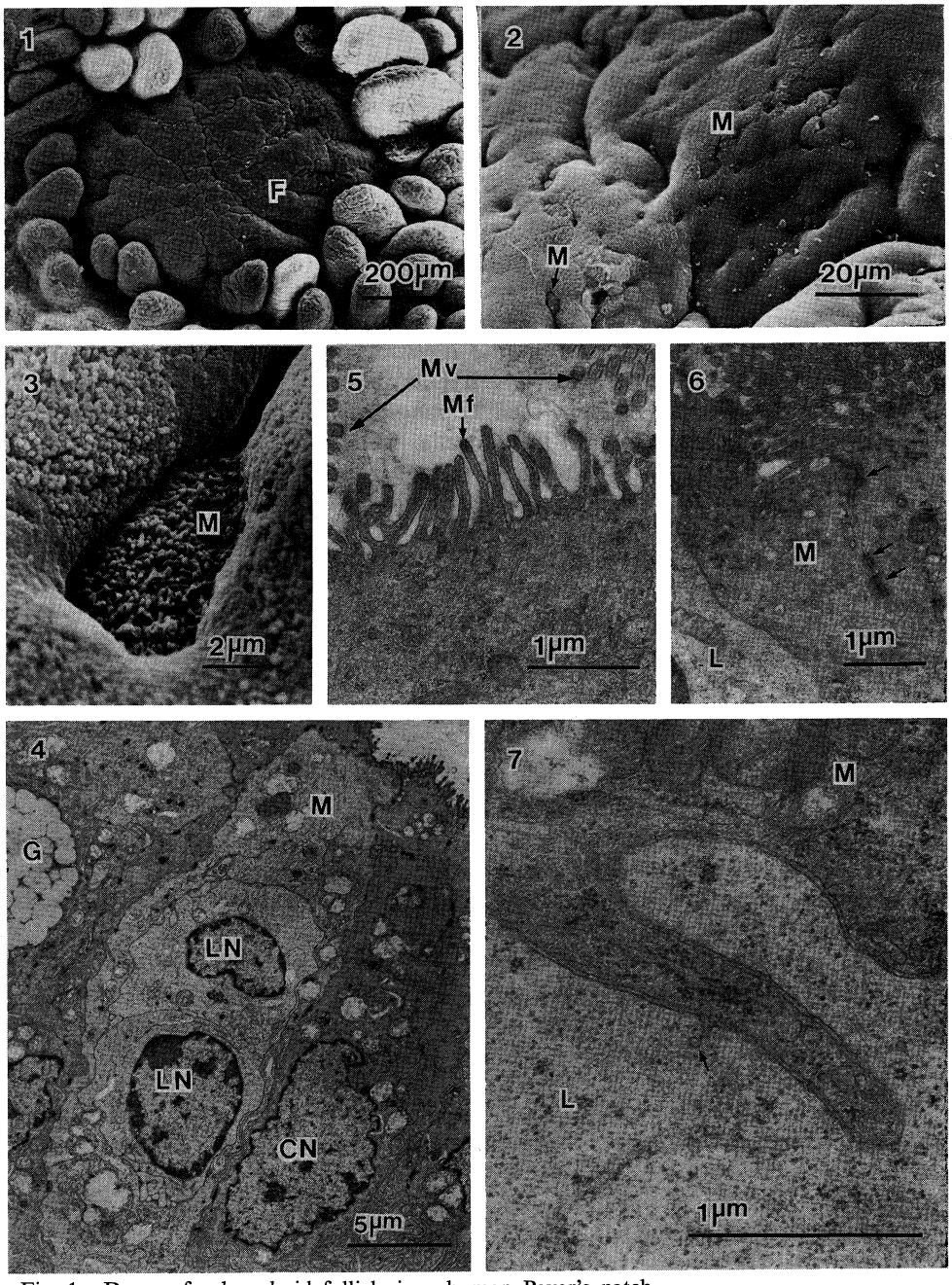

Fig. 1. Dome of a lymphoid follicle in a human Peyer's patch.
Fig. 2. M cells in the lymphoid follicle epithelium of a human Peyer's patch (arrows). M : microfold cell.
Fig. 3. High magnification scanning electron micrograph (SEM) of an M cell in a human Peyer's patch.
Fig. 4. A transmission electron micrograph (TEM) of an M cell in a human Peyer's patch. The M cell encloses two lymphoid cells. MV : microvilli, Mf : microfold, LN : nucleus of the lymphoid cell, CN : nucleus of the columnar cell.

Fig. 5. High magnification of microfolds of an M cell in a human Peyer's patch. Notice the numerous small vesicles in the apical region of the M cell.
Fig. 6. The M cell is connected with adjacent cells by a tight junction and desmosome (arrows).
Fig. 7. Notice the interaction between the cytoplasm of the M cell and the enclosed lymphoid cell. A small vesicle is separating from the cytoplasmic projection of the M cell (arrow). M : microfold cell, L : lymphoid cell.

as a small membraned vesicle (Fig. 7, arrow).

Human M cells, pathologic. The removal of a portion of the ileum from a 34-year-old man with extreme right lower abdominal pain diagnosed as having appendicitis gave the author the opportunity to observe domes of the ileal mucosa. A gastroenteric type of *Yersinia enterocolitica* (biological type 4 ; serum type 0, group 3) was isolated from the intestine of this patient. Transmission electron microscopy revealed the M cell microfolds to bulge above the surface and showed the existence of 3 enclosed lymphoid cells (Fig. 8). Large vesicles appeared in the apical region of the M cells, while small vesicles stretched like long, slender tubes into the cytoplasm (Fig. 9). An extremely long and thin microfold 9 μm in length stretched out from an M cell viewed in serial sections (Fig. 10). A similar observation was had of an M cell from an isolated lymphoid follicle from a 14-month-old girl with congenital stenosis of the jejunum (Fig. 11).

Rat M cells. In one-year-old rats, 15 to 18 Peyer's patches were found in the small intestine from the duodenum on, with greater numbers near the terminal ileum. The number of lymphoid follicles within each Peyer's patch varied from 3 to 24 and tended to increase from the jejunum to the ileum.

In 10-day-old suckling rats, there were only 5 or 6 Peyer's patches distributed in the upper half of the small intestine. Unlike mature rats, the suckling rats did not have Peyer's patches in the ileum. There were 4 to 8 lymphoid follicles in each Peyer's patch. Dome formation was not complete.

Under the light microscope, the absence of a brush border and enclosure of mononuclear cells by M cells was rather obvious in adult rats, but M cells of suckling and young rats had no real distinguishing features.

Characteristics of M cells as revealed by scanning electron microscopy were similar to those of M cells from domes of human specimens. In 20-day and one-year-old rats, the M cells were distributed over the entire dome, while in 10-day-old rats the M cells were scattered only over the upper half of the dome.

Transmission electron microscopy showed the basic structure of the M cells to be the same as in human tissue samples. However, observation of many M cells of adult rats led to the discovery that there were 4 types of surface structures. In Type I, short rod-like microvilli about 0.3 μm long rise densely out of the surface which has a surface coat. The microvilli have central actin filaments which enter the cytoplasm at the apex, but terminal web development is poor (Fig. 13). In Type II, microvilli similar morphologically to those of Type I are arranged irregularly over the surface (Fig. 14). In Type III, the microfolds extend out forming bridges (Fig. 15). In Type IV, there are no microvilli and the cytoplasmic projections clearly are in the form of microfolds (Fig. 16).

Applying this classification to 10-day and 20-day-old rats, many cells

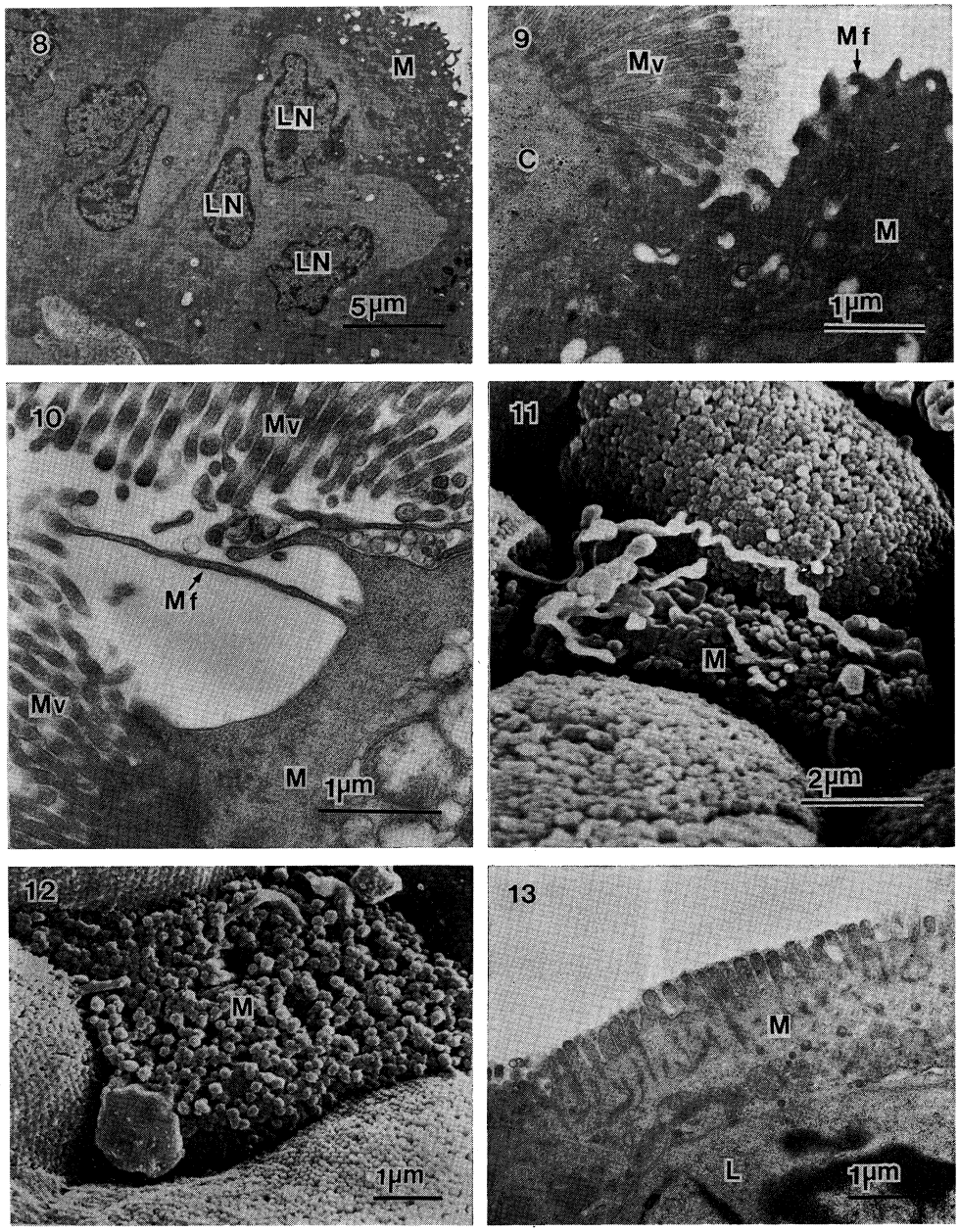

Fig. 8. TEM of an M cell of a case of Yersinia enterocolitis. The M cell encloses three lymphoid cells.
Fig. 9. Apical region of an M cell of a case of Yersinia enterocolitis.
Fig. 10. Section from the same continuous-section series as the section in Fig. 9. Notice the long microfold.
Fig. 11. SEM of an M cell of a 14-month-old girl with congenital stenosis of the jejunum.
Fig. 12. An M cell in the lymphoid follicle epithelium of a rat Peyer's patch.
Fig. 13. Rat M cell surface, Type I : densely arranged short microvilli.

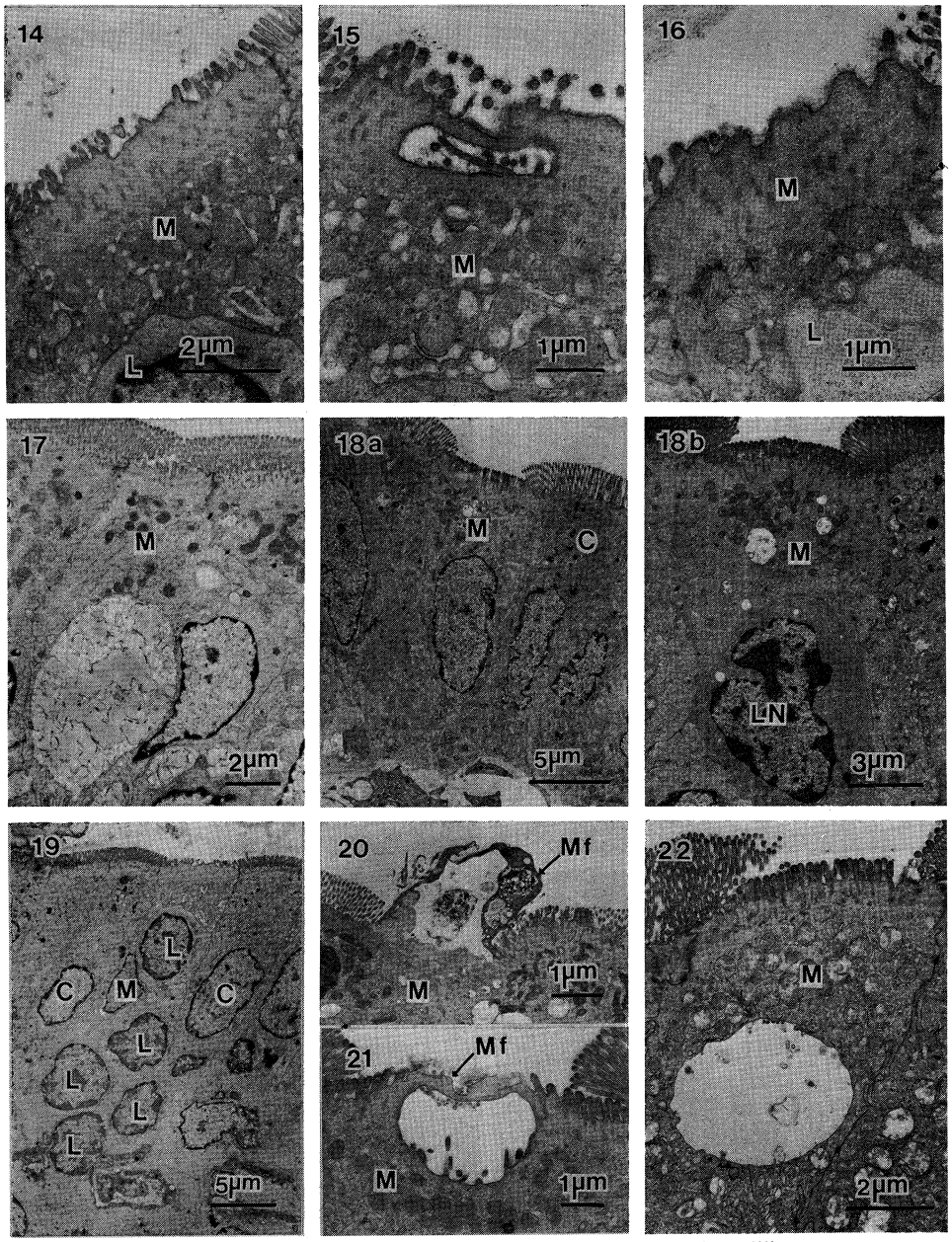

Fig. 14. Rat M cell surface, Type II : irregularly arranged short microvilli.
Fig. 15. Rat M cell surface, Type III : bridging of microfolds.
Fig. 16. Rat M cell surface, Type IV : only microfolds are observed.
Fig. 17. An M cell from a 10-day-old rat has lipid granules, but does not enclose lymphoid cells.
Fig. 18a. An M cell from a 20-day-old rat does not enclose lymphoid cells.
Fig. 18b. An M cell from a 20-day-old rat with one lymphoid cell.
Fig. 19. An M cell from a one-year-old rat with five lymphoid cells enclosed.
Fig. 20. A fold of the M cell impounds macromolecules.
Fig. 21. A large pinocytotic vacuole contains microvilli.
Fig. 22. A large vacuole deep in the cytoplasm of an M cell.

without enclosed lymphoid cells, but having a surface structure different from columnar cells and like M cells were recognized. As shown in Table 1, 84% of the M cells of rats 10 and 20 days after birth were of Type I or II, whereas there were only 5% of Type IV. However, one year after birth, 35% of the M cells were of Type IV, and Type I and II M cells tended to be fewer in number.

TABLE 1. Age-dependent change of microfolds.

Type \ Age	10-day-old	20-day-old	1-year-old
I	47%	81%	12%
II	37%	6%	47%
III	11%	13%	6%
IV	5%	0%	35%

10-day-old : n=19 20-day-old : n=16
1-year-old : n=17 n=number of M-cells

An age difference was also found in the proportion of M cells enclosing lymphoid cells. In 10-day-old rats none of 19 (0%) M cells of any of the 4 types enclosed lymphoid cells, but at 20 days 5 of 16 (31%) and at one year all of 17 (100%) enclosed lymphoid cells. In one-year-old rats, M cells contained from 1 to 6 lymphoid cells, with most of the M cells having 3 lymphoid cells within them (Figs. 17, 18a, b, 19). The existence of lymphoid cells was established by viewing serial sections.

It has been shown that M cells incorporate macromolecules by micropinocytosis,[3,4] but Fawcett[5] reports that uptake of macromolecules also occurs by pinocytosis. In Figure 20 an M cell can be seen which has a long cytoplasmic projection surrounding macromolecules. Within a large vacuole in the apex of the cell formed by a microfold, there are microvilli (Fig. 21). Other micrographs suggest that the large vacuoles move toward the center of the M cell (Fig. 22).

DISCUSSION

The first reports of M cells in man were made by Owen *et al.* who found that M cells existed in the epithelium of lymphoid follicles of the tonsils, Peyer's patches and appendix vermiformis.[1,6] The present author found M cells which were obviously sunken below the columnar cells and had microfolds on their surface in human Peyer's patches. Unlike the observations by Smith *et al.*[7] of mouse M cells, human M cells were fewer in number, and the ratio of M cells to enterocytes in the area of the domes was about 1 to 36. The distribution of M cells of lymphoid follicles from one-year-old rat Peyer's patches was similar to the distribution in mice. In 20-day-old rats the number of M cells was somewhat less, and in 10-day-old suckling rats there were very few M cells. In humans also there were differences in the number and size of solitary lymphoid follicles and Peyer's patches according to age and disease. The number of M

cells, as well as the characteristics of their microfolds and the enclosure of lymphoid cells within them, appeared to be influenced by aging and other external factors.

The microfolds of M cells, confirmed to be so by the enclosure of lymphoid cells within them, of adult rats were found to be of 4 morphological types. While most M cells of young, 10- or 20- day-old rats had short microvilli of about 0.3 μm with central actin filaments, in one-year-old rats, the microvilli disappeared and the cytoplasmic projections formed microfolds. Thus, it became clear that the characteristics of microfolds changed with age in rats. Though somewhat speculative, these differences in the microfolds may be related to the M cells only having brief and weak encounter with antigenic stimulations in young rats so that the cell surface remains rather ordered, but their having taken up various antigens over a long period in adult rats so that the cell surface tends to lose its regularity.

Experiments concerning the function of M cells such as those employing horseradish peroxidase,[3,4] autoradiography,[8,9] *Giardia*,[10] reovirus[11] and *E. coli*[12] have shown M cells to be specialized in the incorporation of antigens. In the 37-year-old male patient with *Yersinia enterocolitica* infection and 14-month-old girl with congenital stenosis of the jejunum, the M cell microfolds stretched out in long projections as if for protection from innumerable antigens.

In suckling rats, M cells did not surround any lymphoid cells, while in 20-day-old rats 31% of the M cells had enclosed lymphoid cells, and all of the M cells observed in adult rats had lymphoid cells within them. Smith *et al.*[7,8] in experiments using thymidine examined the area of cell division and cell renewal in follicle-associated epithelium (FAE) and found that cell division occurs only in the crypts enveloping the FAE and that within the crypts there are no M cells. M cells arise at the base of the FAE and tend to become greater in number higher on the FAE. The distribution of lymphoid cells is similar. The lymphoid cells increase in number from the middle of the FAE to its apex and are always found in close association with the M cells. Evidence has been presented suggesting that M cells are formed from differenciated enterocytes and that lymphoid cells are involved in the differentiation of M cells. From the viewpoint of aging also, M cells and lymphoid cells seem to be intimately related, as seen in the present study where both the numbers of M cells enclosing lymphoid cells and the numbers of lymphoid cells in each M cell increased with age.

It is known that M cells serve as a route for the uptake of macromolecules and microorganisms. Besides micropinocytosis which has been reported in the past, it appears that pinocytosis as described by Fawcett[5] and Hammersen[13] also occurs in the incorporation of large molecules. In response to antigens, a cytoplasmic projection extends from the cell and surrounds the antigens (Fig. 20), and a large vacuole which retains microvilli is formed by the closing of microfolds in the apex of the cell (Fig. 21). The vacuole then moves into the interior of the cell (Fig. 22). Uptake by this process should allow the organism to incorporate very large antigens.

CONCLUSION

1. The ultrastructure of the human M cell was described and discussed.
2. In a patient with intestinal infection, an M cell with a long, thin microfold was noted (Figs. 10, 11).
3. The surface structure of the apex of the M cells of adult rats was classified into 4 types (Figs. 13, 14, 15, 16).
4. With an increase in age, the number of M cells enclosing lymphoid cells and the number of lymphoid cells in each M cell increased.
5. It was shown that besides micropinocytosis, the pinocytosis which Fawcett has reported also plays a role in the incorporation of macromolecules into M cells (Figs. 20, 21, 22).

Acknowledgments

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