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Fine Structure of Mucous Cells in the Skin Epidermis of the Arctic Lamprey, *Lampetra japonica*

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Abstract Mucous cells in the skin epidermis of the arctic lamprey, *Lampetra japonica*, are divided into mid-epidermal and surface mucous cells according to their location in the epidermis. A cytological feature of these cells is the presence of numerous filaments of approximately 7 nm in diameter in their peripheral cytoplasm. In this sense, the lamprey mucous cells, different from typical epidermal mucous cells of teleosts, may be said to be filament-containing cells with mucus. The mucus is released from the surface mucous cell by localized rupturing of its distal plasma membrane fused with the membrane enclosing mucous droplets. Structures considered to be coated vesicles are found in the peripheral cytoplasm immediately adjacent to the plasma membrane of the mucous cells. However, they do not seem to be a structure peculiar to these cells, but may be found universally in various animal cells.

The skin epidermis of lampreys is generally composed of distinct cell types such as mucous, club and granular cells (Pfeiffer and Pletcher, 1964; Damas and Firket, 1966; Bertolini et al., 1968; Downing and Novales, 1971; Andrew and Hickman, 1974; Anderson and Manion, 1977; Lethbridge and Potter, 1980). Among these, the mucous cells are the most numerous and pervade the entire epidermis. Fine structures of the mucous cell in lampreys have been studied by Damas and Firket (1966), Bertolini et al. (1968) and Downing and Novales (1971). The present paper deals with the same problem in adult arctic lamprey, to be compared with the results obtained in the mucous cells of other lampreys and teleosts. In the description of the fine structure, the mucous cells will be divided into mid-epidermal and surface mucous cells depending upon their location in the skin epidermis. As mucous cells are believed to differentiate from basal epidermal cells, this paper will also refer to the fine structures of these cells.

Material and methods

Adult specimens of the arctic lamprey, *Lampetra japonica* (Martens), measuring about 30 cm in total length, were obtained from the Iwaki River flowing into the Sea of Japan. For electron microscopy, small pieces of head skin approximately 1.5 mm^3 were fixed in cacodylate-buffered 4% glutaraldehyde solution for 1.5 hours at 0~4°C, washed overnight in the same buffer, and postfixed in Millonig's phosphate-buffered 1% osmium tetroxide solution for 1.5 hours. Dehydration was accomplished rapidly in a series of graded ethanols, and then embedded in Epon 812, through two changes in propylene oxides. Thin sections were cut on a Poter-Blum MT-I ultramicrotome using glass knives and double-stained with 4% aqueous solution of uranyl acetate and Reynold's lead acetate. Observations were made on a Hitachi HU-12As and HS-7D electron microscope. Semi-thin sections of 1~2 μm were cut with glass knives, mounted on glass slides, and stained with 0.5% toluidine blue solution (pH 7.1). Some paraffin sections prepared with routine histological procedures were also stained by the periodic acid-Schiff technic (PAS) for the identification of mucus.

Results

Basal epidermal cell. The basal layer of the lamprey epidermis consists of elongated cells with pointed apices. These cells are basal epidermal cells, of which the deep surface is attached to a basal lamina. They give no positive reactions to the PAS test, showing little accumulation of mucus. Nuclei of the basal epidermal cells are generally oval with a slightly irregular outline and often deeply infolded. The nucleus is located near the apical region of the cell and the nuclear material is relatively homogenous with

Fig. 1. Perinuclear region of the basal epidermal cell. Structures considered morphologically to be coated vesicles (arrows) are found in the peripheral cytoplasm immediately adjacent to the plasma membrane. ×14,000. er, rough-surfaced endoplasmic reticulum; g, Golgi apparatus; m, mitochondrion; n, nucleus.

a well-defined nucleolus. The cytoplasm may be divided into two morphologically distinct zones: the perinuclear zone and the peripheral zone. The perinuclear zone, particularly basal to the nucleus, contains most of the cytoplasmic organelles, including a good number of mitochondria, rough-surfaced endoplasmic reticula, Golgi apparatus, and numerous ribosomes (Fig. 1). There are also found electron dense bodies looking morphologically like lysosomes. The peripheral zone is filled with a great number of about 7 nm-thick filaments which run all directions (Fig. 2). At the cell periphery, they are compacted into fibrils with a somewhat high electron density, and some of them anchor in desmosomes between two adjacent basal cells. The plasma membrane facing the basal lamina is infolded and lined with hemi-desmosomes (Fig. 2), while the rest of the plasma membrane interdigitates extensively with the membrane of adjacent basal cells, forming numerous desmosomal junctions. Wide intercellular spaces are also found around the basal epidermal cells (Fig. 2). Structures considered morphologically to be coated vesicles are recognized in the peripheral cytoplasm immediately adjacent to the plasma membrane (Figs. 1, 2). The structures mentioned just above seem to be derived from the plasma membrane. Mitotic figures or paired centriols are occasionally seen in the basal epidermal cells.

Mid-epidermal mucous cell. Above the basal epidermal layer are several strata of polyhedral or rhombic cells, giving a positive reaction to the PAS test. These cells are mid-epidermal mucous cells. In electron micrographs these

Fig. 2. Basal portion of the basal epidermal cell. Numerous filaments run in all directions and some of them terminate in hemidesmosomes (hd) lining the basal lamina. Minute spherical ingrowth (arrows) from the plasma membrane suggests that the structure considered to be a coated vesicle may be derived from the plasma membrane. Intercellular spaces are also found around the cell. ×18,000.

cells appear in different stages from the basal epidermal cells (Figs. 3A, 4). During the early stages of differentiation of the cell, its nucleus is almost centrally located, but becomes to assume a more basal postion in the cell, with accumulation of mucous substances. The nucleus usually contains a well-defined nucleolus (Fig. 4). The cytoplasm of the mid-epidermal mucous cell is roughly divided into the perinuclear and the peripheral zones. The perinuclear zone covers a much greater area within the cytoplasm than does the peripheral zone. The perinuclear zone contains a considerable number of mitochondria, numerous free ribosomes, lysosome-like dense bodies, rough-surfaced endoplasmic reticula and prominent Golgi apparatus (Figs. 3A, 4). The latter two organelles are far more developed in the mucous cell than in the basal epidermal cell. Mucous droplets, mucous substances isolated in discrete packets, are also located in the perinuclear zone, especially in the vicinity of Golgi apparatus (Fig. 4). They appear both singly and in groups, and the majority of them are elongated oval. The peripheral zone is occupied by a great number of filaments measuring about 7 nm in diameter and a number of free ribosomes. Ovoid bodies with a moderate electron density and of various sizes, measuring about 1 μm in maximum diameter, are seldom found in the junction between the perinuclear and peripheral zones (Figs. 3A, 5). They are located in such close proximity to the filaments

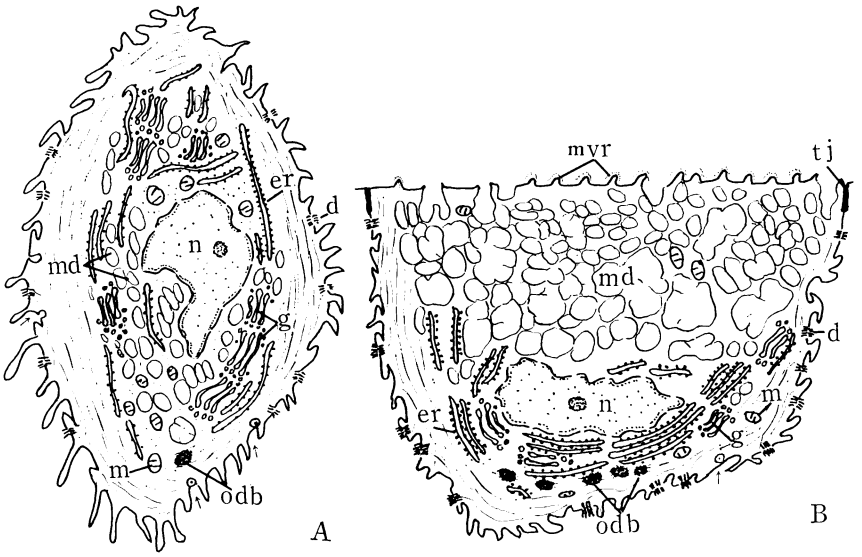

Fig. 3. Schematic illustrations showing the fine structures of the mid-epidermal mucous cell (A) and the surface mucous cell (B). d, desmosome; md, mucous droplet; mvr, microvillar ridge; odb, ovoid body with a moderate electron density; tj, tight junction. Arrow indicates a structure considered to be a coated vesicle. Other letters, vide Fig. 1.

that they seem to be connected with some of the filaments. The number of the bodies contained in a cell is generally one, but sometimes two or more. These bodies are morphologically similar to an electron dense granule observed by Downing and Novales (1971) in club cells of a lamprey, *Ichthyomyzon unicuspis*. The coated vesicles seen in the basal epidermal cell are also found in the peripheral cytoplasm adjacent to the plasma membrane (Fig. 4). The mid-epidermal mucous cells are locked together by considerably complicated interdigitations and also by a considerable number of desmosomes.

Surface mucous cell. The surface layer of the skin epidermis consists of depressed funnel-shaped cells which contain an abundance of mucous substances, giving a PAS-positive reaction. These cells are surface mucous cells, cytologically similar to the mid-epidermal mucous cells. The nucleus of the cell is a flattened oval with a considerably irregular outline, and contains a well-defined nucleolus (Fig. 6). The nucleus is displaced to the basal part of the cell by a marked increase of mucous droplets. The mucous droplets, arranged in parallel arrays or in larger masses, occupy nearly the whole of the apical and supranuclear region of the cell (Figs. 3B, 6). Some cytoplasmic organelles, such as mitochondria and free ribosomes, are wedged between the mucous droplets, but most of these organelles are contained in the subnuclear region, in which are also found well-developed rough-surfaced endoplasmic reticula, Golgi apparatus, and lysosome-like dense bodies (Figs. 3B, 6). The peripheral cytoplasm stretching along the lateral and ventral walls of the cell is filled with numerous filaments which measure about 7 nm in diameter, a few mitochondria and some free ribosomes. The same ovoid bodies as those described in the mid-epidermal mucous cells are occasionally found in the subnuclear region of the surface mucous cell also (Figs. 3B, 6). There are generally only several of these in the cells, but sometimes ten or more can be found. The coated vesicles are also seen in the peripheral cytoplasm adjacent to the plasma membrane of the cell. The free surface of the cell is thrown into numerous microvillar ridges covered by a finely fibrillar substance which is called "fuzz" (Fig. 7). Close to the epidermal surface the plasma membrane of adjacent cells appear to fuse, forming tight junctions. Beneath these tight junctions, the adjacent membranes are lined with a considerable

Fig. 4. Perinuclear region of the mid-epidermal mucous cell containing a good number of mucous droplets (md). ×14,000. nl, nucleolus. Other letters and arrow, vide Fig. 1.

number of desmosomal attachments. The mucous contents in the mature surface mucous cell are released to the outside through a number of openings formed by localized rupturing of the plasma membrane that is fused with the membrane enclosing the mucous droplets (Fig. 8). However, it could not be determined whether or not the cytoplasmic organelles and nucleus were cast from the cell with the release of the mucous contents.

Discussion

The basal epidermal cells of the arctic lamprey are morphologically similar to the filament-containing cells of teleosts and have no mucous droplets; whereas the basal mucous cells, corresponding to the basal epidermal cells, of the silver lamprey, *Ichthyomyzon unicuspis,* do contain such droplets (Downing and Novales, 1971). The formation of the mucous droplets takes place in the mid-epidermal mucous cell in which rough-surfaced endoplasmic reticula and Golgi complexes are more developed than in the basal epidermal cells. These mucous cells, however, contain a great number of filaments measuring about 7 nm in diameter in their peripheral cyto-

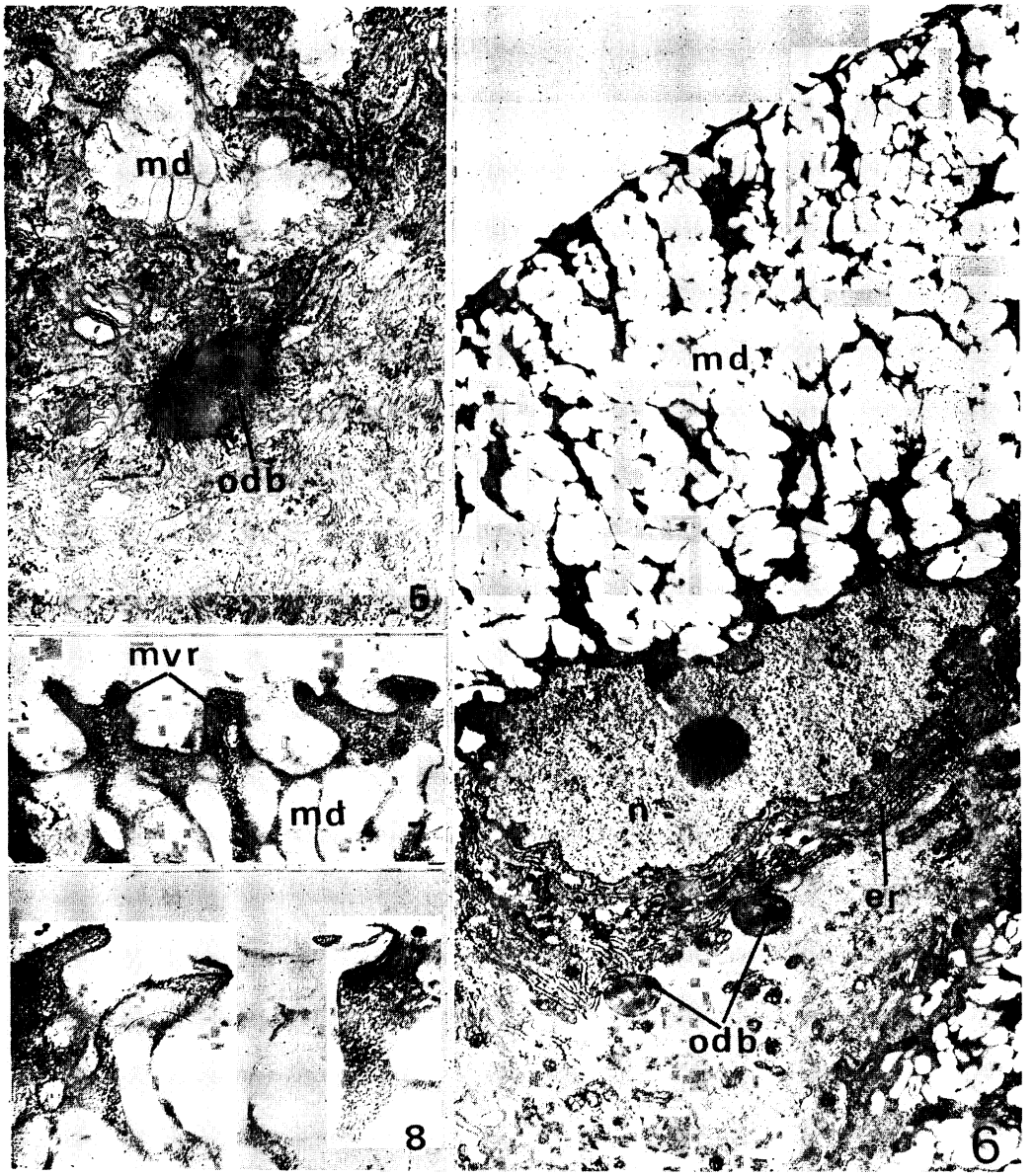

Fig. 5. An ovoid body (odb) found in the junction between the perinuclear and the peripheral cytoplasm of the mid-epidermal mucous cell. ×20,000.

Fig. 6. The fine structure of the surface mucous cell. Numerous mucous droplets (md) occupy most of the apical and supranuclear cytoplasm. Several ovoid bodies (odb) are found near the junction between the subnuclear and peripheral cytoplasm. ×6,000.

Fig. 7. The apical border of the surface mucous cell, possessing microvillar ridges (mvr) covered with fuzzes. ×20,000.

Fig. 8. Electron micrograph showing the release of mucus from the surface mucous cell by localized rupturing of the distal plasma membrane that is fused with the enclosing membrane of mucous droplets. ×42,000.

plasm, differing from typical epidermal mucous cells of teleosts. Even in the mature surface mucous cells, numerous filaments are still recognizable in the peripheral cytoplasm. In this sense, the mucous cells in the lamprey epidermis may be said to be filament-containing cells with mucous substances. Although these mucous cells differ from typical epidermal mucous cells of teleosts, it seems probable that the actual synthesis of mucous substances in the lamprey mucous cells may follow a similar pattern as found in all mucous-secreting cells. This problem was fully discussed by Downing and Novales (1971), so the writer will withdraw it from this paper.

Tiny channels or tubules observed by Downing and Novales (1971) in the apical border of the surface mucous cells of the ammocoete larva of *I. unicuspis* were not recognized distinctly in the apical cytoplasm of the cell of the present lamprey. This may be because all the specimens used in this study were adults in upstream migration. It was already pointed out by Downing and Novales (1971) that the precise recognition of the channels or tubules in the surface mucous cells of adult specimens of *I. unicuspis* is disturbed by congestion and distention of the apical portion of the cell caused by large accumulations of mucus. Fully mature mucous cells in the skin epidermis of teleosts release their contents on the skin surface through an orifice formed on the apical border of the cell (Henrikson and Matoltsy, 1968, and others). However, in the mature surface mucous cells of the lamprey, their mucous contents are not expelled through a single opening, but instead through a number of openings formed by localized rupturing of the plasma membrane that is fused with the enclosing membrane of mucous droplets. According to Brown and Wellings (1970), mucous cells of fish appear to be typical holocrine secretory cells. Although whether or not the lamprey mucous cells discharge mucous contents accompanied by other cellular organelles, and followed by cell exfoliation and death, could not be determined by the present observation, these mucous cells seemed to die and to be shed ultimately. Structures considered morphologically to be coated vesicles, probably derived from the plasma membrane, are found in the mucous cells as well as the basal epidermal cells of the present lamprey, whereas such structures were not reported in the mucous cells of river lamprey *Lampetra fluviatilis* (Damas and Firket, 1966), brook lamprey *L. planeri* (Bertolini et al., 1968), and silver lamprey *I. unicuspis* (Downing and Novales, 1971). However, these structures do not seem to be peculiar to the mucous cell of the present lamprey, but may be found universally in various animal cells.

As mentioned above, most of the skin epidermis of the lamprey consists of cells containing numerous filaments and mucous substances. Accordingly, the skin epidermis of the lamprey may produce greater quantities of mucus which seem to play an important role in the lamprey integument having no scales. The functional significance of the mucous cell and the relation between the cell and other epidermal elements such as granular or club cells will be discussed in subsequent papers which will deal with the fine structures of club, granular and Merkel cells in the lamprey epidermis.

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カワヤツメ皮膚表皮にみられる粘液細胞の微細構造

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