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The Fine Structure of the Epidermis of Two Species of Salmonid Fish, the Atlantic Salmon (*Salmo salar L.*) and the Brown Trout (*Salmo trutta L.*)

II. Mucous Cells

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Summary. The fine structure of epidermal mucous cells of two species of salmonid fish has been described. Mucous cells are, next to filament-containing cells, the most commonly encountered cells in fish epidermis. The development of the cells as they progress to the periphery has been characterised. They are initially difficult to distinguish from filament-containing cells: later, they can be recognised by the presence of much smooth-surfaced E.R. The mucigenesis and the subsequent secretion of mucus has been observed and it is essentially comparable to that which occurs in the mucous cells of the mammalian intestine. The mucous layer of the epidermal surface seems to mainly comprise of the products of these mucous cells and the "cuticle" seen in other species has not yet been observed in the salmonid species investigated here.

Key words: Epidermis – Salmonids – Mucous cells – Mucus – Electron microscopy.

Introduction

The epidermis of all aquatic amniotic vertebrates generally does not keratinize and one of the most distinctive features of the epidermis of fish is the production of mucus, often in considerable amounts (van Oosten, 1957). Furthermore the mucous cell is, second only to the filament-containing cell, the most commonly encountered cell in the epidermis of fish.

Extensive studies of mucous cells at the light microscope level has been undertaken and particular attention has been paid to their histochemistry (Reid, 1894;

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Bertin, 1958; Bolognani *et al.*, 1958; Leppi, 1968; Askawa, 1970). They are readily distinguishable at this level in the mid-epidermal region and a gradual increase in size of the cells can be seen as they reach the peripheral surface. Although it has been presumed by the majority of investigators that the cell products are a major component of the epidermal slime it has not been satisfactorily demonstrated and an attempt has been made here to establish the relationship between them. The development of the cells and the mucigenesis has also been investigated as part of a study of the biology of fish epidermis; the histochemistry of the mucous cells of the species (*Salmo trutta L.*) has been undertaken (Harris *et al.*, 1973) and biochemical analyses of the epithelial mucins of the second species (*Salmo salar L.*) has also been accomplished (Harris and Hunt, 1973). The first paper in the series described the general organization and the filament-containing cells of the epidermis of these two species (Harris and Hunt, 1975).

Materials and Methods

The sources of fish, the preparation of the material and the microscopy have been described by Harris and Hunt (1975). Epidermis from the head, ventral, dorsal and lateral regions of both species were examined.

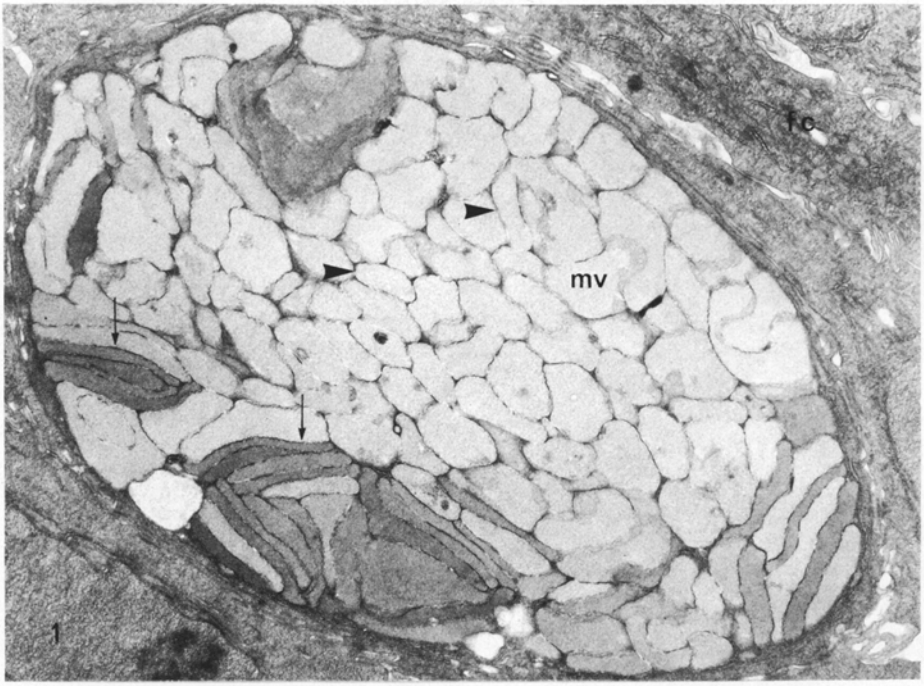
Observations

Mucous cells were found distributed extensively throughout the epidermis of each of the regions examined. The numbers of both the trout and the salmon mucous cells varied in different body regions. There appeared to be more cells associated with the non-scaled areas at the anterior end, although the dorsal, ventral and lateral epidermis were still relatively rich in this type of cell.

Immature mucous cells are difficult to distinguish from filament-containing cells and they could not be recognised in the basal layers. Only in at least the central area of the epidermis did they become readily apparent. One of the major differences between the two cell types is that the nuclei of the mucous cells do not appear to have such extensive indentations as do the nuclei of the filament-containing cells (Harris and Hunt, 1975). Other differences are the relatively few desmosomal attachments between mucous cells and filament-containing cells and there is also significantly more endoplasmic reticulum in the mucous cells.

As the mucous cells "mature" i.e. as they approach the periphery of the epidermis, discrete packets or vesicles of mucus (Fig. 2) are deposited within the cell cytoplasm with the subsequent enlargement of the cell. The greatest diameter of the mucous cells in the mid-epidermal region averaged 6 μ and increased to an average of 13 μ for cells of the peripheral region. The organelles of the mucous cell become very distinctive at this stage. There are relatively few mitochrondia, a number of Golgi vesicles and an abundance of rough-surfaced endoplasmic reticulum (Figs. 3, 4).

The formation of mucus within the cells is, of course, closely associated with the endoplasmic reticulum and the Golgi vesicles. In Fig. 4 the deposition



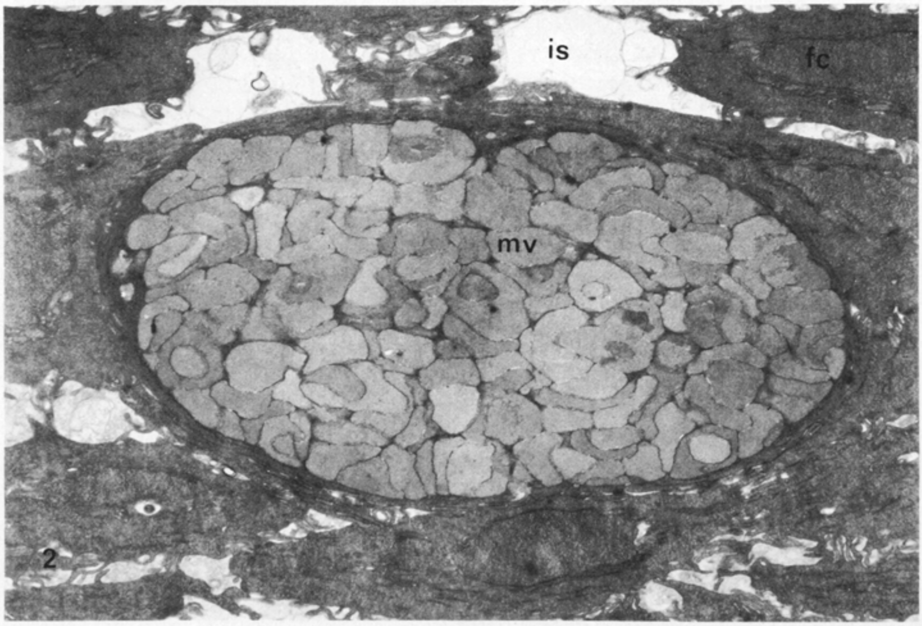


Fig. 1. Mature mucous cell of salmon. Note the two distinct types of vesicles; the longer, thinner variety which is more electron dense (↓) and the shorter more rounded variety which is more electron lucent (▼). *fc*, Filament-containing cell, *mv*, Mucous vesicle. × 11 200

Fig. 2. Mature mucous cell of brown trout. The mucous vesicles of this cell do not demonstrate such marked differences in shape or electron opacities as those of salmon mucous cells. *is*, Intercellular spaces. × 7500

of mucus can be seen quite clearly to be occuring within a Golgi vesicle which appears to swell to form a mucous droplet or vesicle. Furthermore these vesicles contain the same fine fibrillar material which is seen in established mucous vesicles. In Fig. 5 the close association of the endoplasmic reticulum and the development of mucous vesicles is again seen.

In the salmon, at least, there was evidently a number of forms that the vesicles could assume usually varying between the relatively long, ovoid-shaped variety (2.4 μ × 0.6 μ) or the shorter, more rounded type (1.3 μ × 0.75 μ). The shape of the vesicles also seemed to reflect differences in the density of stain between vesicles the longer, thinner forms were often more electron dense than the more rounded forms (Fig. 1). In the trout mucous cells the vesicles tended to be more uniform in electron opacity (Fig. 2).

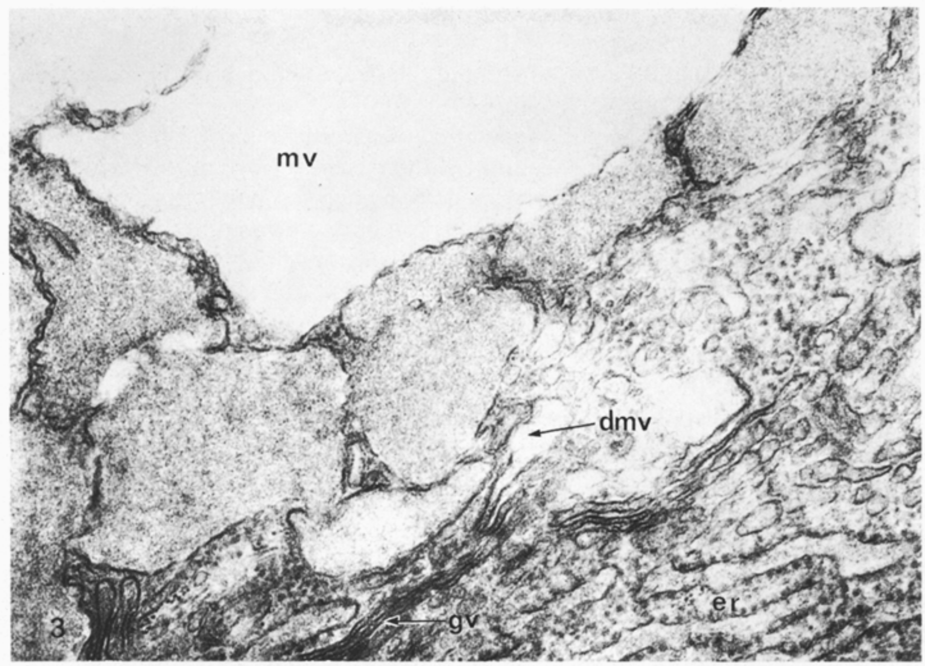
The nearer the periphery of the epidermis the mucous cell is found the more enlarged it becomes with the increasing numbers of mucous vesicles (Figs. 1, 2). The individual mucous vesicles retain their integrity, i.e. they remain as discrete intracellular compartments until they reach the epidermal surface. These large mature mucous cells have the remainder of their organelles restricted to the peripheral margins of the cell (Fig. 4; Harris and Hunt, 1975) directly abutting the cell membrane.

On reaching the surface the mature cell emerges, usually between adjacent filament-containing cells, its cell membrane ruptures at the apical point and the cell contents are released. In some cases individual mucous vesicles are shed onto the surfaces (Fig. 6). In others the vesicular membrane also ruptures simultaneously with the cell membrane releasing the fine fibrillar material directly onto the epidermal surface. At least some of this fibrillar material appears to be associated with the surface microvilli (Fig. 8; Harris and Hunt, 1975).

Discussion

The numbers of mucous cells in the skin from different regions of the fish appeared to vary quite considerably. Although a quantitative study was not carried out there seems to be more cells associated with the non-scaled areas at the anterior end of the fish, particularly the head, as compared with the scaled areas of the fish, and this has been confirmed at least in brown trout in a recent study by Pickering (1974) who found that the highest concentration of mucous cells occurred on the anterior regions of the body and on the body parts with fins there were significantly fewer cells.

Perhaps the difficulty in recognising "immature" mucous cells in the more distal areas of the epidermis can be explained by the suggestion of Downing and Novales (1971) that undifferentiated epidermal cells of fish retain the potential to develop to filament-containing or mucous cells. This bipotency has been well established in higher vertebrates (Zelickson, 1967). Even some of the distinguishing features between the two cell types, such as the presence of non-lobulated nuclei in mucous cells cannot be taken as definitive. Similarly the numbers of desmosomal attachments between mucous cells and filament-containing cells which were found to be very few in these two species studied by Henrickson



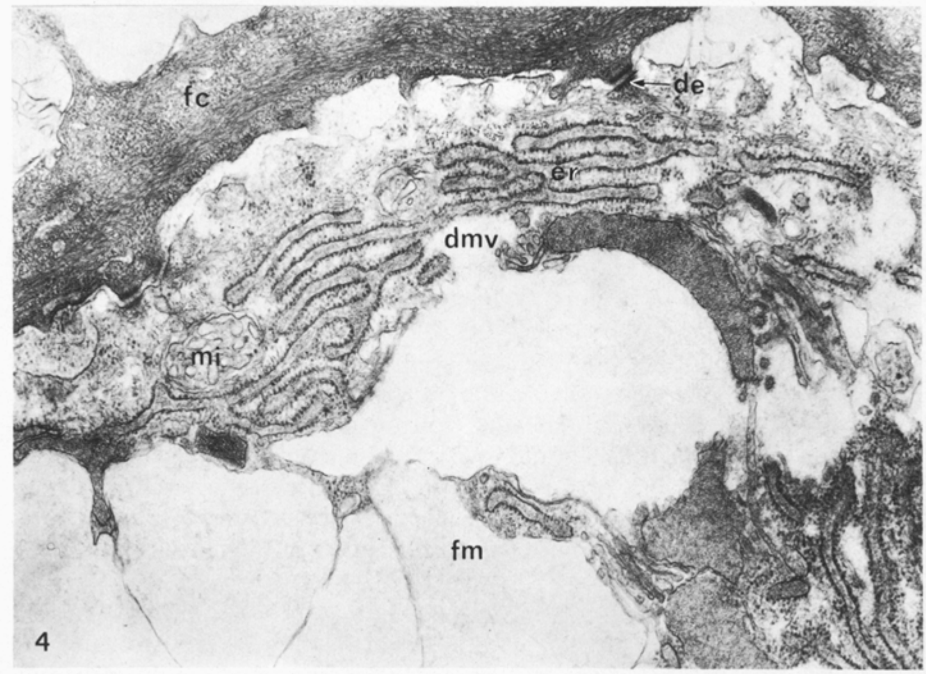


Fig. 3. Deposition of mucus in a developing mucous cell of salmon showing the involvement of the Golgi complex and the endoplasmic reticulum. *dmv*, developing mucous vesicles, *er*, rough-surfaced endoplasmic reticulum, *gv*, Golgi vesicles. × 73 750

Fig. 4. Deposition of mucous vesicles in a salmon mucous cell demonstrating the involvement of the ribosome-studded endoplasmic reticulum. *fm*, fibrillar material, *de*, desmosomes, *mi*, mitochondria. × 52 000

and Matolsty (1968) and likewise here, are relatively "common" in *Protopterus* (Kitzan and Sweeney, 1968).

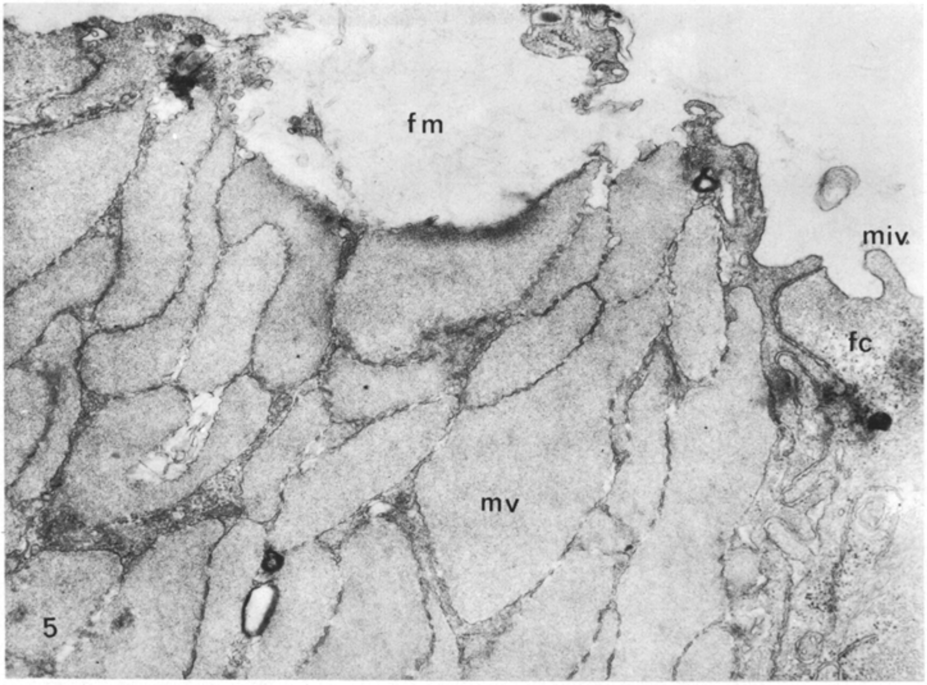
In *Protopterus* three distinct types of mucous cells could be recognised by differences in their histochemistry and in their electron opacities (Kitzan and Sweeney, 1968). In salmon, differences could be recognised in the electron opacities of individual mucous vesicles within a single cell but no gross differences between cells could be seen. These differences in electron opacities of the contents of the mucous vesicles may reflect differences in their carbohydrate and protein content. Protein usually being less osmiophilic than carbohydrate. However, in the trout differences in staining intensities with PAS/Alcian blue technique were recognised in their mucous cell population. These obviously reflect differences in the products of the cells; for example the degree of sulphation or sialic acid content of the glycoproteins (Harris *et al.*, 1973).

Although the structure of teleost mucous cells differs from those seen in mammalian mucous membranes, such as the intestinal goblet cell (Bierring, 1962), mucigenesis is probably fundamentally the same. There is obviously a relationship between the Golgi vesicles and the developing mucous droplets which suggests a transport mechanism operating through the endoplasmic reticulum via the Golgi System to the mucous droplets. Neutra and Leblond (1966a, b) have discussed the formation of mucus in mammalian colonic goblet cells in detail and Freeman (1962) has proposed a mechanism where the protein moiety is synthesized at the rough-surfaced endoplasmic reticulum and at the Golgi vesicles the carbohydrate moiety is introduced. The role of the Golgi vesicle at least, is confirmed by the present study as the vesicle itself becomes distended by mucus and forms an individual mucous droplet.

An extensive study of the skin of several species of Actinopterygian fish has been undertaken by Whitear (1970). She found that the majority of species which she examined bore a distinct cuticle or external coat covering the peripheral epidermal cells. This cuticle showed considerable variation depending on the species; it was found to be approximately 1 μ thick in most species but in the gurnard, *Trigla* it reached a size of 50 μ. In some species notably *Pomatischus mintus*, only fine fibrillar material was seen. In the present study in one of the skin samples of either trout or salmon was a structure corresponding to this cuticle observed, either under the electron or light microscope. Whitear (1970) did not examine the skin of either of these species and in her extensive discussion of earlier work performed at the light microscope level there does not appear to be any mention of a cuticle in salmonid fish. Furthermore Roberts *et al.* (1970) in their study of skin of the head region of salmon did not report any cuticle.

Henrickson and Matolsty (1968) in the guppy (*Lebistes reticulatus*) at least recognised a thin membrane over the microvilli of the peripheral cells which was distinct from surface mucus and suggested that it might represent the remnants of desquamated cells. Whitear (1970) suggests that this is in fact comparable to the cuticle that she has described and that it is not the remnants of desquamated cells but is produced, similarly to the cuticle of all species she has described, by the filament-containing cells of the epidermis.

It would appear that the universal presence of this cuticle of fish epidermis



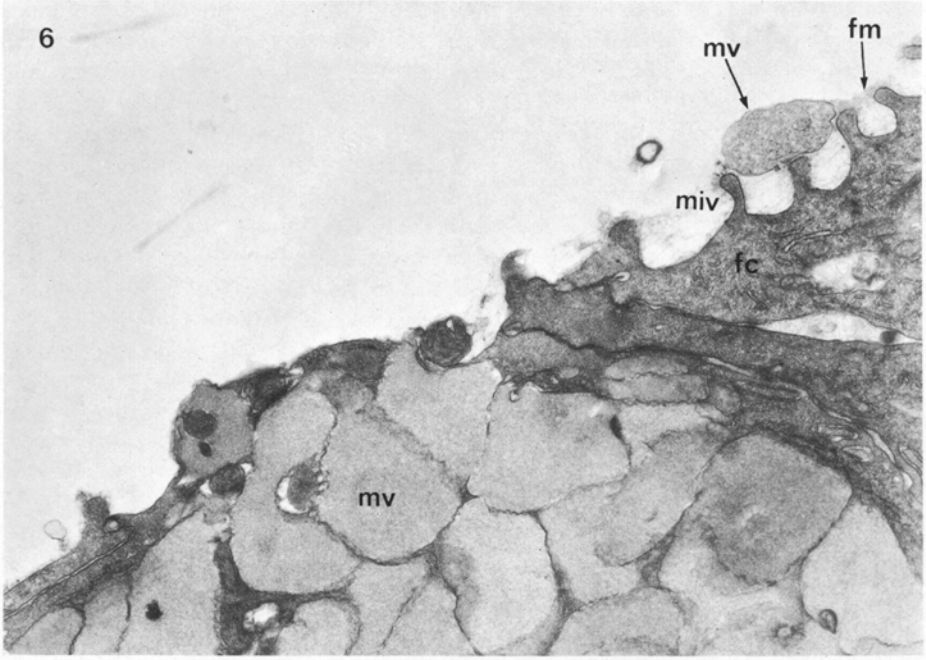


Fig. 5. Mucous cell releasing its contents at the epidermal surface. Here the fibrillar material is released directly onto the surface. *miv*, microvilli. ×28 500

Fig. 6. Actively secreting mucous cell in which at least one of the mucous vesicles has been released intact and is seen attached to surrounding microvilli. Other fibrillar material is seen associated with the microvilli of the adjacent filament-containing cell. ×22 500

is debatable and that there are, at least, "degrees of cuticularization" between species. This is suggested by Whitear (1970) in her extensive discussion of the light microscope evidence. She further suggests that this coat may be a liable layer of mucopolysaccharide but which should nevertheless, retain the description of a cuticle as it is of a "less transient" nature than the mucus produced by the goblet or mucous-producing cells. In the salmonids the external layer appears to be mainly composed of a secretion of similar consistency to that produced by the mucous cells. Both histochemical analyses of mucous cell constituents (Harris, Watson and Hunt, 1973) and biochemical analyses of epithelial mucins (Harris and Hunt, 1973) suggest that the two are very closely related. Furthermore the demonstration in this study that mucous cells do indeed shed their products over the epidermal surface suggests that the excretions of the mucous cells the major constituents of epithelial mucus, at least, of salmonid fish.

The nature of the fine fibrillar material which was found on the external surface of the cells interspersed amongst the microvilli (Fig. 8, previous paper) has an identical appearance to the fibrillar material seen in undischarged mucous vesicles, and also suggests that the mucous cells make a major contribution to the external mucus. Whitear (1970) is of the opinion that the mucous cells can discharge over the surface but that the mucous cell discharge provides "emergency lubrication" only.

The filament-containing cells of the salmonids show no evidence of producing any secretory material and histochemical studies also show that no significant amounts of PAS positive material is found in these cells (Harris, Watson and Hunt, 1973). The suggestions of Henrickson and Matolsty (1968) that the membrane on the epidermis of the guppy may represent desquamated cells is not, perhaps, to be too lightly dismissed. On several occasions peripheral cells in the process of desquamation were probably unrecognisable as such as suggested earlier (Harris and Hunt, 1975). Both Henrickson and Matolsty (1968) and Roberts *et al.* (1970) suggest that microvilli originate from the desmosomes of under-lying cells as the outermost cells are in the process of being sloughed away. During the latter stages of desquamation when these under-lying cells have virtually assumed the role of the most peripheral epidermal cells perhaps the remains of the desquamating cells could be mistaken for a distinct layer.

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