

ARYL-SULFATASE ACTIVITY OF MEMBRANE-COATING GRANULES WITH PARTICULAR REFERENCE TO THEIR ORIGIN

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Abstract. By electronmicroscopic cytochemistry the present study demonstrated that aryl-sulfatase activity was present within membrane-coating granules or keratinosomes as well as in lysosomes of the oral mucosa of rats. In addition, an enzymatic activity was found in several cytoplasmic components and also, in part, in rough endoplasmic reticulum. These data suggest that MCG derive from rough endoplasmic reticulum.

Membrane-coating granules (MCG), first noticed by Selby (15) and Odland (9), are small granules with a characteristic lamellar internal structure, which appear in the cytoplasm of epithelial cells in the upper layer of the epidermis and oral mucosa. The origin and function of these granules remain to be established. It has been suggested, however, that MCG play a specific role in the complex process of keratinization in association with desmosomes, keratohyalin granules, and tonofilaments. Recently, Wolff & Holubar (18) have demonstrated acid phosphatase activity within MCG and therefore considered them to be a special type of epidermal lysosome. However, there is no information on the presence of other enzymes in MCG. Whilst studying these granules by means of electronmicroscopy, we have established the presence of aryl-sulfatase activity within MCG. In addition, we have obtained some data suggesting that MCG originate from the rough endoplasmic reticulum, as will be described below.

MATERIALS AND METHODS

Tissue specimens were obtained from normal oral mucosa of rats. The specimens were cut into thin slices with a

razor blade and placed in 5% glutaraldehyde in 0.05 M cacodylate buffer, pH 7.3, for 2 hours at 4°C.

For the cytochemical demonstration of aryl-sulfatases, the following substrate medium was used according to Hopsu et al. (7) and Goldfischer (4): 5 ml 0.06 M *p*-nitrocatechol sulfate, 5 ml 0.2 M barium chloride, and 10 ml 0.1 M acetate buffer (pH 4.2, 5.2 or 5.6).

Sucrose was added to each medium at a concentration of 7.2%. The incubation time was 1 hour at 37°C. For distinction between sulfatase type A, B and C, each specimen was incubated at different pH levels, 4.2, 5.2 and 5.6, respectively (12). After incubation the slices were briefly rinsed and postfixed in 1% buffered OsO₄ for 30 minutes at 4°C. They were then dehydrated in graded ethanol series and embedded in Epon 812. Controls were processed by the same technique except for the incubation in substrate-free medium. Thin sections, both unstained and stained with uranyl acetate and lead nitrate, were examined in a Hitachi HU-11A electron microscope.

RESULTS

Fine precipitates of the reaction products, barium sulfate, were found to be localized in lysosomes, MCG, and several cytoplasmic components of the epithelial cells, of which the latter will be described in detail later. These precipitates were thought to represent aryl-sulfatase activity because of their absence in control sections. The enzymatic activity varied according to the pH level at which the incubation was carried out. The reaction products at pH 4.2 were very few when compared with those at pH 5.2 or 5.6. Thus the results to be presented are drawn mainly from the sections at the latter two pH levels.

Cytoplasmic MCG showed a characteristic staining pattern in which the enzymatic activity was demonstrated along their unit membrane and internal lamellar structure (Figs. 1, 2, 3, 4, 5)



Fig. 1. Aryl-sulfatase activity at pH 5.6. Dense, long lamellar body, is bounded by a unit membrane and having an internal lamellar structure (arrow). $\times 84\ 000$.

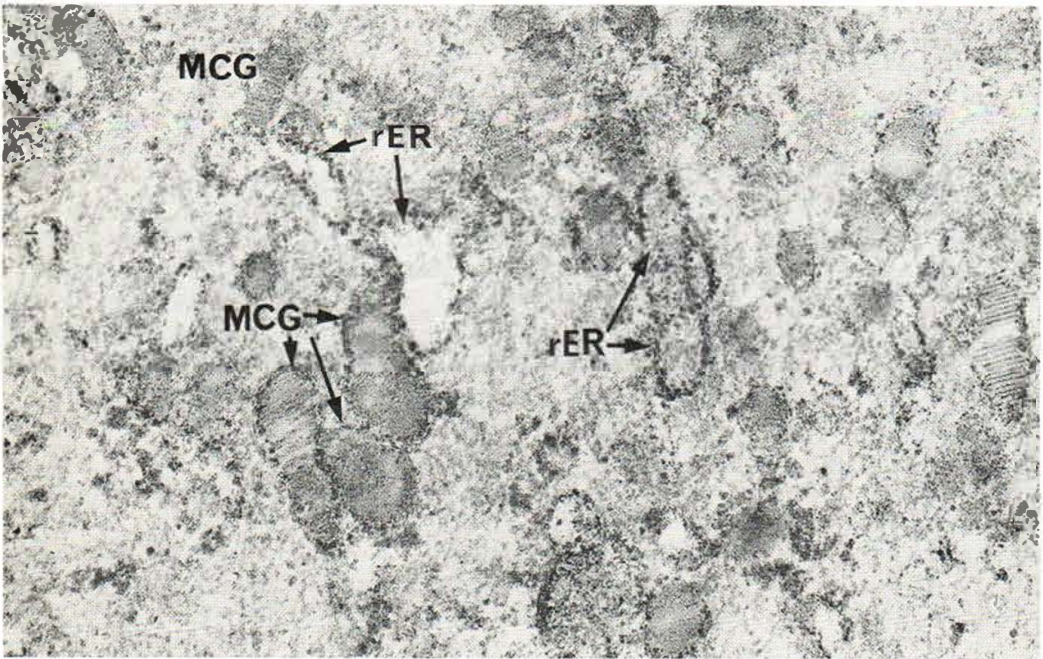


Fig. 2. Aryl-sulfatase activity at pH 5.2. A large number of MCG of varying size and shape can be seen. Membrane of MCG is contiguous with enlarged rough endo-

plasmic reticulum or in close proximity to rough ER. $\times 84\ 000$.

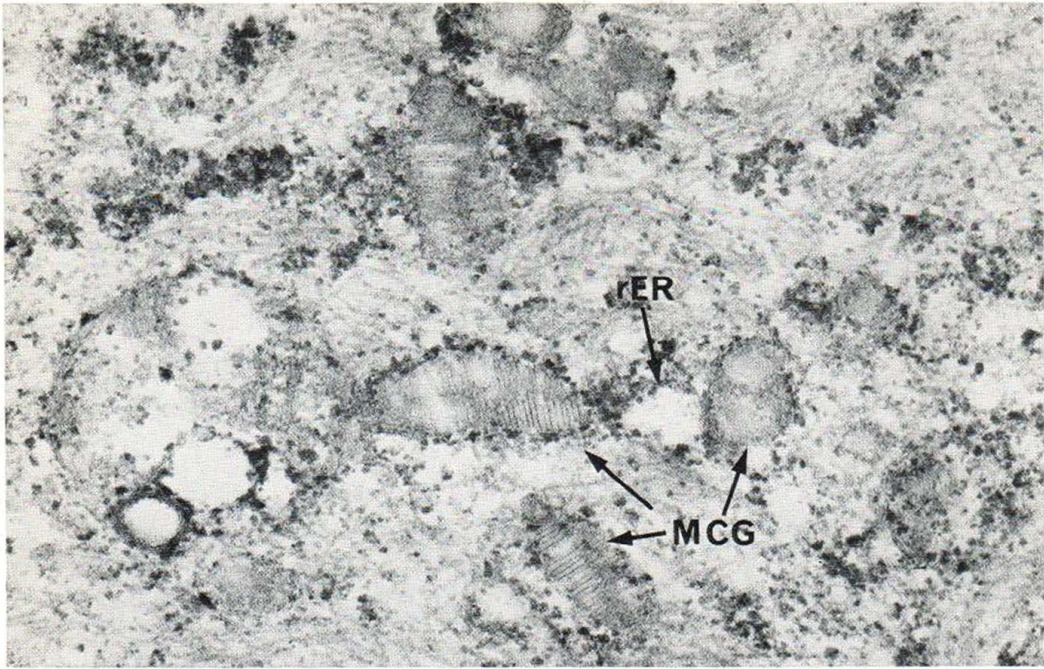


Fig. 3. Aryl-sulfatase activity at pH 4.2. MCG in central portion is contiguous with rER. Enzyme activity is found in cytoplasmic free ribosomes, unit membrane of MCG and lamellar structure of MCG. $\times 84\ 000$.

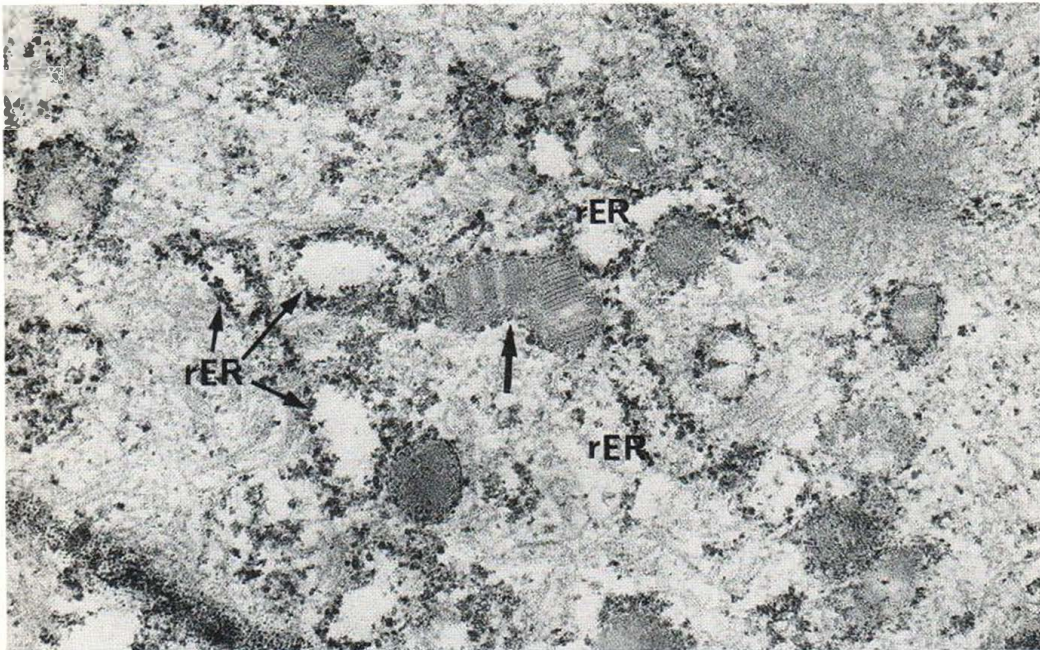


Fig. 4. Aryl-sulfatase activity at pH 5.6. At the central granule, an arrow indicates the irregular-shaped MCG which are bounded by a unit membrane. Internal lamellar structure runs vertically (left) and horizontally (right). $\times 84\ 000$.



Fig. 5. Aryl-sulfatase activity at pH 5.2. Irregular shaped MCG, which have a small protuberance (arrow). Enzyme activity is found on cytoplasmic-free ribosomes and internal lamellar structure of MCG. $\times 84\ 000$.



Fig. 6. Aryl-sulfatase activity at pH 5.6. Enzyme activity is found in intercellular spaces of the upper parts of keratinocytes. The retaining lamellar structure of MCG in the intercellular spaces also displays enzyme activity (arrow). $\times 84\ 000$.

and, in some instances, these granules retaining the enzymatic activity were found in the intercellular space of the granular cells (Fig. 6). In addition, irregularly shaped MCG with occasional protuberances or projections were noted intracellularly (Fig. 5). Some of them had internal lamellar structures running in different directions as shown in Fig. 4. The unit membranes of MCG of irregular shape were noted to be contiguous with rough endoplasmic reticulum or often in close proximity to rough endoplasmic reticulum (Figs. 2, 3, 4). In the vicinity of MCG, elongated bodies were found, approximately 600 nm in length, each bounded by unit membrane and having an internal structure closely resembling MCG (Fig. 1). In addition, the enzymatic pattern similar to MCG was recognized in some parts of rough endoplasmic reticulum. In such areas free ribosomes and some vesicles frequently displayed enzymatic activity (Figs. 2, 3, 4). A small amount of the reaction products was noted in some part of the Golgi lamella.

DISCUSSION

Aryl-sulfatase activity has been reported to be present in various biological materials including human organs. These enzymes were detected by biochemical methods in normal and parakeratotic epidermis of man and hairless mice, especially in lysosomal components (2, 6, 14, 19). By electronmicroscopy, Olson et al. (10) reported that the enzymatic activity was localized in MCG in the upper spinous layer but not in the granular layer, though Rowden (11) failed to detect any activity in MCG of either the granular or upper spinous layers. In the latter organelles, the activity was present along their unit membrane and internal lamellar structure. In addition, some MCG containing the enzymatic activity were found to be located in the intercellular space of the granular layer. The sulfatases demonstrated in MCG appear to be of type B or C according to Roy (12), since the reaction products were very sparse in the sections incubated at pH 4.2, as compared with those incubated at pH 5.2 or 5.6. It is of interest that, by this electron-cytochemical method, irregularly shaped MCG were detected within the epithelial cells in addition to those usually observed and that, in the vicinity of these granules, the same internal structures

were shown in some elongated bodies and in part of the rough endoplasmic reticulum. These findings have not been obtained in previous studies including our own (16) using routine and electron-cytochemical methods for acid phosphatase. Our findings suggest that MCG originate from the rough endoplasmic reticulum, i.e. the formation of MCG began with the enlargement of rough endoplasmic reticulum and development of an internal lamellar structure with aryl-sulfatase activity, thereafter small projections or protuberances became segregated, resulting in well-developed MCG.

There has been a divergence of opinion concerning the origin of MCG. Earlier investigators considered them to be degenerated or fragmented mitochondria, or virus particles, or segregate granules. Later, the following diverse interpretations were proposed: (a) MCG originate from the Golgi region (1, 3, 8); (b) MCG originate by the invagination of and segregation from the plasma membrane (10, 13). As indicated above, our view is in contradistinction to both these previous interpretations.

In this context, some consideration should be given to the function of these granules. Matoltzy & Pagakkal (8) hypothesized that the inner substances of MCG may contribute to the highly resistant protein that is closely associated with the plasma membrane of horny cells. Hashimoto et al. (5) reported that the granules contained polysaccharide material, which may play a role in binding together the horny layers. On the other hand, Wilgram (17) and Bonneville et al. (1) provided evidence that these granules may harbor an enzyme or enzymes that function in the desquamation of epithelial cells by bringing about the breakdown of intercellular cement substances. Wolff & Holubar (18) showed electronmicroscopically the presence of acid phosphatase activity within MCG and their remnants in the extracellular space and considered them to be specialized lysosomes. Our presented previous studies (16) showed that the granules contain aryl-sulfatase as well as acid phosphatase. Additionally, these enzyme activities were found to be present in their remnants in the intercellular spaces of the granular layer. Thus, we believe that MCG produce enzymes which influence the intercellular substances, though their precise nature remains obscure.

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Received May 4, 1973

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