ELECTRON MICROSCOPE STUDY OF A SMALL CYTOPLASMIC STRUCTURE IN RAT ORAL EPITHELIUM

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INTRODUCTION

In recent years electron microscopists have reported small intracytoplasmic granules in epithelial cells of mammalian skin and oral mucosa (4, 6, 8, 10–12, 15, 16). These granules are usually described as small (0.1 to 0.2 micron in diameter), rod-shaped or spherical, membrane-bounded bodies, usually containing electron-opaque material and sometimes (4, 8, 10) showing internal structure. The function of these cytoplasmic components is unknown. It has been suggested that their contents are extruded into the intercellular spaces of the stratum granulosum (4).

It is the purpose of this paper to describe these cytoplasmic structures in greater detail as they appear in the non-gustatory, keratinizing epithelium of the fungiform papilla of the rat tongue, and to discuss the possible significance of the findings. These observations were made during the course of a study on the ultrastructure of the taste bud in the rat fungiform papilla (3).

MATERIALS AND METHODS

Wistar albino rats, 1 week to 6 months old, were anesthetized and the anterior halves of their tongues were removed. The specimens were cut into pieces, 1 to 2 mm^3 in size; the pieces were fixed for 2 hours in ice-cold 1 per cent osmium tetroxide, buffered to pH 7.4 to 7.6 with veronal-acetate (9). Other specimens were fixed *in vivo* by dripping the fixative for 1 hour on the dorsal surface of the tongues of anesthetized rats. These tongues were removed and cut into pieces which were fixed, as described above, for a 2nd hour. After fixation, the tissue blocks were placed in 70 per cent alcohol and individual fungiform papillae were dissected out. The papillae were then dehydrated in a graded series of alcohols, run through several changes of propylene oxide, and embedded in Epon 812 (5). Thick sections (1 to 2 micra) were examined with a phase microscope for orientation. Thin sections, made with glass knives on an LKB ultratome, were placed on Formvar-coated copper grids and stained with uranyl acetate (13) or lead hydroxide (7). They were examined in a Siemens Elmiskop I at original magnifications of from 1800 to 30,000.

RESULTS

The cytological features of rat tongue epithelium do not differ significantly from those described in the epithelial cells in human epidermis (1, 2, 12, 15), human oral mucosa (15, 16), or in the epidermis or oral mucosa of other mammalian species (6, 10, 11). Fig. 1 is a low-power electron micrograph of the lateral surface of the rat fungiform papilla near the taste bud. It demonstrates all the epithelial layers and an intraepithelial, perigemmal nerve process (*n*). Within the cells of the stratum granulosum and the upper layer of the stratum spinosum, electron-opaque cytoplasmic bodies are observed lying, largely, near the part of the cell membrane closest to the epithelial surface. They are frequently seen abutting the plasma membrane of the cells of the stratum granulosum (Fig. 3, arrows), but not at its desmosomal modifications. These bodies are found in much smaller numbers in the cells of that layer immediately below the first layer of cornified cells. Also, it has been observed that some lamellated material, structurally similar to that which comprises the internal structure of the bodies, is found in the intercellular spaces of the stratum granulosum (Fig. 2).

When these cytoplasmic structures are examined for their morphological characteristics, they are seen to be rod-shaped bodies, 0.2 to 0.3 micron in length and 0.1 micron in width, composed of lamellae which traverse part or all of the width of the structures (Figs. 4, 5). Lamellae of high density alternate with lamellae of low density. In some areas, successive dense lamellae are connected at their ends in such a way that they give the appearance of folding back on themselves and partially enclosing the less dense lamellae (Figs. 4, 5). In some parts of the body the more dense and the less dense lamellae are arranged in a regular repeating pattern, *i.e.* a more dense layer, 20 A thick, adjacent to a less dense layer, about 30 A thick. This repeating pattern does not always continue throughout the length of the

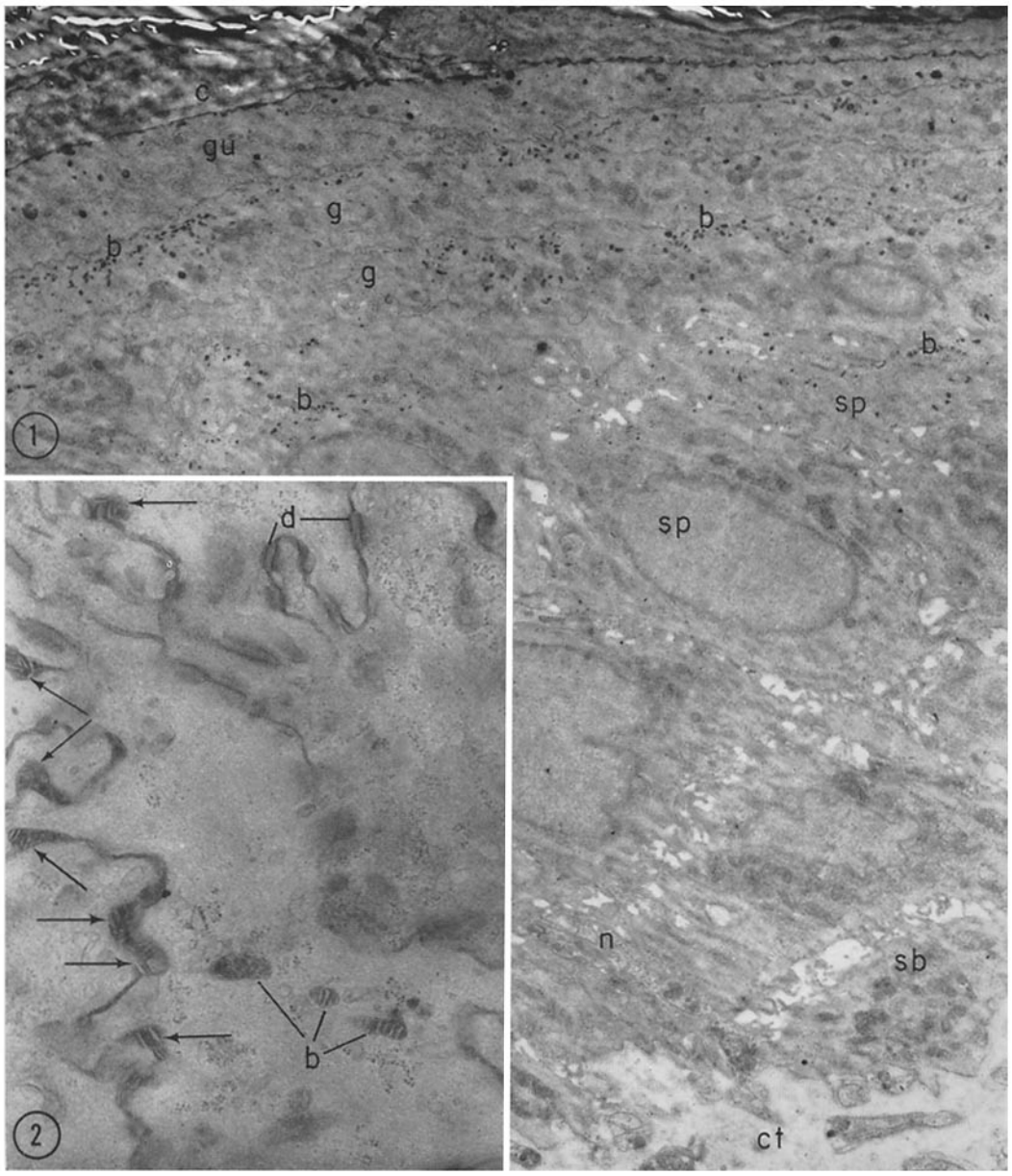


FIGURE 1 Low-power electron micrograph of the fungiform papilla epithelium of tongue from adult rat. In the upper layers of the stratum spinosum (*sp*) and the stratum granulosum (*g*) there are numerous dense bodies (*b*) located largely near that part of the cell membrane closest to the surface. In the uppermost layer of stratum granulosum (*gu*), immediately beneath the stratum corneum (*c*), the number of these bodies is significantly reduced. (*sb*), stratum basale; (*n*), intraepithelial nerve; (*ct*) connective tissue. Pb $(OH)_2$ stain. ×5,000.

FIGURE 2 Parts of epithelial cells from the stratum granulosum. Cytoplasmic lamellated bodies (*b*) are present. Note the intercellular material (arrows) that is structurally similar to the lamellated bodies. (*d*), desmosome. Pb $(OH)_2$ stain. ×28,000.

body, there being areas of variable size where it is interrupted (Fig. 4). It has not always been possible, especially at high magnification, to demonstrate the presence of a limiting membrane around the periphery of the body. Although in some instances (Fig. 2) a limiting membrane appears to be present, at higher resolution (Figs. 4 and 5) no limiting membrane appears in the regions exterior to the peripheral folds of the lamellae.

There is some indication that tiny channels may exist in the dense material composing the lamellae. In cross-sections (Fig. 4) and longitudinal sections (Fig. 5), evidence is seen of electron-transparent channels which pierce the lamellae at right angles and travel longitudinally in the body.

DISCUSSION

The cytoplasmic bodies described above, which have been observed in the epithelium of the fungiform papillae of the rat tongue, are localized primarily near the plasma membrane of cells in the stratum granulosum and upper layer of the stratum spinosum. It is especially noteworthy that these bodies are found largely near that part of the plasma membrane closest to the epithelial surface. High resolution studies have revealed that these bodies have an internal structure consisting of lamellae which traverse the width of the bodies and are arranged in a repeating pattern in which lamellae of high density alternate with lamellae of low density in a regular fashion.

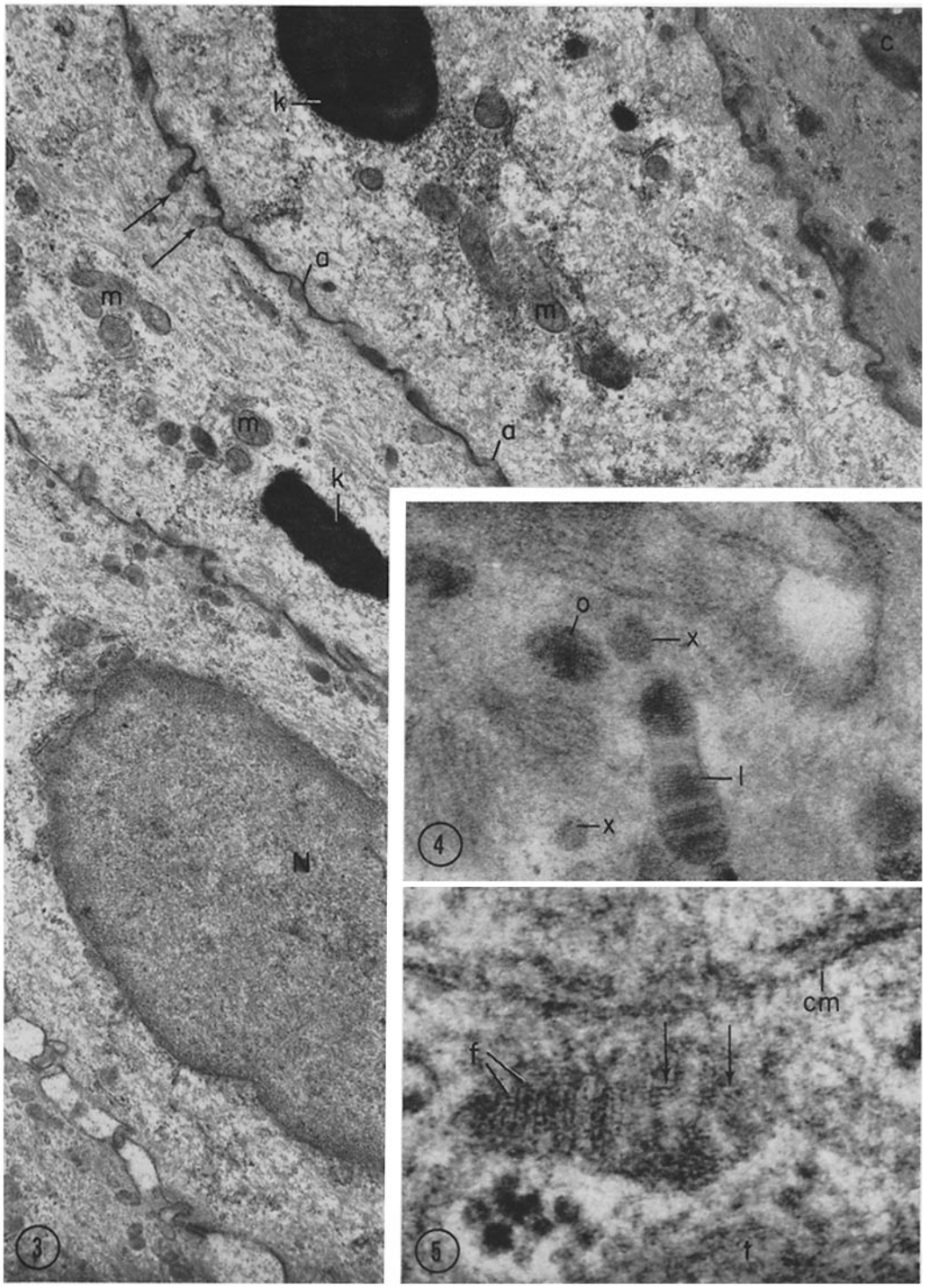
Although other investigators (4, 8, 10, 15, 18) have attributed a complete limiting membrane to small cytoplasmic bodies similarly distributed in keratinizing epithelium, the present study leaves some room for doubt concerning the existence of such a membrane. However, the possibility must not be overlooked that the plane of section was unfavorable for its demonstration despite the fact that examination of many bodies failed to reveal a complete limiting membrane.

There has been some disagreement concerning the origin of similar cytoplasmic bodies that have been described by other workers. Some investigations refer to the bodies as attenuated or fragmented mitochondria (8, 15, 16). However, measurements of the thickness of the lamellae in the bodies observed in the present study reveal that these lamellae are significantly thinner than the membranes that make up the mitochondrial cristae, and, on this basis, it would appear that the bodies are not likely to be mitochondrial fragments. Similar bodies also have been seen associated with the nucleus and Golgi apparatus (4), but no such association was noted in the present study.

It has been speculated that these bodies extrude their contents into intercellular spaces (4). The present study would indicate that this speculation is probable, on the basis of the following observations: (*a*) the bodies are found in large numbers in the upper layer of the stratum spinosum and in the stratum granulosum, but not in the uppermost layer of the stratum granulosum; (*b*) they are found to be contiguous with the cell membrane; and (*c*) material that is structurally similar to that comprising the internal structure of the bodies is found in intercellular spaces.

Other authors have demonstrated certain changes associated with the plasma membrane of cells in the more superficial layers of keratinizing epithelium. The intercellular spaces in these layers become less conspicuous than those in the deeper epithelial layers (2). Some electron-opaque amorphous material (*cf.* Fig. 3) appears to occupy these spaces (1, 4), and fewer desmosomes are present (10). It is not unreasonable to suggest, on the basis of the available evidence, that the cytoplasmic bodies are released into the intercellular spaces where they maintain their structure for a short time and then become amorphous. They may then play a role in the changes associated with plasma membranes during the process of keratinization.

In this regard, it should also be noted that histochemical observations on monkey oral epithelium have revealed that a PAS-positive granular deposit, which is resistant to the action of saliva, appears between cells in the outer epithelial layers (14). The authors of that report state that "the granules appear to be located mainly on the cell surfaces, frequently producing parallel lines of reactive dots." It is tempting to speculate that the bodies described in the present study are related to the PAS-positive material, which is presumably a polysaccharide, and may function as "intercellular cement" or possibly as a barrier to the intercellular passage of materials across the epithelium. Though the evidence is unquestionably circumstantial, it is, nevertheless, suggestive. Further studies of the histochemistry and cytochemistry of oral epithelium are needed to characterize these structures more fully.



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BIBLIOGRAPHY

1. BRODY, I., An ultrastructural study on the role of the keratohyalin granules in the keratinization process, *J. Ultrastruct. Research*, 1959, **3,** 84.
2. BRODY, I., The ultrastructure of the tonofibrils in the keratinization process of normal human epidermis, *J. Ultrastruct. Research*, 1960, **4,** 264.
3. FARBMAN, A. I., Ultrastructure of taste bud in rat fungiform papilla, *Anat. Rec.*, 1963, **145,** 318.
4. FREI, J. V., and SHELDON, H., A small granular component of the cytoplasm of keratinizing epithelia, *J. Biophysic. and Biochem. Cytol.*, 1961, **11,** 719.
5. LUFT, J. H., Improvements in epoxy resin embedding methods, *J. Biophysic. and Biochem. Cytol.*, 1961, **9,** 409.
6. MENEFEE, M. G., Some fine structure changes occurring in the epidermis of embryo mice during differentiation, *J. Ultrastruct. Research*, 1957, **1,** 49.
7. MILLONIG, G., A modified procedure for lead staining of thin sections, *J. Biophysic. and Biochem. Cytol.*, 1961, **11,** 736.
8. ODLAND, G. F., A submicroscopic granular component in human epidermis, *J. Invest. Dermatol.*, 1960, **34,** 11.
9. PALADE, G. E., A study of fixation for electron microscopy, *J. Exp. Med.*, 1952, **95,** 285.
10. RHODIN, J. A. G., and REITH, E. J., Ultrastructure of keratin in oral mucosa, skin, esophagus, claw, and hair, *in* Fundamentals of Keratinization, (E. O. Butcher and R. F. Sognnaes, editors), American Association for the Advancement of Science Symposium No. 70, 1962, 61–94.
11. SELBY, C. C., An electron microscope study of the epidermis of mammalian skin in thin sections, *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 429.
12. SELBY, C. C., An electron microscope study of thin sections of human skin, *J. Invest. Dermatol.*, 1957, **29,** 131.
13. WATSON, M. L., Staining of tissue sections for electron microscopy with heavy metals, *J. Biophysic. and Biochem. Cytol.*, 1958, **4,** 475.
14. WISLOCKI, G. B., FAWCETT, D. W., and DEMPSEY, E. W., Staining of stratified squamous epithelium of mucous membranes and skin of man and monkey by the periodic acid–Schiff method, *Anat. Rec.*, 1951, **110,** 359.
15. ZELICKSON, A. S., Electron Microscopy of Skin and Mucous Membrane, 1963, Springfield, Illinois, Charles C. Thomas.
16. ZELICKSON, A. S., and HARTMANN, J. F., An electron microscope study of normal human non-keratinizing oral mucosa, *J. Invest. Dermatol.*, 1962, **38,** 99.

FIGURE 3 Epithelial cells in the stratum granulosum, showing nucleus (*N*), keratohyalin granules (*k*), and mitochondria (*m*). Note amorphous intercellular substance (*a*). Arrows indicate bodies that are contiguous with that part of the plasma membrane closest to the epithelial surface. (*c*), stratum corneum. Pb $(OH)_2$ stain. ×20,600.

FIGURE 4 High-power electron micrograph of longitudinal (*l*), oblique (*o*), and cross-sections (*x*) of lamellated bodies. Note transverse lamellae, consisting of electron-opaque material, which form loops with adjacent lamellae at their periphery. Note also indications of channels in cross-sections. Uranyl acetate stain. ×98,000.

FIGURE 5. High-power electron micrograph of lamellated body near cell membrane (*cm*). Note folds or loops (*f*) of dense material at periphery of body. Also, note the indication of longitudinal channels (arrows) passing through lamellae. (*t*) tonofilaments. Pb $(OH)_2$ stain. ×220,000.