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THE CONSTITUENTS OF KERATOHYALIN GRANULES OF NEWBORN RAT EPIDERMIS UNDER AN ELECTRON MICROSCOPY

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Abstract. Three-day-old rat skins were treated either by the freezing–thawing method or by the tetraphenylboron technique. After the former procedure, keratohyalin granules were solubilized, leaving empty spaces with an electron-dense or less dense rim. Particles roughly 200 Å in diameter, which were either electron-dense or less dense, were observed in the empty spaces and also seen detaching from the rim. After the freeze–thaw procedure, two types of keratohyalin granule were observed. The one is the same electron-dense granule as keratohyalin granules in vivo and the other is the granule, the electron density of which was identical with tonofilaments. Both granules were granular rather than fibrous in structure.

Key words: Keratohyalin granule; Particles of keratohyalin granules; Heterogeneity of keratohyalin granules; Freezing–thawing procedure

Keratohyalin granules of newborn rat epidermis consist largely of homogeneous, electron-dense granules which are of various shapes and sizes. Besides these homogeneous granules, small, round deposits with higher electron density (dense homogeneous deposits, DHD) are seen at the periphery of keratohyalin granules and in the nuclei of the stratum granulosum cells in epidermis, and also throughout keratohyalin granules in mucous membrane (5–7).

As regards the fine structure of keratohyalin granules, those isolated by Matoltsy et al. and their pH 4.5 precipitates consisted of minute particles ranging from 50 to 250 Å in diameter (8). The precipitate which was obtained by dialysing the deoxycholate-soluble matrix proteins of human plantar stratum corneum against redistilled water consisted of fine, round, electron-dense particles, which stained positively with Pauly reagent (9).

Recently we have developed a new technique by which preferentially solubilizes keratohyalin granules without any need for detergents (4, 10), or high salt buffer (11). The electron microscopic observations on keratohyalin granules obtained using this technique are presented in this paper. Purification and biochemical characterization of keratohyalin granules extracted with this method will be discussed in a separate paper.

MATERIAL AND METHODS

Newborn rats (Sprague-Dawley strain) were obtained from Charles River Japan Inc., Atsugi, Kanagawa and used at 3 days of age. Osmium tetroxide was purchased from E. Merck, Darmstadt, W. Germany. All other chemicals were of reagent or analytical grade, obtained from various commercial sources.

Preparation of tissue

Newborn rats were sacrificed by stunning and decapitation. The skins were treated in two different ways.

(1) The skin was rapidly removed, wrapped in Saran Wrap (Asahi–Dow Chemicals) and stored at −80°C until use (Revco ultra low freezer). After the frozen skins had been thawed in a refrigerator (+4°C) for 24 hours, they were put back in a −20°C freezer and stored for 24 hours. This freezing and thawing procedure was repeated three or four times. The skin was then placed on an iced glass (stratum corneum down) and the dermis rubbed with a scalpel handle. The dermis was easily removed and the epidermis was then rinsed in 50 mM Tris-HCl, pH 8.6, for 10 sec, three times. The epidermal sheets were stirred in the same, fresh buffer for 15 min.

(2) The other preparation required that a few, fresh rat skins were treated in the same way as described in our previous paper (10). Epidermal sheets, which were obtained by the tetraphenylboron technique, were incubated overnight in 50 mM Tris-HCl buffer, pH 8.6, containing 3.3% sodium deoxycholate.

For light microscopy, unfrozen rat skin, the separated epidermis, and the incubated epidermal sheets were fixed in buffered formalin and embedded in paraffin. Sections 4

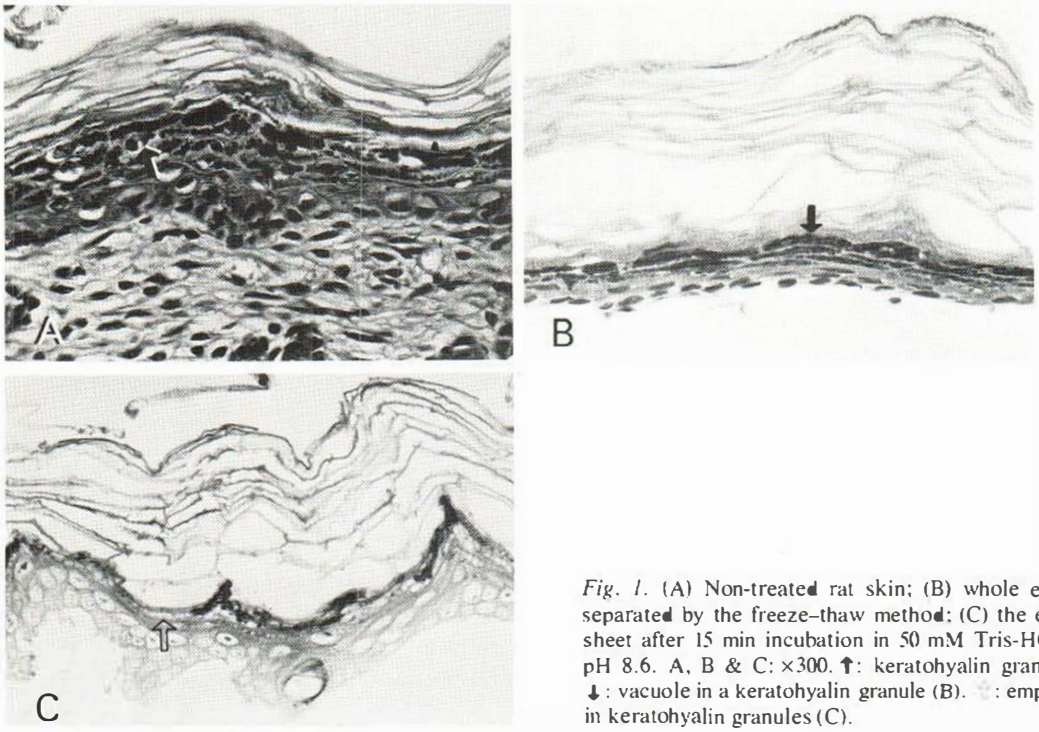

Fig. 1. (A) Non-treated rat skin; (B) whole epidermis, separated by the freeze–thaw method; (C) the epidermal sheet after 15 min incubation in 50 mM Tris-HCl buffer, pH 8.6. A, B & C: ×300. ↑: keratohyalin granules (A). ↓: vacuole in a keratohyalin granule (B). ↑: empty spaces in keratohyalin granules (C).

μm in thickness were used for hematoxylin-eosin staining. The epidermis for the electron microscopic examination was minced to 1 mm² in size, fixed in 2.5% glutaraldehyde for 2 hours, post-fixed in 1% buffered osmium tetroxide, dehydrated and embedded in Epon 812. The material was sectioned with an ultratome (Porter-Blum MT-2), stained with uranyl acetate and lead citrate and examined under a Hitachi Type 12 electron microscope.

RESULTS AND DISCUSSION

The sheets which were separated from the dermis by the freeze–thaw method consisted of the entire epidermis. No dermal components were found. In keratohyalin granules, numerous small vacuolar spaces were observed (Fig. 1B). After epidermal sheets had been incubated for 15 min in 50 mM Tris-HCl buffer, pH 8.6 at 4°C, almost all the keratohyalin granules and the nuclear materials were removed (Fig. 1C). Under electron microscopy, keratohyalin granules were extracted and many nearly empty spaces were observed in the cytoplasm of the stratum granulosum cells, especially in the perinuclear region, leaving either an electron-dense or less dense rim (Fig. 2A). In those spaces numerous small particles remained and some detached from the rims (Fig. 2B). No fibrous structure was found in the empty spaces. These particles were of two kinds: electron-dense (▲) and less dense particles (△). These observations were more distinct at higher magnification (Fig. 3A). In the centers of the spaces, only electron less dense particles remained and at the rims both kinds of particles were seen together. In this area, the electron less dense particles were observed to be grouped in the electron-dense background (Fig. 3A, ↑). The same situation was observed when epidermal sheets, obtained with the tetraphenylboron technique, were incubated overnight in 50 mM Tris-HCl buffer, pH 8.6, containing 3.3% sodium deoxycholate, i.e., there were two kinds of keratohyalin granules in the electron micrograph: the one had the same electron density as keratohyalin granules in non-treated control epidermis, while the other consisted of granules, the electron density of which was lowered to the same electron density as tonofilaments (fig. 4, ↑). These were not fibrous but granular in structure. In the epidermis treated by the freeze–thaw method, there were also rims having the same density as

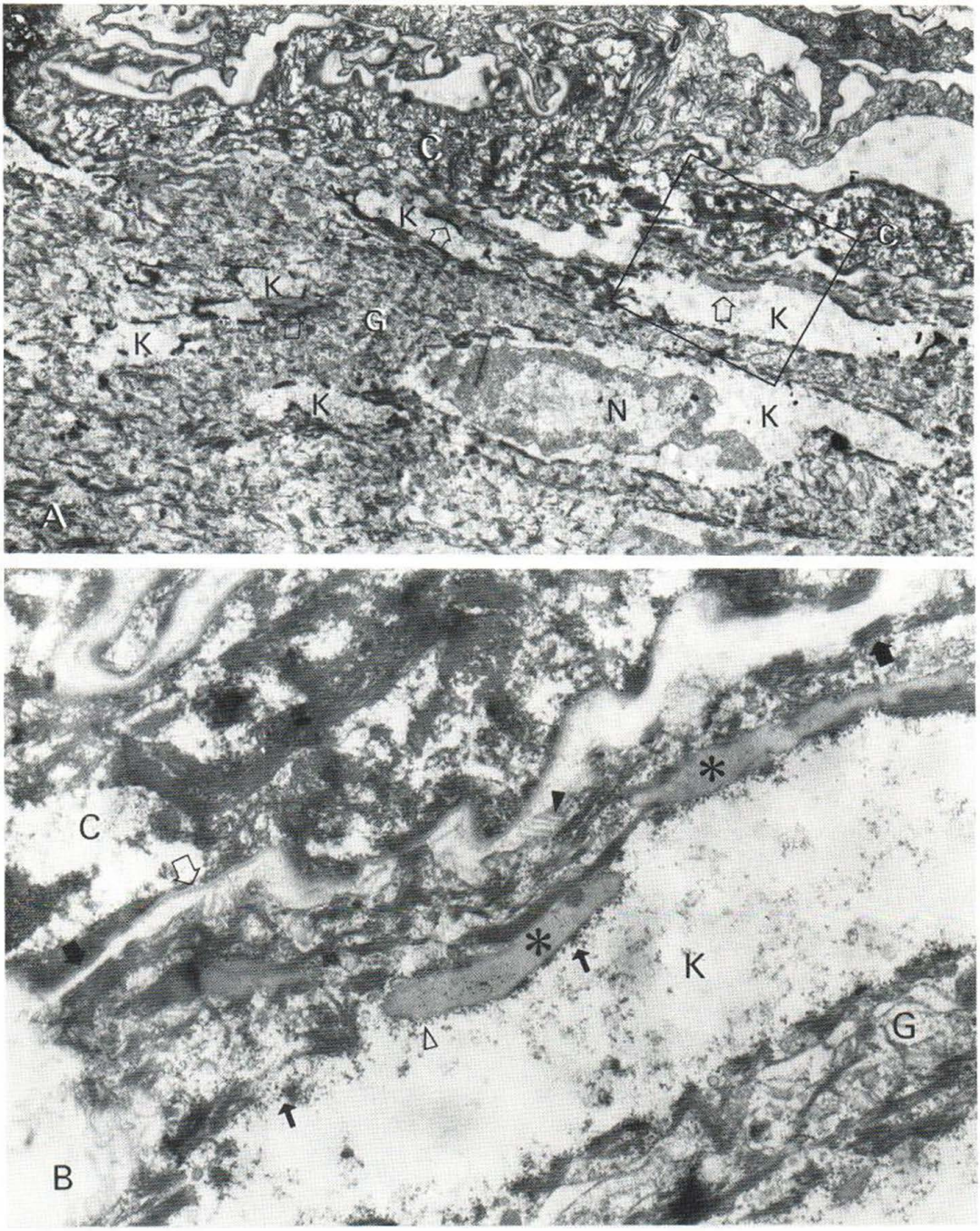

Fig. 2. Electron micrographs. (A) Epidermis after 15 min incubation; C: stratum corneum, G: stratum granulosum, N: nucleus, K: an empty space from which a keratohyalin granule was solubilized. : electron-dense or less dense rims. ×4 800. (B) A higher magnification of a rectangular area in Fig. 2 A. C: stratum corneum, G: stratum granulosum, ✱ : an electron less dense rim, ⬆: electron-dense particles, Δ: electron less dense particles, ⇧: a thickened cellular membrane. ⬆: desmosome, ▲: a lamellar granule. ×23 000.

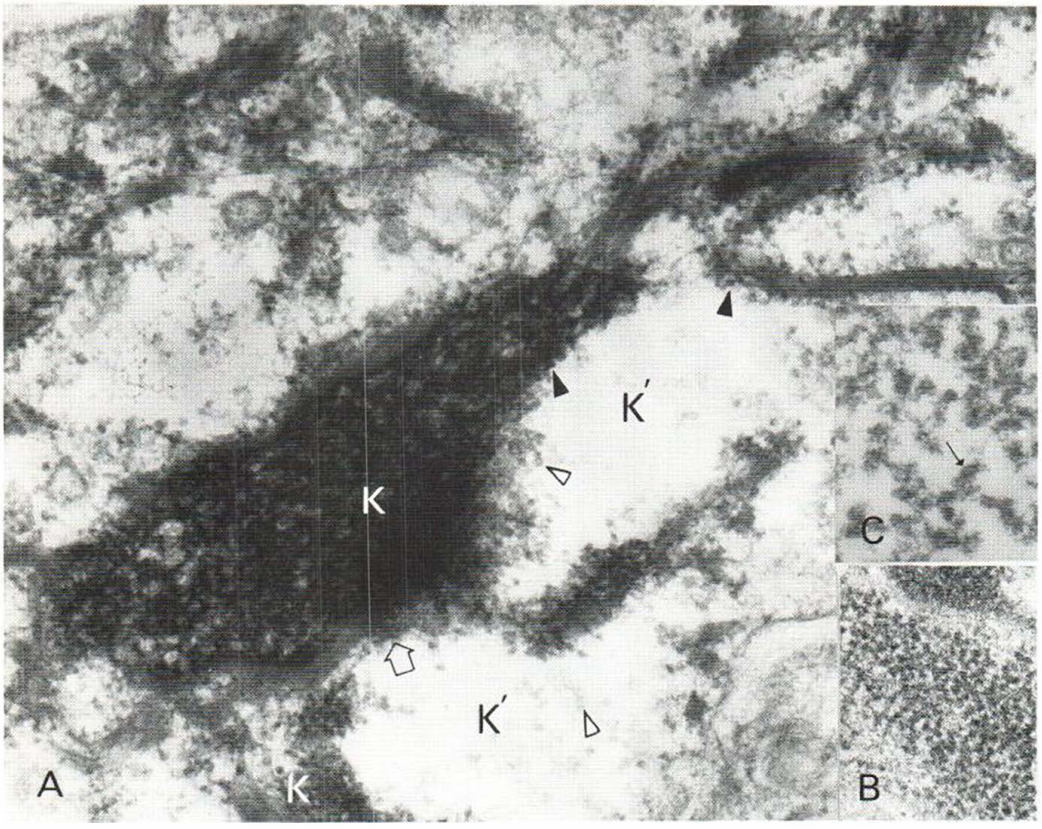

Fig. 3. (A) K: an electron-dense rim, K′: an empty space from which keratohyalin granule was extracted. ▲: an electron-dense particle. △: an electron less dense particle, ⇧: electron less dense particles grouped in electron-dense background. ×50 000. (B) Ribosomes. ×48 000. (C) The pellet which was obtained by dialysing the 270 000 *g* supernatant fraction against redistilled water. ↑: A ribosome-like particle. ×46 000.

tonofilaments. They consisted of particles 200 Å in size. These observations intimate that keratohyalin granules consist of the electron less dense granular material and electron-dense homogeneous substance. The former consists of fine granules whose electron density is similar to that of tonofilaments. The size of the electron-dense or less dense particles seemed to be identical and was around 200 Å in diameter. As compared with the size of ribosomes in vivo (250 Å in diameter) (Fig. 3B), they were slightly smaller. Whether or not these particles were ribosomes, can be decided by their chemical characteristics, but not by morphological ones. These particles collected in the pellet which was obtained by dialysing the 270 000 *g* supernatant fraction against redistilled water (Fig. 3C). The pellet reacted positively with Pauly reagent and contained neither RNA nor DNA, as determined by Orcinol reagent and the diphenylamine method (2, 3). These biochemical data confirmed that these ribosome-like particles were not themselves ribosomes. However, the possibility cannot be denied that the proteins of these particles might derive from ribosomal proteins, as large amounts of a ribonuclease were detected in the histidine-rich fraction from keratohyalin granule fractions (1).

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Fig. 4. Epidermis which was incubated in 50 mM Tris-HCl buffer, pH 8.6, containing 3.3% sodium deoxycholate. C: stratum corneum, G: stratum granulosum, k: a keratohyalin granule, the electron density of which was reduced and was equivalent to that of tonofilament. ✱ : an empty space from which a keratohyalin granule was solubilized. ⇧: a thickened cellular membrane, ⇧: a region of decreased electron density. △: the granular appearance of keratohyalin granule, M: mitochondria, K: keratohyalin granule. ×19 000.

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