A Rapid Fixation Technique of Epidermis for Electron Microscopy

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A rapid processing technique for ultrastructural studies of human epidermis has been devised in order to reduce dislocation of soluble compounds and to make available sections for diagnostic purposes within reasonable time (ca 4 hours). The morphology of cellular components agreed with or improved upon that obtained after commonly used methods. Thus, for example, the substance in the intercorneal space was better preserved and the cytomembrane and certain of its specializations appeared more distinct. *Key words: Intercorneal substance; Cell junctions; Birbeck granules.* (Received January 19, 1985.)

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The manifold steps in routine processing methods for electron microscopy, pre- and postfixation, buffer washing, dehydration in organic solvents, infiltration of plastic solvent(s) and a plastic monomere followed by polymerisation are very time-consuming (generally 70 hours or more). It is known that small molecules dislocate during fixation and buffer washing (1) and that 90% of the membrane lipids can be extracted during the dehydration and embedding steps (2). Thus, rapid processing is crucial to reduce loss of substance from the tissue. Moreover, it is obvious that the diagnostic importance of ultrastructural analyses of various diseases of the skin will continuously enhance in the near future. This development will increase the demand for rapid methods.

The present study shows that by taking advantage of the knowledge of penetration rates of fixatives (3, 4) and by using 2,2-dimethoxypropane as dehydrating agent (5) the processing time can be reduced to three hours without compromising but rather improving the quality.

MATERIAL AND METHODS

Punch biopsies (3 mm) of epidermis were obtained from the forearm of five healthy adult humans. Excessive dermis was trimmed away and the biopsies were divided into several parts so that no side of the specimens exceeded 300 μm. After a number of pilot experiments a final procedure was designed as follows: Primary fixation 15 min in 3% glutaraldehyde dissolved in 0.15 M cacodylate buffer with 3 mM $CaCl_2$ added (+4°C); rinsing in the same buffer during 10 min; postfixation in 2% OsO_4 in the same buffer for 15 min (+4°C) and rinsing during 10 min in cold distilled water. The blocks were stained with 0.5% uranyl acetate in distilled water for 5 min and washed 10 min in distilled water. Dehydration was carried out in acidified 2,2-dimethylpropane (DMP) according to Muller et al. (5) for 10 min followed by infiltration with equal parts of Epon 812 and aceton for 10 min and two changes of Epon 812, 10 min each. The medium was hardened during 45 min at 100°C and cooled with tap water. 10 min after removal from the oven the blocks were trimmed and sectioned on a diamond knife. After-staining was performed in and an LKB ultrastainer (uranylacetate 30 min, lead citrate 4 min), and the sections were examined in a Jeol CX 100.

Part of the specimens were processed according to a previously described method (6), which requires totally around 70 hours.

RESULTS AND COMMENTS

The morphology of various cell components as it appears after the present rapid technique and after a commonly used routine procedure is summarized in a comparative form in Table I. It can be seen that the rapid technique did not introduce any decrease in quality but instead improved the visualization possibilities in certain respects.

Figs. 1–2. The keratinocytic desmosomes and a gap junction (Fig. 2). The central lamellae of the desmosomes are clearly visible. Scale 0.25 μm.

Fig. 3. High power magnification of a part of a Birbeck granule with its tri-laminar membrane and central periodicity. Scale 20 nm.

Fig. 4. Distinct tonofilaments in a basal keratinocyte. The extracellular dense lamellae of the hemidesmosomes and their tiny filaments are clearly depicted. Scale 0.25 μm.

Fig. 5. Tubular subunits of a melanocytic centriole. Scale 50 nm.

Fig. 6. A part of a Langerhans cell showing distinct Birbeck granules and mitochondria with electron dense granules. Scale 2 μm.

Fig. 7. Detail of stratum corneum showing the intercorneal dense substance (*). Scale 0.25 μm.


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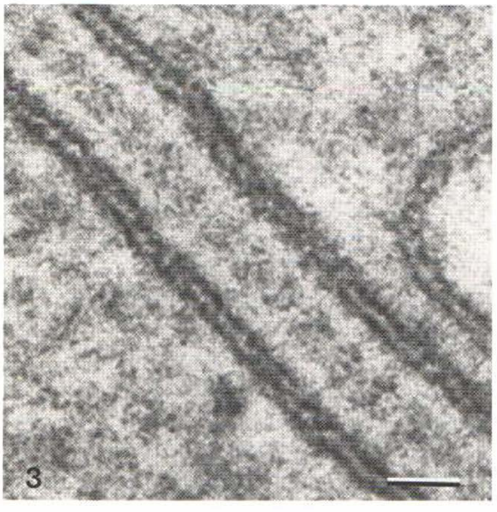
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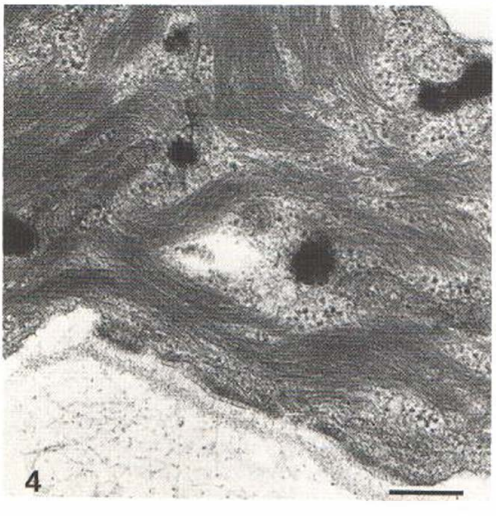
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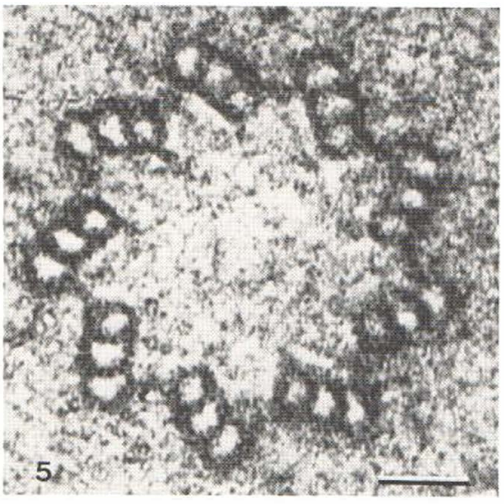
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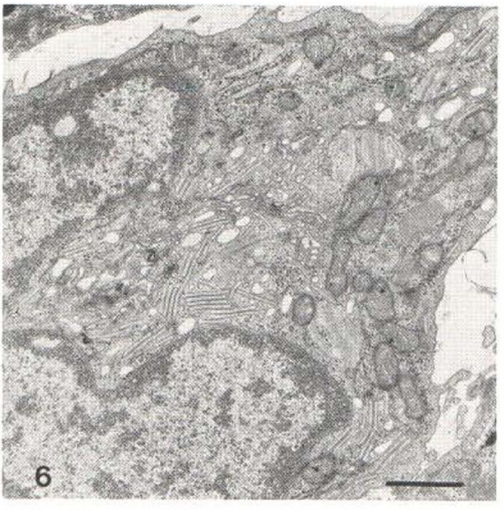
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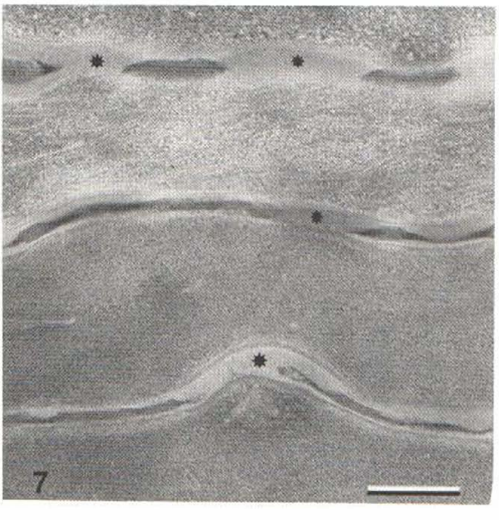
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Table I. *Epidermal fine structure after rapid tissue processing*

	Keratinocytes	Melanocytes	Langerhans cells
Cytomembrane	+	+	+
Desmosomes	+		
Gap junctions	+		
Hemidesmosomes	+		
Melanocytic junctions		+	
Birbeck granules			+
Perinuclear space	=	=	=
Mitochondrial conformation	=	=	=
Mitochondrial matrix	Denser	Denser	Denser
Mitochondrial granules	+	+	+
ER and Golgi conformation	=	=	=
Microfilaments		+	+
Tonofilaments	+		
Microtubuli	+	+	+
Substance in intercorneal space	+		

Improvement of fine structure: +. Same appearance as in control specimens: =.

A very conspicuous improvement was that the intercorneal spaces in all specimens appeared filled with a substance (Fig. 7). This substance has been proposed to consist mainly of lipids and it has been recognized that it is difficult to preserve it for thin section electron microscopy (7). Thus, the rapid technique may open new possibilities to study the corneal barrier in normal and diseased skin.

Moreover, the cytomembrane and certain junctional structures appeared more distinct (Figs. 1, 2, 4). In the desmosomes the central lamellae were unusually conspicuous and a hemidesmosome-like structure probably attaching the melanocyte to the basal lamina was visualized (8). The Birbeck granules of the Langerhan's cell, which by all probability are derived from the cytomembrane (9, 10) were depicted with extraordinary clarity (Figs. 3, 6). Internal membranes also appeared very distinct, for example around vesicles (Fig. 3) and mitochondrial membranes. Electron-dense intramitochondrial granules were often seen (Fig. 6). Other intracellular components such as microfilaments, tonofilaments (Fig. 4) and microtubuli were more well defined, and even the subtubuli of the triplets of the centrioles could easily be detected (Fig. 5).

The different morphologies achieved by the new rapid technique and a commonly used routine procedure can presently not be fully explained. One reason is obviously a minimized redistribution and extraction of soluble substances as evidenced by the preserved intercorneal material. It is possible that a protection from diffusion also of other substances can fully or partially account for the distinctness of other cell constituents as well.

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