notably the part of the cuticular cell which contains low amounts of sulphur. The fact that cuticular swelling results in longitudinal ridging is consistent with the fact that the 8 mm filaments of cuticular cells are in a parallel arrangement and aligned along the axis of the fibre. Since the cuticular filaments are straight, not helically arranged, they have less capacity to take elongation stress than has the helically arranged cortical filaments. At swelling the expansion will therefore take place in a direction at right angle to the fibre axes. The fact that eosin staining does not influence the results of SEM and TEM preparation provides for better selection of specimens.

Our results indicate that human hairs may be attacked in vitro not only by *T. mentagrophytes* but also by *T. rubrum*. These organisms are capable of creating apparently lytic changes in the hair-fibre especially involving the cuticula. From the morphological data our interpretation of these findings is that the lesions caused by these two dermatophytes on experimentally infected hair in vitro are likely to represent different degrees of keratolytic break down rather than different mechanisms of action.

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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 post-fixation, buffer washing, dehydration in organic solvents, infiltration of plastic solvent(s) and a plastic monomer 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 CaCl₂ added (+4°C); rinsing in the same buffer during 10 min; postfixation in 2% OsO₄ 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 1. 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.
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). Interna) 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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Urinary Excretion of Melanocytic Metabolites in Fertile Women


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Pregnant women and women taking oral contraceptives show urinary excretion values of 5-S-cysteinyldopa and of 6-hydroxy-5-methoxyindole-2-carboxylic acid in the same range as nonpregnant women not taking oral contraceptives. The excretion of these melanoma markers can therefore be used in in the biochemical diagnosis of metastatic melanoma in pregnancy and in women taking oral contraceptives. Key words: Pregnancy; Contraceptives; 5-S-Cysteinyldopa; 6-Hydroxy-5-methoxyindole-2-carboxylic acid. (Received February 28, 1985.)

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The urinary excretion of 5-S-cysteinyldopa (5-S-CD) has in recent years been used as a marker for malignant melanoma metastases. In many cases an increase can be noted before metastases become clinically apparent (1, 2).

An indolic metabolite of the eumelanin pathway, 6-hydroxy-5-methoxyindole-2-carboxylic acid (6H5MI-2-C), has been found in melanotic urine (3), and recently a method for detecting this substance in normal urine has been described (4).

Pregnancy, oral contraceptives, and supplementary postmenopausal oestrogen treatment can all induce hyperpigmentation of the face, the areolae, and the ventral midline. The mechanism is unknown, but it seems to result from oestrogen influence (5, 6, 7). Interpretation of increased excretion levels of 5-S-CD and 65MI-2-C in women operated on for malignant melanoma is often uncertain, because the effect of sexual hormones has not been studied.

The excretion of 5-S-CD and 6H5MI-2-C in pregnant women, and in nonpregnant women with and without oral contraceptives was therefore investigated.