Technical methods

Examination of skin window preparations by transmission electron microscopy

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Valuable information concerning the cellular inflammatory response both in normal subjects and in patients with various disorders has been gained from examination of skin window preparations by light and scanning electron microscopy.¹⁻⁴ However, the inability to visualise changes in the fine structure of the exudative cells by standard techniques has until now been a serious limitation. The present report describes a method which has been developed for examining skin window preparations by transmission electron microscopy (TEM).

Material and methods

Skin windows were made on the anterior surface of the non-dominant forearm. After the skin had been cleaned with spirit, the surface layer of epithelium was carefully scraped away with a sterile scalpel blade until exudation was noted; loose squames were wiped off. A sterile, circular (13 mm diameter) coverslip was applied to the abrasion, covered with a protective cardboard square, and secured with surgical tape. The coverslip was removed after 24 hours (day 1 specimen) and replaced with a new one which, in turn, was removed after a further 24 hours (day 2 specimen). Immediately after removal, the specimens were processed for TEM. After an initial wash in isotonic saline (approximately 10 minutes) the cells on the coverslips were fixed in 1.5% glutaraldehyde in phosphate buffer for 1 hour, washed in sucrose phosphate buffer, postfixed in 2% unbuffered osmium tetroxide for 15 minutes, and washed in distilled water. The specimens were then stained in saturated alcoholic uranyl acetate, dehydrated in ascending grades of alcohol, rinsed in epoxy propane, and impregnated for 10 minutes with Araldite resin. The coverslips were inverted and mounted on resin-filled embedding capsules, and the resin was polymerised at 80°C overnight. The cover-

slips were detached from the embedding capsules while still warm (60°C). The resulting blocks were sectioned for light microscopy (to locate the cells) and TEM, the sections being stained with toluidine blue and Reynold's lead citrate respectively. The sections were examined in an AEI 801 electron microscope.

Results

With this technique, ultrathin sections of skin window preparations have been obtained, the cells being sectioned in a plane parallel to the coverslip. Different cell-types were readily identified from their characteristic TEM appearances; a group of macrophages is illustrated in the Figure. The results suggest that the technique gives consistent and reproducible appearances.

Discussion

As far as we are aware, this is the first report of the application of TEM to skin window preparations. Certain technical points seem to be important; the specimens must not be allowed to dry out at any stage, and the initial saline wash results in a cleaner preparation. As the layer of cells is only about 5 µm thick, critical orientation of the block is necessary if a wide field of cells is to be sectioned.

The method is a logical development in the study of skin window specimens and, together with light and scanning electron²⁻⁴ microscopy, completes the standard range of techniques commonly used in the examination of clinical material. Macrophages are plentiful in both day 1 and day 2 preparations, and the statement that the alveolar macrophage (obtained by bronchopulmonary lavage) is the only macrophage readily available for study in man⁵ can now be challenged; furthermore, the results of skin window examination are not dependent on smoking habit⁵ or lung pathology.

Addition of TEM to the range of skin window techniques should considerably increase their potential value in the clinical situation.

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TEM appearance of a group of cells in a 48-hour skin window preparation. Most of the cells can be identified as macrophages, a typical example being the cell at the top left. This cell is over 20 μm in diameter and has an irregular nuclear profile. Its cytoplasm contains many vacuoles with much dense granular material, presumably derived from phagocytosis of melanin, dead cells, and other debris. The cell surface shows a number of fine projections.

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