veloped in other areas of the cell. An ongoing reorganization of the dendrites *in vivo* is probably required for the donation of melanosomes to the newly formed keratinocytes in the epidermal melanin unit (4). Repeated examination of the melanosomes in the dendrites revealed changes in their location, signifying melanosome transport. The aggregates of melanosomes initially observed in the distal part of some dendrites could not always be found at later examinations, in the meantime other dendrites developed similar terminal enlargements. These observations were taken as an indication of an ongoing melansome donation. So far the donation process has not been studied directly

A few melanocytes could not be found at their original location at subsequent examinations. This might be due to dramatic morphological changes or ceased melanin production, but it could also be that the cells had migrated rapidly to quite another location.

In conclusion, we have developed a method by which to study the dynamics of individual melanocytes in a living tissue. The optical resolution in the vital microscope is surprisingly good considering the thickness and the cellularity of the tissue observed. The technique is suitable for studies on functions such as melanocyte migration, dendritic movements and cell-to-cell interactions during the influence of various external or internal factors. Effect will now be made to refine the technique in an attempt to make direct observations on the transfer of pigment from the melanocyte to the kerationocytes.

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Dissociation of Suction Blister Roof Epidermis with Trypsin and Desoxyribonuclease into Viable Single Cells

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Abstract. Human epidermis, obtained *in vivo* by the suction blister method, was dissociated with trypsin and desoxyribonuclease into a single-cell suspension. Autoradiographic analysis of the blister roof epidermis and of the epidermal cell suspension was performed to show that neither the suction procedure nor the enzymatic dissociation affected DNA synthesis of the epidermal cells.

Key words: Dissociation of epidermal cells; Suction blister method; Trypsin; Desoxyribonuclease

Human epidermal cell suspensions are prepared for studies on epidermal cell biology and for cell cultures. The dermo-epidermal separation is usually performed *in vitro* from skin explants either with diluted acetic acid (2) or enzymatically with trypsin (1) or collagenase (3). The isolated epidermis can then be dissociated into single cells with enzymes (1, 3) or by ultrasonication (8).

The *in vivo* method for the dermo-epidermal separation of human skin has been developed by Kiistala (5). Using a special suction blister device it is possible to produce subepidermal blisters in different areas of the skin. This procedure does not

cause the test persons any pain or scarring, and thus provides an easy source of human epidermis.

In this study we enzymatically dissociated adult human epidermis obtained by the suction blister method into single-cell suspensions. Autoradiograms from the blister roof epidermis and the epidermal cell suspension were made and the labelling indices were compared with the labelling index of normal human epidermis.

MATERIAL AND METHODS

Source of the skin. The skin donors were 30 healthy volunteers and patients hospitalized mostly for infectious skin diseases at the Department of Dermatology, Helsinki University Central Hospital. Most of them were young men and women. The test area on the abdomen was healthy in all cases.

Isolation of the epidermis by the suction blister method. The suction blisters were generated on the test persons by the method described in detail by Kiistala (5). In order to produce the blisters, four special suction devices were placed on the skin of the abdomen, and a 200 mmHg vacuum was applied for 2 hours at normal room temperature by warming the test area with electric lamps. Twenty-four subepidermal blisters of approximatley 0.1 cm^3 in volume were generated on each volunteer. The round roofs of the blisters, 5 mm in diameter, containing the whole epidermis were cut off with scissors.

Dissociation of the epidermis. The roofs of the blisters were treated in phosphated-buffered saline containing EDTA 0.02%, glucose 0.02%, trypsin 0.2% (3.5 U/mg, Merck) and desoxyribonuclease 0.02% (Sigma), (10–12 blister roofs in 5 ml of medium) at 37°C and shaken for 15 min at which time the solution became cloudy. The remaining sheets of the stratum corneum were removed with forceps, fixed in 4% formalin and processed by usual histopathological techniques for light microscope investigation. The cell suspensions were centrifugated at 180 *g* for 5 min and washed twice with the same buffer containing desoxyribonuclease 0.01%. Smears or cytocentrifuge preparations were made for microscopical axamination.

Autoradiograms. An autoradiographic analysis of both the whole blister roof epidermis and the epidermal cell suspension was performed in 6 cases. Two roofs as a whole and the other 22 roofs dissociated for the cell suspension were incubated at 37°C for 60 min in 1 ml of Hanks' solution containing 2 μCi of [^{3}H]TdR. After washing twice with Hanks' solution, the blister roofs were fixed in 4% formalin, processed, and sectioned at 4 μm. Cytocentrifuge preparations were made from the epidermal cell suspensions. The preparations were covered with stripping film (Kodak AR-10), exposed for 7 days, and stained with Harris hematoxylin. Labelling indices for the epidermal cells were determined by counting 5000 cells in each specimen and expressing the count as a ratio of the labelled cells to all unlabelled epidermal cells $\times 100$.

RESULTS

Epidermal cell suspensions. Smears and cytocentrifuge preparations of fresh epidermal cell suspensions contained mostly single epidermal cells (Fig. 1). However, small clumps composed of two to eight cells were also present. Most of the cells were rounded as a result of the dissociation procedure. The basal cells appeared as small basophilic cells with scanty cytoplasms, whereas the differentiated cells from the upper stratum malpighi were flat and had large cytoplasms. Furthermore, transitional cell types were seen representing cells from all layers of the epidermis. The histological sections of the keratin sheets revealed that only a few cells from the upper stratum malpighi and the stratum granulosum were still attached to the stratum corneum after the enzymatic dissociation (Fig. 2).

Viability of the blister roofs and the dissociated epidermal cells. To test the viability of both the whole blister roofs and the dissociated epidermal cells, an autoradiographic analysis was performed in 6 cases. The mean labelling indices $\pm$ S.D. for the whole epidermis and for the epidermal cell suspension were $0.55 \pm 0.28\%$ and $0.98 \pm 0.56\%$, respectively.

DISCUSSION

Kiistala & Mustakallio have shown electron microscopically that the suction blister appear at the dermo-epidermal junction subepidermally in the space between the basal lamina and the cell membrane of the basal epidermal cells (6). The blister roof is therefore composed of pure epidermal cells, keratinocytes, melanocytes and Langerhans cells, with no cellular contamination from the dermis. In enzymatic dermo-epidermal separations performed *in vitro*, epidermal cells are easily contaminated by fibroblasts derived from the dermis. Furthermore, prolonged enzymatic incubation may damage epidermal cells more than suction does. Preliminary studies show that human epidermal cells derived from a suction blister roof indeed adhere better to coverslips than do cells derived from enzymatically isolated epidermis (4).

The dissociation of the epidermis into single cells has been widely performed with trypsin (1, 3). In 1967 Briggaman et al. reported a slimy material which appeared in the cell suspension during the procedure (1). The same material has also been found in other tissues during the tryptic incubation,

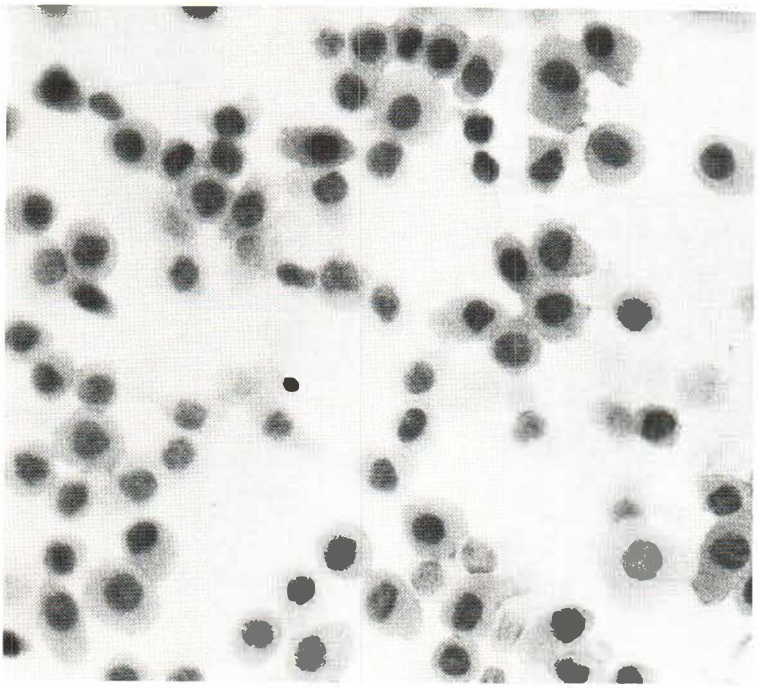

Fig. 1. Cytocentrifuge preparation of human epidermal cell suspension dissociated with trypsin and desoxyribonuclease.

and it has been shown to contain highly hydrated DNA released from the damaged cells at the margins of the tissue (11). We found this material to appear whenever we used only trypsin in epidermal dissociation.

Especially during the centrifugation the already separated epidermal cells became attached to this material, forming gelatinous clumps. We tried, without success, to dissolve the material with chymotrypsin, pronase, panchreatin and col-

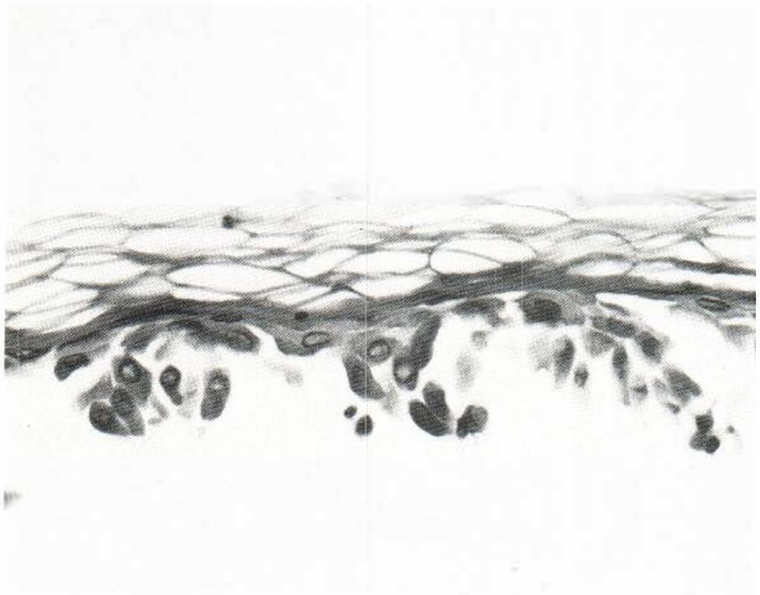

Fig. 2. Sheets of the keratin layer with a few cells from the upper stratum malpighi.

lagenase. Only the use of desoxyribonuclease during the primary tryptic incubation inhibited the formation of this material and the cell clumps.

The autoradiographic analysis showed that the enzymatic dissociation of the epidermis with both trypsin and desoxyribonuclease did not affect DNA synthesis of the epidermal cells. On the contrary, the labelling index in the epidermal cell suspension was higher than the labelling index in the blister roof epidermis before the dissociation. There are many possible explanations for this phenomenon. The total cell count in the cell suspensions is lower than in the whole roofs, because some of the cells in the upper stratum malpighi remain attached to the keratin layer. Obviously, some epidermal cells are destroyed during the dissociation procedure (11). Furthermore, the diffusion of [^{3}H]TdR may be better in the cell suspension than in the whole epidermis. Finally, there is great variation in the labelling indices between different blister roofs, clearly depending of the number of hair follicles and sweat ducts in the area.

According to previous studies the labelling index for normal human skin varies between 2.2 and 5.2 % when calculated for the basal cell layer (7, 12). In order to compare the labelling index of the blister roofs with that of the cell suspensions we had to count the labelled cells for all epidermal cells. Therefore, the labelled cells in higher epidermal layers, presumably Langerhans' cells, are also included in our labelling indices (9). The proportion of labelled Langerhans cells in normal guinea pig epidermis is less than 10% of all labelled cells, and the total number of Langerhans cells in human skin is reported to be about the same as or less than in guinea pig skin (10). Therefore, by correlating our labelling index in blister roof epidermis to the number of epidermal layers in the abdominal skin area (about five) and to the supposed number of Langerhans cells our value is similar to the labelling index of normal human epidermis. Thus it seems that the suction procedure does not interfere with DNA synthesis in fresh suction blister roof epidermis.

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Polymorphonuclear Leukocyte Chemotaxis in Dermatitis Herpetiformis

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Abstract. Polymorphonuclear leukocyte chemotaxis was investigated in 15 patients with dermatitis herpetiformis and was found not to be significantly increased when compared with the polymorphonuclear leukocyte chemotaxis of 15 normal healthy controls. These results indicate that accumulations of polymorphonuclear leukocytes found in the skin of patients with dermatitis herpetiformis are not due to an increased responsiveness of polymorphonuclear leukocytes to a chemotactic factor.

Key words: Dermatitis herpetiformis; Chemotaxis