Acta Dermatovener (Stockholm) 61: 55–92, 1981

SHORT REPORTS

Vital Microscopy of Epidermal Melanocytes

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Received June 13, 1980

Abstract. Vital microscopy has been used to study melanocytes in the mouse. The morphology of the living epidermal melanocyte is described, including dendritic movements, cell division and melanosome transport.

Key words: Melanocyte; Vital microscopy

The epidermal melanocytes constitute a dynamic cell system which is in a continuous state of renewal by mitosis (6, 7, 10). The mitotic activity is influenced by various internal and external factors, such as UV light (6, 7, 10). During mitosis the melanocytes seem to retract their dendrites. The two daughter cells develop new dendritic processes and migrate apart (8). The migration may be governed by repulsive interactions between the cells, possibly mediated by dendritic contacts (9). The dendrites also play an important role in the excretive functions of the melanocytes. The melanosomes are formed in the cell body, are then transported through the dendrites and finally transferred to the keratinocytes. The exact mechanism of pigment donation is not clear, although several models for melanosome transfer have been proposed (2, 3, 5). The spreading of the melanocytes as well as the configuration of the dendritic network are probably of basic importance to ensure an even distribution of pigment in the skin.

Present ideas on the dynamics of the epidermal melanocyte system are based mainly on observations of cells in fixed and sectioned tissue. For further progress in this field, it seems desirable to develop techniques which would make it possible to study the behaviour of the same melanocyte over a long period of time. Tissue cultures can be used for such studies, but in this situation the cells are isolated from their normal environment and an unknown degree of dedifferentiation may take place. We have therefore chosen another approach, which comprises vital microscopic observations of the melanocytes in the intact ear of the C57Bl mouse. This paper describes the method and illustrates its potential for *in vivo* studies on melanocytes.

MATERIAL AND METHODS

The ear skin of C57Bl/bJ mice (Anticimex, Sweden), which provides a well developed epidermal melanocyte system was used. In order to increase the melanin content of the melanocytes, and thus potentiate the contrast against the non-pigmented background, some of the animals were irradiated with UVB light for 3 days prior to examination. The light source was a fluorescent sun lamp (Westinghouse FS) with the main out-put in the UV spectrum between 290 and 350 nm. The daily dose given was 0.1 joule/cm^2. Microscopic observation started the day after the final irradiation.

Before microscopy the mice were anaesthetized with intraperitoneal injections of sodium pentobarbital and diazepam, 1 mg/25 g b.wt. and 0.25 mg/25 g b.wt., respectively. They were then placed head down in a groove on a Frigolit (expanded polystyrene) plate, with one ear spread flat on a polished, light-conducting plexiglass rod (∅ 15 mm, length 19 mm) piercing the Frigolit plate (Fig. 1). The ear was kept immobilized simply by laying a film of immersion oil between the ear and the plexiglass rod.

The mouse was placed on the cross-stage of a Leitz vital microscope, equipped with a long focal distance conden-

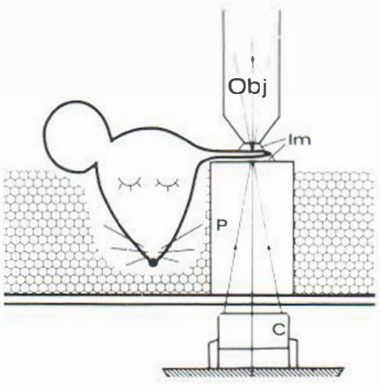

Fig. 1. Drawing of the mouse mounted for microscopy. *C*, Condensor front lens; *p*, plexi-glass rod; *Im*, immersion oil; *Obj*, objective.

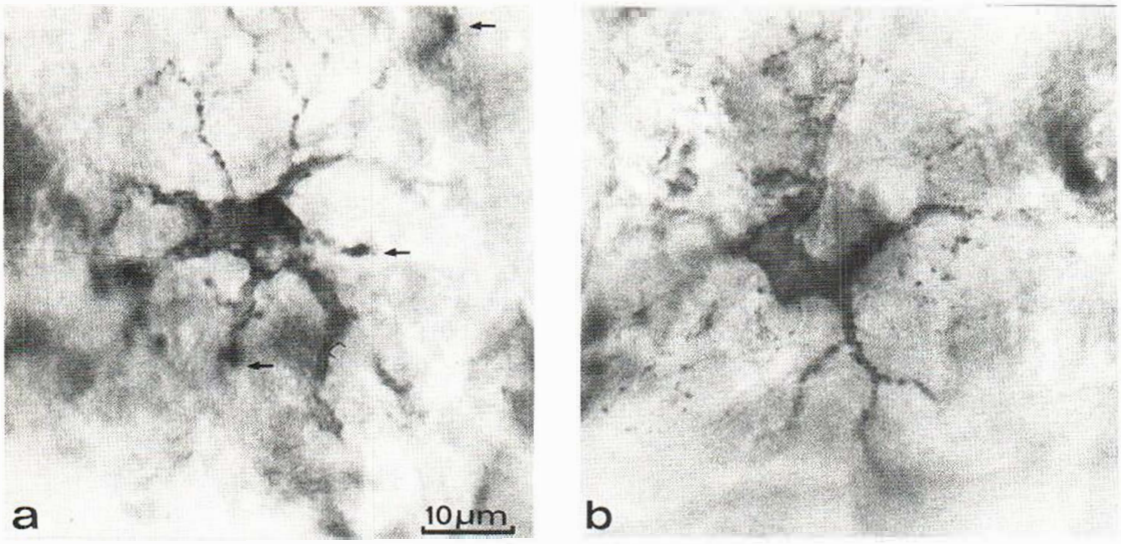

Fig. 2. In vivo photomicrographs of two large epidermal melanocytes in the mouse ear following 3 days of UV irradiation. (*a*) Note the lighter nuclear area in the cell body. Arrows indicate terminal melanosome aggregates. (*b*) Individual melanosomes are demonstrated in the melanocyte dendrites as well as in the neighbouring keratinocytes. (*a*) and (*b*) same magnification.

sor front lens (L20/0.45). Observations were made with Leitz objectives x2.5/0.07, U-O x23/0.55 and eye pieces x15. Several objectives were tested, but the x23 turned out to present the ideal combination of magnification, resolution and focal depth for this particular tissue. The low-power objective was used to establish certain landmarks in the ear—the microvascular network was particularly useful—so that individual melanocytes selected for observations could be identified repeatedly at different times.

Microphotographs were taken with Ilford FP 4, 135 film and a Leica camera operating with a synchronized high intensity flash light (cf. 1). The magnification at the film plane was ×290.

RESULTS AND COMMENTS

The epidermal melanocytes were clearly observed in the vital microscope due to the melanin content which gave them a brown-black colour standing out prominently against the pale non-pigmented background (Fig. 2*a*, *b*). A round, lighter area could be seen in the cell body, corresponding to the location of the nucleus (Fig. 2*a*). In the non-irradiated ears the individual melanosomes were easily distinguished in both the melanocyte dendrites and in the perikaryon. Following UV-irradiation the cell body contained a more homogeneous mass of pigment and the individual melanosomes could no longer be discerned. However, in the dendrites the melanosomes could still be defined as individual organelles. Due to the pigment granules the melanocyte dendrites were fairly easy to follow peripherally. The distal part of the dendrites often showed signs of swelling. These bleb-like terminal enlargements usually contained aggregates of pigment granules (Fig. 2*a*).

A mitotic sequence of a melanocyte is illustrated in Fig. 3. This cell was first seen to retract its dendrites, to become almost round and somewhat darker (Fig. 3*a*, *b*, *c*). The increased staining of the cell body was probably due to an addition of pigment granules from the retracted dendrites. The nuclear region was clearly visible (Fig. 3*b*), but details of the mitotic spindle and the chromatin particles could not be observed. Directly following cytokinesy, light nuclear areas became visible in the two daughter cells (Fig. 3*d*, *e*), which then became irregular in shape and started to put out new dendrites (Fig. 3*f*, *g*, *h*). Twelve hours later the daughter melanocytes were still close together. They now had a few long slender dendrites with secondary ramifications containing a few melanosomes (Fig. 3*i*). The melanosome density of the daughter melanocytes was still sparse compared with surrounding melanocytes. These *in vivo* findings fully confirm earlier observations in fixed and

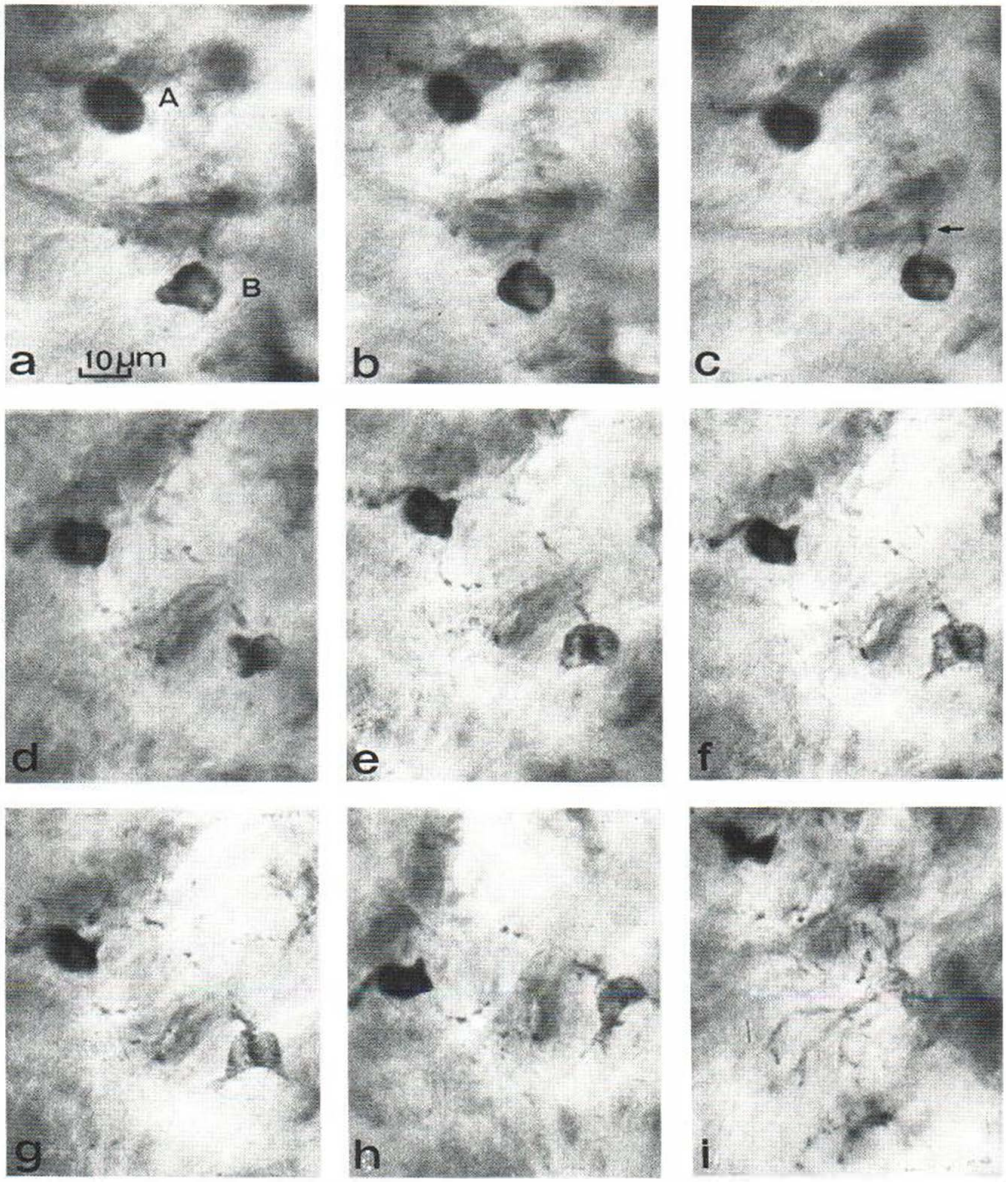

Fig. 3. Sequency of *in vivo* photographs of a dividing melanocyte (B). Melanocyte (A) was used as a marker cell. Most dendrites in cell (B) were retracted, though one short process (arrow) persisted throughout the division (*a–c*). The lighter nuclear region in the cell body developed into two distinct separate areas (*c-d*), after which the cytoplasm was pinched off (*d-e*) and the regrowth of dendrites started in the daughter cells (*f–H*). In (*i*) the newly formed melanocytes demonstrate long slender dendrites and sparsely dispersed melanin granules. Note the gradual change in morphology of the neighbour melanocyte (A), indicating an ongoing division. Note that the mouse ear was somewhat rotated with respect to the camera axes between the different exposures. Same magnification in all photographs.

sectioned skin on teh mitotic sequence of epidermal melanocytes (8).

Some melanocytes were studied repeatedly over a 3-day period. In general there was no problem in identifying the same melanocyte at subsequent examinations. During this period there were obvious changes in the outlines of some cells; a few dendrites had disappeared, while new ones had de-

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.

ACKNOWLEDGEMENT

This work was supported by grants from the Swedish Medical Research Council (B80-12X-00663-156) and the Welander Foundation.

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

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Received June 13, 1980

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