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MORPHOLOGY OF EPIDERMAL MELANOCYTES IN DIFFERENT STAGES OF MITOSIS

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Abstract. The structural changes which take place in epidermal melanocytes during mitosis have been investigated in serial sections of ear skin of C57Bl mice. Melanocytes were DOPA-positive and contained melanosomes in all stages of the mitotic cycle. They retracted their dendrites in prophase so that metaphase cells assumed an almost spherical shape. Signs of new dendrite growth were seen in late telophase and fully developed dendrites first after the division was completed. It is concluded that new epidermal melanocytes are formed in the mouse by division of already differentiated melanocytes.

Key words: Melanocyte; Mitosis; Morphology

It is now clear that the epidermal melanocytes of the mouse ear constitute a dynamic cell population with an ongoing mitotic activity (8, 9). Recently, a number of melanocytes in metaphase have been identified following the injection of a stathmokinetic drug (8). These cells were all DOPA-positive, round, and filled with melanosomes. They also appeared to be entirely devoid of dendrites. Similar cells have been observed also in the rhesus monkey during wound healing and UV irradiation (3).

The morphology of these metaphase cells may indicate that melanocytes retract their dendrites when passing through the mitotic cycle. Alternatively, new melanocytes may be formed by division of certain adendritic melanocyte precursor cells. To clarify this point, melanocytes in different stages of mitosis have been reconstructed from serial sections of mouse ear skin.

MATERIALS AND METHODS

Epidermal melanocytes were studied in the skin on the dorsal side of the ears of C57Bl mice (Försvarets Forskningsanstalt, Stockholm). Only male mice, 12–13 weeks of age, were used.

One group of 4 animals was left completely untreated. Another group of 4 animals had their right ear irradiated with UVB light in order to accelerate the melanocyte proliferation rate. The left ear was covered and constituted a control. The light source consisted of a fluorescent sun lamp (Westinghouse FS) with a wavelenght spectrum of 290–350 nm. The daily UVB dose was about 0.1 joule/cm^2. A detailed description of the irradiation procedure has been given in an earlier report (8). For comparison with earlier findings, a third group of 4 animals received the same UVB irradiation and in addition an i.p. injection of vincristine sulphate (VSC; 2 mg/kg) 3½ hours before being sacrificed.

The ears were cut off at the base and skin from the dorsal side was incubated in buffered DOPA (L-3.4-dihydroxyphenylalanine) (1) and fixed in 10% formol saline or 3% glutaraldehyde. Part of the tissue was not DOPA incubated, so as to serve as a control for the DOPA reaction. After dehydration the tissue was embedded in plastic, cut into 3–5 μm serial sections and stained with haematoxylin or toluidine blue. Skin samples from the ear of 6 irradiated mice were DOPA incubated and processed as "split-skin" preparations following the original method of Staricco & Pinkus (10).

The sections were viewed through a Leitz microscope equipped with an objective of ×100 magnification. Interesting cells were reconstructed with a Leitz binocular tracing device based on the split image principle. For some cells a photomontage reconstruction was made from photomicrographs taken at different focal depths from a few neighbouring sections. AGFA-GEVAERT COPEX PAN film was used for the photomicrographs.

RESULTS

The investigation was concentrated primarily on melanocytes in animals not injected with VCS, since the intention was to describe a normal mitotic sequence. Several thousand epidermal melanocytes were examined in the search for mitotic cells. All cells considered to be melanocytes were DOPA-positive and contained large quantities of pigment granules. As a rule the melanocytes were located along the basal lamina. The overwhelming majority of the cells were in interphase, only 64 cells were in

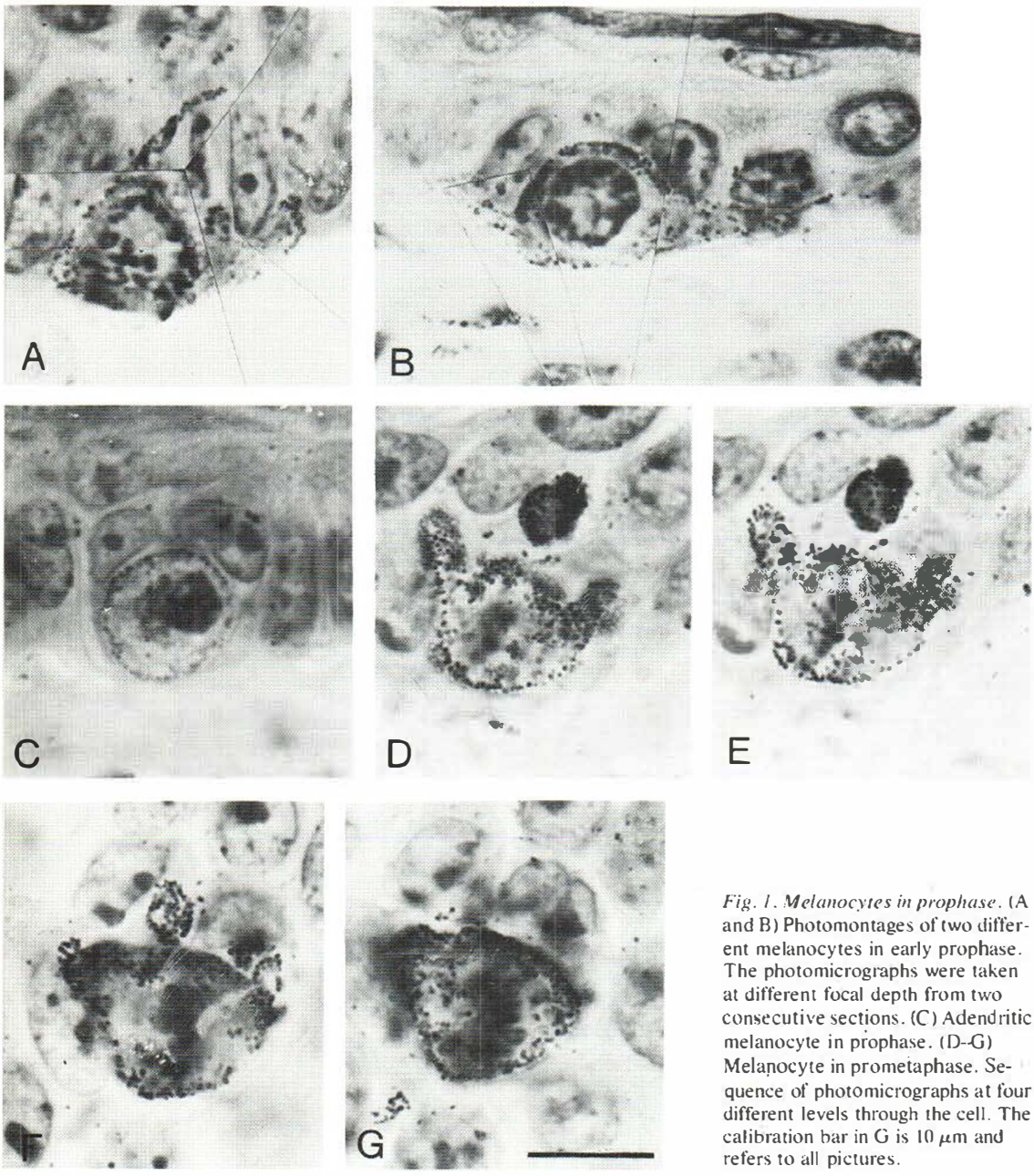

Fig. 1. Melanocytes in prophase. (A and B) Photomontages of two different melanocytes in early prophase. The photomicrographs were taken at different focal depth from two consecutive sections. (C) Adendritic melanocyte in prophase. (D–G) Melanocyte in prometaphase. Sequence of photomicrographs at four different levels through the cell. The calibration bar in G is 10 μm and refers to all pictures.

any of the mitotic stages from early prophase to late telophase.

Most of the mitotic melanocytes were found in sections from the UV-irradiated ears but 12 prophases, 8 metaphases, and 3 ana-telophase cells were seen in completely untreated animals. The melanocytes in the irradiated skin contained more melanosomes and were stained more intensely with DOPA than cells in unirradiated skin. Apart from these differences the cell morphology was the same in these two preparations, and the observations will not be described separately.

Mitotic melanocytes were more common in the skin from the VCS-injected animals and an additional 78 prophase and metaphase cells were found in sections from these mice. As VCS arrests divid-

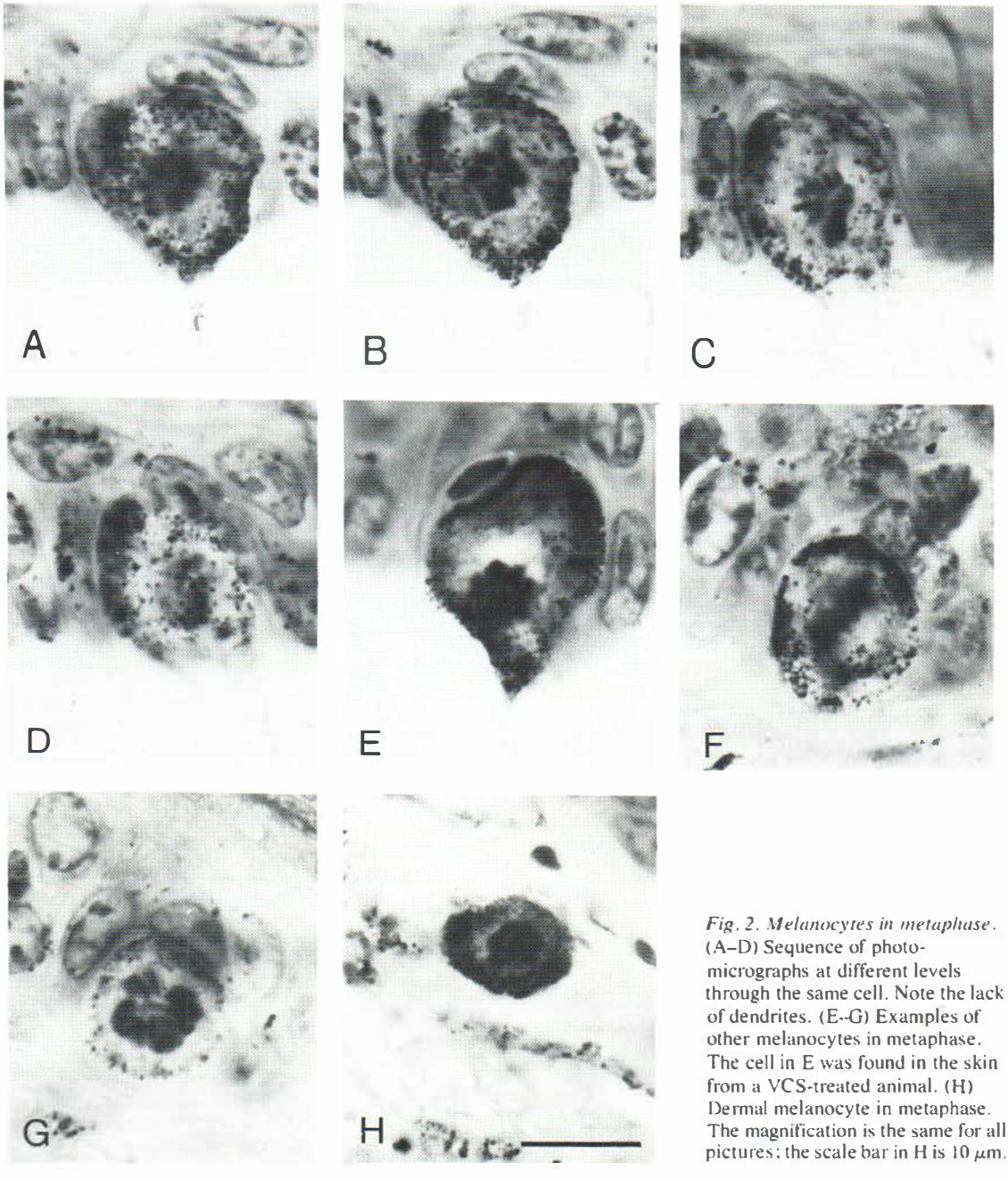

Fig. 2. Melanocytes in metaphase. (A–D) Sequence of photomicrographs at different levels through the same cell. Note the lack of dendrites. (E–G) Examples of other melanocytes in metaphase. The cell in E was found in the skin from a VCS-treated animal. (H) Dermal melanocyte in metaphase. The magnification is the same for all pictures; the scale bar in H is 10 μm.

ing cells in metaphase, no melanocytes in anaphase or telophase were observed in this material. In the VCS-treated animals the metaphase cells seemed to contain more condensed chromosomes than in non-injected animals. Otherwise, there was no apparent difference between the light microscopic morphology of the dividing melanocytes in the two groups of animals. Even so, all illustrated melanocytes with the exception of the metaphase cell in Fig. 2 E were taken from non-injected animals.

Interphase

The melanocytes in interphase had a leptochromatic nucleus. Some melanocytes in the irradiated skin

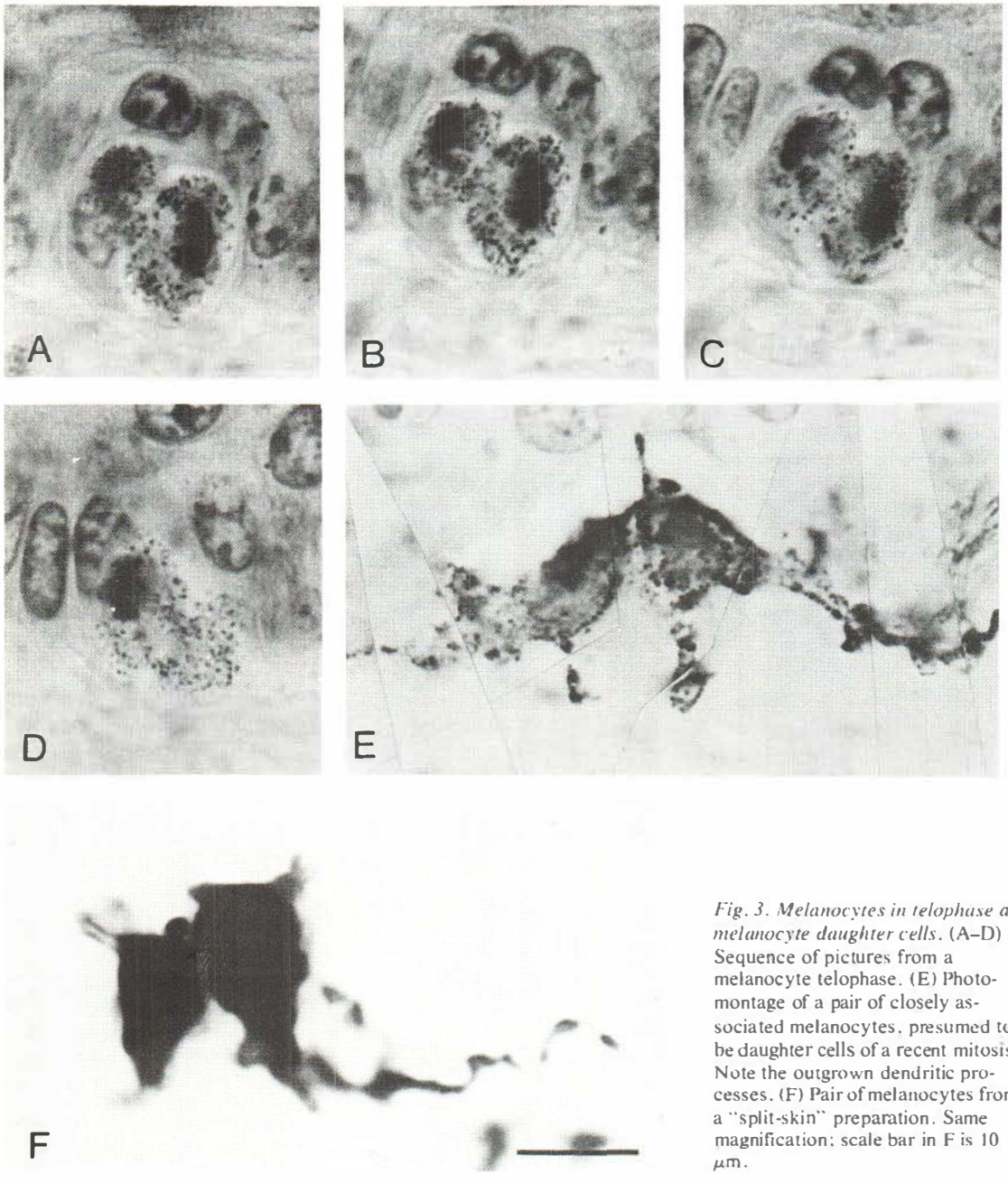

Fig. 3. Melanocytes in telophase and melanocyte daughter cells. (A–D) Sequence of pictures from a melanocyte telophase. (E) Photomontage of a pair of closely associated melanocytes, presumed to be daughter cells of a recent mitosis. Note the outgrown dendritic processes. (F) Pair of melanocytes from a "split-skin" preparation. Same magnification; scale bar in F is 10 μm.

contained more than one nucleus, while such multinucleated cells were rare in the unirradiated skin. The size of the perikaryon of individual nucleated cells was about 8×12 μm. The long axis of most cells was parallel with the basal lamina. Despite a careful histological procedure it was not always possible to prevent a slight shrinkage of the cell body of interphase melanocytes. During this stage the melanosomes were randomly dispersed in the cell body and were densely packed throughout the dendrites. Proximal dendrites were often seen already in single sections through the centre of the cell. In serial sections, two to four arborizing dendrites could be identified, reaching along the basal

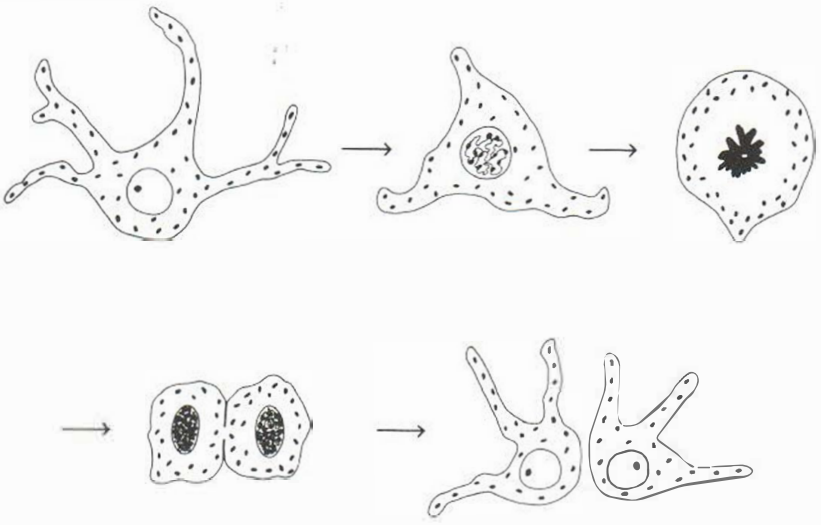

Fig. 4. Schematic illustration of a mitotic sequence of epidermal melanocytes.

lamina and up through the epidermis. The length of the dendrites varied inversely with the melanocyte population density, measuring up to 40–70 μm.

All melanocytes with a nucleus of interphase type seemed to be equipped with dendrites. In an attempt to verify this notion, 100 consecutive melanocytes in each of two unirradiated ears were carefully traced in neighbouring sections. Different landmarks such as blood vessels, hair follicles and the edges of the tissue helped us to trace the same cell in different sections. The relatively low melanocyte population density of the unirradiated mouse ear skin was an advantage in this study. Of the analysed 200 melanocytes, 3 were in the process of mitosis and 194 were interphase cells with well defined dendrites. In the remaining 3 interphase melanocytes it was not possible to identify any dendrites. Two cells could not be found in neighbouring sections even though the cells were definitely cut through the perikaryon; for the other cell, the relevant part of the next section was damaged. In view of the technical difficulties with this type of investigation, it seems reasonable to assume from these figures that there is normally no significant pool of adendritic interphase melanocytes in mouse ear skin.

Prophase

The melanocytes in prophase presented typical nuclear changes with an increase in the number and density of stainable chromatin particles and later the disappearance of the nuclear membrane (Fig. 1). Some cells in late prophase had distinguishable chromosome threads, which had started to concentrate in the centre of the cell. The transition between the prophase and the metaphase was not easy to determine in stained sections and the classification of cells in this intermediate stage was rather arbitrary.

The melanocytes in prophase had cell bodies which were somewhat larger than the average size in interphase. The melanosomes were mostly situated in the periphery of the perikaryon, so that a narrow clear zone was seen around the chromatin particles. The cells had dendrites although they were shorter and clumpier than in interphase (Fig. 1 A, B). This was especially notable for melanocytes in late prophase, which had very stubby, non-arborized processes (Fig. 1 D–G). As can be seen in Fig. 1 A and D–G, the ends of the dendritic processes were often swollen and well demarcated by an aggregate of pigment granules. An occasional prophase melanocyte was found without any detectable dendrites (Fig. 1 C).

Metaphase

In metaphase, the chromosomes were all well condensed and easily visualized in the centre of the melanocyte (Fig. 2). The cell bodies were almost spherical, with a diameter of about 15 μm, and were thus clearly larger than the perikaryons of interphase melanocytes. The shape of the melanocytes in metaphase was generally better preserved than that of interphase cells, indicating that the melanocytes in this stage were more resistant to shrinkage during the histological procedure. The cytoplasm

was slightly DOPA-stained and the melanosomes were all displaced towards the periphery of the cell, leaving a wide melanosome-free space around the chromosomes. There seemed to be considerably more melanosomes in the cell body of metaphase melanocytes than in the perikaryon of neighbouring interphase melanocytes.

As the point of focus of the microscope slowly traversed through the metaphase cells it was seen that most melanocytes at this stage were completely without dendritic protrusions (Fig. 2A–D). A few cells had a single short dendritic branch pointing laterally. In addition many melanocytes had a cone-shaped process pointing towards the basal lamina (Fig. 2A, E). It is possible that this basal process served to anchor the melanocyte in metaphase to the basal part of the epidermis.

A few dermal melanocytes were observed in metaphase. Like the epidermal melanocytes these metaphase cells were round, lacking any detectable dendritic processes (Fig. 2H).

Ana-telophase

In ana-telophase the chromosomes were separated towards the opposite poles of the melanocyte and appeared to fuse with each other. In later stages the dense, nuclear masses were surrounded by a new nuclear membrane. When the chromosomes were widely separated the cells took on a more ellipsoid shape and an equatorial constriction was sometimes visible in the centre (Fig. 3A–D). In early ana-telophase the melanosomes were still found mainly in the periphery of the melanocyte; in later telophase they were more randomly dispersed in the perikaryon. Some cells in late telophase had small lateral processes, which presumably represented outgrowing dendrites.

Daughter cells

Several melanocytes with a rather small and dense nucleus were observed close together in pairs. Most likely, such pairs of melanocytes constitute daughter cells of a recent mitosis. The cells in such pairs demonstrated different degrees of dendritic development. Some had only a few short processes, while others had well developed dendrites of typical interphase length (Fig. 3E, F). Most dendrites extended laterally from the cell. Frequently there were some dendritic processes directed up through the epidermis, but there were usually no dendrites on the side where the two cells faced each other.

The "split-skin" preparations

When the melanocyte shape in different mitotic stages was clarified, several DOPA-incubated "split-skin" preparations were scanned in search of similar cells. All cell forms identified in the sections were found in the "split-skin" preparations too. Thus, DOPA-positive cells with short stubby dendrites—like prophase melanocytes—and cells with large, spherical, adendritic cell bodies—like melanocytes in metaphase—were seen. The nuclear elements were unstained by the DOPA reaction and were seen as a clear zone in the perikaryon. Attempts to reveal the chromosome pattern by counterstaining with haematoxylin or paracarmine were unsuccessful.

The "split-skin" preparations seemed to be especially suitable for cells in telophase, and for presumed daughter cells. An example of such a cell pair is shown in Fig. 3F. The cell to the right had one long dendrite and some short processes, while the left cell had only two rudimentary dendrites (partly out of focus). Such an asymmetry in the development of the dendrites was frequently seen in similar melanocyte pairs. No attempt was made to estimate the frequency of different mitotic cells in the "split-skin" preparations, since individual cells could only tentatively be assigned to a particular mitotic category.

DISCUSSION

Epidermal melanocytes in all stages of mitosis were identified in the mouse ear skin. The melanocytes were stained with the DOPA reaction (1) and they contained large amounts of melanosomes throughout the mitotic cycle. In principle, the melanocytes seemed to follow Flemming's classical description of the mitotic sequence in vertebrate cells (2).

Characteristic of the melanocyte was the progressive change in the outlines of the cell, when passing from one mitotic stage to another. The dendrites became shorter during the prophase and most melanocytes in metaphase were round and adendritic. Rudimentary dendrites were seen again in late telophase and fully developed dendrites in pairs of closely associated interphase melanocytes. As mentioned earlier, it is assumed that such cell pairs represent the daughter cells of a recent melanocyte mitosis.

There was a certain variation in the outlined mitotic sequence. For example, some prophase

melanocytes seemed to be without dendrites, while some metaphase cells had one stubby process. Such a variability is not surprising, when considering that the traditional mitotic stages constitute an arbitrary partition of a continuous process.

The progressive change in the melanocyte structure during mitosis lends strong support to the idea that new epidermal melanocytes are formed in the mouse ear by division of differentiated melanocytes (8). The likely sequence from a mature melanocyte to two daughter cells is illustrated schematically in Fig. 4. It is reassuring that all the indicated mitotic forms are seen also in "split-skin" preparations. In the sections, there was no evidence for a significant pool of adendritic melanocytes in interphase. If present, such cells might be melanocyte precursor cells (cf. Introduction). This negative result is also in keeping with the idea that new melanocytes are formed by division of fully differentiated melanocytes.

The size of the melanocyte perikaryon increased during the early stages of the mitosis. A similar change has been observed also for other cell types. The general concept is that the increase in cell volume during mitosis is due to changes in membrane permeability and cell hydration. The synthesis of new cytoplasm seems to start first after the cytokinesis (6). With respect to the melanocytes, it is likely that the increase in the size of the perikaryon is partly caused by an addition of cytoplasm from the retracted dendrites.

The metaphase melanocytes contained more melanosomes than the cell body of interphase cells. It is suggested that even this difference is due to retraction of the dendrites, thereby contributing melanosomes to the perikaryon. The localization of the pigment granules in the periphery of the melanocyte during mitosis would prevent the melanosomes from interfering with the development of the mitotic spindle and the movement of the chromosomes. This displacement of the melanosomes outside the mitotic apparatus is in accordance with the findings for other subcellular organelles (7).

It has been suggested that melanocytes in vitro cease to produce melanin during mitosis (4, 5, 11). The present observation that melanocytes *in vivo* remain DOPA-positive and contain melanosomes during mitosis cannot be taken as an indication of an ongoing melanin production; DOPA might be oxidized non-specifically and the cells presumably discontinue their donation of melanosomes as they retract their dendrites. Thus, it is possible that epidermal melanocytes even *in vivo* undergo different stages of activity, with alternating melanin production and proliferation. This is in contrast to basal keratinocytes, which stop to divide when they differentiate.

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