

PIGMENT DONATION *IN VITRO*\*

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For many years it has been generally accepted that the formation of pigment is confined to the specialized pigment cell or melanocyte. Billingham (1) traced the development of this concept and described the appearances of melanocytes in skin, their relationship with the epidermal cell (keratinocyte) and in particular the passage of melanin granules through the cytoplasm of the epidermal cell. Masson (5) coined the term cytochrome for this activity, implying that the melanocyte was the active partner secreting its granules into another cell. The exact physical state of the membranes of each of these cells during donation has not been described, although Charles (2) has produced electron microscope photographs of a melanin granule which might be passing through a gap in the cell membrane.

In a previous study of the behavior of melanocytes and keratinocytes in culture, release of pigment granules into the culture medium and their subsequent phagocytosis was observed. In addition, cells also appeared to take part in an active pigment transfer process (3, 4). By means of time-lapse cinemicrography it has now been possible to make more detailed observations of this process.

MATERIALS AND METHODS

Cell suspensions were prepared as previously described from the epidermis of guinea pig ear and human skin, after incubation in 0.15% trypsin and cultured in special chambers (3, 4). Time-lapse cinemicrographic observations were made on selected fields using 16 mm Kodak special cinemicrographic film at 3 frames per minute.

RESULTS

It appears that transfer of pigment occurs chiefly in two ways. In the first, the end of the dendrite is approximated at right angles to the edge of the epithelial cell membrane. The end of the dendrite splays out and the cytoplasm becomes more fluid as judged by the rapid movement of the melanin granules, a clump of granules escape and are engulfed (Fig. 1). The activity of the epithelial membrane attempting to clasp the dendrite, particularly if it attempts to withdraw, is striking.

When a dendrite lies in apposition to an epithelial cell, pigment donation does not inevitably take place—the pigment cell may retract its process intact. The melanocyte may donate to more

than one epithelial cell at the same time and conversely, one epithelial cell may receive from more than one melanocyte. It is of interest that at no time has the transfer of a single granule been observed.

In the second process the dendrite lies along the surface of the epithelial cell, the granules move towards the end of the dendrite and the distal portion becomes separated off, forming a clump of granules. These similarly show rapid dancing movements and are taken into the epithelial cell as a group, probably still surrounded by a film of cytoplasm. The remaining length of the dendrite is then withdrawn rapidly. These processes, which are illustrated by prints from 16 mm film take about 1 to 2 hours (Fig. 2 a-e).

COMMENT

These observations provide direct evidence for the concept of pigment donation. The very strong impression is given that the epithelial cell is the active partner in the process—in effect removing the melanin granules from the living melanocyte. It could be, that having attached itself, the epithelial cell releases proteases which digest the cytoplasm of the dendrite prior to the “phagocytosis” process. The somewhat prolonged time required would support this hypothesis. It has been impossible to find out what factors determine whether donation will occur or not. The age of the culture did not appear to matter. The donation was, however, most frequently observed in cultures of lightly pigmented skin. The melanocytes from black skin which were very full of granules appeared less mobile, did not survive so well, and rarely donated pigment.

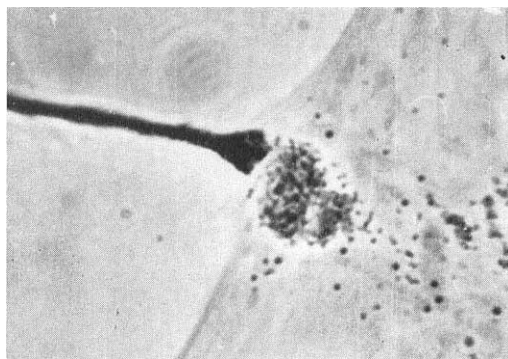


FIG. 1. Guinea pig ear skin culture—(13 days). Illustrates clump of granules passing from end of dendrite into epithelial cell.

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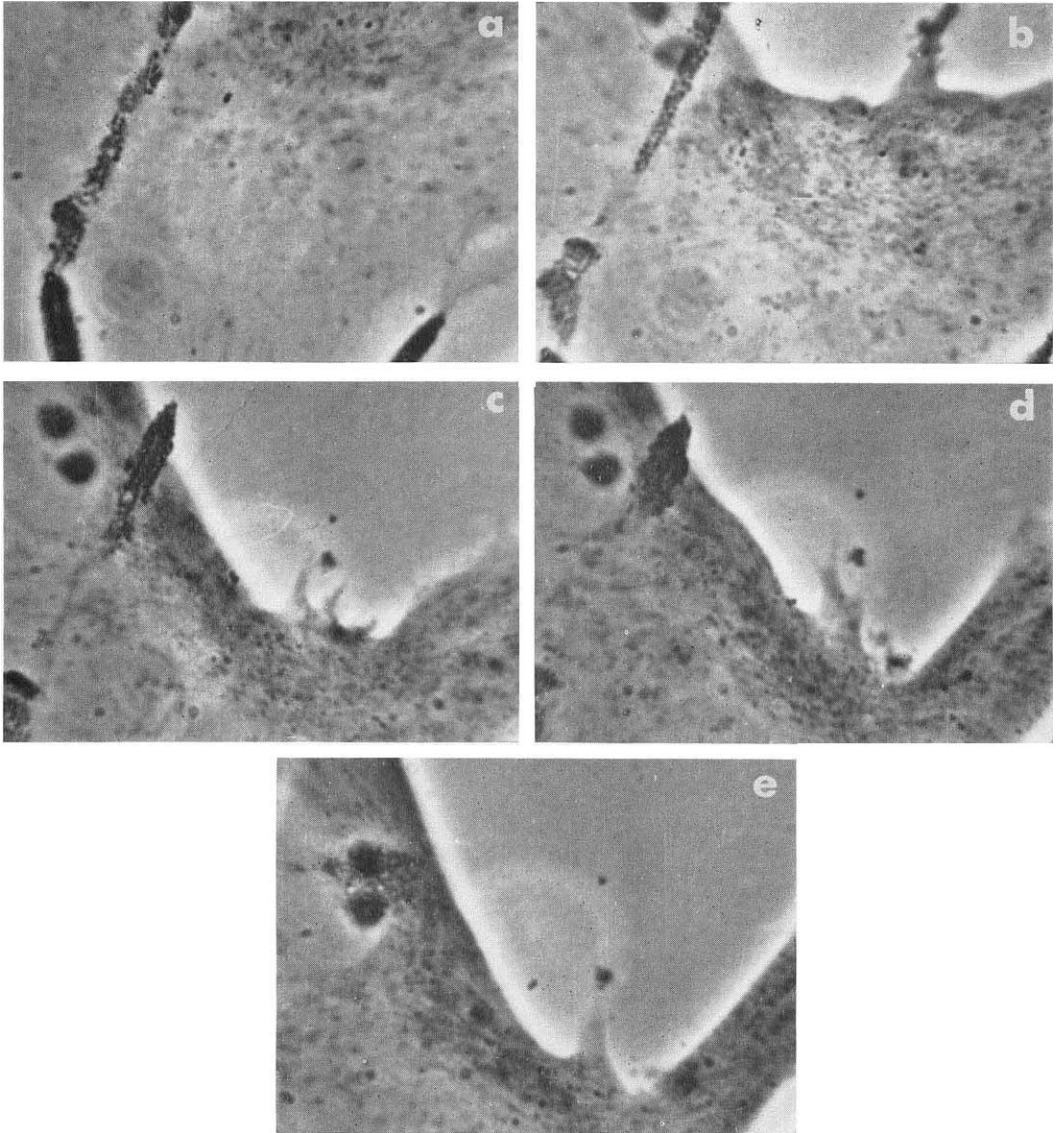


FIG. 2a-e. Guinea pig ear skin culture—(35 days). In Fig. 2a the dendrite is lying across the epithelial cell and the cytoplasm is becoming more fluid (0 mins.). In Fig. 2b the dendrite is severed (29 mins.). In Fig. 2c the nucleus of the epithelial cell appears top left (49 mins.), and in the remaining photographs the clump of granules is engulfed (d, 62 mins.; e, 76 mins.).

#### SUMMARY

The process of pigment donation *in vitro* has been studied by time-lapse cinemicrography. As judged by activity of the cell membrane the epithelial cell appeared to play a more active part. Granules were transferred as clumps rather than as individuals.

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