Melanocyte–Keratinocyte Interactions in Vivo:
The Fate of Melanosomes.

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Studies on pigment donation in tissue culture (1–5) indicate that melanosome transfer is a cytophagic process during which a portion of a melanocyte dendrite is pinched off by the epidermal cell so that melanosomes and melanocyte cytoplasm are incorporated into the keratinocyte. At the ultrastructural level, one would expect to see, at this stage, a cluster of melanosomes embedded in a cytoplasmic matrix surrounded by two membranes: one derived from the melanocyte and one belonging to the epidermal cell (Fig. 1). Although such images have not been published in reports on the electron microscopy of tissue culture (which readily reveals “cytophagocytosis”-phenomena at the light microscope level) they have been observed in in vivo specimens of hair bulbs (6), developing fowl feathers (7), and epidermis (Fig. 1). The melanosome complex, however, as it usually appears within keratinocytes, is limited by only one membrane. It has been suggested (6, 7) that the inner (the melanocytic) membrane is rapidly decomposed but since double membrane-delimited structures occur only in the cell periphery they could equally well represent cross-sectioned dendrites of melanocytes bulging into the cytoplasm of the epidermal cell (Fig. 1). Also, if the matrix of a melanosome complex represents melanocyte cytoplasm it is surprising that it never contains identifiable cytoplasmic residues, such as mitochondria, microfilaments, or similar structures. In heterophagic and autophagic vacuoles such organelles may persist for a considerable time before they are decomposed (8, 9).

The question also arises how cytophagocytosis explains the simultaneous occurrence of both melanosome complexes, which supposedly contain melanocyte cytoplasm, and dispersed melanosomes, which do not. It has been suggested that melanosome complexes break and thus release the individual melanosomes which are dispersed throughout the cell (6, 7). Accordingly, the melanosome complex is considered a precursor of singly dispersed melanosomes and this could correspond to the dispersion of melanin granules observed, at the light microscope level, in tissue culture (4). It does not, however, explain the truly uniform (complexed or dispersed) distribution patterns of melanosomes that are seen in some species,

1 Supported, in part, by Fonds zur Förderung der wissenschaftlichen Forschung, Vienna, Schering AG, Berlin and Squibb and Sons, New York.
FATE OF MELANOSOMES

FIG. 1. "Melanosome complex" surrounded by two concentric membranes (arrows). This could reflect the earliest stage after cytophagocytosis but could equally well represent a cross-sectioned melanocyte dendrite bulging into the keratinocyte. Human epidermis. ICS: Intracellular space. ×91000.

races, or individuals (10, 11, 17). Also, if aggregation and dispersion were two consecutive stages of a uniform process one would expect complexes to occur in the vicinity of melanocyte dendrites and dispersion in more distant and older cells, i.e., higher up in the epidermis. This is not the case, and even after the stimulation of pigmentation by uv light the general patterns are maintained (although melanosomes increase in number, and complexes become larger, and are more numerous in the suprabasal layers (12–14)). Finally, since large melanosomes are usually dispersed whereas small ones are complexed (10, 15–17), and since purely uniform (complexed and discrete) distribution patterns exist, it seems unlikely that all melanosomes are initially complexed and secondarily dispersed, because the size of the melanosome is determined within the melanocyte long before transfer occurs.

These discrepancies have stimulated experiments which are summarized in this paper. They were performed to determine the kinetics and fate of melanosomes once they have entered the epidermal cell.

MATERIALS AND METHODS

Black, red, white-spotted, and albino guinea pigs were used. To induce interactions between keratinocytes and melanosomes or melanosome-like particles suction blisters were produced in the animals and injected with suspensions of such particles (18). As described elsewhere in detail, the melanosomes employed were isolated from B16 mouse melanomas and C57 bi mouse hair bulbs (19); latex beads of selected sizes were used to simulate melanosomes (20). The uptake and inter-
cellular fate of these particles were followed in time-sequence studies of the regenerating epidermis at the electron microscope level. Thorotrast was employed as a marker to label lysosomes and was injected intracutaneously (21). Acid phosphatase activity was demonstrated at the electron microscope level by a histochemical Gomori-type procedure (22). The uv experiments were performed on guinea pigs kept in the dark, injected with Thorotrast, and irradiated with 10–20 MED of erythemogenic uv light delivered by a Kromayer lamp (23).

RESULTS AND DISCUSSION

I. Uptake of Melanosomes

Since the uptake of melanosomes by epidermal cells in some way involves phagocytosis we searched for phagocytosis-models which would permit a closer scrutiny of such a process.

Latex beads appeared suitable for this purpose as they can be selected to resemble melanosomes in shape and size and guinea pig epidermis was stimulated to interact with such beads in vivo (18, 20). The beads were incorporated into the cytoplasm of epidermal cells (Fig. 2) and it became apparent that their intracytoplasmic distribution depended mainly on the uptake mechanisms involved. Small beads (0.1 \( \mu \)m) accumulated on the cell surface and were engulfed in groups. The phagosomes thus formed contained aggregated, multiple particles (Fig. 2a) and preferentially moved to the perinuclear region. By contrast, large beads (0.8 \( \mu \)m) were incorporated as singles and remained singly dispersed within the cytoplasm (Fig. 2b). They were also transported to the perinuclear region (18). These distribution patterns were maintained throughout the life cycle of the cells. The selectivity of the uptake mechanisms appeared to be the decisive factor for the distribution of melanosomes and not a limitation for the potential volume of the phagosomes, since keratinocytes are perfectly capable to form, by fusion, large or even giant phagosomes which could easily accommodate multiple large beads (18, 24).

This model indicated that the uptake of formed particles into keratinocytes depends on their size and it was postulated that the uptake and distribution of melanosomes might follow similar rules (20).

Consequently, melanosomes were isolated from the anagen hair bulbs of C57 bl mice and from B16 mouse melanomas and were permitted to interact, in vivo, with albino and white spotted guinea pig epidermis (19). Large C57 melanosomes (1.3 \( \times \) 0.9 \( \mu \)m) were taken up singly by the keratinocytes and remained singly dispersed within the cytoplasm (Fig. 3). Small B16 melanosomes (0.5 \( \times \) 0.3 \( \mu \)m) were taken up in groups and remained as membrane-delimited aggregates within epidermal cells (Fig. 4). The discrete, single melanosomes and their relationship to the surrounding cytoplasm were morphologically identical with the single melanosomes which naturally occur in epidermal cells (Fig. 3b); the aggregated, small melanosomes bore all morphological features of naturally occurring melanosome complexes (Fig. 4) and, just as their natural counterparts, moved to the perinuclear region (19) (Fig. 4a). Thus, epidermal cells are capable of taking up melanosomes both as singles and as complexes. Small melanosomes form complexes whereas large melanosomes remain singly dispersed. It appears that the size of the melanosome determines the appropriate uptake mechanism and this, in turn, the distribution pattern of pigment organelles within the epidermal cell. These findings confirm and explain the results of studies on pigmented human skin in which
Fig. 2. Two different distribution patterns of latex beads (arrows) which were taken up by epidermal cells in vivo. The small beads were engulfed as a group (2a) and occur as a membrane delimited aggregate within the cytoplasm. The large beads were taken up singly and are singly dispersed in the perinuclear region (2b). Guinea pig epidermis. N: nuclei ×26000.
FIG. 3. Uptake of C57 bl mouse melanosomes into albino guinea pig keratinocytes in vivo. Note that these large melanosomes are taken up singly (3a) and remain singly dispersed within the cytoplasm (3b). ECS: extracellular space. N: nucleus. ×26000.

the size of melanomas was correlated with their distribution pattern and in which it was observed that large melanosomes tend to be singly dispersed whereas small ones occur in the aggregated form (16, 17).
Fig. 4. B16 mouse melanoma melanosomes within the cytoplasm of albino guinea pig keratinocytes in vivo. These small melanosomes were taken up in groups forming membrane-delimited aggregates (arrows) within the cytoplasm. They are indistinguishable from the melanosome complexes which naturally occur in pigmented epidermis. N: nucleus. 4a: ×26000 4b: ×45000.

These experiments utilized model systems and, therefore, cannot claim to reflect the entire, complex, melanosomal transfer in vivo. They do, however, disclose several mechanisms which may well be operative in the in vivo situation. Surprisingly, they indicate that melanosomal complexes can be formed in a system which bypasses the melanocyte and which thus excludes the cytophagocytosis of melanocyte dendrites. The possibility should, therefore, be considered that the cellular canni-
balism (4) of cytophagocytosis, which by itself represents a unique phenomenon, may not be the only answer to the transfer problem in vivo.

II. The Fate of Melanosomes within Keratinocytes

After transfer is completed single and aggregated melanosomes can be shown to be associated with acid hydrolase activity (9, 13, 25–29). This has been taken to suggest that they are contained within lysosomal structures (9, 13, 26–29) which derive their acid hydrolases from primary lysosomes transferred to them from the

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**Fig. 5.** (a) Secondary lysosomes (L) of guinea pig keratinocyte labeled by Thorotrast ×26000. (b) Lysosomes and melanosome complexes have fused and the complexes are labeled by the lysosomal marker (arrows). Guinea pig epidermis injected with Thorotrast. ×26000.
Golgi region (29). To substantiate this hypothesis we have induced the formation of secondary lysosomes in keratinocytes in vivo and have labeled them with an electron microscopic tracer (30) (Fig. 5a). Lysosomes are known to interact with each other and such interactions were regularly observed between labeled lysosomes and melanosome complexes and/or single melanosomes within keratinocytes. Lysosomes fused with and spilled the lysosomal marker into melanosome complexes and/or single membrane delimited melanosomes (Fig. 5b). Labeled melanosome complexes were thus obtained. Since fusions occur only between like organelles, the observed fusions between melanosomes and lysosomes were considered additional evidence that melanosome complexes belong to the lysosomal system.

Less frequently, interactions were also noted between melanosomes and melanosome complexes labeled by the tracer: fusions of single melanosomes occurred, generating new melanosome complexes, and it was even possible to observe the shifting of melanosomes from one lysosome to another (Fig. 6). It is difficult to quantitate such phenomena with the electron microscope but, whereas fusions between melanosomes and lysosomes occurred so frequently that they appeared to

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**Fig. 6.** Interactions of two heavily labeled melanosome complexes which contain melanosomes in various stages of melanization (M). The complexes 1 and 2 have fused at the point marked by the arrow and a melanosome (asterisk) is about to cross over from 1 to 2. Guinea pig epidermis injected with Thorotrust. ×91000.
be the rule (Fig. 5b), interactions between preexisting melanosome complexes were rare and were seen only after a massive stimulation of the phagosomal apparatus of keratinocytes by the injection of large amounts of the tracer. This indicates that although the general distribution patterns of melanosomes are maintained throughout the lifespan of these cells, a small proportion of melanosomes can cycle from a dispersed to complexed state, and may even cycle between different complexes.

The lysosomal nature of the vacuoles containing aggregated or single melanosomes implies that the melanosomes should be subject to degradation. Such degradation does take place and can best be demonstrated in melanosomes labeled by the tracer which permits their identification even after they have lost their characteristic morphology (30); their characteristic outlines disappear, they are fragmented and broken up to a granular or amorphous debris (Fig. 7). The decrease of the number of melanosomes which is observed as one proceeds from the basal layer of the epidermis to the str. corneum is thus not only owing to a dilution effect by cell division and the horizontal flattening of horny cells but also (and perhaps most importantly) to a true degradation of melanosomes. Thus, it appears that it is the natural fate of melanosomes to be degraded within the epidermal cell.

This also applies to the single, discrete melanosomes and is found in black guinea
Fig 8. (a) Guinea pig epidermis, 5 days after the labelling of melanosome complexes. There is no diffuse tracer within the granular cell and even in the str. corneum the tracer forms well defined aggregates some of which still contain identifiable melanosomes (M). K: keratohyalin. ×26000. (b) Higher magnification of a melanosome complex within the str. corneum, 4 days after labelling. Partly degraded melanosomes (M) can be recognized which are associated with the tracer. There is no spilling of tracer into the matrix of the horny cell. ×91000.

pigs and, in humans, in Negroids. In the latter, however, the number of nondegraded melanosomes found in the str. corneum is much higher than in less-pigmented species and races (31) and, at present, we do not know whether this is solely because of the established higher rate of melanosome production or to a
reduced degrading activity. Preliminary results suggest that lysosomal activity does not differ between Caucasoids and Negroids.

If melanosomes are broken up in lysosomes the question arises what happens to the melanin which is known to be resistant to enzymatic attack. It has been suggested that melanosome dust (the final product of melanosome degradation) is spilled into and dispersed within the cytoplasm of epidermal cells. In such a dispersed state it could provide a protective screen of high effectiveness against uv irradiation. Unfortunately, this hypothetical melanosome dust is not identifiable with electron microscope techniques and indirect approaches have to be used to examine this problem. The labeling of melanosome complexes with Thorotrast (30) reveals that although lysosomes do occasionally lyse within the epidermis the membranes of the melanosome complexes usually do not break in sufficient frequency to account for a release of appreciable amounts of melanosome dust into the cytoplasm. If this were a phenomenon which occurs regularly within the epidermis the labeled melanosome complexes should spill the lysosomal marker which should be found diffusely within the cells of the upper epidermal layers. This does not occur (Fig. 8a); the membranes of single and aggregated melanosomes remain intact and, with rare exceptions, the tracer also remains membrane bound. Even in the str. corneum it is confined to well defined focal aggregates
or is associated with foci of partly degraded melanosomes surrounded by the remnants of a membrane (Fig. 8). Thus it appears unlikely that a dispersion of melanosome dust occurs in the viable layers of the epidermis.

After irradiation with uv light the envelopes of some melanosome complexes do break and spilling of the tracer into the cytoplasm occurs (Fig. 10). But this is seen to a sufficient degree only in cells which already shows signs of cytoplasmic damage several hours after 10–20 MED of uv-irradiation (23); it thus appears to be a secondary phenomenon which accompanies uv injury of the epidermis. This parallels the behavior of other, "ordinary," epidermal lysosomes which also show a significant amount of lysis only after cytoplasmic damage has become apparent (23). In the early stages after irradiation the labeled melanosome complexes and melanosomes appear unaltered even in the presence of incipient cytoplasmic damage and are thus just as intact as those of unirradiated controls (Fig. 9).

All this indicates that throughout its life span within epidermal cells, melanin remains membrane-delimited and that, at least in the viable layers of the epidermis, it exerts its protective effect while it is confined to the melanosome or the melanosome-degradation products surrounded by their respective membranes.

SUMMARY

The uptake of melanosomes into keratinocytes appears to be a size-dependent phenomenon which determines the overall distribution patterns of melanosomes within epidermal cells. Large melanosomes are taken up as singles and remain singly dispersed whereas small melanosomes are engulfed as multiples and form aggregates (melanosome complexes). Although the general distribution patterns are maintained a small proportion of melanosomes cycles from a dispersed to complexed state, and vice versa, and also from one melanosome complex to another.

Aggregated and discrete melanosomes of keratinocytes are contained within lysosomal membranes. As keratinocytes differentiate and move towards the str. cor-
neum melanosomes are broken up and degraded but the decomposition products remain within the confines of their delimiting membrane and, at present, there is no evidence for a dispersion of "melanosome dust." Such a spilling of melanosome debris may occur after uv irradiation but only after considerable cytoplasmic damage has occurred.

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