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ARE MELANOSOME COMPLEXES LYSOSOMES?*

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ABSTRACT

The formation of secondary lysosomes was induced in keratinocytes of guinea pig skin. Lysosomes labeled by an electron microscopic tracer (Thorotrast) were observed to fuse with and spill their contents into melanosome complexes and membrane bound single melanosomes within keratinocytes. Since fusions occur only between like or functionally related organelles it is concluded that melanosome complexes and single melanosomes within keratinocytes represent secondary lysosomes. This is supported by the observation that these structures contain acid hydrolases and that melanosome degradation takes place in the labeled melanosome complexes.

Fusions were also observed between labeled and non-labeled melanosome complexes and single melanosomes, respectively, which suggests that, in a given population of melanosome complexes, not all are complexed during pigment transfer but that some are formed *de novo* within keratinocytes as the product of fusion phenomena.

Melanosomes within the cytoplasm of keratinocytes are always surrounded by a single membrane (1, 2). Depending on the species and race (3) they either occur singly or in groups and the term "melanosome complex" has been used for the compound structures (4). Since it is held that melanosomes are transferred to keratinocytes by a phagocytic process (5, 6, 7) the melanosome complexes are, by definition, analogous to the phagosomes of phagocytic cells. They contain acid hydrolases (1, 2, 8–11) and Olson *et al.* (12) have shown that ferritin injected into the skin can be reidentified within the confines of ther membrane. These findings have been interpreted to indicate that melanosome complexes belong to the lysosomal system (1, 2, 9, 12). The present investigation was performed to test this hypothesis.

The rationale of our experiment was to induce heterophagy and thus the formation of secondary lysosomes within keratinocytes and to label the newly formed lysosomes with an electron microscopic tracer. Thorotrast was chosen for this purpose as it is an inorganic moiety which cannot be decomposed by the cells; it thus represents a long term label which provides the opportunity to study the entire life cycle of lysosomal organelles (13). Since lysosomes are known to interact with each other (14) it was anticipated that interactions between labeled lysosomes and melanosome complexes would provide further information on the relationship between the two cell components.

MATERIAL AND METHODS

Three red and four black guinea pigs were used in these studies. Aliquots of 0.1 ml of Thorotrast (a colloidal solution of thorium dioxide stabilized with dextrin; obtained from Fellows Testagar, Inc., Detroit, Mich.) were injected intracutaneously into multiple sites on the backs of the ears and shave biopsies of the injected sites were performed after 5 min., 1 hour, 3, 12, 24, and 48 hours, and 3 and 4 days, respectively. The specimens were minced in a formaldehyde-glutaraldehyde fixative (15), fixed 5 hours at room temperature and rinsed overnight in three changes of 0.1 M cacodylate buffer, pH 7.2. They were postfixed 1½ hours in 3% osmic acid in distilled water, "stained" en bloc in 0.5% uranyl acetate in veronal acetate buffer, pH 7.3, dehydrated rapidly in alcohols and embedded in Epon 812. Ultrathin sections were cut with glass knives on LKB Ultrotome III and Reichert OM U2 ultramicrotomes, contrasted with lead citrate or uranyl acetate followed by lead citrate and examined with a Zeiss EM 9S electron microscope.

Cytochemistry. Thin strips of tissue (0.5 × 0.5 × 3.0 mm) were fixed in the paraformaldehyde-glutaldehyde fixative for three hours at room temperature and washed in buffer as described above; nonfrozen 50 micron sections were cut with a Smith and Farquhar tissue chopper and incubated 1 hour at 35° C in a Gomori-type incubation medium for the demonstration of acid phosphatase (16). The controls included omission of the substrate from the medium, incubation of sections pretreated with 0.1 m sodium fluoride and of heat inactivated sections. After the cytochemical incubation the sections were post-fixed and embedded according to the procedure described above.

RESULTS

Morphology. In red guinea pig epidermis more than 50% of the melanosomes within keratinocytes were confined to melanosome complexes comprising 3 to 7 or more individual melanosomes. A considerable number of melanosomes were also found to be dispersed singly within the keratinocyte cytoplasm but they were always surrounded by a single membrane. In the black animals most melanosomes were singly dispersed

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within the keratinocytes and the melanosome complexes observed contained only up to five individual melanosomes. In the black animals all melanosomes were heavily melanized, they appeared larger, and were by far more numerous than those of red skin. Since the observations to be described below were essentially the same in the red and black animals they will be discussed together.

Tracer studies. Immediately after injection the tracer permeated the intercellular spaces of the epidermis and the first signs of uptake into the cells were apparent after one hour. This process has been described elsewhere in detail (13). As has been our experience with Thorotrast (13) and other tracers (9, 17) a considerable number of lysosomes were formed which were labeled by the tracer.

Cytochemistry. Acid phosphatase was demonstrated in lysosomes, Golgi cysternae and Golgi vesicles and within the confines of the delimiting membranes of both melanosome complexes and singly dispersed melanosomes of keratinocytes (Fig. 1). This confirms the results of previous investigations (1, 2, 9).

The following observations appear pertinent to the aim of our study: Interactions between labeled lysosomes and membrane delimited single and compound melanosomes were regularly and frequently observed both in the basal and suprabasal layers of the epidermis. Lysosomes fused with and spilled their contents into the melanosome complexes or membrane delimited single melanosomes and composite structures were formed which contained melanosomes, the lysosomal marker and, occasionally, vesicles and residues of other organelles. Figures 2 and 3 illustrate these events.

Another finding concerns the fate of melanosomes within these composite structures, as observed within the suprabasal layers of the epidermis during the advanced stages of the labeling process (i.e. 12 hours after the injection of Thorotrast and thereafter). The composite fusion products still contained Thorotrast which permitted their identification; the melanosomes, however, gradually lost their morphologic characteristics, they appeared fragmented, or were replaced by a granular or amorphous debris. The membranes of these structures invariably remained intact even

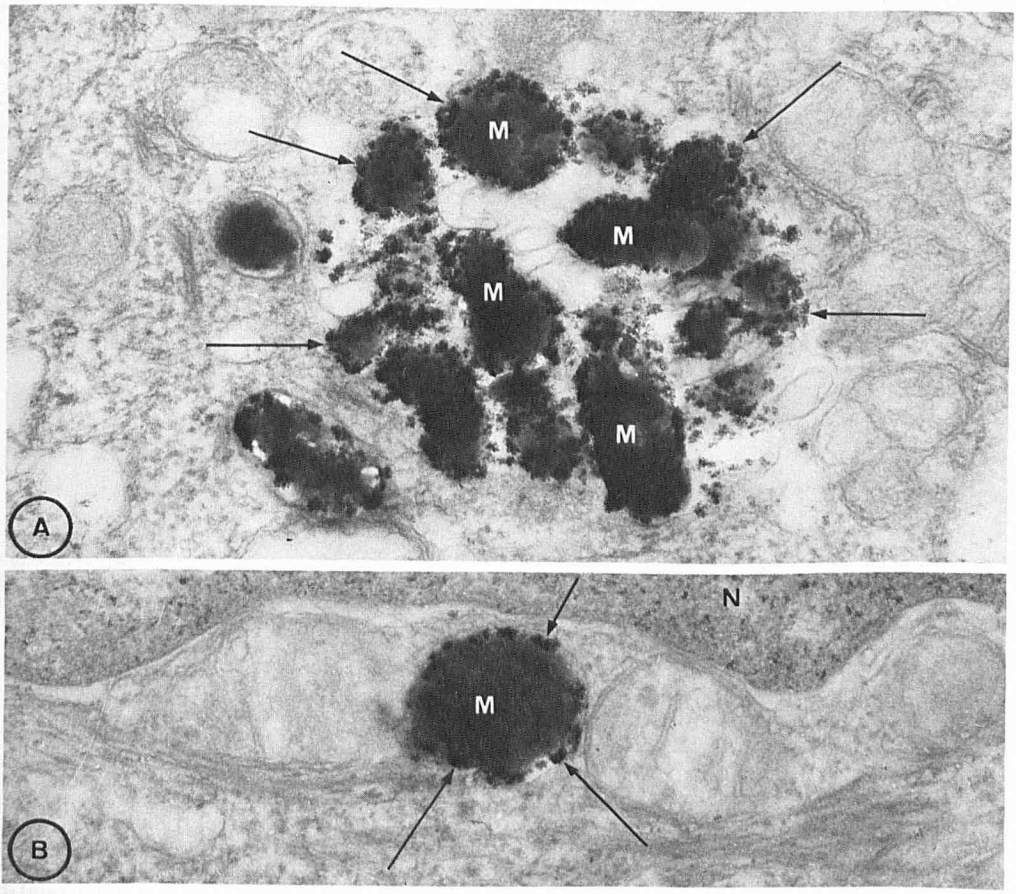

FIG. 1. Demonstration of acid phosphatase activity within a melanosome complex (A) and a single melanosome (B) of guinea pig keratinocytes. The arrows denote the electron dense reaction product which indicates enzymatic activity. M: melanosomes, N: nucleus. Red guinea pig. A and B × 49,500.

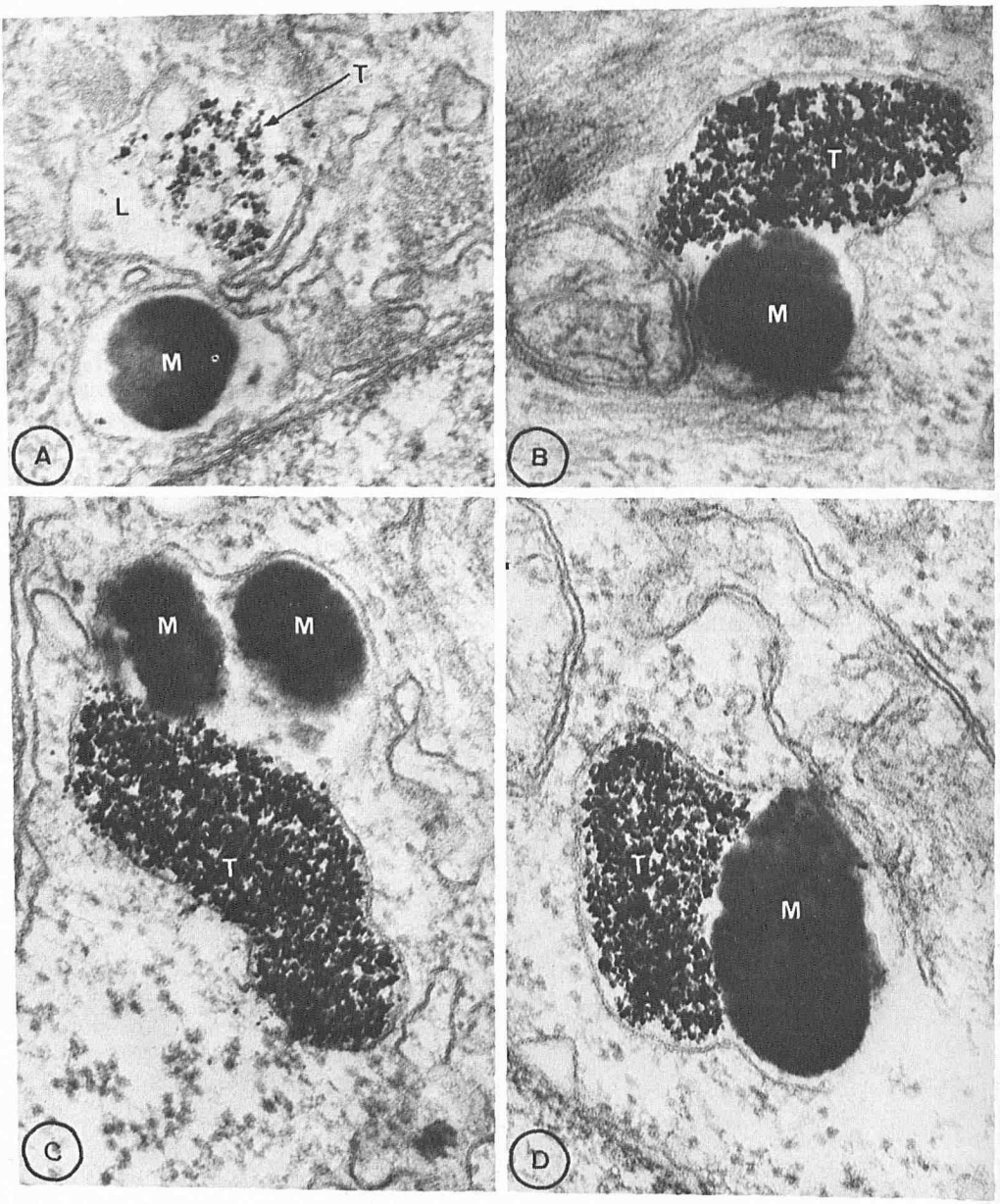

FIG. 2. Electron micrographs showing fusions between labeled lysosomes and single or complexed melanosomes (black guinea pig). In (A) a lysosome (L) containing Thorotrast (T) is seen in close proximity of a membrane delimited melanosome (M) within a keratinocyte. This could represent a stage immediately prior to fusion of the two organelles. In (B), (C), (D) fusion has occurred and composite structures have been formed which are surrounded by a common membrane and contain melanosomes (M) and the tracer (T). Note that at this stage, there is still a polarity in the distribution pattern of melanosomes and the tracer and that the original outlines of the fused organelles (i.e. lysosome and membrane delimited melanosome) are still maintained. (A)–(D) × 91,200.

in the uppermost layers of the viable epidermis and spilling of Thorotrast into the cytoplasm was not observed. Figure 4 documents this process. Images like these show that melanosomes are degraded within the confines of the membranes which delimit compound and single melanosomes within keratinocytes. However, not all melanosomes were decomposed and in the black animals, pigment granules still associated with the lysosomal marker were observed within the *stratum corneum (Fig. 5).*

The third finding of significance concerns interactions between single and compound melanosomes. Both were frequently seen in relationship to each other and images were observed which suggested fusions between Thorotrast-labeled and

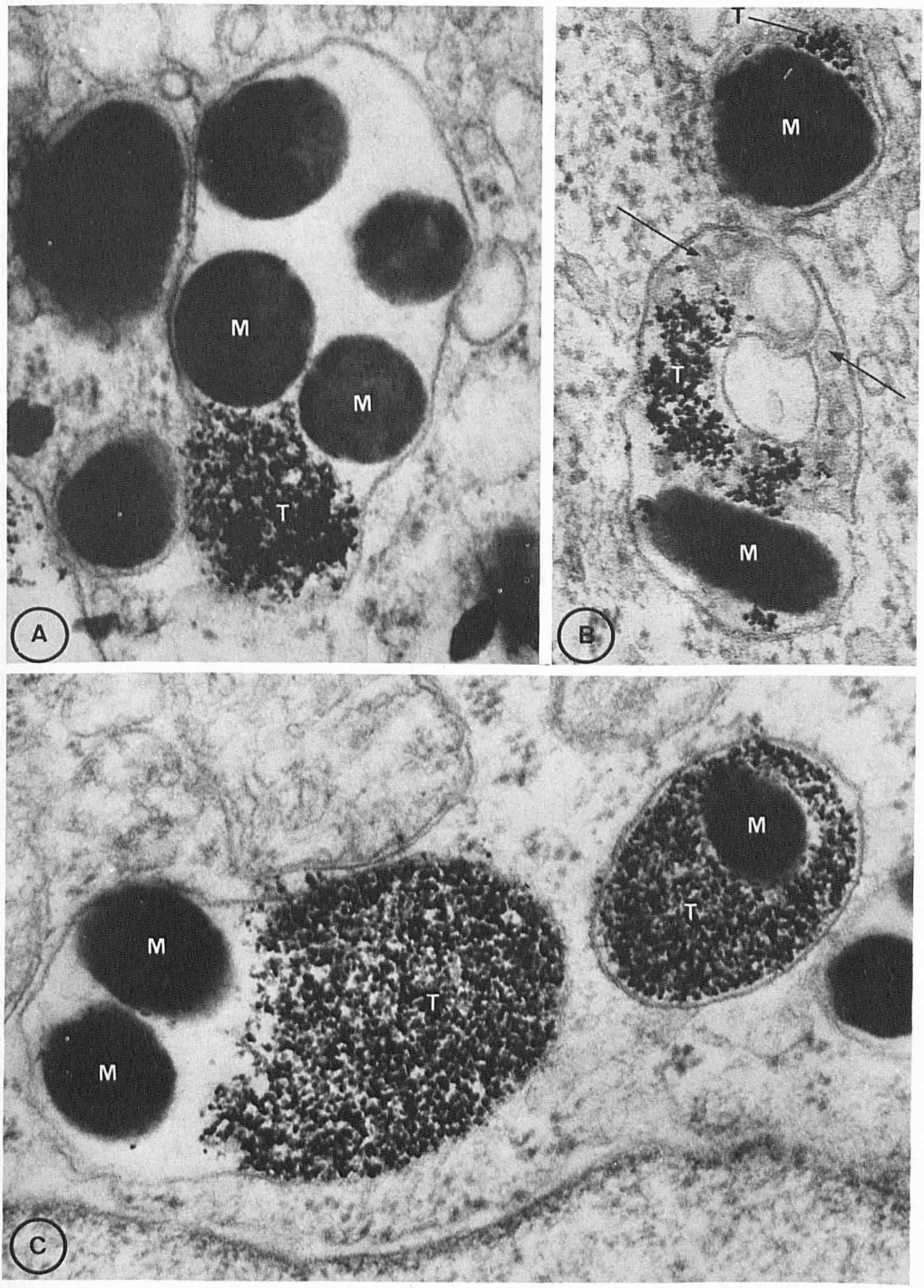

FIG. 3. Composite structures formed by fusion of lysosomes with melanosome complexes or single melanosomes. These labeled melanosome complexes contain both melanosomes (M) and the tracer (T). In (B) the composite structure also contains small vesicles (arrows). The outlines of the two original components are no longer evident and the polarity in the distribution of the tracer and melanosomes is disappearing (C, right). (A)–(C) × 91,200.

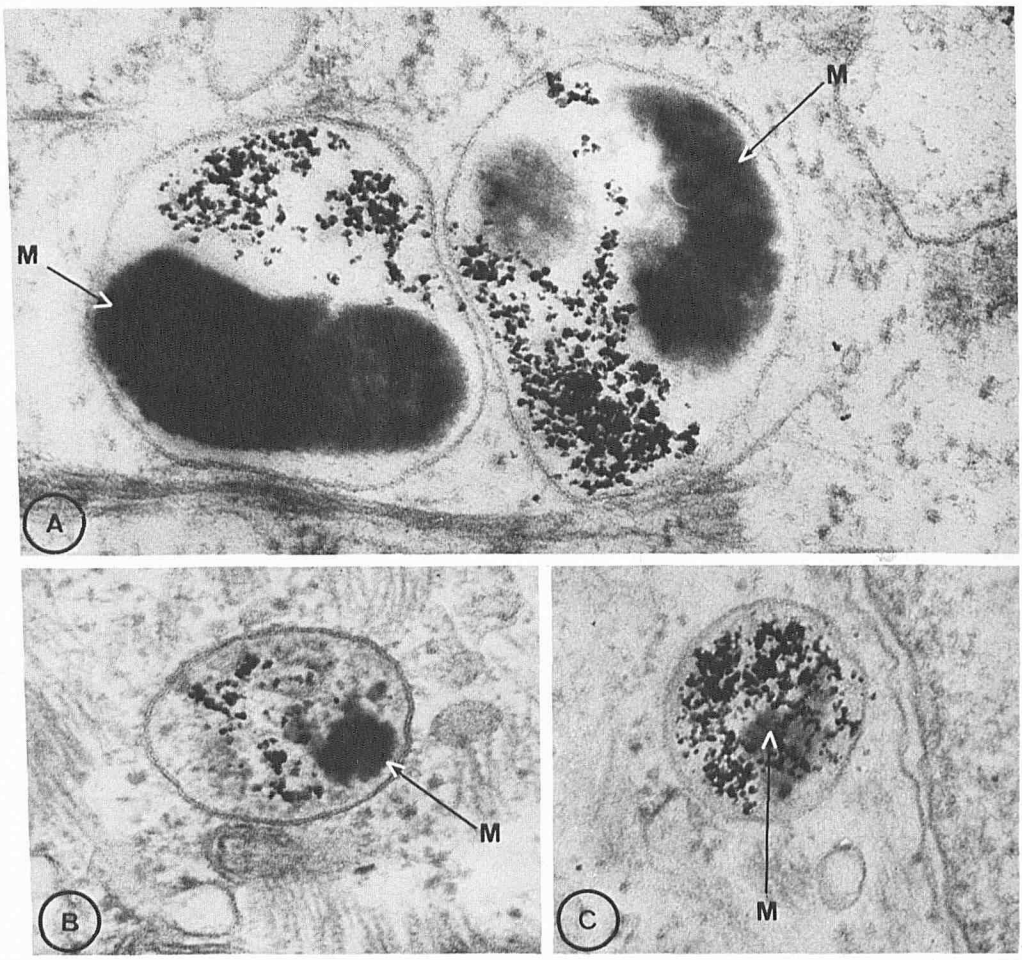

FIG. 4. Different stages of melanosome degradation within labeled complexes. The organelles retain an intact membrane and the tracer remains (optically) unaltered. The melanosomes (M), however, progressively loss their outlines and morphology. Upper spinous layers. (A): 24 hr, (B) and (C): 48 hr biopsy specimens. × 91,200.

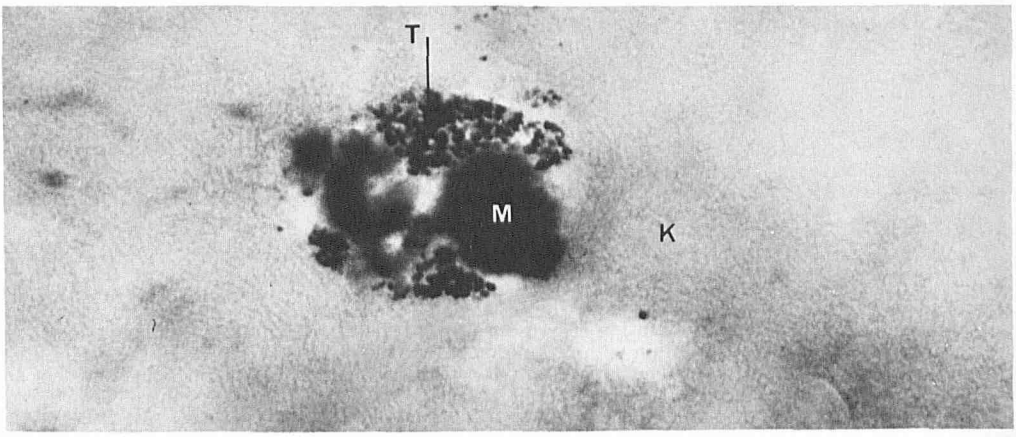

FIG. 5. Non-degraded melanosomes within the stratum corneum, 3 day biopsy specimen, black guinea pig. Note that, although the delimiting membrane is no longer visible, the tracer is still associated with the melanosome fragments (M). K: keratin pattern of the horny cell. × 91,200.

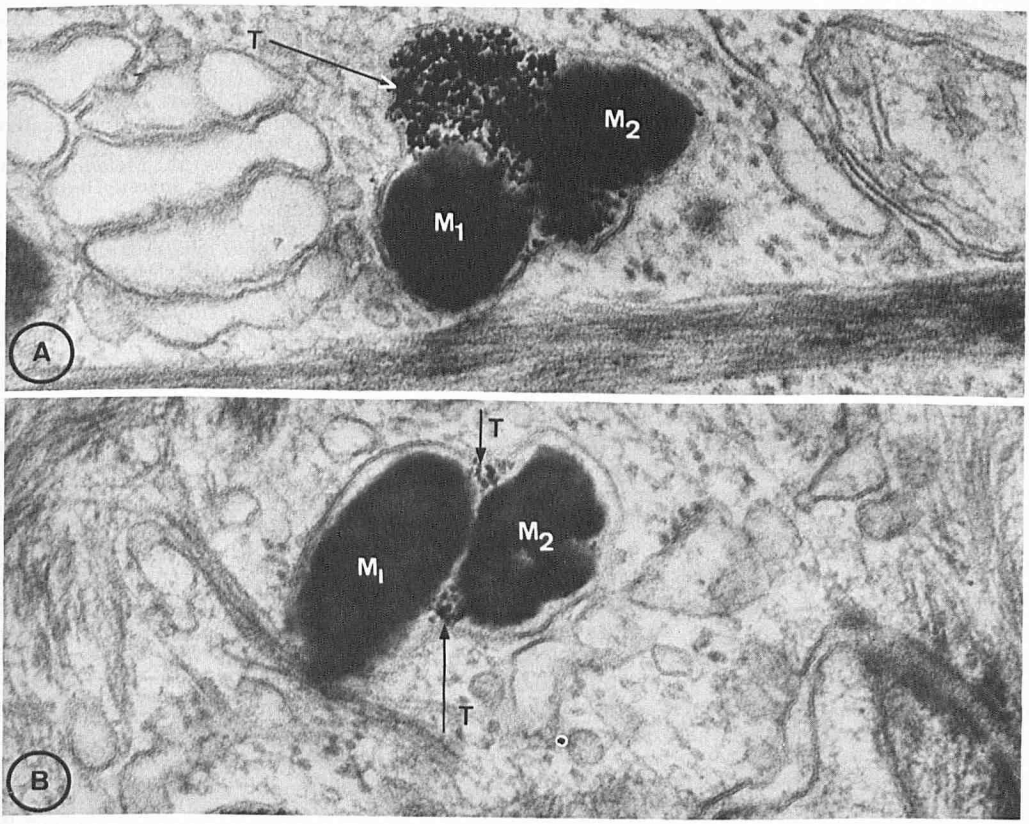

FIG. 6. Electron micrographs suggesting fusions of labeled and non-labeled (or labeled) single melanosomes within guinea pig keratinocytes M: melanosomes, T: tracer. × 91,200.

non-labeled melanosome complexes and single melanosomes, respectively (Fig. 6). This indicates that, in a given population of melanosome complexes, not all have been complexed from the beginning but that some are formed do novo as the product of fusion phenomena.

DISCUSSION

The implications of our findings are threefold: Since fusions occur only between like or functionally related organelles (18) and since labeled lysosomes and melanosome complexes were observed to fuse it can be concluded that lysosomes and melanosome complexes belong to the same class of organelles. Taken together with the fact the melanosome complexes and single melanosomes within keratinocytes contain acid hydrolases (1, 2, 8, 9, 10, 11) and that they incorporate injected ferritin (12), it can be further concluded that they belong to the lysosomal system and represent secondary lysosomes. We assume that acid phosphatase and other lysosomal hydrolases are transferred to them by primary or secondary lysosomes through similar fusion phenomena. This is also suggested by the localization of the acid hydrolase within the complexes as has been discussed elsewhere in detail (2).

If melanosome complexes and single melanosomes of keratinocytes are lysosomal structures they should represent the sites of melanosome degradation. Recent studies have shown that melanosomes from mouse melanomas are decomposed by liver lysosomes *in vitro* (19) and in our experiment such a degradation was observed in melanosome complexes labeled by the tracer. This, however, indicates that the melanosome complex, *per se*, may represent the final stage in the life span of melanosomes within keratinocytes. Support for this interpretation is also derived from the observation that non-digested melanosomes were still associated with the lysosomal marker within complexes in the stratum corneum. Our conclusion does not support the view that melanosome complexes *always* represent the earliest intracellular stage after melanin transfer is completed, that they are subsequently broken up and that, eventually, melanosomes are dispersed singly within the cytoplasm (5, 7, 20). Our findings do show that within the viable epidermis, transferred melanosomes always remain delimited by a membrane, whether they are digested or not, and that melanosome complexes do not break to release the decomposition products of melanosomes (i.e. "melanosome-dust") into the cytoplasm. If this were a phenomenon occurring regularly within the epidermis the lysosomal

marker should be spilled into the cytoplasm and this was not the case. In this context it appears important to stress that, by referring to digestion, we are not implying that melanin, as chemical compound, is degraded but that the melanosome, as a cell particle, is broken down into smaller units.

The observation of fusions between membrane-limited melanosomes suggests that the presence of melanosome complexes does not exclusively indicate a melanosome transfer in bulk but that melanosome complexes may also arise *de novo* within keratinocytes by the fusion of melanosomes which had been taken up individually; This appears particularly interesting in view of the differences that exist in the complex formation and distribution of melanosomes within keratinocytes in different races (3). There is now good evidence that these different distribution patterns depend on the sizes of the individual melanosomes (21, 22).

It is evident that the findings, presented in this paper raise several points which are somewhat at variance with some of the current views on the uptake, intracellular fate and degradation of melanosomes within keratinocytes. They suggest that the mechanisms involved in these processes are more complex than has been realized so far.

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