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AN ELECTRON MICROSCOPIC STUDY OF MELANIN IN THE HAIR AND HAIR FOLLICLES*

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ABSTRACT

The distribution of melanin pigment in all black guinea-pig hairs and anagen hair follicles was studied with the electron microscope. Melanocytes were identified in the upper part of the hair bulb, the outer root sheath and in the dermis adjacent to the follicle. In the immature medullary and cortical cells large numbers of melanosomes were seen among the other cytoplasmic structures. The cuticle cells contained a few melanosomes. As the different cell lines matured and underwent keratinization the melanosomes became embedded in the keratin. In the cortical cells the melanosomes were surrounded by a dense amorphous material which at many sites was attached to the inner surface of the plasma membrane. No melanin was seen in the cells of the inner root sheath. In the upper part of the outer root sheath the distribution of melanin closely resembled that found in the epidermis. In the lower part of the sheath the majority of the cells contained only a small amount of melanin pigment.

The fine structure of melanocytes in the hair follicles was first examined by Birbeck, *et al* (1). Since that time other investigators have studied melanocytes at this site and have contributed much to our understanding of the process of melanogenesis and the donation of the melanin pigment to the hair cells in the hair bulb (2, 3, 4, 5). The subsequent fate of the melanosomes once they enter the hair cells has, however, received scant attention. The purpose of this report is to trace, in an anagen hair, the melanin pigment from the region of the hair bulb to the emergent hair. A brief description of the melanin pigment in the cells of the outer root sheath is also included.

MATERIALS AND METHODS

Skin biopsies were taken from the scalp area of black mature male guinea-pigs. The specimens were immersed immediately in cold 6.25% glutaraldehyde in 0.1 M cacodylate buffer and were trimmed under a dissecting microscope. The specimens were then placed in fresh cold glutaraldehyde for two hours. The skin fragments were transferred to ice cold 1% osmium tetroxide buffered to pH 7.4 with veronal acetate buffer and further fixed for one hour. The tissues were dehydrated in ethanol and embedded in epoxy resins according to the method of Luft (6). Additional cut hairs were individually fixed in osmic acid for two hours prior to embedding in epon. Serial transverse sections of hair follicles and hairs were cut with an L.K.B. Ultratome, using a diamond knife and "stained" with uranyl acetate and lead citrate. In this study an R.C.A. E.M.U. 3F Electron Microscope was used.

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RESULTS

For purposes of description the findings in anagen follicles will be described at four planes of transverse section: 1) The follicle at the level of the upper part of the bulb, 2) The follicle above the bulb but below the exit of the sebaceous gland, 3) The follible above the level of the sebaceous gland, 4) The mature hair.

Plane 1. The melanocytes were found in large numbers in the bulb over the upper half of the dermal papilla. They were easily recognized since they had dendritic processes, desmosomes were absent, few filaments were present in the cytoplasm and numerous melanosomes were seen at different stages of development scattered throughout the cytoplasm. The dendritic processes of the melanocytes were seen between the cells of the upper bulb in the gaps between the plasma membranes.

Small groups of melanosomes were found in the neighboring bulb cells. Occasionally a group of melanosomes was seen to be surrounded by a plasma membrane.

Plane 2. At this level the bulb cells were already differentiated into those of the medulla, cortex and cuticle of the hair. The cells of the three layers of the inner root sheath, namely the cuticle, Huxley's layer, Henle's layer were identifiable. In the peripheral part of the follicle the cells of the outer root sheath could be recognized.

Medulla of hair. Smooth surfaced melanosomes either occurring singly or in small groups were seen lying free between the irregular shaped electron dense cytoplasmic granules found characteristically in these cells (Fig. 1). Many ribosomes, some smooth or rough surfaced vesicles, and a few filaments were also noted in the cytoplasm. In the more mature cells of this region the melanosomes and other cytoplasmic organelles were seen to be compressed together by the en-

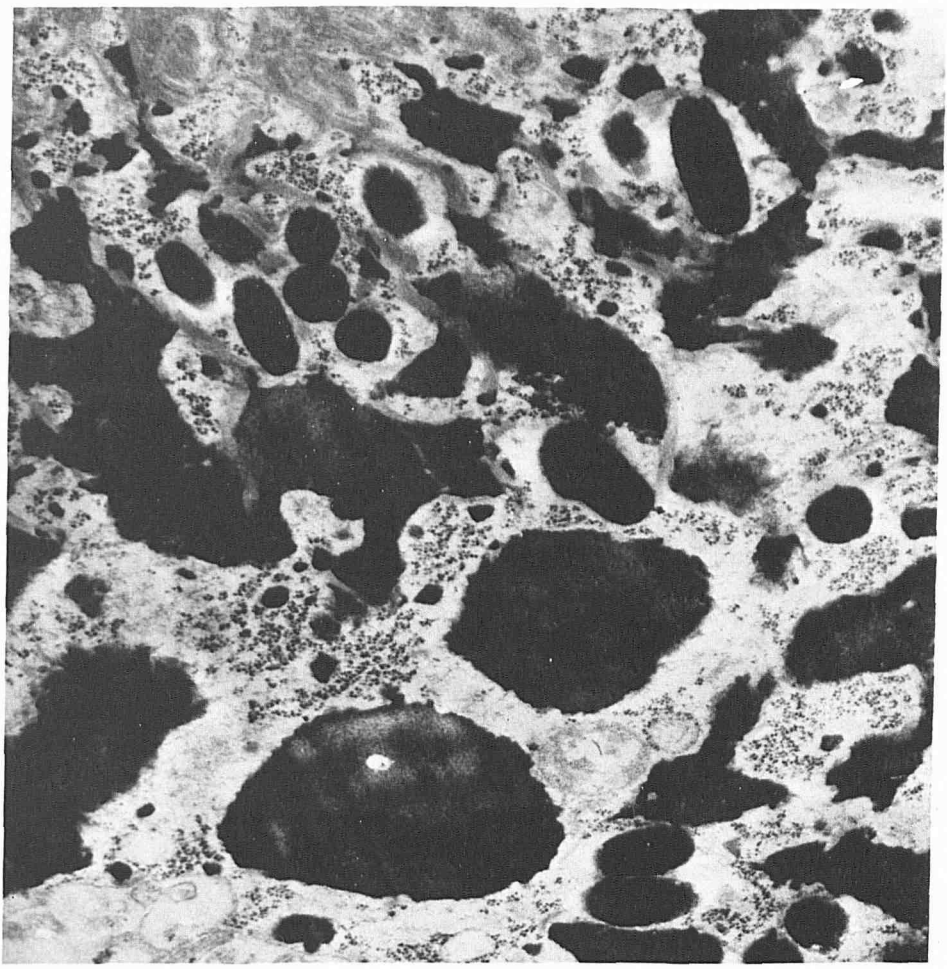

FIG. 1. Transverse section of part of a medullary cell (Plane 2) showing many smooth surfaced round or oval shaped melanosomes (MG) scattered throughout the cytoplasm. Note the presence of large numbers of irregular shaped electron dense granules (T), the bundles of filaments (F) and many ribosomes (R). Vesicle (V). × 29,900.

largement and fusion of the electron dense cytoplasmic granules.

Cortex of hair. Free melanosomes were seen scattered among the bundles of cytoplasmic filaments (Fig. 2). In the mature cells the melanosomes and ribosomal and nuclear remains were seen to be compressed by the fusion of the expanding bundles of cytoplasmic filaments.

Cuticle of hair. An occasional melanosome was seen within the cytoplasm of these cells.

Inner root sheath. No melanosomes were observed in any of the layers of the inner root sheath (Fig. 3).

Outer root sheath. The majority of cells at this level contained only a few melanosomes. However, an occasional cell showed a large group of melanosomes (Fig. 5).

Numerous melanocytes were seen in the dermis adjacent to the outer root sheath. These cells showed multiple dendritic processes which extended out between the connective tissue cells and fibers. The cytoplasm contained large numbers of melanosomes (Fig. 4) which were rounded, oval, or rod shaped; only a few premelanosomes were recognizable.

Plane 3. Medulla of hair. The melanosomes together with the remaining cell organelles were seen to be surrounded by a finely granular material of moderate electron density. This material occupied the greater part of each cell and the large electron dense masses were no longer present. Large intracellular vacuoles were also seen. In the less mature cells (Plane 2–3) the melanosomes and cytoplasmic remains appeared to be lying free within irregular intracellular spaces (Fig. 6).

Cortex of hair. The melanosomes and the remains of other cell organelles were surrounded by densely packed filaments embedded in matrix. In the less mature cortical cells, as in the cells of the medulla, the melanosomes and cytoplasmic remains appeared to be lying free within intracel-

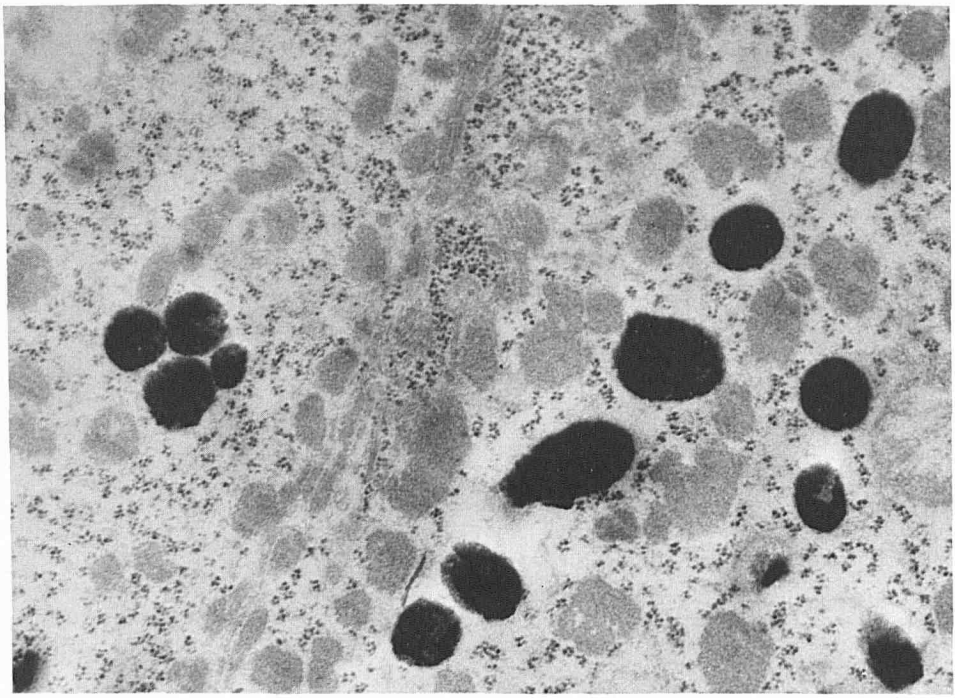

FIG. 2. Transverse section of parts of two cortical cells (Plane 2) showing numerous melanosomes (MG) in the cytoplasm. Note the presence of bundles of filaments (BF) embedded in matrix cut in cross section. Ribosomes (R); Mitochondrion (M): Plasma Membranes (arrowed). × 26,000.

lular spaces (Fig. 7).

Cuticle of hair. An occasional melanosome was identified.

Inner root sheath. This was no longer recognizable as a definite layer and was seen as cellular debris lying outside the hair. No melanosomes were identified.

Outer root sheath. The sheath at this level resembled the stratified epithelium of the epidermis. Melanocytes were seen in the basal layer and melanosomes were present in the adjacent keratinocytes and also in the keratinocytes of the more superficial layers. In the desquamating cells of the stratum corneum numerous melanosomes could be identified (Fig. 8).

Plane 4 (Hair only). *Medulla of hair.* Small groups or individual melanosomes were seen embedded in a dense granular material. No cell organelles were recognizable. In some of the hairs examined the medullary cells were absent in short segments of the hair and were replaced with air spaces or cortical cells.

Cortex of hair. Melanosomes, usually in groups, were seen to be embedded in irregular areas of electron dense material (Figs. 9 and 10). At many sites this material was seen to be attached to the inner surface of the thickened plasma membranes. The remainder of each cell was filled with tightly packed filaments and matrix.

Cuticle of hair. A few single melanosomes were found in the flattened cuticle cells (Figs. 9 and 10). Each granule was seen to be lying in an electron dense material situated close to the inner surface of the plasma membrane facing the cortical cells.

DISCUSSION

In the hair bulb of the anagen hairs of the guinea pig the matrix cells multiply and the daughter cells differentiate into the various cell lines which form the hair and the inner root sheath. The detailed fine structural changes which occur in a growing guinea pig hair have been described elsewhere (7). The morphological changes which occur in the cells of the developing inner root sheath have also been described by Parakkal and Matoltsy (8).

The fine structural characteristics of the melanocytes seen in the hair bulb in the present study confirm the findings of previous workers (4, 5, 9). The dermal melanocytes seen close to the outer root sheath contained many more melanosomes in their cytoplasm although only a few premelanosomes were seen. The relative absence of premelanosomes is presumably due to the fact that dermal melanocytes rarely if ever donate their melanin to other cells.

The immature medullary cells of the hair receive their melanin from the melanocytes in the upper part of the hair bulb. The dendritic processes of these melanocytes insinuate themselves between adjacent medullary cells and the donation process would appear to take place in a manner similar to that described for the epidermis (10, 11, 12). Many of the melanin granules

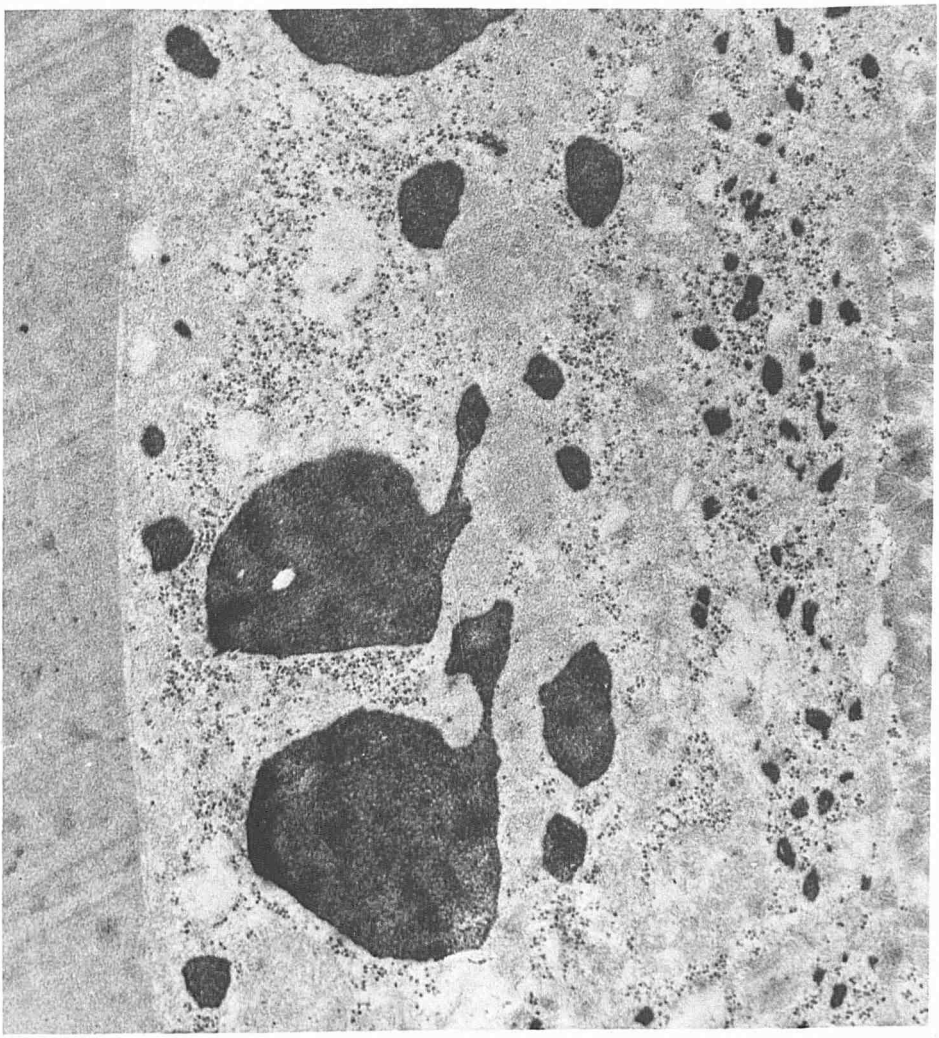

FIG. 3. Transverse section of the inner root sheath seen in the lower part of plane 2. Shows parts of cells of Henle's layer (HE), Huxley's layer (HU) and the cuticle of the inner root sheath (CIRS). On the right of the micrograph a small area of a cuticle cell of the hair (CH) is also shown. No melanosomes are seen in any of the cells. Keratin (K); Trichohyalin Granules (T); Granules in Cytoplasm of Hair Cuticle (GR). X 22,500.

are transferred in groups and are initially surrounded by a membrane which later disappears. As the medullary cells mature the melanin granules together with the other cytoplasmic remains become surrounded and finally embedded in keratin.

The immature cortical cells of the hair also receive their melanin from the melanocytes of the hair bulb. With maturation of the cortical cells the melanosomes become trapped between the bundles of filaments and matrix which appear in the cytoplasm. As the process of keratinization continues, the melanin, along with other cytoplasmic remnants, becomes embedded in a dense amorphous material which at many sites is seen to be attached to the inner surface of the thickened plasma membranes. The origin of the amorphous material is of interest. It is possible that it is a new material which has been produced during the final stages of keratinization within the cells or it may merely represent the final breakdown products of the cytoplasmic remnants. Another point of even greater interest is the significance of the attachment of this material to the inner surface of the plasma membrane. An examination of the electron micrographs shows that at the majority of sites where this occurs the attachment is narrow and discrete. The impression is gained that clusters of melanosomes are suspended from the plasma membranes by these attachments. One explanation is that the dense amorphous material is in the process of being extruded through the plasma membrane during the final stages of keratinization of the cortical cells. The possible relationship of this material to the amorphous material found between adjacent cortical

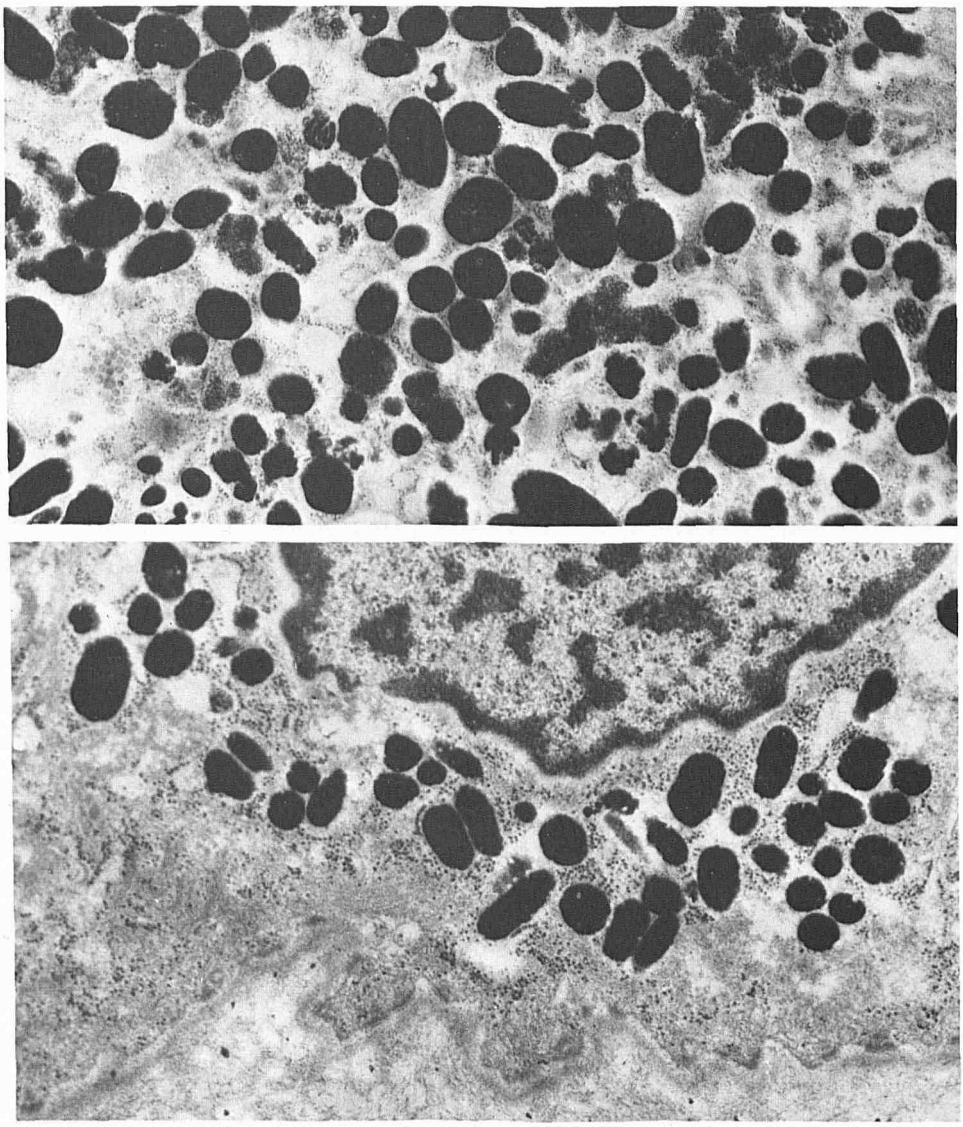

FIG. 4. Transverse section of part of a dermal melanocyte situated close to the external root sheath (Plane 2). Shows the cytoplasm to be filled with melanosomes of different shapes and sizes. Note the presence of an occasional premelanosome (arrows). × 15,800.

FIG. 5. Transverse section of part of cell of the external root sheath (Plane 2). Shows many melanosomes within the cytoplasm. Dermis (DE); Basement Membrane (BM); Nucleus (N); Vesicle (V). × 20,800.

cells and the thickening of the plasma membranes is fully discussed elsewhere (7).

Montagna (13) while describing the general structure of the hair noted that the cells of the cuticle, in contrast to those of the cortex, contain no melanin. In the present work on the guinea pig a few melanosomes were consistently seen in the cuticle cells. As the cuticular cells of the hair become keratinized, the melanosomes become embedded in the electron dense material found close to the inner surface of the plasma membrane facing the cortical cells. The origin and significance of this dense material is open to question. Some authorities believe it is derived from the remains of cytoplasmic organelles (14).

Progressing in a centrifugal direction, the inner root sheath consists of the cuticle, Huxley's layer and Henle's layer. In this study no melanosomes were observed in any of these layers, an observation in agreement with the light microscopic findings of other workers (15). The cells of the inner root sheath arise from the peripheral and central mass of cells of the matrix. It is interesting to note that the melanocytes thrust their dendritic processes between the passing stream of cortical and medullary cells and donate melanin to them but they do not, under normal conditions, donate melanin to the migrating cells of the inner root sheath. According to Chase (16) it is possible to displace melanocytes experimentally so that they

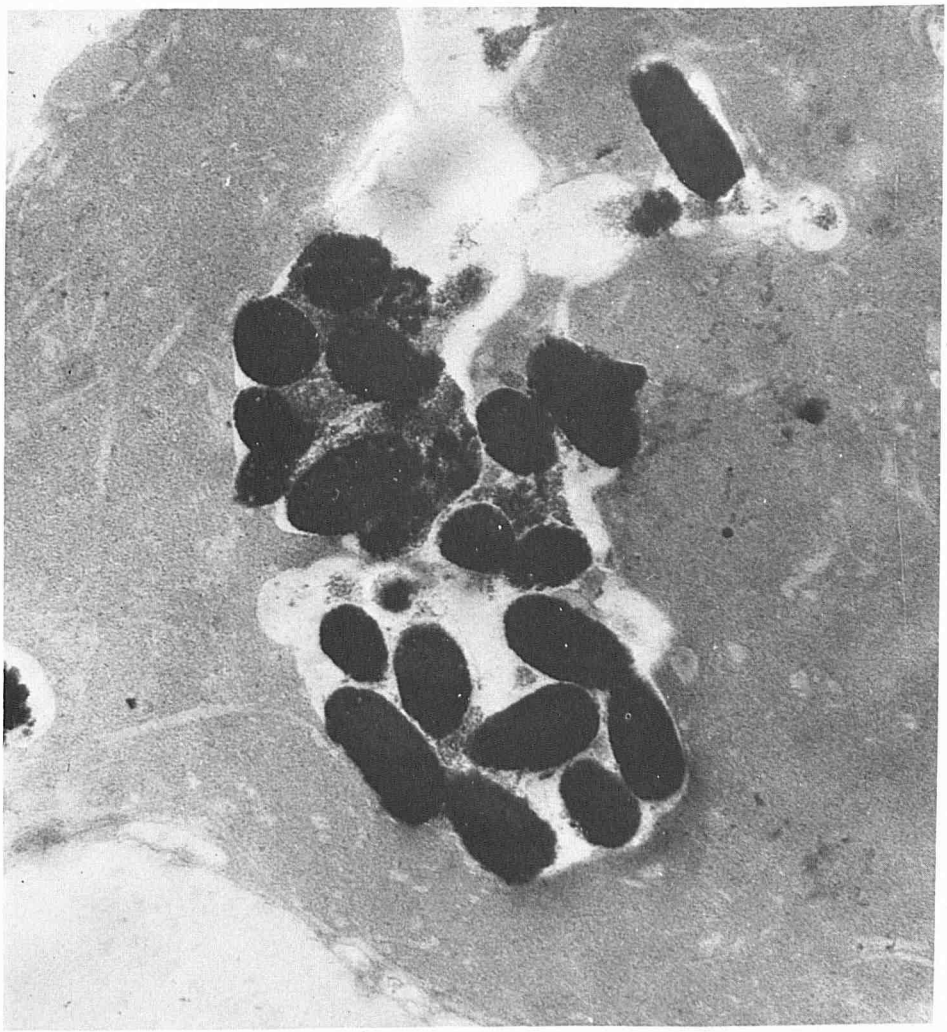

FIG. 6. Transverse section of part of a medullary cell (Plane 2–3). Shows a large group of melanosomes (MG) and remnants of cytoplasmic organelles (CR) lying free in an irregular intracellular space. Note that the greater part of the cell is filled by a finely granular material of moderate electron density (K). Vesicle (V). × 37,200.

descend to the lower bulb. Under these conditions melanin is found in the inner sheath cells during the next day's growth. It would thus appear that the relationship between the dendrites of a melanocyte and the streaming daughter cells of the matrix is a precise one which may have both a spatial and temporal significance. The actual transfer of pigment may involve contact between specialized regions of the cells and the recipient cells may have to be at a critical stage of maturity during which the melanosomes may be phagocytized. It is conceivable that by the time the cells of the inner root sheath reach the upper part of the bulb where the melanocytes are normally located, they have matured to such an extent that they are unable to engage in phagocytic activity.

The outer root sheath may be divided into upper and lower halves. The upper half above the opening of the sebaceous gland is relatively permanent and resembles the surface epidermis. The donation of melanin by the melanocytes in this region takes place in precisely the same manner as that described for the epidermis (12, 17, 18). The lower half is a transient structure which degenerates and disappears during catagen. Montagna and Van Scott (15) were of the opinion that the outer root sheath cells were non-pigmented. It was interesting to find in the present work that the majority of the cells in the lower part of the sheath possessed only a few melanosomes.

In the above account of the fine structure of guinea pig black hair the melanosomes have been traced from their origin in the melanocytes of the hair bulb up to and including the emergent hair. The relationship of the pigment to the cytoplasmic structures within the various maturing hair cells has been described. In the mature hair most of the melanin pigment was found in the cortex and the medulla and only a small amount was present in the cuticle.

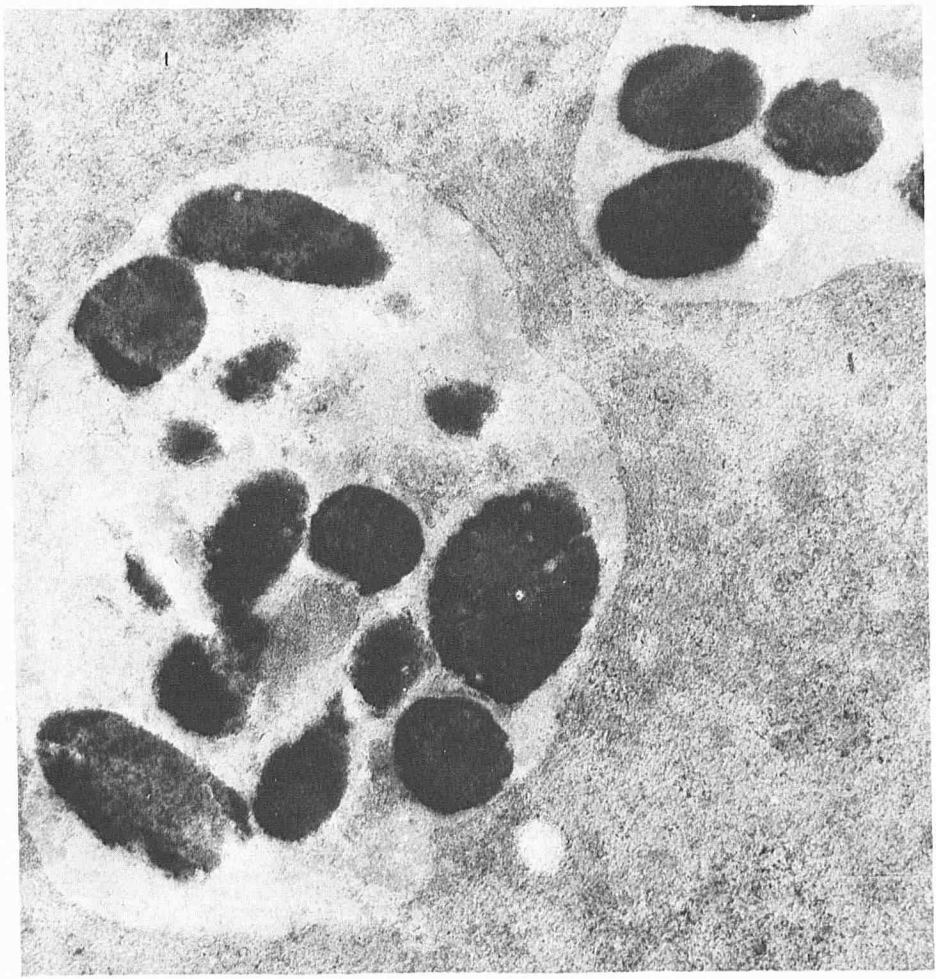

FIG. 7. Transverse section of part of a cortical cell (Plane 2–3). Shows two groups of melanosomes (MG) and remnants of cytoplasmic organelles (CR) lying free in two intracellular spaces. The remainder of the cell is filled with densely packed filaments cut in cross section embedded in matrix (K). The individual filaments cannot be readily identified at this magnification and with this staining technique. × 55,200.

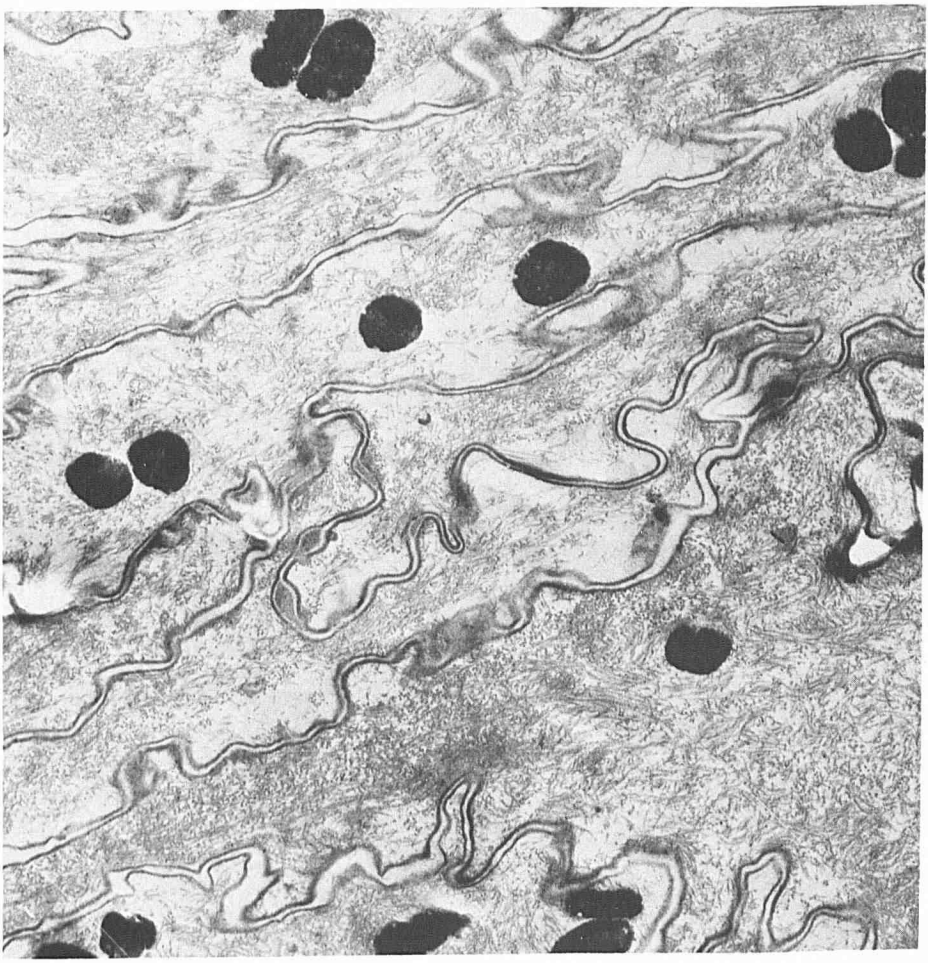

FIG. 8. Transverse section of the superficial cells of the external root sheath (Plane 3). Numerous melanosomes (MG) are present in the keratinized cells. Note the identical appearance of these cells to those of the stratum corneum of the surface epidermis. Thickened Plasma Membranes (PM); Keratin (K); Ovoid Bodies of Desmosomes (arrowed). × 24,000.

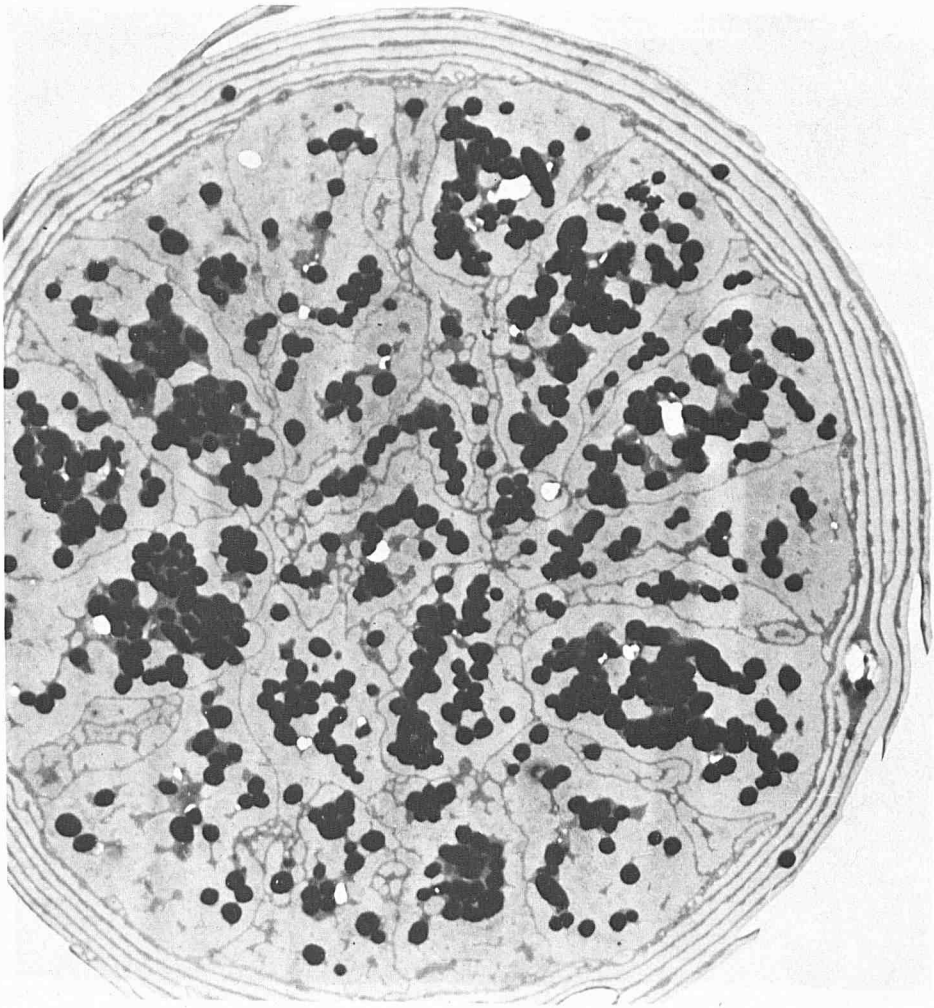

FIG. 9. Transverse section of a mature black hair (Plane 4) showing a few melanosomes (MG) in the cuticle (CH) and large numbers of melanosomes in the cortical cells (C). In the cortical cells the black melanosomes are embedded in irregular areas of electron dense material (DM). At several sites (arrowed) the dense material is attached to the inner surface of the thickened plasma membranes. No medullary cells are present in this section. × 8,500.

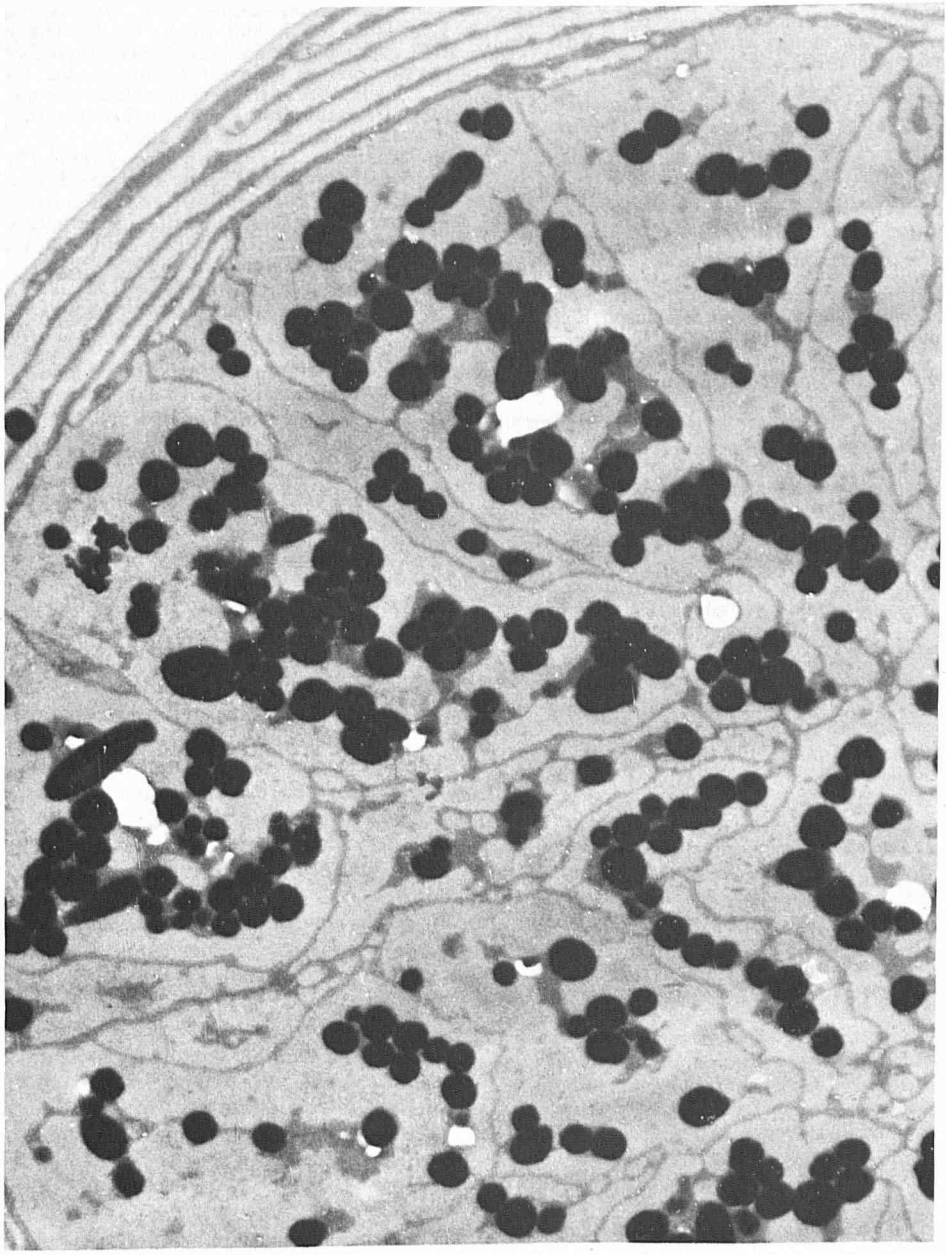

FIG. 10. High power electron micrograph of part of the mature black hair seen in Fig. 9 shows a melanosome (MG) in the cuticle (CH) and very many melanosomes in the cortical cells. (C). In the cuticle cell, the melanosome is partially embedded in a band of electron dense material which is adjacent to the inner plasma membrane. In the cortical cells the melanosomes are embedded in irregular areas of electron dense material. Note that at several sites (arrowed) the dense material is attached to the inner surface of the thickened plasma membranes. × 16,700.

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