ELECTRON MICROSCOPE STUDIES OF EPIDERMAL MELANOCYTES, AND THE FINE STRUCTURE OF MELANIN GRANULES

P. DROCHMANS, M.D.

From the Laboratoire de Cytologie et de Cancérologie Experimentale, Université Libre de Bruxelles, Belgium

ABSTRACT

Melanocytes and melanin granules have been studied by electron microscopy in normal human and cat skin, and in hyperplastic human skin lesions. The melanocytes have always been found as free cells within the epidermis, *i.e.*, on the epidermal side of the dermal membrane. Melanocytes frequently rest on the dermal membrane or bulge towards the dermis. In such cases the uninterrupted dermal membrane is uniformly thin and smooth in appearance, in contrast with the regions alongside Malpighian cells, where it appears appreciably thicker and seemingly anchored to the basal cell layer. Two types of melanin granules have been distinguished according to their location in the melanocytes and to morphological characteristics which may only express different stages in the maturation of the granules: (*a*) light melanin granules in which a structure resembling a fine network is apparent; (*b*) dense melanin granules which, in osmium-fixed preparations, appear as uniformly dense masses surrounded by a coarsely granular, intensely osmiophilic shell. Treatment of sections of osmium-fixed tissues with potassium permanganate has revealed within the dense granules the existence of an organized framework in the form of a regular, crystalline-like lattice. It is suggested that this basic structure is protein in nature and may include the enzymatic system capable of producing melanin. The existence is reported of fine filaments located in the cytoplasm of melanocytes and morphologically distinct from the tonofilaments found in Malpighian cells.

The melanocyte of the mammalian epidermis is currently thought of as distinct from the Malpighian cell, both as regards its morphology and its biochemical properties (5, 2, 18). This view has been based on numerous studies by light microscopy, and especially on histochemical findings by means of the dopa reaction (5). Marking with radioactive substances (12) has helped to characterize the melanocyte as the specific and active element in the melanization of the epidermis.

Electron microscope studies on the normal epidermis have strengthened this view. Odland (15) noticed that the melanocyte is free of the fibrillar material (tonofibrils and tonofilaments) typical of the Malpighian cells, and that intercellular bridges between it and the other cells of the epidermis are also absent. Clark and Hibbs (7) confirmed these observations and directed attention to the low density of the cytoplasm of the melanocyte and the significant differences in the size and shape of its mitochondria and endo-

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plasmic reticulum compared to those of the epidermal cell. Charles and Ingram (6) raised the question of the mobility of the melanocyte. The latter authors describe positions of the melanocytes which they interpret as describing its passage through the dermo-epidermal junction.

The fine structure of the melanin granules has been studied by Birbeck, Mercer, and Barnicot (4) in the melanocytes of human hair. According to these workers the melanin granule and its precursor, the so called pregranule found in the Golgi zone, appear as concentrically folded membranes in cross-sections, and as parallel strands in longitudinal sections (3). Charles and Ingram (6) noticed in the human epidermis a periodic structure in the granules consisting of alternating dense and light bands. Barnicot, Birbeck, and Cuckow (1) have isolated these melanin granules from human hair by centrifugation. Sections made of embedded granules show an internal granular structure very resistant to alkali and acids.

The present paper reports on the examination by electron microscopy of melanin-pigmented tissues obtained from four different sources, each type of tissue being selected because of a more favorable demonstration of characteristic features of melanin pigmentation. The four tissues studied were: (*a*) normal human skin from the vulvar region: the pigmentation of this region undergoes an important increase in the course of pregnancy, thus affording an opportunity to study a physiologically active phase of the process of pigmentation; (*b*) normal skin from the nose of the cat, where melanocytes are rich in pigment; (*c*) hyperplastic human skin lesions, diagnosed as venereal papillomas (condylomas); in this case the variation in the degree of melanization was particularly advantageous for the study of certain aspects of melanin formation and transfer; and (*d*) a hyperkeratic lesion of human skin in which the later stages of the process of pigmentation, *i.e.*, the accumulation of pigment in the dendrites and in the Malpighian cells was evident. To our knowledge, the problem of melanization of the Malpighian epithelium, in cases of hyperplastic skin lesions, has not previously been studied by electron microscopy. Becker (2) examined these lesions under the light microscope and found the melanocytes (which he called melanoblasts), undistinguishable from those of normal skin.

The findings reported in this paper concern only melanocytes, their dendrites, and the structure of the melanin granules found in these cells. The transfer of melanin granules from melanocytes to Malpighian cells is an aspect of skin pigmentation which will be treated separately.

MATERIAL AND METHODS

Material

1. *Normal Human Skin:* (*a*) Human skin was obtained from a normal 41-year-old white woman, who presented an over-all normal skin color. A fragment of skin, 0.5 × 0.5 mm. in size was taken by biopsy on the cutaneous side of the genital labia minora after local anesthesia; (*b*) Normal human skin from a subject in the 4th month of pregnancy; the material for study was obtained likewise from the cutaneous side of the genital labia minora, and presented the intense pigmentation which is typical at this stage of pregnancy.

2. *Normal Cat Skin:* The hairless region of the nose of an 8-day-old cat was used. The skin of that particular region was a greyish-black color.

3. *Condyloma:* This hyperplastic lesion of the skin had developed in the inguinal region of a 60-year-old male, over a period of about 2 years. When first examined preliminary to these studies, the growth appeared as a lesion 1 cm. in diameter and raised about 1.5 cm. above the surface of the normal skin. The fleshy papillomatous growth was of uniform height, with normally keratinized surface, healthy in appearance, without ulcerations or signs of gross inflammatory or degenerative processes. Under the light microscope, the lesion was found to consist of a hyperplasia of the Malpighian layer with a regular, papillary arrangement of the epithelium. The pigmentation was poor and only located in the basal layers. From the pathological point of view, the lesion would be classified as a benign growth. Preparations of condyloma tissue were obtained from the patient at different times: (*a*) before the tissue had been subjected to any treatment and (*b*) 10 hours after a local application of podophyllin.

4. *Hyperkeratic Papilloma:* This hyperplastic skin lesion was removed from the nose of a 20-year-old male. The small tumor, 0.5 mm. in diameter, was raised about 0.3 mm. above the surface of the surrounding skin. The pigmentation was moderate.

Methods

Light Microscope Techniques: Tissue slices approximately 1 mm. thick were fixed in Bouin-Hollande and in Zenker-formol fluids. Haematoxylin-eosin and Masson's trichrome were used as routine staining techniques. The inherent opacity of the melanin was increased by means of silver impregnation,

using the Masson's ammoniacal silver nitrate method (13); carmine was used for counterstaining.

Electron Microscope Techniques: (*a*) Specimens from the lesions were obtained under local novocain anesthesia except for the cat which had been sacrified with chloroform. Small fragments, about 1 $mm.^3$ were subjected to fixation for 24 hours at room temperature, in two different solutions: (1) a 1 per cent osmium tetroxide solution in acetate-veronal buffer (16), (2) a 1 per cent osmium tetroxide solution in distilled water (9). The fixation was followed by washing in distilled water, and dehydration in 70, 94, and 100 per cent ethanol, each for three periods of 20 minutes. Embedding was performed in a 95 per cent butyl-5 per cent methyl- methacrylate mixture.

(*b*) Staining of tissue fragments of each series was carried out at the absolute ethanol stage of dehydration, by immersion of the tissue in a 1 per cent solution of phosphotungstic acid in this solvent, for 48 hours (14).

(*c*) Staining of sections was performed by floating on a 5 per cent potassium permanganate solution for 20 minutes and for special purposes for 40 minutes. This method of staining is described in detail in another paper (10).

OBSERVATIONS AND RESULTS

The subject of the following report will be the study of the melanocyte cell body, and the melanocyte dendritic processes. Two characteristic cytoplasmic components of the melanocyte, the melanin granules and filamentous elements, will be described.

Light Microscopy

In the normal human skin the special feature to be noted is the projection of the melanocyte cell body into the dermis. In the case of the vulvar skin obtained from a pregnant woman, the melanocytes were sparsely pigmented and the dendrites difficult to identify. Pigmentation in the Malpighian epithelium was mainly located in the basal layers. In the case of the cat skin, the melanocytes appeared loaded with pigment, but were otherwise comparable to those of the human skin. The optical microscope observations just described have not been illustrated in this paper.

The hyperplastic skin lesions were examined under the light microscope, after appropriate staining and impregnation. Under these conditions, the melanocytes appeared as dendritic cells, rich in melanin pigment. The light micrographs shown in Figs. 1 and 2 illustrate melanocytes and extended dendrites as they are found in the condyloma. In Fig. 1, the melanocyte (*ME*) is located between epidermal basal cells, close to the basement membrane. In Fig. 2, the melanocyte (*ME*) lies between the first and second basal layers of epidermal cells. The melanocyte nucleus is irregular in form and its limits are frequently masked by superimposed melanin granules. These pigment granules are found in the perikaryon as round or oval bodies of sizes, varying from 0.5 to 1.5 μ in diameter.

Slender dendrites extend from the perikaryon. Two types of dendrites may be distinguished by their shape and length: (*a*) long, slender dendrites (Fig. 1, *DE*), generally oriented perpendicularly to the surface of the skin and extended either upward or downward between the Malpighian cells. These dendrites are seen in intercellular spaces and appear to weave up and down between intercellular processes present along their path; (*b*) shorter dendrites (Fig. 2, *DE*) which terminate with club-shaped endings (arrows) at the level of the basal cells.

The present electron microscope study has been restricted to an examination of the morphology of the melanocyte, with the first type of dendrites described, and to that of the melanin granules. The second type of dendrite, and the related problem of the transfer of melanin must be treated together, and will be the subject of a subsequent study.

Electron Microscopy

The following observations are concerned with the electron microscope study of melanocytes as they appear in normal and hyperplastic epidermis, and of the dendritic extensions of type I, *i.e.*, those illustrated and described in the light micrograph of Fig. 1.

The Melanocytes

The melanocytes contained in the vulvar skin are regularly arranged along and on the epidermal side of the dermo-epidermal junction. The perikaryon, bulging deeply into the dermis, was always found to be separated from the dermal connective tissue by the uninterrupted dermal (or basement) membrane. Fig. 3 shows such a melanocyte (*ME*), depressing the dermal membrane (*DM*), and completely surrounded by it.

It is noteworthy that in this tissue the dermal membrane appears uniformly thin when in contact with the melanocyte, but that it appears to be thicker and reinforced when adjacent to Malpighian cells, where tonofilaments reach the cell membrane (*IM*). On the upper part of the melanocyte, the cytoplasm forms irregular extensions resembling pseudopodia (*CE*). Details regarding the cytoplasmic organelles will be described in more favorable micrographs of melanocytes observed in hyperplastic skin.

In the condyloma, the melanocytes lie either on the dermal membrane, or between epidermal cells of the Malpighian layer. Figs. 4 and 6 give a general view of a melanocyte lying between the dermal membrane and Malpighian cells. Fig. 4 shows the perikaryon resting directly on the dermal membrane (*DM*), the profile of the cellular membrane parallel to the latter remaining quite distinct. In addition, part of the body of the same melanocyte appears also in Fig. 6, with a slender dendrite extending upward (*d3*) between two Malpighian cells. The following inclusions, which it shares with other cell types, may be recognized in the cytoplasm of the melanocyte: (*a*) mitochondria, more numerous at both poles of the cell; (*b*) elements of the endoplasmic reticulum, with attached Palade granules; and (*c*) melanin granules.

It is to be noted that melanin granules are found in greater abundance at the periphery of the melanocyte, *i.e.*, in the dendrites and, characteristically, in points of the perikaryon where pseudopodia appear to emerge (Fig. 4, *d1*; and Fig. 6, *d2*).

The elements found in the centrosphere region of the melanocyte illustrated in Fig. 8 are: (*a*) in the center, diplosomes disposed at right angles to each other (*ce*), and therefore seen respectively in lengthwise and cross-section; (*b*) around the centrosome, numerous smooth membranes and vesicles, presumably part of the Golgi complex (*gg*); (*c*) at the periphery, numerous melanin granules of relatively uniform size and ovoid shape,

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Light micrographs of human condyloma.

FIGURE 1

View of a section perpendicular to the epidermal surface. From the lower part to the top: (*a*) a small portion of the dermis, (*b*) a portion of the basement membrane (faintly stained), and (*c*) three to four basal cell layers of the epidermis. The melanin granules play the role of tracers for the dendrites (*DE*) which thus may be followed as they weave between the cells, in front and back of intercellular junctions. In this picture, the same dendrite appears to extend as far as the length of two to three basal cell layers. The melanocyte perikaryon (*ME*), which lies close to the basement membrane, is loaded with fine melanin granules.

Fixation: Bouin-Hollande; staining: ammoniacal silver nitrate (Masson), counterstaining: carmine.

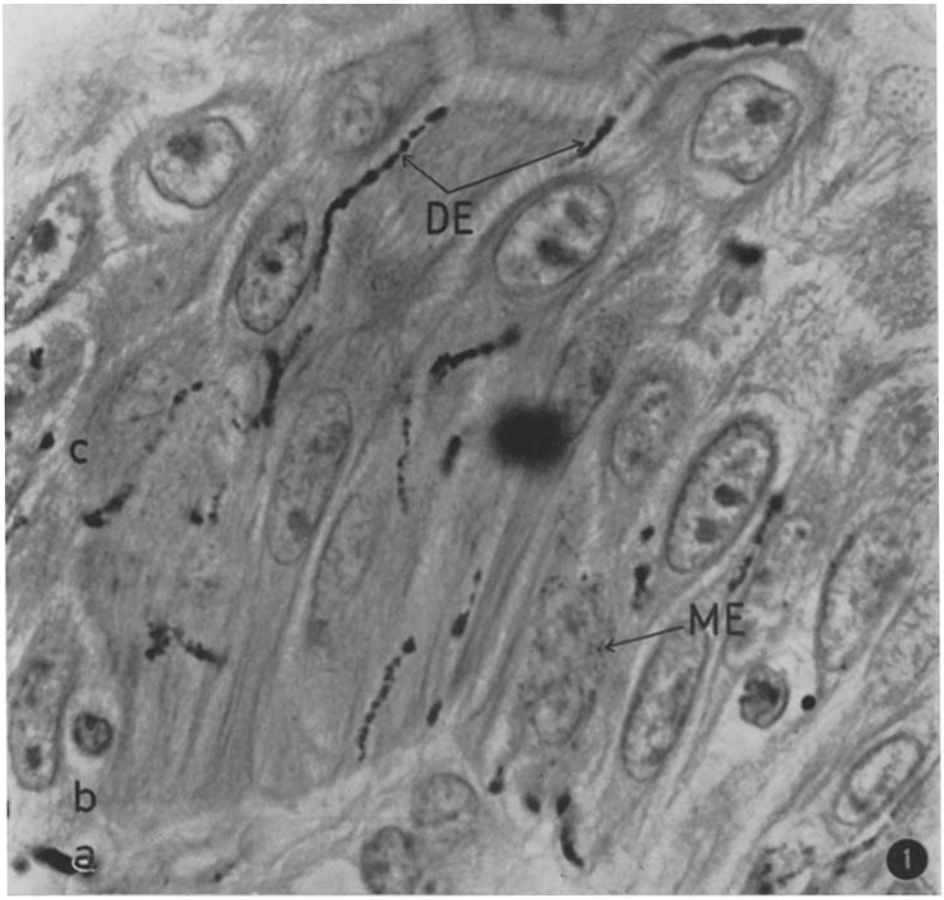
Light micrograph taken at × 800, enlarged to 1400.

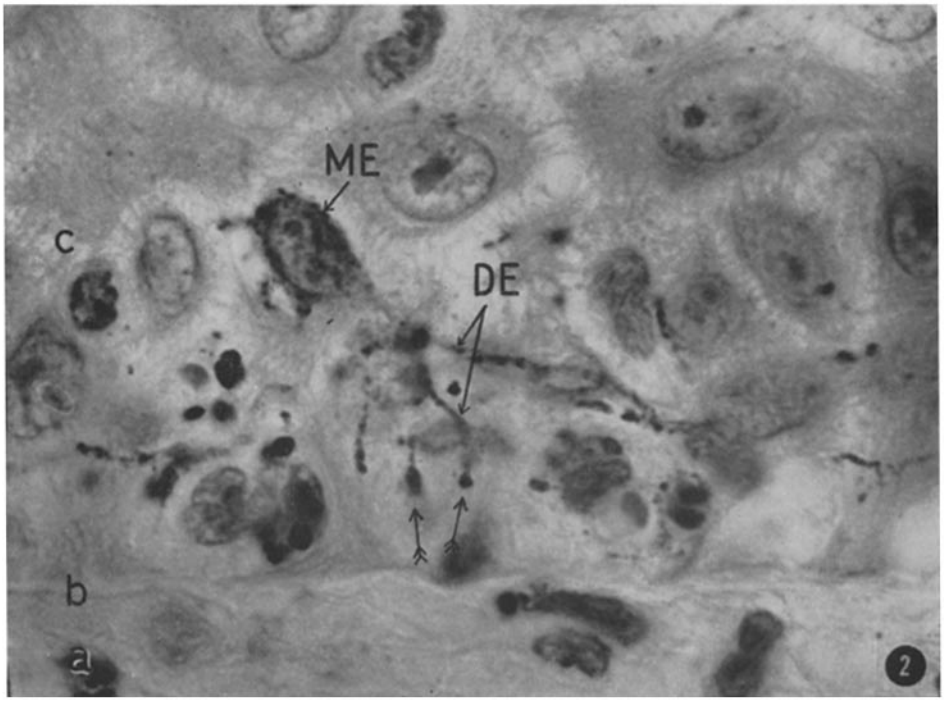
FIGURE 2

View of a section perpendicular to the surface of the epidermis and showing from the lower part to the top: (*a*) a portion of the dermis, (*b*) the basement membrane (faintly stained), (*c*) a zone composed of several layers of basal and spinous cells with their intercellular connections. Left to the center of the picture, a melanocyte (*ME*) is seen between spinous cells, with its dendrites (*DE*) extended in various directions. Two of the downward directed dendrites terminate with a club-shaped ending (arrows) at the level of a basal cell. The melanin is seen in the shape of granules disposed all along the dendrites and in the perikaryon. Segments of dendrites are visible in the intercellular spaces.

Fixation: Bouin-Hollande; staining: ammoniacal silver nitrate (Masson); counterstaining: carmine.

Light micrograph taken at × 800, enlarged to 1400.


DE
c
ME
b
a
1


ME
c
DE
b
a
2

which may be recognized, especially those (*mg*) in the upper part of the picture, by their characteristic, crystalline-like structure; (*d*) fine filaments (*fl*) which appear in a loose matting in the upper left corner of Fig. 8; (*e*) and other elements, such as swollen mitochondria (lower left corner of the picture) and a few dense bodies which may correspond to the cytosomes or microbodies of other authors (17).

The Dendrites

The dendrites of melanocytes consist of slender cytoplasmic processes extending at relatively great length from the perikaryon, and penetrating between the Malpighian cells. Their wavy course may be best observed in light micrographs where they may be seen to extend through intercellular spaces and around intercellular bridges (Fig. 1). Because of their irregular path and of the thinness of the sections, the dendrites are usually evident only as segments in any given electron micrograph. The beginning of a dendrite, with an accumulation of melanin granules, is seen in Fig. 4 (*d1*) and in Fig. 6 (*d2*). A slender dendrite extending from the same melanocyte but from an opposite cell region, is shown in Fig. 6 (*d3*); this dendrite may be followed from the melanocyte upward between two Malpighian cells, identified by the presence of tonofilaments in their cytoplasm. As shown in Fig. 6 the tip of the slender dendrite ends next to an intercellular bridge, or may have been deflected by the latter to another level. The composition of the dendrites can best be observed in cross-sections such as shown in Fig. 5. In this picture, the melanocyte process is seen lying in an intercellular space. In such dendrites, the melanin granules usually accumulate at the periphery, close to the extension of the limiting cell membrane, and groups of mitochondria may be found, centrally located in the axis of the process (*mi*).

The Melanin Granules

Since in the melanocytes examined under the electron microscope the individual melanin granules range from 0.1 to 0.4 μ in size, it is probable that most of the melanin inclusions, as detected usually under the light microscope, are not single, but groups of melanin granules (Figs. 1 and 2). For present purposes, the melanin granules detected in melanocytes may be said to occur in two extreme types, one light and the other dark or dense. The difference appears to result from the variable degree of deposition of melanin on a supporting basic framework. In melanocytes, these melanin bodies always appear singly, as individual organelles: they do not aggregate into compound inclusions, as may be the case in Malpighian cells or in pigmented cells of the dermis.

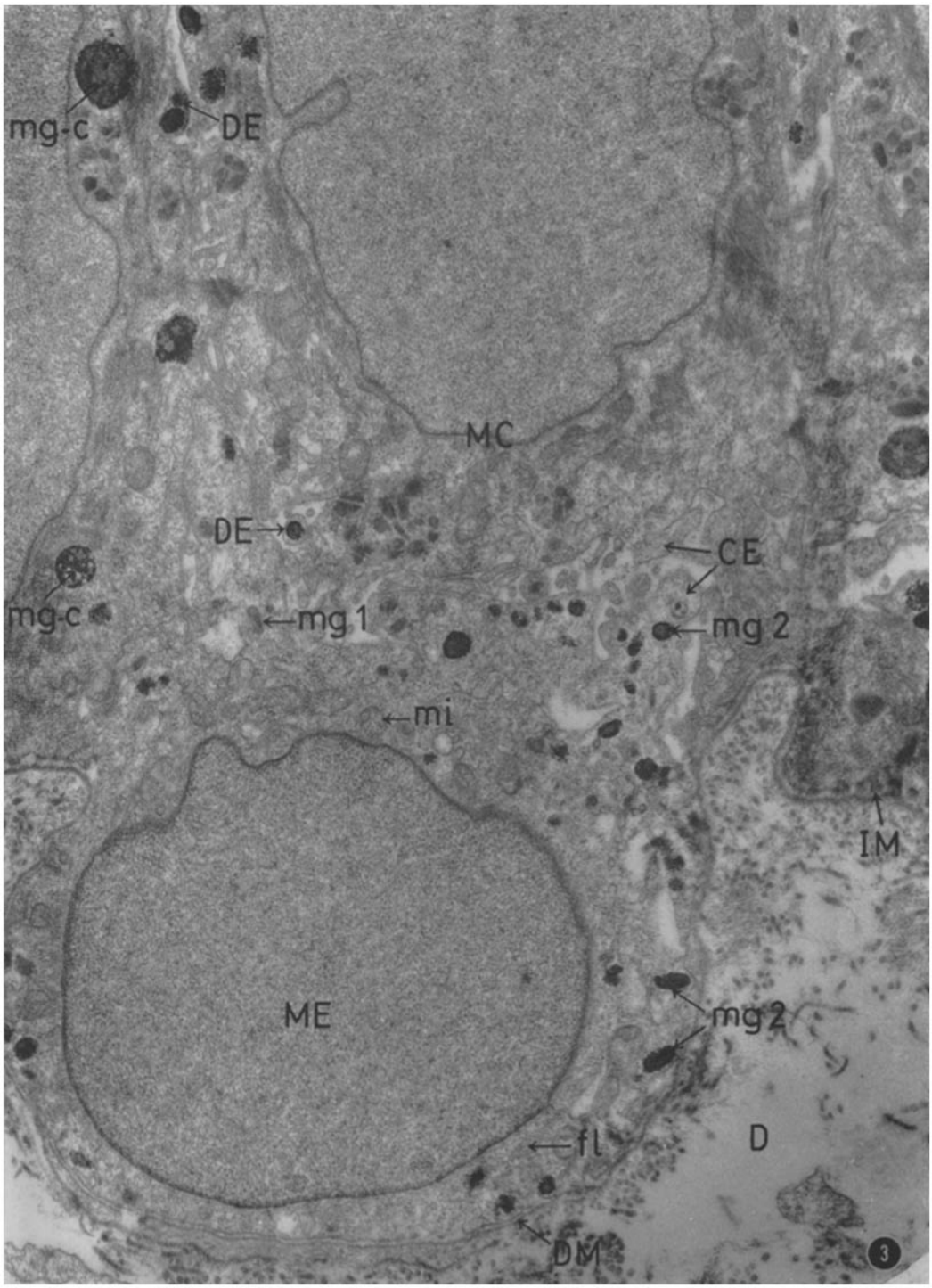
(*a*) *Light Melanin Granules:* Although the light melanin granules are commonly found in normal and hyperplastic skin, their fine morphology is best illustrated in the latter type of tissue. The

FIGURE 3

Human skin of the vulvar region: This electron micrograph shows a melanocyte bulging into the dermis (*D*). In the lower half of the picture, the round-shaped melanocyte (*ME*) is lined on its dermal surface by a uniformly thin dermal membrane (*DM*). On the right side of the picture this dermal membrane appears thickened when in contact with epidermal basal cells, and shows reinforcements (*IM*) characteristically located opposite tonofilament endings of the Malpighian cells. The cytoplasm of the melanocyte contains mitochondria (*mi*), oblique sections of filaments (*fl*) close to the nucleus, melanin granules of the light type (*mg 1*) and of the dense type (*mg 2*). The melanocyte on its epidermal side, close to the base of the epidermal basal cell (*MC*) shows numerous irregular cytoplasmic extensions (*CE*) containing melanin granules. In the intercellular spaces, between the basal cells, dendrites (*DE*) are sectioned obliquely. The melanin inclusions (*mg-c*) in the epidermal basal cells are composed of agglomerations of elementary granules.

Fixation: 1 per cent osmium tetroxide in distilled water; stained with PTA.

Electron micrograph taken at × 12,000, enlarged to 28,000.


mg-c
DE
MC
DE
CE
mg-c
mg 1
mg 2
mi
IM
ME
mg 2
fl
D
DM
3

following observations are related to structures observed in melanocytes or melanocyte dendrites of non-stained sections of the condyloma. It appears that the material making up the light granules constitutes the main structural component of the melanin granule. In thin sections cut parallel to the long axis of the granule, this component is seen to have an organization reminiscent of a crystalline lattice.

(*b*) *Dense Melanin Granules:* Regarding the more opaque melanin granules, two features may be noted which distinguish them from the granules of lighter type: (*a*) in the so called dense granules the striated pattern characteristic of the lighter granules is also present, and with a line spacing of the same order (about 70 A); in this case, however, the basic framework readily demonstrated in the light granules no longer appears distinct but seems to have embedded in it a diffuse, grayish substance responsible for the compact, dense appearance of the granule; (*b*) in contrast with the melanin granules of the lighter type, the dense melanin granules appear to be surrounded by a shell of relatively coarse, non-organized granular material.

The appearance of the dense granules under a variety of experimental conditions seems to throw some light on the relationship between the two types of granules, and on their basic constitution. The following examples illustrate the general morphology exhibited by melanin granules in tissues fixed in osmium tetroxide (*a*) directly, *i.e.*, without staining; (*b*) stained with phosphotungstic acid; and (*c*) treated with potassium permanganate for different lengths of time.

In osmium-fixed, non-stained sections of normal and hyperplastic skin, the dense melanin granules appear as moderately dense bodies surrounded by a denser shell composed of coarsely granular, osmiophilic material (Figs. 5 and 9, *mg2*). Examined at higher magnification, the rod-shaped melanin granule of the *cat skin* appears as an elongated mass of uniform density, likewise surrounded by a relatively thinner, osmiophilic shell made up of more finely granular material. Since the structural differences are slight, illustration of the unstained cat melanin granules has been omitted.

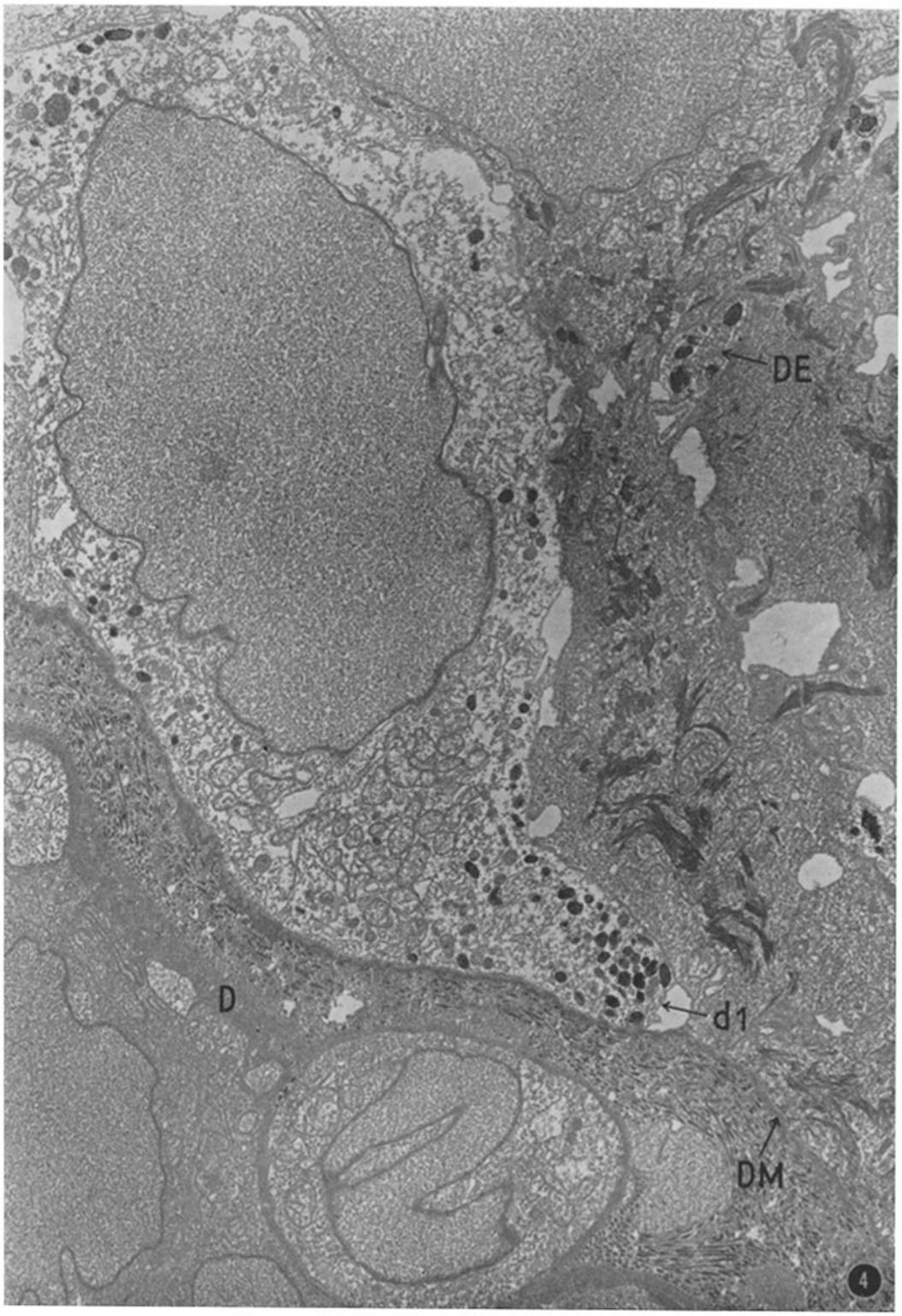
After phosphotungstic impregnation, especially in ultrathin sections, a striated pattern may be detected in the body of the dense granules, with an apparent orientation parallel to the long axis of the granules. This aspect of phosphotungstic-treated material is illustrated in the case of the

FIGURE 4

Condyloma: This electron micrograph illustrates the characteristic difference in density to electrons, between the perikaryon of the melanocyte, which appears relatively light, and the dense cytoplasm of the Malpighian cells, loaded with tonofilaments. The lower left corner of the picture shows a portion of the dermis (*D*) which contains a wandering cell. The dermis proper is separated from the epidermis by a layer of dark collagen fibers and the dermal membrane (*DM*). On the upper left part of the picture a melanocyte rests directly on a portion of the dermal membrane. No tonofilaments cross the melanocyte nor reach the dermal membrane. On the right part of the electron micrograph, the dermal membrane is in contact with Malpighian cells. There, the dermal membrane is reinforced on the sites opposite to the tonofilaments endings. The melanocyte contains in its basal pole numerous mitochondria, and vesicles of the endoplasmic reticulum. The melanin granules are of various densities but of relative constant size. They are preferentially situated at the periphery of the cell. In the lower right part of the cell there is an accumulation of melanin granules probably located at the origin of a dendrite (*d1*). The upper left part of this melanocyte is represented in the next figure. On the right of the melanocyte, in the intercellular spaces, dendrites are sectioned obliquely (*DE*). They are well limited with a membrane and contain melanin granules of the same density as those of the perikaryon. Other cellular components are present. The limits of the Malpighian cells may be followed owing to the enlarged intercellular spaces and the intercellular bridges.

Fixation: 1 per cent osmium tetroxide buffered at pH 7.4; stained with PTA.

Electron micrograph taken at × 5000, enlarged to 12,000.


DE
D
d1
DM
4

melanin granules of the human condyloma (Fig. 10), and in the case of the melanin granules of cat skin (Fig. 13). In both cases a longitudinal striation is apparent, but the contrast remains generally poor.

The effect of permanganate on osmium-fixed melanin granules is illustrated in Figs. 11, 12, and 14. Fig. 11 shows an electron micrograph of a section of condyloma tissue treated with potassium permanganate, for a period of 20 minutes. Figs. 12 and 14 illustrate respectively, sections of human vulvar skin and cat skin, which have been exposed to the action of potassium permanganate for a somewhat longer period, *i.e.*, 40 minutes.

The action of potassium permanganate on the appearance of the dense melanin granules is striking: it affects two aspects of the granules that were described in the cases of osmium-fixed, unstained preparations, namely, (*a*) the dense, osmiophilic shell surrounding the granules; and (*b*) the compact and uniformly dense condition of the body of the granules. Staining with phosphotungstic acid left the osmiophilic shell unchanged, or else slightly increased in density. On the other hand, phosphotungstic acid had a definite differentiating effect on the mass of the granule, as indicated by the appearance of a striated pattern (Figs. 10 and 13).

The results of prolonged treatment of tissue sections with permanganate leaves no doubt that the dense melanin granules are structurally organized, as already suggested by their appearance after phosphotungstic acid staining. After permanganate treatment, the dense melanin granules appear to be occupied by a crystalline-like lattice while the dense, osmiophilic shell, conspicious in osmium and phosphotungstic acid-treated preparations, is no longer apparent (Figs. 12 and 14).

The effects observed might find their explanation in the fact that permanganate possesses the capacity to oxidize low oxides of both osmium and melanin, and thus permits the removal of the more soluble products from the preparation.

FIGURE 5

Condyloma: This electron micrograph shows a transversal section of a dendrite (*DE*) located in an intercellular space. In the center of the dendrite are assembled mitochondria (*mi*) and at the periphery melanin granules (*mg2*), which remain separate. The dense shell surrounding each granule corresponds to a deposit of osmium.

Fixation: 1 per cent osmium tetroxide in distilled water; no stain.

Electron micrograph taken at × 10,000, enlarged to 25,000.

FIGURE 6

Condyloma: This electron micrograph illustrates the upper left part of the melanocyte of Fig. 4. In the lower left corner, the dermis is limited by the reinforced dermal membrane in contact with Malpighian cells. In the lower right corner, the picture shows the melanocyte nucleus and perikaryon (*ME*) with the origin of a dendrite (*d2*). The perikaryon is prolonged by a long dendrite (*d3*), containing melanin granules (*mg*). This dendrite lies in between two Malpighian cells and is directed perpendicularly to the dermal membrane. At the distal extremity of the dendrite, the intercellular space is narrowed and then interrupted with an intercellular bridge. (*D*): dermis; (*er*): endoplasmic reticulum.

Fixation: 1 per cent buffered osmium tetroxide; stained with PTA.

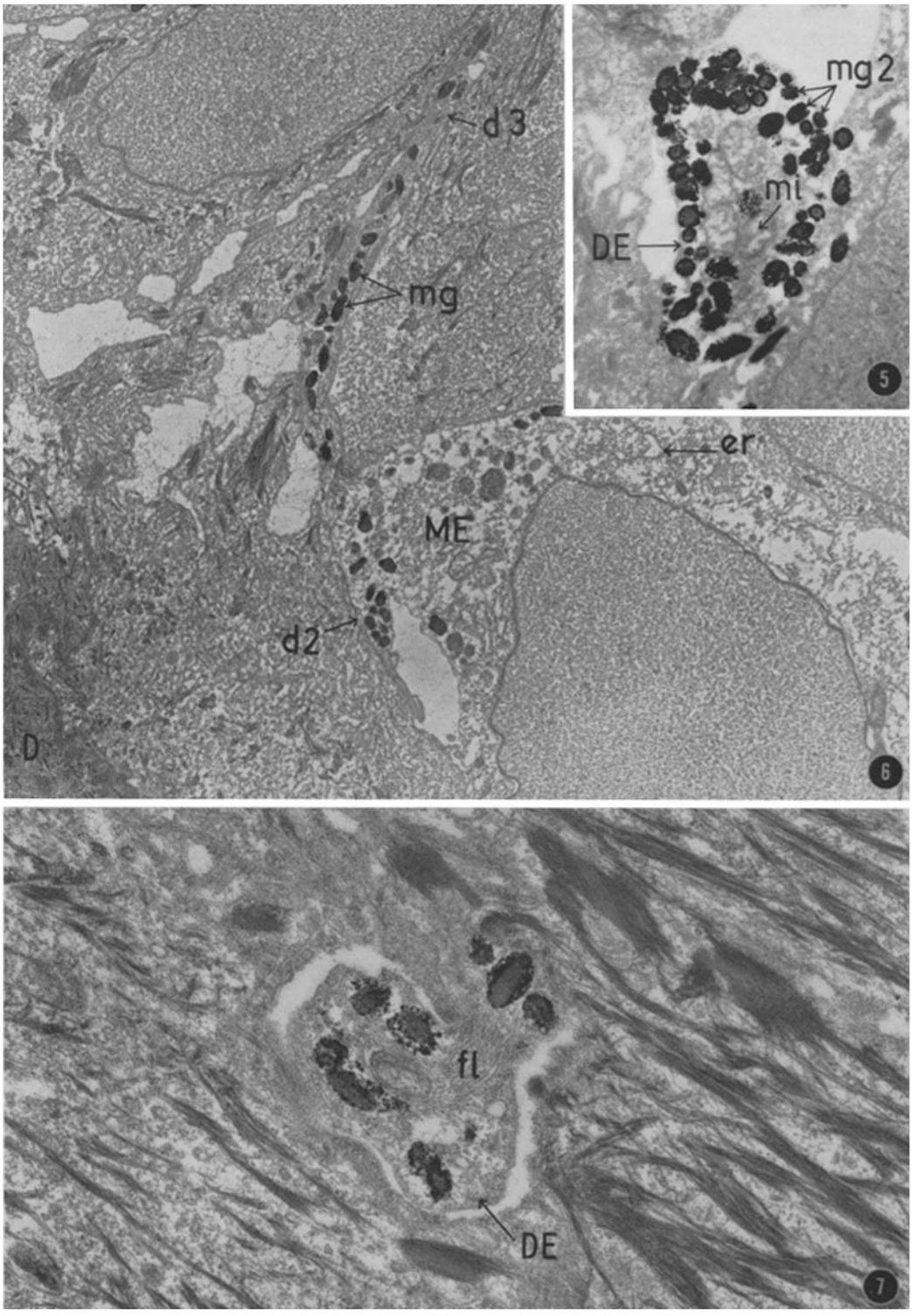
Electron micrograph taken at × 5000, enlarged to 12,000.

FIGURE 7

Hyperkeratic papilloma: This electron micrograph of an oblique section through a weaving dendrite (*DE*) shows the filaments (*fl*) characteristic of the melanocyte. These filaments, by their disposition in loose bundles, may be distinguished from the tonofilaments which form the dense tonofibrils and intercellular bridges of the surrounding Malpighian cells.

Fixation: 1 per cent buffered osmium tetroxide, stained with PTA.

Electron micrograph taken at × 13,500, enlarged to 33,000.


d3
mg
mg2
ml
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5
er
ME
d2
D
6
fl
DE
7

The removal of osmium from tissue preparations by the action of permanganate may be demonstrated by observations on similarly treated sections in the electron microscope. On the other hand, the bleaching action of permanganate on melanin is well known and has been applied in clearing up preparations of melanin for ordinary microscopy (13).

The striated structure, readily apparent in the so called light melanin granules, and the crystalline-like framework clearly demonstrated by permanganate action are not unlike in appearance and it may be inferred that they represent the same, basic component of melanin granules.

The Melanocyte Filaments

The fine filaments found in the cytoplasm of melanocytes have been detected only in preparations stained with phosphotungstic acid. These filaments, about 70 A thick, are readily distinguished from the tonofilaments of Malpighian cells by a looser arrangement and the lack of aggregation to form tonofibrils; unlike tonofilaments, they do not show the tendency to approach or contact the cell membrane, as in the case of intercellular bridges. In certain regions of the cell their abundance may produce a relatively dense matting (Fig. 8, upper left corner). Similar filaments are also found in small melanocyte dendrites, in close association with melanin granules (Fig. 7, *fl*). In the present study, a possible relation between the melanocyte filaments and the constitution of melanin granules has not been demonstrated although such a possibility is not ruled out.

DISCUSSION AND CONCLUSIONS

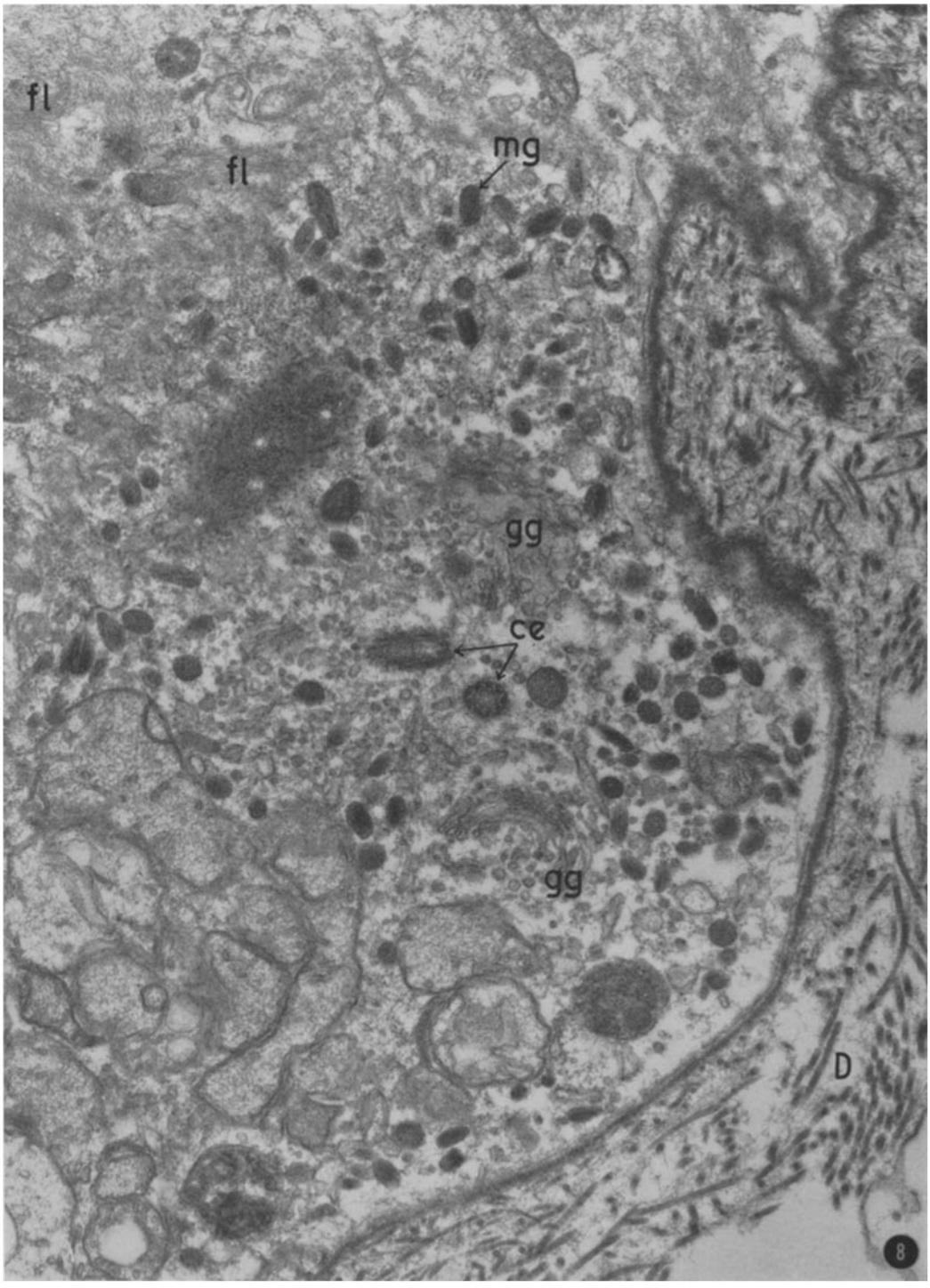
The pigment cell under study, referred to as the melanocyte, may be characterized morphologically by its localisation in the epidermis and its pronounced polymorphism, especially its tendency to form long and slender dendrites. Previous histochemical studies have demonstrated that melanocytes are endowed with the capacity to produce melanin pigment, a property associated with the presence of oxidative enzymes (tyrosinases) in loci which appear to correspond to the melanin granules (5, 11, 12). The higher resolving power afforded by electron microscopy has permitted confirmation of the view advanced by light microscopists that the melanocyte is a cellular component of normal mammalian epidermis (7, 6, 1). Staining the fixed tissues with phosphotungstic acid (14), as used in the work just reported, increases appreciably the contrast of the dermal membrane, a situation which permits one to ascertain readily the limiting boundary between the dermis and the epidermis. In such preparations, the entire melanocyte, *i.e.*, the main body and its dendrites, has always appeared to be located above the dermal membrane, and as a rule among the Malpighian cells of the basal layers. The possibility that melanocytes may pass through the dermal membrane has been suggested in a recent paper by Charles and Ingram (6). Since no such passage has been observed in the present study, nor in electron microscope observations carried out in parallel in this laboratory in other cases of human epidermis, we are not prepared at present to confirm this hypothesis. The histochemical and morphological information so far available seems to reinforce the view that the melanocyte is an integral constituent of the epidermis. On the other hand, the melanocyte appears as a "free" cell, *i.e.*, not structurally involved in the constitution of the epidermis. The melanocytes may be distinguished further from Malpighian cells by the absence of tonofibrils and of connecting intercellular bridges.

FIGURE 8

Condyloma: This electron micrograph illustrates the cell center of a melanocyte: in the center of the picture, the centrosome is composed of two centrioles (*ce*), one sectioned transversally. the other obliquely to the long axis. The centrosome is surrounded by the Golgi complex (*gg*) formed of flattened curved vesicles. Outside this area, many melanin granules are scattered in the cytoplasm. Some granules show a coiled texture (*mg*), but the fine details do not appear in this micrograph. In the upper left corner, fine filaments (*fl*), distinct from tonofilaments, form a loose matting. (*D*): dermis.

Fixation: 1 per cent buffered osmium tetroxide, stained with PTA.

Electron micrograph taken at × 12,000, enlarged to 30,000.


fl
fl
mg
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ce
gg
D
8

FIGURE 9

Condyloma: This electron micrograph shows sections of two dendrites which contain melanin granules at different stages of development. The right dendrite (*DE*) contains mitochondria (*mi*) and a melanin granule of the light type (*mg 1*). The structured character of this granule is clearly apparent. The second dendrite (left of the picture) shows two melanin granules of the dense type (*mg 2*) which appear composed of a uniformly dense mass associated with coarsely granular, osmiophilic material.

Fixation: 1 per cent osmium tetroxide in water; no stain.

Electron micrograph taken at × 10,000, enlarged to 41,000.

FIGURE 10

Condyloma: This electron micrograph illustrates the fine texture of the melanin granules of the dense type. The larger granule (*mg 3*) is sectioned parallel to its main axis. It shows longitudinal striae made apparent by phosphotungstic acid staining. This texture appears embedded in a structureless material.

Fixation: 1 per cent buffered osmium tetroxide in distilled water; stained with PTA.

Electron micrograph taken at × 20,000, enlarged to 50,000.

FIGURES 11 and 12

Human vulvar skin: Sections exposed to potassium permanganate for 20 and 40 minutes respectively.

Fixation: 1 per cent osmium tetroxide in distilled water; stained with PTA.

Electron micrographs taken at × 25,000, enlarged to 62,000.

FIGURE 11 Treatment with permanganate for 20 minutes has revealed a texture resembling that seen in Fig. 10, *i.e.*, a striation parallel to the long axis of the granule. In addition, the action of permanganate has resulted in the disappearance of the osmiophilic shell. It may be significant that the outer region, previously occupied by osmiophilic material (Fig. 10) is now of lesser density than the center of the granule.

FIGURE 12 Treatment with permanganate for 40 minutes reveals the existence of a basic structure, grid-like in appearance, and involving the entire granule.

FIGURE 13

Cat skin (nose): The rod-like shape of the melanine granules shown in this, and the following micrograph, is typical for those found in the cat skin. Following PTA staining these granules were found to exhibit, but faintly, the longitudinal striation observed in the case of the human vulvar skin (Fig. 10).

Fixation: 1 per cent osmium tetroxide in distilled water; stained with PTA.

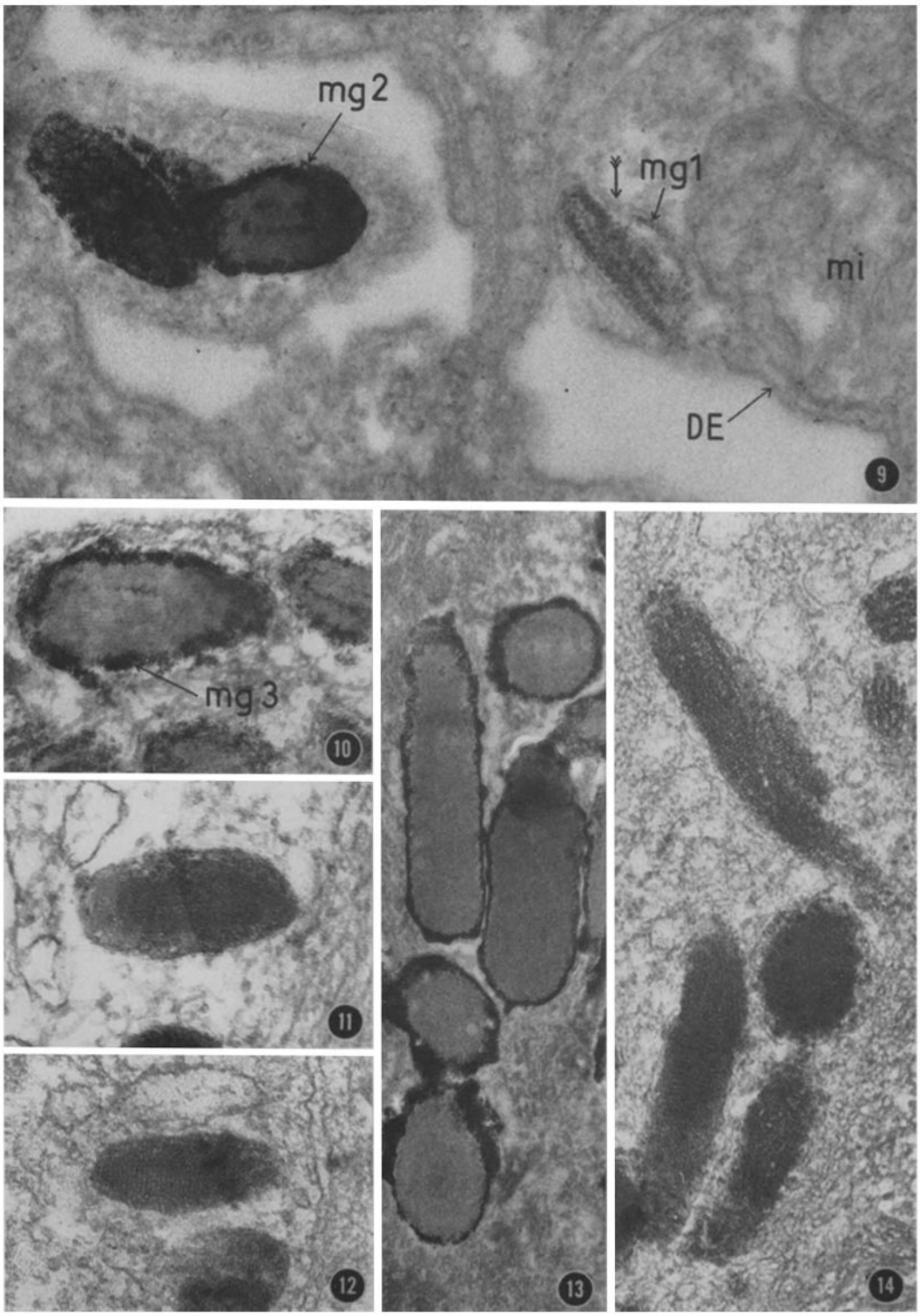
Electron micrograph taken at × 15,000, enlarged to 82,000.

FIGURE 14

Cat skin (nose): As in the case of the granules shown in Fig. 12, treatment of the section with permanganate for 40 minutes demonstrates that the melanin granules possesses a basic organized component in the shape of a crystalline-like lattice.

Fixation: 1 per cent osmium tetroxide in distilled water; stained with PTA; potassium permanganate, for 40 minutes.

Electron micrograph taken at × 20,000, enlarged to 82,000.


mg2
mg1
mi
DE
9
mg3
10
11
12
13
14

The particular morphology of the melanin granules, based on the observations reported in the present work, appears to result from the presence of two distinct constituents: (*a*) a framework, the regularity of which gives a crystalline-like appearance to the structure, especially in sections parallel to the main axis of the granule (Fig. 9); (*b*) a diffuse component which, under present conditions of resolution, seems to be structureless; this diffuse constituent possesses a relatively high density and appears to fill the space between the lattice-work of the granule (Figs. 10 and 13); (*c*) a non-organized shell on which the osmium is deposited and which, after removal, appears as a granular substance; (*d*) a limiting single membrane.

The hypothesis which might be ventured at the moment, based on presently available histochemical and electron microscope information, is that the basic framework of the granule is essentially protein in nature and may include among its constituents the enzymatic system capable, under proper conditions, of producing melanin. The diffuse component deposited in this framework, presumably melanin, might be the result of this activity. Tyrosinases involved in the production of melanin pigments are known to be copper protein enzymes. The demonstration by Claude (8) that copper is present in certain melanin granules isolated by differential centrifugation would be in agreement with the present working hypothesis.

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