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# Aging of Melanocytes

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**Choroidal melanocytes of the eyes of postnatal animals are classified as postmitotic terminally differentiated cells. They have specific granules, the melanosomes, which undergo changes qualitatively and quantitatively correlated to the animal's increasing age. Epidermal melanocytes, which normally divide only on demand or by stimulation, are classified as intermittent mitotic cells. During their development, lentigines and nevi of the skin show progressive ultrastructural and cytochemical changes similar to those in the choroidal cells, and thus may be considered as aging populations of skin melanocytes. These facts have led to the conclusion that choroidal melanocytes may be used advantageously as a model for studying changes in cells from maturation to senescence.**

Diploid human fibroblasts in culture change from rapidly replicating (phase I), to slowly replicating (phase II), to nonreplicating (phase III) cells [1]. Their life span, in terms of the number of population doublings, depends upon the age of the donor [2,3]. Fibroblasts from young individuals proliferate more times than those from old people, but the sum total of their population doubling is a constant characteristic of the species. Although investigators have proposed several hypotheses to explain this observation [4,5], so far no one has adequately explained the mechanisms of aging operating in all cells. Most investigators have emphasized changes in the ability of cells to divide as indicators of the aging process. However, not all postnatal mammalian cells divide continuously; some replicate only intermittently, and some stop dividing early in postnatal life.

Basal epithelial cells of the epidermis divide continuously because they differentiate into a dead product, keratin. When epidermis is grown in culture, the mitotic rate generally is not in keeping with the rate of keratinization, and the culture dies. Given an optimal environment, however, these cells can proliferate continuously [6].

Fibroblasts and epidermal melanocytes *in vivo* are intermittently mitotic, but both fibroblasts and melanocytes, in certain stages of their lives (for instance, during embryonic development), must be rapidly replicating if the organism is to acquire the population of these cells it requires. At certain stages of development the rate of proliferation changes; the cells become differentiated and acquire the specific properties that characterize the particular cell types. Only at this time do these cells become intermittently mitotic. After this stage has been reached, they are required to replicate only when cell injury or death occurs or when extra demands are placed upon their numbers or products.

Muscle and nerve cells are nonmitotic. These cells also must go through the continuously mitotic stage until they reach sufficient numbers. Only then do they cease division, become organized as specialized tissue, and serve their specific functions. Apparently the organism does not require periodic replenishment of these cells, which, once formed, are expected to last for the life of the organism.

We have demonstrated that choroidal melanocytes of adult eyes are postmitotic, terminally differentiated cells [7]. Obviously, we could not use the cell's inability to divide as the indicator of aging changes. Because melanocytes synthesize a specific enzyme and produce a characteristic product, the melanosomes, we logically assumed that a decline in these well-recognized functions was associated with aging.

## MATERIALS AND METHODS

### *Eyes of Rhesus Macaques*

Eyes from rhesus (*Macaca mulatta*) fetuses of gestational ages varying from 65 days to 165 days (term) and eyes from newborn, infant, adolescent, young adults, and old adults of different ages were used. All eyes were removed aseptically, soaked in several changes of Hanks' balanced salt solution with antibiotics, and cut anteroposteriorly into 4 parts. After the removal of the crystalline lens and the vitreous, the pigmented membrane (the iris, ciliary body, and choroid with their corresponding pigment epithelium) was exposed.

For histological study, a strip of the pigmented membrane was fixed in 10% buffered neutral formalin and prepared for paraffin sectioning. Another strip was frozen and sectioned for enzyme studies.

For electron microscopy, strips were fixed in 2.7% glutaraldehyde in Millonig's phosphate buffer [8] at pH 7.4 for 1 hr. While in the fixative, the tissue was divided into iris, ciliary body, and choroid-retina and cut into pieces (about 1 $mm^3$). One-half of these pieces were incubated in 0.1% L-3,4-dihydroxyphenylalanine (dopa, Sigma Chemical Company) in phosphate buffer at pH 7.4 for 5 hr and postfixed for 90 min in cold 1% $OsO_4$ in cacodylate buffer [9]. Control pieces were incubated in the buffer without dopa and were processed in an identical manner. The pieces were then washed, dehydrated in a series of graded alcohol solutions, and embedded in Spurr [10]. One-micrometer sections were cut and stained with toluidine blue so that specific areas or cells could be selected for ultrathin sectioning with a Porter-Blum MT-2 ultramicrotome. Ultrathin sections were stained with uranyl acetate and lead citrate [11], and examined with a Philips 200 electron microscope.

### *Human Skin*

Biopsy samples were taken from lesions diagnosed clinically as lentigo simplex; lentigo senilis; multiple lentigines syndrome; nevus spilus; junctional, compound, and intradermal nevus; lentigo maligna; and superficial malignant melanoma. Each sample was cut into halves. One was processed routinely and stained with hematoxylin-eosin; the other was processed for electron microscopy. Like the rhesus eye tissues, the latter specimens were again divided into 2 parts. One part was incubated in dopa reagent and the other was used as a control. Otherwise both were processed in an identical fashion.

## RESULTS

### *Choroidal Melanocytes*

Melanocytes of all ages were filled with brown or brownish black melanin pigment granules (melanosomes) that appeared dark green in toluidine-blue- or Giemsa-stained sections. In the young animals, e.g., newborns to 3-yr-olds, these granules were uniform in color, size, and shape (Fig 1). Melanosomes at age 7 under a light microscope were still uniform. But under an electron microscope we observed that a few of these granules had lost some electron density. Adults (12 to 13 yr of age) had considerable numbers of melanosomes that varied in size and

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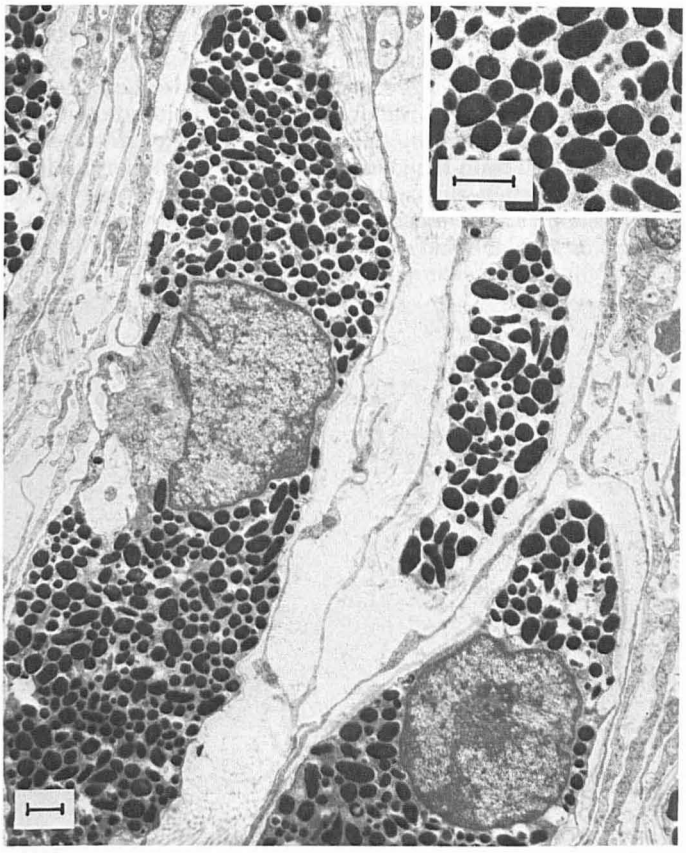

FIG 1. Choroidal melanocytes from the eye of a 3-yr-old rhesus macaque. The melanosomes appear uniform in size and electron density. *Bar* = 1 μm. *Inset:* a higher-magnification photograph showing melanosome details.

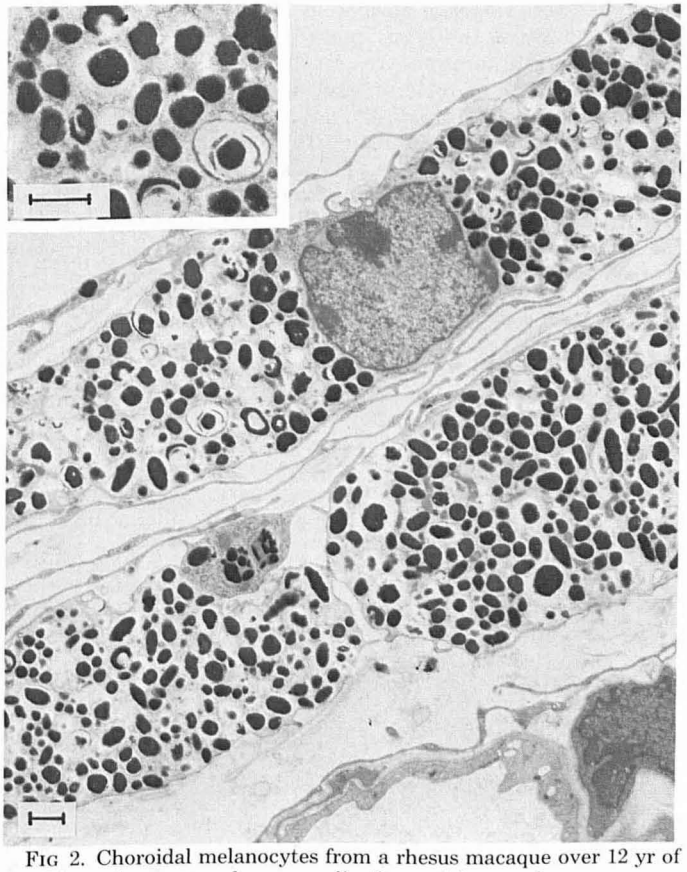

FIG 2. Choroidal melanocytes from a rhesus macaque over 12 yr of age. The melanosomes show vacuolization and form melanosome complexes. *Bar* = 1 μm. *Inset:* a higher-magnification photograph showing melanosome details.

in color and that appeared vacuolated (Fig 2). In toluidine-blue-stained sections, the granules did not stain uniformly dark green, but rather varied from dark green, to light green, to brown, to light brown.

In the eyes of animals at least 15 yr of age, more than 60% of the melanocytes showed extensive alterations in the melanosomes; almost all cells had some altered granules. Under an electron microscope, the changes were more obvious. Melanosomes showed extensive loss of electron density and resembled vacuoles filled either completely or partially with a homogeneous material of a low density. Some vacuoles had electron-dense cores of varying size and shape, some had dense or lighter cores, and some had cores that were half-dense, half-light. Some melanosomes appeared fused together as a melanosome complex enclosed by a unit membrane (Fig 3). Table I* shows the percentage of melanocytes that had extensively altered melanosomes. About 10 to 15% of melanocytes had changes at age 7, 50% at age 13, and more than 60% at 15. Indeed, in animals more than 15 yr of age, almost all melanocytes had some altered melanosomes, and more than 60% had extensive changes.

### *Lentigo Simplex, Lentigo Senilis, Multiple Lentigines Syndrome, and Nevus Spilus*

Lentigo simplex tissue showed a slight elongation of the rete ridges, an increase in the concentration of melanocytes in the basal layer, an increase in the amount of melanin in both the melanocytes and the basal keratinocytes, and melanin-containing macrophages in the dermis [12]. These changes became exaggerated in lentigo senilis, which had elongated and hyper-

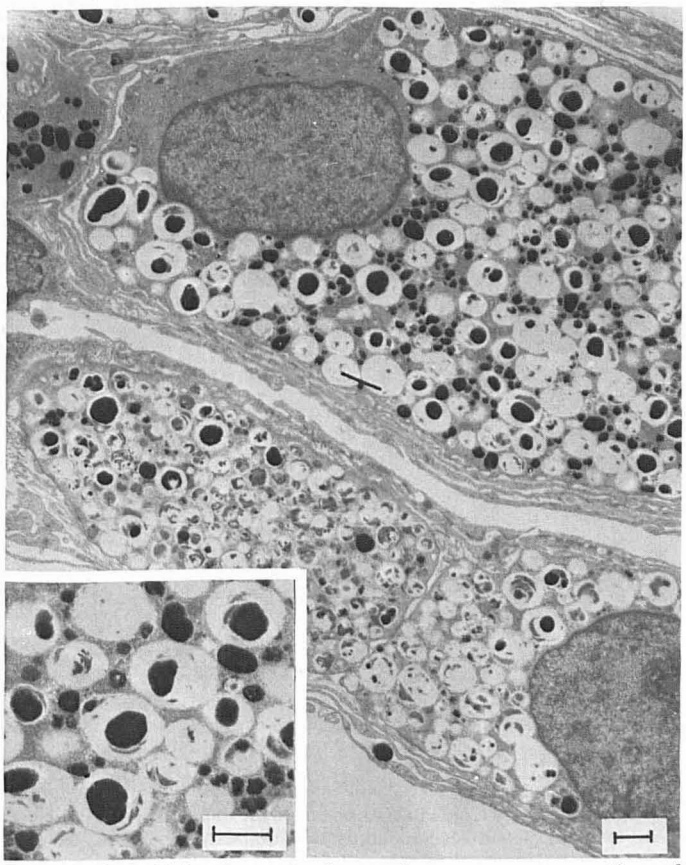

FIG 3. Choroidal melanocytes from a rhesus macaque over 15 yr of age. There is marked vacuolization. *Bar* = 1 μm. *Inset:* a higher-magnification photograph showing melanosome details.

* The age of the adult animals is an approximation only. Because the adults were not born or raised at the Oregon Regional Primate Research Center, their ages were initially estimated at the time of procurement and then were adjusted as time passed.

pigmented rete ridges, a marked increase in the concentration of melanocytes in the basal layer as well as higher up in the epidermis. Both keratinocytes and melanocytes contained increased amounts of melanin and appeared more darkly pigmented than the surrounding uninvolved skin. Electron microscopic examination revealed that the keratinocytes were filled with melanosome complexes containing many melanosomes. Most of these contained from 2 to 10 melanosomes within a unit membrane. (Melanosome complexes are normally seen in Caucasian and lightly pigmented skin [13], but usually they are seen as 2 to 5 melanosomes enclosed by a unit membrane.)

TABLE I. *Choroidal melanocytes with altered melanocytes in different age groups*

| Age (yr) | Melanocytes with altered melanosomes (%) |
|---|---|
| 3 | 0 |
| ≥7 | 10–15 |
| ≥13 | 50 |
| >15 | 60–70[a] |

[a] Some large cells may be completely filled with vacuoles.

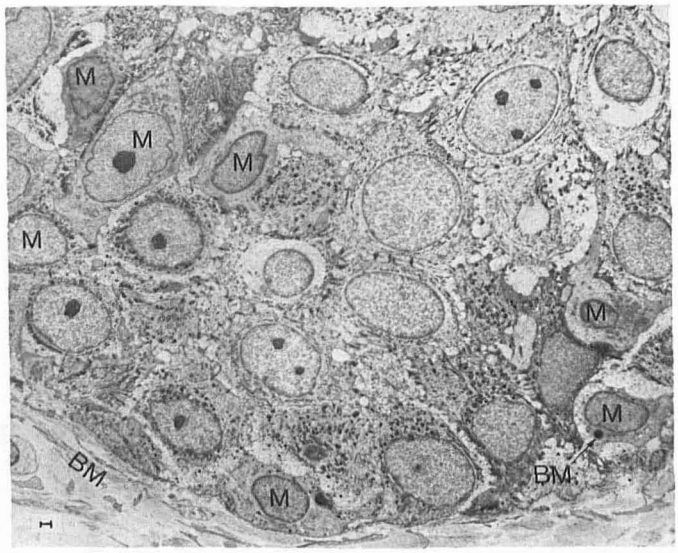


FIG 4. A section of a lentiginous lesion from a patient with multiple lentigines syndrome. Note the increased number of melanocytes (*M*) in the epidermis and a melanocyte with a giant melanosome (*arrow*). *BM* = basement membrane. *Bar* = 1 μm.

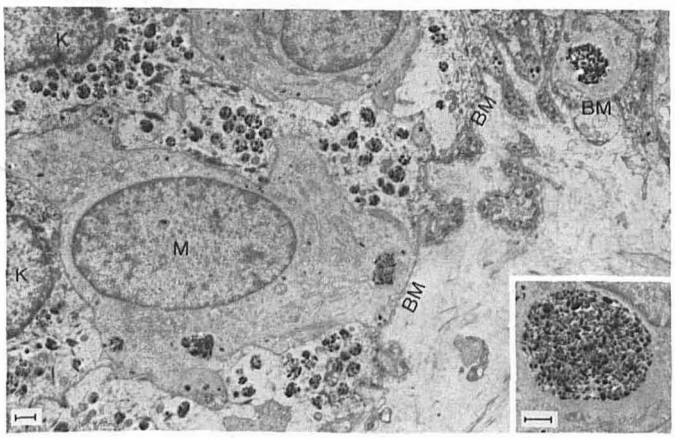


FIG 5. Epidermal melanocytes (*M*) with normal-sized as well as giant melanosome complexes. Note the many melanosome complexes in the keratinocytes (*K*). The cytoplasm of the melanocytes is relatively clear, with only scattered melanosomes and large melanosome complexes. *BM* = basement membrane. *Bar* = 1 μm. *Inset:* a giant melanosome complex containing hundreds of melanosomes.

In tissue of multiple lentigines syndrome or nevus spilus [14], in addition to the increase in the concentration of melanocytes and the increase in melanosome complexes in the keratinocytes (Fig 4), there were a few giant melanosome complexes containing up to hundreds of melanosomes bound by a single membrane (Fig 5). These sometimes were in the melanocytes as well as in the keratinocytes. In addition, there were a few giant melanosomes, which were characteristically round in shape and varied tremendously in size; some were a few times larger than normal, and others were as much as 30 to 50 times

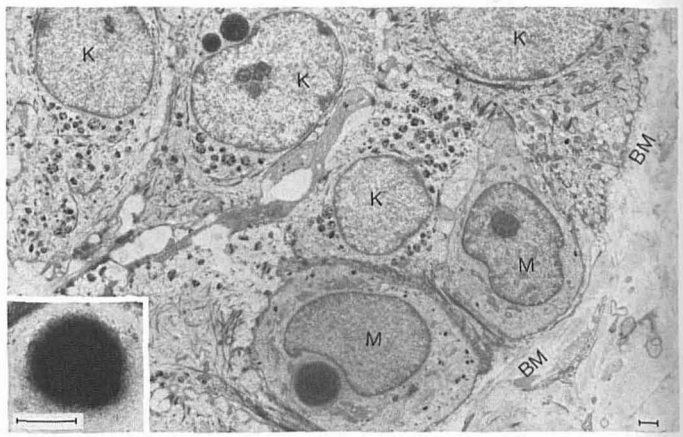


FIG 6. Epidermal melanocytes (*M*) and keratinocytes (*K*). One melanocyte has a giant melanosome and a small number of normal-sized melanosomes. One keratinocyte has 2 giant melanosomes and some melanosome complexes. *BM* = basement membrane. *Bar* = 1 μm. *Inset:* a giant melanosome. Note the electron-dense core, rows of electron-lucent vesicles and fibrillar materials at the periphery.

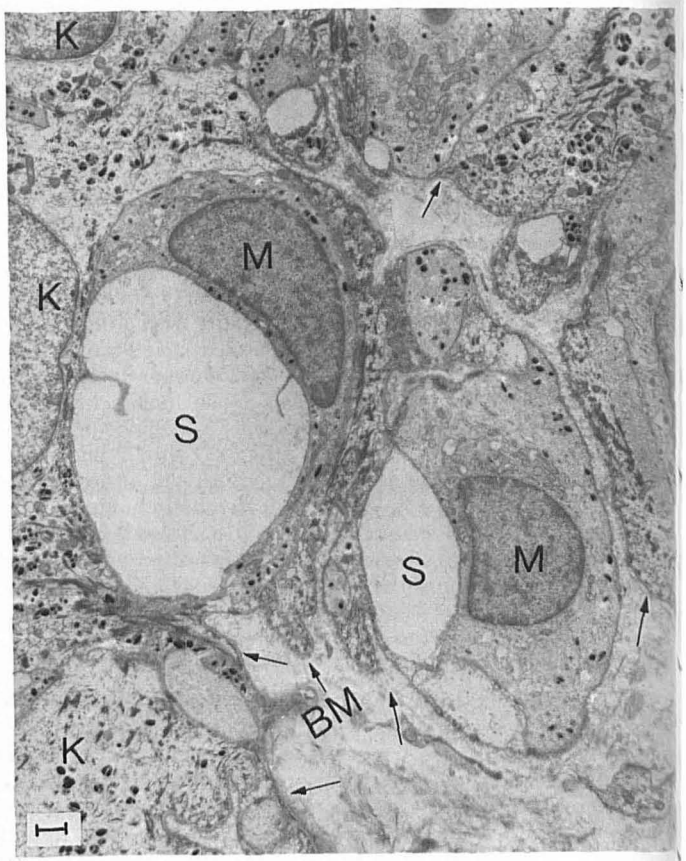


FIG 7. Basal melanocytes (*M*) in the epidermis. There are large spaces (*S*) next to the melanocytes. *Arrows* indicate basement membrane. *BM* = basement membrane; *K* = keratinocytes. *Bar* = μm.

larger (Fig 6). They were extremely electron-dense and appeared solid black, especially in the center. At the periphery there were some flocculent or fibrillar materials and sometimes concentric rows of electron-lucent vesicles similar to melanosomes seen in black hair [15]. No definite membrane was resolved. Melanocytes often appeared to be retracted from their neighboring keratinocytes, i.e., we saw large spaces around them (Fig 7 and 8). The cell membranes of the melanocytes appeared intact. In or at the border of the spaces we saw tufts of tonofilaments, and we found no definite cell membrane for the keratinocytes (Fig 8). For the moment, the significance of these findings is unknown.

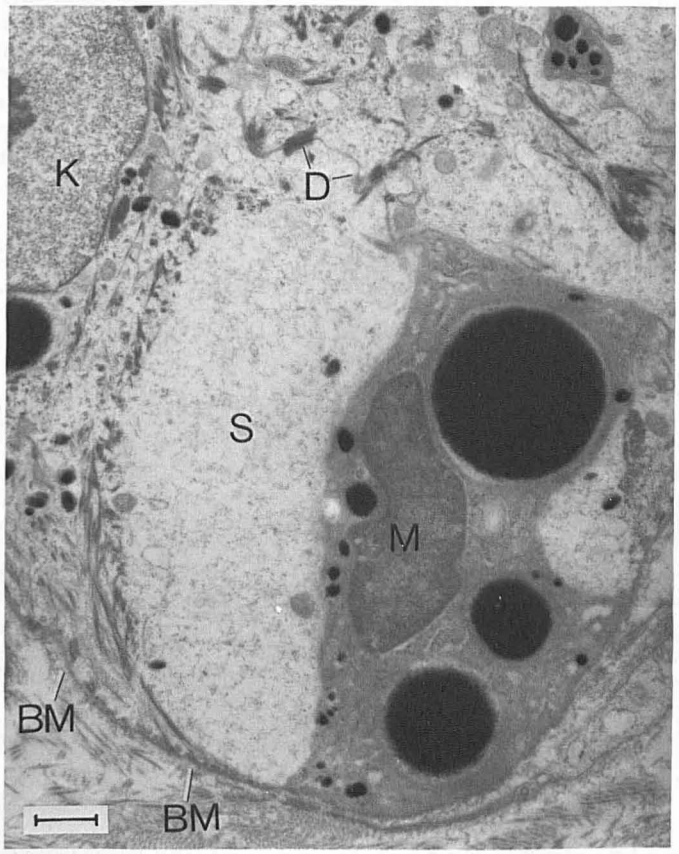


FIG 8. One melanocyte (*M*) containing 4 giant melanosomes of widely different sizes. The cell appears to be pushed away from the neighboring keratinocytes (*K*) by large spaces (*S*) containing flocculent material. Note the tonofibrils of the keratinocytes next to the edge of the space; there is no evidence of a definite cell membrane. A few intact desmosomes (*D*) are identifiable. *BM* = basement membrane. *Bar* = 1 μm.

TABLE II. *Ultrastructure and enzyme activity in epidermal melanocytes and nevus cells*

| Melanocytes | Nevus cells |
|---|---|
| Melanosomes in Stages I-IV | Melanosomes in Stages III and IV or abnormal premelanosomes somewhat similar to pheomelanosomes (granular background, irregular pattern of fibrils, myelin-like, ring-shaped or half-moon-shaped, etc.) |
| Single | Predominantly complexes |
| Dopa-positive | Positive in junctional area and upper dermis, negative lower down |
| Vacuoles or lipid-like droplets rare | Vacuoles or lipid-like droplets common |
| Occasional cilia | Dermal pigment cells often have cilia (intradermal nevus cells, melanocytes of blue nevus, and nevus of Ota) |

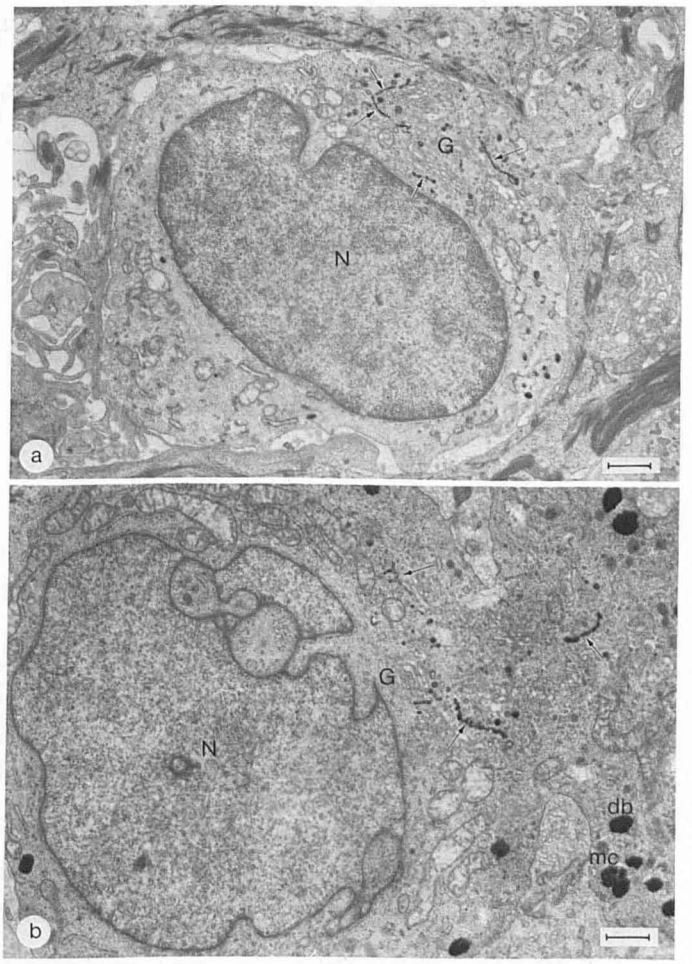


FIG 9. Comparison of an epidermal melanocyte and a dermal nevus cell incubated in dopa. *Bar* = 1 μm. *a*, melanocyte. There are only a few stage III or stage IV melanosomes in the cytoplasm, active Golgi complexes (*G*), and dopa reaction product in the nearby vesicles and cisternae (*arrows*). *b*, nevus cell. Note the large, irregular nucleus and dopa reaction product in the Golgi-associated vesicles and cisternae (*arrows*). No typical melanosomes, except some residual bodies (*db*) with granular background and melanosome complexes (*mc*), are identifiable. Note the large size of the nucleus (*N*) as well as of the whole cell.

### *Junctional and Intradermal Nevi*

Nevus cells usually had larger cell bodies and shorter and thicker processes than epidermal melanocytes. Table II compares the ultrastructure and tyrosinase activities of nevus cells and normal epidermal melanocytes. Both junctional nevus cells and epidermal melanocytes were dopa-positive (Fig 9).

The ultrastructure of junctional nevus cells was similar to that of melanocytes in lentigines. They contained mostly melanosome complexes, and there were spaces next to the keratinocytes, which contained many melanosome complexes. They differed from the epidermal basal melanocytes of skin (which normally has no melanosome complexes [Fig 9]).

In intradermal nevi, the nevus cells immediately below the dermoepidermal junction, both ultrastructurally and cytochemically, resembled the junctional nevus cells. Often we saw more residual bodies with granular matrices of uneven electron densities and vacuolization instead of the typical melanosome complexes that predominated in the junctional nevus cells (Fig. 10*a*). Some of the dermal nevus cells were still dopa-positive, but their reactions usually were not as strong as those of the latter. Those in the lower dermis were nonreactive (Fig 10*b*). The dermal nevus cells quite often had cilia (Fig 11*a*); some-

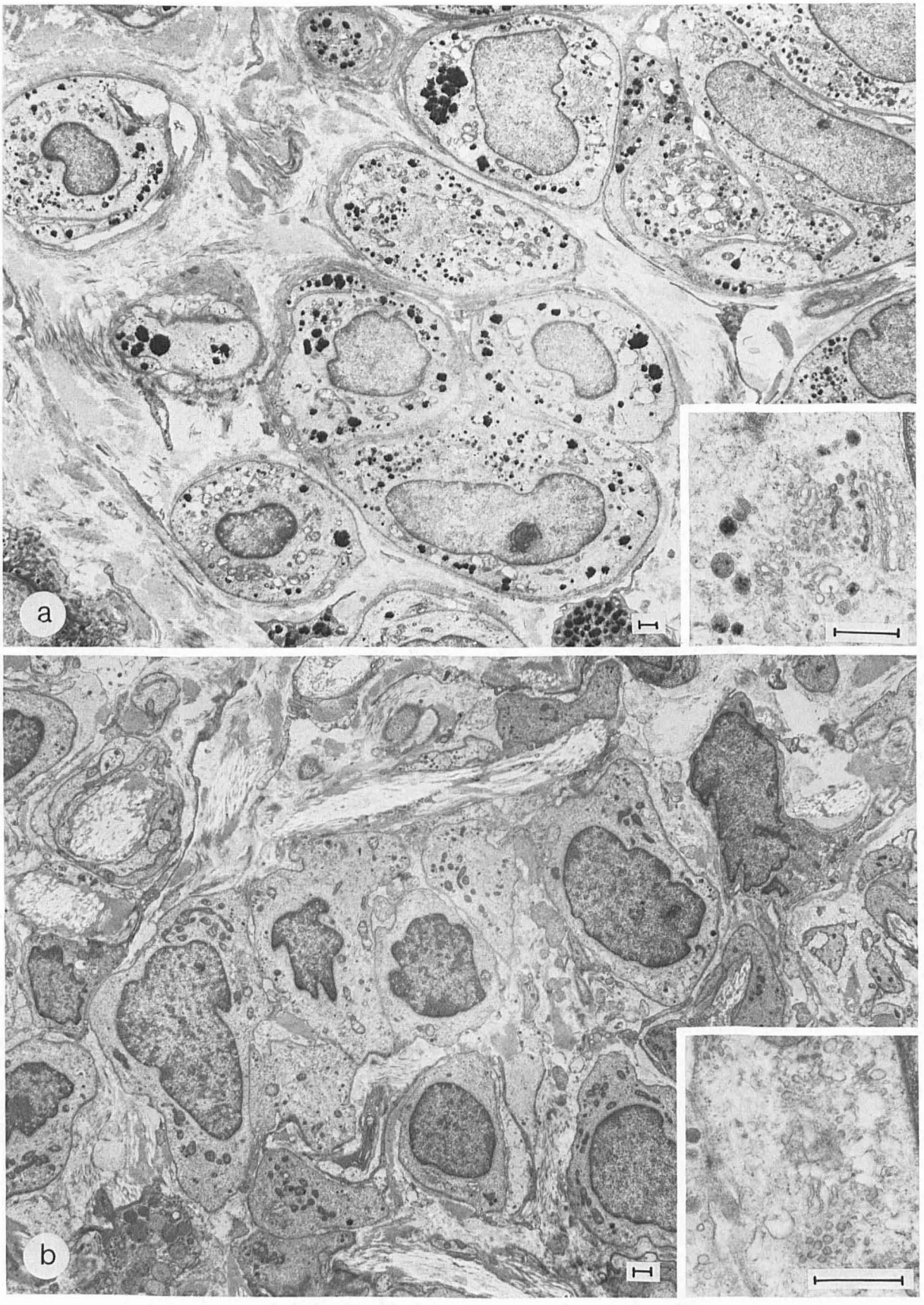


FIG 10. An intradermal pigmented nevus incubated in dopa. *Bar* = 1 μm. *a*, nevus cell nests in the upper dermis. Note the predominant melanosome complexes of varying sizes in all the nevus cells. *Inset:* higher-magnification photograph of a nevus cell showing dopa reaction product in the Golgi-associated vesicles and cisternae and a few residual bodies with granular background of an uneven electron density. *b*, nevus cell nests in lower dermis. Note the lack of pigmented granules. *Inset:* higher-magnification photograph showing the absence of dopa reaction product in the Golgi areas. There are a few residual bodies with granular background similar to those seen in *a*.

times these cilia were also present in their epidermal counterparts. Lower down in the dermis, the nevus cells became less pigmented or were not pigmented. They contained few or no melanosome complexes, and usually were dopa-negative. Often these nevus cells contained a few residual bodies with granular matrices, lamellated membranes, or both.

Even though the intracytoplasmic organelles of dermal nevus cells differed somewhat from those of dermal melanin-containing macrophages (for instance, we rarely saw macrophages with active Golgi complexes [Fig 11*b*] but often saw nevus cells with such complexes), we could not easily differentiate these 2 cells types because, like macrophages, the nevus cells contained melanosome complexes and were, at times, also dopa-negative. The most important difference was that macrophages were usually found in the dermis singly and were widely separated from each other by collagen and elastic fibers, whereas nevus cells occurred in aggregates and were well delineated from the surrounding connective tissues. Both macrophages and nevus cells had melanosome complexes. In addition, some had melanolysosomes and lipid-like droplets. Some of these droplets were fused with melanosomes to form melanolysosomes (Fig 11*b*).

A special type of nevus, the so-called balloon cell nevus [12,16], contained melanosome complexes and residual bodies. Some of these cells were partially or completely filled with vacuole-like or lipid-like organelles (Fig 12). These granules were not true vacuoles because they were partially and sometimes completely filled with materials of a low electron density (Fig 12*a*, *b*). Some contained small vacuoles as well as irregular lamellated membranes of varying sizes and shapes. At higher magnification, we saw that some of these vacuole-like granules contained fibrils in a loose and disorderly arrangement (Fig 12*c*). Some of the fibrils exhibited a periodicity similar to that seen in the melanosomes (Fig 12*d*). Some of these vacuoles were fused to materials similar to melanin and of the same extreme electron density (Fig 12*b*). Close to the nest of nevus cells there were large spaces, filled with flocculent materials (Fig 12*a*), similar to the spaces seen adjacent to the epidermal melanocytes (Figs 7 and 8).

### *Lentigo Maligna and Malignant Melanomas*

A wide variety of changes were seen in lentigo maligna melanosomes. Many of the changes were similar to those seen in lentigines, but were more intense (Fig 13). In parts where dermal invasion had occurred (i.e., in truly malignant melanomas), the melanoma cells showed similar, but more intense and varied, changes in the melanosome complexes, melanolysosomes, and vacuoles. The vacuoles were partially or completely filled with granular materials of varying densities (Fig 13*a*, *b*). In some cells, we saw large vacuole-like structures with only remnants of melanosomes in a light background of flocculent materials and membranes (Fig 13*b*). In 1 epidermal melanoma cell, we found melanosomes, melanosome complexes, and vacuole-like structures of varying densities in the nucleus (Fig 13*c*). In another superficial melanoma, the melanosomes appeared ring-like (Fig 13*d*) and resembled those described first by Mishima [17]. Other melanoma cells had organelles containing fibrils similar to those seen in the balloon cell nevus (Fig 14).

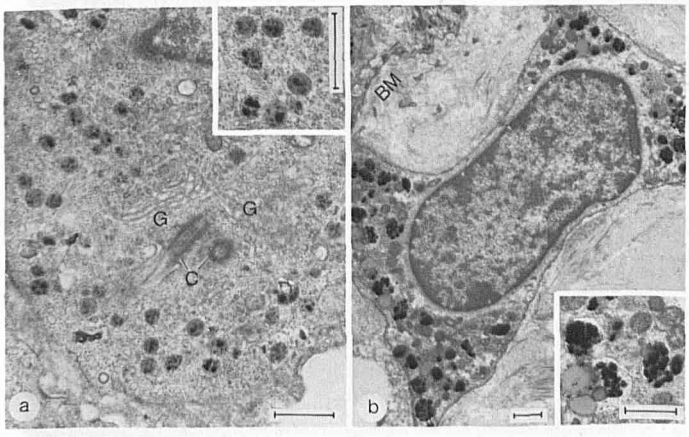


FIG 11. Comparison of a dermal nevus cell and a macrophage, both incubated in dopa. Bar = 1 μm. *a*, nevus cell. Note the lack of dopa reaction product in the Golgi-associated vesicles and cisternae. There are no typical melanosomes except some residual bodies with uneven granular background and lysosome-like granules (melanolysosomes). A pair of cilia, (*C*), 1 cut tangentially and 1 cut longitudinally, are protruding from the cell surface. *Inset:* residual bodies with granular background of an uneven density. *b*, macrophage. Note the many melanosome complexes, residual bodies, and lipid-like droplets, which appear to have fused with melanosomes to form melanolysosomes. *BM* = basement membrane; *G* = Golgi complex. *Inset:* high-magnification photograph showing residual bodies and melanolysosomes.

## DISCUSSION

Both melanocytes of the skin and those of the uvea of the eye are derived from the neural crest [18, 19]. During embryonic development they migrate and then settle down, mainly in the basal layer of the epidermis and in the stromata of the iris, ciliary body, and choroid of the eye. In addition, they have been found in the dermis in mongolian spots, blue nevi, nevi of Ota, and nevi of Ito [12]. These conditions are collectively known as dermal melanocytosis.

In the epidermis, melanocytes do not appear to actively engage in DNA synthesis or division unless they are stimulated, as in the case of wounding and exposure to actinic rays. During one's lifetime, nevi and lentigines develop in the skin. The latter are commonly known as liver spots or age spots because they appear in older individuals and increase in number and size with advancing years. We can identify the stage of development of these lesions on the basis of their histological features.

Lentigo simplex starts in a younger age group as a slight elongation of the rete ridge and an increase in the number of melanocytes in the basal layer. The changes become exaggerated in lentigo senilis, which is seen in older individuals and which probably develops from lentigo simplex. These changes are probably manifestations of the response of the epidermis to various environmental stimuli and insults over time. So far keratinocytes and melanocytes respond in like manner, i.e., both show increases in number and activities. In lentigo maligna the melanocytes increase further in number, so that as time goes on they form aggregates or nests [12]. The epidermis does not increase further in thickness; rather, it becomes flattened, an indication that the keratinocytes have not been keeping pace with the proliferative activities of the melanocytes and that they are outnumbered by the melanocytes in the basal layer of the epidermis. This loss of the normal spatial relationship between keratinocytes and melanocytes suggests a loss of growth control in the melanocytes, a prelude to malignant transformation. When individual or nests of melanocytes show pleomorphism of nuclei and size, an increase in the nucleus-cytoplasm ratio, and abnormal melanocytes in the upper epidermis, lentigo maligna melanoma *in situ* is the diagnosis. Invasion of the dermis by these abnormal melanocytes is a characteristic sign of malignancy. The condition is commonly designated as a lentigo maligna melanoma or a superficially spreading malignant melanoma.

In nevus cell nevi or common moles, the nevus cell nests at the epidermodermal junction also are the result of melanocytic proliferation. These cells increase in number; expand as they develop in all directions; and push aside, without invading, the connective tissue along the way to form the intradermal nevus. These cells often remain in aggregates. Deeper in the dermis, they lose their proliferative activity as well as their melanogenic activity, a phenomenon indicated by their loss of dopa reactivity. In other words, they grow old. In the development of nevi, epidermal melanocytes change from small dendritic, enzymatically active, functioning cells into large epithelioid forms in the upper dermis, into small lymphocytoid, and finally into spindle

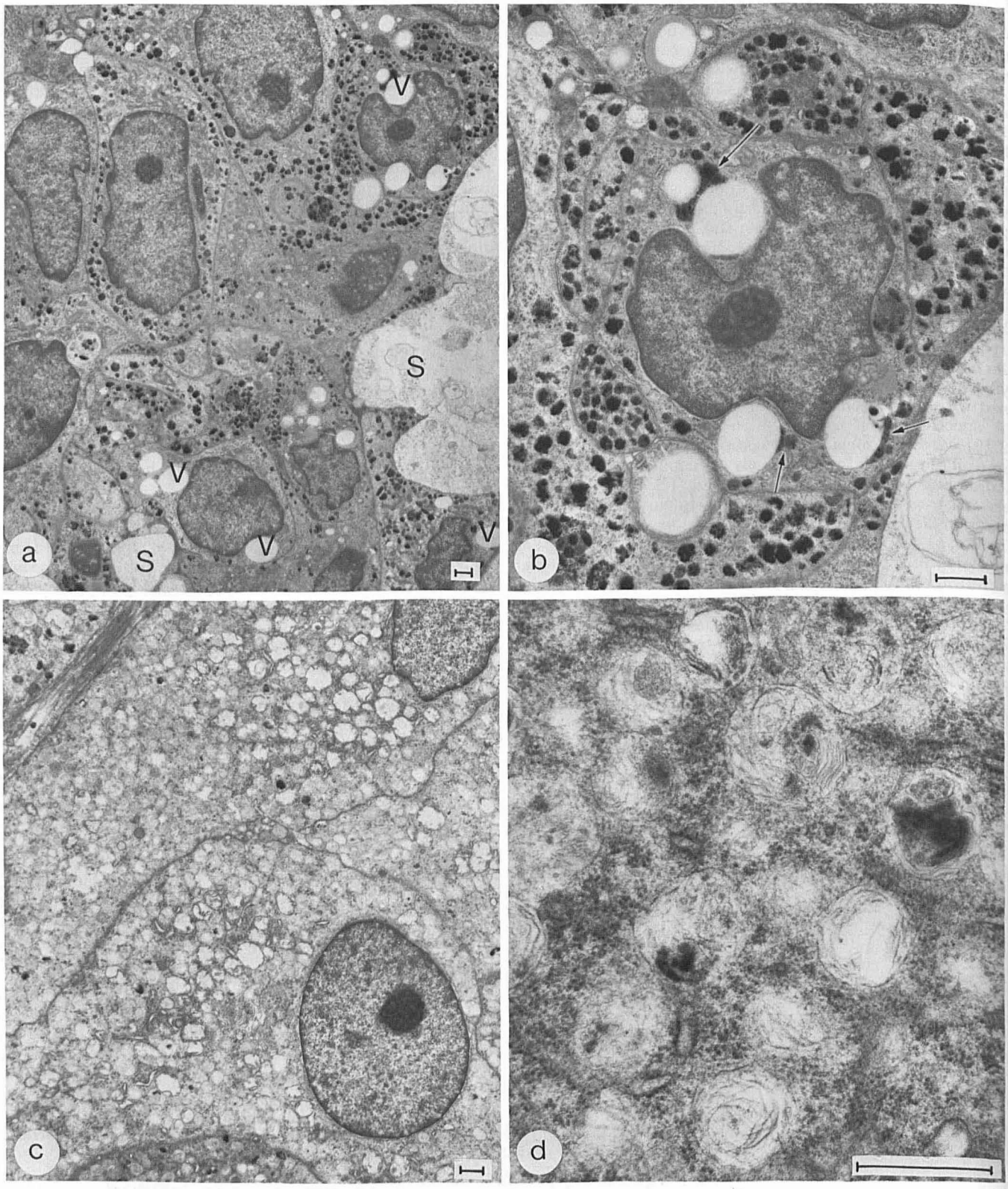


FIG 12. Balloon cell nevus. *Bar* = 1 μm. *a*, a balloon cell nevus nest with many melanosome complexes and large vacuoles (*V*) in some of the nevus cells. Note the large spaces (*S*) filled with flocculent material—similar to that seen in the epidermal melanocytes of lentigines—next to the nevus cells at the periphery of the cell nest. *b*, high-magnification photograph of a nevus cell with vacuoles. The vacuoles are filled with homogeneous material of a very low electron density. Some are partially or completely rimmed with a high-density material, and some are fused with material having the same density as melanin (*arrows*). *c*, balloon cells with vacuoles, many of which contain fibrillar material of an uneven density. *d*, high-magnification photograph of the vacuoles. Note the fibrils with a periodicity similar to that in stage II melanosomes.

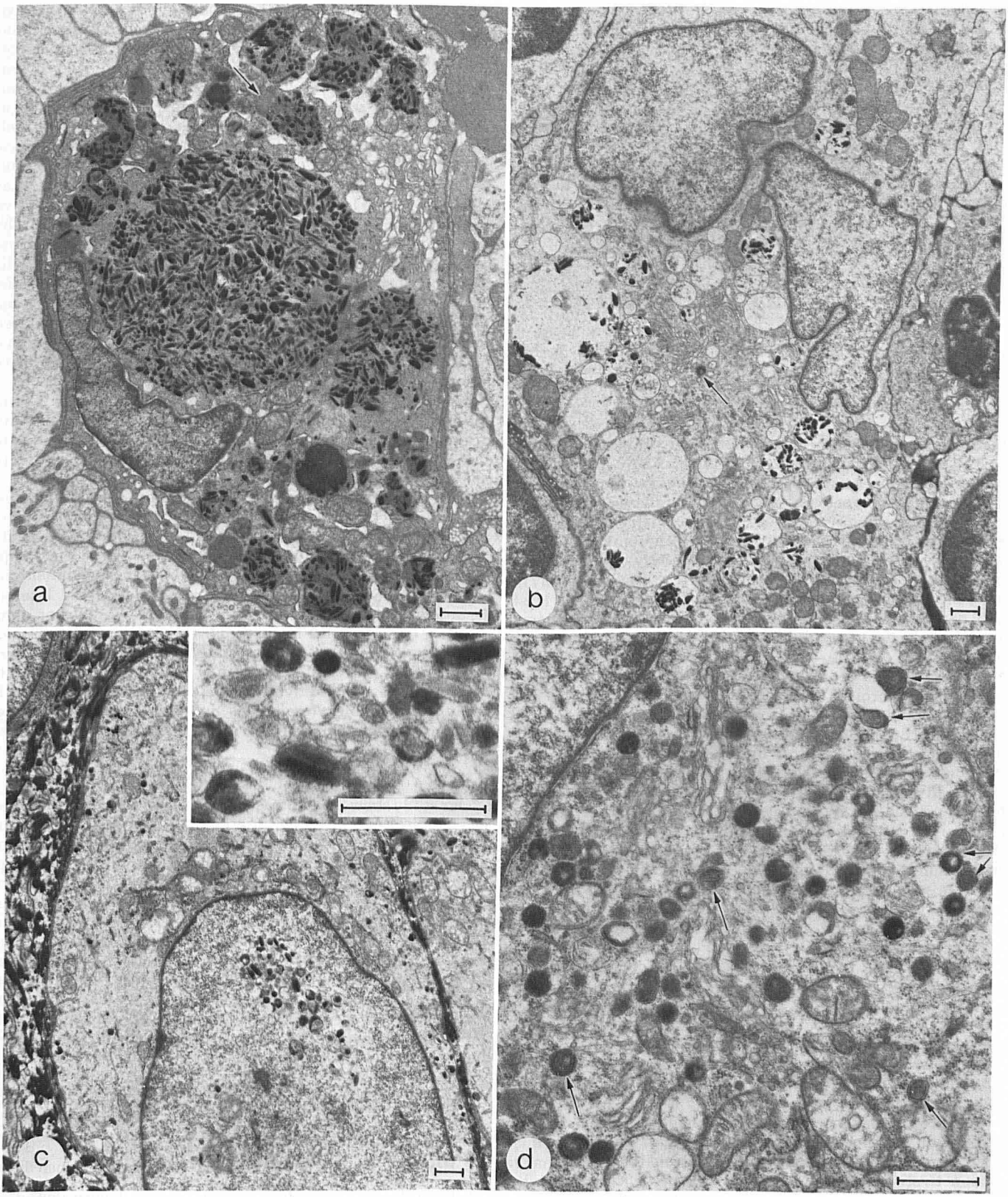


FIG 13. Melanoma cells. *Bar* = 1 μm. *a*, a dermal melanoma cell with melanosome complexes. Note the giant melanosome complex and the lipid-like droplet fused with the smaller melanosome complex (*arrow*). *b*, a nest of dermal melanoma cells with large vacuoles filled with a light flocculent material as well as some melanosomes. A centriole (*arrow*) is surrounded by Golgi vesicles. *c*, an epidermal melanoma cell surrounded by the tonofilaments of keratinocytes. There are melanosome-like granules in the nucleus. *Inset:* high-magnification photograph of a part of the nucleus. There are stage II and stage III melanosomes exhibiting regular periodicity, a few stage IV melanosomes, and some ring-like melanosomes. *d*, an epidermal melanoma cell with ring-like melanosomes and round bodies with lamellated membranes (*arrows*).

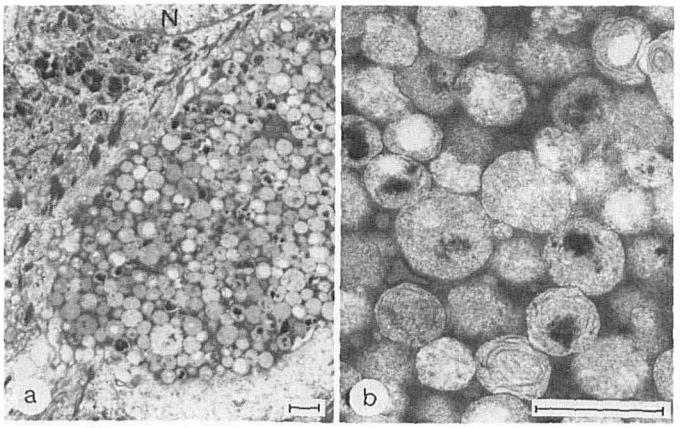


FIG 14. An epidermal melanoma cell. *Bar* = 1 μm. *a*, the cell contains organelles with varying amounts of granular and fibrillar materials of an uneven density. *N* = nucleus of a keratinocyte. *b*, higher-magnification photograph. Some of the fibrils exhibit a periodicity similar to that seen in balloon cell nevi.

or neuroid forms deep in the dermis; these are also known as type A, B, and C nevus cells, respectively [20]. The neuroid type cells no longer have pigment and are dopa-negative.

The stromal melanocytes of the uvea of a rhesus macque eye appear as nonpigmented dopa-positive small bipolar cells in the iris between days 60 and 80 of gestation, become pigmented between days 140 and 145, and increase greatly in number and in melanin content from then on to birth (term = 165 days). After birth their melanin content continues to increase at least until early adulthood. They appear later in the choroid stroma on about day 105 of gestation and become pigmented near the time of birth. As they develop, they increase in size, their dendrites become more complex, and they become heavily pigmented. Choroidal melanocytes become functionally and developmentally mature soon after birth and remain so until adolescence or young adulthood (about 3 yr of age [9,21]). That these cells are fully matured and terminally differentiated at this stage is supported by the following facts: (i) they have fully melanized melanosomes, (ii) they have little tyrosinase activity, (iii) they have never been seen to divide, (iv) they do not incorporate $^3$H-thymidine (autoradiographic evidence), and (v) they survive in culture medium containing high levels of cytosine arabinoside (which kills DNA-synthesizing, actively dividing cells such as fibroblasts and endothelial cells in the same culture [7]).

When we examined these cells from the eyes of rhesus macaques of different ages, we found changes such as: (i) a tendency for the melanosomes to fuse into complexes, (ii) uneven melanization, (iii) melanolysosomes in the form of vacuoles containing irregular fibrillar or myelin-like lamellated membranes, and (iv) lipid-like granules completely or partially filled with homogeneous material of a minimal electron density. We have shown that these changes in melanosomes are quantitatively and qualitatively correlated to the ages of the animals.†

The activity of the melanogenic enzyme tyrosinase is at its peak when melanocytes reach their prime of life and declines soon after the cells grow old. In adult choroid these cells change from actively melanin-synthesizing cells to melanin-containing cells without any tyrosinase activity or the ability to proliferate. Thus, they become a nonreplicative, terminally differentiated cell population in the same category as neurons and muscle cells.

In many ways nonreplicative choroidal melanocytes resemble intradermal nevus cells. The development of epidermal junction nevus cells into intradermal type A, B, and C nevus cells changes them from a replicative population to a nonreplicative one. There are similar sequential changes in ultrastructure and enzyme activity in the choroidal melanocytes. The progression of lentigo simplex to lentigo senilis is characterized by an increase in the proliferative activities of both keratinocytes and melanocytes as well as an increase in melanogenic activity in the melanocytes and an increase in the active transfer of melanosomes from melanocytes to keratinocytes. These changes, I believe, are the result of epidermal melanocytes and keratinocytes responding to repeated environmental insults such as actinic rays, mild irritation, or trauma (which are often effective in stimulating cell proliferation and other processes). When the stimulus is repeated long enough or when the injury is severe enough to cause irreversible damage to the cells, the cells undergo changes resembling those in aging cells and finally die. The development of lentigo maligna and malignant melanoma is a deviation in the usual sequence of changes. Stimulated melanocytes, instead of growing old and dying, become transformed into a cell population with an abnormal proliferative activity. The mechanism of this transformation could be a mutational event or the loss of growth regulation due to unknown causes. The high incidence of cancer in the aged population suggests some causal relationship between these 2 conditions. Their ultrastructural changes also are similar, except that they are more pronounced in malignant cells.

In another study we examined a γ-glutamyl transfer enzyme, i.e., γ-glutamyl transpeptidase (GGT), in the pigment cells of eyes from rhesus macaques of different ages. The GGT activity appeared in the retinal pigment epithelium early in fetal life, but was not detectable in the eyes of newborn animals; its activity in the iridial stromal melanocytes appeared later in gestation, but persisted through adulthood. We did not demonstrate GGT activity in choroidal melanocytes at any age.‡ This enzyme appears to be associated with active protein synthesis and the early differentiation of pigment cells. It ceases to function as terminal differentiation sets in. Their presence in adult iridial melanocytes and absence in choroidal melanocytes indicate the relative stages of differentiation of these 2 cell types.

## CONCLUSIONS

Our study shows that ultrastructural changes in melanosomes of postmitotic, fully differentiated choroidal melanocytes are quantitatively and qualitatively related to aging. We found similar changes in human epidermal melanocytes in lentigo senilis, intradermal nevus, multiple lentigines syndrome, and lentigo maligna. Biochemically there are at least 2 enzymes, tyrosinase and GGT, in addition to the lysosomal enzymes, that are related to the differentiation and aging of these cells. Even though these changes are not specific (similar changes are seen in certain pigmentary disturbances in response to environmental factors or injuries), as markers they are useful in the evaluation of aging processes. I believe that the choroidal melanocyte system is an excellent model for studies on cell changes from maturation to senescence.

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