THE ULTRASTRUCTURE OF CONJUNCTIVAL MELANOCYTIC TUMORS

BY *Frederick A. Jakobiec,* MD

INTRODUCTION

THE PAST DECADE WITNESSED A REMARKABLE GROWTH IN OUR UNDERSTANDING OF the biologic features of cutaneous melanomas and related conditions. In addition to an exponentially expanding literature of original scientific articles, seven monographs published in English during the past 8 years summarize these new developments.[1-7] Because of the apparent relationship of mucosal melanomas to cutaneous melanomas,[8-13] ophthalmic pathologists have attempted to apply some of the insights derived from cutaneous pathology to the diagnosis and prognosis of conjunctival melanomas. The transference of diagnostic and prognostic categories from the skin to the conjunctiva, however, has not been notably successful.[14-22] This failure may in part be due to differences in the anatomic soil, differences in the intrinsic biologic behavior of cutaneous and conjunctival tumors, sampling errors, or the small size of ophthalmic series. The comparative rarity of conjunctival melanomas (they develop about one-fortieth as often as uveal melanomas[23,24]) prevents the accumulation in most laboratories of large numbers of cases for statistically significant retrospective and prospective studies of the different diagnostic classes of lesions.

To date, the ultrastructural characteristics of conjunctival melanotic tumors have not been adequately studied. Surprisingly, there is only a single—and not a very detailed—ultrastructural report on a conjunctival melanoma.[25] Morphology remains the most important method for analysis and interpretation of cutaneous and conjunctival melanocytic disorders; it would seem logical and scientifically sound to expand our appreciation of the morphologic aspects of conjunctival melanotic tumors by means of electron microscopy. This thesis, which is based on an examination of almost 50 benign and malignant lesions, is the first systematic ultrastructural study aimed at clarifying the biologic features and improving the diagnosis of common conjunctival melanotic tumors.

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The main purpose of this study is to refine diagnostic precision in the therapeutically problematic area of primary acquired melanosis of the conjunctiva—a flat, pigmentary condition occurring in middle-aged or elderly persons, which may be static for many years, regress, slowly spread, or rapidly culminate in invasive nodules of melanoma. Many, but not all, lesions of primary acquired melanosis represent the earliest precursor phase (radial or horizontal growth phase) of melanomas, analogous to the intraepidermal phase ("in situ melanoma") of cutaneous lentigo maligna, superficial spreading, and acral lentiginous melanomas. The ability to better judge the malignant potential of conjunctival primary acquired melanosis, whether or not the latter condition(s) can be successfully categorized according to the cutaneous scheme, might well be served by electron microscopy. To appreciate the full spectrum of atypical or aberrant cells participating in primary acquired melanosis, a baseline is needed for comparison; therefore, lesions of benign epithelial melanosis and ordinary melanocytic nevi of the conjunctiva have also been studied to establish the morphologic features of benign melanocytes and nevus cells.

BACKGROUND

Because conjunctival melanocytic lesions are generally believed to bear a striking similarity to related conditions of the skin and other mucous membranes (in contrast to choroidal melanocytic disorders[20]), it is necessary to review the salient features of the normal intraepidermal melanocyte, analogous cutaneous tumors, and the ultrastructure of cutaneous neoplasms. The last item is particularly important in view of the dearth of published electron microscopic studies on conjunctival melanocytic disorders. Not all published studies can be cited in this summary; many review articles have been utilized, which direct the reader to the literature for complicated and extensive topics.

THE MELANOCYTE

The melanocyte is defined in terms of its ability to produce the pigment melanin, which accumulates within specific cytoplasmic inclusions or granules referred to as melanosomes.[26-31] Within the melanosome is incorporated the enzyme tyrosinase; it converts tyrosine to dopa, which in turn is converted to dopa-quinone. Dopa-quinone, through several intermediate oxidative steps, forms a variety of indoles and quinones that polymerize and deposit as granules on the lamellar-filamentary substruc-

ture of the melanosome. This is the common type of melanin and is referred to as eumelanin; it confers a brown or black appearance, and it is responsible for most human pigmentation. Phaeomelanin, by contrast, involves the covalent linkage of cysteine to dopa-quinone, to confer a yellow or auburn clinical pigmentation; it is responsible for red hair.

The melanosome derives from vesicles associated with the Golgi complex; tyrosinase is incorporated as a moiety, perhaps contributed by the rough-surfaced endoplasmic reticulum. The formation of the melanosome has been divided into four stages in the skin: Stage I melanosomes are spherical or oval membrane-limited vesicles containing only a few melanofilaments. Stage II melanosomes are elliptic or rod-shaped organelles and contain many more filaments producing cross-linkages and displaying a distinct periodicity. Stage III melanosomes demonstrate the early deposition of electron-dense homogeneous melanin granules that partially obscure the internal filamentous substructure. Stage IV melanosomes are those in which melaninization is complete and a uniform electron density totally obliterates the internal filamentous architecture. In the case of melaninization of phaeomelanin, the melanosomal substructure differs from that previously described, in that the melanosomes of phaeomelanin are round and the protein-filamentary substructure has a more tangled appearance, resulting in scroll-like filaments or flocculogranular material with electron density that rarely totally obliterates the melanosomal substructure. Teleologically, melanin is protective against the absorption of damaging ultraviolet irradiation; another hypothesis, which is less secure, is that the degree of cutaneous pigmentation may modulate in different latitudes the conversion of 7-dehydrocholesterol to vitamin D_3, thereby preventing vitamin D toxicity and disease-inducing hypercalcemic states.[28]

The melanocyte originates from the neural crest[31-33] and undergoes extensive migrations before lodging among the basal epithelial cells of the epidermis or mucosal membranes. Melanocytes make their appearance within the dermis of the skin of black persons around the tenth week of gestation, and during the ensuing 3 to 4 weeks they complete their migration into the epidermis.[34] By the 20th week this migration is virtually complete, and, except for formation of a sacral mongolian spot or various hamartomatous persistences, at the time of birth few dermal melanocytes can be identified. Ultrastructurally, intraepidermal melanocytes rest upon the basement membrane of the epidermis nestled among the basal germinal cells of this structure.[26,27] The melanocytes are dendritic in appearance, extending long cellular processes from the perikaryon to arborize and insinuate among a group of keratinocytes (epidermal-

melanin unit) to which they transfer their pigment. This phenomenon is accomplished by means of a poorly understood cannibalistic mechanism that is generally felt to be due to the nibbling off of the tips of the dendrites by the keratinocytes. The melanocyte appears as a clear cell on light microscopy and as relatively electron-lucent on electron microscopy, owing to the absence in the cytoplasm of tonofilaments possessed by the surrounding keratinocytes, although intermediate-type filaments (now known to be vimentin[35,36]) can be detected in the cytoplasm. S-100 protein also has been histochemically detected in melanocytes and nevus cells.[37] The melanosomes in the perikaryon arise in the Golgi zone and associated smooth vesicles of the endoplasmic reticulum (GERL), but melaninization is completed in the dendritic processes. Desmosomes are not made between melanocytes and keratinocytes, although hemidesmosomes without inserting cytoplasmic filaments are manifested along the basal plasmalemma of the melanocytes where they abut the epidermal basement membrane.

For many years there was considerable debate about the relationship of the intraepithelial dendritic melanocyte to the more rounded nevus cell that participates in the formation of garden-variety melanocytic nevi or moles. Based on ultrastructural and embryologic considerations, Mishima[38,39] forcefully championed the concept that, from the earliest stages of the breakup of the neural crest, even before phenotypic expression of melanogenesis can be demonstrated, a dichotomy exists between cells destined to become nevus cells (nevoblasts) and those destined to become dendritic melanocytes (melanoblasts). Postembryonically, nevoblasts were alleged to be responsible for the formation of nevus cells and nevocytic nevi, whereas melanoblasts were destined to become dendritic melanocytes of the epithelium and to participate in the formation of the mongolian spot, blue nevus, and cellular blue nevus. This concept was carried one step further: Mishima[39] also postulated that cutaneous melanomas could be divided into those which arose from nevus cells (nevocytic melanomas) and those that arose from dendritic melanocytes (melanocytic melanomas), each displaying different ultrastructural and clinical features.

Because of its simplicity and symmetry, Mishima's hypothesis regarding the dual origin of cutaneous melanomas held sway for about a decade; its critical examination is therefore central to some of the objectives of the present work. Regarding the distinction between dendritic melanocytes and nevocytes, Mishima was in the august company of the famous pathologist, Pierre Masson,[40] who felt that the deepest cells in a dermal nevus, which frequently assumed spindled or neuroid characteristics, were de-

rived from Schwann cells, also neural crest progeny. Cells of this character are referred to as type C nevus cells; on light microscopy they are devoid of a melanin granule content, fail to stain by the dopa-oxidase histochemical reaction, and display nonspecific cholinesterase reaction.[41] In failing to demonstrate dopa reaction within type C cells, Mishima[42] had utilized formalin-fixed tissue, whereas Thorne and co-workers,[43] using glutaraldehyde as a fixative, were able to demonstrate dopa-oxidase activity even in type C cells. Electron microscopic studies of neuroid structures deep in dermal nevi have also revealed ultrastructural features more in keeping with spindle nevus cells rather than Schwann cells, including the identification of extremely small melanosomes.[44] Finally, myelin-basic protein, which is regularly present in Schwann cells, has not been found in any of the cell types, and in particular type C cells, that participate in the formation of melanocytic nevi.[45] Present opinion in dermatopathology,[46] with which the morphologic findings in this particular study are essentially in agreement, would seem to indicate that the nevus cell is merely a modified form of the dendritic melanocyte. The dendritic melanocyte, therefore, is the paradigmatic melanogenic cell, which is responsible for the vast majority of cutaneous lesions as well as for all of the lesions investigated in this study.

The elaborate dendritic system of the intraepithelial melanocyte, which certainly signifies a neural origin for this cell, can be best brought out by silver stains or in dopa-reacted split-skin preparations of epidermis.[46] Racial pigmentary differences are felt to be due not so much to a differential distributional intensity of dendritic melanocytes among the races, but rather to the degree of melanogenic activity within the cells and to the manner in which the melanin is transferred to the neighboring keratinocytes.[27] In black persons the melanin granules are particularly large and heavily melaninized (predominantly type IV melanosomes), and upon transfer to surrounding keratinocytes, the melanosomes are singly dispersed within the cytoplasm. In Caucasians and Orientals the melanosomes are smaller and less uniformly melaninized, and upon transfer to the surrounding keratinocytes, they are frequently packaged in phagolysosomal complexes. In the latter site partial degradation of the melanoprotein filamentous complex is accomplished, although melanin itself is indigestible by keratinocytic lysosomal enzymes. Suntanning brings about an increased synthesis of melanin, but not immediately an increased density of melanocytes.[47] Chronic exposure to sunlight in Caucasians, however, does bring about an enlargement of the dendritic processes and approximately a twofold increase in the number of melanocytes.[48,49] In albinism, the absence of the enzyme tyrosinase prevents the

deposition of melanin upon the matrix of the melanosomes, which nonetheless are identifiable and retain their intact substructure. In vitiligo, melanocytes within the involved zones disappear.[46]

The dendritic melanocyte must be distinguished from other clear cells that inhabit the epidermis. The most important of these is the Langerhans' cell, which is referred to as a high-level clear cell within the epidermis because it tends to reside in the middle of the malpighian layer in a suprabasilar position.[26] Rather than exhibiting melanin granules within its cytoplasm, it possesses an intracytoplasmic granule referred to as Langerhans' granule (also called Birbeck granule or racquet body).[50] Its distinctive cytoplasmic organelle has been variously attributed to modifications of the Golgi vesicular system versus invagination and application of apposed leaves of the cell surface membrane. Once believed to be an effete or worn-out melanocyte, the Langerhans' cell is now well recognized to be a species of histiocyte.[51,52] It has also been hypothesized that it plays a role in allergic reactions by fixing and processing antigen, which is subsequently presented to lymphocytes.[53,54] Histiocytic proliferations within the histiocytosis X spectrum of diseases are constituted by Langerhans'-type cells, which frequently exhibit an invasive epidermotropism. The Merkel cell has also been identified as a clear cell within the epidermis; its distinctive subcellular marker is the neurosecretory-type dense-core granule. Merkel cells are consequently a component of the neurosecretory cellular diaspora described by Pearse[55] as the APUD system. This cell is believed to have a role in sensation and has been incriminated as the cell of origin for undifferentiated round cell carcinomas (trabecular carcinomas), which frequently are confused with amelanotic melanomas. Tumors of Merkel cell origin have been described in the skin of the lid,[56,57] but neither the Merkel cell itself nor tumors derived from it have been described in the conjunctiva.

The second most common melanocytic cell type of the skin, which if one accepts the unitary hypothesis mentioned previously is actually a variant dendritic melanocyte, is the melanocytic nevus cell. This cell is also a clear cell initially residing within the epidermis, but rather than occuring in isolation, it tends to form small junctional nests, or theques. It is a proliferative but benign melanocyte. In the production of common nevi or moles, multiple junctional nests (so termed because the nevus cells are situated within little cavities resting along the basement membrane of the epidermis) occupy a field of involved epidermis and generally make their appearance during youth. As long as the nevocytic nests are restricted to the epidermis, these lesions are referred to as junctional nevi. The cells within intraepidermal junctional nests tend to drop off into

the pupillary and upper reticular dermis (abtropfung phenomenon), at which stage a compound nevus has developed. As more and more junctional cells drop off into the dermis, those that had earlier colonized the upper dermis are pushed farther and farther into the depths of the connective tissue of the dermis. Within the third or fourth decades of life, most of the junctional intraepidermal nests have ceased to proliferate; all that generally remains is the dermal component, which may have acquired considerable thickness to produce polypoid and verrucous lesions.[31,38,46]

The cells within the epidermal junctional nests, as well as those immediately beneath the epidermis in the papillary or upper reticular dermis, are usually somewhat epithelioid in shape and have been referred to as type A nevus cells.[38] Proceeding downward within the substance of a dermal nevus component, the cells become smaller, lose their pigment, and are frequently called lymphocytoid cells. This intermediate dermal group has been termed "type B" nevus cells. Finally, in the depths of a dermal nevus, the constituent cells may assume spindled or Schwannian characteristics. They can even form organoid structures mimicking Meissner tactile corpuscles, and these nevus cells are referred to as type C cells. As mentioned previously, it is now believed that these neuroid cells are final modifications of an initially intraepidermal junctional nevus cell. Cells within the dermal component of a nevus generally are nonpigmented (except for the most superficial cells) because they are out of reach of incident ultraviolet radiation. In their resting stage they do not display tyrosinase activity, but this can be induced by intensive ultraviolet irradiation of nevi prior to excision, as proven by histochemical enzymatic reactions.[58] If a melanoma arises within a preexistent nevus, with the rarest of exceptions[59,60] this does not occur within the dermal component, but rather from persistent and progressively dysplastic intraepithelial junctional activity. In the course of a lifetime, there is a cycle of nevus formation and disappearance: there are few nevi at birth, and those acquired in adolescence and early adulthood eventually disappear, perhaps through fibrotic effacement.[61,62]

In comparison with the work that has been performed during the past two decades on the melanocytic system of the skin, the paucity of studies on the normal melanocyte of the conjunctiva is embarrassing. The intraepithelial dendritic melanocyte has been described in anatomic studies of the conjunctiva[63,64]; in anatomic studies of normal conjunctiva I have rarely come upon dendritic melanocytes (Fig 1). More deeply situated, elongated melanocytes in the perilimbal episclera and substantia propria of dark-complexioned persons have also been described.[65,66] These deep-

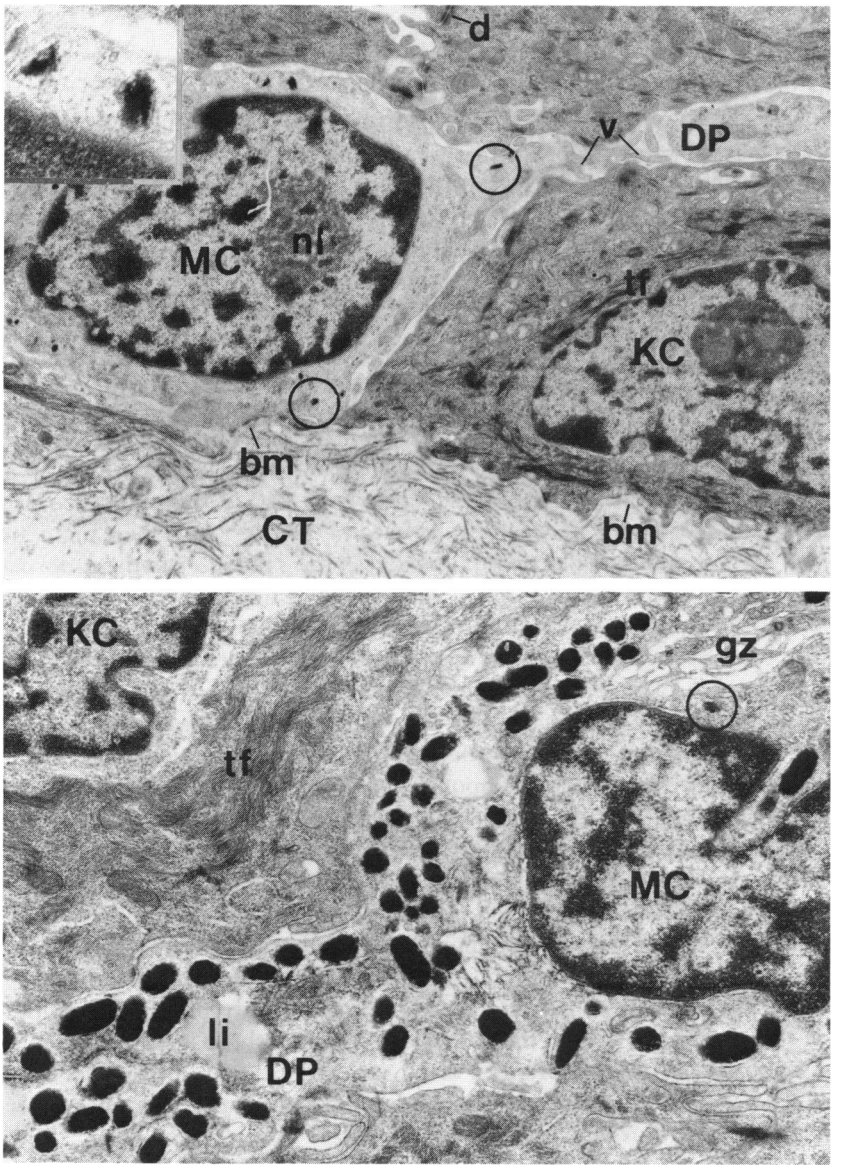

FIGURE 1

TOP: Conjunctival dendritic melanocyte (MC) with a nucleolus (nl) from a Caucasian child displays dendritic processes (DP) from which project smaller villi (v). The melanosomes in cytoplasm (*circles*) are small (200 × 70 nm) (× 12,000). INSET: Displays that they are only partially melaninized. The surrounding keratinocytes (KC) are more electron-dense because of presence of tonofilaments (tf). Desmosomes (d) join adjacent keratinocytes, but are not formed by melanocyte. Both melanocyte and keratinocyte are separated by a basement membrane (bm) from underlying connective tissue (CT) (× 49,400). BOTTOM: Conjunctival melanocyte from a black patient displays much larger and intensely melaninized melanosomes, measuring 200 × 500 nm. An active Golgi zone (gz) shows early formation of melanosomes (*circle*). In the dendritic process (DP) of melanocyte is a lipid (li) droplet. This surrounding keratinocyte (KC) has abundant tonofilaments (tf) (× 20,000).

ly situated cells are present in increased numbers in melanosis oculi; they do not proliferate by and large, and they are not responsible for conjunctival melanomas. Henkind[67] established in guinea pigs that conjunctival melanocytes can migrate and persist in the corneal epithelium only if corneal vascularization occurs. McCracken and Klintworth[68] confirmed these findings in another animal model. Normal conjunctival dendritic melanocytes have been identified in two ultrastructural studies of juxtalimbal squamous cell carcinomas in black patients.[69,70] It is known that the number of intraepidermal dendritic melanocytic clear cells in the skin is approximately one to every ten keratinocytic basal cells[71]; a similar ratio has been discovered in the oral mucosa of Caucasians.[72] No anatomic determinations of this sort have been made in the conjunctiva. A considerable topographic variation in the distribution of dendritic melanocytes within the conjunctival epithelium is likely; unlike in the skin, however, there is probably a differential racial distribution in this tissue. In order for these data to be determined, large geographic portions of conjunctiva would have to be treated in a fashion similar to that in the skin, with split-thickness preparations of fresh conjunctival epithelium that are incubated in the dopa-reaction system. This, of course, could only be done with postmortem material.

Masson[31] characterized deeply situated mesenchymal melanocytes as continent, in contrast to the incontinent dendritic melanocytes of the epithelium. By this he meant that the former are postmitotic and melanogenically inactive; they rarely give a positive histochemical reaction for tyrosinase and, if injured, release their pigment into the connective tissue but do not replenish it or transfer their own cytoplasmic melanin to other cells without being injured. Such continent melanocytes are dramatically different from the dendritic melanocytes of the epithelium, which are incontinent in terms of being able to transmit their melanin to the surrounding keratinocytes, and are additionally capable of replenishing their cytoplasmic melanin content. The deeply situated mesenchymal melanocytes are responsible for persistent mongolian spots, the nevus of Ito, the nevus of Ota, melanosis oculi with epibulbar pigmentation, and benign proliferation such as blue nevi and cellular blue nevi. The latter two entities are rarely encountered in the ocular adnexa, and blue nevi of the conjunctiva have only recently been the subject of a published report.[73] Deep, hamartomatous orbital melanocytes[74,75] may rarely cause primary orbital melanomas.

COMPARISON OF CUTANEOUS AND CONJUNCTIVAL MELANOCYTIC LESIONS

Over 99% of conjunctival melanocytic lesions, including all those re-

ported in this work, stem from either the dendritic melanocyte or its close relative, the more rounded nevus cell. The more deeply situated, incompletely migrated mesenchymal melanocytes, responsible for melanosis oculi with episcleral pigmentation (and the associated uveal melanocytic excess), blue nevi, and cellular blue nevi, are accountable for the remainder of pathologic pigmentations beneath the conjunctival epithelium. Jay,[76] Henkind,[24] Henkind and Friedman,[77] and Zimmerman[16,20] have provided overviews of the comparative nomenclatures of conjunctival and cutaneous melanocytic lesions. Textbooks on ophthalmic pathology[21,78,79] additionally provide illustrative examples of the major types of conjunctival melanocytic disorders. In the following summary the significant pathologic differences between cutaneous and conjunctival disorders are stressed. The four major types of conjunctival melanocytic lesions that are related to cutaneous lesions and to intraepithelial dendritic melanocytes or nevus cells are: (1) benign epithelial melanosis, (2) benign melanocytic nevi, (3) primary acquired melanosis (analogous to the radial or horizontal growth phase of cutaneous melanosis—"in situ melanoma"), and (4) invasive malignant melanoma.

Benign epithelial melanosis of the conjunctiva is characterized by flat, uninflamed, nonvascularized, and finely to coarsely granular brown pigmentation, generally occurring in the interpalpebral zone. The pigmentation moves with the conjunctiva, thereby establishing that the pigment resides within the epithelium (or less likely, the substantia propria). This pigmentation therefore differs from that of more deeply situated melanocytes in melanosis oculi or the nevus of Ota, in which case there is more of a slate-gray cast to the pigmentation, and movement of the conjunctival membrane does not cause any displacement of the pigmentation. Although benign conjunctival epithelial melanosis may be congenital, more often it is an acquired lesion that is typically encountered in black patients, possibly due to climactic or traumatic irritation of the conjunctiva. Such pigmentation may also be seen in association with irradiation, arsenic poisoning, Addison's disease, chloasma of pregnancy, and following chronic conjunctivitis in heavily pigmented persons (such as in trachoma). Regardless of the cause and of whether or not the condition is congenital or acquired, the histopathologic picture is uniform. Heavy pigmentation is generally restricted to the basal cell layer of the conjunctival epithelium; the responsible clear melanocytes are extremely difficult to discern. There is no nest formation, nor are there any high-level clear cells. Inflammation of the substantia propria is inconspicuous, apart from the normally resident lymphocytic population of this tissue, which may be accompanied by a scattering of histiocytic melanophages.

The conjunctival lesion bears a close histopathologic resemblance to cutaneous ephelides, which are garden-variety freckles acquired early in life and are generally not caused by sun exposure. It is impossible to relate benign epithelial melanosis of the conjunctiva to other flat macular pigmentations of the skin, such as lentigo simplex and lentigo senilis. In each of these latter conditions, there is elongation of the rete pegs of the epidermis, a feature which is anatomically absent from the conjunctiva.[46] In lentigines there are increased numbers of dendritic melanocytes, and in lentigo simplex there may be nests of nevoid cells at the tips of the rete pegs. This last finding certainly suggests a direct relationship between rounded nevus cells and dendritic melanocytes, as mentioned previously. The development of conjunctival benign epithelial melanosis obligates the presence of dendritic melanocytes on a normal anatomic basis within the conjunctival epithelium, although obviously this is a racially dependent anatomic variable. In a study of 300 patients at the Montefiore Hospital in New York, Henkind[24] reported that benign epithelial melanosis, as demonstrated by penlight or as visible in room light, was detected in 4.9% of Caucasians, 92.5% of blacks, 28% of Hispanics, and 35.7% of Orientals. Whether these percentages might be increased by the use of slit lamp examination or by the use of Wood's lamp ultraviolet irradiation would, of course, be of interest. An extremely useful clinical finding that the I discovered on clinical examination of patients with benign epithelial melanosis is its almost constant bilateralism, even if this is asymmetric. Bilateralism, coupled with a relatively stationary character, helps to distinguish benign epithelial melanosis from potentially more serious proliferative conditions of the melanocyte (primary acquired melanosis).

After benign epithelial melanosis, conjunctival melanocytic nevi are the second most common conjunctival pigmentary condition. Like cutaneous nevi, conjunctival epithelial nevi appear within the first two decades of life; like benign epithelial melanosis, they display a predilection to be located in the interpalpebral fissure from the limbus to the caruncle. In my experience, I have never seen a histopathologically documented, bona fide nevus located in the fornices or palpebral conjunctiva, although this clearly could happen; lesions in the latter sites should therefore be looked upon with more suspicion and should probably be excised, which is not routinely necessary for lesions developing in the interpalpebral fissure. Thirty percent of conjunctival nevi are very poorly pigmented or nonpigmented.[77] A distinctive feature of conjunctival nevi, not shared by cutaneous nevi, is the formation of epithelial inclusion cysts. The cysts may be seen grossly but are probably best discerned with the slit lamp, appear as clear areas in the midst of either pigmentation or solidity, and

superficially resemble lymphatic channels. In fact, the cysts may be so prominent as to invite the mistaken diagnosis of a lymphangioma.[80] Conjunctival nevi are generally thick and upraised at the time of detection; this distinguishes them from the flat pigmentation of benign epithelial melanosis, which furthermore lacks epithelial inclusion cysts. Like benign epithelial melanosis, conjunctival nevi are freely movable over the surface of the globe, because they are exclusively situated within the substantia propria and do not involve the deeper episcleral or Tenon's-level tissues.

Junctional activity tends to disappear by the third or fourth decades of life. Conjunctival nevi show some maturation from more superficial type A epithelioid cells into more deeply situated type B lymphocytoid cells. I have not seen type C neuroid or Schwannian formations in the depths of subepithelial conjunctival nevi, nor has such a change been described in the literature. Pigmentation disappears among the cells in the depths of the lesion, although scattered populations of nevus cells may retain a melanin content within their cytoplasm, possibly due to the thinness of the lesions that allows access of incident ultraviolet irradiation.

While highly vacuolated benign balloon cell nevi[81-83] and blue nevi, but not malignant variants of these, have been described in the conjunctiva—halo nevi encountered in the skin (regressing nevi mediated by a lymphocytic inflammatory infiltrate), combined nevi (a mixture of an ordinary mole with a blue nevus), spindle cell nevi, and epithelioid-spindle cell nevi of the Spitz variety[84-87] have not been well documented in the conjunctiva; they probably occur but are rarely recognized. Likewise, congenital nevi,[88-90] which have recently been well characterized in the skin by virtue of their periadnexal, perineural, and perivascular nevoid clusters of cells, have not been described in the conjunctiva. Indeed, these features would be hard to elucidate in the latter site, in view of the general absence of adnexal structures as well as the absence of thick bundles of collagen which constitute the dermis of the skin and which permit characteristic single-file nevocytic dispositions. Dysplastic nevi of the B-K syndrome, in which malignant melanomas may arise in preexistent nevi in genetically predisposed persons, have not been identified in the conjunctiva; intraocular melanomas, however, have been discovered to occur in this syndrome.[91-95] Deposition of bone, replacement by adipose tissue, and ultimate regression by progressive fibrosis are transformations of cutaneous nevi that do not seem to have their analogs in lesions of the conjunctiva.[61,62]

Primary acquired conjunctival melanosis is an acquired proliferative condition of melanocytes situated initially within the epithelium of the

conjunctiva. This entity or group of entities has engendered considerable diagnostic and therapeutic controversy within ophthalmology.[14-22,96,97] Unlike benign epithelial melanosis, which has a distinct predilection for dark-complexioned persons, primary acquired melanosis has a predilection for Caucasians (it is rarely seen in blacks), is always unilateral, and develops in the middle aged and elderly. The pigmentation is not restricted to the interpalpebral zone but may begin anywhere and spreads irregularly, sometimes showing evidence of waning in the older areas while waxing into new areas. With induced movement of the conjunctiva, the pigment will move with it, indicating again that the affection is initially situated within the epithelium. The color is virtually always golden brown, although nonpigmented variants occur rarely.[98] Biopsies of these lesions reveal increased numbers of melanocytes, which in the milder forms of hyperplasia are restricted to the epithelial basement membrane region. The milder forms of hyperplasia are probably benign and analogous to lentiginous hyperplasias of the skin,[46,99] but because of the absence of rete peg elongation, a marker that is useful for the identification of senile and simple lentigines of the skin, attention must be scrupulously paid to the identification of any melanocytic atypicality in the conjunctiva. There is no question that at the more proliferative end of the spectrum, in which cytologic atypia is clearly discerned among the participating melanocytes, one is dealing with a formal precursor of malignant melanoma.[100-104]

Reese,[96] who first thought that primary acquired melanosis was most kindred to Hutchinson's melanotic freckle of the skin or precancerous melanosis of Dubreuilh, termed the lesion in the conjunctiva precancerous melanosis and recommended exenteration, even for the flat lesions before nodule formation. Zimmerman[97] felt that this was inordinately aggressive and unnecessarily radical surgery for lesions that, in his experience, had an uncertain and often slow progression. He recommended the term "benign epithelial melanosis" for intraepithelial proliferations of melanocytes and cancerous melanosis only if and when invasion of the subepithelial connective tissues occurred. Zimmerman[16] gave eloquent testimony to the nature of the debate between himself and Reese when he delivered the first Algernon B. Reese Lecture. Reese[96] ultimately reported that, of the cases of flat intraepithelial melanocytic lesions he had seen as a clinician, nodules formed in 17% after 5 years and, of those patients with nodules, 40% died. It is my opinion that the former statistic is too low, because in reviewing file material in the pathology laboratory at the Harkness Eye Institute, I discovered that Reese had included lesions of nonproliferative benign melanosis among his cases of flat "pre-

cancerous melanosis."[17]

It is in the area of primary acquired melanosis—a term which is acceptable to me because it neither overly cancerizes the condition nor falsely reassures (which may occur by substituting the word benign for primary)—that the recent advances in the analysis of the radial growth phase of cutaneous melanoma might have some application. The work of Clark,[4] McGovern,[1,6] and others[105-111] led to a tripartite classification of cutaneous melanoma: (1) superficial spreading melanoma, (2) lentigo maligna melanoma, and (3) nodular melanoma. The first two lesions are characterized by a radial, centrifugal, or horizontal growth phase within the epidermis, after which a vertical growth phase supervenes with invasion of melanoma cells into the underlying papillary and reticular dermis. Nodular melanoma differs from the other two in failing to exhibit a prominent radial growth phase. A fourth division of melanoma has been added—the acral lentiginous melanoma, which also has a radial growth phase but has a predilection to develop on the glabrous skin of the digits, palms, and soles as well as allegedly in mucosae.[9-11,112] Seventy percent of cutaneous melanomas are diagnosed as superficial spreading, 8% as acral lentiginous, 5% as lentigo malignant, and 15% as nodular; about 2% are unclassifiable according to the previously designated categories.[46]

Superficial spreading melanoma grows in a pagetoid fashion, wherein atypical melanocytes not only form nests at the dermal-epidermal junction but additionally permeate and percolate throughout the upper limits of the epidermis to confer a buckshot appearance to the involved segments of epidermis.[102,108] The melanocytes are generally rounded or epithelioid. Superficial spreading melanoma develops on both sun-unexposed and sun-exposed skin, and the radial intraepidermal growth phase before nodular invasive melanoma supervenes has been estimated to last from one to several years.[46] In lentigo maligna, atypical dendritiform cells proliferate singly and then in small nests along the dermal-epidermal junction; the permeation of higher levels of the epidermis by the neoplastic cells is not prominent. The atypical melanocytes may involve the outer epithelium of the adnexal structures (sweat ducts and pilar epithelial canals), a feature not displayed by superficial spreading melanoma. Solar elastotic degeneration of the skin is prominent, because lentigo maligna (melanoma) develops preferentially on sun-exposed skin. This melanoma is far more indolent in its progression, and the radial growth phase may last 10 to 15 years before a vertical invasive nodule of melanoma occurs, in which case spindle cells frequently typify the invasive nodule (contrasting thereby with the invasive epithelioid cells of superficial spreading melanoma). Acral lentiginous lesions share many features with lentigo maligna

melanoma, including intraepithelial dendritiform atypical cells and an invasive spindle cell component when melanoma eventuates, but it appears to be a more aggressive lesion with a shorter radial growth phase and a higher propensity for metastasis. In nodular melanoma, an initially intraepidermal proliferation of atypical melanocytes apparently transpires for only a short while without extensive radial spread within the epidermis, so that a vertical phase develops early in the evolution of this lesion.

Bernardino and co-workers[14] were the first to attempt to apply the cutaneous classification to conjunctival lesions. They reported 23 cases, of which they were able to classify 5 as lentigo maligna melanoma (with a good prognosis) and 10 as superficial spreading melanoma (with a poor prognosis). Eight lesions appeared to be unclassifiable, because of the suboptimal nature of the pathologic material with which to evaluate the radial growth phase surrounding a nodular invasive component. Other investigators[10,15-22] have not been as successful as Bernardino and associates,[14] and they have cited differences in the anatomy of the conjunctiva and the skin. The conjunctival epithelium is comparatively delicate and thin compared with the epidermis, there is an absence of adnexal structures, and elastotic degeneration is frequently present in the interpalpebral substantia propria of the conjunctiva in early life as a result of actinic exposure. Silvers and associates[15] studied 20 lesions of preinvasive intraepithelial melanocytic proliferation of the conjunctiva and 28 other lesions with a radial growth phase accompanied by invasive nodules of melanoma. They were unable to systematically classify their lesions according to the cutaneous scheme but felt that, based on clinical behavior, the lesions resembled superficial spreading melanoma more than the other types. Crawford,[19] in a study of 19 conjunctival melanomas, could only classify 6 according to the skin classification and recommended the term "atypical intraepithelial melanocytic hyperplasia" be employed as suggested by Silvers and associates.[15] Liesegang and Campbell,[18] in a study of 42 conjunctival melanomas, found that they were unable to use the skin classification for the analysis of the radial growth phase of the conjunctival lesions. Zimmerman, in his Reese Lecture,[16] also reported that he was unable to use the skin classification in the interpretation of cases of conjunctival primary acquired melanosis on file at the Armed Forces Institute of Pathology. It is anomalous that all of these workers with the exception of Zimmerman have failed to address themselves to the identification of acral lentiginous melanoma of the conjunctiva, inasmuch as many pathologists feel that this is the most typical kind of melanoma with a radial growth phase to arise in squamous mucosal membranes.[9-13] Zim-

merman, himself,[20] believes that acral lentiginous melanoma is elusive in the conjunctiva. It is unfortunate that it is not possible, according to the present state of ophthalmic pathologic practice, to divide primary acquired melanosis into the various types of radial growth phase manifested by cutaneous melanoma, since in those cases that are judged to be a lentigo maligna, a slower rate of progression would be expected. Not only is there a prolongation of the radial growth phase, but also, once invasion occurs, it appears that lentigo maligna melanoma is less likely to metastasize than similarly invasive superficial spreading melanoma.[111] The use of cryotherapy[113-116] or radiotherapy[117-119] for primary acquired melanosis might be held in abeyance if the low-risk patients for nodule formation could be identified.

The subject of invasive conjunctival melanoma, in which a frank nodule of melanoma forms within the connective tissue of the substantia propria, deeper episcleral tissues, the sclera itself, or the connective tissues of the lids and fornices, is also somewhat controversial. The controversy lies not in the ability to diagnose these lesions but rather in interpreting how often they arise in preexistent primary acquired melanosis or a nevus and in whether metastatic potential can be predicted from any characteristics of the invasive nodule. Zimmerman[16] stated that, in 50% of the conjunctival melanomas he has seen at the Armed Forces Institute of Pathology, he was unable to ascribe an origin in either primary acquired melanosis or a preexistent nevus; in the remaining 50%, those arising in nevi outnumbered those arising in primary acquired melanosis by a ratio of 2:1. In other words, he found that 33% of melanomas arose in nevi, 17% arose in primary acquired melanosis, and the remainder arose in previously unblemished conjunctiva.

In Jay's series[76] of 104 melanomas from the Institute of Ophthalmology in London, only 14% of the melanomas arose in association with a preexistent nevus, but in comparison with nevi, primary acquired melanosis was responsible for melanoma by a ratio of 4:1. Of 42 malignant melanomas from the Mayo Clinic, Liesegang and Campbell[18] discovered that 15 developed in the soil of primary acquired melanosis and 9 in association with a nevus. Of some interest was the finding that two of their patients with primary acquired melanosis had a previous excision of a nevus, indicating that primary acquired melanosis may also arise in association with this entity. Crawford,[19] in his review of 19 conjunctival melanomas, found only 3 cases with evidence of a preexistent nevus in the form of subepithelial collections of nevus cells. Nine of the 19 melanomas were associated with primary acquired melanosis, but no mention is made of whether any of the nevi coexisted with the primary acquired melanosis.

Silvers and associates,[15] in their study of 48 preinvasive and invasive melanocytic lesions with a radial growth phase, found no evidence of a preexistent nevus.

The high incidence of evidence of a preexistent nevus in the melanomas on file in the Armed Forces Institute of Pathology may be the result of a selection bias, in that pathologists would be more apt to submit confusing material for consultation in which both benign and malignant populations of subepithelial cells are found. Apart from any histogenetic implications of the association of a nevus with a melanoma, there is the finding in some series of a better prognosis for melanomas associated with nevi. This was definitely established in Jay's series[76] as well as in that of Liesegang and Campbell[18]; Crawford,[19] on the other hand, did not feel that this was an important prognostic feature.

Very often, in the excision of invasive nodules of malignant melanoma of the conjunctiva, much of the flat surrounding component is left behind to allow for preservation of the function of the globe; therefore, an entire lesion is not available for step sections to elucidate different melanocytic components. This problem is easily circumvented in the skin, where a wide local excision is performed for every lesion. Ackerman and Su[102] discovered that in the skin 50% of superficial spreading melanomas have an associated intradermal nevus. In the skin all types of melanomas may arise in association with a preexistent nevus: in one series,[105] 35% of superficial spreading melanomas, 25% of nodular melanomas, and 5% of melanomas arising in Hutchinson's melanotic freckle had evidence of a preexistent dermal nevus in the excised specimen. I have seen 3 cases of nevi in association with 21 cases of either primary acquired melanosis or invasive malignant melanoma, which is consistent with the findings of Jay,[76] Liesegang and Campbell,[18] and Crawford.[19]

Another significant contribution of dermatopathologists regarding the prediction of outcome and fatality of cutaneous melanoma has been the discovery of a relationship between depth of invasion of the nodule and development of metastases. Clark and co-workers[107-109] initially developed a leveling system wherein superficially invasive lesions restricted to the papillary dermis had virtually a 100% survival rate, whereas lesions impiniging on the upper reticular dermis, permeating through the reticular dermis, and finally reaching the subcutaneous fat had increasing rates of fatality. The anatomy of the skin varies from zone to zone; for example, in the eyelid there is no demonstrable papillary dermis and the reticular dermis is very thin, while in the conjunctiva these distinctions have no anatomic meaning. Breslow[120] was the first to explore the value of an actual thickness measurement taken with an ocular micrometer as a prog-

nosticator of metastasis. He found that invasive nodules less than 0.75 mm thick were almost always associated with an excellent, metastasis-free prognosis, whereas progressively thickening lesions became increasingly likely to metastasize, so that lesions measuring 3 mm or more in thickness were associated with a 78% rate of metastasis.

Silvers and co-workers[15] were the first to employ Breslow's micrometer measurements to determine the thickness of invasive conjunctival nodules as a prognostic index. Of 28 patients with invasive melanoma, they discovered that the 16 patients who survived their disease (regardless of whether local excision or exenteration had been performed) had an average nodular thickness of 0.7 mm, with a range of 0.4 to 1.95 mm. The 12 patients who died of their disease, again regardless of the type of therapy, had an average thickness measurement of 3.0 mm, with a range of 1.8 to 15.0 mm. These investigators felt that the transition from nonmetastasizing to metastasizing lesions occurred in the range of 1.5 to 2.0 mm of thickness. McGhee and co-workers[22] corroborated these findings on 25 melanomas at the Massachusetts Eye and Ear Infirmary; metastases developed in one of eight patients with thickness measurements less than 1.5 mm but in 50% of patients with lesions greater than 1.5 mm. Liesegang and Campbell[18] found that they could not successfully utilize thickness measurements to predict outcome, and Crawford[19] made a similar observation. These other investigators seem to verify Jay's[76] earlier finding that both superficially and deeply invasive nodules of conjunctival melanoma are capable of metastasizing. All of these investigators recognize that the number of cases they are dealing with in the conjunctiva is quite small and that with larger numbers of lesions it might be possible to observe a more definite trend toward metastasis with thicker lesions. Several series[12,121] on mucosal melanomas of other sites (anorectal, vaginal) have established that lesions measuring less than 2 mm in depth of invasion are compatible with survival, whereas thicker lesions (unfortunately the overwhelming majority) have been uniformly fatal regardless of therapy.

Crawford[19] analyzed several other prognostic features in conjunction with conjunctival melanomas that have been considered valid with respect to cutaneous melanomas: age of patient at initial presentation, topographic location of the lesion in the conjunctival sac, degree of inflammation associated with the invasive nodules of melanoma, and number of mitotic figures observed in 40 high-dry microscopic fields. His findings, which were subjected to statistical analysis, showed that patients younger than 37 years of age had an increased incidence of metastases, those with a light lymphocytic infiltrate surrounding their lesions were

more apt to have metastases than those with a heavy infiltrate, those patients with four or more mitotic figures per 40 high-dry fields had increased rates of metastasis, and those patients whose lesions were situated in the caruncle, fornix, or palpebral conjunctiva were at greater risk for metastasis than those with epibulbar lesions. With respect to the last finding, Crawford is in agreement with findings reported by Jay[76] and Silvers and co-workers.[15] Other studies may verify the value of these prognostic parameters described by Crawford.[19]

The one bright spot in comparing conjunctival melanoma with cutaneous and other mucosal melanomas has been the discovery of an overall better prognosis for melanomas arising in the conjunctiva. Jay[76] found a 78% survival rate, and Liesegang and Campbell[18] had an 86% 5-year survival rate. Whether or not this feature is due to differences in biology, differences in the anatomy of the conjunctiva, or the fact that conjunctival melanomas are frequently discovered early because of their occurrence on the white sclera is open to speculation. It should be pointed out, however, that Crawford[19] discovered that 8 of his 19 patients with conjunctival melanoma died; he also emphasized that 5-year follow-up may not be adequate to determine survival, because several of his patients died 10 to 15 years after treatment of their conjunctival melanomas.

ULTRASTRUCTURE OF MELANOCYTIC LESIONS

With the exception of a single lesion of invasive conjunctival melanoma studied ultramicroscopically,[25] melanotic lesions of this tissue have not been subjected to electron microscopic study. Before the ultrastructural features of conjunctival melanocytic lesions are evaluated, the relevant data derived from electron microscopic studies of cutaneous lesions will be reviewed.

Ultrastructural studies have shown that lentigo simplex,[122-124] lentigo senilis,[125,126] and lentigo maligna[127-129] (Hutchinson's melanotic freckle) are essentially proliferative lesions of the dendritic melanocyte. The first two lesions are benign, while lentigo maligna is considered a premalignant lesion that can evolve into lentigo maligna melanoma. In these lesions increased numbers of dendritic melanocytes are present among the basal cells of the epidermis and continue to extend long cellular processes that donate pigment to the surrounding keratinocytes. In the case of lentigo senilis, there appears to be melanocytic hyperactivity, with increased numbers of melanosomes being transferred to the surrounding keratinocytes; in the latter cells, there is more prominent formation of melanosome complexes in comparison with uninvolved skin. Lentigo simplex has been proven to display an occasional giant melanin granule

(rounded structures measuring up to 5 μm in diameter), which have a distinctive vesicular substructure and are most typically encountered in the café au lait spots of von Recklinghausen's disease[130] and Albright's disease.[131] Giant melanosomes, however, have now been described in a wide variety of benign and malignant cutaneous lesions.[132-139] It is of interest that in ophthalmic pathology, giant melanosomes have been found in melanocytomas of the uvea,[140] in X-linked albinism in the retinal pigment epithelium,[141] and in iris melanocytes in uveal melanocytosis[141a]

Despite the common occurrence of garden-variety nevi, it is surprising how few thorough ultrastructural studies of these cutaneous lesions have been published.[38,44,58,137,142-150] Most previous studies have dealt with compound or intradermal nevi. Within the intraepidermal junctional nests the melanocytic nevus cells stand apart from the keratinocytes by virtue of their paler cytoplasm, which is due to the absence of tonofilaments. Desmosomal junctions between nevus cells, as well as between nevus cells and the surrounding keratinocytes, are also absent. As the cells in the intraepidermal nests emerge to drop into the dermis, the epidermal basement membrane becomes thinned. The nevus cell has a more rounded appearance than the dendritic melanocyte, but the former cell nonetheless continues to display blunter dendritic processes and villi, which can break through the basement membrane region into the upper dermis. It is presumably by these structures that nevus cells are able to communicate their pigment to the surrounding keratinocytes, although less efficiently than is the case with dendritic melanocytes.

Heterochromatin is generally clumped at the nuclear membrane of nevus cells, and nucleoli may be prominent. Nuclear indentations and surface irregularities are common, but large pseudoinclusions or sequestrations of cytoplasm within the nucleus are not typical. Within the cytoplasm there are frequently numerous mitochondria and scattered cytoplasmic filaments that have recently been identified as vimentin,[35,36] and there may be short segments of rough-surfaced endoplasmic reticulum and scattered cytoplasmic monoribosomes. Where the nevus cells abut the basement membrane region they may form poorly developed hemidesmosomes. The melanosomes may be either short rods or more spherical structures that display disorderly melanofilamentary substructures with fewer cross-linkages. The melanosomes are usually only partially melaninized. Upon dropping off from the epidermis to the dermis, the uppermost dermal nevus cells continue to display melanin synthetic activity but adopt a feature never displayed by intraepidermal melanocytes, namely, basement membrane formation. The basement membrane surrounds the outermost aspects of the nevus cell clusters but tends not to

dissect within the clusters (ie, not between the adjacent plasmalemmas of nevus cells). Villi, suggesting dendritic processes, frequently are formed by intradermal nevus cells, and these have been seen by both transmission and scanning electron microscopy.[58,143,147] Mitochondria are common and cytoplasmic filaments can be prominent.

The type A nevus cells, which are located superficially in the dermis, are polygonal, have spacious amounts of cytoplasm, and appear to retain the ability to reactivate melanogenesis upon exposure to ultraviolet irradiation.[58] The smaller, type B lymphocytoid cells deeper in the dermis have diminutive and sparser melanosomes. Most of the melanosomes within the intradermal nevus cells are round, although short rod-like structures may also be seen. Type C nevus cells have elongated Schwannian processes that may become imbricated into organoid arrangements reminiscent of Meissner corpuscles.[44] Scattered rudimentary melanosomes and the absence of axons are morphologic features that have been used to defend the nevus rather than the Schwann cell origin for type C cells. It has recently been recognized that microfibrillar precursors of elastic fibers, which lack the deposition of amorphous elastin itself, are frequently associated with nevus cell clusters.[151] The ultrastructural features of benign nevus cells are clearly different from those of invasive malignant melanoma cells; these features have allowed Erlandson and Rosen[152] to distinguish an ectopic deposit of nevus cells in the capsule of a lymph node from metastatic melanoma cells. In the Spitz nevus (epithelioid-spindle cell nevus or juvenile melanoma),[142,144,148,150] many of the same features, including basement membrane formation, are present as in more common nevi, except that mitochondria are more prominent. Balloon cell nevi have highly vacuolated cytoplasm, which is felt to be due to an abnormality in melanosome formation, resulting in large cytoplasmic vacuoles with disoriented melanofilaments on which virtually no melanin is deposited.[153-155] On the other hand, in choroidal melanomas balloon cell transformation is the result of cytoplasmic accumulation of lipid droplets.[156-158]

The greatest controversy has been in the area of the ultrastructure of malignant melanoma. Mishima's work,[38,39,42] which at first held considerable promise for differentiating melanomas derived from nevus cells from those derived from dendritic melanocytes, presented a neat dichotomy and was predicated on putative biologic differences between melanomas arising from nevus cells and dendritic melanocytes. Nevocellular melanomas were held to be more malignant, whereas melanocytic melanomas were felt to be more indolent and more amenable to radiotherapy. Mishima's distinction between these two classes of melanoma hinged on mela-

nosomal morphology. In the nevocellular melanomas he described football-shaped melanosomes, measuring 500 nm × 150 nm, whereas in the melanocytic melanomas (which he referred to as examples of Dubreuilh's precancerous and cancerous melanosis), he described rod-shaped to round melanosomes, occasionally displaying a ring configuration with a central lucency, measuring 500 nm × 200 nm. He felt, however, that in the early and later stages, more elongated melanosomes resembling those seen in normal melanocytes and measuring 700 nm × 150 nm could be encountered.

Subsequent investigators[143,159-163] have by and large not been able to confirm Mishima's findings regarding melanosomal polymorphism as an indicator of cell of origin and therefore of biologic behavior. Much of the problem may derive from the fact that Mishima's study predated the definitive work of McGovern and Clark, leading to the major subdivisions of cutaneous melanoma described previously and now almost universally employed for diagnostic and prognostic purposes. For example, Mishima[39] described his nevocellular melanomas as arising from junctional nevi,[164,165] but it would seem more likely that these lesions were already in the radial growth phase of precursor melanoma lesions. He implied that the precancerous melanosis of Dubreuilh showed evidence of regression, but this obviously is a feature that has now been well recognized in superficial spreading melanoma. Some of Mishima's lesions of precancerous melanosis, therefore, would probably now be called lentigo maligna melanomas, whereas others might be referred to as superficial spreading melanomas. Mishima seemed to feel that nest formation was not a feature in keeping with lentigo maligna or precancerous melanosis of Dubreuilh, but it has been well recognized that nest formation is well within the province of these lesions. His published photomicrographs also call into question the accuracy of this light microscopic diagnoses according to present standards, in that hyperplasia and elongation of the rete ridges are described in a case of Dubreuilh's melanosis, which would suggest that this particular lesion was more kindred to superficial spreading melanoma than to lentigo maligna melanoma. The published illustrations of Dubreuilh's precancerous melanosis show cells at high levels of the epidermis by means of the tyrosinase-dopa staining technique, suggesting a closer similarity to superficial spreading melanoma. No clinical information is supplied on the patients studied, no numbers of cases are given, and it is not clear whether light microscopic illustrations were taken from the same cases on which electron microscopy was performed. In short, attractive though Mishima's hypothesis initially seemed to be, and despite the amount of effort that has been spent to confirm it, it appears to

be on very shaky footing in terms of our inability to transfer the ultrastructural melanosomal morphologic findings into present categories of melanoma diagnosis.

Clark and co-workers[160] devised a different descriptive scheme for melanosomal morphology in melanomas and described five types of melanosomes. Some tumors, particularly the lentigo maligna melanomas, displayed normal melanosomes that were essentially ellipsoidal in character but might be larger or smaller than those found in nonneoplastic melanocytes. The internal structure of the individual melanofilaments was discerned to have distinctive periodicity, and cross-striations were typically found. These well-formed melanosomes were characteristic of lentigo maligna melanoma. Abortive melanosomes were more spherical organelles and displayed more evidence of melanofilamentary disorganization, with less tendency to cross-linkage. Granular melanosomes were spherical in outline and contained melanin deposited in a granular fashion. Lamellar melanosomes were spherical organelles characterized by an internal array of disorganized, concentrically arranged whorls, also sometimes accompanied by clusters of vesicles. The last type was vacuolar melanosomes, which were empty spherical organelles with a limiting membrane. They never exhibited deposits of melanin, but dopa-positivity could be established in histochemical stains performed on tumors with these melanosomes. Other investigators[162] have also supplied evidence that these morphologically indifferent structures are premelanosomes. All of the other types of melanosomes were partially or erratically melaninized. The more rudimentary and abortive melanosomes were characteristic of superficial spreading and nodular melanomas but could not be used to distinguish them. The simple premelanosomal vacuoles were found to the exclusion of other melanosomal types in the extremely primitive cells of nodular melanoma.

Hunter and co-workers[161] attempted to apply the melanosomal types of Clark and associates[160] to their study of 40 malignant melanomas examined ultrastructurally, both in the radial growth phase and in the invasive components; their lesions were fortunately also diagnosed according to the Clark-McGovern classification. They discovered that lentigo maligna melanoma displayed a preponderance of ellipsoidal melanosomes with a normal substructure. On the other hand, abortive granular and lamellar organelles were found predominating in the superficial spreading and nodular melanomas, with the vacuolar melanosomes seeming to be encountered more frequently in superficial spreading melanoma than in other types—a feature somewhat, but perhaps not very importantly, divergent from that of Clark and associates.[160] Drzewiecki[166] studied 28

invasive lesions of melanoma, of both the nodular and superficial spreading types, and could not distinguish between them based on subcellular and melanosomal features. In Hunter and associates' study,[161] melanomas arising in association with nevi displayed all of the melanosomal typologies, a finding in keeping with our present knowledge that all types of melanomas appear to be able to arise in association with preexistent nevi,[105] a feature not recognized by Mishima.

The dichotomy in melanosomal polymorphism (well-formed-elongated versus abortive-spherical) described by Clark and associates[160] and Hunter and co-workers[161] has also been borne out by Klug and Gunter,[159] who studied 32 melanomas; however, these last cases were not diagnosed according to the Clark-McGovern classification. Klug and Gunter[159] described type A melanosomes as cigar-shaped, exhibiting atypical internal periodicity, and probably corresponding to the type of melanosome predominating in lentigo maligna melanoma. Type B melanosomes were spherical and tended to show no internal fibrillary structure, probably corresponding to the granular type. Curran and McCann[143] studied the ultrastructure of 16 malignant melanomas, again not classified according to the Clark-McGovern system. Of their 16 melanomas, 13 displayed ellipsoidal or cigar-shaped melanosomes, with an internal periodicity. They also described circular melanosomes as well as those appearing to have derangements of internal organization, but these are not well described in terms of the melanosomal system of Clark and associates.[160] Bleehen and co-workers[128] studied eight cases of lentigo maligna melanoma in both the radial and vertical growth phases. They found a preponderance of well-formed elongated melanosomes in the intraepidermal surrounded cells, but in the invasive nodules, granular and abortive melanosomes made their appearance attendant upon a dedifferentiation. Lupulescu and co-workers[129] described the ultrastructure of a "lentigo maligna" of the finger tip, a lesion that probably would now be diagnosed as an acral lentiginous melanoma. In this case, the authors described by electron microsoopy distinctive dendritic melanocytes, with cytoplasmic melanosomes having a distinctly elongated or cigar-shaped appearance with an internal periodicity, suggesting the neoplastic counterpart of dendritic melanocytes. Finally, 8 years after Mishima's first report,[39] Mishima and Matsunaka[139] published an electron microscopic study of 23 examples of pagetoid premalignant melanoma with invasive melanoma, diagnosed by McGovern or Pincus on the basis of slides that were sent to them. In this series they discovered that granular-spheroidal melanosomes were predominant. There is a curious obliquity in the failure of these workers to compare their later findings with those of Mishima's

earlier work. Of some interest was the discovery of giant spherical melanosomes in some of their superficial spreading melanomas.

There is, therefore, a consistent body of evidence that certain fundamental differences exist in the melanosomal morphologies of the different clinical types of melanoma, but it is by no means clear that there is any fundamental histogenetic implication to this observation. Mintzis and Silvers[163] somewhat confused the lines emerging from these other studies in their electron microscopic evaluation of 27 lesions of preinvasive intraepithelial melanocytic hyperplasia; 17 were associated with malignant melanoma and 10 were benign simulants of melanoma as judged by light microscopy. These workers, who were unaware of some of the studies previously mentioned, were unable to find any distinctive cytologic or melanosomal differences to segregate true melanoma precursors from benign melanocytic hyperplasias. They found a tremendous overlap of all the melanosomal morphologic findings of earlier studies and concluded there was no pathognomonic melanosomal type to separate benign from malignant tumors. Unfortunately, in their data and illustrations they did not provide precise diagnoses to correspond to the electron micrographs. Additionally, they described the juxtaposition of extremely atypical cells with more normal-appearing cells, the latter containing well-formed elongated melanosomes, but they did not take into account the possibility that a neoplastic population of cells may frequently collide with preexistent benign dendritic melanocytes of the epidermis. Furthermore, there was no attempt in this particular evaluation to examine nuclear characteristics or the type and number of nonmelanosomal cytoplasmic organelles.

The fixation of some pathologists on melanosomal morphology as a clue to the histogenetic origin of cutaneous melanoma has not side-tracked other investigators from codifying nonmelanosomal subcellular features of benign and malignant melanocytic proliferations that might be of diagnostic value. Curran and McCann[143] were the only previous investigators to systematically compare the morphologic features of benign melanocytic nevi with those of malignant melanoma. They believed that electron microscopy was a useful adjunct in the interpretation of these disparate classes of lesions but, by and large, was not necessary for clinical diagnosis and mostly served investigational purposes. Other workers[159-161] also observed subcellular aberrations in melanoma cells that are diagnostically useful. The nuclei of malignant melanoma cells are particularly bizarre, with an evenly dispersed or open chromatin pattern, frequent nuclear lobations, deep nuclear envelope indentations, cytoplasmic intranuclear sequestrations or herniations (pseudoinclusions), and pronounced nucleoli. The cytoplasm of malignant cells has been found to be endowed

with more polyribosomes, malformed mitochondria, and abortive forms of melanosomes than that of benign dendritic melanocytes and nevus cells.

The accumulation of large phagolysosomal complexes in the cytoplasm of malignant cells tends to distinguish them from benign cells, although the latter may also display smaller phagolysosomal collections. Annulate lamellae, tubular inclusions within profiles of rough-surfaced endoplasmic reticulum, exaggerated amounts of the rough-surfaced endoplasmic reticulum, desmosomes with filamentary insertions, and cytoplasmic pseudolumens are unusual features, which, when present, additionally reinforce the impression of malignancy.[167-171] Basement membrane formation has been described sporadically as partially surrounding invasive malignant melanoma cells, and this feature may also be observed in metastatic foci. Both scanning and transmission electron microscopy have demonstrated the presence of villous processes, filopodia, and dendrites in invasive nodules of melanoma as well as in their metastases.[166,171,172] Based on the present state of our knowledge about the ultrastructure of invasive melanoma cells, it is not possible to predict which tumors will metastasize and which will not, because well-differentiated types of melanosomes, basement membranes, and the persistence of other subcellular features identical to those of the invasive nodules can be seen in metastases. Morphology clearly cannot account for the imponderableness of host immunity and the diversity of the cell surface antigens in morphologically inseparable melanoma cells. These latter factors probably play a crucial role in the late stages of the evolution of cutaneous and conjunctival melanomas in predisposing to metastases.[173]

The last group of lesions that has been studied in dermatopathology is the melanocytic hamartomas of the dermis, including nevus of Ito, the mongolian spot, blue nevi, cellular blue nevi, and melanotic clear cell sarcomas of tendon sheath origin.[174-185] The morphologic features described in many of these lesions include a more spindle cell constituent population, frequently elongated melanosomes with an internal melanofilamentary architecture, spotty basement membrane formation, and tangles of cellular processes. Many authors consider these lesions to be derivative of bona fide melanocytes, but others interpret some of them as instances of pigmented melanotic Schwannomas (either benign or malignant).[186-189] In view of the fact that type C nevus cells may form extremely elaborate imbricated delicate processes[44] and are still believed to be nevus cells rather than Schwann cells, and that such processes can even be seen in metastatic melanomas,[171] encountering similar elaborate tangles of processes in some of the proliferative deeply situated pigment-

ed lesions may not be a basis on which to designate such tumors as being of Schwann cell origin. The debate about pigmented Schwannoma versus a deeply situated melanocytic hamartoma may be more academic than useful, in view of the common origin of both cells from the neural crest, which would allow the rare possibility for Schwann cells to phenotypically express melanin synthesis. The expression of both vimentin and S-100 protein in the cytoplasms of neurogenic and melanocytic cells further substantiates the considerable overlap between these two cellular types.[35-37]

Melanin has also been identified in unusual spindle cell melanoma variants, such as desmoplastic melanoma[190,191] and neurotropic melanoma[192,193]; these lesions are generally believed to have an initial origin from intraepidermal melanocytes. Spindle cell melanoma nodules are frequently spawned by lentigo maligna, acral lentiginous, and mucosal melanomas; they therefore should be distinguished from the foregoing spindle cell tumors arising from deeply situated melanocytes. Overall, clinicopathologic correlation, rather than electron microscopy, creates the basis for such distinctions.

PRESENT STUDY

MATERIALS AND METHODS

Patients with pigmented lesions involving the epibulbar, forniceal, plical-caruncular, or palpebral portions of the conjunctiva were examined, and the clinical appearance of the lesions was photographed. Specimens from patients with benign racial epithelial melanosis were obtained at the time of cataract extraction, and their small biopsies were entirely utilized for electron microscopic evaluation. In all of the other cases in which the lesions were removed for diagnostic or therapeutic reasons, portions of the lesions were submitted for both light and electron microscopic evaluation. Focal or nodular lesions (eg, nevi or invasive nodules of melanoma) were bisected; one half was submitted for light microscopy and the other half for electron microscopy. In those patients who had extensive flat pigmentation of the conjunctiva, usually as the result of primary acquired melanosis, multiple biopsies (two to four) were obtained from areas of different intensities of pigmentation and at varying locations away from a nodule of invasive melanoma, if present. The multiple biopsies were carefully charted on the patient's record so that light microscopic and electron microscopic correlations could be made with clinical appearances. When a biopsy of primary acquired melanosis was made, one half

of the specimen was submitted for light microscopy and the remainder for electron microscopy. The accuracy of all light microscopic pathologic diagnoses was confirmed by an ophthalmic pathologist not connected with the study.

For electron microscopy, tissues were fixed with 3.5% glutaraldehyde in 0.05 N phosphate buffer (pH 7.4) for more than 1 day, postfixed with 1% osmium tetroxide in Caulfield's buffer (pH 7.4) for 2 hours, dehydrated with graded alcohols, and embedded in resin (Durcupan ACM). First, 1-μ sections were cut and stained with methylene blue-azure II for preliminary light microscopy. Desired areas selected in the light microscopy were then trimmed, thin-sectioned by an ultramicrotome (Porter-Blum), stained with uranyl acetate and lead citrate, and examined by electron microscopy (Zeiss EM9S, Siemens Elmiskop I and JEOL, JEM-100S).

RESULTS

BENIGN EPITHELIAL MELANOSIS AND PIGMENTED SQUAMOUS PAPILLOMA

Eleven lesions with benign melanocytes were studied. In six elderly black patients undergoing cataract extraction, a biopsy specimen of a portion of clinically pigmented epibulbar conjunctiva (Fig 2) next to the conjunctival flap was obtained for electron microscopic study. In these patients the pigmentation was most intense at the limbal zone and frequently extended horizontally in the epibulbar conjunctiva across the interpalpebral fissure. The pigmentation was dark to golden-brown, occasionally fine, but more often coarsely granular. The pigmentation always moved with the conjunctiva. A 46-year-old black woman complained of bilateral and symmetrical inferior epibulbar and forniceal pigmentation, which she had been aware of for 4 to 5 months and which appeared to vary in intensity from time to time. An 18-year-old pregnant Hispanic woman presented with bilateral inferior forniceal pigmentation (Fig 3, top), which disappeared after childbirth. A 71-year-old Caucasian woman had an epibulbar lymphoid mass with a faint overyling epithelial pigmentation. Finally, two middle-aged black men each had a juxtalimbal or plical pigmented lesion in which an underlying vascular pattern suggesting a pigmented squamous papilloma could be identified (Fig 3, bottom).

When the 1-μ plastic sections were examined by light microscopy, an intense deposition of melanin was seen among the basal cells of the conjunctival epithelium (Fig 4) in all patients except the two with the pigmented squamous papillomas. Mononuclear inflammation and melanophages were not conspicuous in the subjacent substantia propria. It was

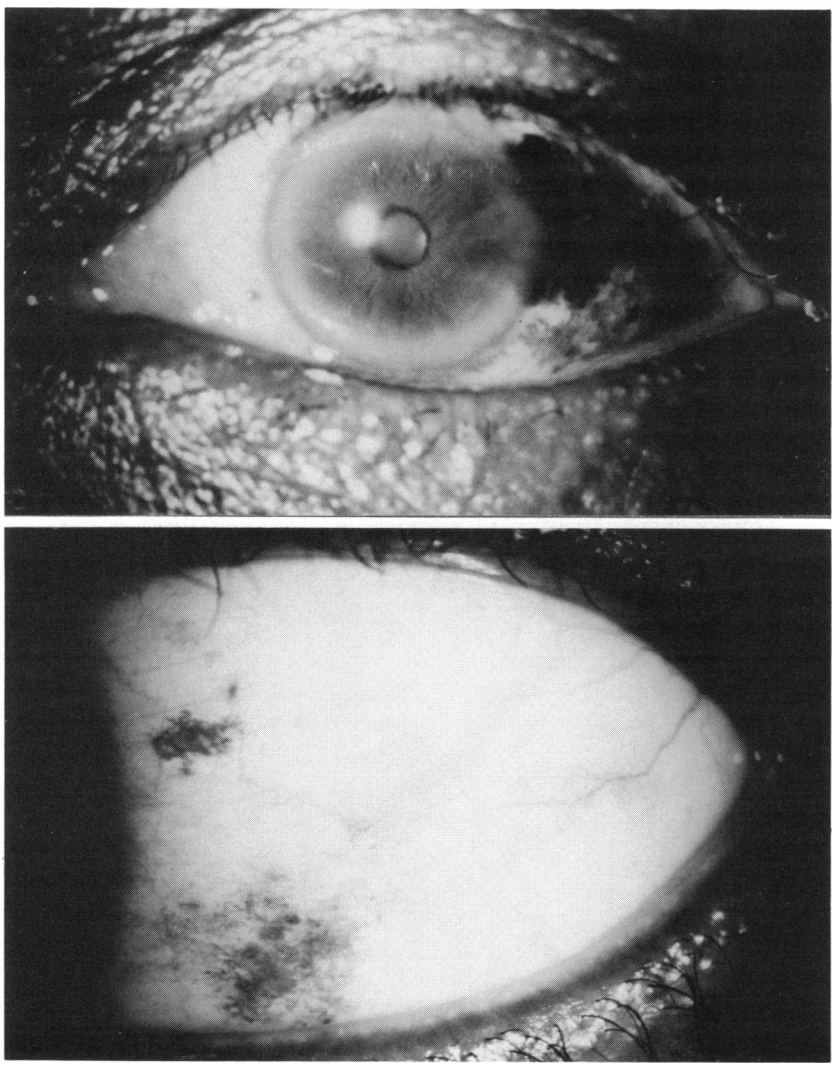

FIGURE 2
Benign epithelial melanosis in two black patients.

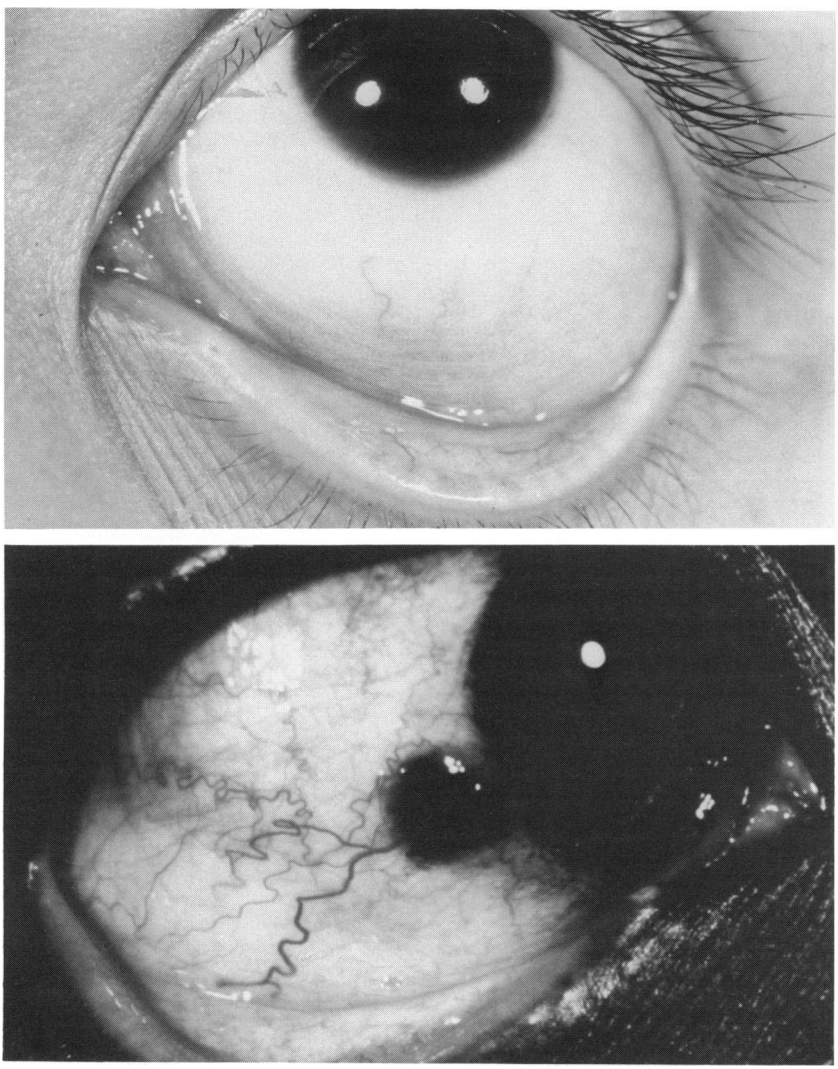

FIGURE 3
TOP: Faint inferior forniceal pigmentation in Hispanic female. The pigmentation appeared during pregnancy and disappeared after childbirth (chloasma). BOTTOM: Juxtalimbal pigmented lesion in a black male that was a pigment squamous cell papilloma.

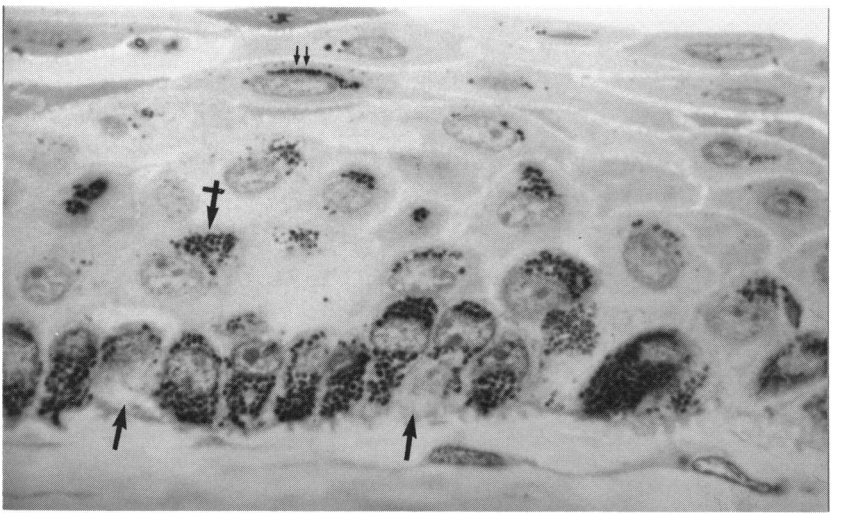

FIGURE 4
Benign epithelial melanosis displays abundant melanosomes within basal keratinocytes. Between every four to five of the latter cells is lodged a melanocyte (*arrows*) with somewhat retracted cytoplasm and less cytoplasmic melanin. Suprabasilar keratinocytes rearrange melanin granules into supranuclear position (*crossed arrow*), until finally most superficial squamous cells exhibit a more elongated disposition of melanin granules (*double small arrows*) (1-μ plastic section, methylene blue-azure II, × 280).

extremely difficult to distinguish melanocytes from the basal keratinocytes. Pigmentation was also observed in the suprabasilar conjunctival keratinocytes, frequently assuming a supranuclear aggregation (supranuclear pigment caps). The two patients with bilateral inferior forniceal pigmentation displayed scattered melanocytes in the suprabasilar epithelium, and their dendritic processes extended to extremely high levels of the epithelium (Fig 5). In the two pigmented squamous papillomas, very little pigment deposition was seen among the basal cells surrounding the papillary cores, and only a small amount of pigment appeared to have been exchanged to the surrounding keratinocytes (Fig 6). Instead, plump dendritic processes of intermixed melanocytes were seen scattered among the proliferating benign keratinocytes of the papilloma. At the tumor-free edges of the specimens, the undisturbed conjunctival epithelium showed the same pattern as that in the cases of benign racial melanosis.

At the ultrastructural level, scattered among every five or six basal keratinocytes was a dendritic melanocyte, with a comparatively clear cytoplasm due to the absence of tonofilaments (Fig 7). The nucleus dis-

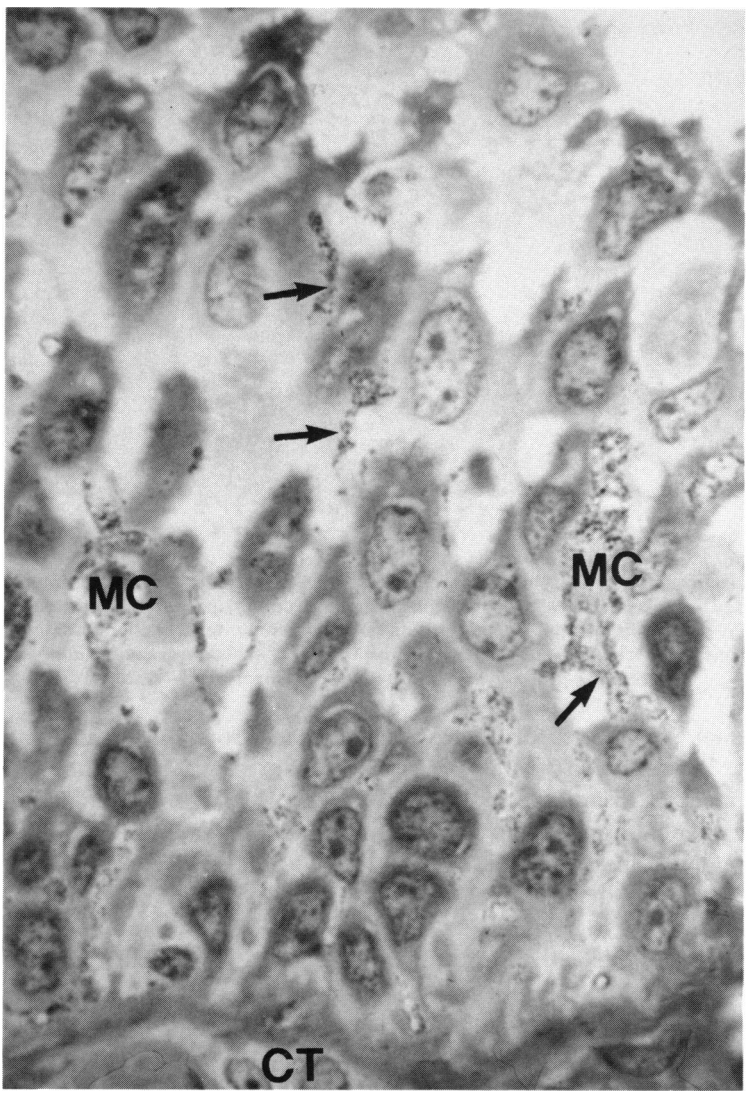

FIGURE 5

Benign epithelial melanosis in pigmentation of pregnancy shows mild melanocytic (MC) hyperplasia with widely separated melanocytes at higher locations within epithelium. Dendritic processes (*arrows*) extend from melanocyte to insinuate at high levels between keratinocytes. CT subepithelial connective tissue (1-μ plastic section, methylene blue-azure II, × 120).

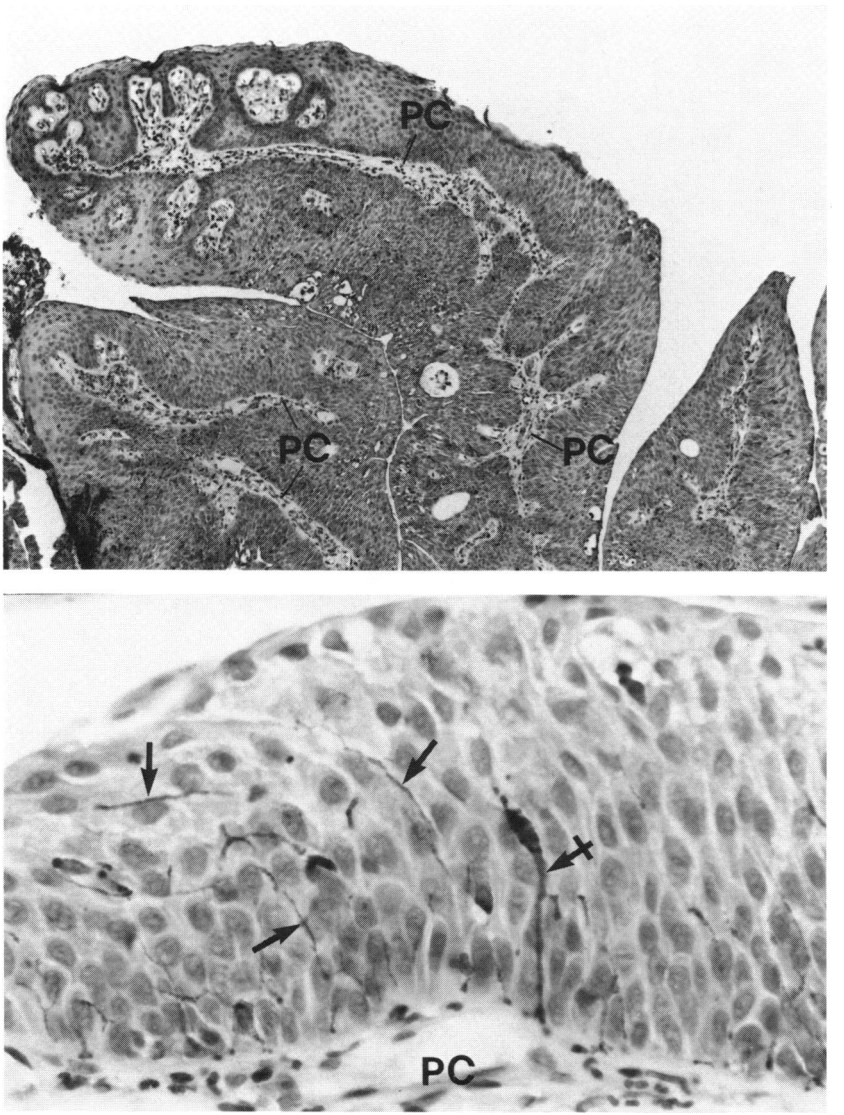

FIGURE 6

TOP: Pigmented squamous cell papilloma displays benign epithelium covering fibrovascular papillary cores (PC) (hematoxylin-eosin, × 90). BOTTOM: Cell body of melanocytes (*crossed arrow*) is not restricted to basal layer, but can be found at all levels of hyperplastic epithelium. Small dendritic processes (*arrows*) ramify throughout epithelium, which is not notably pigmented. PC, papillary core (hematoxylin-eosin, × 200).

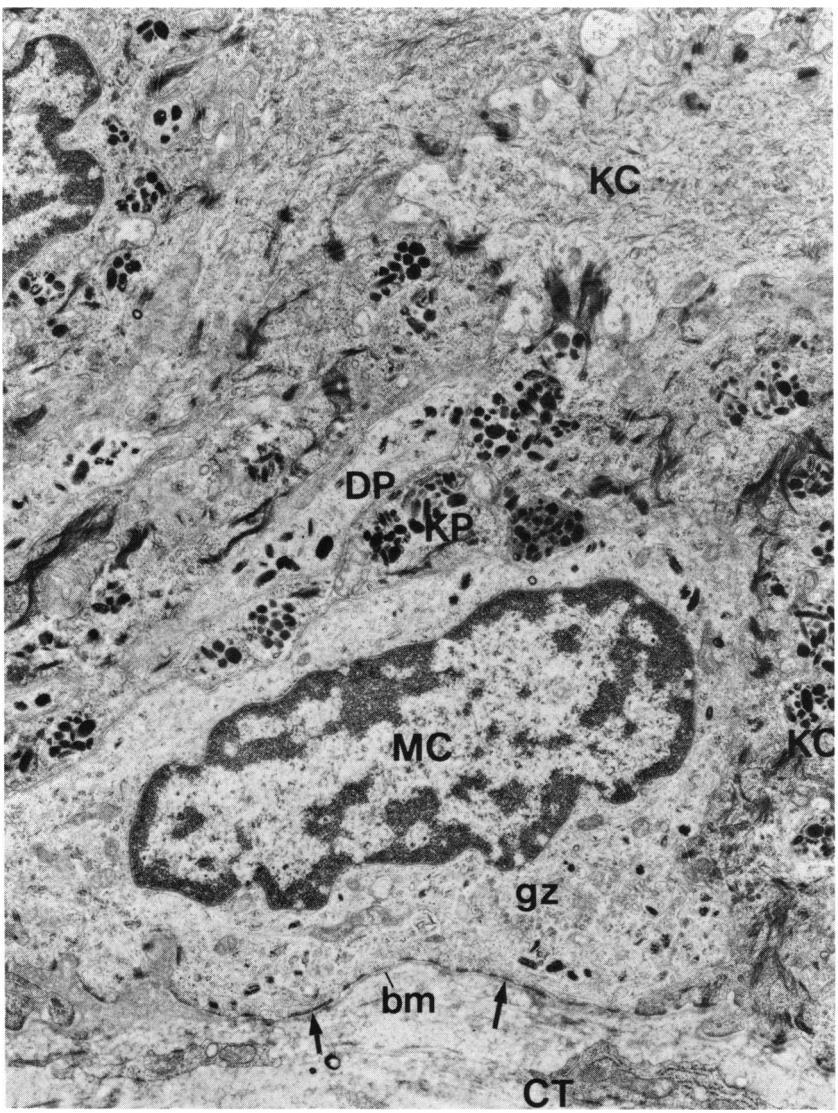

FIGURE 7
Benign epithelial melanosis. Melanocyte (MC) sits on epithelial basement membrane (bm) and focally displays hemidesmosomes (*arrows*). Perikaryon of melanocyte displays small numbers of melanosomes, originating in Golgi zone (gz). Dendritic process (DP) of melanocyte is more electron-lucent in comparison with that of surrounding keratinocytic processes (KP), and contains fewer melanosomes. KC, keratinocyte; CT, subepithelial connective tissue (× 13,000).

played clumped heterochromatin at the nuclear membrane, and the nucleolus was thickly textured. The nuclei were at least 20% smaller than those of the keratinocytes. The melanocytes rested on the epithelial basement membrane and formed focal hemidesmosomal plasmalemmal densifications. Within the cytoplasmic region of the perikaryon were scattered stage 2 or stage 3 melanosomes; numerous smaller vesicles appearing to acquire melanin deposition were concentrated in the vesicles of the Golgi zone. Most of the stage 4 melanosomes were aggregated into the dendritic processes of the melanocyte, which insinuated between basilar and suprabasilar keratinocytes. The dendritic processes of the melanocytes (Figs 7 and 8) could be distinguished from the keratinocytic processes because the former tended to have evenly dispersed melanin granules, whereas the latter had more aggregated melanosomal granules. Furthermore, wisps of tonofilaments were identified in the keratinocytic cytoplasm, and desmosomes and tonofilaments were never seen in the dendritic processes of the melanocytes. The most dramatic electron microscopic feature of these lesions was the intense accumulation of melanin granules in the cytoplasm of some of the keratinocytes (Fig 8). These cells at first glance appeared to be melanocytes, but they showed bundles of tonofilaments and formed desmosomes with adjacent basilar keratinocytes. Basilar and suprabasilar keratinocytes totally packed with melanin granules (Fig 9) often had the aspect of a macrophage and might be termed keratinocytic melanophages. The melanin granules were characteristically clustered in groups with and without a distinct limiting membrane that failed to display evidence of melanosomal degradation. Rarely there was evidence of the true formation of phagolysosomal complexes in the keratinocytes (Fig 10), with the presence of a limiting membrane and a darker background material including granular debris of melanosomal degradation.

In three of the cases, an unusual feature was the accumulation of lipid vacuoles in the cytoplasm of the dendritic melanocytes (Fig 11). Exceptionally, a single melanocyte was dislocated into a suprabasilar position and showed the same morphologic features as the melanocytes located along the basement membrane region (Fig 12, top). The keratinocytes in the intermediate zones of the epithelium displayed collections of melanosomes set among the cytoplasmic tonofilaments (Fig 12, bottom); the apical cells bordering the conjunctival sac (Fig 13) frequently had a supranuclear collection of melanin granules. The melanosomes among the black patients were all elongated, cigar-shaped, and measured on the average 200 nm × 500 nm. In the one Caucasian patient with benign melanosis secondary to an underlying lymphoid infiltrate in the substantia

propria, the granules were somewhat rounder and were incompletely melaninized; they measured 400 nm × 160 nm (Fig 11, bottom).

The two patients with bilaterally symmetric pigmentation of the inferior cul-de-sac and inferior epibulbar conjunctiva displayed features very similar to those just described, except for more frequent disposition of melanocytes in a suprabasilar location (Fig 14); their dendritic processes extended to high levels of the epithelium and tended to be plump. Never were two melanocytes identified back-to-back. An intact functional relationship was maintained with the surrounding keratinocytes, which accepted pigment from the melanocytes and frequently displayed supranuclear caps of pigment in the superficial levels. Melanophagic keratinocytes among the basal cells were also observed. In the two patients with pigmented squamous papillomas, melanocytes were not restricted to the basement membrane region of the papillary cores (Fig 15) but were frequently located at all levels of the acanthotic epithelium (Fig 16). Pigment was communicated to the surrounding benign proliferating keratinocytes, but not as intensely as in benign racial epithelial melanosis; keratinocytic melanophages were not observed. The morphology of the melanocytes was identical to that in benign racial melanosis, in that the nuclear chromatin was clumped and dendritic processes extended among the proliferating keratinocytes. The melanin in the keratinocytes was dispersed as single granules rather than being aggregated into small clusters or segregated into phagolysosomes.

MELANOCYTIC NEVI

Sixteen patients with conjunctival melanocytic nevi underwent excision of their lesions. Eight patients were male and eight were female. Fifteen patients were Caucasians and 1 was a black female. Patients were aged 8 to 75 years at the time of excision, with a median age of 29 years. All but one of the lesions were located in the interpalpebral zone from the limbus to the caruncle or on the epibulbar surface (Fig 17); the one exception was a peripunctal cystic-appearing nonpigmented lesion on the right lid margin. None of the lesions was located in the fornices or on the palpebral conjunctiva. All lesions were thickened, elevated, and sharply demarcated with respect to the adjacent conjunctiva. The most common reason for excision was enlargement or a change in pigmentation, causing either a cosmetic blemish or concern about a possible malignant transformation. Nine lesions had cysts (Fig 18) discernible on slit lamp examination, and all were freely movable over the epibulbar underlying connective tissues. Two lesions were intensely pigmented, and six lesions were lightly to moderately pigmented; eight lesions appeared to either be nonpigmented

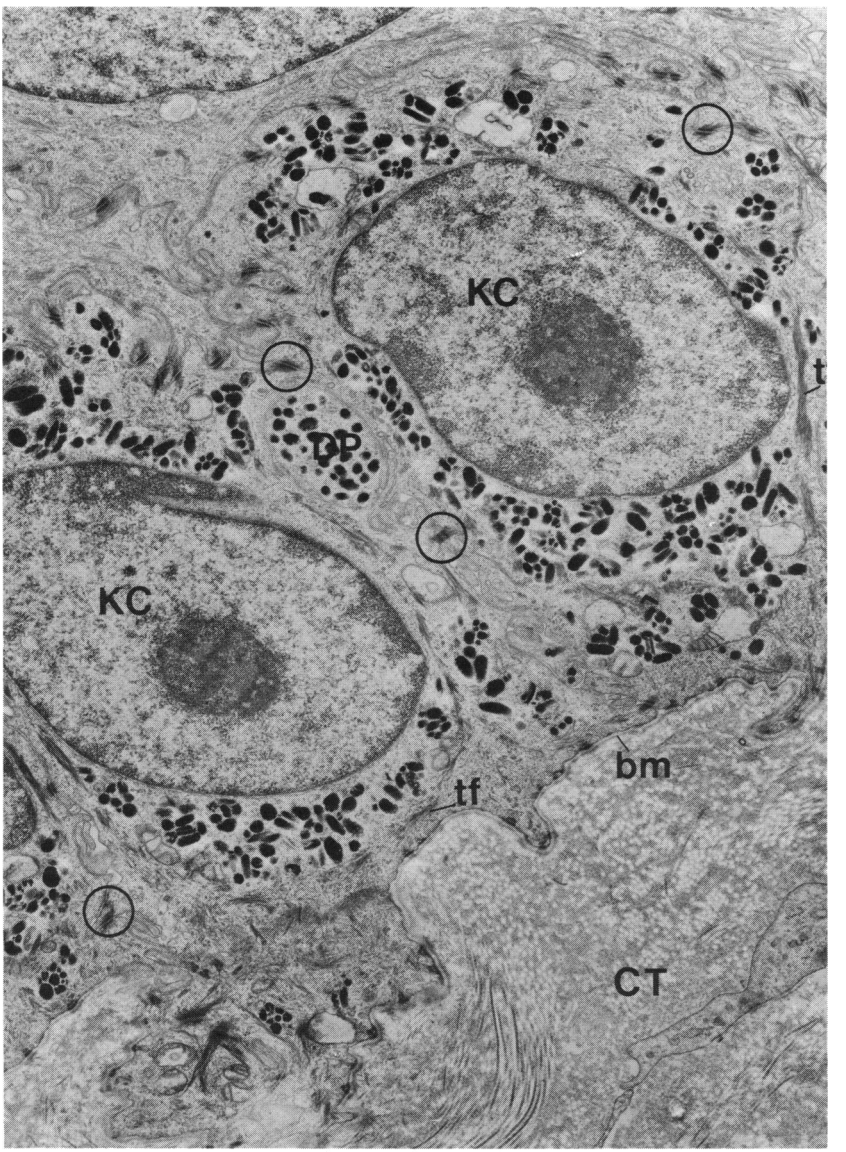

FIGURE 8
Basal keratinocytes (KC) look almost like melanocytes because of their heavy content of cytoplasmic melanin granules, but they form intercellular desmosomes (*circles*) and possess cytoplasmic tonofilaments (tf). A melanocytic dendritic process (DP) is insinuated between two keratinocytes. bm, basement membrane; CT, connective tissue of substantia propria (× 10,500).

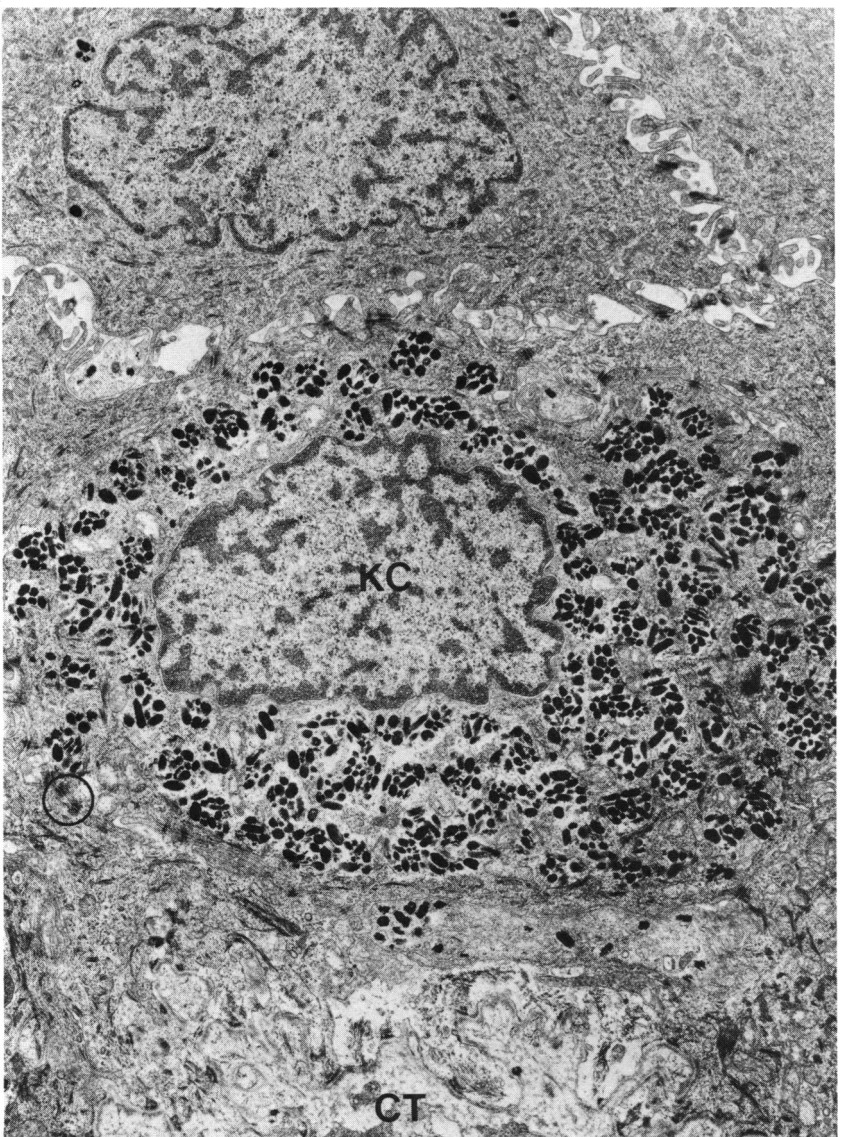

FIGURE 9
A keratinocyte (KC) is so engorged with melanin granules that it is truly a melanophage. Cell forms desmosomes with adjacent less melaninized cells (*circle*). CT, connective tissue (× 10,500).

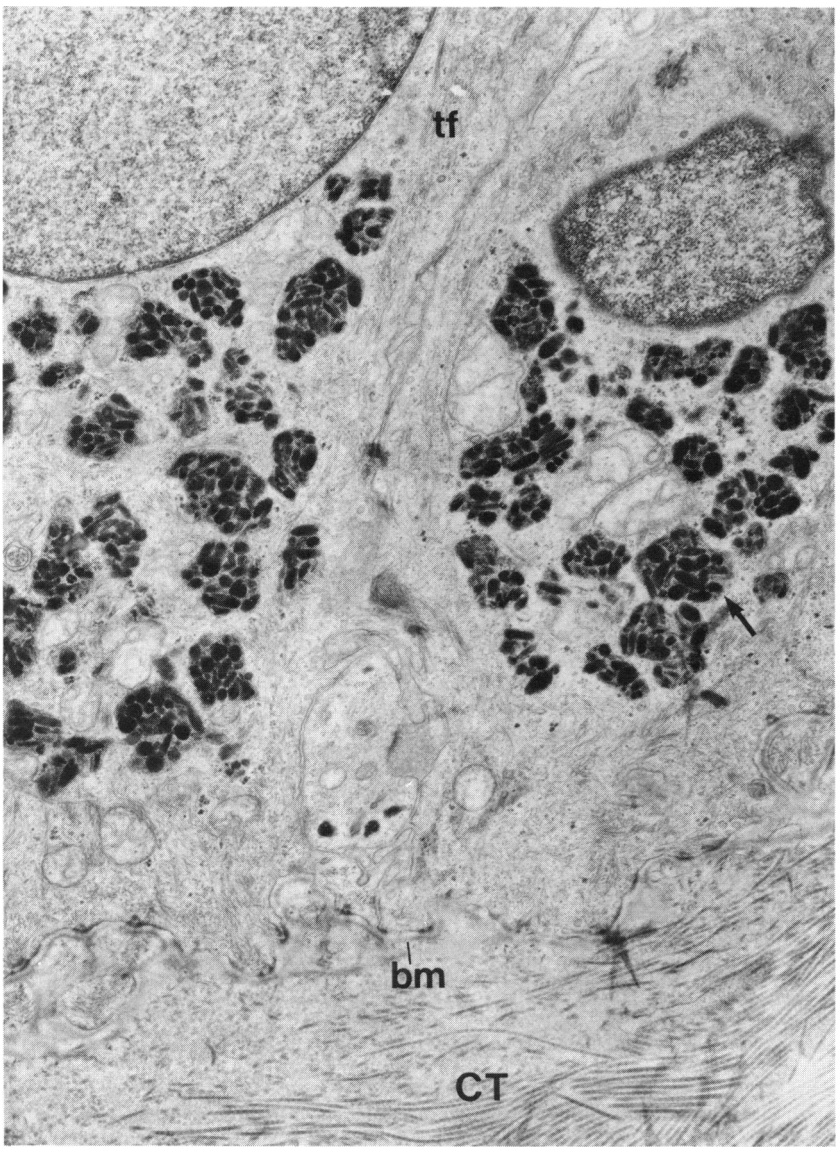

FIGURE 10
Basal keratinocytes contain cytoplasmic melanin organized into phagolysosomes (*arrow*) with an electron-dense background. Tonofilaments (tf) are present in cytoplasm. bm, basement membrane; CT, connective tissue (× 15,000).

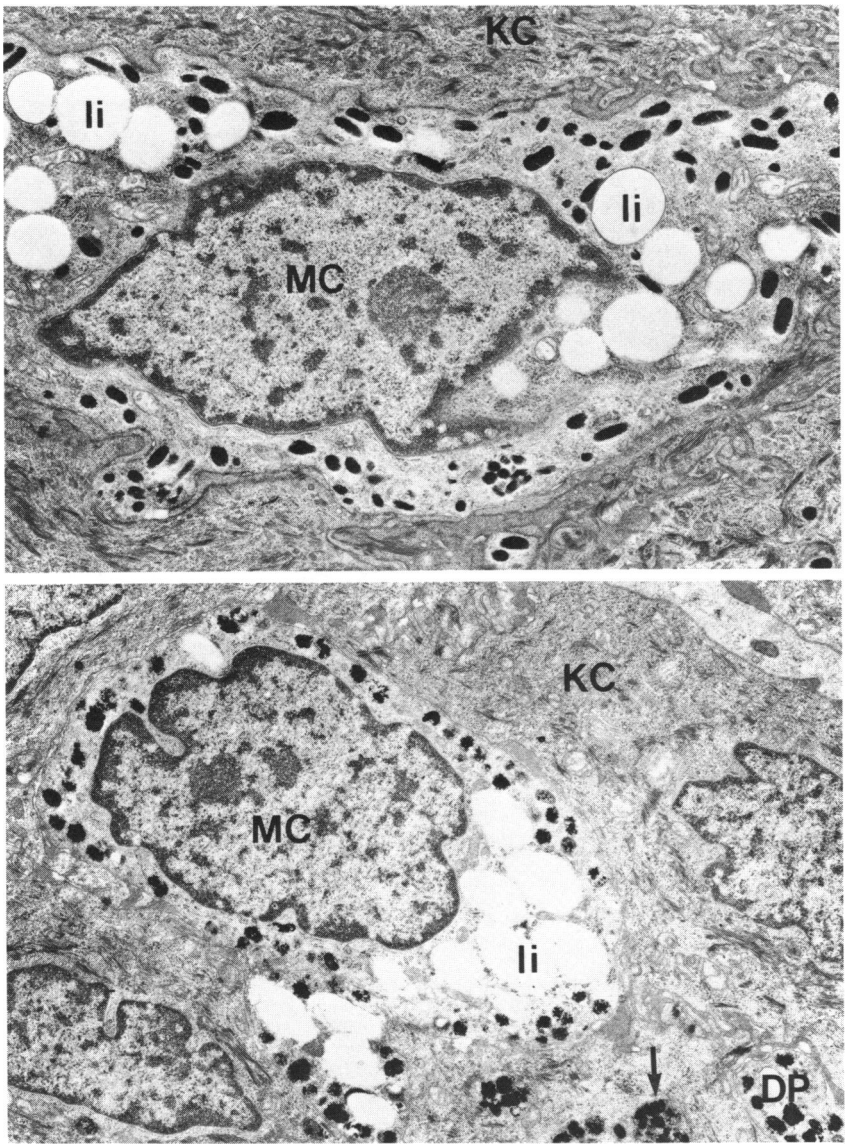

FIGURE 11

TOP: Melanocyte (MC) shows prominent accumulation of lipid droplets (li) within cytoplasm. KC, keratinocyte. BOTTOM: Melanocyte (MC) from a Caucasian patient with melanosis displays less totally melaninized melanosomes compared with those from black patient shown above, and melanosomes are smaller than those in blacks, measuring in this case 400 × 160 nm. Cytoplasm also displays lipid droplets (li). Phagolysosomes (*arrow*) appear in cytoplasm of surrounding keratinocytes (KC). A dendritic process (DP) extends from melanocyte between keratinocytes (× 10,000).

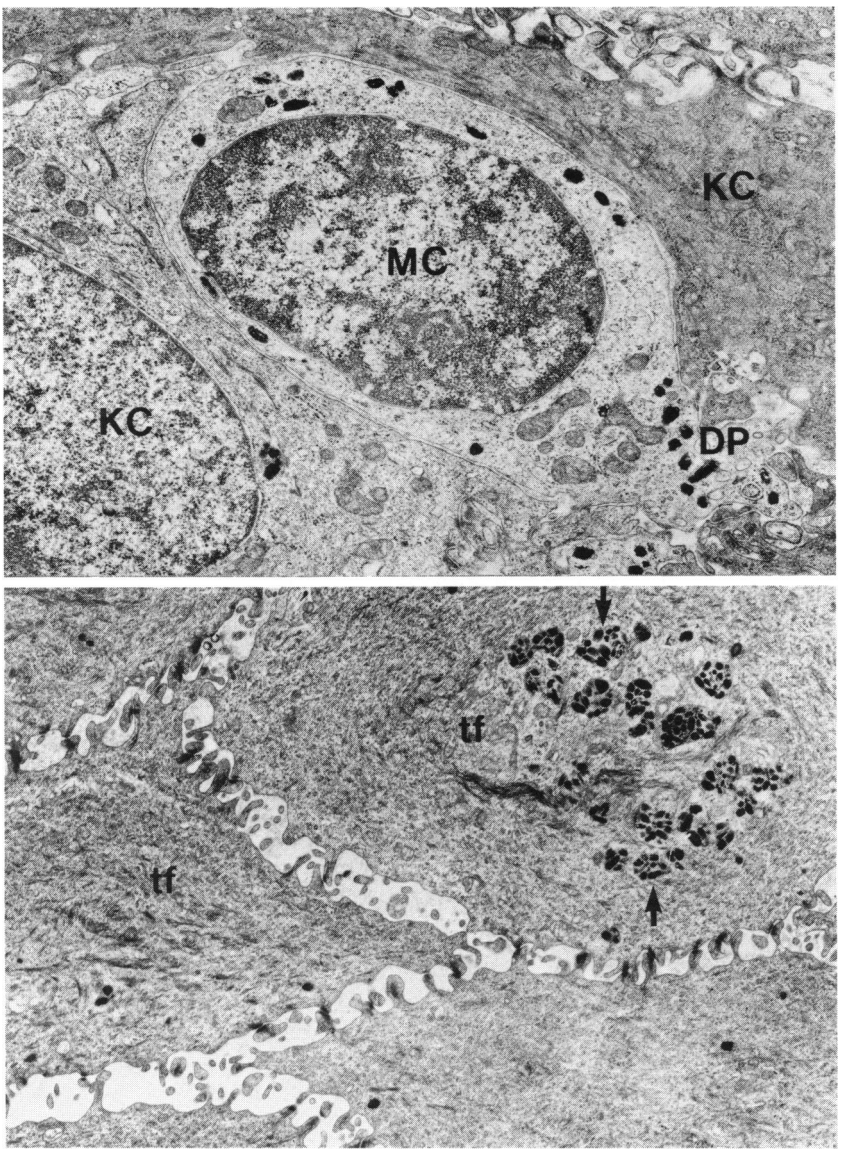

FIGURE 12

TOP: An individual melanocyte (MC) is dislocated above basilar layer and resides between suprabasilar keratinocytes (KC). A dendritic process (DP) extends beyond perikaryon of melanocyte (× 12,500). BOTTOM: Melanosomes are segregated into phagolysosomes (*arrows*) in many suprabasilar keratinocytes, which contain tonofilaments (tf). Note widened intercellular space with bridging desmosomes (× 10,000).

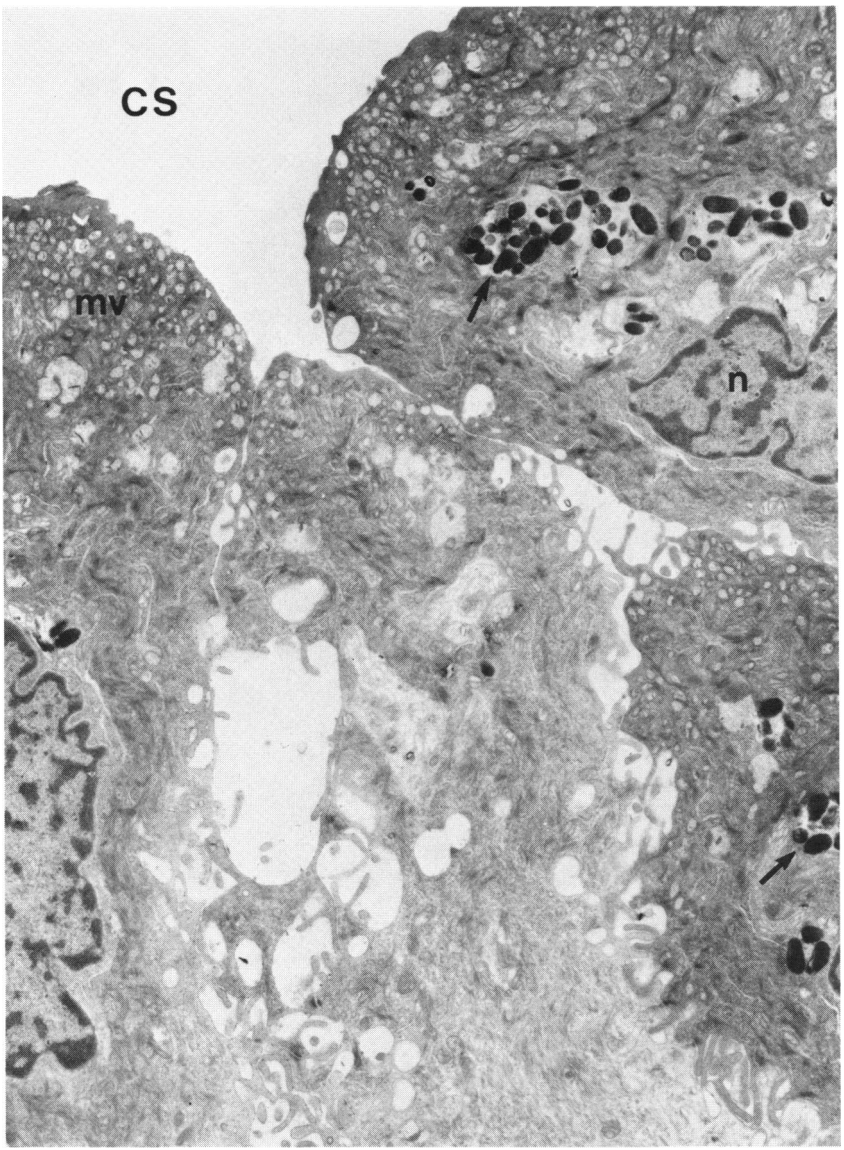

FIGURE 13
Melanin granules (*arrows*) form a supranuclear cap within most superficial keratinocytes. These latter cells display small mucus vesicles (mv). CS, conjunctival sac; n, nucleus (× 10,800).

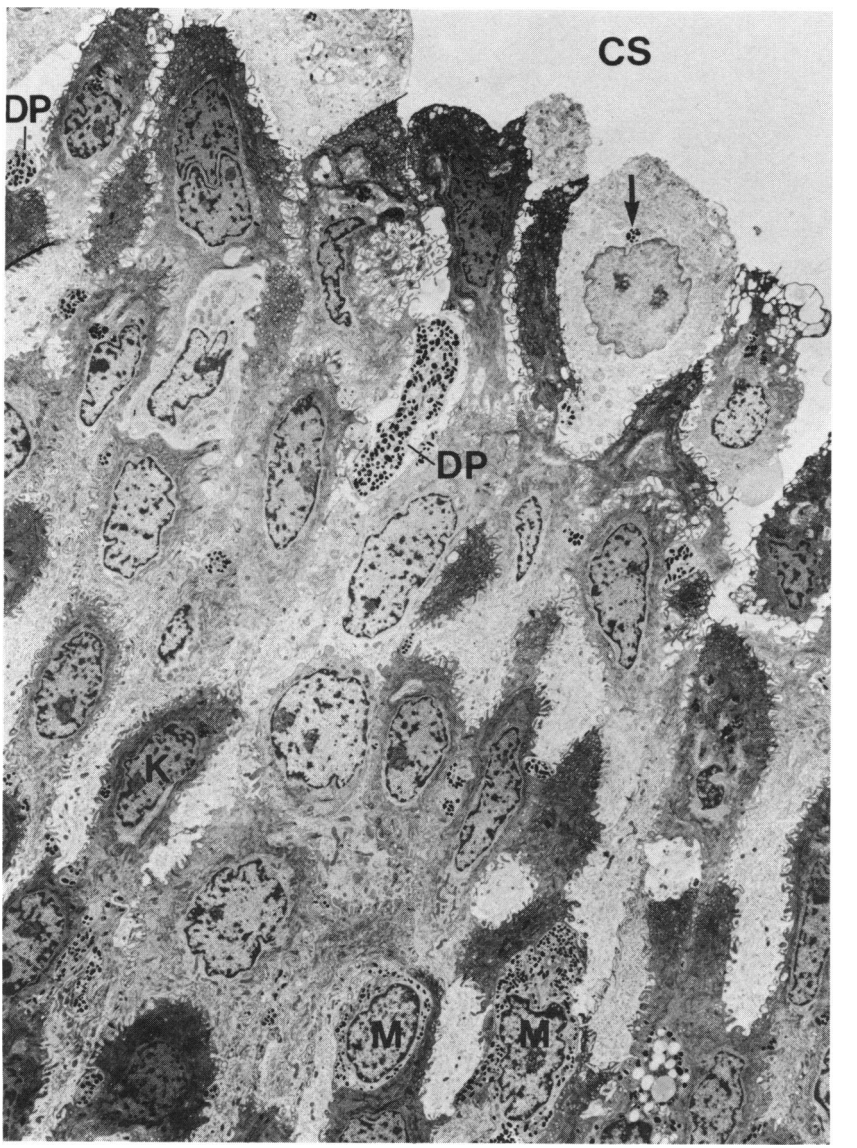

FIGURE 14

Benign epithelial melanosis showing mild melanocytic (M) hyperplasia, with melanocytes situated in suprabasilar epithelium, but still separated from each other by processes of keratinocytes. Large and somewhat plump dendritic processes (DP) extend to high levels of epithelium. Melanin has been transferred to surrounding keratinocytes, and is normally disposed as supranuclear collections (*arrow*). K, keratinocyte; CS, conjunctival sac (× 23,000).

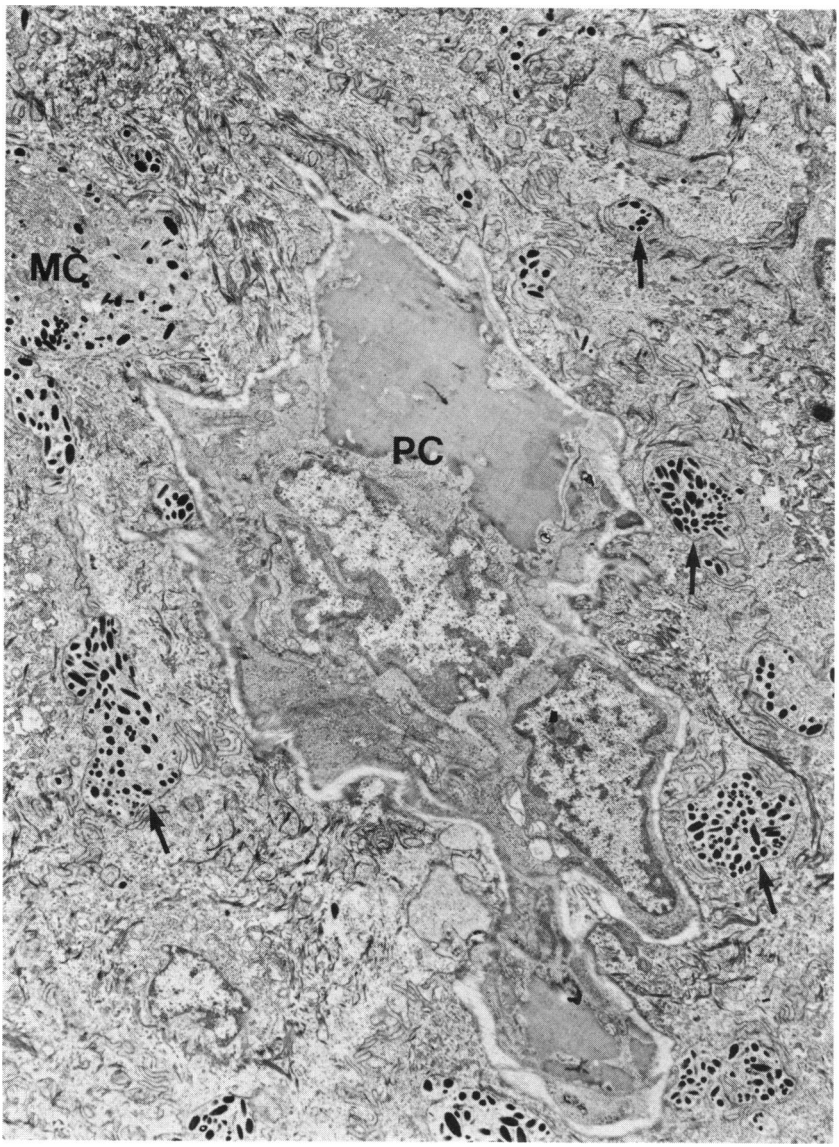

FIGURE 15

Pigmented squamous cell papilloma showing proliferating cells around a connective tissue papillary core (PC). A melanocyte (MC) is located just above basilar keratinocytic cell processes, and multiple dendritic processes (*arrows*) of melanocytes insinuate among keratinocytes. Note sparse transfer of melanin to cytoplasm of keratinocytes (× 5600).

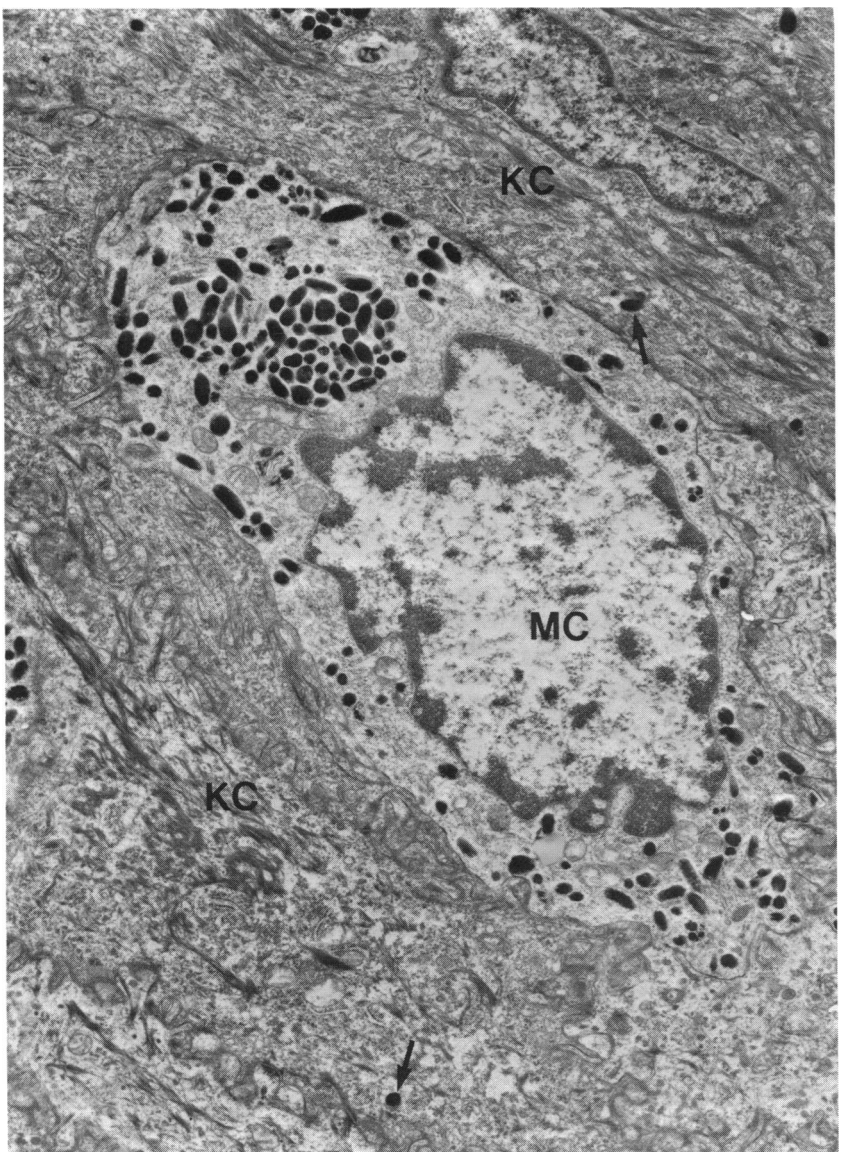

FIGURE 16
Melanocytes (MC) in pigmented squamous cell papilloma located at all levels of hyperplastic benign epithelium. Keratinocytes (KC) possess bundles of tonofilaments and sparse numbers of individually disposed melanin granules (*arrows*) (× 11,000).

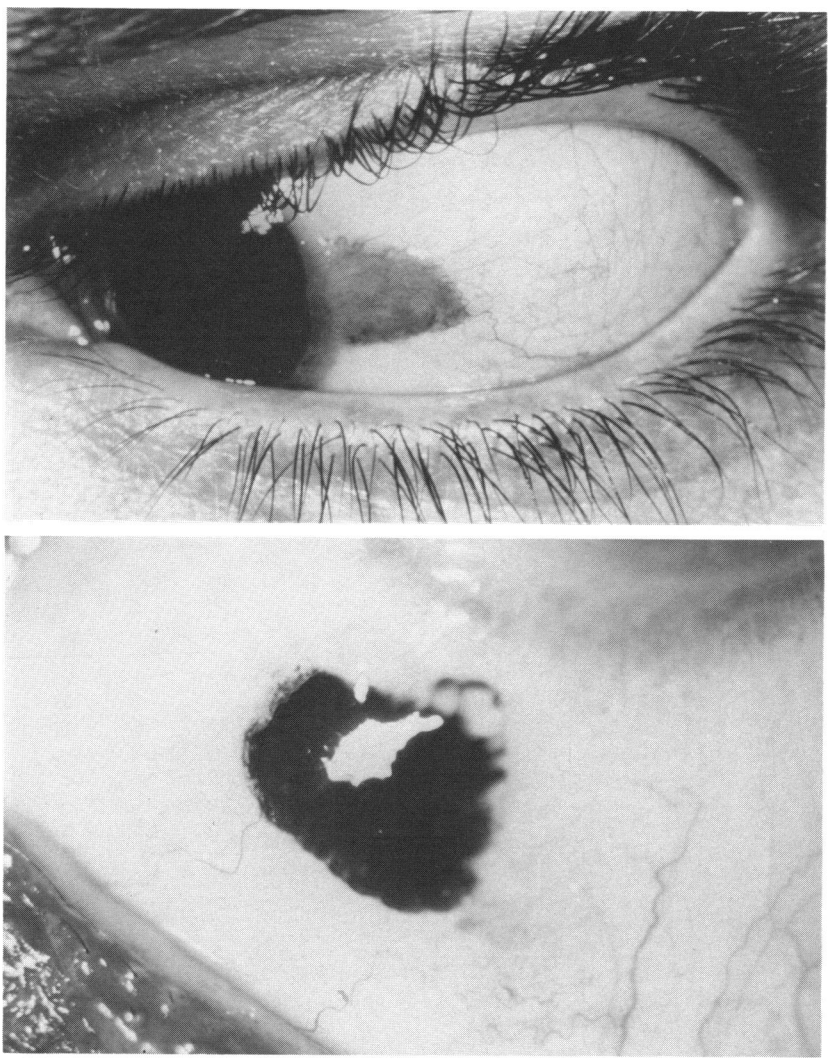

FIGURE 17
TOP: Juxtalimbal flesh-colored nevus in a 23-year-old Caucasian male who reported slow growth over a 2- to 3-year period. BOTTOM: Heavily pigmented epibulbar nevus in a black patient. Note cysts at upper inner edge of lesion with surrounding pigmentation.

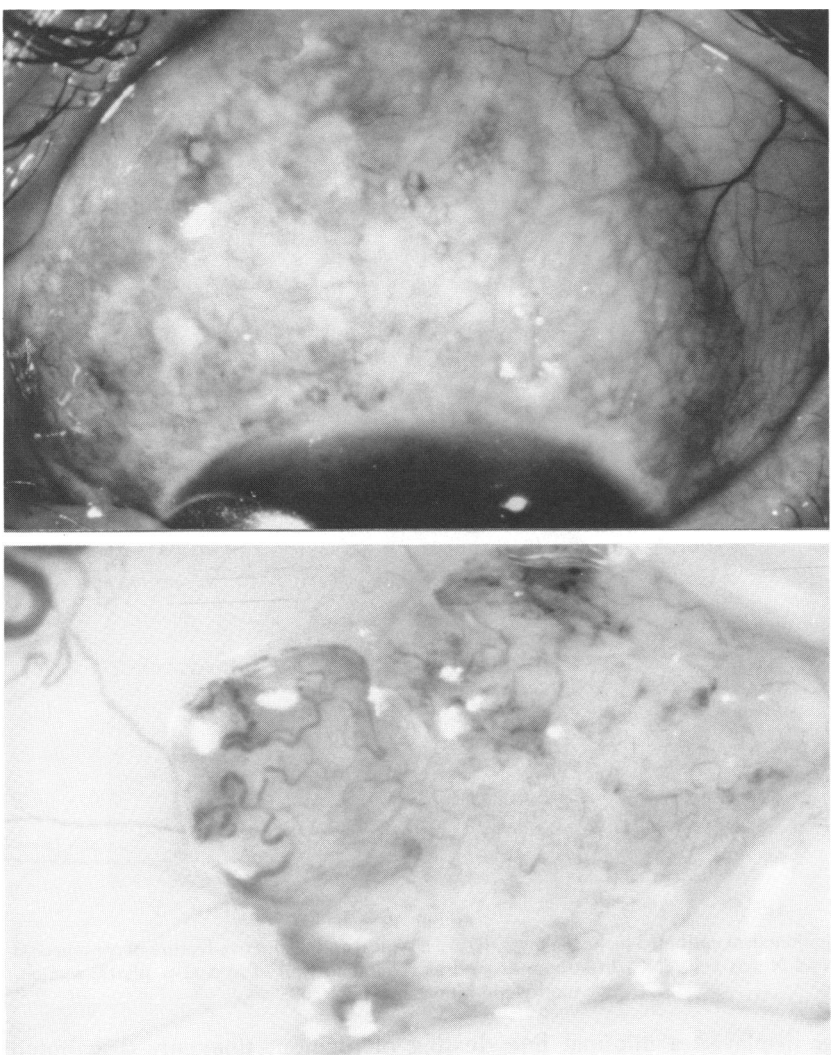

FIGURE 18
TOP: An epibulbar, spottily pigmented nevus covers most of upper half of eyeball. Note sharp demarcation and slight elevation of lesion. BOTTOM: A cystic nevus of plica.

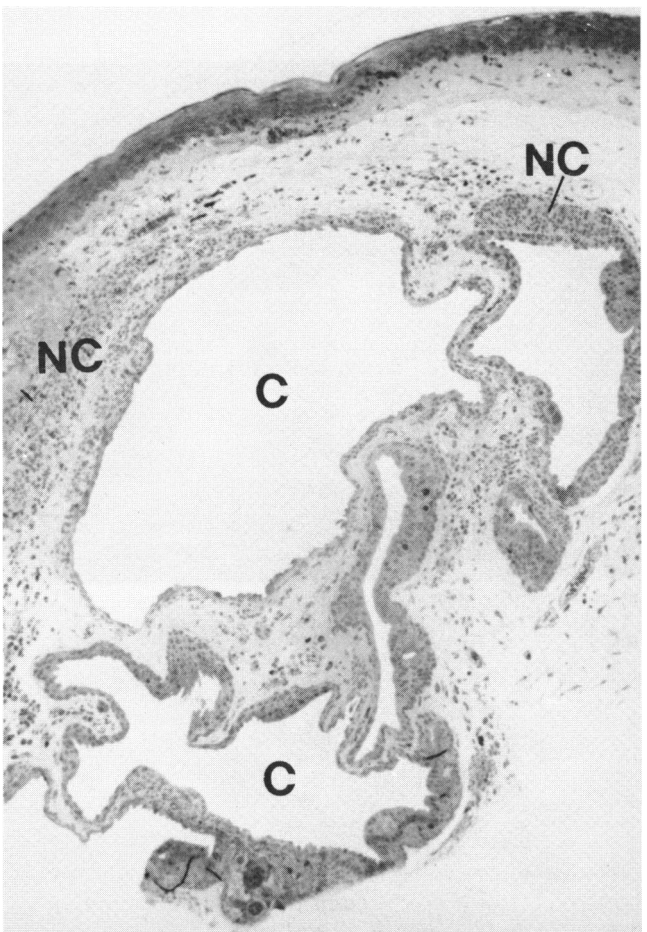

FIGURE 19
Conjunctival epithelial inclusion cysts (C) are a typical and distinctive feature of conjunctival nevi. Nevus cells (NC) in stroma as well as related to wall of cyst (1-μ plastic section, methylene blue-azure II, × 60).

or displayed a minimal fine dusting of pigment unevenly distributed throughout the mass.

Light microscopic evaluations failed to show that any of the lesions was a pure junctional nevus. Epithelial cystic inclusions were identified in 13 of the 16 lesions (Figs 19 and 20); junctional nests were occasionally observed in the epithelium of these cysts. One lesion in a younger subject showed prominent junctional nests and the early formation of subepithe-

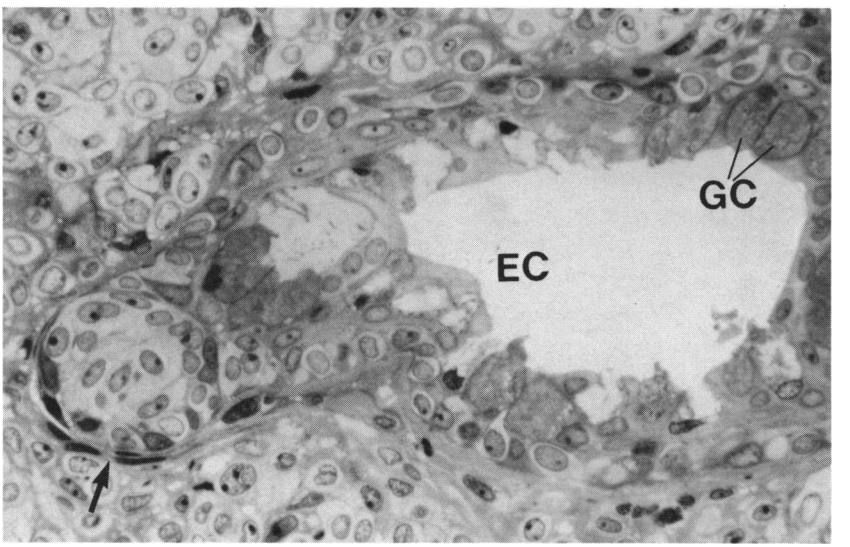

FIGURE 20
Smaller epithelial inclusion cyst (EC) contains goblet cells (GC) in epithelial lining . Cyst is surrounded by stromal nevus cells, but a small junctional nest (*arrow*) is present within epithelial lining of cyst (1-μ plastic section, methylene blue-azure II, × 220).

lial nests of nevus cells within the superficial substantia propria (Fig 21). Eight lesions were predominantly subepithelial but exhibited a significant degree of junctional activity. Five lesions were predominantly subepithelial with only minimal or difficult-to-discover junctional activity. Two lesions appeared to be totally subepithelial with a grenz zone of connective tissue separating the epithelium without junctional nests from the underlying collections of nevus cells in the substantia propria.

The 1-μ plastic sections demonstrated the exquisitely circumscribed nature of the junctional nests, with a sharp edge created by the bordering keratinocytes (Fig 22). No individual cells distributed singly among the keratinocytes were identified outside the junctional nests. The cells within the junctional nests had irregularly shaped nuclei, prominent nucleoli, and a variable presence of cytoplasmic melanin, which was frequently coarse. Small amounts of melanin were found in the cytoplasm of the neighboring keratinocytes. No mitotic figures were observed. In one lesion, lentiginous hyperplasia of the dendritic melanocytes within the epithelium was observed next to a compound nevus. Cells within the underlying connective tissue of the substantia propria showed pigment within the most superficially located nevus cells in six cases, and these

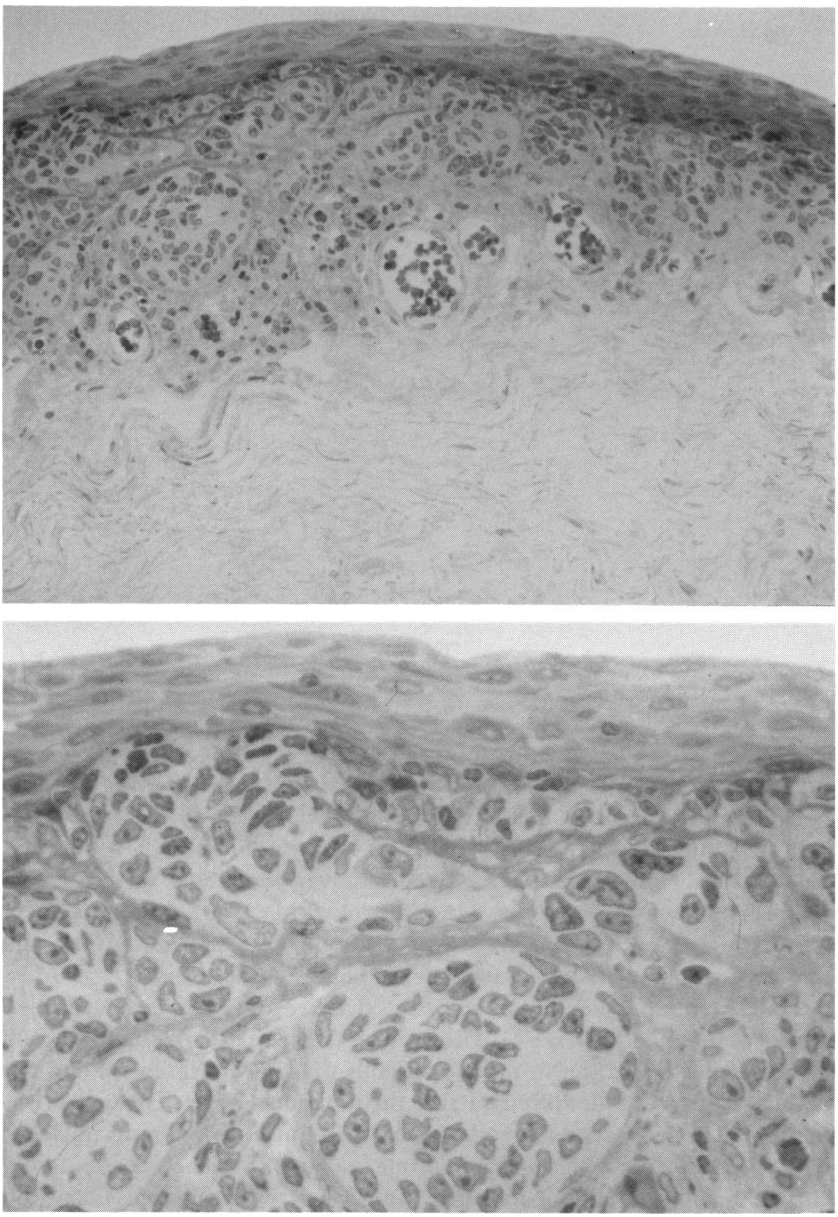

FIGURE 21

TOP: An early compound nevus in a 9-year-old child shows junctional nests merging with formation of stromal nests in upper substantia propria. Notice most of substantia propria shown below has yet to be occupied by nevus cells (1-μ plastic section, methylene blue-azure II, × 60). BOTTOM: Nevus cells have angulated nuclei, small nucleoli, and display same characteristics in both junctional nests and in immediately subadjacent stromal nests (1-μ plastic section, methylene blue-azure II, × 220).

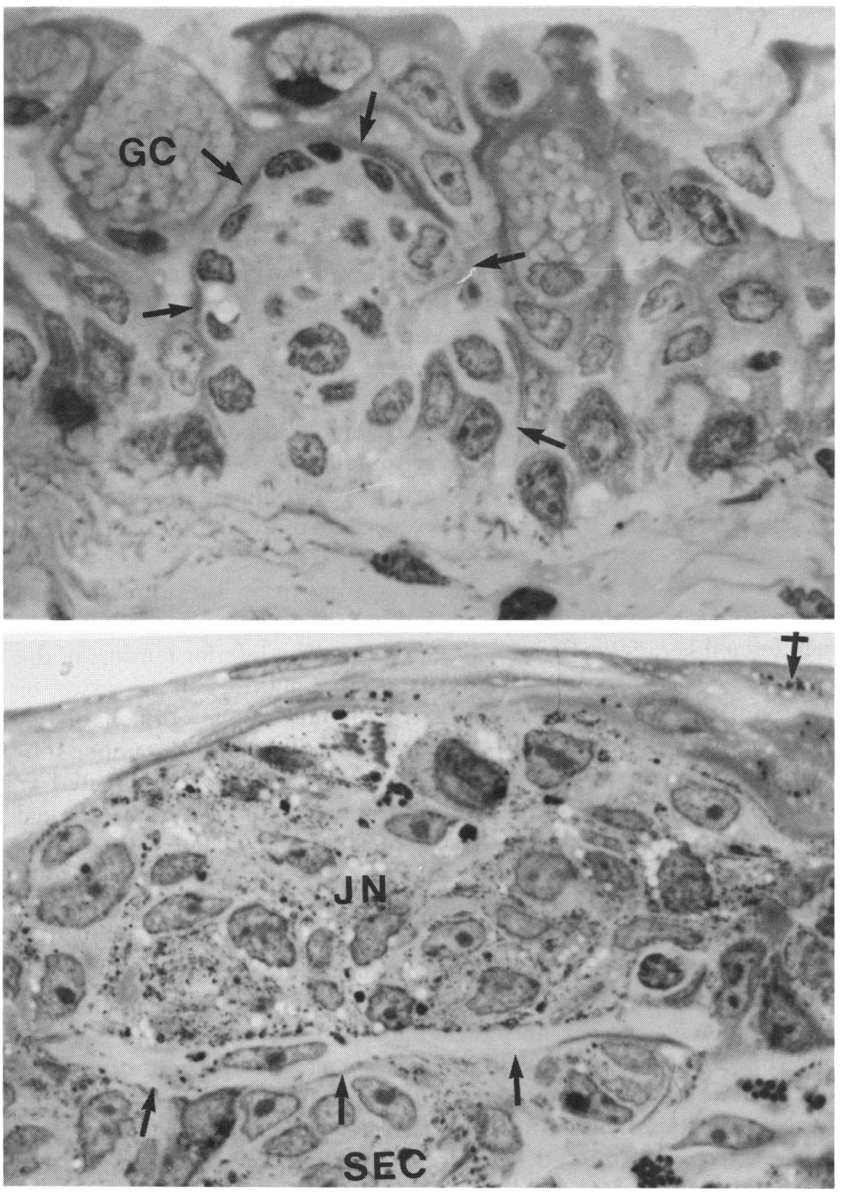

FIGURE 22

TOP: A small intraepithelial junctional nest shows clear demarcation (*arrows*) from surrounding keratinocytes. GC, goblet cell. BOTTOM: A large junctional nest (JN) contains cells with cytoplasmic melanin. A few melanin granules (*crossed arrow*) have been transferred to surrounding keratinocytes. Junctional nest is separated from subepithelial nevus cells (SEC) by a thin lamina of connective tissue containing a fibroblast (*arrows*) (1-μ plastic sections, methylene blue-azure II, × 210).

cells were virtually identical in morphologic characteristics to those within the epithelial junctional nests. In the deeper half of the subepithelial component of the nevi, the nevus cells formed variably sized nests (Fig 23), frequently invested by a prominent connective tissue fiber system. Pigment was not identified within the cytoplasm of these cells. The nuclei appeared to be smaller and more quiescent, possessed punctate nucleoli, and showed either a delicate nucleoplasm with dispersed chromatin or a moderately but not coarsely clumped chromatin pattern (Fig 24). Cells with these different nuclear chromatin patterns were clustered into segregated populations. In two lesions pigmented and nonpigmented cells were intermixed side-by-side. One completely subepithelial lesion was remarkable for containing round, poorly pigmented nevoid cells in clusters bordered by heavily pigmented spindle cells (Fig 25). The nevus occurring in a black patient was most heavily pigmented. No neuroid collections of spindle-shaped cells were located in the depths of any of the lesions.

At the ultrastructural level, the cells comprising the intraepithelial junctional nests were tightly clustered and demarcated outwardly by a well-defined keratinocytic layer (Fig 26, top). The inferior borders of the junctional nests were delimited by small keratinocytic cell processes, or else the participating nevus cells rested directly on the epithelial basement membrane. When nests of nevus cells bulged into the upper substantia propria, the basement membrane region became attenuated or disappeared (Fig 26, bottom). The cellular outlines of the intraepithelial nevus cells were generally polygonal, but both villi and thicker cellular processes could be identified (Fig 26, top). The nucleus was frequently irregular and indented, but intranuclear sequestrations or herniations of cytoplasm were usually inconspicuous and not very large (Fig 27). The nuclear chromatin was clumped at the nuclear membrane and distributed as heterochromatin aggregates throughout the nucleoplasm. The nucleoli were prominent, but the nucleolonema was tightly wound and coarsely ropy (Fig 27). Within the cytoplasm the major cytoplasmic organelles were well-developed Golgi zones with associated vesicles, many mitochondria, scattered ribosomes and rare polyribosomes, and only short segments of rough-surfaced endoplasmic reticulum (Figs 27 and 28). No desmosomes were identified between nevus cells or between nevus cells and the keratinocytes. Cytoplasmic filaments were only rarely seen and were not clustered into prominent bundles. Melanin granules were rudimentary, poorly developed with a disorganized internal structure, and only lightly melaninized by small, noncoalescent melanin particles. Internal striations of well-registered melanofilaments were not observed.

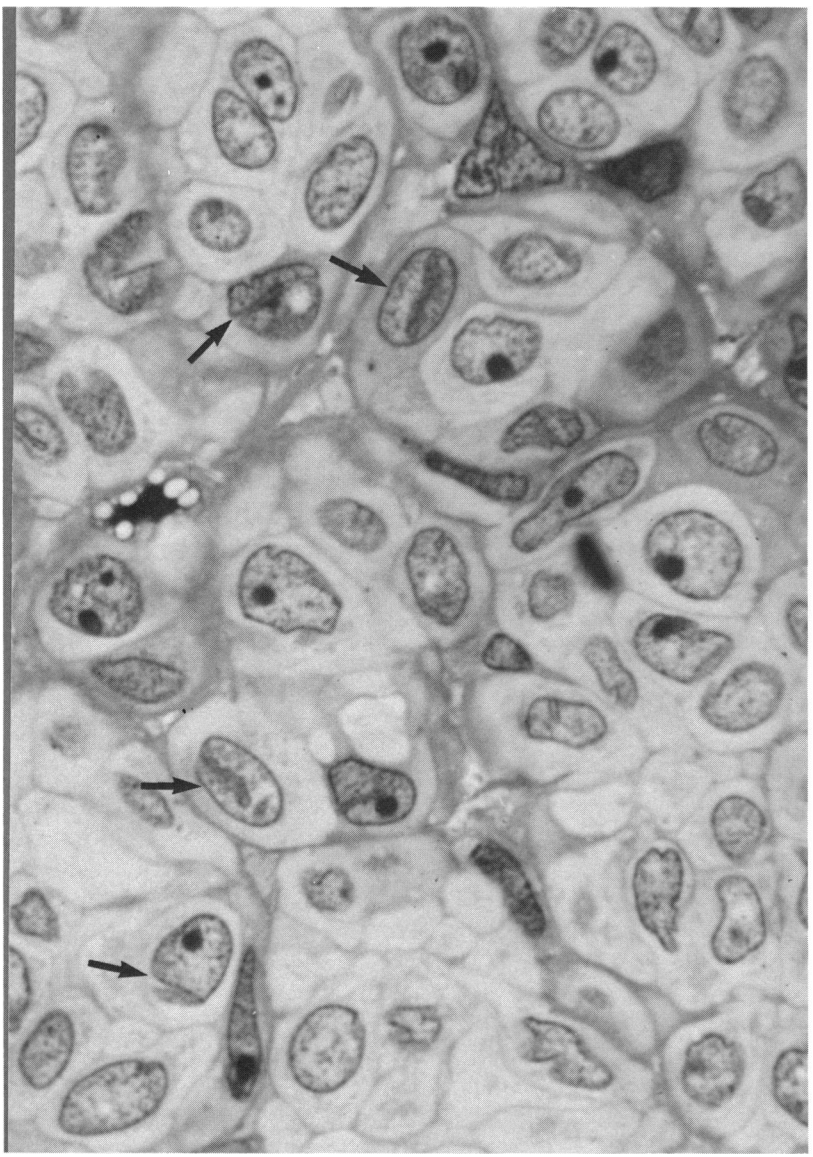

FIGURE 23
The stromal nevus cells are aggregated into small clusters by thin strands of connective tissue. Many nevus cells show nuclear indentations or folds (*arrows*), somewhat imitating nuclear grooves of spindle A uveal melanocytic proliferations (1-μ plastic section, methylene blue-azure II, × 290).

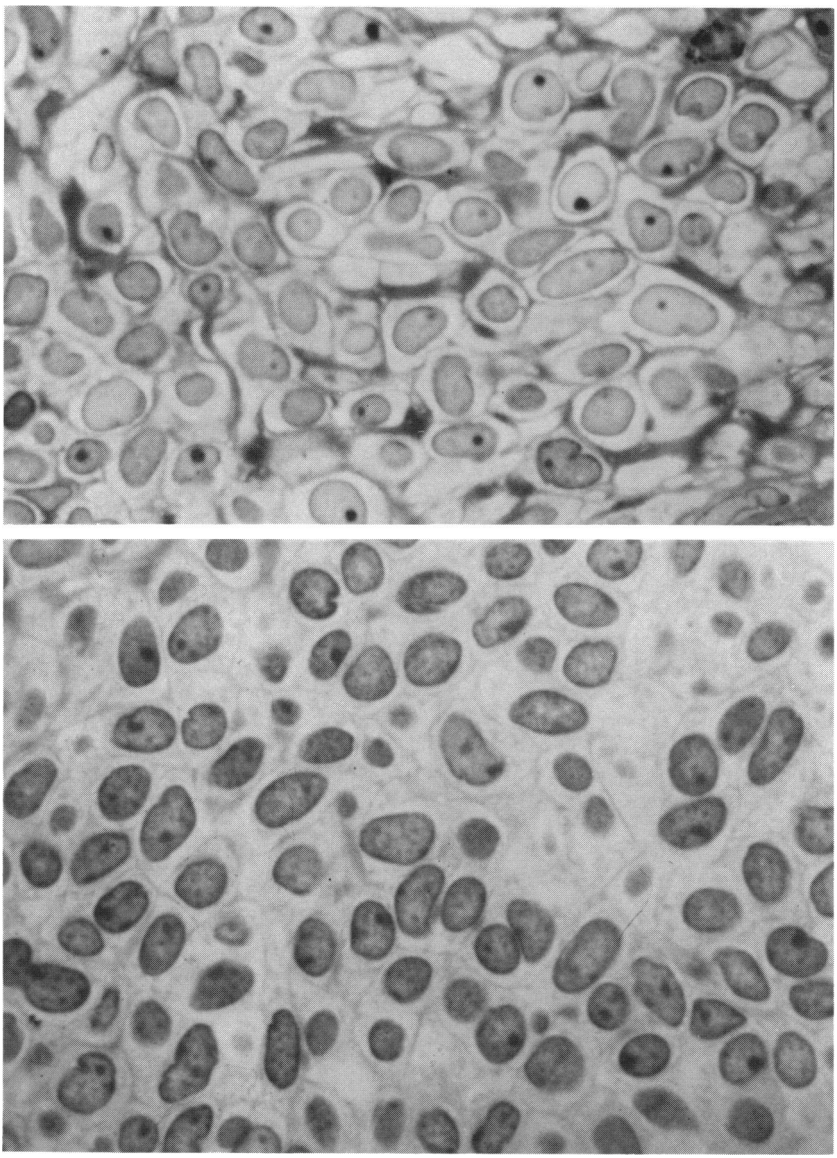

FIGURE 24

Subepithelial nevus cells displaying two different chromatin patterns. TOP: Nuclear chromatin is finely divided to impart appearance of a vesicular or delicate nucleus. Nucleoli are small and stand out from surrounding pale nucleoplasm. BOTTOM: Chromatin is more clumped, but there are no coarse granules in this compact type of chromatin organization. Small punctate nucleoli are still in evidence (1-μ plastic sections, methylene blue-azure II, × 270).

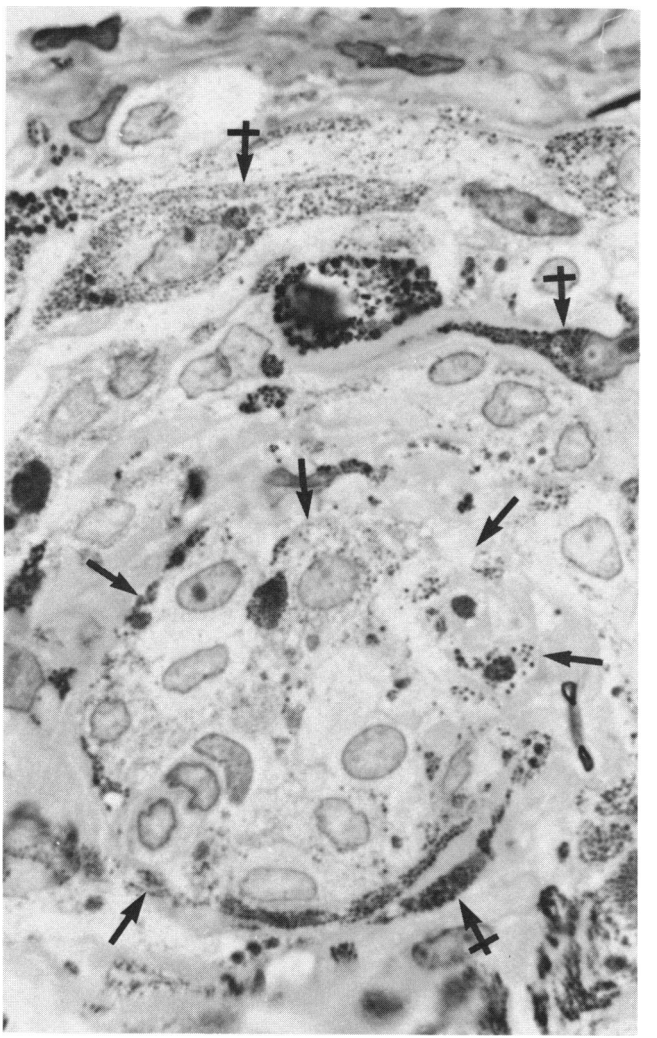

FIGURE 25

Subepithelial nevus cells from a plical lesion. A rounded nest of cells is identifiable (*arrows*), outside of which are more elongated, spindle cells (*crossed arrows*) with a heavy distribution of cytoplasmic melanin. While this lesion may appear to be a mixed or combined nevus (a routine nevus plus a blue nevus component), ultramicroscopically the nested cells and the more spindle cells showed identical organization and melanosomes (1-μ plastic section, methylene blue-azure II, × 260).

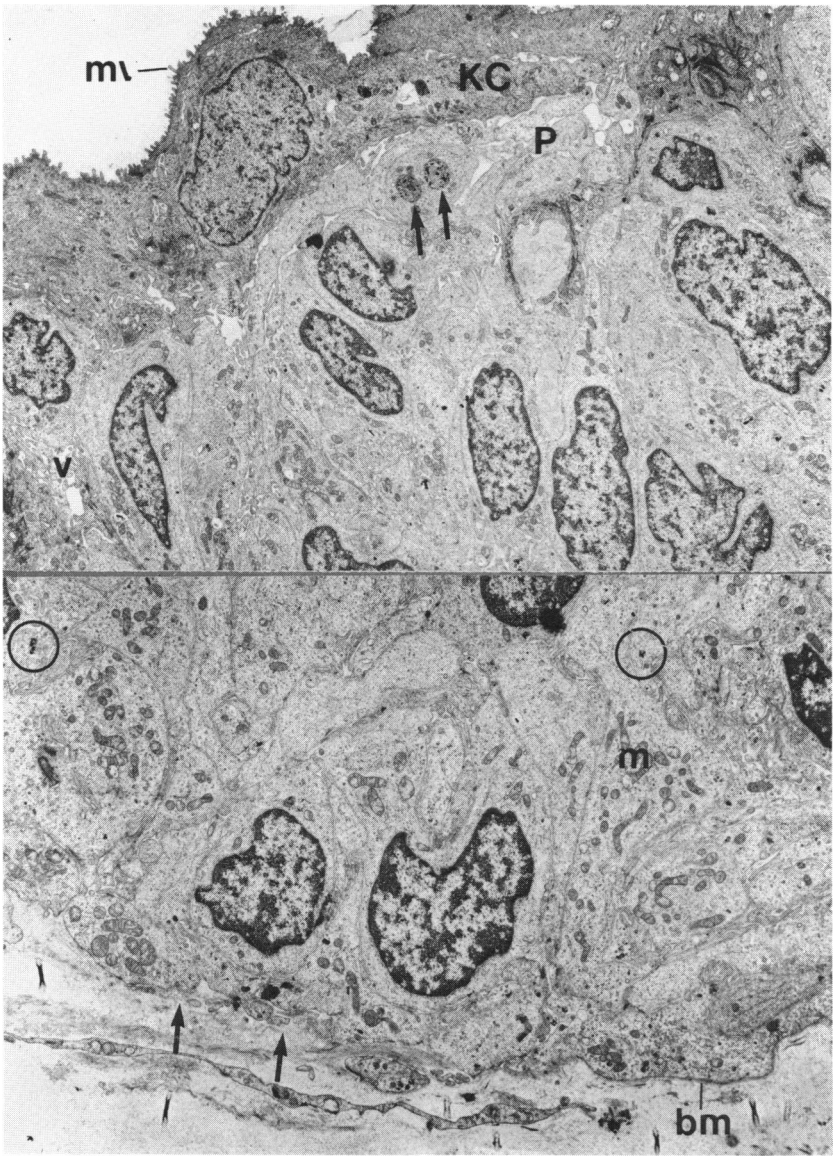

FIGURE 26

TOP: A large junctional intraepithelial nest of melanocytic nevus cells is sharply delimited by a surface bordering layer of keratinocytes (KC) displaying surface microvilli (mv). Note that nuclear chromatin is clumped and marginated at nuclear membrane. Both thick cytoplasmic processes (P) and thinner villi (v) are formed by nevus cells. The *arrows* indicate two phagolysosomes in cytoplasm of nevus cells (× 38,000). BOTTOM: Junctional nests bordering stroma are separated from it by an intact basement membrane (bm), which focally becomes interrupted (*arrows*), portending dropping off of cells into connective tissue. Small melanosomes (*circles*) can be seen in cytoplasm, and mitochondria (m) are numerous in cytoplasm (× 57,000).

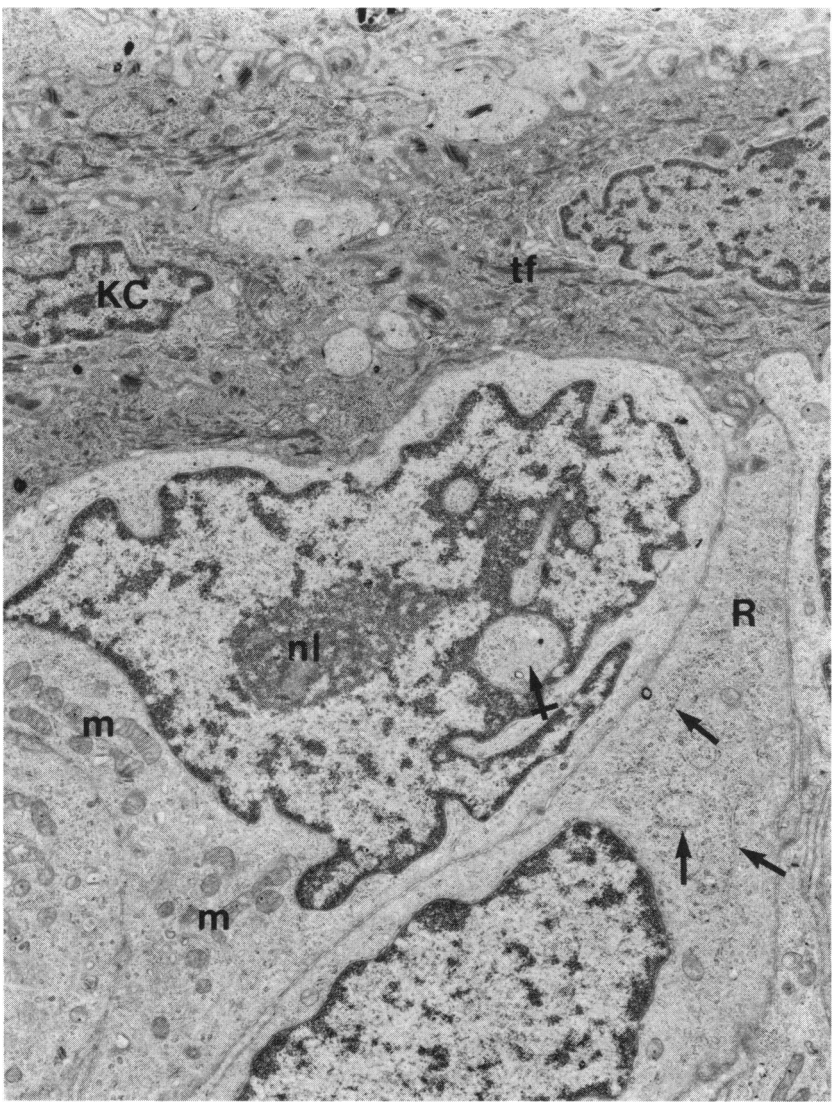

FIGURE 27
Nevus cells have paler cytoplasm compared with surrounding keratinocytes (KC) because latter cells have abundant tonofilaments (tf), which are lacking in nevus cells. Notice tightly coiled, and coarsely ropy nucleolus (nl) of nucleus, which additionally features a small herniation of cytoplasm (*crossed arrow*). Nevus cell cytoplasm is endowed with ribosomes (R), mitochondria (m), and short profiles of rough-surfaced endoplasmic reticulum (*arrows*) (× 11,500).

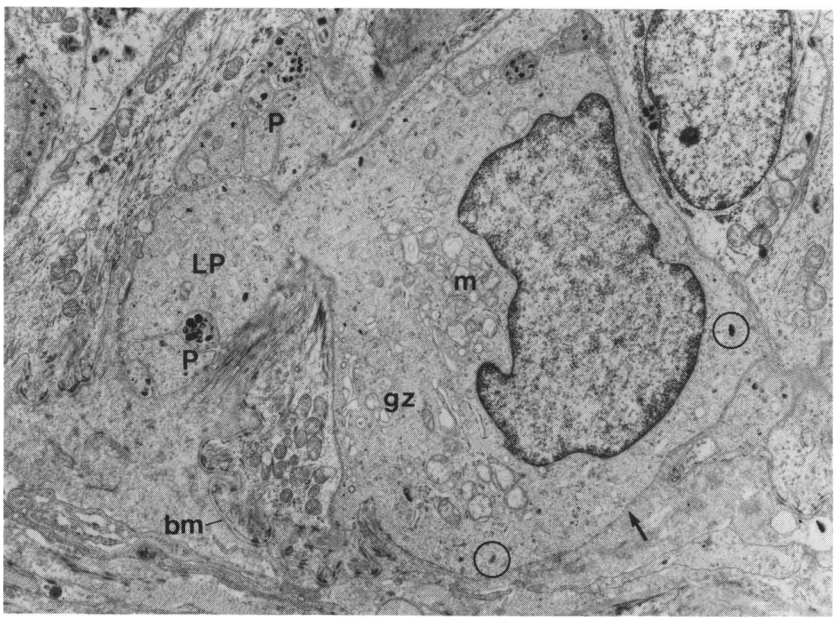

FIGURE 28
A nevus cell has abundant cytoplasm, with an active Golgi zone (gz). Numerous mitochondria (m) are present in cytoplasm. Small melanosomes (*circles*) are scattered in perikaryon. Notice large dendritic process (LP) as well as smaller dendritic processes (P) containing melanin granules. Basement membrane (bm) surrounding a basilar keratinocyte; *arrow*, attenuation of basement membrane as nevus cell bulges into upper substantia propria. Melanin granules measure 180 × 70 nm (× 11,000).

The melanin granules varied greatly in size, with the average measurement 180 nm × 70 nm. Occasionally, granules reached 230 nm × 150 nm. Rarely, small clusters of phagolysosomes were observed in the nevus cells themselves (Fig 26, top). When cytoplasmic processes were found, they contained more melanin than localized in the perikaryon region, and the melanosomes were more heavily melaninized (Fig 28). Only a light scattering of melanosomes was found in the keratinocytes adjacent to the junctional nests of nevus cells, and the melanin tended to be distributed as single granules rather than in phagolysosomes. In a child, there was a prominent lentiginous dendritic melanocytic hyperplasia (Fig 29) within the epithelium adjacent to the junctional nests. The dendritic melanocytes showed larger and more heavily melaninized elongated melanosomes compared with the nevus cells, and their myriad dendritic processes had managed to donate considerable pigment to the surrounding keratinocytes in contrast to the behavior of the adjacent nevus cells.

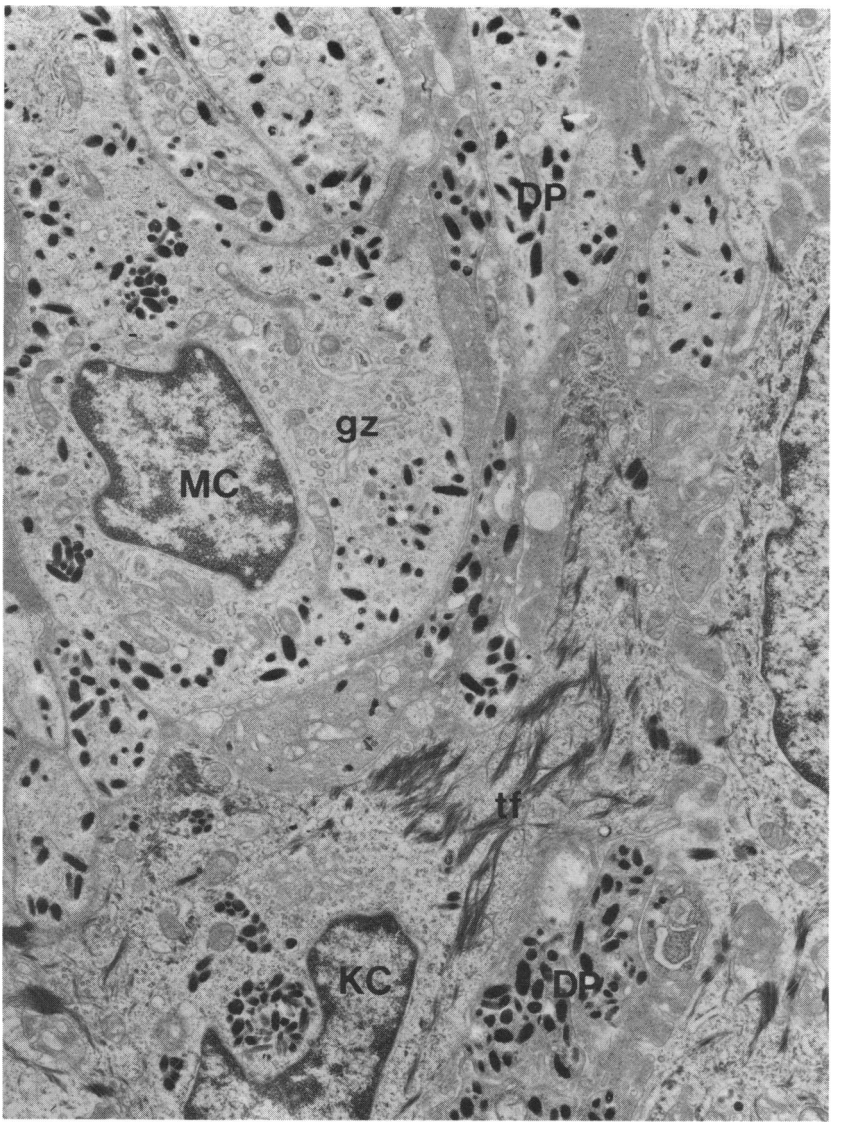

FIGURE 29

Lentiginous hyperplasia of dendritic melanocytes adjacent to junctional nevus cell nests in a young individual. Notice dendritic melanocyte (MC) has more abundant melanin granules than typifies nevus cells, and additionally extends far more prominent and well-developed dendritic processes (DP). Melanin granules are elongated as would be expected in a dendritic melanocyte, thereby differing from more rounded melanosomes of nevus cells. Golgi zone (gz) is well developed, and melanosomes around it are smaller than those in dendritic processes. An adjacent keratinocyte (KC) has abundant cytoplasmic tonofilaments (tf) and additionally shows a collection of ingested melanin granules in a recess of nucleus. This individual was a Pakistani child, thereby accounting for intense melaninization of melanosomes (× 11,000).

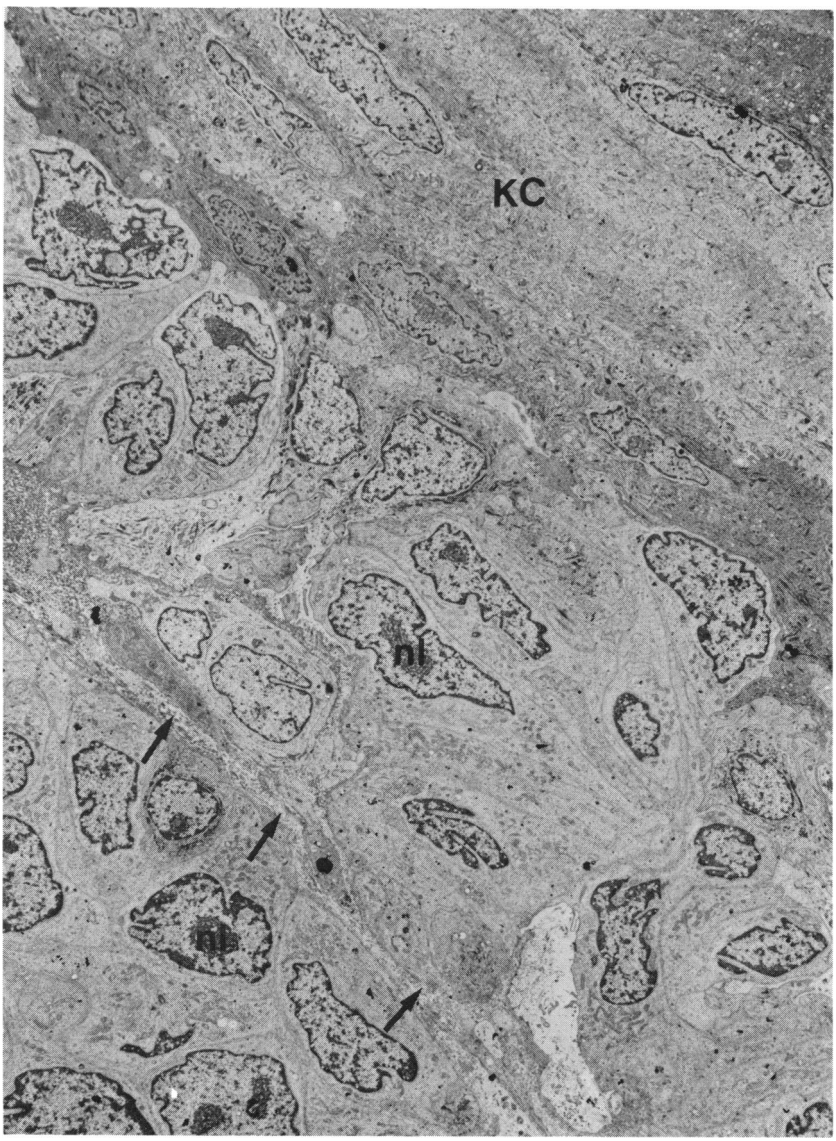

FIGURE 30
Beneath surface keratinocytes (KC) are several junctional nests of nevus cells, which in turn are separated from subepithelial nevus cells by a thin connective tissue strand (*arrows*) containing fragments of basement membrane material. Nevus cells of both junctional nest and subepithelial component are morphologically identical, including a clumped chromatin pattern and a large but coarsely ropy nucleolus (nl) (× 2600).

The cells in the upper subepithelial region of compound nevi were separated from the junctional nests by a thin connective tissue lamina with interrupted fragments of basement membrane material (Fig 30). The nuclear, cytoplasmic, and melanosomal features were virtually identical between the junctional nest cells and those in the upper substantia propria, with the exception that cells in the latter site were frequently invested by basement membranes on the outer plasmalemma bordering the stroma (Fig 31). The cells in the middle and lower portions of the connective tissue of the conjunctiva were frequently arranged in nests (Fig 31) or else formed monomorphous sheets devoid of large amounts of connective tissue (Fig 32). The nuclei of these smaller, somewhat lymphocytoid cells displayed either a fine coalescence of the chromatin (Fig 33, top) or a fine dispersion of the chromatin, with only a thin margination of heterochromatin on the inner aspect of the nuclear membrane (Fig 33, bottom).

Both the larger, more epithelioid superficial cells and the deeper, more lymphocytoid cells were organized similarly in their nevoid nests. Villi and cellular processes frequently projected into small intercellular spaces, suggesting abortive dendrite formation (Fig 34). Intercellular desmosomal contacts were extremely rare, but these were encountered in one case with particularly florid villi and processes (Fig 35). In many of the nevus cells, the melanosomes were extremely rudimentary and sparse, but in others they assumed more of a granular and rounded configuration (Fig 36). Filamentary, granular, and scroll-like melanosomes were found in the same cells (Fig 37). Mitochondria were the most conspicuous cytoplasmic organelle, but a Golgi zone was invariably present; only a few strands of rough-surfaced endoplasmic reticulum could be seen. Monoribosomes rather than polyribosomes were scattered throughout the cytoplasm. When individual cells were displaced outside of a nevoid collection, they were almost always completely surrounded by basement membrane (Fig 37). Binucleated and trinucleated cells were rarely identified among the mononuclear cells and, like other cells, were surrounded by basement membranes; the multinucleated cells had more prominent mitochondria and fewer, poorly melaninized melanosomes (Fig 38). In most of the lesions, the pigmented and nonpigmented cells were segregated into different nests, but in two lesions they were juxtaposed, showing similar nuclear and cytoplasmic features but different levels of melanogenic activity (Figs 39 and 40). The melanosomes were round and displayed a granular deposition of melanin upon disordered melanofilaments. One lesion in a black patient was remarkable: it was composed entirely of rounded nevus cells with abundant, heavily mela-

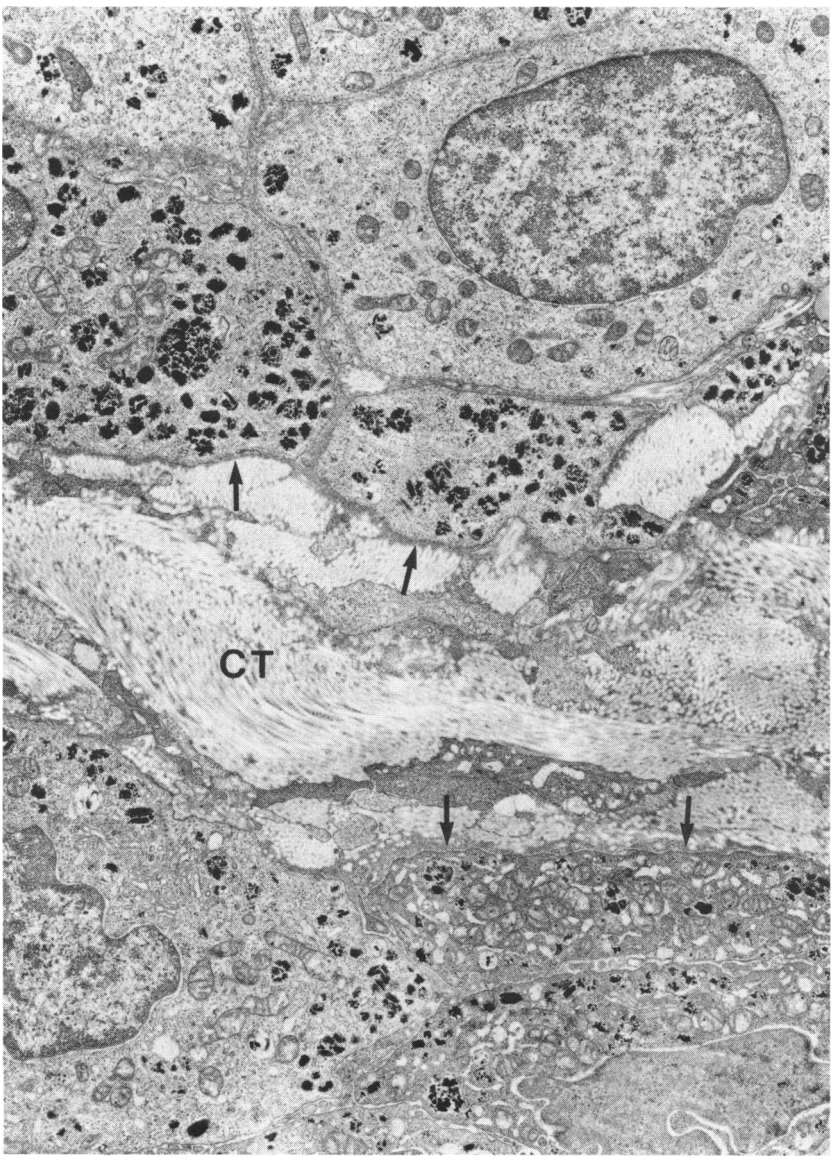

FIGURE 31
Two separate clusters of nevus cells in subepithelial zone show basement membrane material (*arrows*) on plasmalemmas bordering a strand of connective tissue (CT) (× 11,000).

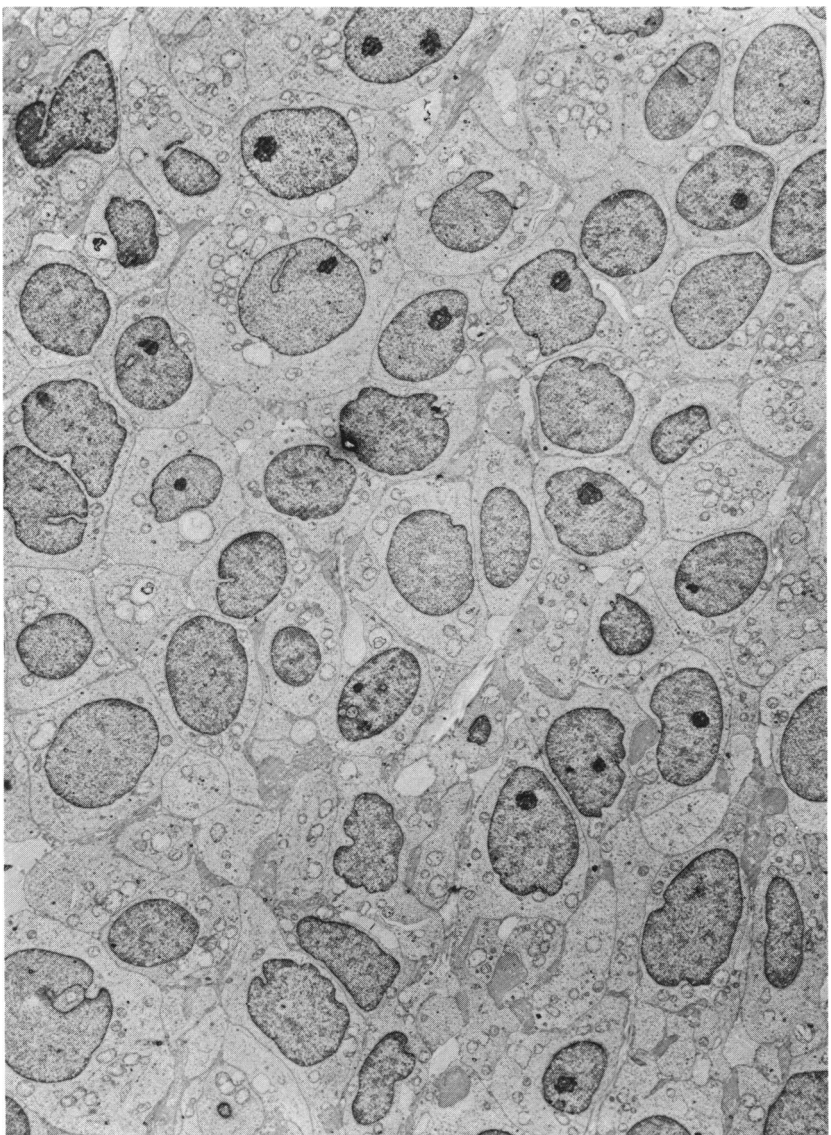

FIGURE 32
Tightly packed monomorphic nevus cells form a sheet with inconspicuous amounts of interstitital collagen (× 2400).

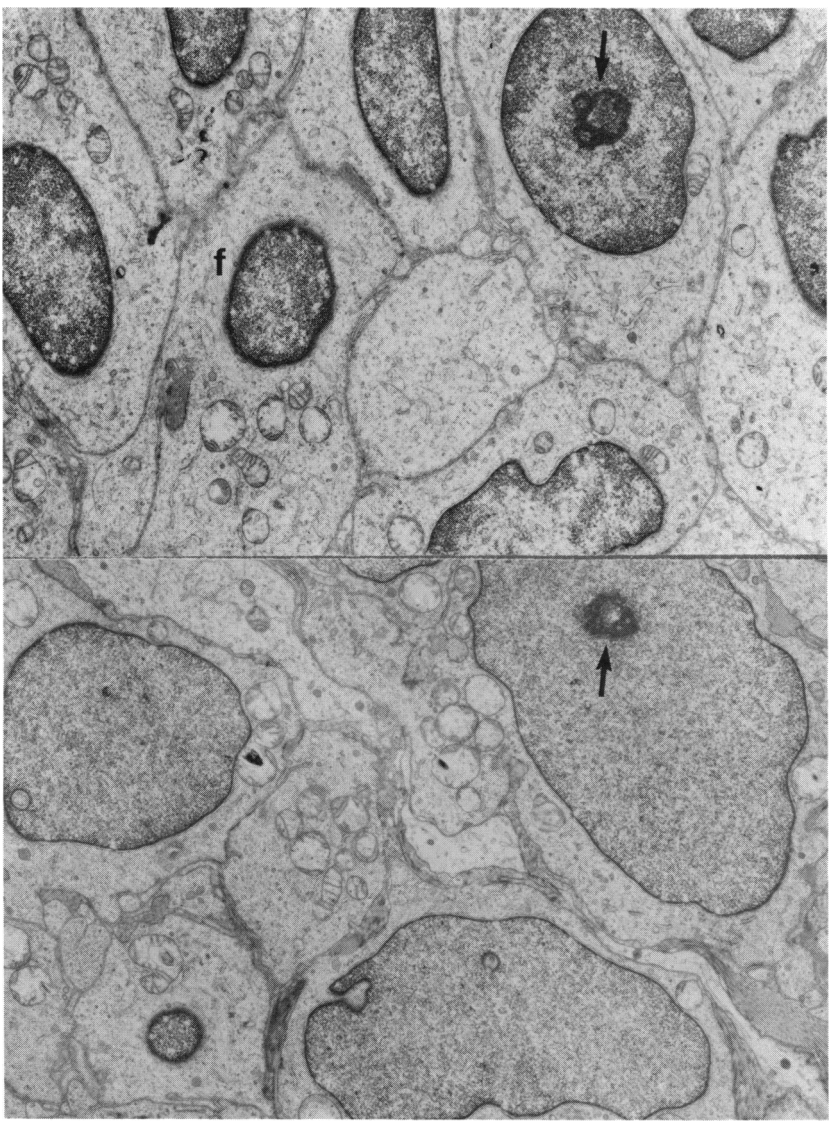

FIGURE 33

Two different chromatin patterns of subepithelial nevus cells. TOP: Chromatin is compact, without coarse clumping. *Arrow* indicates a nucleolus. Cytoplasm contains mitochondria as well as filaments (f). BOTTOM: Chromatin in this pattern is finely divided and not clumped, with a thin lamina of heterochromatin on inner aspect of nuclear membrane. A small nucleolus (*arrow*) is featured again (× 8500).

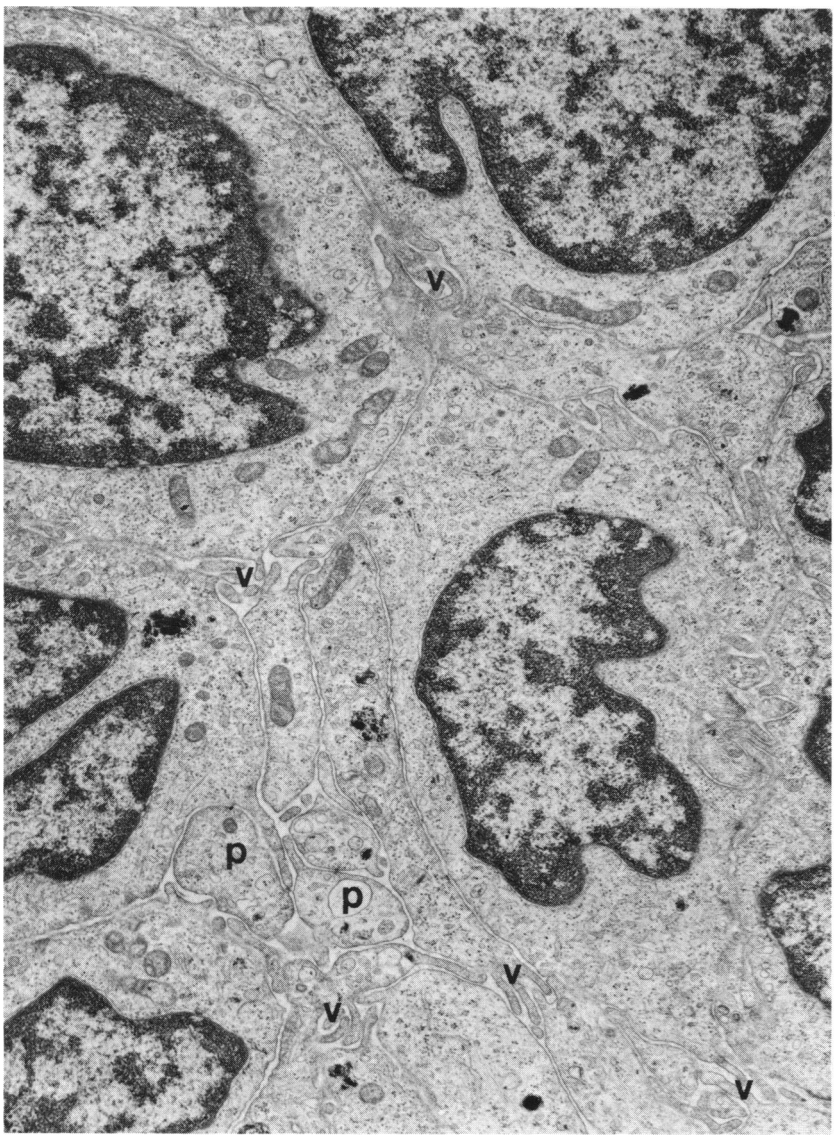

FIGURE 34
Nevus cells display thick margination of heterochromatin at nuclear membrane and dispersed aggregates of heterochromatin in nucleoplasm. Numerous small villi (v) as well as larger cytoplasmic processes (p) interdigitate in focal widenings of intercellular space in middle of the nevus cell nest. Scattered poorly formed melanosomes are identifiable (× 13,500).

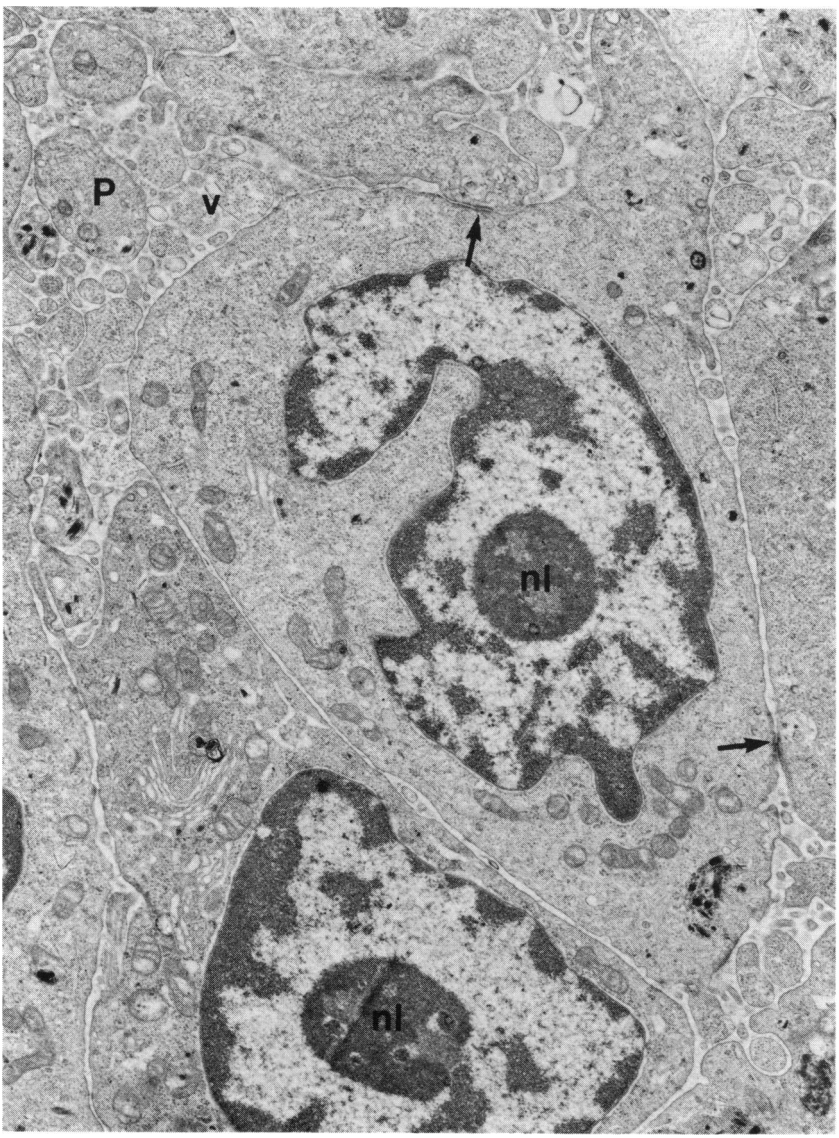

FIGURE 35

Subepithelial nevus cell nest displaying a particularly florid development of intercellular villi (v) as well as thicker processes (P). Poorly developed desmosomes (*arrows*) are formed at strikingly prominent widenings of intercellular space. Nuclear chromatin is clumped at nuclear membrane, and while nucleoli (nl) are quite large, they are composed of tightly wound and coarse strands (× 13,500).

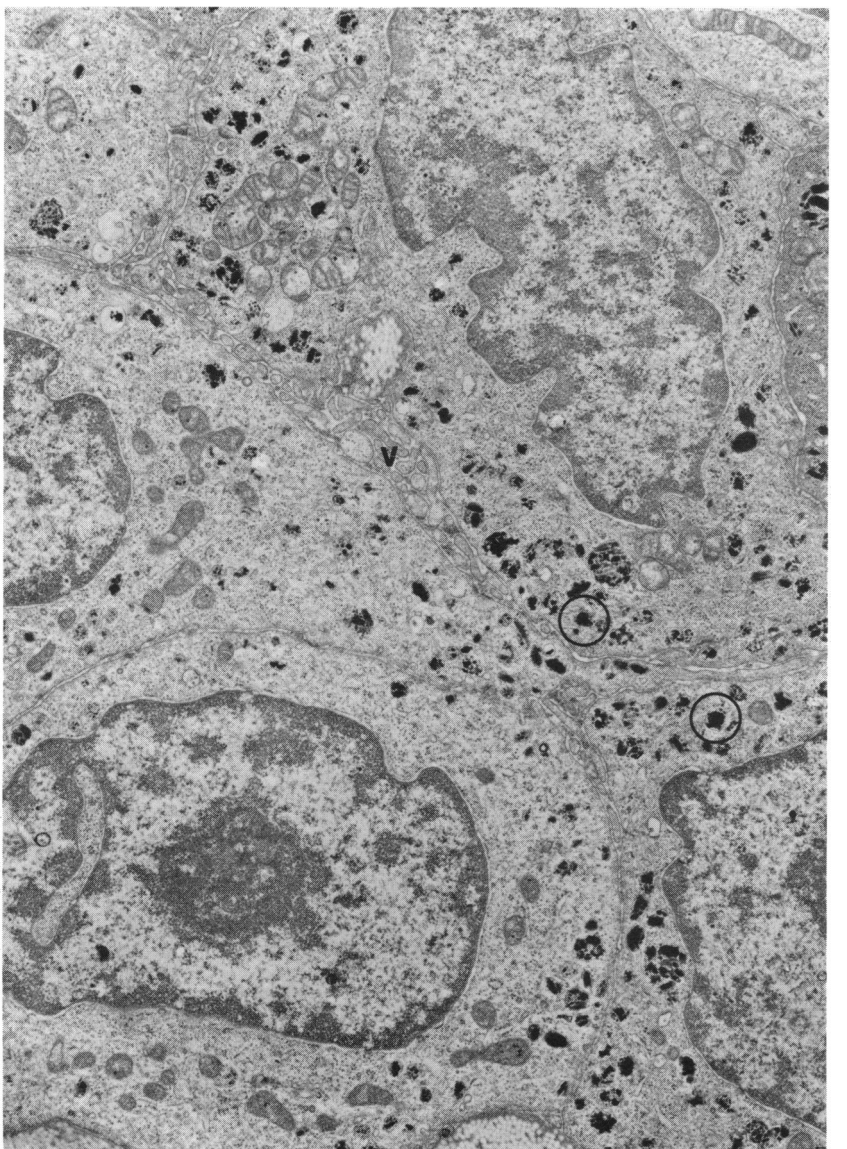

FIGURE 36
A comparatively heavily melaninized set of subepithelial nevus cells displays granular and poorly formed melanosomes (*circles*). Villi (v) project into focal widenings of intercellular space (× 12,500).

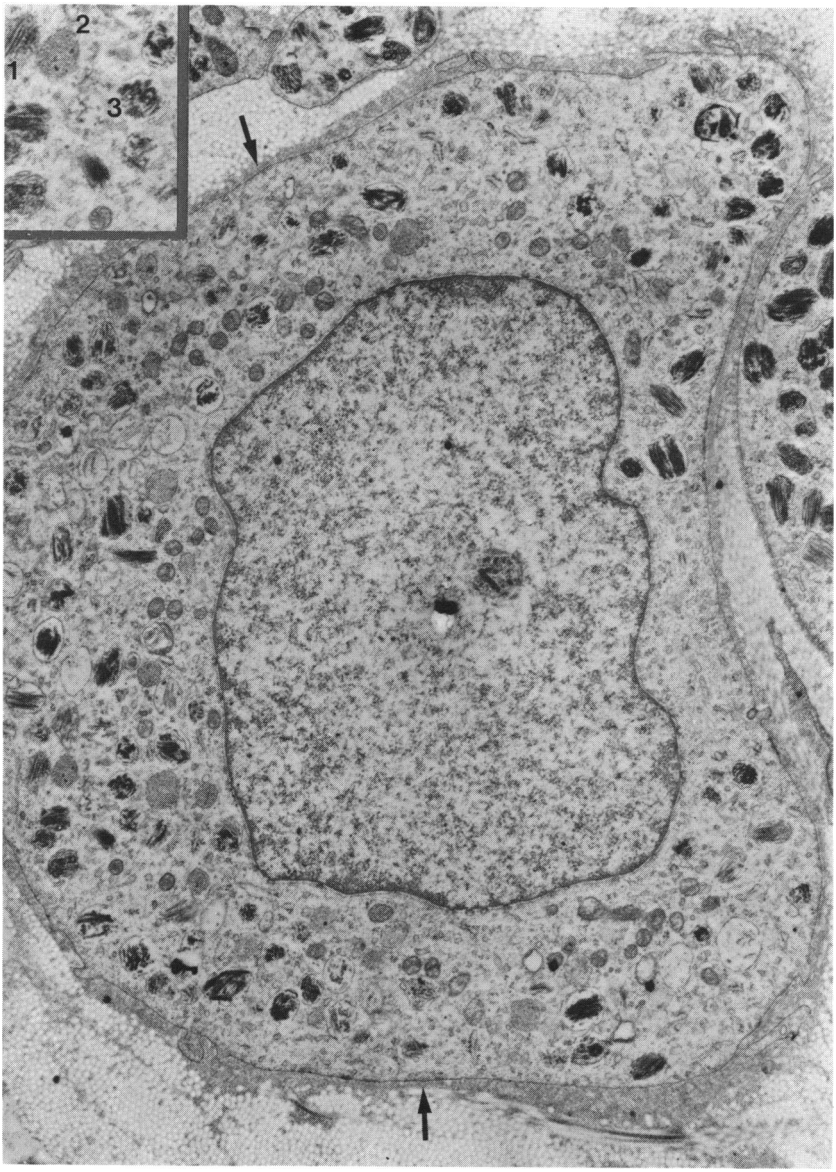

FIGURE 37

A nevus cell displaced into stroma away from a nest is surrounded on all sides by a thick basement membrane (*arrows*). Melanosomes are poorly melaninized. INSET: Diversity of architecture of the melanosomes is displayed. Melanosomes are typically rounded in shape rather than elongated, and there may be an internal poorly organized melanofilamentary substructure without cross-striations (1), a granular matrix (2), or a scroll-like substance (3). Mixtures of all these types also occur (× 11,000; INSET, × 19,000).

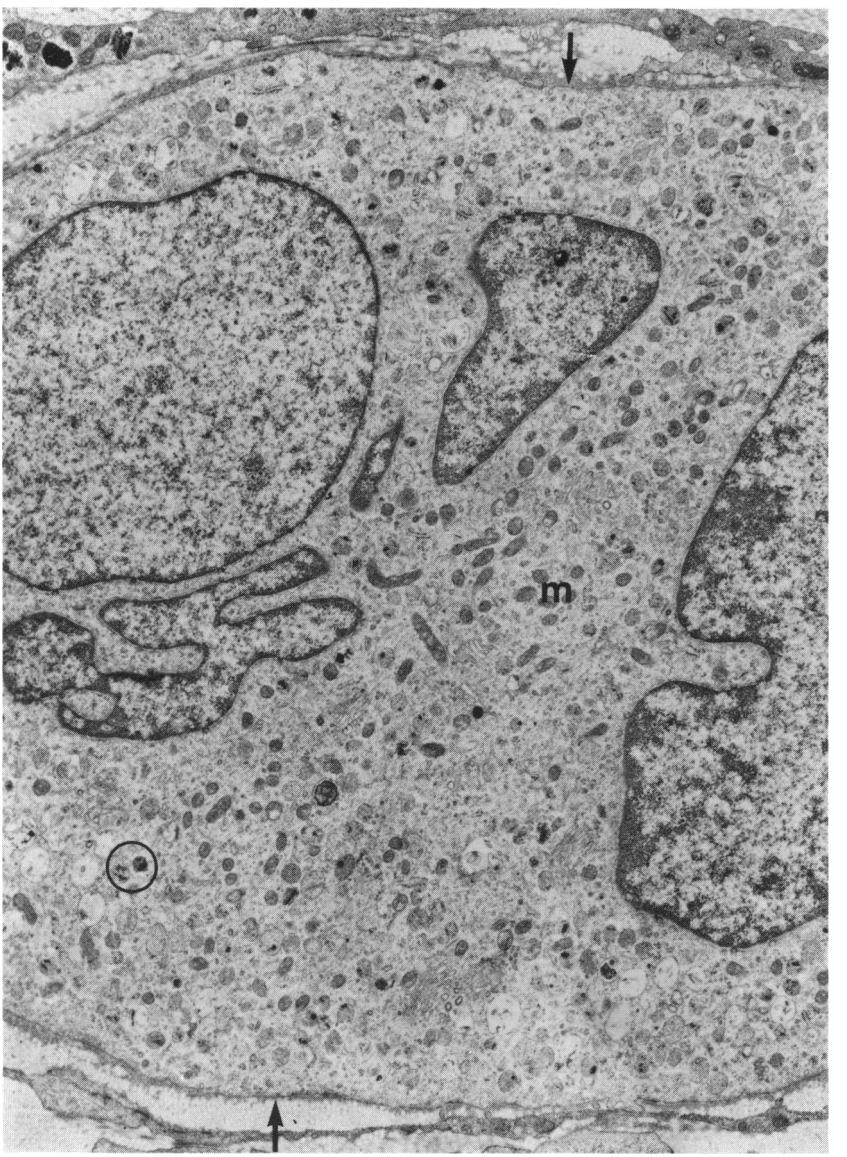

FIGURE 38
A multinucleated subepithelial nevus cell is surrounded by basement membrane material (*arrows*), and displays more numerous but small mitochondria (m) than typical nevus cells. Melanin granules are particularly rudimentary (*circle*) (× 11,000).

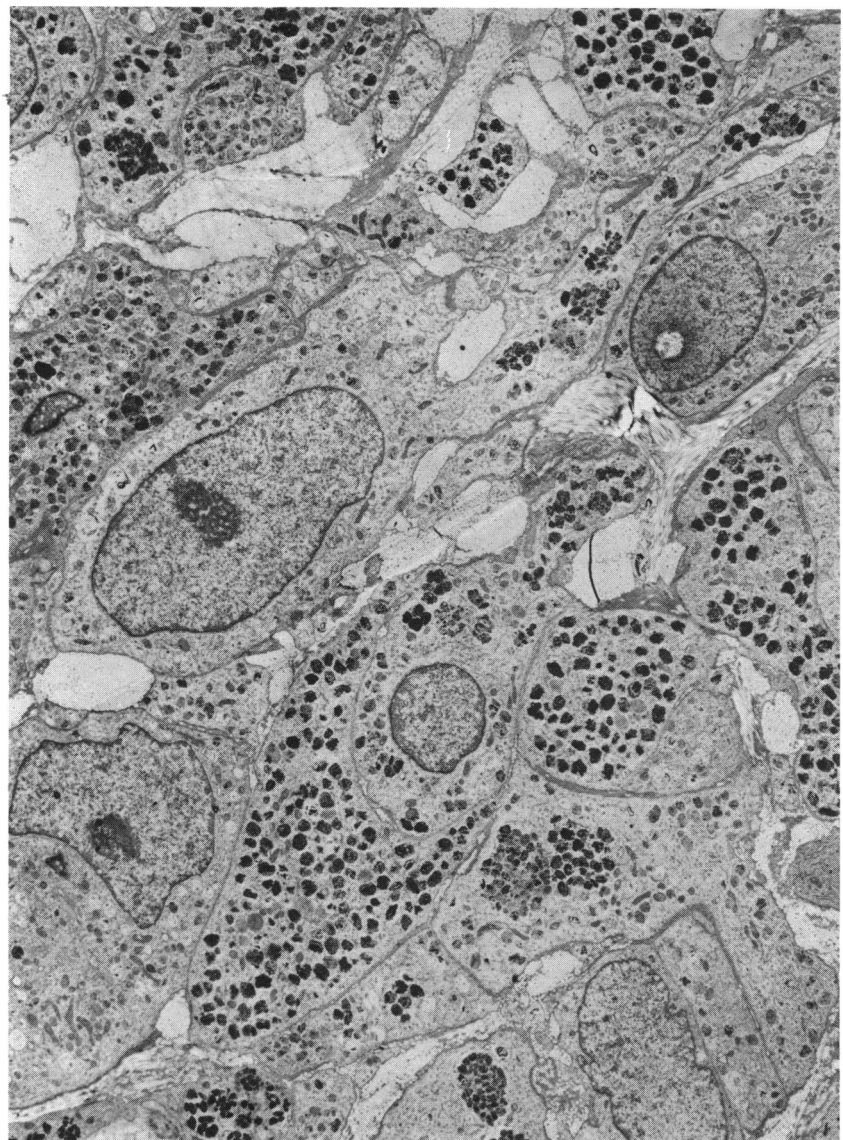

FIGURE 39
An unusual subepithelial nevus of plica shows double population of cells, namely, those that are poorly pigmented and those that are heavily pigmented (× 4800).

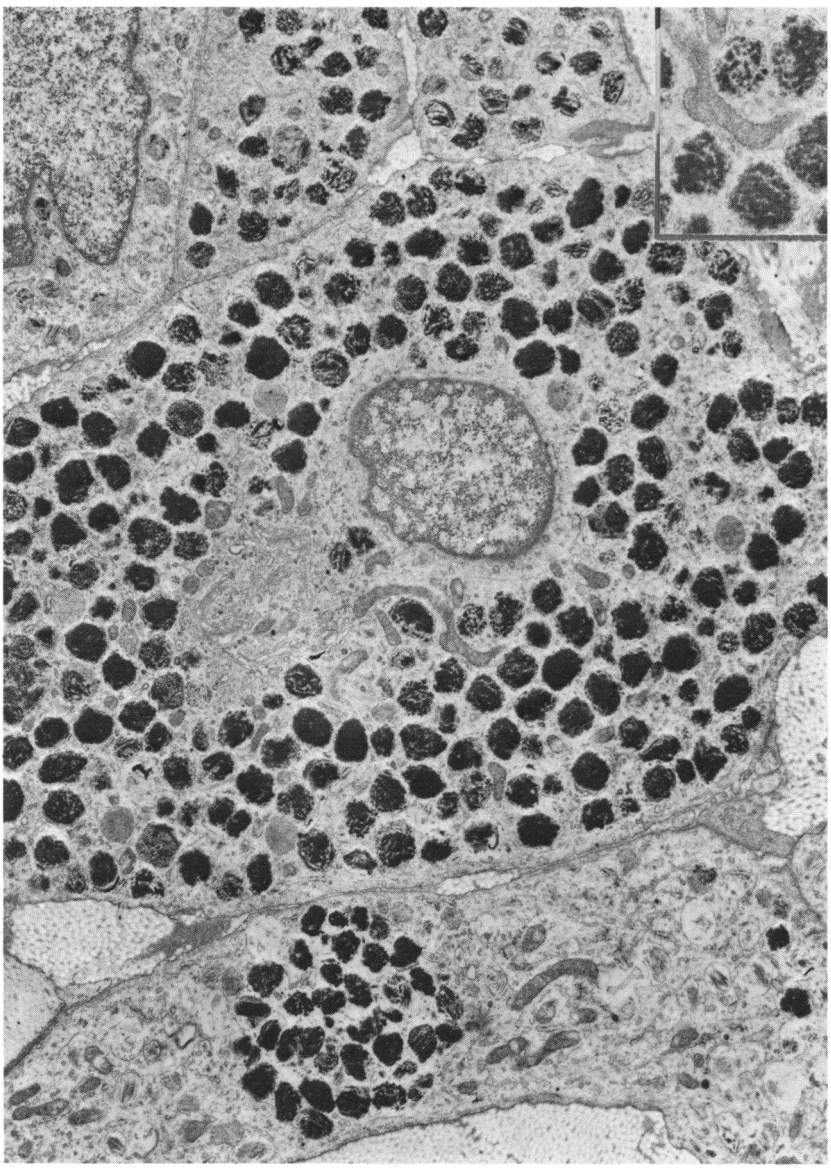

FIGURE 40
Both poorly pigmented and heavily pigmented nevus cells contain varying numbers of melanosomes of same granular type, organization of which is displayed in INSET (× 11,000; INSET, × 19,000).

ninized round melanosomes measuring 200 nm × 400 nm (Fig 41). The individual cells and clusters of cells in this case were surrounded by basement membranes, and there were prominent villous processes interdigitating between adjacent cells (Figs 41 and 42, top). Melanophages were more prominent in this particular lesion than in the other poorly pigmented nevi (Fig 42, bottom).

A feature that was variably present in all lesions was the presence of interstitial microfibrillar material outside of the basement membranes (Fig 43). This fibrillar material was occasionally present in cytoplasmic recesses or in enclosures of cellular processes (Fig 43, top) but usually formed aggregates that hugged the perimeters of some of the nevus cell clusters (Fig 43, bottom). This microfibrillar material was occasionally identified between cells in the middle of a cellular nest (Fig 44). This material was more prominent in the deeper reaches of the nevus cell population (Fig 45). The more deeply situated cells also showed cytoplasmic filaments (Fig 45), which were more prominent than in epithelioid cells of the junctional nests and of the superficial connective tissue component. No evidence of neuronevus formation was seen in the depths of the lesions; one lesion was remarkable for showing in its depths cells with melanin granules (Fig 46), which are not normally observed in deeply situated nevocytes. In another lesion, the nondescript, oval-to-spindled, deeply situated cells resembled mesenchymal cells, except that they formed focal basement membranes, possessed cytoplasmic filaments, and were associated with extracellular aggregates of microfibrillar material, thereby suggesting that they were dormant nevus cells (Fig 47).

PRIMARY ACQUIRED MELANOSIS AND MALIGNANT MELANOMA

In this study, 22 patients had primary acquired melanosis, primary acquired melanosis associated with a malignant nodule of invasive melanoma, or a nodule of melanoma without a conspicuous surrounding of flat primary acquired melanosis. Most patients were over 50 years of age at the time of presentation. Because their clinical courses were occasionally complicated and are not amenable to a tabular summary, brief clinical histories will be provided.

CASE REPORTS

CASE 1

A 52-year-old man complained of redness of the eyes for 6 weeks, which led him to discover a diffuse brown discoloration of his left eye. On physical examination all of the epibulbar, forniceal, and tarsal conjunctiva was universally involved by a

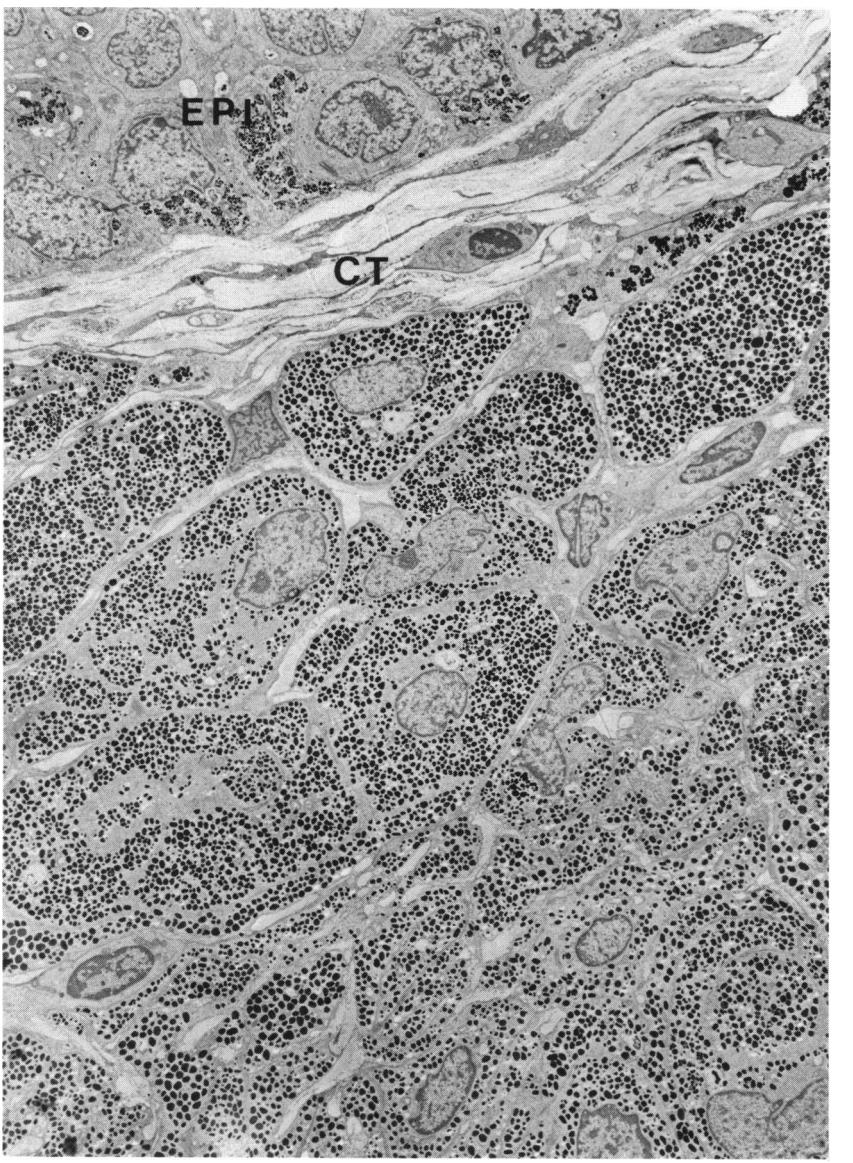

FIGURE 41
Heavily melaninized subepithelial nevus cells from a nevus in a black patient. Epithelium (EPI) shows benign racial melanosis. Nevus cell clusters are separted from epithelium by a band of connective tissue (CT) displaying numerous elongated fibroblasts. Notice uniform and heavy melaninization of melanosomes of nevus cells (× 2200).

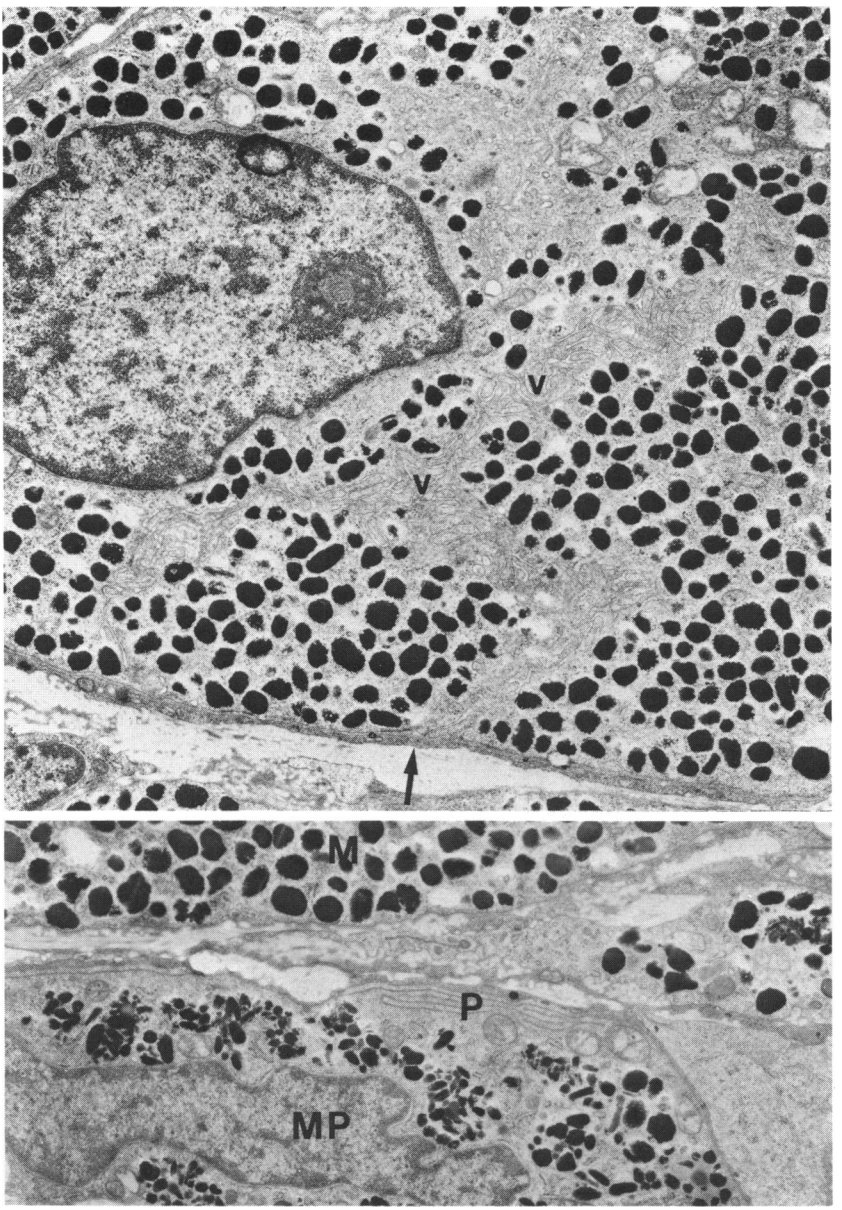

FIGURE 42

TOP: A nevus cell nest is surrounded on all sides by basement membrane material (*arrows*). Notice large size, round shape, and uniform melaninization of cytoplasmic melanosomes. Intercellular space is widened and occupied by numerous interdigitating villi (v) (× 11,000). BOTTOM: Contrast between clumped melanin granules in cytoplasm of a melanophage (MP) and individually dispersed melanin granules within cytoplasm of a melanocyte (M). Melanophagic histiocytic cell shows numerous interdigitated ruffled cytoplasmic processes (P). Melanophage is not covered by basement membrane material (× 11,500).

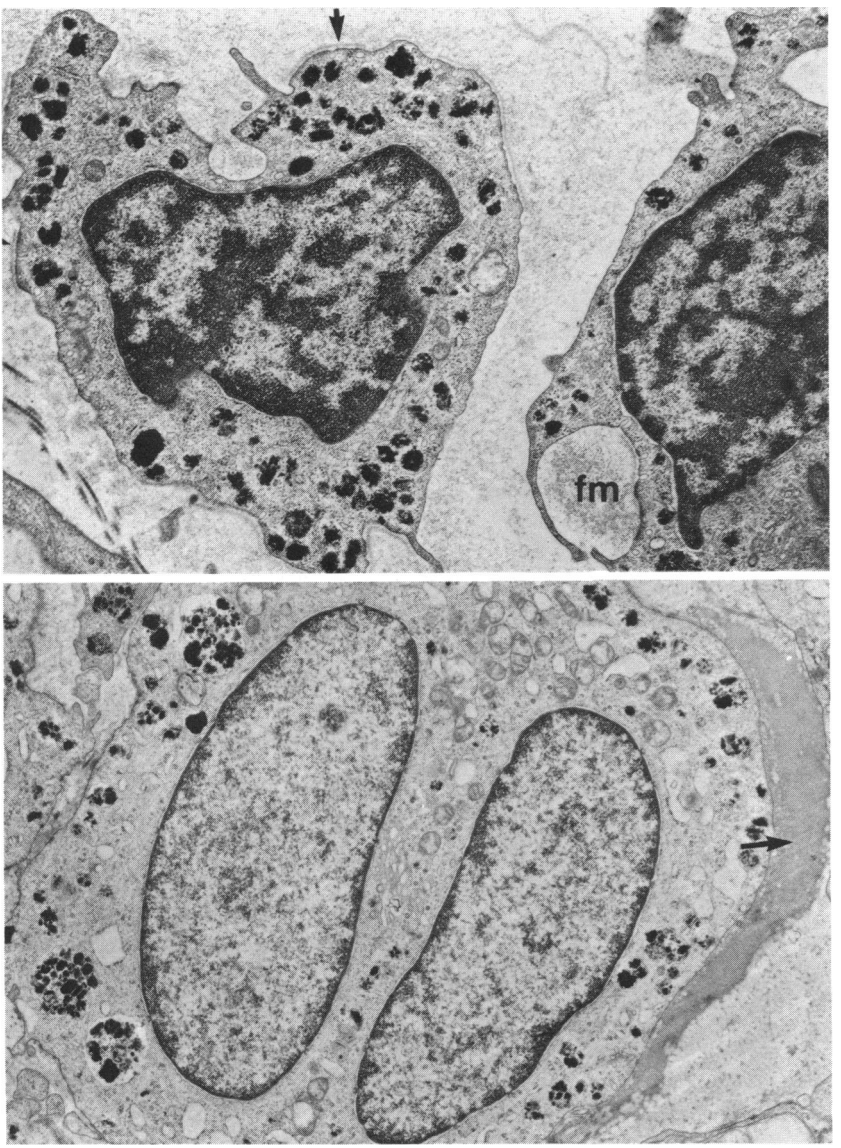

FIGURE 43

TOP: Subepithelial pigmented nevus cells are partially covered by linear segments of basement membrane (*arrows*). Fibrillar material (fm) is prominent in a cytoplasmic recess of melanocyte on right, and is additionally present in a light dispersion in interstitium (× 13,500). BOTTOM: A binucleated melanocyte is ensheathed by a bundle of fibrillar material (*arrow*) (× 9500).

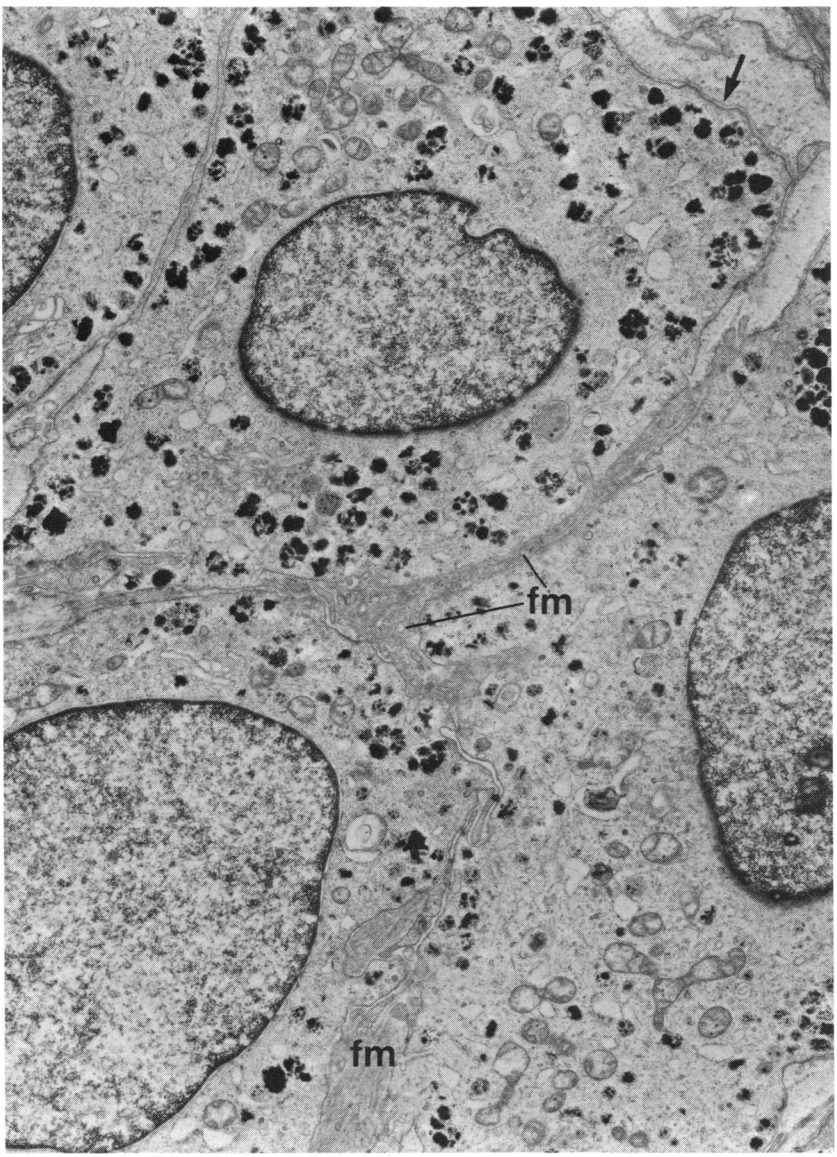

FIGURE 44
A nest of nevus cells covered on outside by basement membrane material (*arrow*) additionally displays fibrillar material (fm) in the intercellular space within center of nevus cell nest (× 11,000).

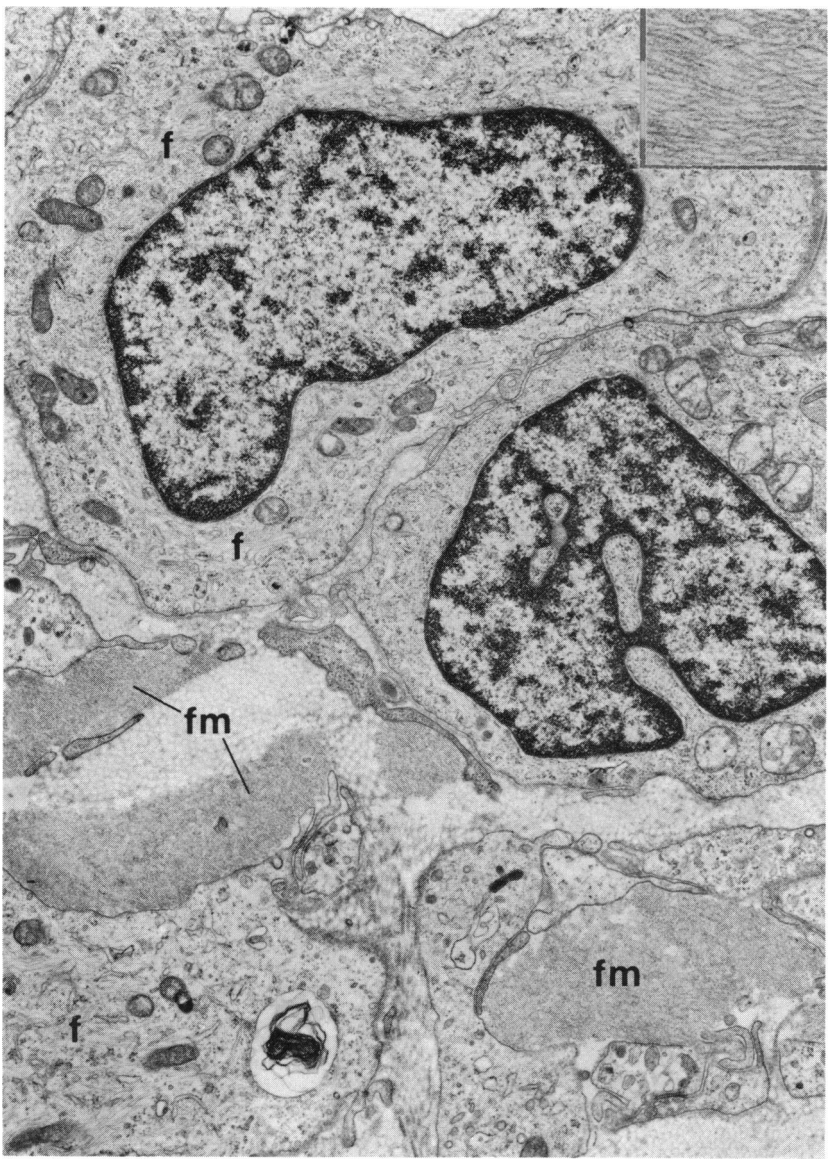

FIGURE 45

Nevus cells with cytoplasmic filament (f) are intimately related to aggregates of extracellular fibrillar material (fm). INSET: Demonstrates straight and hollow-centered nature of this fibrillar material, which measured 100 nm in diameter and failed to have any longitudinal periodicity (× 12,500; INSET, × 41,000).

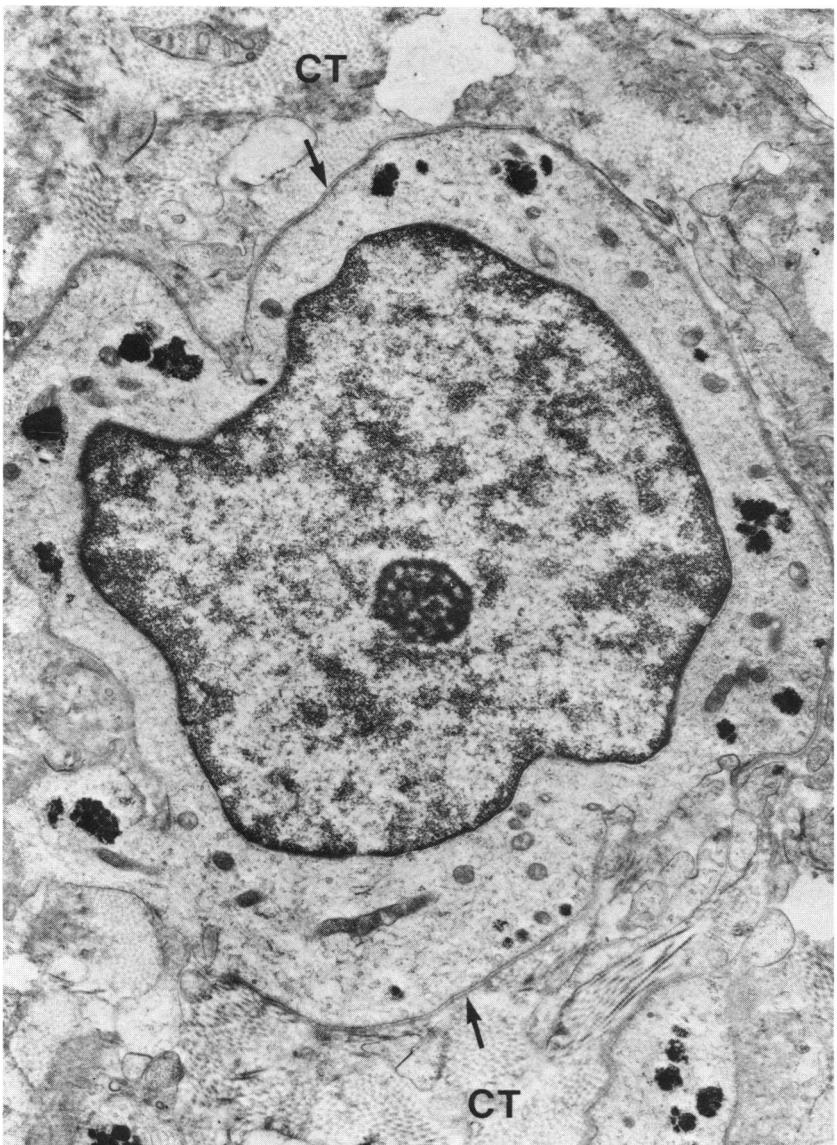

FIGURE 46
A deeply situated subepithelial nevus cell anomalously continues to produce melanin, and is surrounded on all sides by linear basement membrane material (*arrows*). CT, bundles of collagen in deep substantia propria (× 12,500).

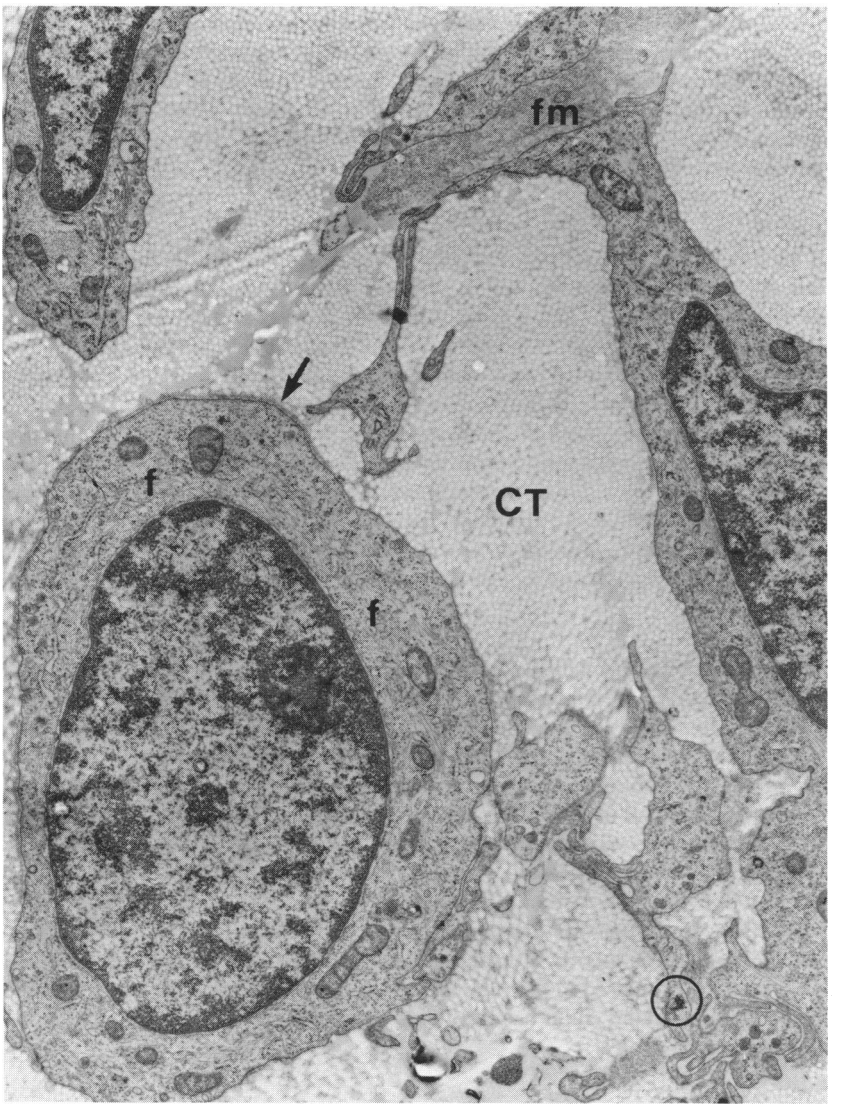

FIGURE 47

In deepest portions of a subepithelial nevus nondescript cells with cytoplasmic filaments (f) are focally surrounded by linear basement membrane material (*arrow*). Extracellular aggregates of fibrillar material (fm) are related to processes of some of these cells. Connective tissue (CT) is comprised of thick collagen fibers. These cells are interpreted as dormant nevus cells. Process of one of these cells contains a small and poorly melaninized melanosome (*circle*) (× 12,000).

flat pigmentation (Fig 48, top), which additionally extended onto the cornea. No nodule formation was found. Multiple biopsies of the conjunctiva showed melanocytic proliferation within the epithelium. Several sessions of cryotherapy were required to treat all of the epibulbar and lid involvement. Three years following treatment, the patient has faint, scattered conjunctival pigmentation, but no nodules have developed.

CASE 2

A 62-year-old man noted a pigmented growth for 4 to 6 months in the corner of his left eye. Examination disclosed a pedunculated, lobulated, and cystic lesion protruding beyond the lid margin with its base at the caruncle; flat pigmentation extended across the plica to involve most of the epibulbar surface (Fig 48, bottom). The polypoidal lesion was excised, to reveal multiple cysts of invaginating conjunctival epithelium, in the walls of which were proliferating atypical melanocytes. Two other biopsy specimens from the plica and epibulbar conjunctiva disclosed intraepithelial melanocytic proliferation of a less florid nature than that in the caruncle. After the caruncular lesion was completely excised, cryotherapy was delivered to all of the epibulbar pigmentation on two occasions. Two years after completion of treatment, no new nodules have formed and only a small focus of faint pigmentation is present in the superotemporal epibulbar conjunctiva.

CASE 3

A 79-year-old woman with early senile dementia presented with left epibulbar pigmentation of unknown duration. Flat pigmentation overriding the cornea and extending temporally in the conjunctiva was noted in the left eye from the 1- to the 6-o'clock position. Multiple biopsies revealed intraepithelial melanocytic proliferation. The patient failed to return for follow-up; when she was next seen 2 years later, she had extensive perilimbal flat pigmentation extending from the 2- to the 6-o'clock position, involving all of the temporal epibulbar conjunctiva and reaching the inferior cul-de-sac. A nodule of melanoma overrode the limbus from the 4- to the 6-o'clock position (Fig 49, top). The nodule was excised, followed by cryotherapy to the surrounding conjunctiva. Total thickness of the excised nodule was 0.8 mm. Three months later the patient returned with extensive flat pigmentation that had recurred in the inferotemporal quadrant of the epibulbar conjunctiva. No new nodules could be identified. The patient is scheduled for reexcision and treatment.

CASE 4

A 68-year-old woman had been aware of pigmentation of the right lower lid for almost 15 years. She had undergone several biopsies, all of which were reported as benign melanosis. Continuing spread of the flat pigmentation from the right inferior cul-de-sac led to involvement of the inferior tarsal conjunctiva, and eventually the pigmentation emerged across the lid margin and onto the cutaneous aspect. Several biopsies showed intraepithelial atypical melanocytic proliferation.

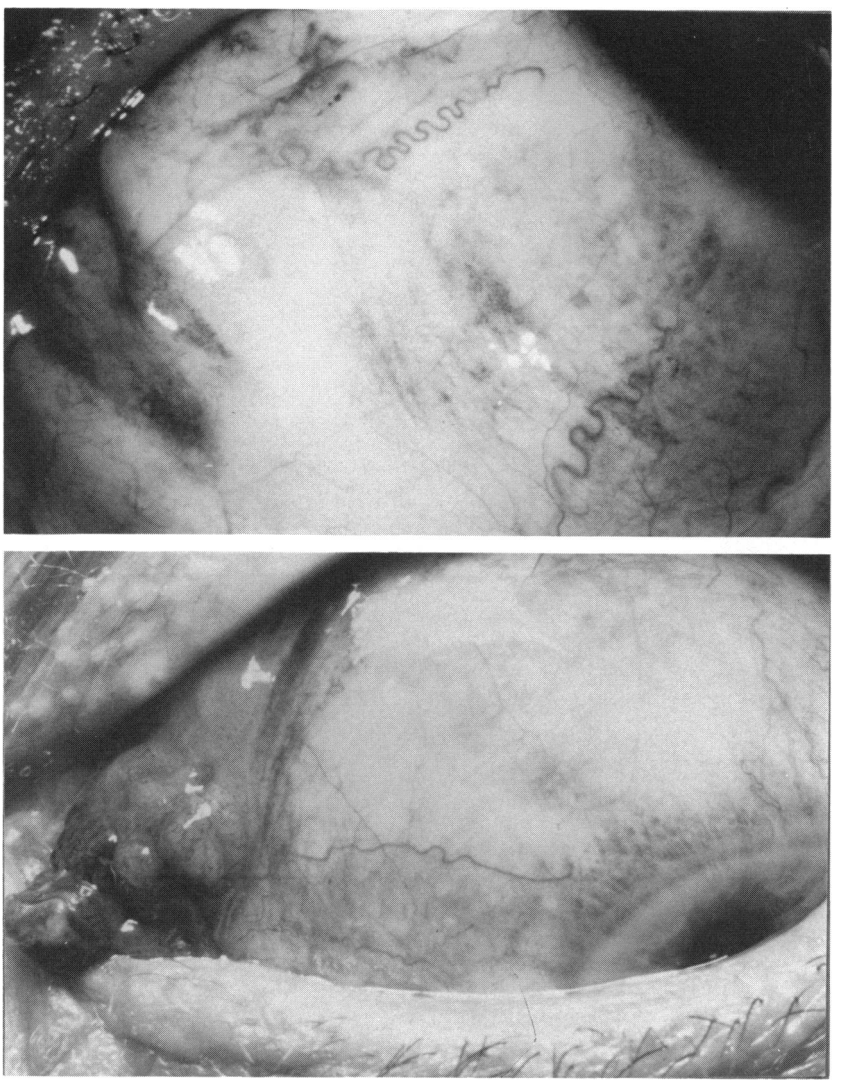

FIGURE 48

TOP: Primary acquired melanosis of conjunctiva (case 1) unassociated with a nodule of invasive malignant melanoma. Epibulbar surface displays variable and patchy intensities of flat pigmentation, with more uniform pigmentation of plica and caruncle. BOTTOM: Flat primary acquired melanosis of epibulbar surface is associated with a polypoidal multilobular caruncular lesion. Latter was freely moveable over underlying tissues. Caruncular component was discovered after pathologic examination to be composed of epithelial inclusion cysts with florid proliferation of markedly atypical cells in their walls (case 2).

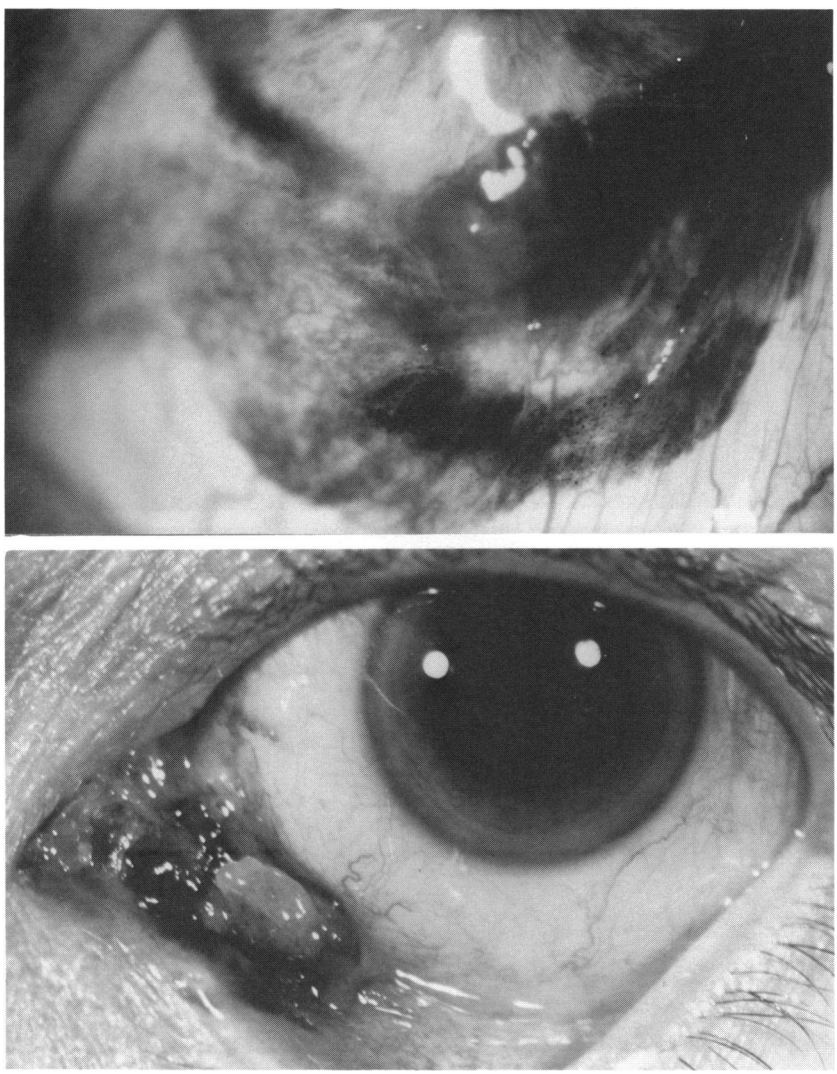

FIGURE 49
TOP: Primary acquired melanosis of inferior epibulbar surface coexists with a nodule of invasive melanoma straddling limbus (case 3). BOTTOM: A poorly pigmented nodule of inferior tarsal conjunctiva is surrounded by extensive pigmentation of primary acquired melanosis (case 12).

Cryotherapy to both the conjunctival and cutaneous aspects of the lesion was performed, with resolution of the clinical pigmentation. Nine months later a small black nodule appeared in the inferior cul-de-sac, which was excised. The pathologic specimen revealed a small nodule of melanoma composed of epithelioid cells as well as a lobule of lacrimal gland tissue, within the acini of which were atypical melanocytic cells. During 2 years of follow-up the patient has had no recurrent disease.

CASE 5

A 72-year-old man had been aware of pigmentation of his right lower lid for about 1 year. Examination revealed flat pigmentation on the tarsal conjunctiva of the right lower lid, with spillover of the condition across the lid margin onto the cutaneous portion of the lid. Biopsies of the conjunctiva and the lid revealed intraepithelial melanocytic proliferation. Two sessions of cryotherapy were required to eradicate the pigmentation, which has not reappeared during a 4-month follow-up.

CASE 6

A 76-year-old woman had been aware for 20 years of a pigmentary condition involving the skin of the right lower lid. Initial surgical excision had been followed by five cautery treatments over the ensuing 20 years; these treatments controlled most of the pigmentation on the lid skin. The patient presented with flat pigmentation of several years' duration involving the temporal and inferior epibulbar surface was well as intense pigmentation on the cutaneous aspect of the right lower lid. Biopsies of the conjunctiva revealed mild proliferation of intraepithelial melanocytes, while in the lid skin there was more intense intraepidermal melanocytic proliferation. The lower lid skin lesion was excised, but no treatment was given to the conjunctival portion of the process because of its quiescent nature.

CASE 7

A 57-year-old Caucasian male had noted for 2 years a pigmented spot near the caruncle of his left eye. His optometrist believed it was a freckle. Two years later an ophthalmologist examined the patient and noted extension of the pigmentation onto the surface of the eye. Multiple biopsies of the caruncle, plica, and nasal epibulbar surface disclosed mild intraepithelial melanocytic proliferation. Cryotherapy was delivered to the remainder of the clinically pigmented conjunctiva. Two years later the patient is free of disease.

CASE 8

A 47-year-old Caucasian woman noted a pigmented spot on the caruncle of the left eye, which she felt had been getting larger over a 7-month period. Examination disclosed flat pigmentation of the lid margin on the medial aspect of the left lower lid, with additional pigmentation of the medial tarsal conjunctiva, plica, and caruncle. Biopsies were taken of the tarsal and caruncular portions of the lesion,

which showed intraepithelial melanocytic proliferation. Cryotherapy was delivered to the clinically involved areas; after 2 years of follow-up there has been no recurrent disease.

CASE 9

A 52-year-old woman presented with epibulbar pigmentation, particularly prominent temporally, including intense pigmentation of the inferior fornix and right inferior tarsal conjunctiva as well as at the lid margin. Five years earlier the patient had had a nodular melanoma excised from the tarsus of the right upper lid. The biopsy showed invasive epithelioid cells with a thickness of 1.3 mm. The patient had been recommended to undergo exenteration of the orbit, but she sought other opinions. Multiple biopsies of the epibulbar and tarsal conjunctiva revealed intraepithelial melanocytic proliferation. Three sessions of cryotherapy to the epibulbar and lid areas of involvement were conducted, with almost complete disappearance of pigmentation. One and a half years later the external aspect of the right lower lid skin became lightly pigmented and was subjected to cryotherapy. Only a small area of pigmentation of the right lower lid skin remains, with no evidence of metastases or epibulbar recurrence 4 years after initial cryotherapy.

CASE 10

A 79-year-old black woman had a 10-year history of pigmentation on the surface of the left eye; a biopsy in 1975 revealed a "pigmented dermal nevus" of the conjunctiva. The pigmentation continued to extend throughout the entire palpebral and epibulbar conjunctiva, being particularly intense in the tarsal and forniceal conjunctiva. Multiple biopsies were taken of the epibulbar tarsal and forniceal regions. Intraepithelial melanocytic proliferation was noted on the epibulbar and tarsal surfaces, but in the fornix an invasive nodule of spindle cell melanoma cells was found. The invasive nodule was 0.8 mm thick. The patient underwent one session of cryotherapy but died 4 months later of a ruptured aortic aneurysm.

CASE 11

A 79-year-old man had pigmentation of his right caruncle and upper tarsal conjunctiva for 5 years. Biopsy of several thickened tarsal areas revealed invasive malignant melanoma, the greatest thickness of which was 0.9 mm. Seven years after onset of disease, a large, pigmented, plaque-like lesion of the lateral aspect of the right upper tarsal conjunctiva was noted as well as two small nodules in the medial tarsus. Flat pigmentation was diffusely present throughout the rest of the superior tarsal and forniceal conjunctiva. Excision of the right upper lid tumor with plastic repair revealed an invasive nodule of melanoma 2.1 mm thick and intraepithelial melanocytic proliferation. Biopsy of nodules recurring 6 months after the plastic repair showed small nodules of invasive melanoma measuring less than 0.5 mm. Two years after the large excision of the upper lid, the patient has residual disease but no metastases.

CASE 12

A 57-year-old man had a 3-year history of pigmentation of the caruncular area of the left eye. Three months prior to presentation he felt that the pigmentation had increased in intensity. His ophthalmologist discovered a nonpigmented nodule in the medial aspect of the fornix of the left lower lid (Fig 49, bottom) associated with flat pigmentation extending into the plica, caruncle, superior fornix, medial epibulbar conjunctiva, and inferior tarsal conjunctiva. Excision of the nodule led to the discovery of an epithelioid-cell invasive malignant melanoma, with a thickness of 2.4 mm. Multiple biopsies of the other areas disclosed intense proliferation of intraepithelial atypical melanocytes. Cryotherapy was delivered to the bed of excision as well as to the surrounding pigmented areas. No recurrence of pigment has developed during a 4-month follow-up.

CASE 13

A 69-year-old woman had noticed 5 years earlier a brown spot on the outer aspect of the left eyeball. An excision was performed, and the diagnosis of melanoma of the conjunctiva was made. Another excision of recurrent temporal pigmentation was performed 1 year later. Five years after initial presentation the patient noticed a very prominent pigmentation on the left side of the eye. Slightly elevated pigmented conjunctiva hugged the limbus from the 6:30- to 8:30-o'clock position on the left eye. Excision of this area revealed a nodule of malignant melanoma measuring 0.5 mm. Cryotherapy was delivered to the area of involvement, and no recurrence has been noted during a 6-month period.

CASE 14

A 64-year-old Caucasian woman had been aware of a caruncular mass in the left eye for about 8 years, but over the preceding 2 to 3 months she felt that the area had become more pigmented. Clinical examination disclosed a cystic, brown lesion in the caruncle, with flat pigmentation extending onto the epibulbar surface, onto the medial portion of the tarsal conjunctiva, and culminating in an elevated nodule at the lower lid margin adjacent to the punctum (Fig 50, top). Excision of the caruncular lesion displayed a benign subepithelial nevus with typical inclusion cysts, but the overlying epithelium was remarkable for increased numbers of atypical melanocytes. Biopsies of the medial epibulbar and inferior tarsal conjunctiva also revealed intraepithelial melanocytes. The lid nodule was an invasive malignant melanoma 0.8 mm thick. Cryotherapy was delivered to all areas of involvement, and 1 year following treatment there has been no recurrence of disease.

CASE 15

A 70-year-old Caucasian man noted a pigmented area in the left inferior fornix 6 years earlier. Slowly and inexorably thereafter the pigmentation crept in a flat fashion to involve all of the epibulbar, palpebral, and forniceal conjunctiva. Multiple biopsy specimens of thickened epibulbar areas disclosed invasive epithelioid

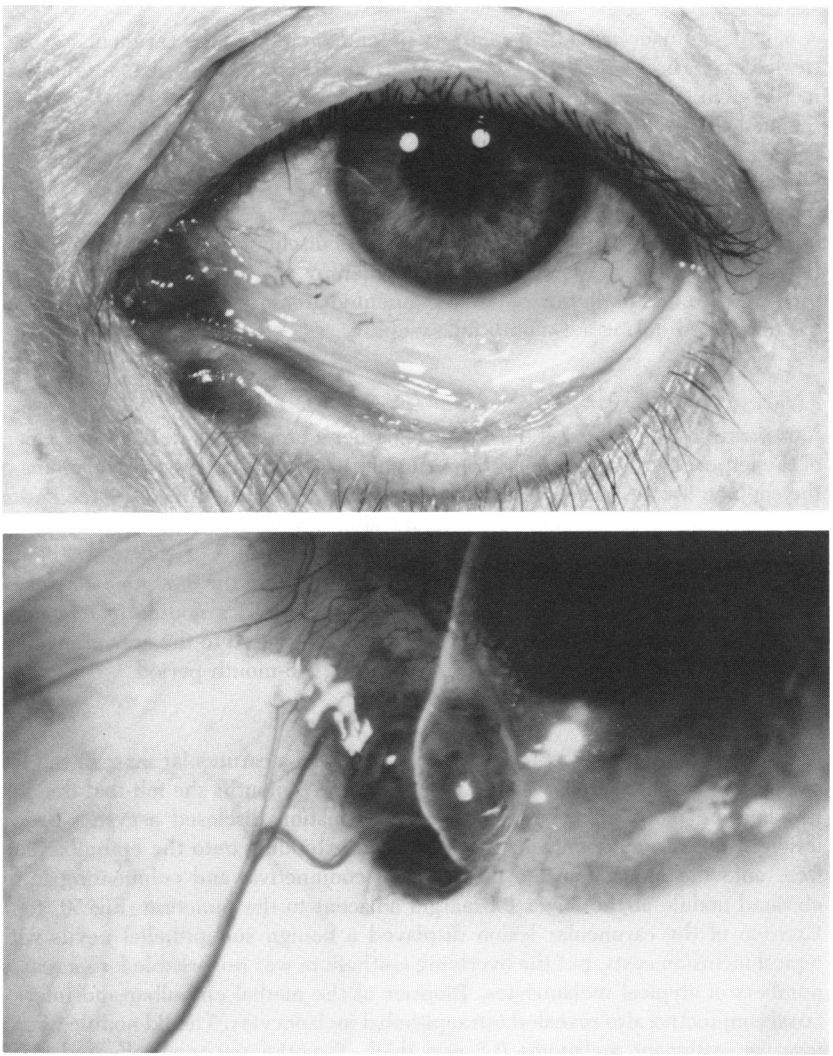

FIGURE 50

TOP: A cystic pigmented lesion of caruncle discovered histopathologically to be a nevus is associated with flat pigmentation of primary acquired melanosis extending into inferior cul-de-sac and inferomedial tarsal conjunctiva. Nodular lesion at lid margin was proved histopathologically to be a melanoma (case 14). This was the only case in the series to be a proven melanoma arising in association with a nevus. BOTTOM: A 59-year-old man had a 10 year history of a pigmented temoral epibulbar lesion of the right eye that developed into a nodular lesion (case 18). Mass recurred four times. Notice extension of juxtalimbal translucent tissue, which was created by florid intraepithelial atypical melanocytic proliferation with subadjacent inflammation.

cell melanoma. Multiple biopsies were taken of the flat and invasive nodules for electron microscopy. The thickest nodule measured 1.5 mm. Because of the extent of the disease process, exenteration was performed. One year later the patient is free of metastases.

CASE 16

A 56-year-old man had known for 10 years of pigmented spots on the conjunctiva of the left eye near the medial canthus. A local ophthalmologist diagnosed the condition as conjunctival nevi. Two years later an excisional biopsy was performed, but this again was followed by recurrence 2 years later. The patient was lost to follow-up for 4 years, after which he returned with flat pigmentation of the caruncle, plica, medial epibulbar surface, and superior fornix and an elevated, pigmented superior tarsal nodule. Excision of the tarsal nodule revealed a spindle cell proliferation of invasive melanoma, achieving a depth of 1.4 mm. Biopsies of several of the flat areas of pigmentation on the medial epibulbar surface, as well as in the caruncle and plica, showed intraepithelial melanocytic proliferation. Cryotherapy was delivered to all areas of pigmentation including the bed of excision of the left upper lid nodule; the patient has been free of disease during 2 years of follow-up.

CASE 17

A 70-year-old Caucasian man believed he had had a pigmented spot on the surface of the right eye since childhood. Two years earlier a reddened area in the temporal epibulbar juxtalimbal conjunctiva was excised and was read as a "compound nevus." The lesion recurred 6 months later and was reexcised followed by cryotherapy. Again 6 months later, flat pigmentation was noted and treated with excision and cryotherapy. In each of these biopsies, intraepithelial melanocytic proliferation was accompanied by superficially invasive nodules of melanoma, measuring no thicker than 0.8 mm. Recurrent elevation occurred 6 months later with pigmented tissue hugging the limbus from the 9- to the 12-o'clock position; scarred temporal epibulbar conjunctiva at the 9-o'clock position was noted, and there was faint pigmentation at the 6-o'clock position. A small pigmented nodule in the midtarsus of the right upper lid was observed. Excision with cryotherapy of the juxtalimbal tissue through 360° was performed, to reveal minimally invasive malignant melanoma. Two months later the patient returned with a massive superior tarsal nodule, which was excised and treated with cryotherapy. This tarsal nodule measured 2.8 mm thick. Two months later the epibulbar surface from the 10- to the 12-o'clock position was involved with a multinodular, poorly pigmented lesion. Biopsy revealed extensive invasive epithelioid cell melanoma, with a thickness of 1.8 mm. Because of the extent of disease, an exenteration was performed.

CASE 18

A 59-year-old man had a 10-year history of pigmentation on the temporal surface of the right eye. Eight years ago, excision of a temporal epibulbar pigmented

lesion that was suspicious revealed superficially invasive malignant melanoma. Two years later, an excision of recurrent flat pigmentation was performed. Recurrent pigmented tissue at the inferior temporal limbus of the right eye developed on three separate occasions during a 1-year period, and the patient was referred for definitive management. Examination revealed an elevated epibulbar pigmented tumor at the right limbus centered at the 8-o'clock position (Fig 50, bottom). Translucent, poorly pigmented tissue extended circumferentially at the limbus from the 12- to the 7-o'clock position. The pigmented nodule was excised, as was the juxtalimbal tissue through 360°; all the involved regions were treated with cryotherapy. The invasive nodule of melanoma measured 2.1 mm; only intraepithelial melanocytic proliferation with subadjacent inflammation was noted in the rest of the juxtalimbal conjunctiva. No recurrence has developed during a 5-month period.

CASE 19

A 68-year-old woman had had a 25-year history of pigmentation on the conjunctival aspect of the left lower lid. Eight years ago the first excision was performed, to reveal intraepithelial melanocytic proliferation. On two occasions at 2-year intervals, reexcision of suspicious conjunctival pigmentation was performed, each time disclosing intraepithelial proliferation. Two years ago an inferior tarsal nodule appeared, which was excised and submitted in part for light and electron microscopic examination. This lesion was composed of spindle cells, with a thickness of 1.4 mm. One year later a small nodule was reexcised, and 6 months after that a massive nodule of invasive melanoma arose, composed of spindle cells that displayed neuroid features. A full-thickness lid resection with reconstruction of the left lower lid was performed. One year later the patient has no recurrent disease and is free of metastases.

CASE 20

A 36-year-old Caucasian woman has noticed 14 years earlier a pigmented lesion on the temporal aspect of the right eye. Five years later it was excised; it quickly recurred and was reexcised. While the initial pathologic impression was malignant melanoma, a consultation was obtained and the pathologic diagnosis was revised to a nevus. Six years later a recurrent epibulbar pigmented lesion was noted at the inferior temporal limbus. The lesion was excised with cryotherapy to the base of excision and a generous surrounding of uninvolved conjunctiva. The lesion was a "small cell" epithelioid cell invasive melanoma 1.7 mm thick. Four months later the patient is free of recurrent disease.

CASE 21

An 87-year-old senile Caucasian woman was noted in a nursing home to have a pigmented lesion at the 8-o'clock position of the right limbus. An excision was performed, but 4 months later the lesion recurred. A reexcision was performed, and again 5 months later a large pigmented nodule developed at the 10-o'clock

juxtalimbal position. Reexcision was performed, and the lesion measured 1.6 mm. The patient died 2 years later of unknown causes.

CASE 22

A 66-year-old Caucasian woman noted 9 years ago a dark spot in the upper outer quadrant of her right eye. Two years ago a biopsy was performed, and the lesion was diagnosed as benign. The lesion recurred 1½ years later in the same area. An excisional biopsy was performed followed by cryotherapy, and invasive malignant melanoma was diagnosed. The lesion recurred yet again as a poorly pigmented temporal epibulbar mass. An excision was performed with cryotherapy; the excised specimen showed invasive malignant melanoma with a thickness of 0.6 mm. Nine months after this last excision there has been no recurrence of the lesion.

Eight patients (cases 1 through 8) had primary acquired melanosis unassociated at the time of initial presentation with a nodule of invasive melanoma. The 1-μ plastic sections studied by light microscopy revealed varying degrees of melanocytic proliferation within the epithelium. In the mildest lesions (cases 6 and 7), scattered melanocytes were identified within the basal cell layer and immediately suprabasilarly (Fig 51). The cells appeared bland, had round nuclei slightly smaller than those of the surrounding keratinocytes, and possessed small nucleoli. Pigment had been transferred to surrounding keratinocytes. In the remaining six patients in this group, somewhat larger but not markedly atypical cells were identified in greater numbers along the basilar region (sometimes confluently), at higher levels of the epithelium, and occasionally forming nests (Fig 52). These cells had variable pigment, some of which had been transferred to the surrounding keratinocytes. A moderate to intense mononuclear infiltrate was found in the subjacent substantia propria, where melanophages were prominent. In two patients (cases 2 and 4), in addition to the cells just described, markedly atypical cells were present with abundant cytoplasm, dendritic processes, and prominent nucleoli (Fig 53). One of these cases was quite remarkable (case 2 and Fig 48, bottom) in that the three areas sampled for electron microscopy showed different morphologic features of the participating cells. At the farthest remove from the area of maximal involvement at the caruncle, the cells were mildly atypical (Fig 54, top), whereas progression toward the caruncle showed more markedly atypical cells (Fig 54, bottom). In this case the polypoidal lesion in the caruncle was found to be composed of invaginated cysts of epithelium, in the walls of which were bizarre and mitotically active epithelioid-type cells (Fig 55).

Ten patients (cases 9 through 18) had primary melanosis associated with invasive nodules of melanoma. (In addition, cases 3 and 4 went on to

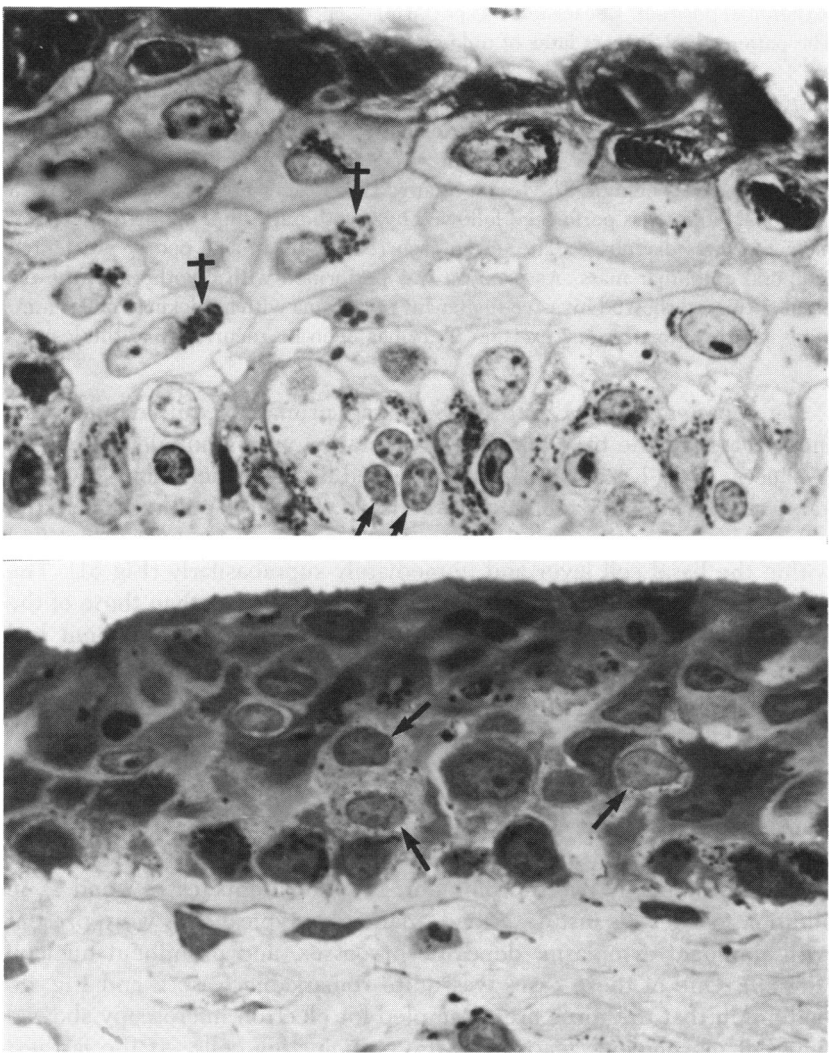

FIGURE 51

Primary acquired melanosis produced by mild hyperplasia of bland melanocytes. TOP: A cluster of melanocytes (*arrows*) nestled among basilar epithelial cells have small nuclei two-thirds the size of surrounding keratinocytes, and clumped nuclear chromatin. Integrity of melanocytic-keratinocytic relationship is established by orderly transfer of pigment to surrounding keratinocytes (*crossed arrows*) (1-μ plastic section, methylene blue-azure II, × 260). BOTTOM: Several suprabasilar melanocytes (*arrows*) display benign nuclear characteristics. Presence of back-to-back melanocytes establishes diagnosis of true hyperplasia (1-μ plastic section, methylene blue-azure II, × 250).

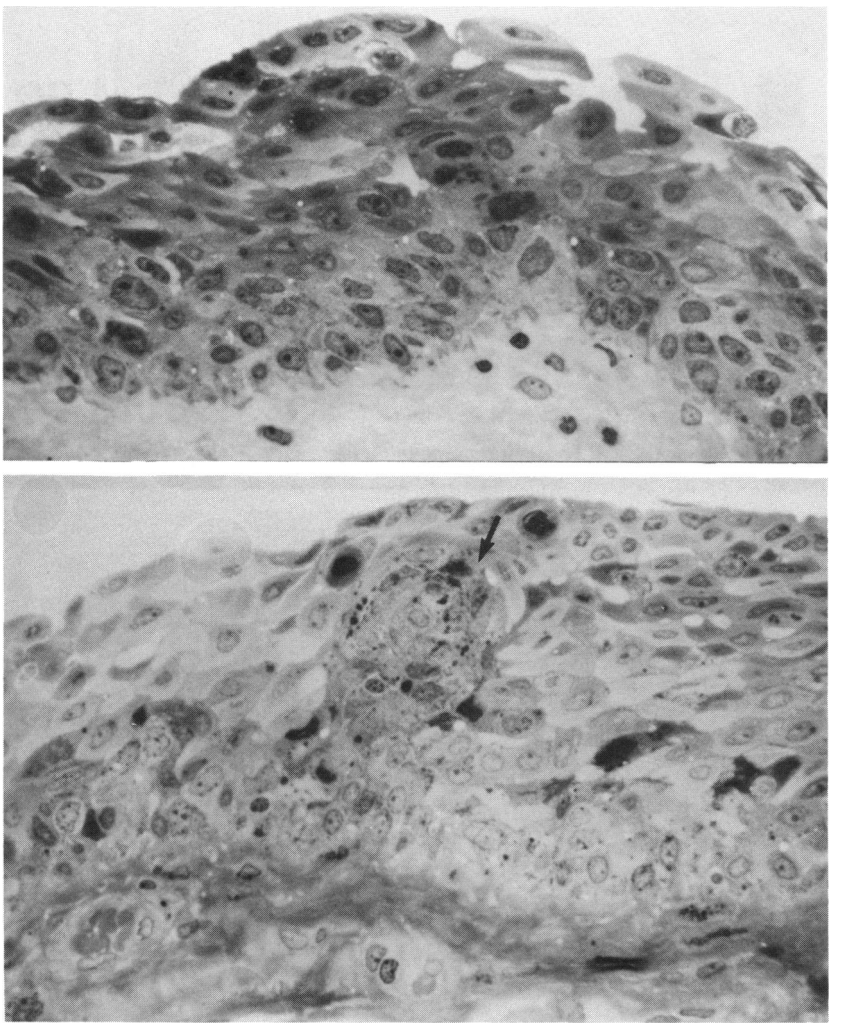

FIGURE 52

TOP: Atypical melanocytes proliferating along basement membrane of epithelium. Many cells have small nucleoli. BOTTOM: Melanocytes proliferating along basement membrane region have mushroomed upward (*arrow*) to higher levels of epithelium (1-μ plastic sections, methylene blue-azure II, × 220).

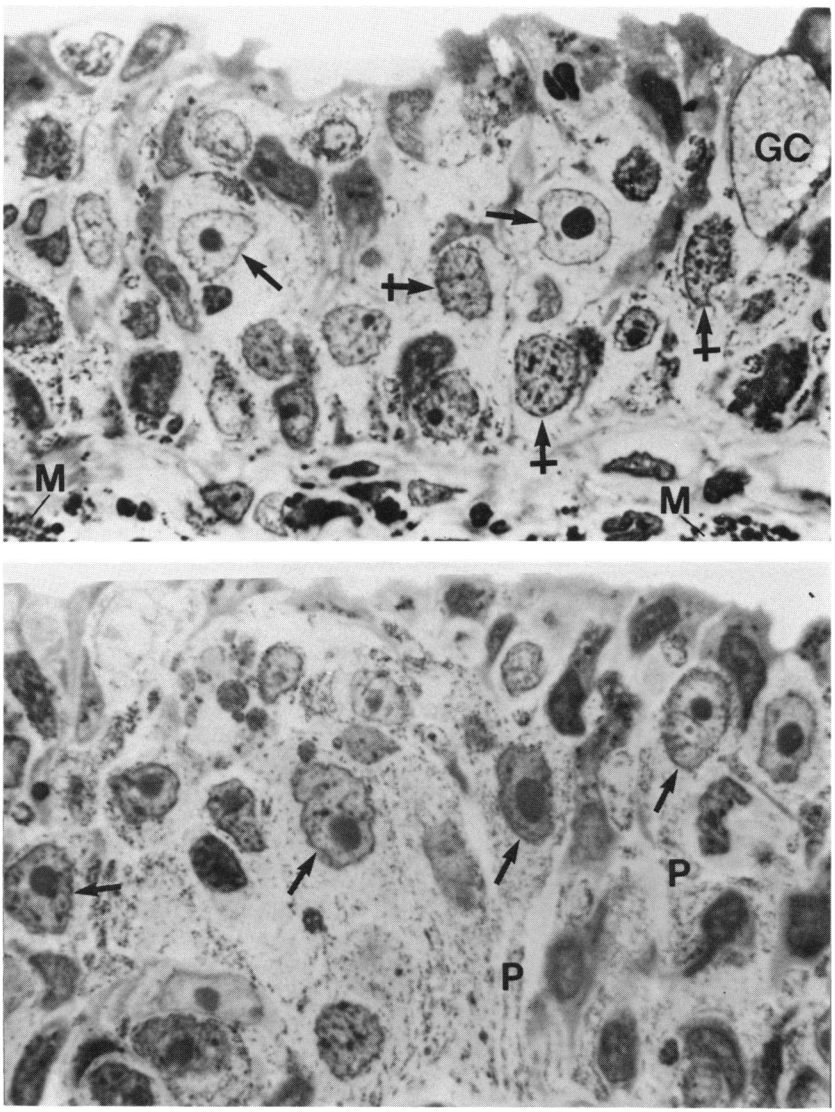

FIGURE 53

TOP: Primary acquired melanosis created by markedly atypical cells (*arrows*) with giant nucleoli and vesicular nuclei. Other less atypical melanocytes (*crossed arrows*) show more clumping of nuclear chromatin and smaller nucleoli. GC, goblet cells; M, melanophages in substantia propria. BOTTOM: Markedly atypical intraepithelial melanocytes (*arrows*) have many dendritic processes (P) containing abundant melanin (1-μ plastic sections, methylene blue-azure II, × 320).

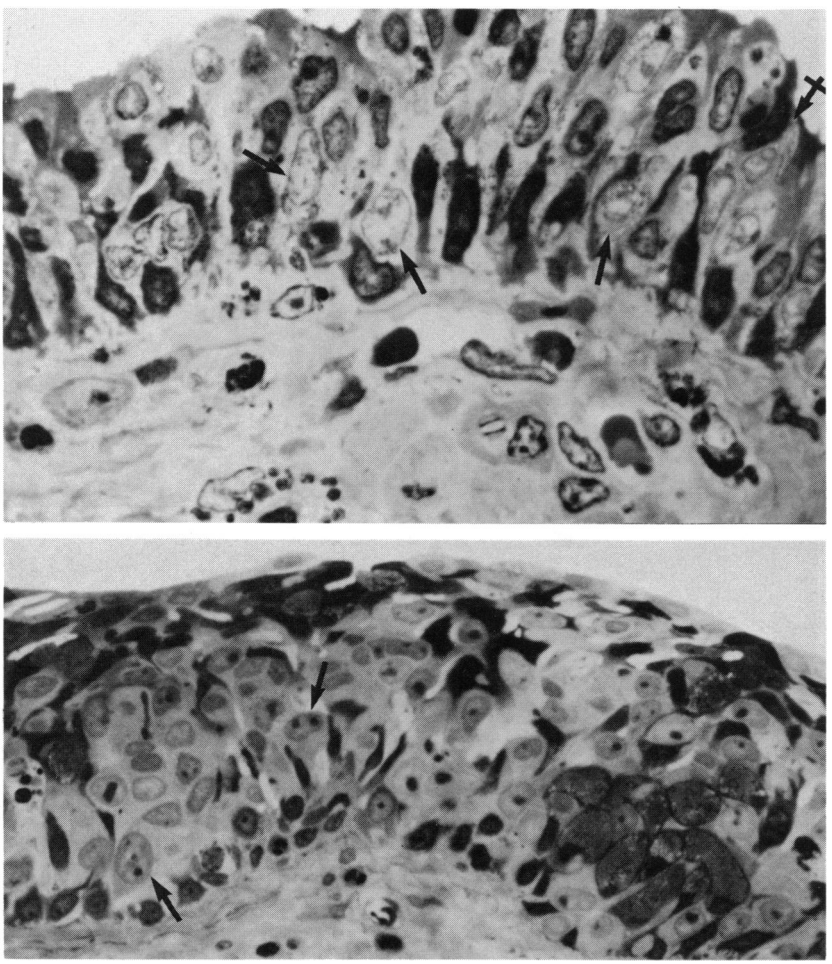

FIGURE 54

Biopsies from two different regions of primary acquired melanosis display different cytologic features (case 2). TOP: Intraepithelial melanocytes at periphery of a primary acquired melanosis show clumping of nuclear chromatin (*arrows*). There is a subpopulation of smaller melanocytes (*crossed arrows*). BOTTOM: Approaching center of lesion cellular characteristics of primary acquired melanosis change, with larger epithelioid cells demonstrating prominent nucleoli and a more vesicular nucleoplasm (*arrows*) (1-μ plastic sections, methylene blue-azure II, × 290).

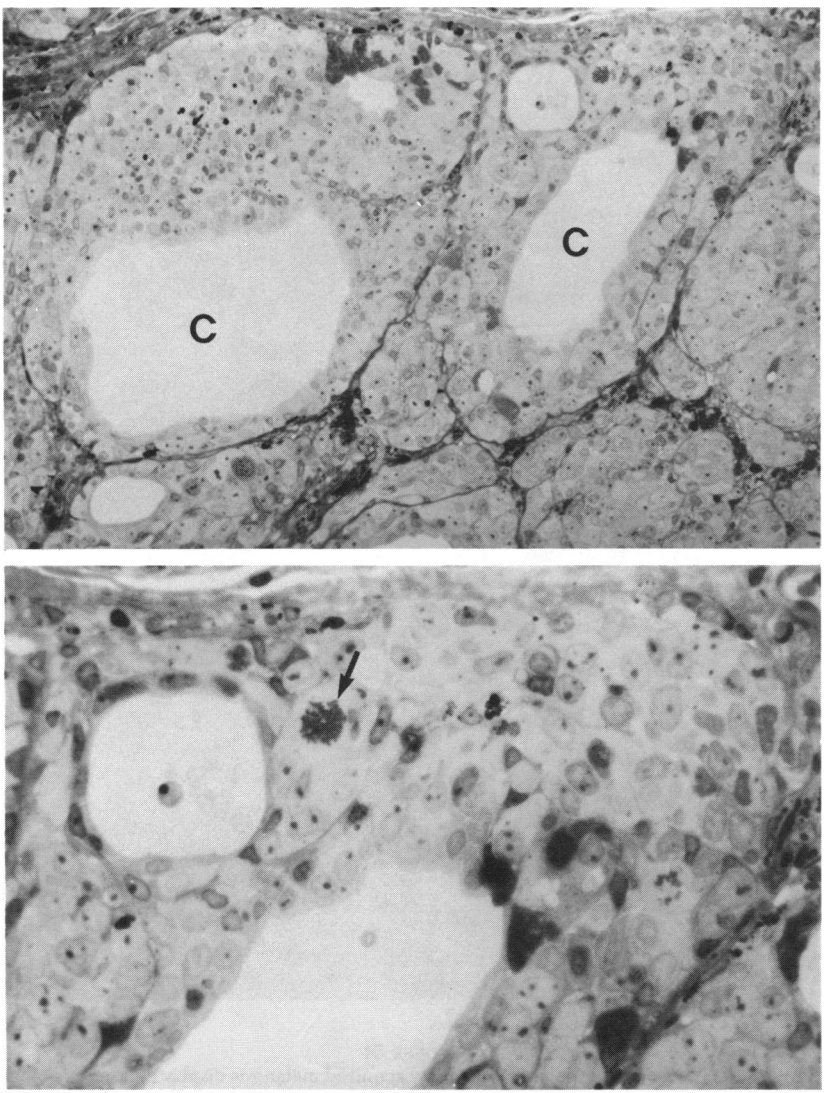

FIGURE 55

TOP: Main polypoidal lesion of case 2 is constituted by epithelial inclusion cysts (C) in walls of which are proliferating piled-up atypical melanocytes (1-μ plastic section, methylene blue-azure II, × 80). BOTTOM: Atypical melanocytes are polyhedral and have prominent nucleoli set in a vesicular nucleus. *Arrow*, bizarre mitotic figure (1-μ plastic section, methylene blue-azure II, × 300).

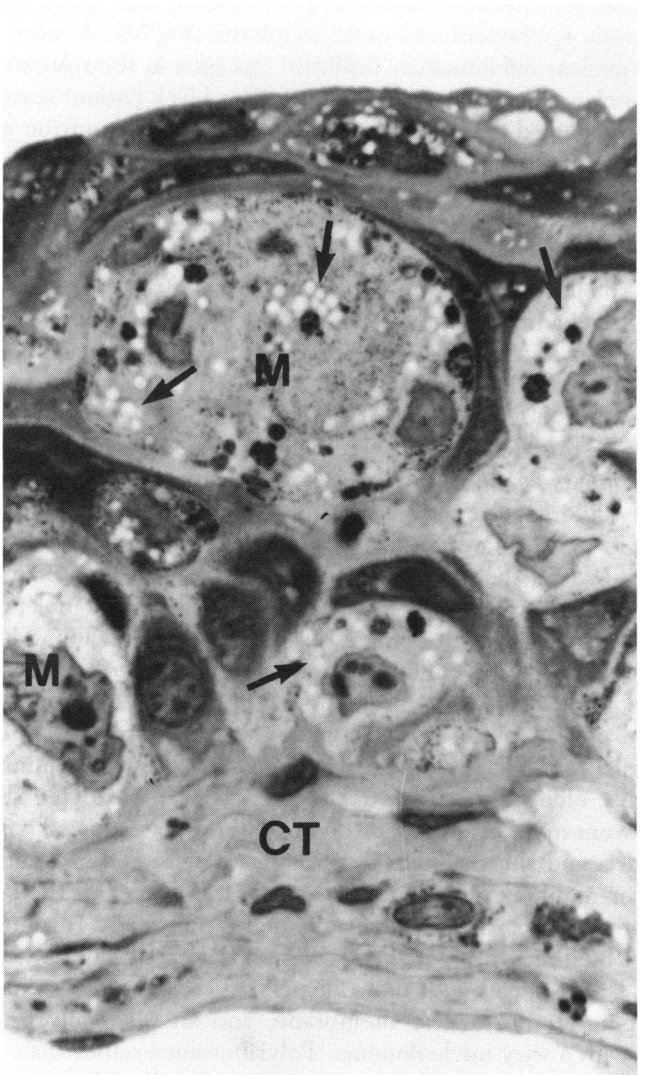

FIGURE 56
Bizarre intraepithelial melanocytes (M) display cytoplasmic lipid droplets (*arrows*). A lymphatic channel is present below. CT, subepithelial connective tissue; L, lymphatic channel (1-μ plastic section, methylene blue-azure II, × 320).

invasive melanoma.) These lesions manifested consistently more severe degrees of intraepithelial melanocytic proliferation, and the melanocytes were generally epithelioid and quite anaplastic (Fig 56). A more prominent mononuclear inflammatory infiltrate was seen in the substantia propria, and melanophages were prominent. The black patient (case 12) in this group displayed massive, atypical intraepithelial dendritic melanocytes (Fig 57). Finally, 11 nodules of invasive melanoma were sampled for electron microscopy, and all but 2 were composed of markedly atypical epithelioid cells (Fig 58). In one case the invasive component was a spindle cell proliferation (case 19 and Fig 57, top), and in the other, the cells were small, "nevoid" epithelioid cells (case 20 and Fig 59, bottom). Three patients had nodules of melanoma studied by electron microscopy that were unassociated with a significant surrounding of primary acquired melanosis (cases 20 through 22). Only a minimal amount of inflammation was observed at the margins of the nodules of invasive melanoma.

At the electron microscopic level, the melanocytes constituting the lesions of primary acquired melanosis with or without invasive nodules of melanoma could be characterized according to a three-part descriptive scheme. Grade 1 cells were indistinguishable from normal dendritic melanocytes, that is, they displayed clumped nuclear heterochromatin, coarse and ropy nucleoli, numerous well-formed elongated melanosomes, and well-developed dendritic processes; they transferred abundant melanin granules to the surrounding keratinocytes. Grade 2 cells had less condensation of the nuclear chromatin at the nuclear envelope; the nucleoli were more prominent, the nucleus was 20% larger than those of grade 1 cells, the melanin granules were less uniformly melaninized but were usually elongated with a filamentary substructure, the dendritic processes were more erratic, and the cytoplasm was endowed with more mitochondria and short segments of rough-surfaced endoplasmic reticulum. Lesser numbers of melanosomes were transferred to the keratinocytes. Whereas in certain instances distinguishing grade 1 from grade 2 cells might be difficult, grade 3 cells were remarkably different, possessing much larger nuclei with more dispersion of the nuclear chromatin, less clumping at the nuclear membrane, and an extremely prominent nucleolus with a wiry nucleolonema. Polyribosomes rather than monoribosomes were present in the cytoplasm, mitochondria were more abundant than in grades 1 and 2, and the melanosomal morphology was more disturbed, with frequent round, abortive, or rudimentary melanosomes. Dendrites were not prominent but were sometimes present. There was much less transfer of melanin to the surrounding keratinocytes when grade 3 cells predominated.

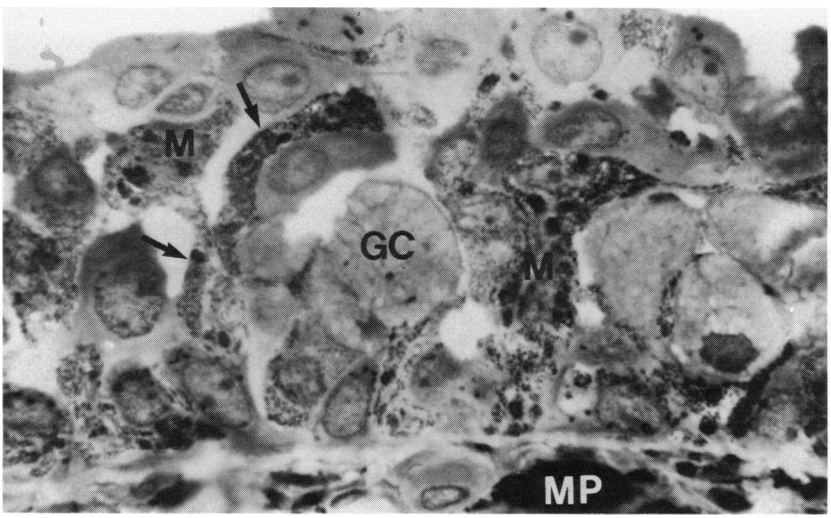

FIGURE 57
Primary acquired melanosis in a black patient. Proliferating melanocytes (M) are present at all levels of epithelium, and extend enormous swollen dendritic processes loaded with pigment (*arrows*). GC, goblet cells; MP, subepithelial melanophage (1-μ plastic section, methylene blue-azure II, × 260).

Of the eight patients with primary acquired melanosis initially not associated with nodules of invasive melanoma, two lesions were distinguished by virtue of increased numbers of melanocytes almost entirely of the grade 1 type, with only a few grade 2 cells intermixed (Fig 60). The melanocytes were located either among the basal cells or in the two keratinocytic layers above the basal cells. The melanosomes were elongated, had an intact melanofilamentary substructure, and were frequently well melaninized. Considerable pigment was transferred to the surrounding keratinocytes, occasionally assuming the character observed in benign racial melanosis (Fig 61). There was clear-cut melanocytic hyperplasia, however, because back-to-back melanocytes were frequently encountered (Fig 62), a feature never observed in benign racial melanosis. In these cells, the nuclei displayed clumped heterochromatin, and the nucleoli were only of moderate size (Fig 62). The essential integrity of the melanocytic-keratinocytic unit was demonstrated in terms of the organization of the melanin into supranuclear caps in the keratinocytes at the higher levels of the conjunctival epithelium (Fig 63), but the melanocytic hyperactivity was manifest by the large amounts of melanin that were so aggregated. Even at higher levels of the epithelium melanocytic dendritic

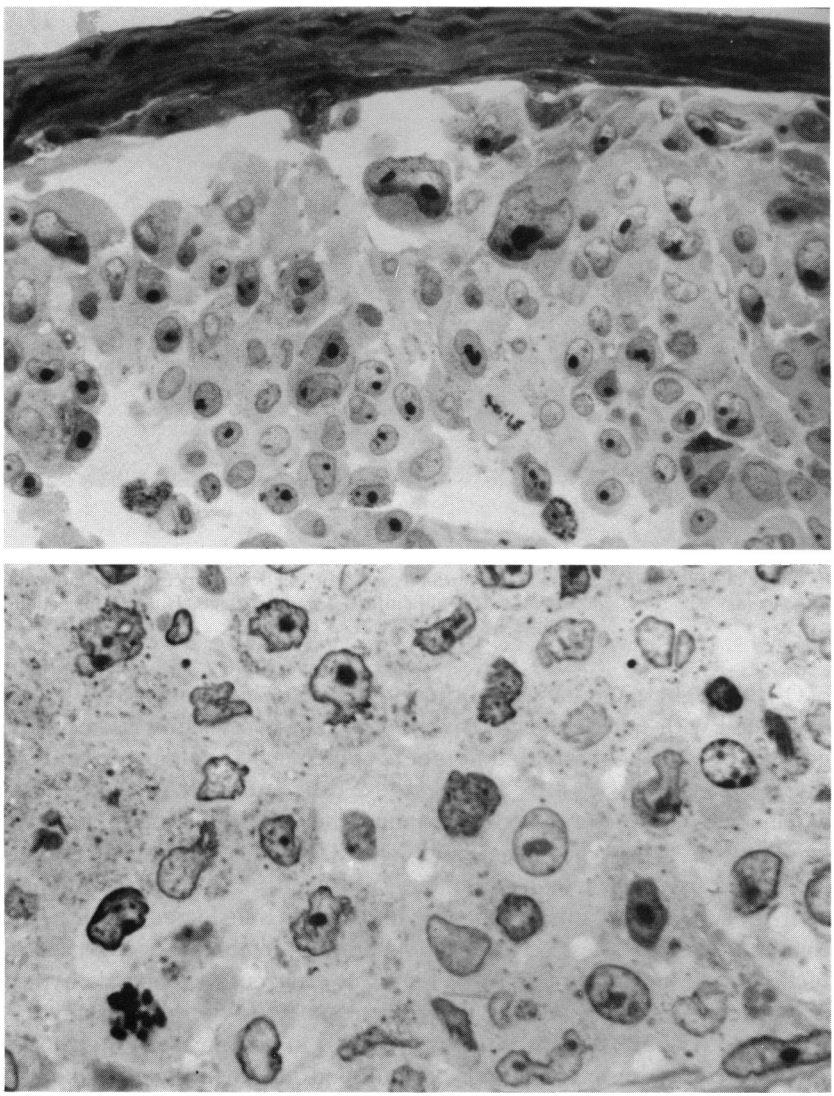

FIGURE 58

TOP: An invasive nodule of malignant melanoma is composed of epithelioid cells with bizarre giant forms lifting up overlying epithelium (1-μ plastic section, methylene blue-azure II, × 220). BOTTOM: Deep in infiltrating nodule of another lesion are pleomorphic melanocytes with large nucleoli and coarsely clumped cytoplasmic melanin. Toward bottom left is a mitotic figure (1-μ plastic section, methylene blue-azure II, × 300).

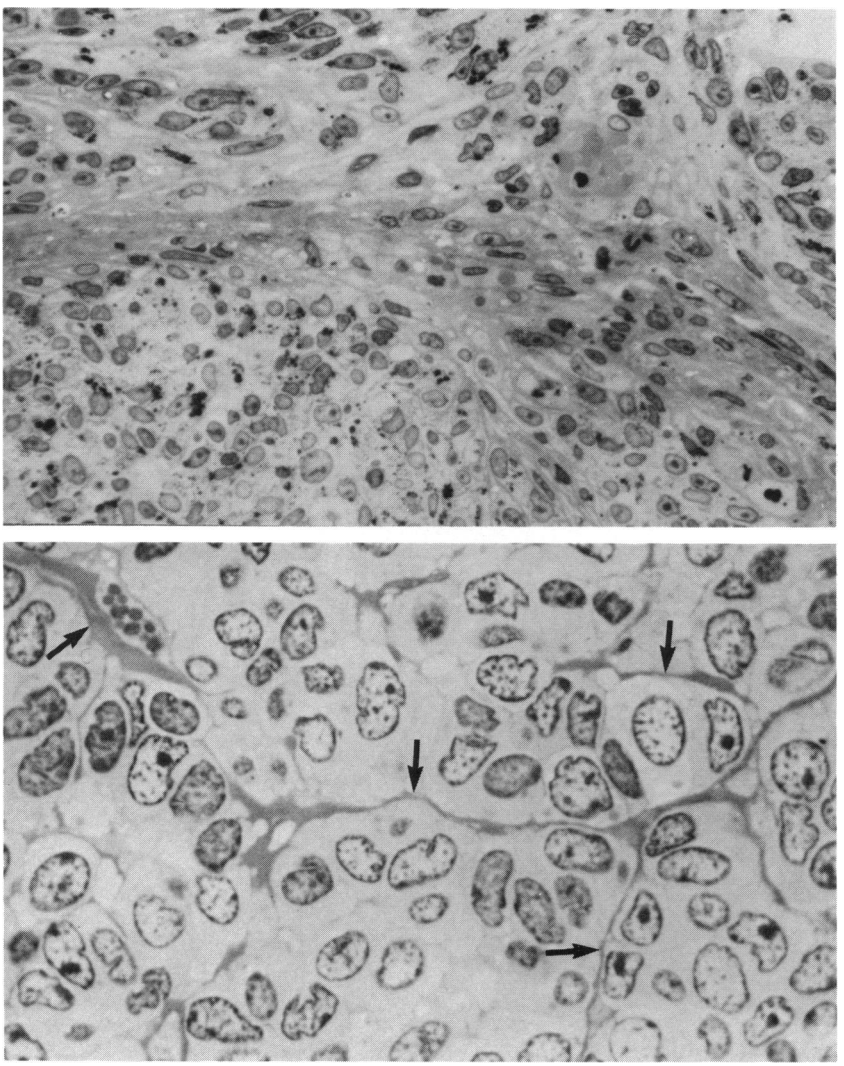

FIGURE 59

TOP: Invasive nodule of malignant melanoma is composed of spindle cells (case 19) (1-μ plastic section, methylene blue-azure II, × 180). BOTTOM: Invasive nodule of melanoma composed of nevoid epithelioid cells, segregated into large nests by connective tissue septa (*arrows*). Nuclei have a stippled chromatin pattern, and scattered cells have prominent nucleoli (case 20). Compare these nuclear features with those shown in Fig 58, bottom (1-μ plastic section, methylene blue-azure II, × 300).

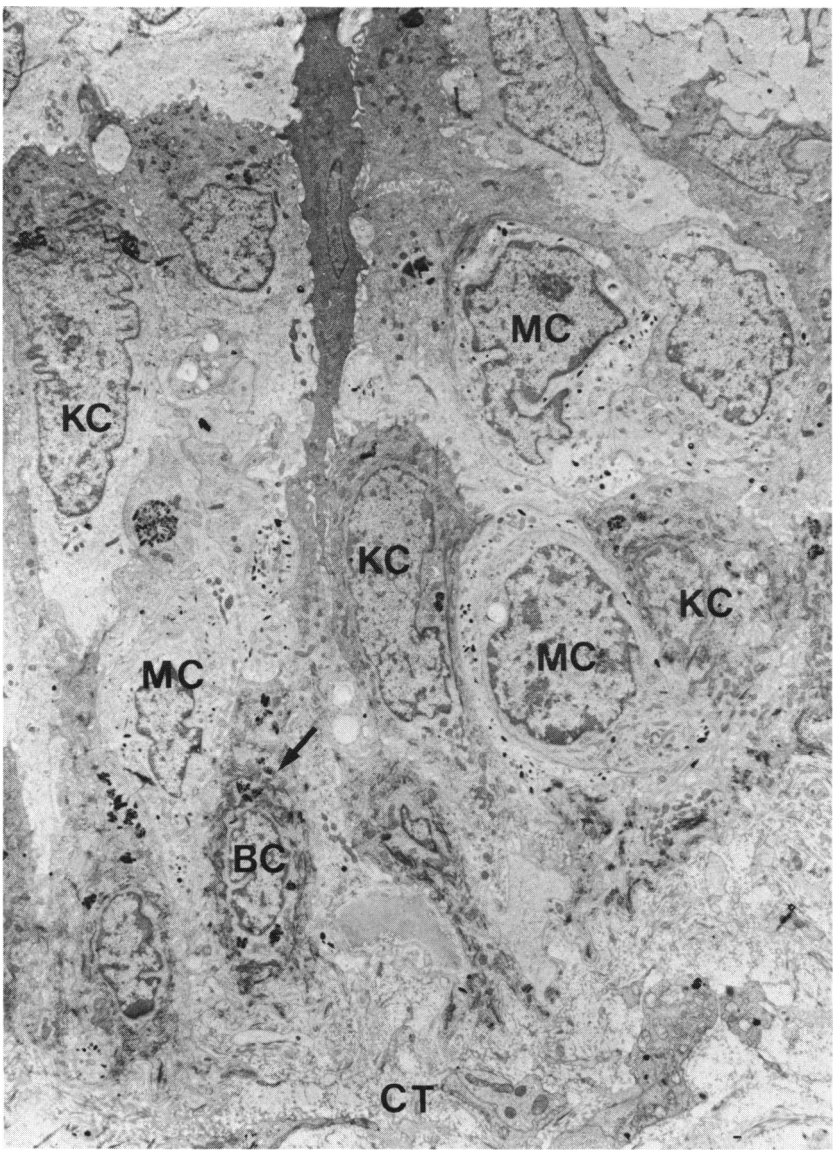

FIGURE 60
Primary acquired melanosis composed of melanocytes (MC) with clumped nuclear chromatin. There has been a transfer of melanin (*arrow*) to surrounding basal cells (BC) and suprabasilar keratinocytes (KC) (× 2100).

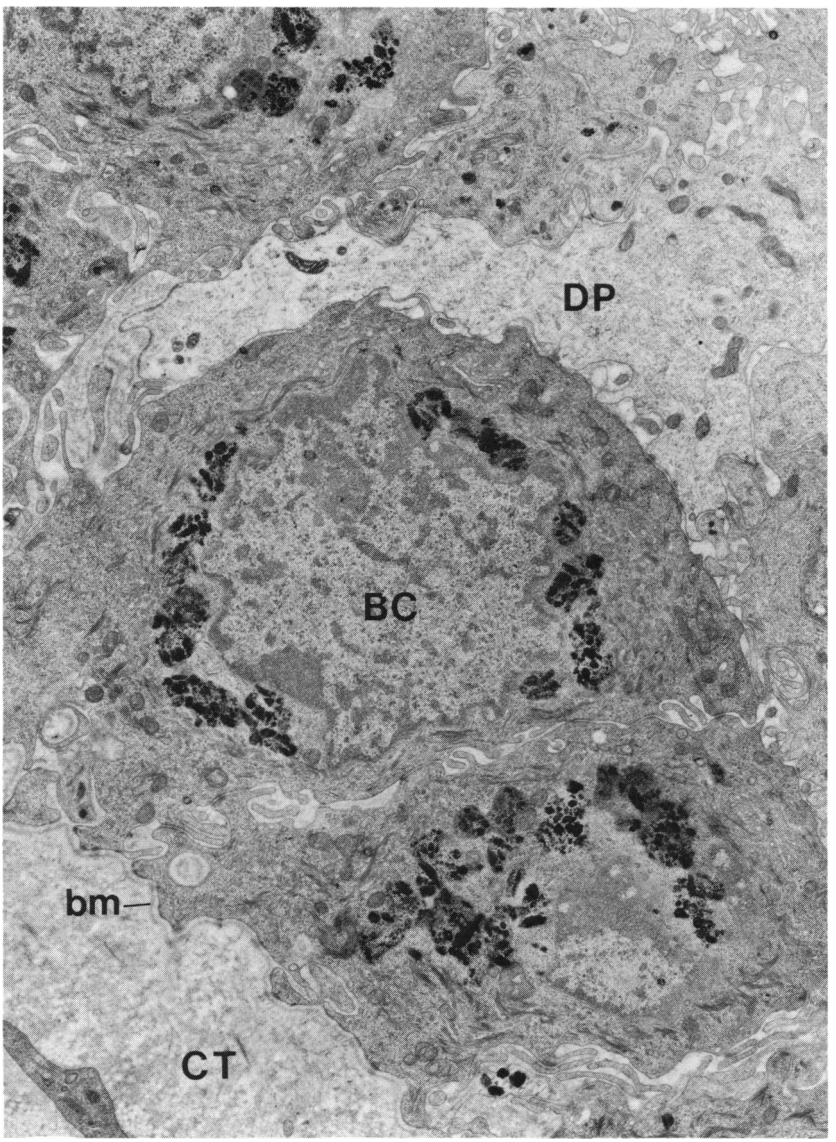

FIGURE 61
Melanin granules are present in phagolysosomes in perinuclear region of a basal cell (BC) keratinocyte. Clear dendritic process (DP) of an adjacent melanocyte is shown. bm, basement membrane; CT, connective tissue (× 12,000).

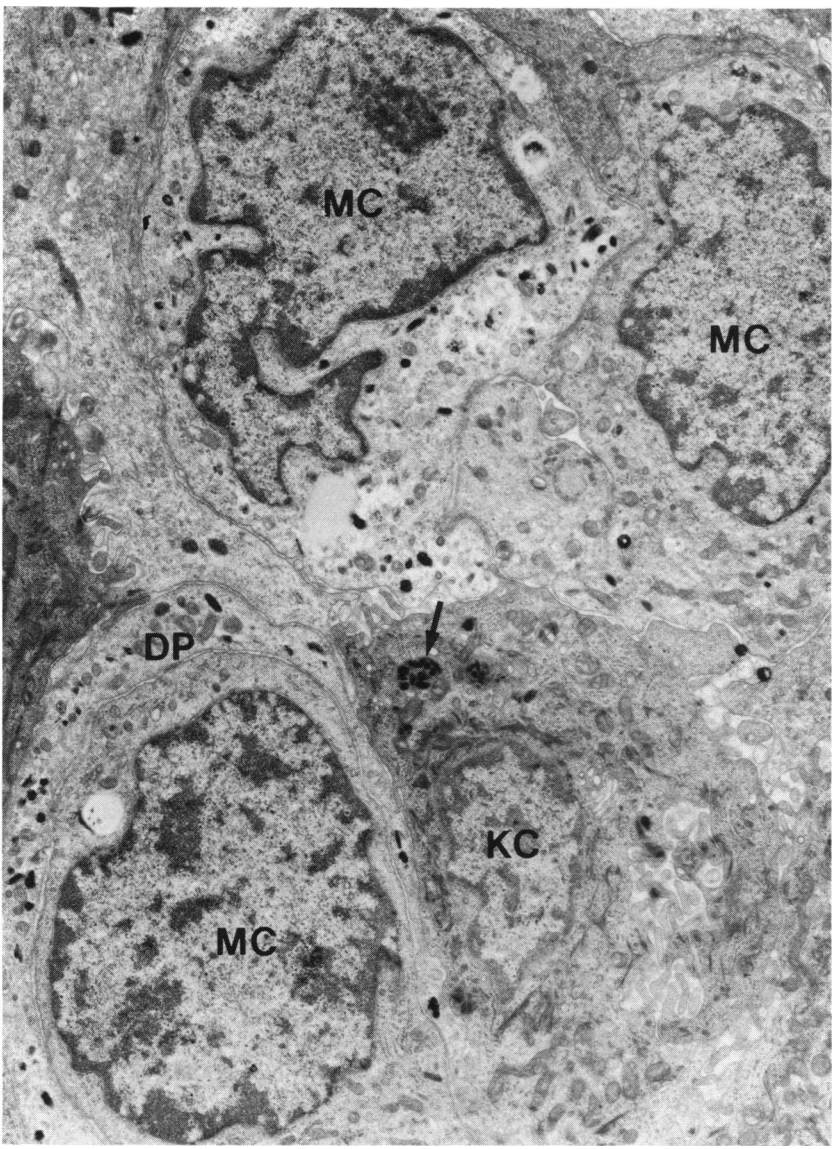

FIGURE 62

Three benign-appearing melanocytes are present in a back-to-back fashion. These are grade 1 melanocytes of primary acquired melanosis, which are virtually indistinguishable from normal dendritic melanocytes except for their increased number. Melanocytes (MC) have clumped nuclear chromatin, elongated melanosomes, and insinuate dendritic processes (DP) among keratinocytes. Melanin granules (*arrow*) are present in supranuclear region of the keratinocytes (KC) (× 5000).

processes were obvious (Fig 63). In these two cases, the underlying connective tissue failed to display significant melanophagic activity, bespeaking an efficient transfer of melanin from the melanocytes to the keratinocytes, and minimal numbers of mononuclear inflammatory cells were observed.

Four of the other cases in this group displayed greater degrees of melanocytic hyperplasia. Both grade 1 and grade 2 cells were present, the latter frequently preponderant. Melanocytes reached all levels of the epithelium (Figs 64 and 65), continued to extend dendritic processes, and communicated melanin to the surrounding keratinocytes (Fig 66). The melanin granules were usually elongated and showed recognizable and intact longitudinal melanofilaments, which had been impregnated with moderate amounts of melanin. Two patients (cases 2 and 4) differed from the others in displaying grade 3 cells in their lesions, which were intermixed with the grade 2 cells (Fig 67). The degree of melanocytic proliferation was most intense in these cases, with confluence of the melanocytes along the basement membrane and basilar regions. Individual cells were also displaced higher up in the epithelium (Figs 67 and 68). An intact basement membrane could be identified where the melanocytes had replaced the basilar keratinocytes, thereby establishing the absence of microinvasion (Fig 69). Both elongated and rounded melanosomes were seen in these grade 3 cells. An unusual feature of two of the eight lesions was the presence of prominent lipidization within the cytoplasm of the proliferating melanocytes (Fig 70).

Eight of the 10 patients with primary acquired melanosis associated with a nodule of malignant melanoma displayed a mixture of grade 2 and 3 cells, frequently with a preponderance of grade 3 cells. In the three patients whose specimens of primary acquired melanosis displayed mixtures of grade 1 and 2 cells, the biopsy specimens of the flat component were taken at a considerable distance from the invasive nodules (cases 9, 10, and 16). One of these patients (case 10) was a black patient in whom the proliferating melanocytes adopted a bizarre, hyperplastic, intensely dendritic appearance (Figs 71 and 72). Extremely plump dendritic processes loaded with heavily melaninized elongated granules were present at all levels of the epithelium; the nuclear chromatin pattern was rather clumped. The type 3 cells predominating in the majority of lesions exhibited more abundant cytoplasm, were more rounded, and had a greater tendency to confluence and nest formation than the type 2 cells (Figs 73 and 74). The cells were more epithelioid in character and had rare and inconspicuous cellular processes; there was only minimal to moderate communication of melanin to surrounding keratinocytes. Many of the

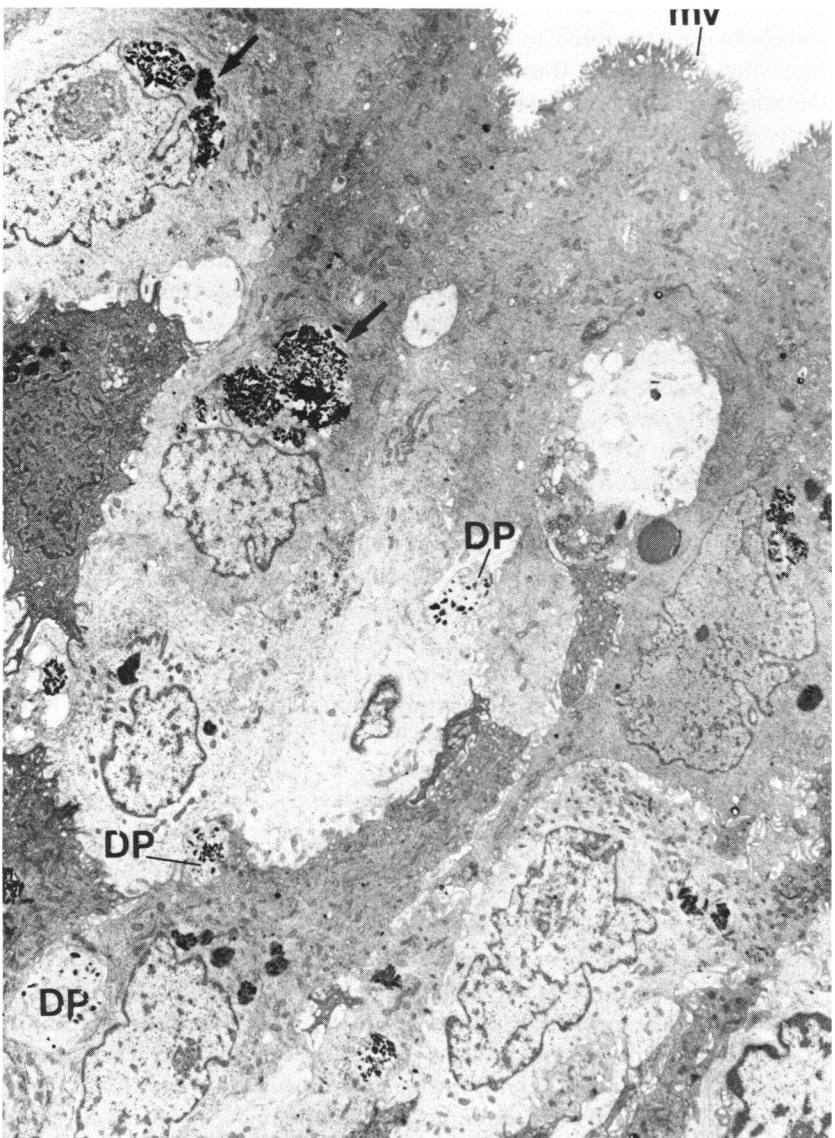

FIGURE 63
Dendritic processes (DP) are present at high levels of conjunctival epithelium. Notice masses of melanosomes that have been aggregated as supranuclear caps in keratinocytes (*arrows*); mv, surface microvilli (× 2000).

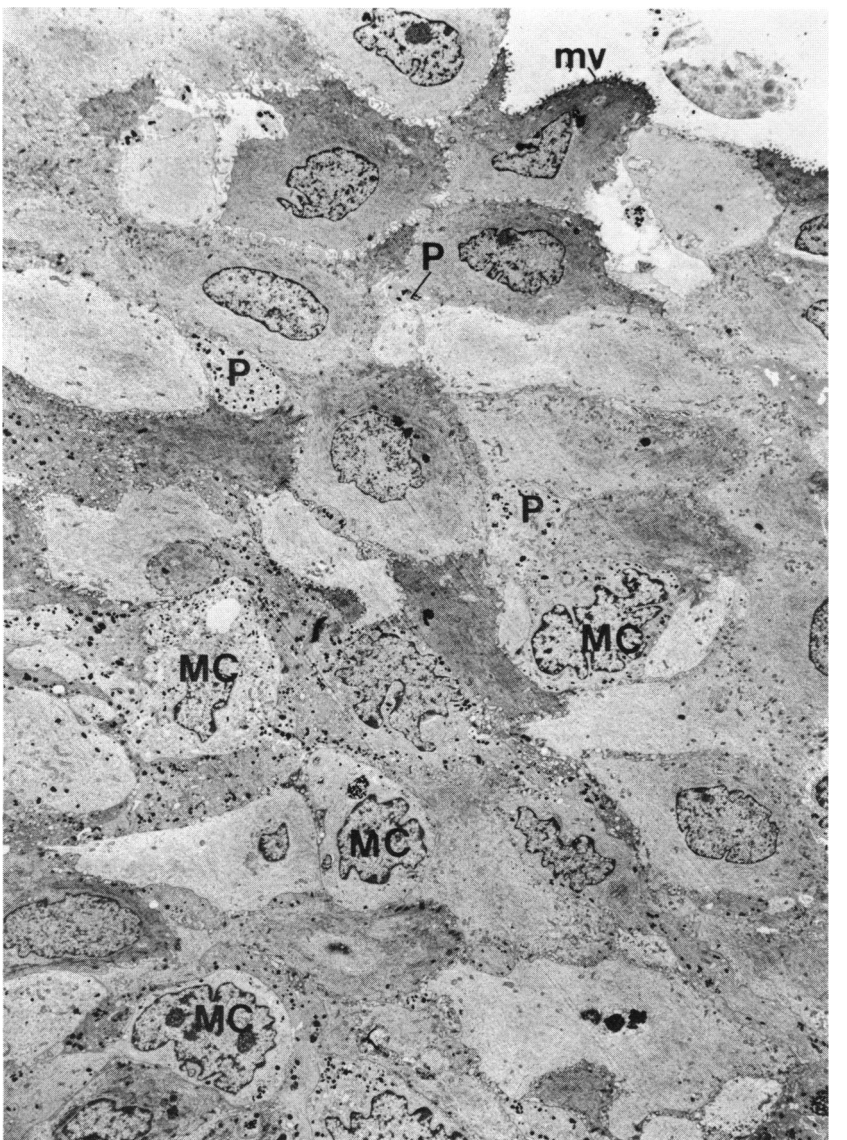

FIGURE 64
Grade 2 melanocytes (MC) of primary acquired melanosis are present at high levels of epithelium, and extend plump dendritic processes (P) among keratinocytes. Nuclear chromatin is somewhat less clumped at nuclear membrane; mv, surface microvilli of epithelium (× 2300).

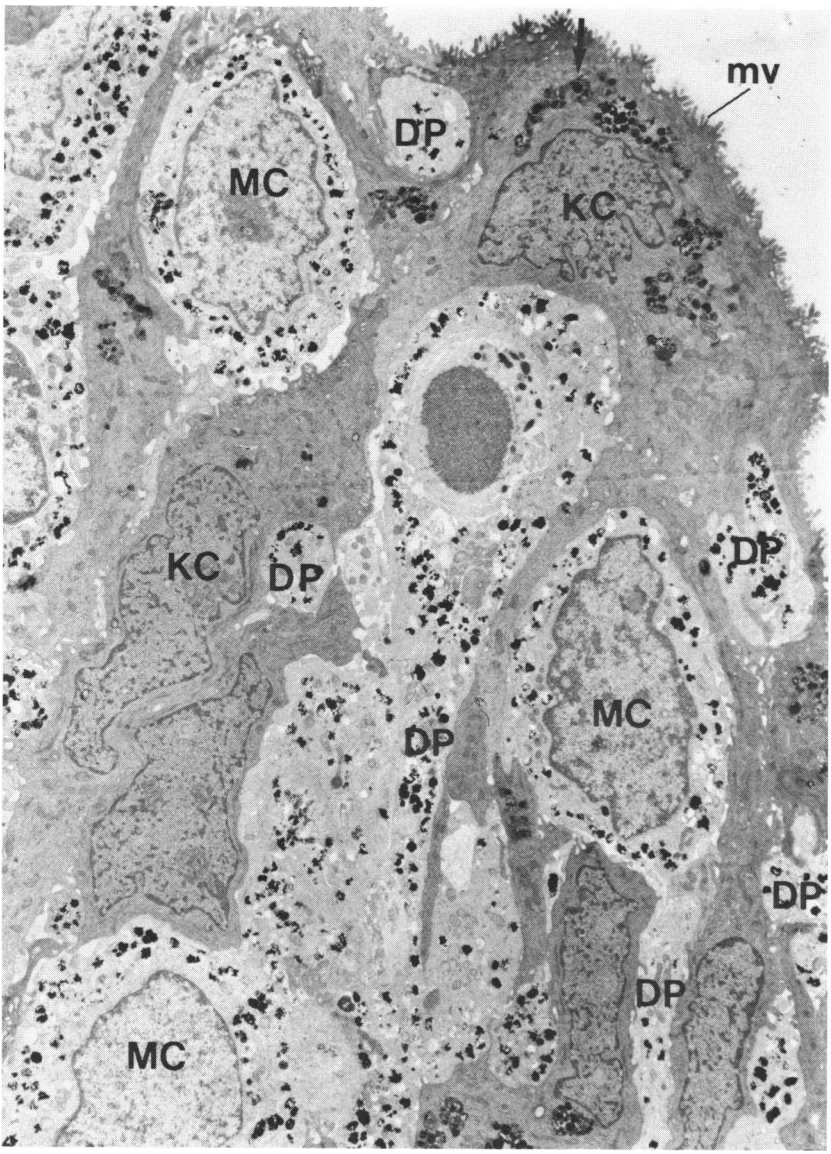

FIGURE 65
Grade 2 melanocytes (MC) with granular melanosomes retain prominent dendritic processes (DP), and are functionally intact by virtue of being able to transfer considerable amounts of melanin (*arrow*) to surrounding keratinocytes (KC); mv, surface microvilli (× 5600).

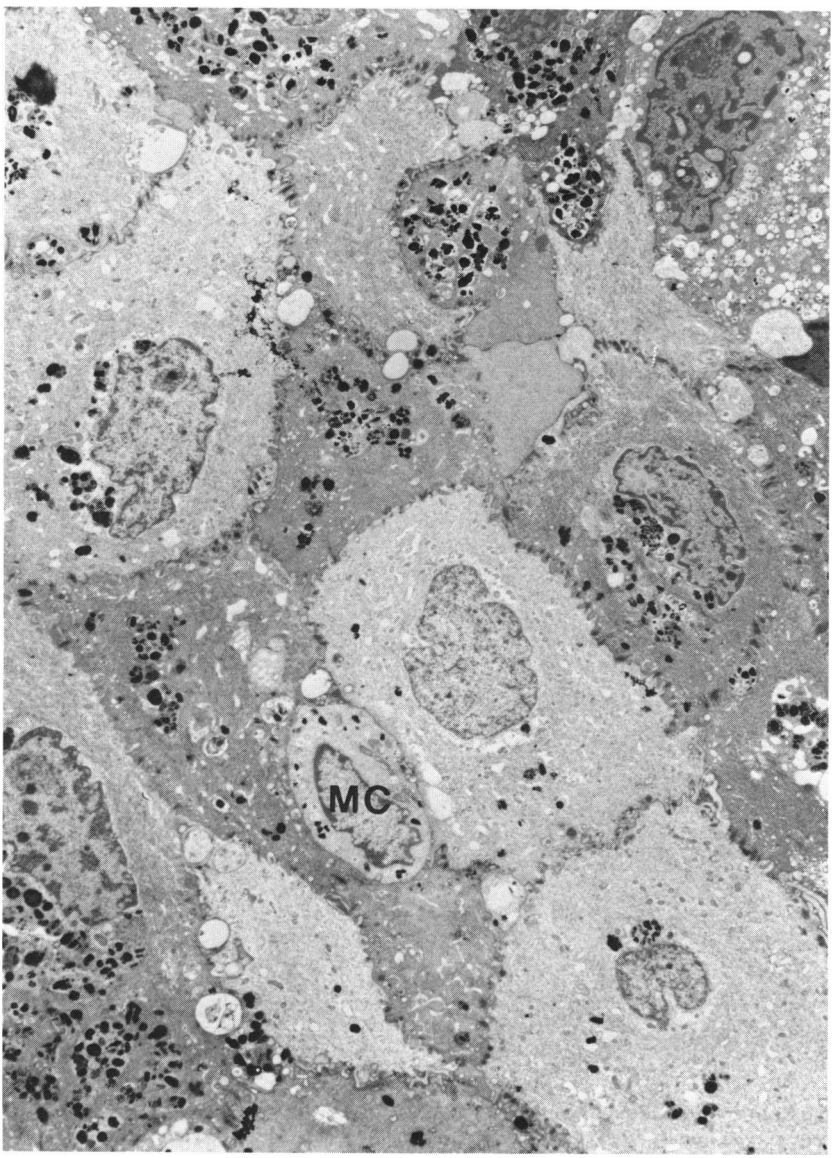

FIGURE 66
Considerable amounts of melanin have been donated to keratinocytes. A solitary melanocyte (MC) is lodged among keratinocytes (× 4600).

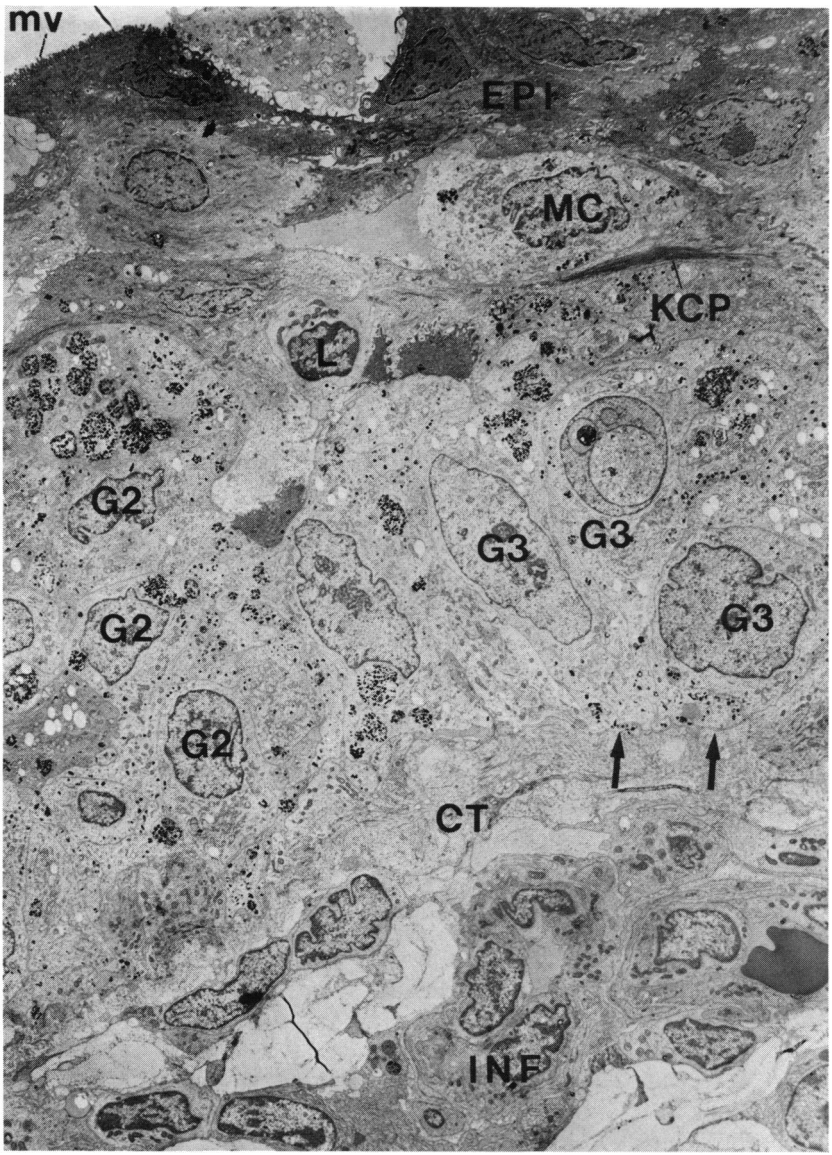

FIGURE 67

A mixture of grade 2 cells (G2) and grade 3 cells (G3) of primary acquired melanosis. Grade 3 cells have more dispersed nuclear chromatin, and one cell features an intranuclear sequestration of cytoplasm. Cells are confluent and form back-to-back nests, but a solitary atypical melanocyte (MC) is present among surface epithelial cells (EPI). Keratinocytic cell process (KCP) is loaded with tonofilaments; infiltrating lymphocyte (L) within epithelium; *arrows*, intact basement membrane of epithelium; CT, connective tissue; INF, mononuclear inflammatory infiltrate; mv, surface microvilli (× 2500).

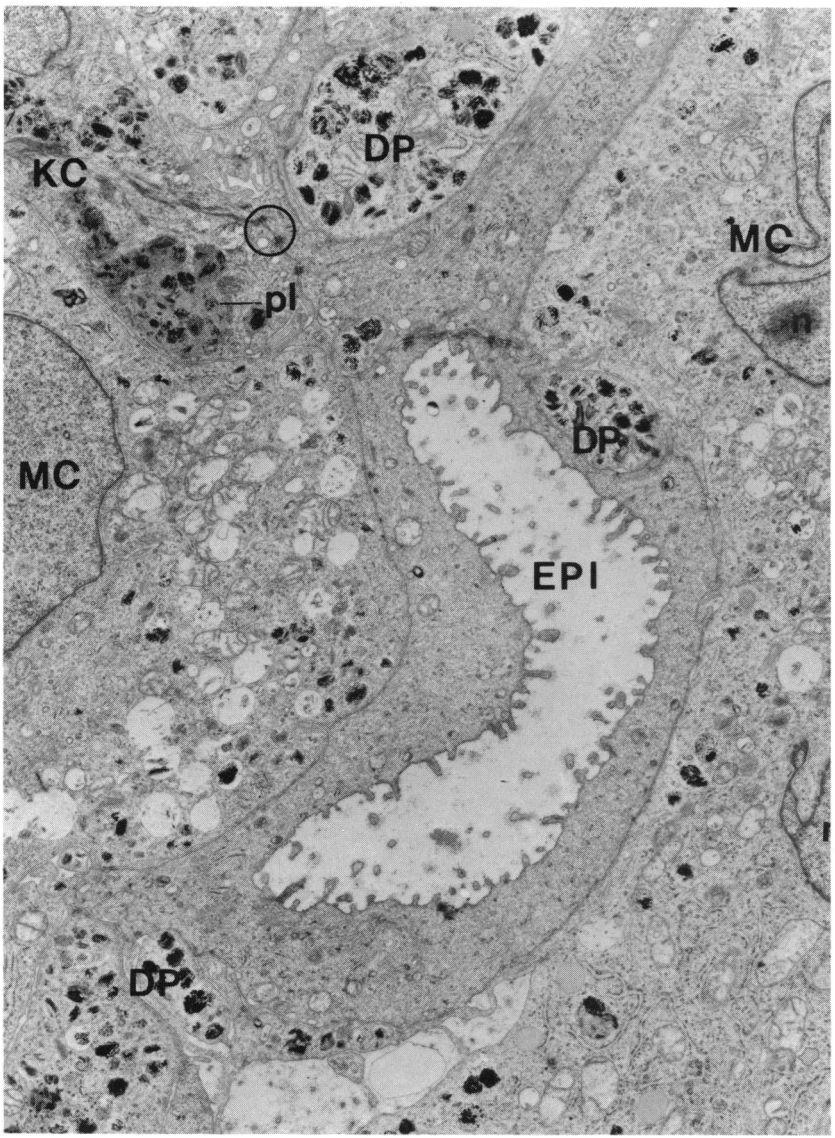

FIGURE 68

Markedly atypical grade 3 melanocytes (MC) are present in wall of an epithelial invagination (EPI). A phagolysosome (pl) is present in keratinocytic process (KC), as indicated by desmosomal junctions (*circle*). Despite their atypicality, neoplastic melanocytes produced dendritic processes (DP), thereby effecting melanin transfer to keratinocytes; n, bilobed nucleus of grade 3 melanocyte (× 8800).

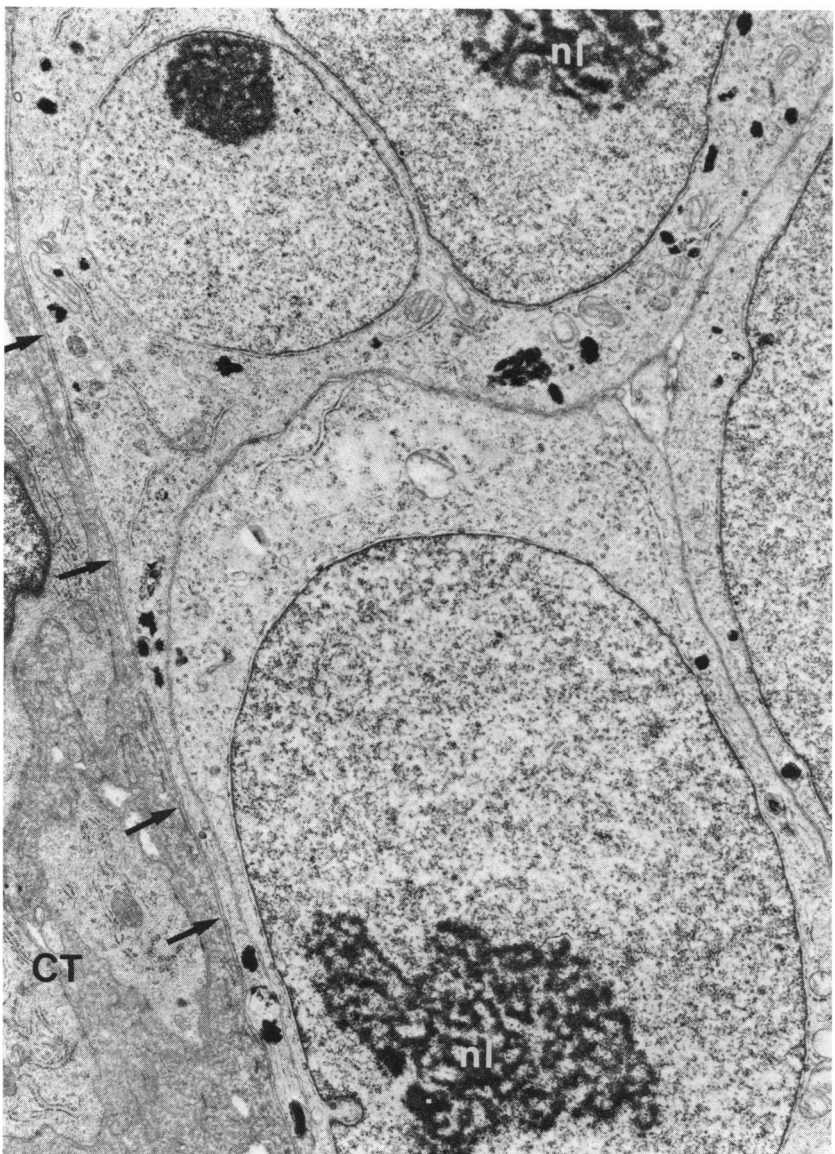

FIGURE 69
Grade 3 atypical melanocytes with dispersed nuclear chromatin and gigantic wiry nucleoli (nl). Cells have not yet invaded connective tissue, as evidenced by an intact basement membrane (*arrows*). CT, subepithelial connective tissue (× 12,000).

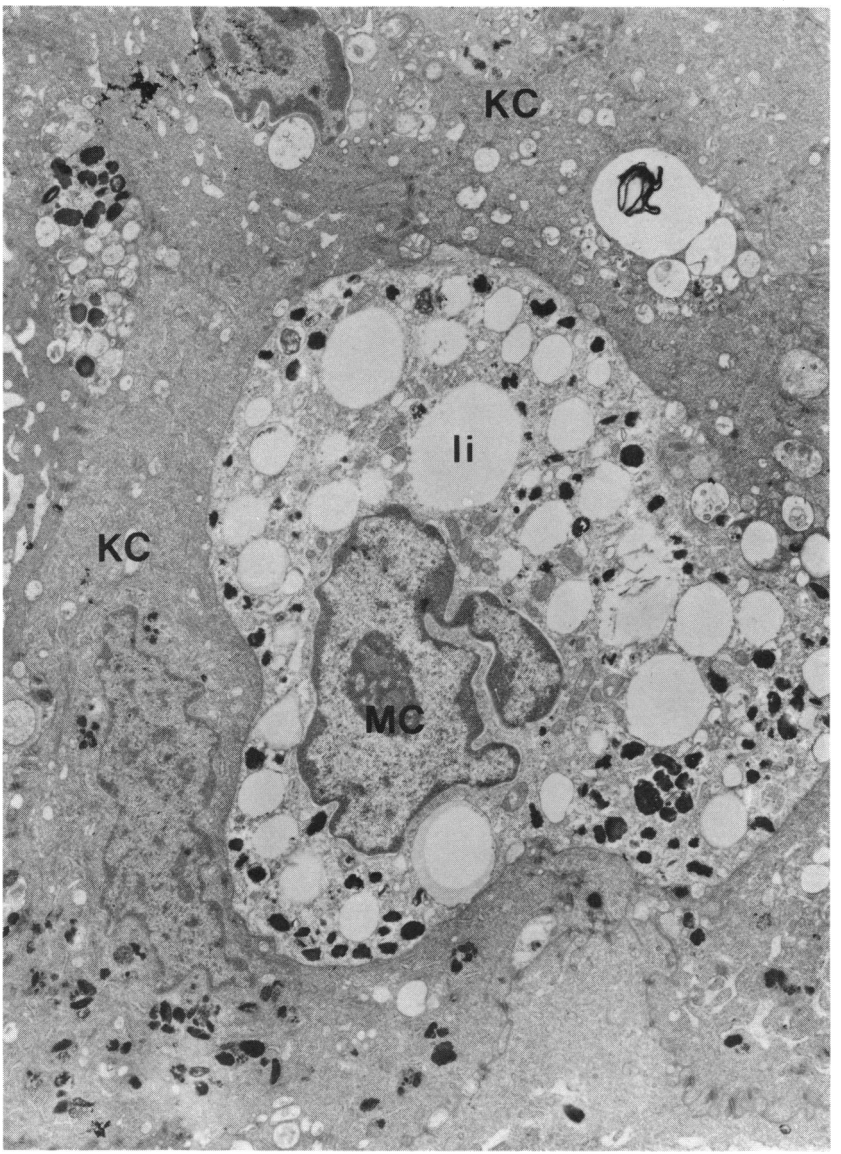

FIGURE 70
A heavily lipidized (li) grade 1 melanocyte (MC) situated among keratinocytes (KC) with abundant cytoplasmic melanin. Lipidization was also seen in grade 2 and grade 3 cells (× 8800).

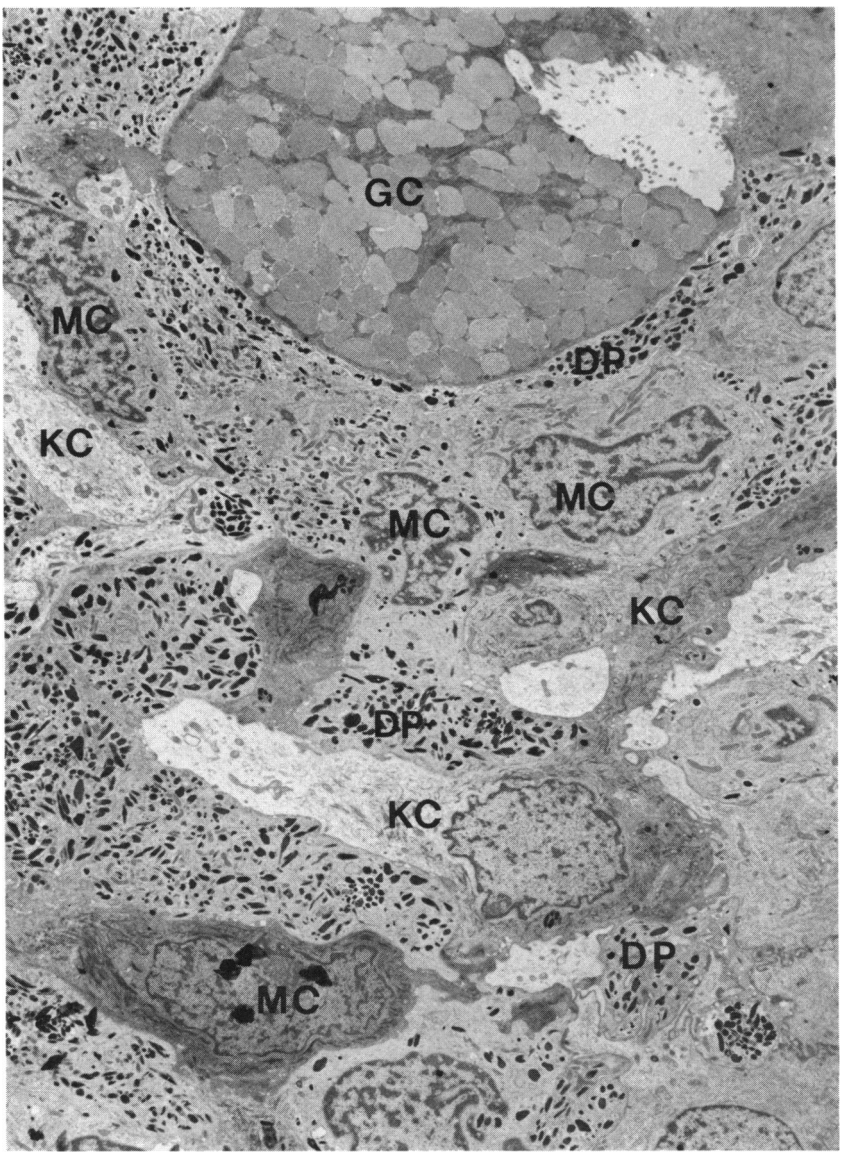

FIGURE 71
Hyperplastic and hypertrophic grade 2 dendritic melanocytes (MC) in a case of primary acquired melanosis in a black patient (case 10). Dendritic processes (DP) are massive. There is a relative blockade of melanin transfer to surrounding keratinocytes (KC). GC, goblet cell (× 4400).

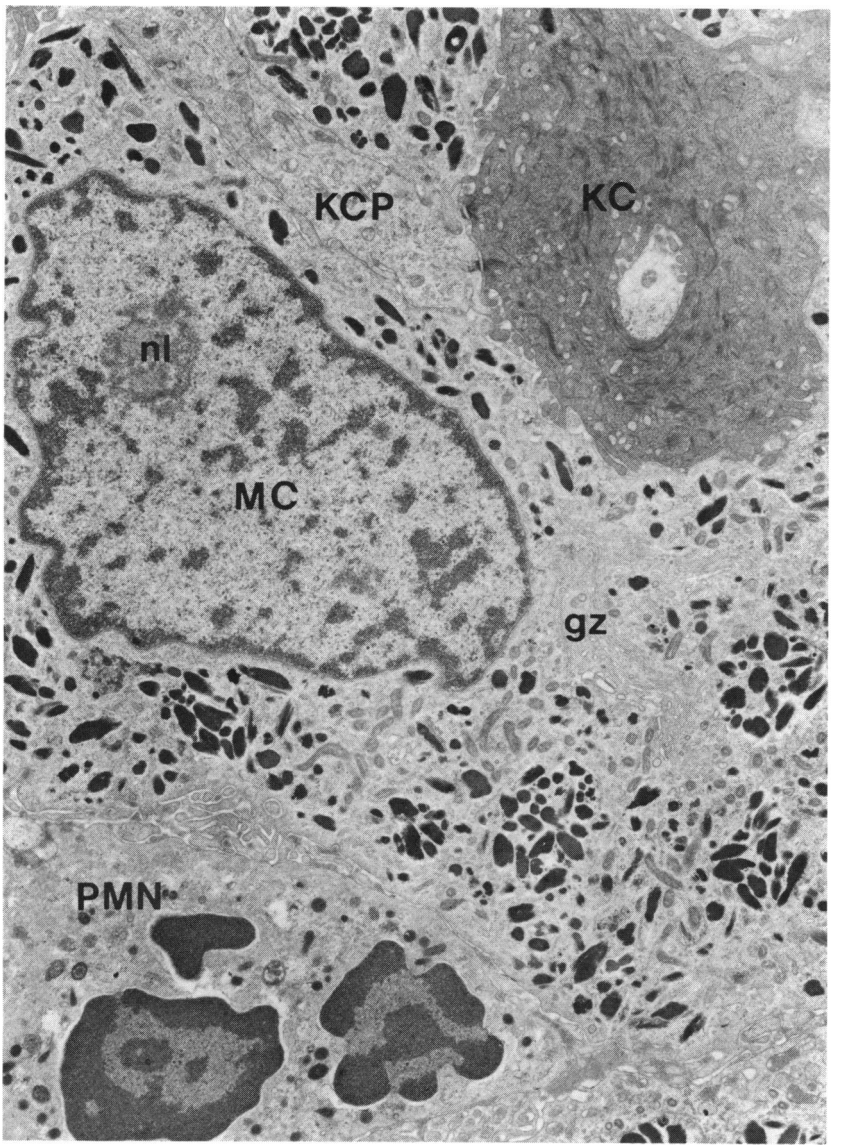

FIGURE 72
Neoplastic dendritic melanocyte (MC) with a small nucleolus (nl). Melanin granules in this black patient's neoplastic melanocytes are large and elongated. gz, Golgi zone; KCP keratinocytic cell process; KC, keratinocytic process; PMN, infiltrating polymorphonuclear leukocyte (× 11,500).

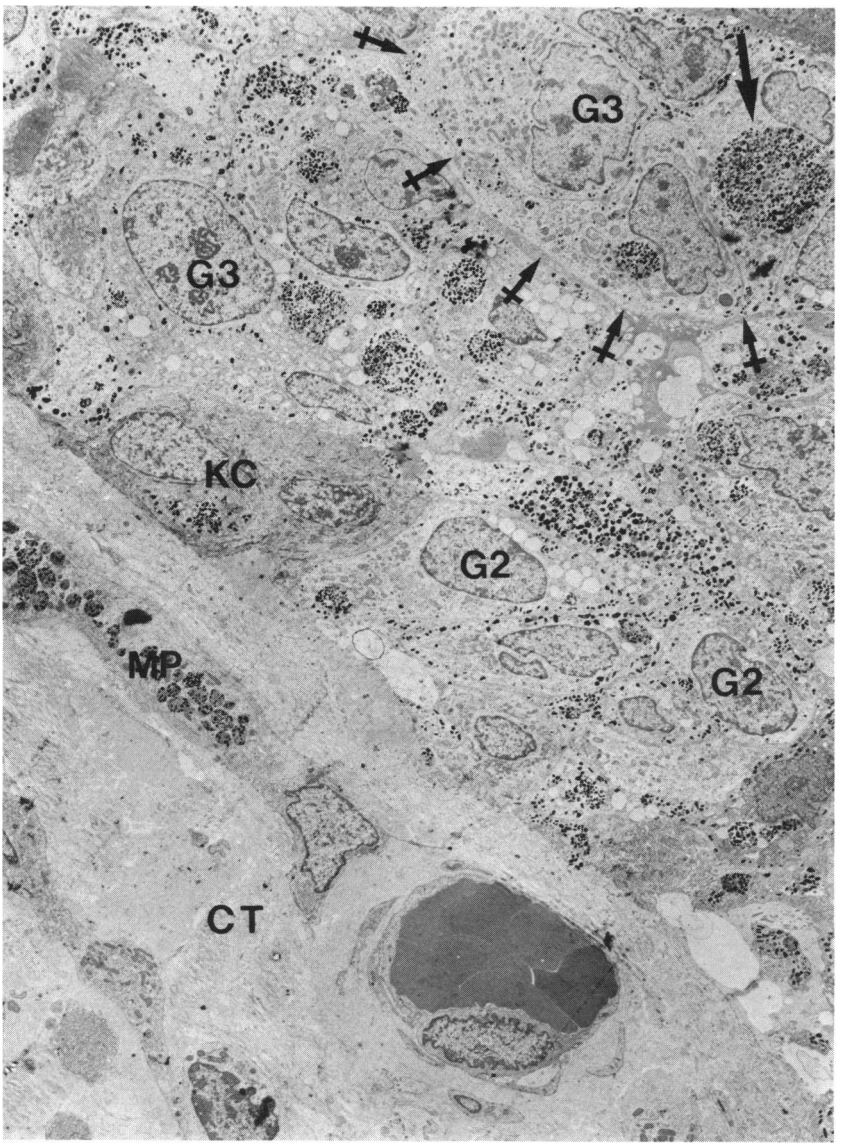

FIGURE 73
A mixture of grade 2 (G2) and grade 3 (G3) melanocytes in primary acquired melanosis. A high level nest composed of grade 3 cells (*crossed arrows*) displays a massive collection of autophagocytosed melanin granules (*heavy arrow*). A keratinocyte (KC) contains ingested melanin granules. MP, subepithelial melanophage; CT, connective tissue (× 2400).

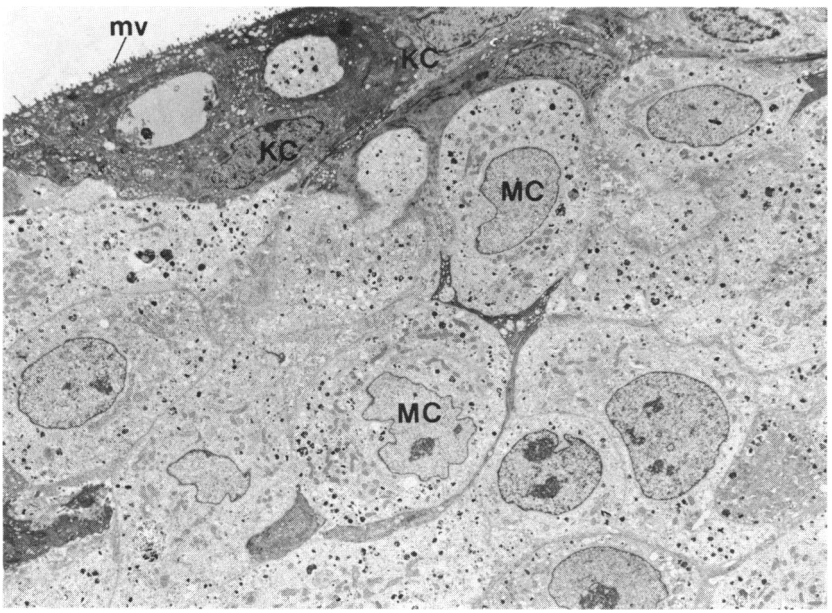

FIGURE 74
A continuous sheet of intraepithelial grade 3 polyhedral melanocytes (MC) has lifted up surface keratinocytes (KC) with surface microvilli (mv) (× 3000).

cells showed evidence of large phagolysosomes of melanin granules in the cytoplasm (Fig 75), and there was an abundance of mitochondria and polyribosomes. In one patient the spillover of pigmentation onto the cutaneous aspect of the lower lid margin (case 14) showed similar grade 3 cells, admixed with epidermal keratinocytes with more clear-cut bundling of the cytoplasmic tonofilaments in a perinuclear location than is usually exhibited by conjunctival keratinocytes (Fig 76). The melanosomes within the type 2 cells were either elongated or round with a moderate amount of melanin deposition, whereas the melanosomes in the type 3 cells were round, rudimentary, and tended to have only spotty melanin deposition. Lipidization of the melanocytes was noted in two of the cases (cases 15 and 16).

The ultrastructural features of the cells of the invasive nodules of melanoma were studied in 11 cases, 8 patients having had an associated radial growth phase of primary acquired melanosis (studied in all lesions except case 19), and 3 patients in whom this feature was not clinically observed or pathologically identified. The cells were, with two exceptions

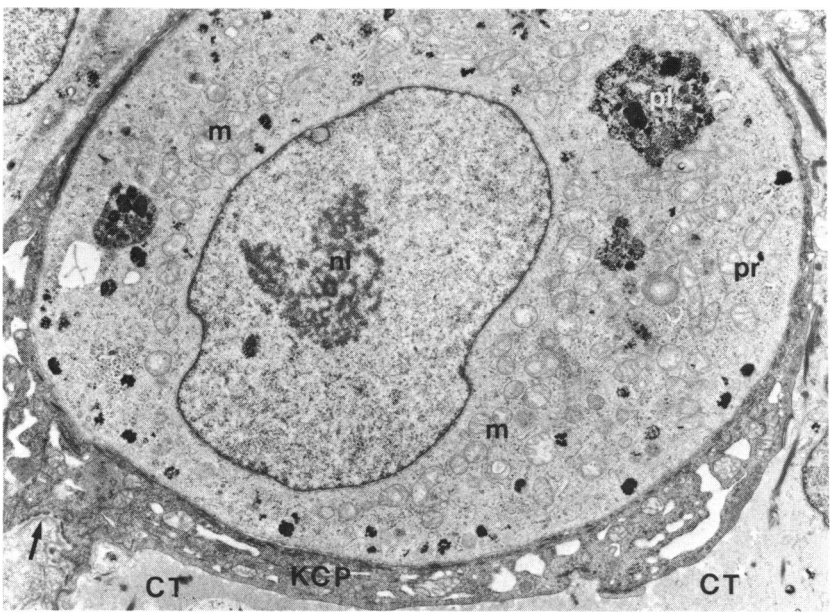

FIGURE 75
A grade 3 atypical melanocyte has a wiry nucleolus (nl), numerous mitochondria (m), scattered polyribosomes (pr), and phagolysosomes containing melanin (pl). A slender keratinocytic cell process (KCP) restrains atypical melanocyte from invading connective tissue (CT). *Arrows*, basement membrane of basilar keratinocyte, which is absent under keratinocytic cell process (× 11,000).

to be described below, epithelioid in character; they manifested nuclei with finely dispersed chromatin, minimal clumping of heterochromatin at the nuclear membrane, large to gigantic wiry nucleoli, and numerous features of cytoplasmic disorganization (Figs 77 and 78). In the heavily pigmented lesions there were frequent collections of melanin in autophagosomal depots (Fig 79). The Golgi zone was active and multifocal, and frequently had associated numerous vesicles that had failed to undergo organization into melanosomal structures (Fig 80). Polyribosomes predominated over monoribosomes in the cytoplasm, and mitochondria were often the dominant cytoplasmic organelle (Fig 81). Short segments of rough-surfaced endoplasmic reticulum were less frequently observed and were never a conspicuous feature. Lipid droplets were seen in the cytoplasm but were never a striking finding as in some of the intraepithelial melanocytes. Intranuclear sequestrations of cytoplasm were often observed (Figs 81 and 82). Cytoplasmic filaments were variably present and

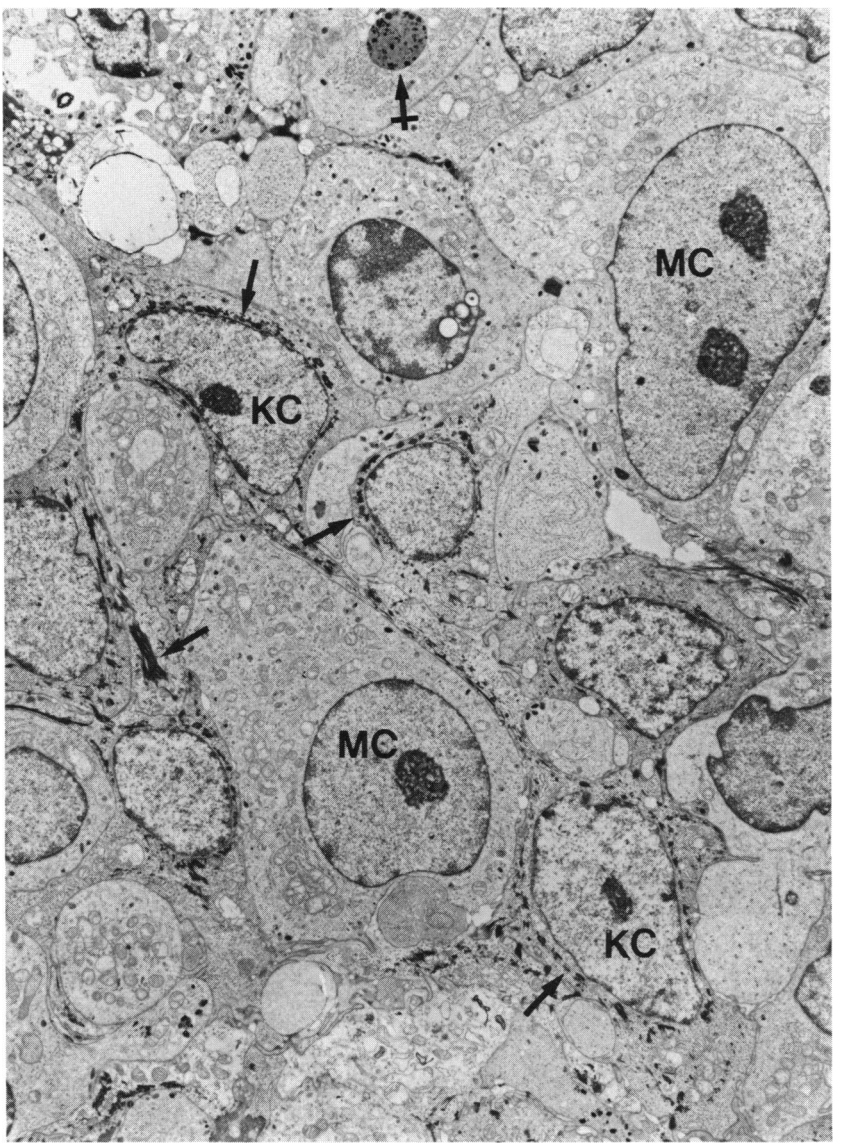

FIGURE 76
Grade 3 atypical melanocytes (MC) within epidermis of lid margin in association with primary acquired melanosis of conjunctiva. Keratinocytes (KC) have more features of epidermis by virtue of thickly bundled tonofilaments arranged in a perinuclear disposition (*arrows*). *Crossed arrow*, phagolysosome in a melanocyte (× 4500).

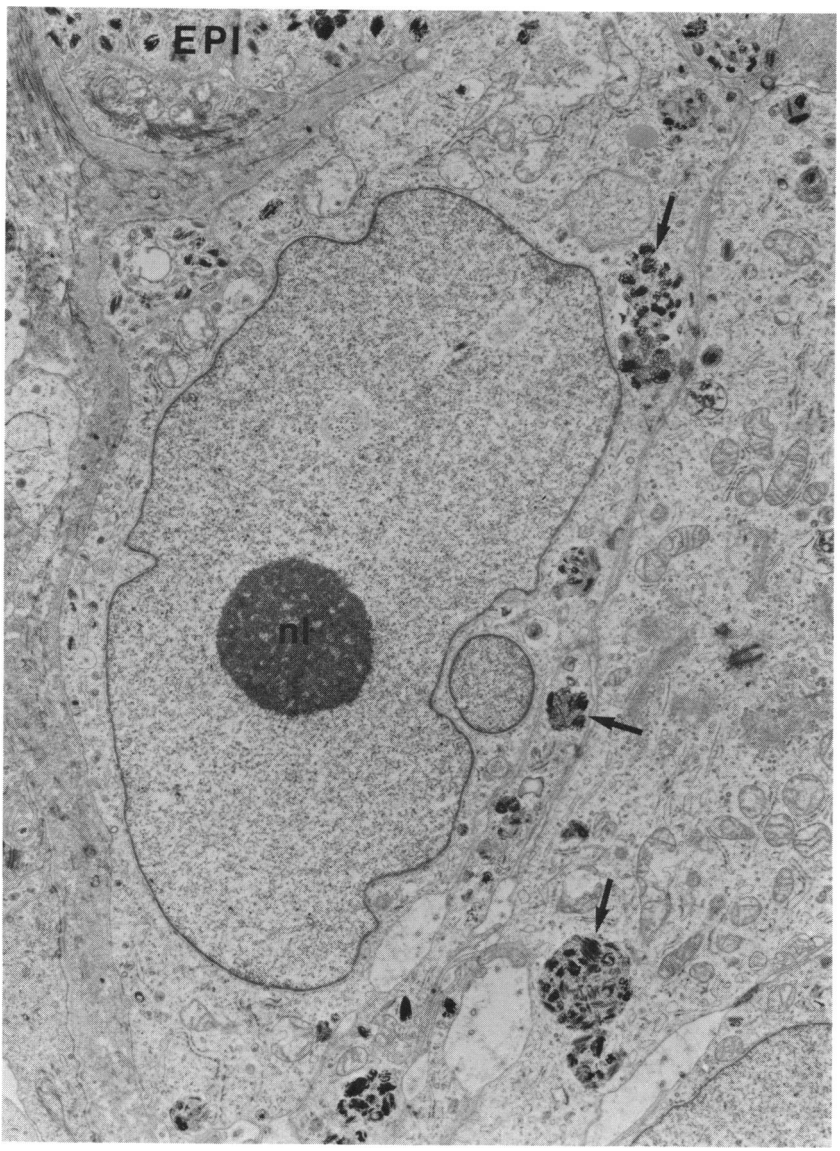

FIGURE 77
An invasive malignant melanoma cell beneath surface epithelium (EPI) has a large nucleolus (nl) and a dispersed nuclear chromatin pattern lacking significant margination at nuclear membrane. Numerous cytoplasmic phagolysosomes with melanin are present in neoplastic cells (*arrows*) (× 8800).

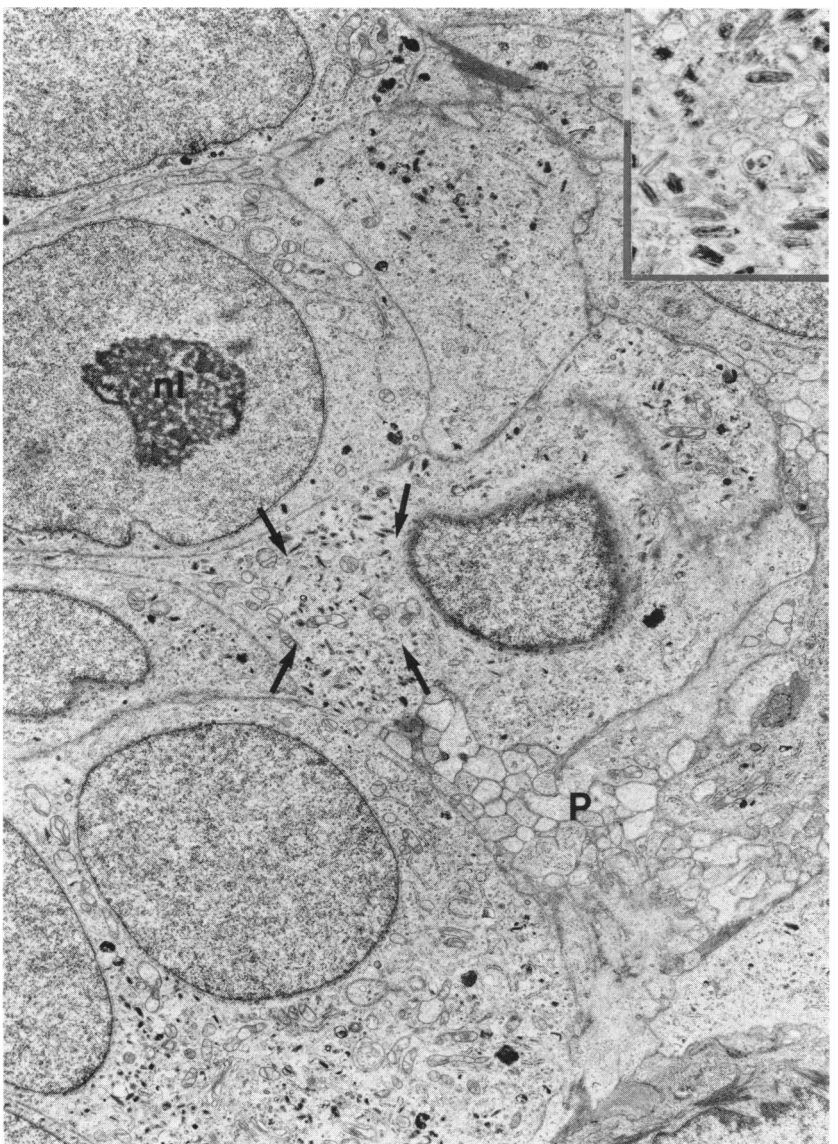

FIGURE 78

A cluster of infiltrating malignant melanoma cells with dispersed nuclear chromatin and large wiry nucleoli (nl). In cytoplasm are numerous elongated melanosomes (*arrows*), which in INSET are shown to have a melanofilamentary substructure. Tangles of thick cellular processes (P) are insinuated between tumor cells (× 6600).

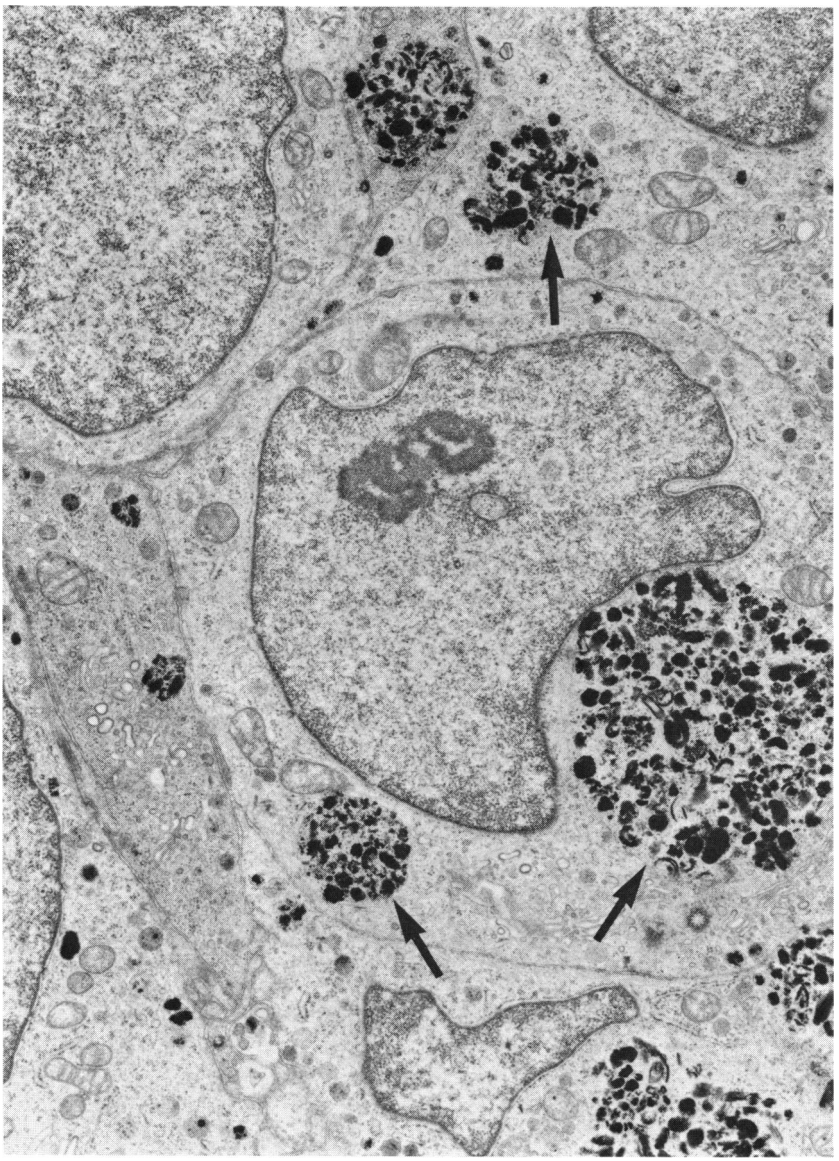

FIGURE 79
Tumor cells with prominent autophagolysosomal collections (*arrows*) of melanin. Such prominent segregation of melanin is rare in nevus cells (× 13,000).

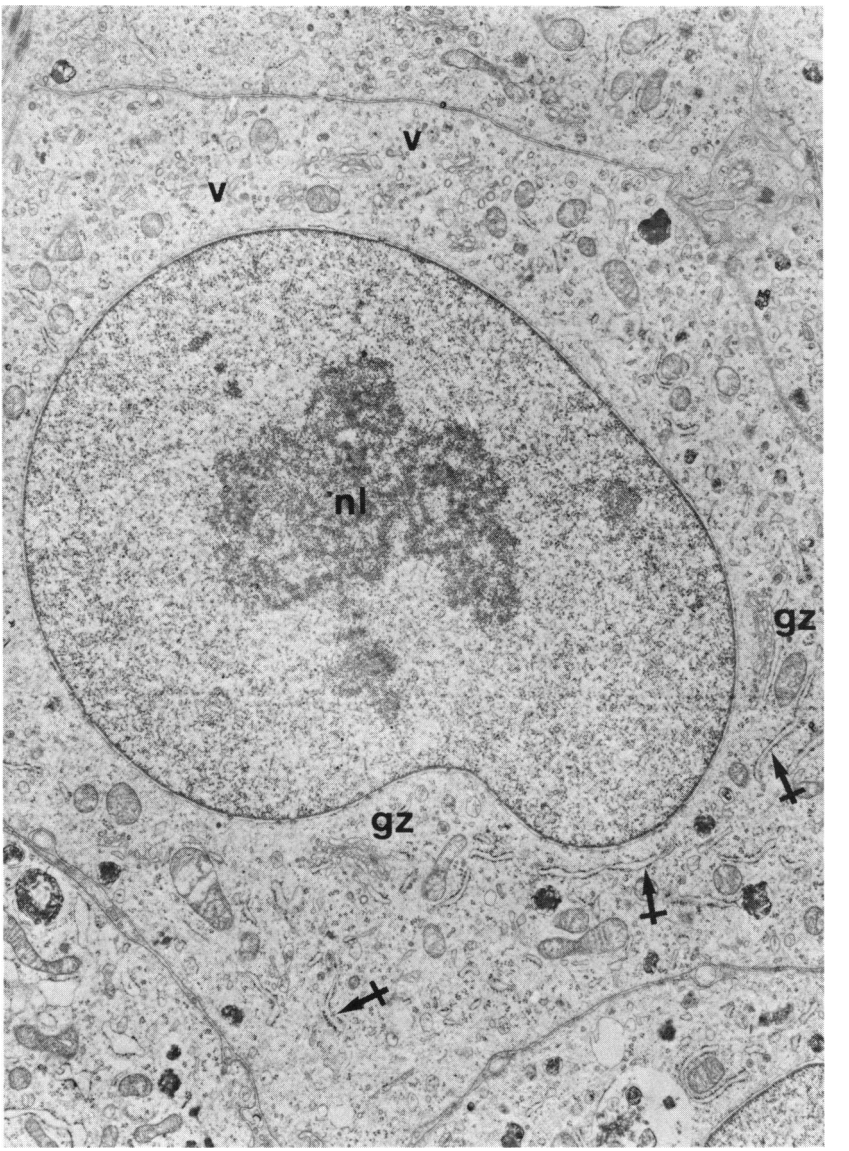

FIGURE 80
A tumor cell with a large nucleolus (nl) features numerous cytoplasmic vesicles (v), active Golgi zones (gz), and segments of rough-surfaced endoplasmic reticulum (*crossed arrows*) (× 11,500).

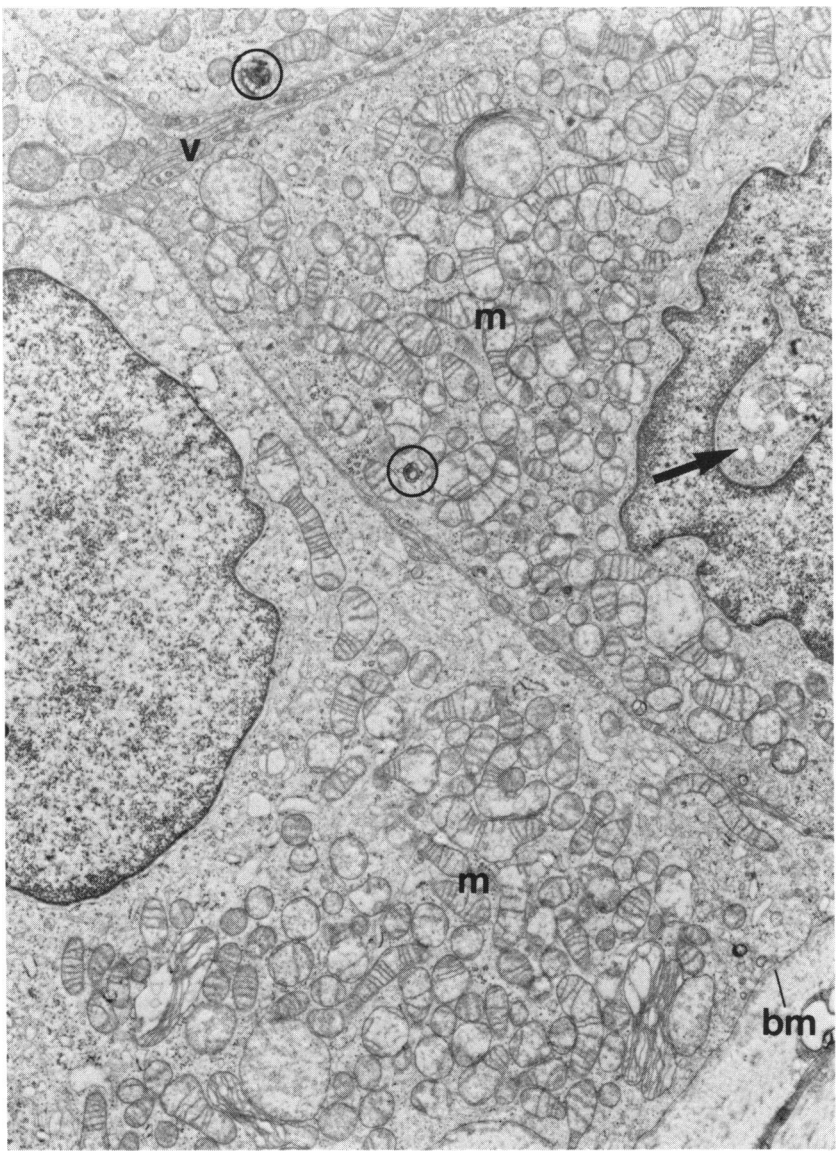

FIGURE 81

Polyhedral invasive tumor cells display massive collections of mitochondria (m). A few intercellular villi (v) are seen, as well as are cytoplasmic abortive melanosomes (*circles*). An attenuated basement membrane (bm) covers outer aspects of plasmalemma. *Arrow*, small intranuclear sequestration of cytoplasm (× 12,000).

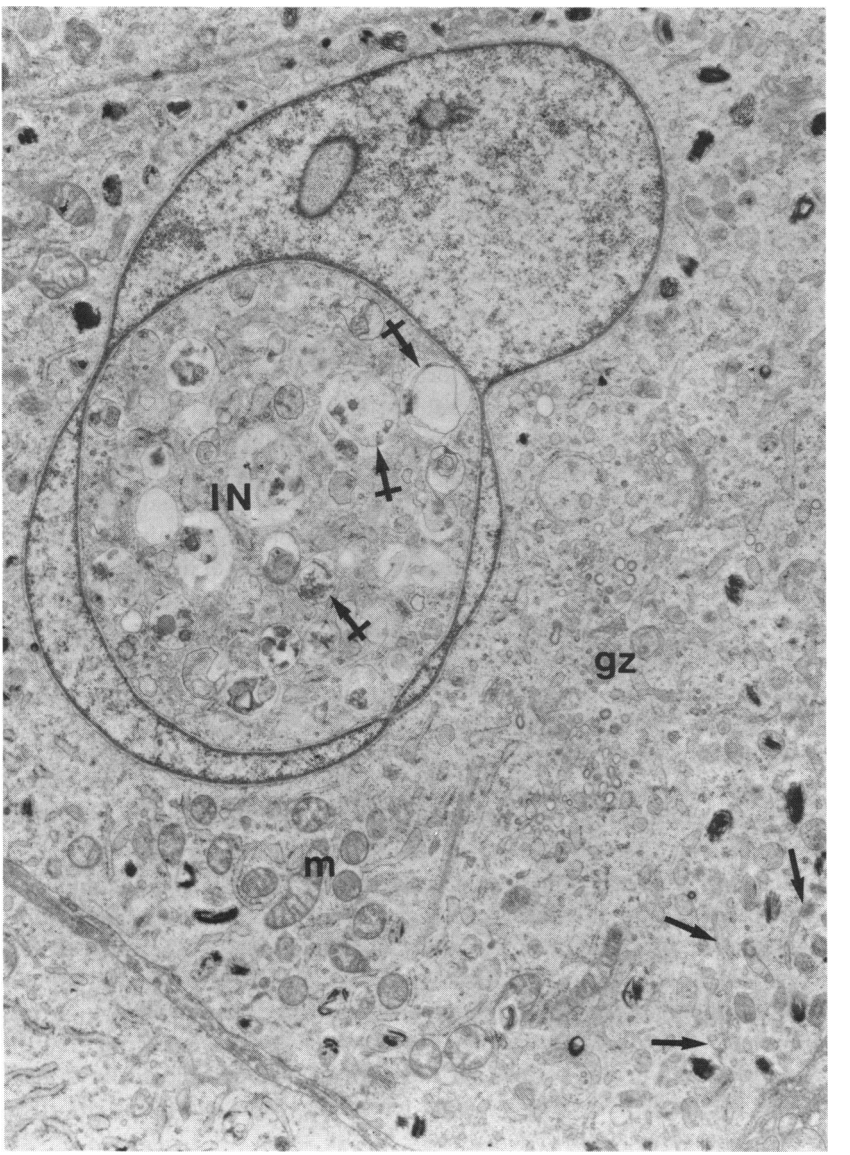

FIGURE 82
A large intranuclear sequestration of cytoplasm (IN) contains abortive melanosomes (*crossed arrows*) and lysosomes. The cytoplasm has several prominent Golgi zones (gz), and numerous mitochondria (m). A cluster of poorly melaninized melanosomes is shown toward bottom right (*arrows*) (× 11,000).

only rarely prominent (Fig 83). Tangles of thick intercellular processes and a few villi were present in many lesions (Fig 78), but elaborate interdigitations of villi characteristic of nevus cells were absent. One lesion (case 12) was remarkable for the presence of straight tubular inclusions (300 nm in diameter) within profiles of dilated rough-surfaced endoplasmic reticulum (Fig 84), and this was also one of two lesions to display segments of basement membrane surrounding some of the cellular nests. In one case (case 20) studied by electron microscopy, the invasive nodule was composed of spindle cells; the nuclei were not particularly bizarre and had a finely clumped chromatin pattern, the cytoplasm had scattered filaments, and mitochondria were the chief cytoplasmic organelle (Fig 85). (Two other patients in this series—cases 10 and 16—displayed spindle cells in an invasive nodule that was not sampled for electron microscopy.) In one patient (case 14), benign nevus cells (Fig 86) were found in the substantia propria of the caruncle in association with epithelial inclusion cysts; the overlying epithelium showed grade 3 atypical melanocytes proliferating in an acquired melanosis component, culminating in the invasive nodule of melanoma along the medial aspect of the right lower lid margin. In case 20, the invasive nodule was composed of small epithelioid cells, having many features of nevus cells. The cells formed cellular nests (Fig 87) but did not form basement membranes; no interstitial microfibrillar aggregates were observed. The main evidence of malignancy was the finely clumped nature of the central nuclear chromatin without heavy margination of heterochromatin at the nuclear membrane as in nevus cells, the presence of cytoplasmic annulate lamellae (Fig 88), and numerous mitochondria and polyribosomes (Fig 89). Very few villi typical of nevus cells were seen. The melanosomes of the invasive nodules of melanoma were generally abortive or rudimentary, particularly in comparison with those within the intraepithelial cells. In 4 of the 11 cases, more elongated shapes with disorganized melanofilaments were observed (Fig 78, inset), usually admixed with round abortive forms, whereas in the rest of the tumors, the melanosomes were uniformly round, often granular, and highly disordered internally (Fig 84). It is worth mentioning that the one melanoma in this series that was proved histopathologically to have arisen in association with a nevus (case 14) displayed round-abortive, rather than elongated, melanosomes.

DISCUSSION

The investigations reported in this thesis were undertaken in order to improve our knowledge of the morphologic aspects of melanocytic lesions

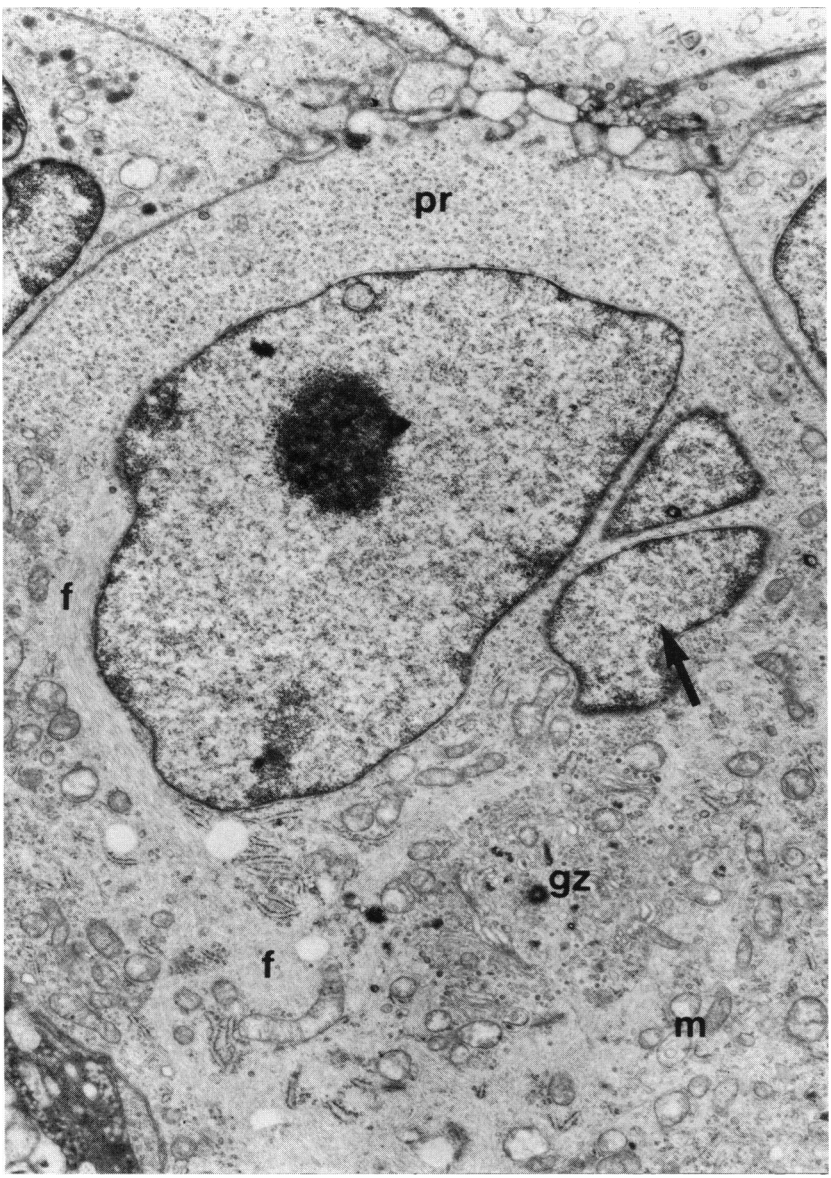

FIGURE 83
Tumor cell with abundant polyribosomes (pr), cytoplasmic filaments (f), and multilobation of the nucleus (*arrow*). gz, Golgi zone; m, mitochondria (× 11,000).

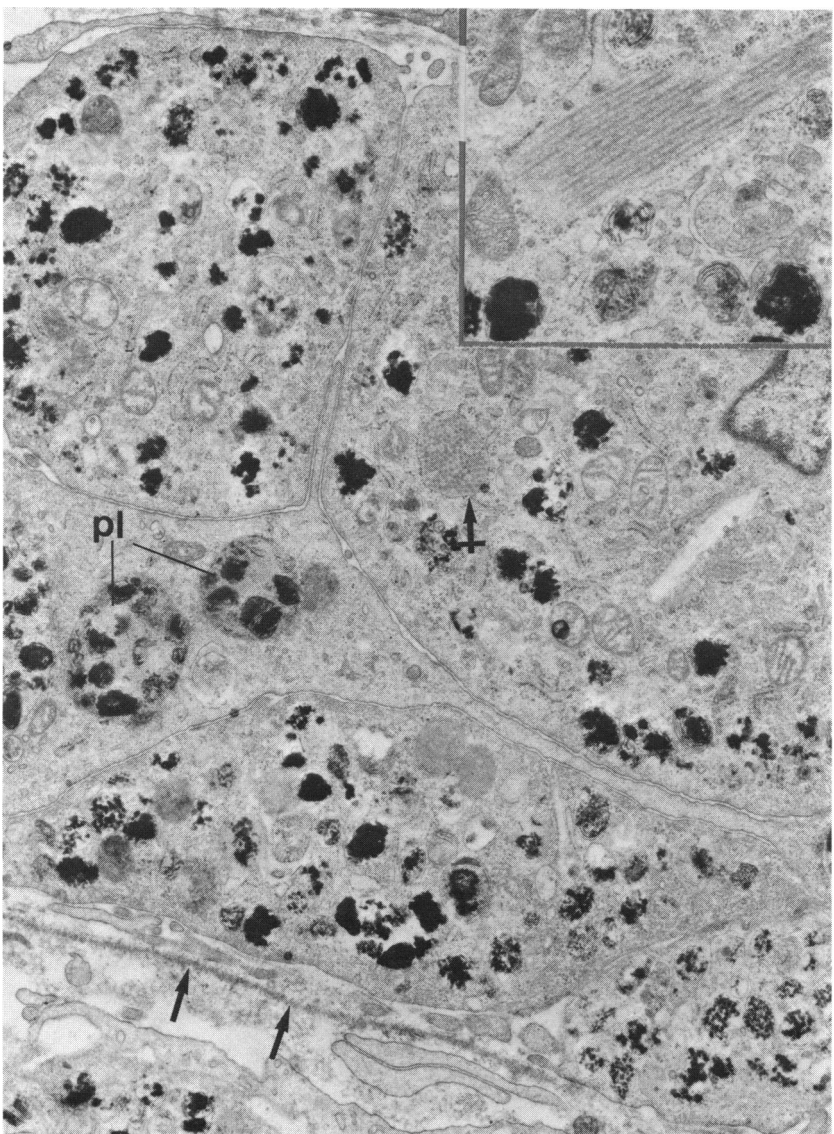

FIGURE 84
Nest of tumor cells showing granular melanosomes as well as phagolysosomes (pl). Linear basement material (*arrows*) surrounds outer aspect of cellular nest. Within cytoplasm of tumor cells are tubular inclusions (*crossed arrow*), shown longitudinally in INSET to be delimited by rough-surfaced endoplasmic reticulum (× 14,000).

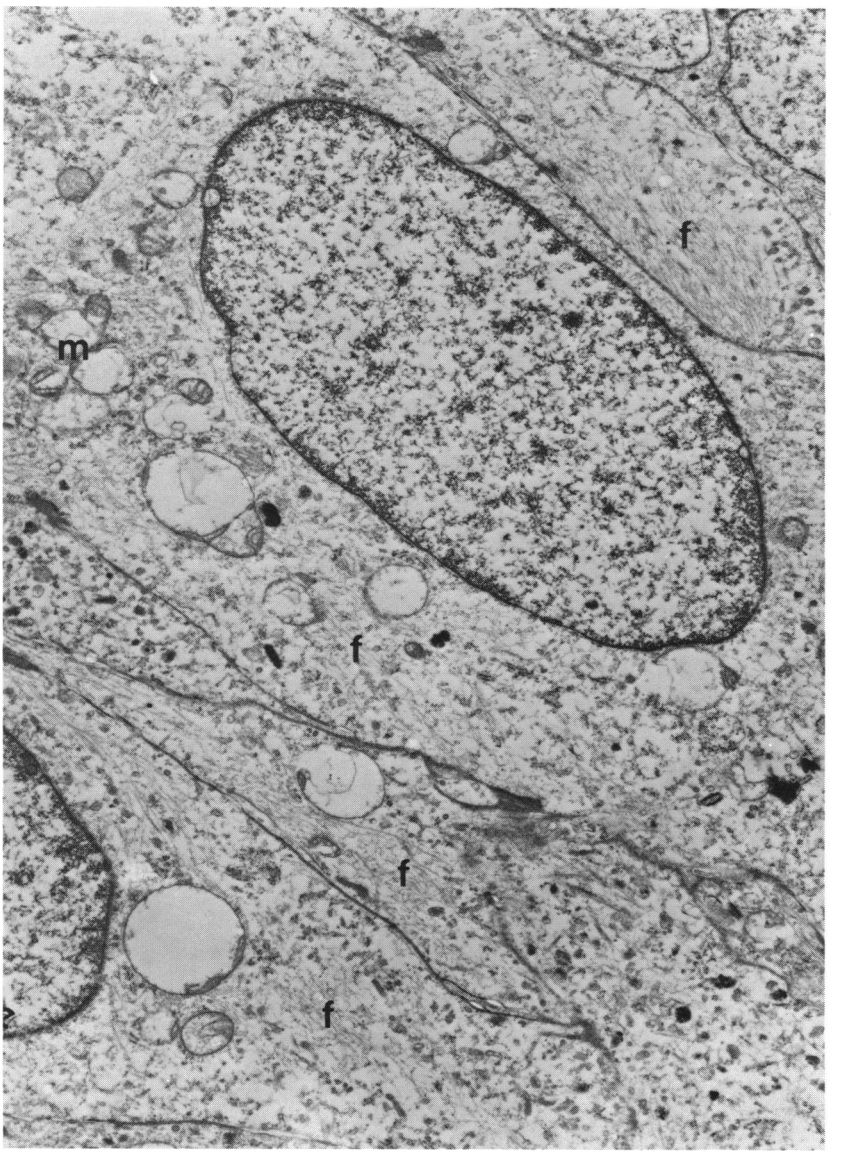

FIGURE 85
Infiltrating spindle melanoma cells have cytoplasmic filaments (f), mitochondria (m), and small melanin granules, shown toward bottom right. Nuclear chromatin pattern is finely clumped, without thick margination at nuclear membrane (× 12,500).

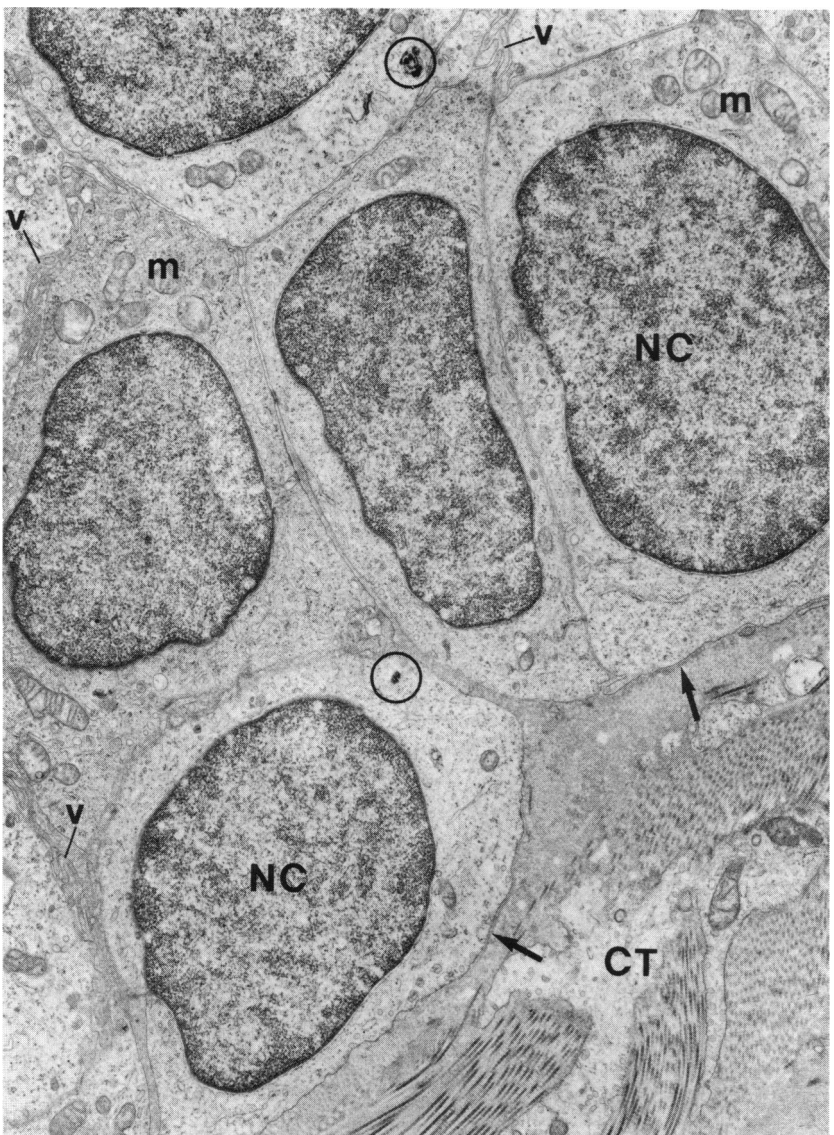

FIGURE 86
Subepithelial nevus cell (NC) nest in association with a malignant melanoma. Small abortive melanosomes (*circles*) and a scattering of mitochondria (m) are characteristic of nevus cells. Basement membrane material (*arrows*) insulates outer aspects of nevus cells from connective tissue (CT). Villi (v) are present in intercellular zone between nevus cells. Note that nuclear chromatin pattern is compact (× 12,000).

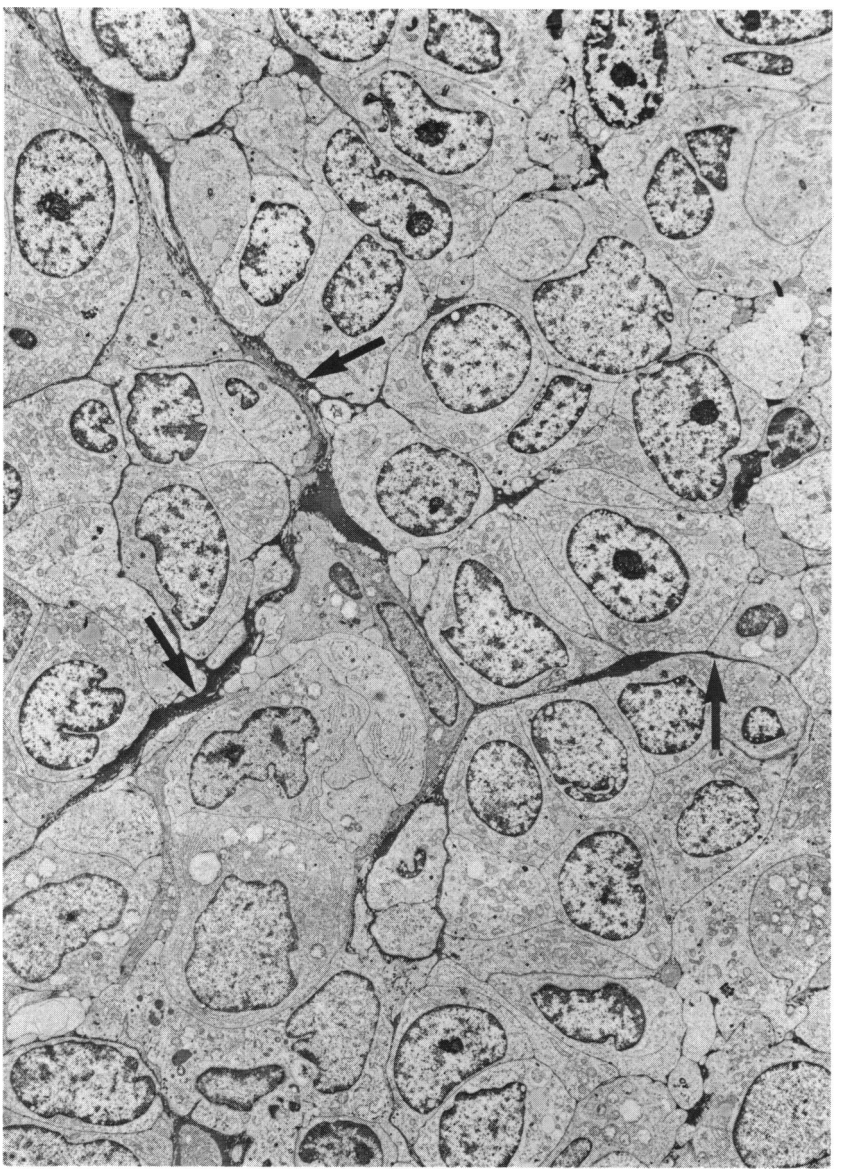

FIGURE 87
Malignant melanoma composed of nevoid-type of epithelioid cells. Connective tissue septa (*arrows*) subdivide infiltrating tumor cells into large nests. Note finely clumped chromatin in contrast to more evenly compacted chromatin of nevus cells shown in Fig 86 (× 2500).

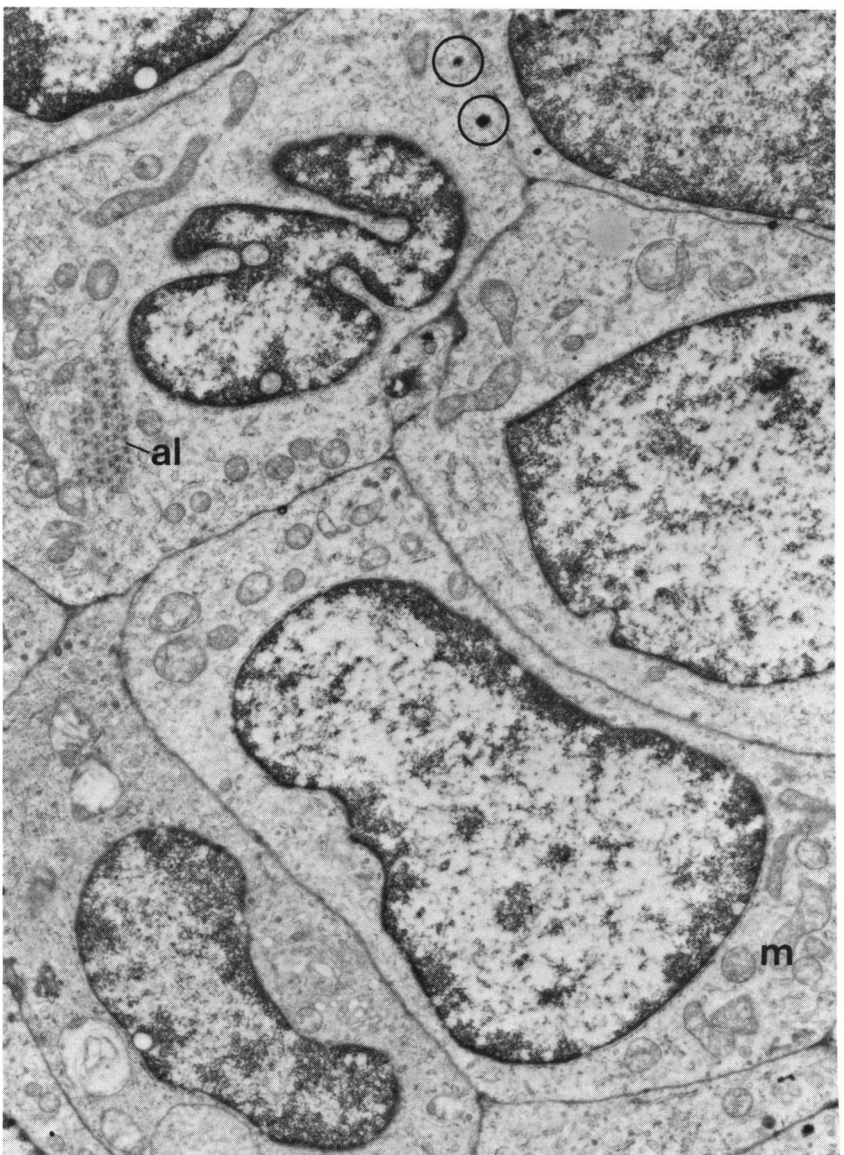

FIGURE 88
The tumor cells contain mitochondria (m) and small abortive melanosomes (*circles*). Presence of annulate lamellae (al) in cytoplasm is a helpful feature indicating that these are malignant cells (× 12,000).

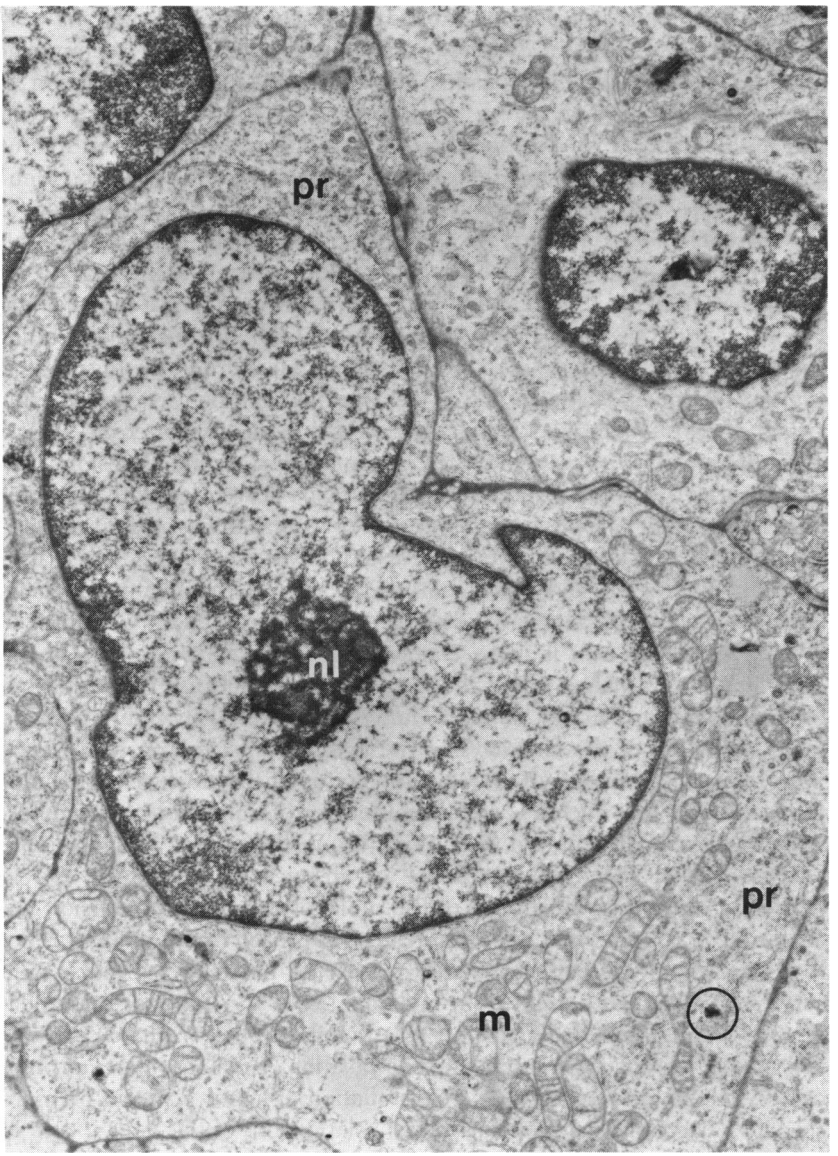

FIGURE 89
Tumor cell with nucleolus (nl) has many cytoplasmic mitochondria (m) and scattered polyribosomes (pr), suggesting malignant nature of cells. A rudimentary melanosome is *circled* (× 11,500).

of the conjunctiva, to refine diagnostic categories, and to codify any new features of these lesions previously unstudied by electron microscopy that might have prognostic importance. The light microscopic evaluation of conjunctival primary acquired melanosis has proved to be controversial and problematic; it would therefore be a step forward if electron microscopic characteristics with diagnostic and prognostic value could be identified. In this study a broad spectrum of common melanocytic disorders of the conjunctiva was investigated by electron microscopy, which led to the discovery of several valuable morphologic and prognostic findings.

In illustrating the light microscopic features of conjunctival melanocytic lesions in this thesis, 1-μ plastic sections stained with methylene blue-azure II were primarily used. The cytologic detail afforded by this method (routine paraffin sections are 4 μ to 6 μ thick) is in some ways far superior to that of routine paraffin sections stained with hematoxylin-eosin. Plastic sections open up a new world for the interpretation of difficult lesions, but the features they reveal should nonetheless be correlated with standard light microscopic morphologic features. For example, in routine preparations, malignant cells very often appear to be hyperchromatic, whereas in glutaraldehyde-fixed and plastic-embedded tissues, malignant cells have vesicular nuclei with an evenly dispersed chromatin pattern. Clumping of the nuclear chromatin is more characteristic of benign lesions. On the other hand, information about the cytoplasm is sometimes lost in plastic-embedded tissues, so that paraffin-embedded material should be utilized for examination of the character of the cytoplasm. Electron microscopy itself offers more information about the status of the nuclear chromatin, the nature of the nucleoli, and the identity of subcellular organelles, including their distribution and quantity. With respect to melanoma, analysis of cell shape (the presence or absence of dendrites), and in particular of the polymorphism of the melanosomes, is a dimension not accessible to routine light microscopy. The approach in this study was not to try to identify a pathognomonic feature of melanocytes that would serve to distinguish benign from malignant, but to survey as many features of benign and malignant cells as possible (nuclear characteristics, cell shape, cytoplasmic organelle composition, melanosomal morphology), with the expectation that a constellation of features would be more reliably discriminatory. The functional relationship of the melanocyte to the surrounding keratinocytes, which can be gauged by the degree of transfer of melanin into the surrounding keratinocytes, might provide diagnostically useful information.

Benign epithelial melanosis of the conjunctiva is essentially a nonproliferative disorder of the dendritic melanocyte, which is probably present

in varying densities in the conjunctiva of different races. This lesion was studied in seven black patients, one Caucasian patient, and one Hispanic. The ultrastructural features were remarkably consistent among these patients. In blacks, the dendritic melanocytes distributed among the basal epithelial cells were obviously hyperactive, contributing abundant melanin granules to the surrounding basilar and suprabasilar keratinocytes. This transfer is accomplished through the delicate dendritic processes, the tips of which are probably decapitated by the keratinocytes.[194,195]

In blacks, the melanin granules generally remained in nonmembrane limited clusters or singly within the cytoplasm, whereas in the Caucasian patient and rarely in blacks, the melanin granules became sequestered in phagolysosomes. The extent of melanin uptake by the keratinocytes could be so great as to result occasionally in almost complete cytoplasmic occupation by the melanin granules, suggesting a condition of keratinocytic melanophagia. At higher levels of the conjunctival epithelium, the more flattened squamous cells aggregated the melanin to form a supranuclear cap—teleologically to protect the nucleus from incident ultraviolet radiation. The melanosomes were elongated, cigar-shaped structures and generally completely melaninized in the black patients.

In one black patient and in a pregnant Hispanic with symmetrical inferior forniceal pigmentation, an extremely mild hyperplasia of morphologically normal dendritic melanocytes was observed, with occasional suprabasilar melanocytes extending processes to higher reaches of the epithelium. Despite this hyperplasia, there was no evidence of back-to-back disposition of melanocytes, which were always separated by adjacent keratinocytes. High-level melanocytes were not seen. These cases therefore differed from the others in representing a minimal hyperplasia, with retention of the morphologic features of benign melanocytes.

In the cases of benign epithelial melanosis and benign melanocytic hyperplasia, the absence of significant numbers of melanophages and mononuclear inflammatory cells beneath the epithelium may be a helpful finding that points toward a functionally intact melanocytic-keratinocytic transfer mechanism. The absence of these cells perhaps also indicates the lack of any immunologic surface irregularities that could attract inflammatory cells in a neoplasia. By contrast, in cases of primary acquired melanosis, numerous melanophages and increased numbers of lymphocytes and plasma cells were situated in the superficial substantia propria. The melanophages were commandeered to engulf melanin granules that had been inadeptly and inefficiently transferred to the keratinocytes by the neoplastic melanocytes. The falling down of melanin granules into the sub-

stantia propria is an intriguing morphologic marker of a relative breakdown in melanin transfer.

The morphologic features of the benign dendritic melanocytes in the pigmented squamous papillomas were identical to those in benign racial melanosis, except that the melanocytes were generally not restricted to the region of the basal cells, but instead were located at all levels of the acanthotic epithelium. The dendritic processes of these displaced melanocytes transferred very little melanin to the surrounding keratinocytes. These findings are in contrast to those in two previously reported pigmented squamous cell carcinomas of the limbal conjunctiva, in which the malignant cells displayed an avidity for the uptake of melanin granules.[69,70] The melanocytes, however, displayed benign morphologic features, thereby establishing that they were merely fellow-travellers in the tumors.

Most authorities now regard the nevus cell as an altered or abnormal form of the intraepithelial dendritic melanocyte.[46,143] The observations in the present study on 16 conjunctival nevi further support this premise. While the nevus cells within the epithelial junctional nests and in the subepithelial connective tissue zone typically appear by light microscopy to be rounded, electron microscopy reveals that there are short villous processes as well as some thicker, abortive dendritic processes. The latter are distinguishable from the villi by virtue of being broad enough to contain melanin granules. The nevus cell is abnormal in the sense of its altered shape, but more so in the sense that it is not so successful in transferring its pigment to the surrounding keratinocytes. Its melanin granules exhibit a wide range of aberrant morphologies, including more rounded shapes, internal filamentary disorganization, and partial melaninization. These differences were well demonstrated in the case of a compound nevus in a Pakistani child who had a benign lentiginous hyperplasia of dendritic melanocytes immediately adjacent to junctional nests. The dendritic melanocytes had elongated, well-melaninized melanosomes, whereas the rounded nevus cells manifested smaller, round, poorly melaninized melanosomes.

Within the conjunctival epithelium the nevus cells are virtually never found in isolation outside the junctional nests, which are clearly demarcated by an umbrella of sharp-edged keratinocytes. On the other hand, in primary acquired melanosis many individual melanocytes invade or percolate through all levels of the epithelium. In routine light microscopic sections, these higher-level melanocytes are easily overlooked and in fact may be inseparable from pigment-containing keratinocytes. The nevus cell, like the benign dendritic melanocyte, has clumped nuclear hetero-

chromatin, but the former sports a more prominent nucleolus, probably because it is more metabolically active and undergoing slow mitotic activity. The cytoplasm contains an active Golgi zone, which is the source of production of the melanosomes, and the most conspicuous organelle is the mitochondrion; short profiles of rough-surfaced endoplasmic reticulum and scattered monoribosomes (extremely rare, polyribosomes) are also featured. Cytoplasmic filaments are not particularly prominent in the intraepithelial nevus cells.

The subepithelial nevus cells were normally grouped into small nests or clusters, on the outside aspect of which basement membranes were identified. Within the center of the cellular nests small villi projected into widened intercellular spaces, but broader cellular processes containing melanin and perhaps representing abortive dendrites were sometimes observed. In dark-complexioned persons the most superficial cells contained melanin granules, which were generally round and had a granular substructure, but deeper in the substantia propria melanogenic activity fell off and only small abortive melanosomes were seen. Cytoplasmic filaments were a conspicuous feature of more deeply situated cells in the nevoid nests. Two patterns of nuclear chromatin in subepithelial nevus cells were observed in this study: either an extremely delicate, finely and evenly dispersed chromatin pattern, or a compact chromatin pattern without significant clumping. Moderate-sized, tightly coiled nucleoli were seen in each cell type. A distinctive feature of nevus cells within the connective tissue was their association with microfibrillar aggregates, sometimes formed between nevus cells in the center of the nests but more typically found as bundles outside the basement membrane region. These fibers may represent elastic precursor or oxytalan fibers,[196] as has recently been demonstrated in an ultrastructural study of dermal nevi.[151] Similar fibers are also seen in benign nevoid melanocytic lesions and "leiomyomas" of the iris.[197] No evidence of neuroid formation was identified in the deepest portions of the conjunctival nevi that were investigated in this study. Some of the cells did adopt a more spindled appearance, and in the absence of any melanosomal differentiation, their identity as nevus cells could be inferred only from interrupted segments of basement membranes, cytoplasmic filaments, and associated extracellular microfibrillar aggregates.

The delineation of the foregoing ultrastructural findings for both benign epithelial melanosis and benign melanocytic nevi should facilitate the identification of distinctive cytologic abnormalities in lesions of primary acquired melanosis. Unlike benign epithelial melanosis, which is a condition of increased production and transfer of melanin to surrounding kera-

tinocytes, primary acquired melanosis (precancerous melanosis of Reese,[96] benign acquired melanosis of Zimmerman,[97] atypical melanocytic hyperplasia of the dermatopathologists[100]) has long been recognized as a proliferative condition of the intraepithelial melanocytes. The proliferation could represent benign hyperplasia, atypical hyperplasia, or melanoma in situ. Well-defined light microscopic criteria have not been established for distinguishing the relatively innocent lesions from those that may evolve into full-fledged invasive nodules of malignant melanoma.

The terminology employed in connection with potentially premalignant intraepithelial melanocytic proliferations of the conjunctiva is at variance with the diagnostic vocabulary of dermatopathology. Reese[96] favored the term "precancerous melanosis," but Zimmerman[97] argued that this term overly cancerized a condition that may only rarely result in invasive malignant melanoma. Zimmerman therefore recommended the term "benign acquired melanosis" for all intraepithelial melanocytic proliferations and "cancerous melanosis" when invasion of the substantia propria supervened. The problem with employing the adjective "benign" for the preinvasive condition is that it may mislead clinicians into believing that none of these lesions is a formal precursor of malignant melanoma. In his Reese Lecture, Zimmerman[16] adopted the neutral term "idiopathic acquired melanosis," which seems more acceptable. Folberg and associates[198] recently completed a major review of proliferative conjunctival melanocytic disorders on file at the Armed Forces Institute of Pathology; they recommended the term "primary acquired melanosis" for intraepithelial melanocytic proliferations, which can be further characterized according to the degree of cellular atypicality. Realizing that a series of important papers will issue from this research, I adopted the term "primary acquired melanosis" in this thesis. Regardless of the term that is ultimately used, many lesions comprising primary acquired melanosis parallel the radial growth phase of cutaneous melanomas (lentigo maligna, superficial spreading, and acral lentiginous melanomas[99-112]). As mentioned in the "Background" section, conjunctival primary acquired melanosis has proved to be refractory to the cutaneous diagnostic system in the hands of most ophthalmic pathologists. One problem in transposing the concept of a radial or horizontal growth phase of cutaneous lesions is posed by the anatomy of the conjunctiva. The cutaneous radial growth phase includes occupation of the papillary dermis[109]; in the conjunctiva, the substantia propria does not have a papillary component, and therefore all lesions just breaking through the basement membrane are regarded as microinvasive melanomas.

Probably the most clinically important portion of the present study was

the delineation of fine structural features of primary acquired melanosis, which seem to have predictive value regarding which lesions might evolve into nodules of invasive melanoma. In 18 patients with primary acquired melanosis—8 without associated nodule formation when first studied and 10 initially presenting with a nodule of invasive melanoma—at least one and generally two or more biopsies were taken of extensive areas of flat pigmentation. The atypicality of the melanocytes participating in these lesions was graded into three categories: grade 1, in which the cells showed features virtually identical to those of the normal dendritic melanocyte; grade 2, in which the nuclear chromatin pattern was less clumped, the nucleus was somewhat larger, and the nucleolus was more prominent, but a dendritic form could still be identified ultrastructurally; and grade 3, in which a large nucleus displayed a more delicate and finely distributed chromatin pattern associated with a prominent, frequently wiry nucleolus, and the cell exhibited more ample polygonal cytoplasm with few dendrites. Melanosomal morphology also varied with the degree of atypicality. The grade 1 and grade 2 melanocytes frequently possessed elongated melanosomes, with an intact melanofilamentary substructure that was variably melaninized; these cells showed a considerable retention of their capacity to transfer melanosomes to keratinocytes through their preserved dendrites. Some of the grade 2 melanocytes had more rounded and abortive melanosomes that were variably melaninized, whereas the grade 3 melanocytes usually possessed rudimentary, disorganized, and only partially melaninized or totally nonmelaninized granules.

In the group of eight patients with primary acquired melanosis initially unassociated with a nodule of invasive malignant melanoma, grade 1 and 2 cells predominated in all but two lesions. These cells sometimes reached higher levels of the epithelium than was the case with benign epithelial melanosis. Nest formation was occasionally observed, but was generally not prominent. Case 6 is particularly instructive. This patient had been followed up for 20 years with progressive pigmentation of the lower lid skin requiring five treatments with cautery. The temporal epibulbar and inferior forniceal conjunctiva became slowly pigmented over a 10-year period. Because of recent rapid progression, a biopsy of the cutaneous lesion was performed, which disclosed prominent grade 3 epithelioid melanocytes; the epibulbar biopsy specimen, on the other hand, displayed an intraepithelial proliferation composed of mostly grade 1 and a few grade 2 melanocytes. In the conjunctiva the melanocytes were located either among the basal cells or suprabasally, but not at high levels. Back-to-back melanocytes, however, were observed, a feature not

seen in benign epithelial melanosis. In the conjunctiva the melanocytes successfully transmitted their pigment via their arborizing dendrites to the surrounding keratinocytes, in which the pigment became organized into supranuclear caps at higher levels of the epithelium. The indolent course of the epibulbar lesion in comparison with the cutaneous lesion indicates that a different clone of cells was probably proliferating in the former site, and the morphologic aspects of the conjunctival lesion were indicative of a high degree of differentiation and functioning.

Topographic differences in the radial growth phase of conjunctival primary acquired melanosis were also underscored by case 2. In this patient a pigmented, polypoidal lesion of the caruncle coexisted with flat melanosis of the plica and most of the epibulbar surface. A biopsy of the medial epibulbar conjunctiva disclosed grades 1 and 2 intraepithelial melanocytes, while the flat plical component showed grade 3 cells. Finally, the caruncular lesion was composed of masses of anaplastic melanocytes growing in cystic invaginations of the epithelium, certainly reaching the stage of melanoma in situ. This case and the preceding one point out the necessity of obtaining multiple biopsies of primary acquired melanosis to discover if clonal expansion has occurred and possibly reached a worrisome condition. In case 4 of primary acquired melanosis, the initial biopsy showed many grade 3 cells, and a nodule of invasive melanoma occurred 4 months later. One patient (case 3) with mostly grade 2 cells refused further treatment after a conjunctival biopsy was performed, and she returned 3 years later with a nodule of invasive melanoma arising in primary melanosis and exhibiting grade 2 and 3 cells. This last case supports the contention that the cellular characteristics of primary acquired melanoma can change over time.

Eight of the 10 patients who presented with primary acquired melanosis in association with a nodule of invasive melanoma had large numbers of grade 3 cells in the radial growth phase component of the lesion immediately adjacent to the nodule of melanoma. In three patients, only grade 2 cells were observed intraepithelially, but their primary acquired melanosis was sampled for electron microscopy at some distance from the invasive nodule and not immediately adjacent to it. For example, in one patient (case 9) who initially experienced an invasive nodule of melanoma situated in the superior tarsus, epibulbar, inferior forniceal, and inferior tarsal flat primary acquired melanosis developed several years later. Biopsies of both the epibulbar and the inferior palpebral conjunctiva showed only grade 1 and 2 cells. Another case was quite remarkable, namely, that of primary acquired melanosis in an elderly black female of 10 years' duration (case 10) that universally involved all aspects of the conjunctival

sac. A spindle cell invasive nodule of melanoma finally developed in the inferior fornix. The ultrastructure of the flat epibulbar component, which again was unfortunately taken at some distance from the fornix, showed enormous hyperplastic and hypertrophic bloated dendritic melanocytes, which contained extremely well-melaninized elongated melanin granules that were apparently not capable of being transferred in large numbers to the surrounding keratinocytes. This lesion was morphologically unlike any of the others and may represent a distinctive and favorable type of primary acquired melanosis analogous to lentigo maligna. Conjunctival melanoma is rare in blacks but has been previously documented.[199,200]

Based on the foregoing results, electron microscopy seems to hold considerable promise for analyzing the potential of a lesion of primary acquired melanosis to go on to nodule formation. Lesions displaying only grade 1 and 2 cells probably represent a benign process, whereas those showing the emergence of grade 3 cells should be viewed with more concern. In addition to displaying more atypicality, the grade 3 cells frequently formed confluent nests within the epithelium, lifting up the most superficial keratinocytes, which formed only an umbrella over the masses of melanocytes proliferating above the basement membrane region. Lesions composed mostly of grade 1 and 2 cells showed less intense melanocytic proliferation, which was usually pronounced along the basilar region, although individual cells sometimes drifted to higher levels of the epithelium. Judgments about the nature of a primary acquired melanosis can only be predicated upon multiple biopsies of the full extent of the lesion, since some areas of the epithelium may harbor less atypical cells whereas others may exhibit extremely worrisome cells. These findings are in essential agreement with a recent Armed Forces Institute of Pathology light microscopic study on 41 cases of primary acquired melanosis.[198] These cases could be divided into two groups: those that did not display atypicality and those that did. The latter atypical lesions frequently exhibited epithelioid cells and progressed to invasive nodules of melanoma in over 75% of cases. Lesions without atypical cells did not progress on clinical follow-up.

The majority of the nodules of invasive malignant melanoma in this series (11 were examined) showed extremely atypical epithelioid cells, with a dispersed nuclear chromatin pattern, convoluted wiry nucleoli, irregular nuclear shapes including cytoplasmic invaginations or sequestrations within the nucleus, abortive melanosome formation, abundant mitochondria, predominance of polyribosomes over monoribosomes, prominent autophagic pools of melanin granules, and the general absence of basement membrane formation (discovered in only two lesions as inter-

rupted segments). Cytoplasmic filaments were less conspicuous than in benign nevus cells, although in one case (case 21) they were more common. These intermediate filaments have been characterized immunohistochemically as vimentin.[35,36] (Melanocytes also stain positively for S-100 protein.[37]) Most of the invasive cells in the nodules resembled the grade 3 intraepithelial cells, but the latter were often intermixed with grade 1 and 2 cells. One lesion was composed of spindle cells (case 19) and developed in a patient with a 10-year history of multiple surgeries on primary acquired melanosis of the inferior fornix and palpebral conjunctiva. Another patient was remarkable for showing a nevoid or small epithelioid cell composition to her recurrent melanoma (case 20), which had caused confusion in an earlier biopsy when a nevus was diagnosed. Even on recurrence, the small epithelioid cells closely mimicked those of a nevus, with nest formation, small nucleoli, and a more clumped chromatin pattern by light microscopy.

The ultrastructural features of this case, however, when compared with those of benign nevus cells, were confirmatory of the malignant nature of the lesion, which of course had also been more than intimated by the multiple clinical recurrences. The chromatin pattern was unlike that of the other invasive epithelioid melanoma cells. It was organized into small clumps, different from the finely dispersed chromatin of classic large epithelioid cells. Compared with benign subepithelial nevus cells, the nucleoli were larger, the cytoplasm failed to show filaments, and there were many more mitochondria. No evidence of basement membrane formation, extracellular microfibrillar aggregates, or focal villous interdigitations in the center of the nests could be discerned; these features are normally encountered in benign nevus cells. The discovery of pigment granules in the most deeply situated cells also militated for the diagnosis of melanoma, since in a nevus the deeper cells tend to lose their melanogenic activity. Finally, annulate lamellae were found in the cytoplasm—a marker for a diversity of mitotically active and frequently malignant cell types.[167,168] This case therefore exemplifies the value of ultrastructural criteria for distinguishing between nevus cells and mimicking melanoma cells. It is well known that in cutaneous melanomas, including conjunctival and other mucosal melanomas, the small nevus-type[10,12,17,19,201] melanoma cell may cause considerable diagnostic confusion. In one study their presence did not alter the prognosis when lesions were matched for depth of invasion.[202]

Two other ultrastructural features observed in this study deserve comment. A curious feature of both benign and neoplastic intraepithelial dendritic melanocytes was the occasional accumulation of large numbers

of lipid droplets in their cytoplasm (three cases of benign epithelial melanosis and four cases of primary acquired melanosis). Lipidization of normal intraepidermal dendritic melanocytes has been documented in ultrastructural studies of cutaneous xanthomas, in which the overlying epidermal melanocytes were shown to imbibe lipid.[203] None of the nevus cells or invasive melanoma cells in this series manifested this finding. Similar cytoplasmic lipidization occurs in choroidal balloon cell melanomas.[156-158] Conversely, balloon cell nevi and melanomas of the skin are produced by vacuolar disorganization of the melanosomes rather than by lipid accumulation.[153-155] The second unusual finding was the presence of straight, longitudinal tubules in the cisternae of the rough-surfaced endoplasmic reticulum in one case (case 12). The nature of these tubular inclusions is unclear, but in one study they were found in 6 of 100 invasive melanomas studied by electron microscopy. In amelanotic round cell tumors their discovery may be diagnostically useful.[169-171]

It seems unlikely, according to present knowledge, that ultrastructure has anything to offer in predicting which nodules of invasive melanoma are going to metastasize. This could not be tested in the present study because of the small number of cases, the short follow-up periods, and the absence of any known tumor deaths despite the development of deeply invasive nodules in some patients. Ultrastructural studies of metastatic cutaneous melanomas have established that the metastases recapitulate the full gamut of morphologic features observed in the primary, which does not leave much room for isolating distinctive morphologic features portending metastasis.[166,171]

In closing this discussion, it is worthwhile to return to two of the controversies explored in the "Background" section. The present electron microscopic studies did not help to resolve the issue of whether superficial spreading, acral lentiginous, and lentigo maligna melanomas can be identified in the conjunctiva. In this study, the majority of cases of primary acquired melanosis showed features indicative of an origin from the dendritic melanocyte. These features included the coexistence of types 1 and 2 dendritic cells with emergent grade 3 cells, the ability of the neoplastic melanocytes to communicate considerable numbers of melanin granules to surrounding keratinocytes, and the morphologic aspects of the melanin granules, which were frequently elongated with an internal filamentary structure. In the one earlier ultrastructural study of conjunctival melanoma arising in primary acquired melanosis, the melanosomes were also proved to be elongated.[25] No ultrastructural criteria have yet been adduced to differentiate lentigo maligna from acral lentiginous melanoma of the skin, both of which result from the neoplastic transformation of

dendritic melanocytes. Morphologic studies of cutaneous melanomas have also established the participation of dendritic melanocytes in the radial growth phase of superficial spreading melanoma, and these same dendritic features can be discovered in metastases of superficial spreading and nodular melanomas.[166,171,172,204] It seems safest for the present to regard conjunctival primary acquired melanosis as a type of mucosal melanoma precursor most often arising from the dendritic melanocyte and avoid forcing these lesions into the cutaneous nosology.

The controversy over whether electron microscopic features of the cells in the radial growth phase or invasive nodules of malignant melanoma can determine which lesions arose from a nevus or from the dendritic melanocyte may be quite meaningless and arid. If the nevus cell is a benign morphologic variant of the dendritic melanocyte, then a malignant variant of the dendritic melanocyte might also display many of the features of a nevus cell, particularly with respect to melanosomal morphology. The presence of elongated-filamentary melanosomes characteristic of normal dendritic melanocytes suggests an origin from dendritic melanocytes, but rounded melanosomes cannot be interpreted in favor of an origin from a nevus. In the cases in this study, only one lesion was known histopathologically to have arisen in association with a caruncular cystic nevus, and the radial growth phase component of this lesion showed melanosomes with a rounded and disorganized melanosomal structure. If a lesion arises in a nevus—and the dysplastic nevus syndrome is an ideal model of this[91-95]—it seems logical that the neoplastic melanocytes would continue to show the rounded melanosomes[149] of the nevus cell of origin rather than revert to the more highly ellipsoidal differentiated melanosomal structure of the dendritic melanocyte. Predicating biologic behavior on melanosomal morphology, however, is far too restrictive and fails to take into account the more valuable information about neoplastic potential provided by examination of the nuclear characteristics and the degree of cytoplasmic disorganization.[159-161]

A final word should be said about the management of conjunctival melanoma, although this subject has not been the major focus of this investigation. It is generally recognized that the earlier a melanoma is detected, the better the outlook. Much of the progress made in the management of cutaneous melanoma has resulted from earlier detection, preferably in the horizontal or radial growth phase. While dermatopathologists recognize various types of intraepidermal atpyical melanocytic hyperplasias that are difficult to diagnose as precursors of melanoma,[99,100,103,104] the wide local excision of these lesions cures the disease and allows for comfortable pathologic rumination about the true nature of

the process. Therapy for the late-stage, deeply invasive melanoma of the skin has not greatly improved. Because their surface location allows earlier detection, conjunctival melanomas have a better outlook than other mucosal melanomas[16-18,76]; oropharyngeal, anorectal, vulvar, and vaginal lesions generally fare poorly, because they are bulky at the time of discovery.[12,121]

In the past, patients with flat conjunctival lesions of primary acquired melanosis have been treated conservatively, because excision of large areas of conjunctiva can create major cosmetic and functional deficits,[97] a problem that is not confronted in most excisions of skin. Some physicians have recommended radiotherapy[117-119] or cryotherapy[113-116] for the treatment of early stage conditions. Nodules have been excised, but regional lymph node dissections have not been routinely recommended unless clinical signs of lymphatic involvement are present.[15-17,205,206] If other workers are able to support the findings reported here on the increased likelihood of invasive nodules appearing in the setting of high-grade intraepithelial anaplasia, then a sound foundation has been established to identify those patients in need of early treatment.

SUMMARY AND CONCLUSIONS

The ultrastructure of conjunctival melanocytic lesions in 49 patients was evaluated to find significant differences between benign and malignant cells. The patients studied included 9 with benign epithelial (racial) melanosis, 2 with pigmented squamous cell papillomas, 16 with conjunctival nevi, 18 with primary acquired melanosis, and 11 with invasive nodules of malignant melanoma.

In benign epithelial melanosis, dendritic melanocytes were situated along the basement membrane region of the conjunctival epithelium, with one basilar dendritic melanocyte lodged among every five or six basilar keratinocytes. The dendritic melanocytes extended arborizing cellular processes between the basilar and among the suprabasilar keratinocytes, which manifested considerable uptake of melanin granules into their cytoplasm. The benign dendritic melanocytes possessed nuclei with clumped heterochromatin at the nuclear membrane, small, tightly wound nucleoli, and large, elongated, fully melaninized melanin granules. In two patients with benign hyperplasia of the dendritic melanocytes, occasional dendritic melanocytes were located in a suprabasilar position, but were always separated from each other by keratinocytes or their processes. In the two black patients with benign pigmented squamous papillomas, the benign dendritic melanocytes were located hapharzardly at all levels of the acanthotic epithelium and not just along the basement mem-

brane region. Melanin uptake by the proliferating keratinocytes was minimal.

In benign melanocytic nevi of the conjunctiva, nevus cells within the intraepithelial junctional nests displayed a more rounded cellular configuration; short villi and broader cellular processes suggestive of abortive dendrites were found. The nuclear chromatin pattern was clumped at the nuclear membrane, but the nucleoli were somewhat larger than those of benign dendritic melanocytes in epithelial melanosis. The melanosomes were smaller and rounder than those in dendritic melanocytes and exhibited more haphazard arrangements of the melanofilaments, which were only partially melaninized. Mitochondria were more numerous than in dendritic melanocytes, and monoribosomes predominated over polyribosomes. Cytoplasmic filaments were inconspicuous. Cells in the immediate subepithelial connective tissue zone had features identical to those of the cells within the junctional nests. Smaller, lymphocytoid cells with less numerous and more rudimentary melanosomes were found in the middle and deeper portions of the lesions. The nuclear chromatin pattern of the subepithelial cells displayed one of three patterns: thick margination at the nuclear membrane, a fine dispersion throughout the nucleoplasm, or an even, compact arrangement. Nucleoli were small. The cytoplasm was notable for intermediate filaments and scattered mitochondria. The melanosomes were rudimentary and partially melaninized. Individual cells and cellular nests in the connective tissue were surrounded by basement membranes. Microfibrillar aggregates presumed to be elastic fiber precursors were often associated with the cellular nests. In the middle of the cellular clusters, villi projected into widened intercellular spaces. No neuroid formations were discovered in the deepest regions of the nevi, but occasionally cells with extremely rare melanosomes became spindled. They could be recognized as quiescent nevus cells by virtue of segments of basement membrane material, cytoplasmic filaments, and extracellular aggregates of microfibrillar material next to their processes.

The cells participating in the lesions of primary acquired melanosiss were graded according to a three-part system. Grade 1 cells were indistinguishable from normal dendritic melanocytes; grade 2 cells were obviously dendritic in character, but had larger nuclei, less clumping of the nuclear chromatin, and larger nucleoli; grade 3 cells were more epithelioid in character, with rare or retracted dendritic processes, had a finely divided and dispersed chromatin pattern in which was set a large wiry nucleolus, and displayed many abortive, rudimentary, and variably melaninized melanosomes.

Of the eight patients who presented with primary acquired melanosis

unassociated with an invasive nodule of melanoma, six patients displayed a predominance of grade 1 and 2 cells in their lesions. The two patients who showed progression of their disease with the subsequent development of invasive melanoma had intermixed grade 3 cells. Among the ten patients who initially presented with primary acquired melanosis associated with nodules of invasive malignant melanoma, seven showed a predominance of grade 3 cells in their intraepithelial lesions of primary acquired melanosis. The other three patients with grade 2 cells had their primary acquired melanosis sampled at some distance away from the nodules of malignant melanoma. The ultrastructural features of primary acquired melanosis showed variable features depending upon the topographic site sampled for electron microscopy.

Of the 11 nodules of invasive melanoma that were studied by electron microscopy, 9 were composed of classic epithelioid cells, featuring large nuclei with a dispersed heterochromatin pattern, large wiry nucleoli, and frequent cytoplasmic sequestrations or herniations within the nucleus. The cytoplasm contained many mitochondria, polyribosomes predominated over monoribosomes, and the melanosomes were generally rudimentary in nature. Cytoplasmic filaments were observed in one case and interrupted segments of basement membrane material in two cases. One patient's lesion was composed of small nevoid-type epithelioid cells, but ultrastructurally several features of malignancy were discernible, including the presence of annulate lamellae and a finely clumped chromatin pattern. The final patient's lesion was composed of neoplastic spindle cells. An unusual finding in one of the invasive nodules of melanoma was the presence of longitudinal tubules within dilated cisternae of rough-surfaced endoplasmic reticulum, a feature also rarely encountered in cutaneous melanomas.

Because a broad spectrum of benign and malignant lesions was systematically investigated in this study, the nuclear and cytoplasmic features of benign dendritic melanocytes and nevus cells could be contrasted with the atypical features of the cells in primary acquired melanosis and invasive malignant melanoma. Whether present within the epithelium or in invasive melanoma nodules, the atypical cells showed a finer and more dispersed nuclear chromatin pattern with larger nucleoli as well as more disorderly, aberrant melanosomes. The most important and clinically relevant finding in this study was that cases of primary acquired melanosis could be divided into two groups, primarily on the basis of whether grade 3 atypical melanocytes were observed within the epithelial proliferation. The presence of grade 3 cells suggests a more aggressive lesion, either coexisting with invasive nodules of melanoma or portending their subse-

quent development. The ability to subdivide cases of primary acquired melanosis according to this scheme provides a rational basis for early treatment in cases with markedly atypical cells.

REFERENCES

1. McGovern V: *Malignant Melanoma, Clinical and Histologic Diagnosis*. New York, John Wiley & Sons, 1976.
2. Milton GA: *Malignant Melanoma of the Skin and Mucous Membrane*. New York, Churchill-Livingston, 1977.
3. Kopf A, Bart RS, Rodriguez-Sains R, et al: *Malignant Melanomas*. New York, Masson Publishing, 1979.
4. Clark W, Goldman I, Mastrangelo M (eds): *Human Malignant Melanoma*. New York, Grune & Stratton, 1979.
5. Ackerman AB: *Pathology of Malignant Melanoma*. New York, Masson Publishing, 1981.
6. McGovern VJ: Melanoma, in *Histological Diagnosis and Prognosis*. New York, Raven Press, 1983.
7. Roses DF, Harris MN, Ackerman AB: *Diagnosis and Management of Cutaneous Malignant Melanoma*. Philadelphia, WB Saunders, 1983.
8. Regezi JA, Hayvvard JR, Pickens TN: Superficial melanomas of oral mucous membranes. *Oral Surg* 1978; 45:730-740.
9. Clark W, Bernardino V, Reed R, et al: Acral lentiginous melanomas, in Clark W, Goldman L, Mastrangelo M (eds): *Human Malignant Melanoma*. New York, Grune & Stratton, 1979, pp 109-124.
10. Lopansri S, Mihm MC Jr: Clinical and pathological correlation of malignant melanoma. J Cutan Pathol 1979; 6:180-194.
11. Kerl H, Hodl S, Stettner H: Acral lentiginous melanoma, in Ackerman AB (ed): *Pathology of Malignant Melanoma*. New York, Masson Publishing, 1981, pp 217-242.
12. Wanebo HJ, Woodruff JM, Farr GH, et al: Anorectal melanoma. *Cancer* 1981; 47: 1891-1900.
13. McDonald JS, Miller RL, Wagner W, et al: Acral lentiginous melanoma of the oral cavity. *Head Neck Surg* 1983; 5:257-262.
14. Bernardino VB, Naidoff MA, Clark WH: Malignant melanomas of the conjunctiva. *Am J Ophthalmol* 1976; 82:383-394.
15. Silvers DM, Jakobiec FA, Freeman TR, et al: Melanoma of the conjunctiva: A clinicopathologic study, in Jakobiec FA (ed): *Ocular and Adnexal Tumors*. Birmingham, Ala, Aesculapius Publishing Co, 1978, pp 583-599.
16. Zimmerman LE: The histogenesis of conjunctival melanomas, in Jakobiec FA (ed): *Ocular and Adnexal Tumors*. Birmingham, Ala, Aesculapius Publishing Co, 1978, pp 600-630.
17. Jakobiec FA: Conjunctival melanoma: Unfinished business. *Arch Ophthalmol* 1980; 98:1378-1384.
18. Liesegang TJ, Campbell RJ: Mayo Clinic experience with conjunctival melanomas. *Arch Ophthalmol* 1980; 98:1385-1389.
19. Crawford JB: Conjunctival melanosis: Prognostic factors. A review and an analysis of a series. *Trans Am Ophthalmol Soc* 1980; 78:467-502.
20. Zimmerman LE: Melanocytic tumors of interest to the ophthalmologist. *Ophthalmology* 1980; 87:497-502.
21. Campbell RJ: Tumors of the eyelids, conjunctiva and cornea, in Garner A, Klintworth GK (eds): *Pathobiology of Ocular Disease: A Dynamic Approach, Part A*. New York, Marcel Dekker, 1982, pp 601-633.

22. McGhee CN, Ni C, Albert DM, et al: Conjunctival melanoma. *Int Ophthalmol Clin* 1982; 22:35-56.
23. Scotto J, Fraumeni JF, Lee JAH: Melanomas of the eye and other noncutaneous sites: Epidemiologic aspects. *JNCI* 1976; 56:489-491.
24. Henkind P: Conjunctival melanocytic lesions: Natural history, in Jakobiec FA (ed): *Ocular and Adnexal Tumors*. Birmingham, Ala, Aesculapius Publishing Co, 1978, pp 572-582.
25. Pialat J, Grochowicki M, Dumas C, et al: Reese's conjunctival melanosis: Two anatomico-clinical observations with ultrastructural study in one case. *J Fr Ophtalmol* 1982; 5:805-811.
26. Zelickson A: Melanocyte, melanin granule and Langerhans cell, in Zelickson A (ed): *Ultrastructure of Normal and Abnormal Skin*. Philadelphia, Lea & Febiger, 1967, pp 163-182.
27. Hu F: Melanocyte cytology in normal skin, melanocytic nevi, and malignant melanoma: A review, in Ackerman A (ed): *Pathology of Malignant Melanoma*. New York, Masson Publishing, 1981, pp 1-22.
28. Nordlund JJ: Genetic basis of pigmentation and the disorders of pigmentation, in Ackerman AB (ed): *Pathology of Malignant Melanoma*. New York, Masson Publishing, 1981, pp 23-46.
29. Frenk E: Pigment cell biology and its relation to disorders of melanin pigmentation (review). *Dermatologica* 1975; 159:185-194.
30. Jimbow K, Quevedo WC Jr, Fitzpatrick TB, et al: Some aspects of melanin biology (review). *J Invest Dermatol* 1976; 67:72-89.
31. Masson P: Melanogenic system: Nevi and melanomas. *Pathol Annu* 1967; 00:351-397.
32. Weston JE: The migration and differentiation of neural crest cells. *Adv Morphog* 1970; 00:41-96.
33. Noden D: Periocular mesenchyme: Neural crest and mesodermal interactions, in Jakobiec FA (ed): *Ocular Anatomy, Embryology and Teratology*. Philadelphia, Harper & Row, 1982, pp 97-119.
34. Zimmerman AA, Becker SW: Precursors of epidermal melanocytes in the Negro fetus, in Gordon M (ed): *Pigment Cell Biology*. New York, Academic Press, 1959, pp 159-170.
35. Caselitz J, Janner M, Breitbart E, et al: Malignant melanomas contain only the vimentin type of intermediate filaments. *Virchows Arch* [Pathol Anat] 1983; 400:43-51.
36. Osborn M: Intermediate filaments as histologic markers: An overview. *J Invest Dermatol* 1983; 81:104s-109s.
37. Cochran AJ, Wen DR, Herschman HR, et al: Detection of S-100 protein as an aid to the identification of melanocytic tumors. *Int J Cancer* 1982; 30:295.
38. Mishima Y: Melanotic tumors, in Zelickson AS (ed): *Ultrastructure of Normal and Abnormal Skin*. Philadelphia, Lea & Febiger, 1967, pp 388-424.
39. ———: Melanocytic and nevocytic malignant melanoma. *Cancer* 1967; 20:632-649.
40. Masson P: My conception of cellular nevi. *Cancer* 1951; 4:9-38.
41. Winkelmann RK: Cholinesterase nevus: Cholinesterases in pigmented tumors of the skin. *Arch Dermatol* 1960; 82:17-23.
42. Mishima Y: Macromolecular changes in pigmentary disorders. *Arch Dermatol* 1965; 91:519-557.
43. Thorne EG, Mottaz JH, Zelickson AS: Tyrosinase activity in dermal nevus cells. *Arch Dermatol* 1971; 104:619-624.
44. Niizuma K: Electron microscopic study of nevic corpuscle. *Acta Derm Venereol* 1975; 55:283-289.
45. Penneys NS, Mogollon R, Kowalczyk A: A survey of cutaneous neural lesions for the presence of myelin basic protein. *Arch Dermatol* 1984; 120:210.
46. Lever WF, Schaumburg-Lever G: *Histopathology of the Skin*. Philadelphia, JB Lippincott Co, 1983, pp 14-19; 440-444; 681-725.

47. Breathnack AS, Wyllie LM: Electron microscopy of melanocytes and melanosomes in freckled human epidermis. *J Invest Dermatol* 1964; 42:389-394.
48. Quevedo WC, Szabo G, Virks J, et al: Melanocyte populations in UV-radiated human skin. *J Invest Dermatol* 1965; 45:295-298.
49. Gilchrest BA, Blog FB, Szabo G: Effects of aging and chronic sun exposure on melanocytes in human skin. *J Invest Dermatol* 1979; 73:141-143.
50. Hashimoto K: Langerhans' cell granule: An endocytic organelle. *Arch Dermatol* 1971; 104:148-160.
51. Katz SI: The role of Langerhans cells in immunity (editorial). *Arch Dermatol* 1980; 116:1361-1362.
52. Chu A, Eisinger M, Lee JS, et al: Immunoelectron microscopic identification of Langerhans cells using a new antigenic marker. *J Invest Dermatol* 1982; 78:177-180.
53. Rodrigues MM, Rowden G, Hackett J, et al: Langerhans cells in the normal conjunctiva and peripheral cornea of selected species. *Invest Ophthalmol Vis Sci* 1981; 21:759-765.
54. Gillette TE, Chandler JW, Greiner JV: Langerhans cells of the ocular surface. *Ophthalmology* 1982; 89:700-711.
55. Pearse AGE: The APUD cell concept and its implications in pathology. *Pathol Annu* 1974; 9:27-41.
56. Beyer C, Goodman M, Dickersin G, et al: Merkel cell tumor of the eyelid: A clinicopathologic case report. *Arch Dermatol* 1983; 101:1098-1101.
57. Lamping K, Fischer MJ, Vareska G, et al: A Merkel cell tumor of the eyelid. *Ophthalmology* 1983; 90:1399-1402.
58. Gottlieb B, Brown AL Jr, Winkelmann RK: Fine structure of the nevus cell. *Arch Dermatol* 1965; 92:81-87.
59. Warner TF, Seo IS, Bennett JE: Minimal deviation melanoma with epidermotropic metastases arising in a congenital nevus. *Am J Surg Pathol* 1980; 4:175-183.
60. Hendrickson MR, Ross JC: Neoplasms arising in congenital giant nevi: Morphologic study of seven cases and a review of the literature. *Am J Surg Pathol* 1981; 5:109-135.
61. Stegmaier OC: Natural regression of the melanocytic nevus. *J Invest Dermatol* 1959; 32:413-419.
62. Maize JC, Foster G: Age-related changes in melanocytic naevi. *Clin Exp Dermatol* 1979; 4:49-58.
63. Jakobiec FA, Iwamoto T: The ocular adnexa, in Fine B, Yanoff M (eds): *Ocular Histology: A Text and Atlas*, ed 2. Hagerstown, Md, Harper & Row, 1969, pp 289-342.
64. Srinivassan BD, Jakobiec FA, Iwamoto T: The conjunctiva, in Jakobiec FA (ed): *Ocular Anatomy, Embryology and Teratology*. Philadelphia, Harper & Row, 1982, pp 733-760.
65. Last RJ: *Wolf's Anatomy of the Eye and Orbit*. Great Britain, WB Saunders Co, 1969, p 215.
66. Hogan MJ, Alvarado JA, Weddell JE: *Histology of the Human Eye: An Atlas and Textbook*. Philadelphia, WB Saunders Co, 1971, pp 128-131.
67. Henkind P: Migration of limbal melanocytes into the corneal epithelium of guinea-pigs. *Exp Eye Res* 1965; 4:42-47.
68. McCracken JS, Klintworth GK: Ultrastructural observation on experimentally produced melanin pigmentation of the corneal epithelium. *Am J Pathol* 1976; 85:167-182.
69. Jauregui HO, Klintworth GK: Pigmented squamous cell carcinoma of cornea and conjunctiva: A light microscopic, histochemical and ultrastructural study. *Cancer* 1976; 38:778-788.
70. Salisbury JA, Szpak CA, Klintworth GK: Pigmented squamous cell carcinoma of the conjunctiva. *Ophthalmology* 1983; 91:1477-1481.
71. Cochran AJ: The incidence of melanocytes in normal skin. *J Invest Dermatol* 1970; 55:65-70.
72. Soames JV: The morphology and quantitative distribution of dopa-positive melanocytes in the gingival epithelium of Caucasians. *Oral Surg* 1974; 38:254-258.

73. Eller AW, Bernardio VB: Blue nevi of the conjunctiva. *Ophthalmology* 1983; 90:1468-1471.
74. Jakobiec FA, Ellsworth R, Tannenbaum M: Primary orbital melanoma. *Am J Ophthalmol* 1974; 78:24-39.
75. Coppetto JR, Jaffe R, Gillies CG: Primary orbital melanoma. *Arch Ophthalmol* 1978; 96:2255-2258.
76. Jay B: Naevi and melanomata of the conjunctiva. *Br J Ophthalmol* 1965; 49:169-204.
77. Henkind P, Friedman AH: External ocular pigmentation. *Int Ophthalmol Clin* 1972; 11:87-111.
78. Hogan M, Zimmerman LE: *Ophthalmic Pathology: An Atlas and Textbook*. Philadelphia, WB Saunders Co, 1962, pp 266-273.
79. Yanoff M, Fine B: *Ocular Pathology: A Text and Atlas*. Philadelphia, Harper & Row, 1982, pp 800-805.
80. Rosenfeld SI, Smith ME: Benign cystic nevus of the conjunctiva. *Ophthalmology* 1983; 90:1459-1461.
81. Pfaffenbach DD, Green WR, Maumenee AE: Balloon cell nevus of the conjunctiva. *Arch Ophthalmol* 1972; 87:192-195.
82. Jao W, Fretzin D, Sundaran L, et al: Balloon cell nevus of the conjunctiva. *Arch Pathol* 1973; 96:124-126.
83. Schlaeeter PE, Daicker B: Balloon cell nevi of the conjunctiva. *Klin Monatsbl Augenheilkd* 1975; 166:798-802.
84. Buchner A, Hansen LS: Pigmented nevi of the oral mucosa: A clinicopathologic study of 32 new cases and review of 75 cases from the literature. *Oral Surg* 1980; 49:55-62.
85. Reed RJ, Ichinose H, Clark W, et al: Common and uncommon nevi and borderline melanoma. *Semin Oncol* 1975; 2:119-147.
86. Kamino H, Misheloff E, Ackerman AB, et al: Eosinophilic globules in Spitz's nevi: New findings and a diagnostic sign. *Am J Dermatopathol* 1979; 1:319-324.
87. Fletcher V, Sagebiel R: The combined nevus: Mixed patterns of benign melanocytic lesions must be differentiated from malignant melanomas, in Ackerman AB (ed): *Pathology of Malignant Melanoma*. New York, Masson Publishing, 1981, pp 273-284.
88. Penman HG, Stringer HCW: Malignant transformation in giant congenital pigmented nevus. *Arch Dermatol* 1971; 103:428-432.
89. Mark GJ, Mihm MC, Liteplo MG, et al: Congenital melanocytic nevi of the small and garment type. *Hum Pathol* 1973; 4:395-418.
90. Kopf AW, Bart RS, Hennessey P: Congenital nevocytic nevi and malignant melanomas. *J Am Acad Dermatol* 1979; 1:123-130.
91. Bellet RE, Shields JA, Soll DB, et al: Primary choroidal and cutaneous melanomas occurring in a patient with the B-K mole syndrome. *Am J Ophthalmol* 1980; 89:567.
92. Abramson DH, Rodriguez-Sains RS, Rubman R: B-K mole syndrome: Cutaneous and ocular malignant melanoma. *Arch Ophthalmol* 1980; 98:1397.
93. Greene MH, Sanders RJ, Chu FC, et al: The familial occurrence of cutaneous melanoma, intraocular melanoma, and the dysplastic nevus syndrome. *Am J Ophthalmol* 1983; 96:238.
94. Rodriguez-Sains RS: Uveal findings in patients with ocular and cutaneous melanoma (letter). *Am J Ophthalmol* 1983; 96:257.
95. Elder DE, Greene MH, Bondi EE, et al: Acquired melanocytic nevi and melanoma: The dysplastic nevus syndrome, in Ackerman AB (ed): *Pathology of Malignant Melanoma*. New York, Masson Publishing, 1981, pp 185-216.
96. Reese AB: Precancerous and cancerous melanosis. *Am J Ophthalmol* 1966; 61:1272-1277.
97. Zimmerman LE: Criteria for management of melanosis. *Arch Ophthalmol* 1966; 76: 307-308.
98. Griffith WR, Green WR, Weinstein GW: Conjunctival malignant melanoma originating in acquired melanosis sine pigmento. *Am J Ophthalmol* 1971; 72:595-599.

99. Kamino H, Ackerman AB: The problem of interpreting the meanings of atypical melanocytes and unusual patterns of melanocytes within the epidermis, in Ackerman AB (ed): *Pathology of Malignant Melanoma*. New York, Masson Publishing, 1981, pp 129-158.
100. Sagebiel R: Histopathology of borderline and early malignant melanomas. *Am J Surg Pathol* 1979; 3:543-552.
101. Ackerman AB: Carcinoma in situ. *Hum Pathol* 1979; 10:127-128.
102. Ackerman AB, Su W: The histology of cutaneous malignant melanoma, in Kopf A, Bart R, Rodriguez-Sains R, et al (eds): *Malignant Melanomas*. New York, Masson Publishing, 1979, pp 25-147.
103. Reed RJ, Clark WH Jr, Mihm MC: Premalignant melanocytic dysplasias, in Ackerman AB (ed): *Pathology of Malignant Melanoma*. New York, Masson Publishing, 1981, pp 159-184.
104. Kamino H, Ackerman AB: Malignant melanoma in situ: The evolution of malignant melanoma within the epidermis, in Ackerman AB (ed): *Pathology of Malignant Melanoma*. New York, Masson Publishing, 1981, pp 59-92.
105. McGovern VJ: Melanoma: Growth patterns, multiplicity and regression, in McCarthy WH (ed): *Melanoma and Skin Cancer*. Sydney, Australia, Blight, 1972, pp 95-106.
106. McGovern VJ, Mihm MC Jr, Bailly C, et al: The classification of malignant melanoma and its histologic reporting. *Cancer* 1973; 32:1446-1457.
107. Clark WH Jr, Ainsworth AM, Bernardino EA, et al: The developmental biology of primary human malignant melanomas. *Semin Oncol* 1975; 2:83-100.
108. Elder D, Ainsworth A, Clark W: The surgical pathology of cutaneous malignant melanoma, in Clark W, Goldman L, Mastrangelo M (eds): *Human Malignant Melanoma*. New York, Grune & Stratton, 1979, pp 109-124.
109. Clark W, Folbert R, Ainsworth A: Tumor progression in primary human cutaneous malignant melanomas, in Clark W, Goldman L, Mastrangelo M (eds): *Human Malignant Melanoma*. New York, Grune & Stratton, 1979, pp 15-31.
110. McGovern V, Shaw H, Milton G, et al: Prognostic significance of the histological features of malignant melanoma. *Histopathology* 1979; 3:385-393.
111. ———: Is malignant melanoma arising in Hutchinson's melanotic freckle a separate disease entity? *Histopathology* 1980; 4:235-242.
112. Arrington JH, Reed RH, Ichinose H, et al: Plantar lentiginous melanoma: A distinctive variant of human cutaneous malignant melanoma. *Am J Surg Pathol* 1977; 1:131-143.
113. Brownstein S, Jakobiec FA, Wilkinson RD, et al: Cryotherapy for precancerous melanosis (atypical melanocytic hyperplasia) of the conjunctiva. *Arch Ophthalmol* 1981; 99:1224-1231.
114. Jakobiec FA, Brownstein S, Wilkinson RD, et al: Adjuvant cryotherapy for focal nodular melanoma. *Arch Ophthalmol* 1982; 100:115-118.
115. Jakobiec FA, Brownstein S, Albert W, et al: The role of cryotherapy in the management of conjunctival melanoma. *Ophthalmology* 1982; 89:502-515.
116. Jakobiec FA, Iwamoto T: Ultrastructural effects of cryotherapy for intraepithelial conjunctival melanocytic proliferations. *Arch Ophthalmol* 1983; 101:904-912.
117. Lederman M: Radiotherapy of cancerous and precancerous melanosis. *Trans Ophthalmol Soc UK* 1958; 78:147-164.
118. Lederman M: Discussion of pigmented tumors of the conjunctiva, in Boniuk M (ed): *Ocular and Adnexal Tumors: New and Controversial Aspects*. St Louis, CV Mosby Co, 1964, pp 32-40.
119. Lommatzsch P: Beta irradiation of conjunctival melanomas. *Trans Ophthalmol Soc UK* 1977; 97:378-380.
120. Breslow A: Thickness, cross-sectional areas, and depth of invasion in the prognosis of cutaneous melanoma. *Ann Surg* 1970; 172:902-908.
121. Chung AF, Casey MJ, Flannery JT, et al: Malignant melanoma of the vagina: A report of 19 cases. *Obstet Gynecol* 1980; 55:720-727.

122. Hirone T, Eryu Y: Ultrastructure of giant pigment granules in lentigo simplex. *Acta Derm Venereol* 1978; 58:223-229.
123. Bhawan J, Purtilo DT, Riordan JE, et al: Giant and "granule melanosomes" in leopard syndrome. *J Cutan Pathol* 1976; 3:207-216.
124. Horikoshi T, Jimbow K, Sugiyama S: Comparison of macromelanosomes and autophasic giant melanosome complexes in nevocellular nevi, lentigo simplex and malignant melanoma. *J Cutan Pathol* 1982; 9:329-339.
125. Braun-Falco O, Schoefinius HH: Lentigo senilis. *Hautarzt* 1971; 22:277-283.
126. Montagna W, Hu F, Carlisle K: A reinvestigation of solar lentigines. *Arch Dermatol* 1980; 116:1151-1154.
127. Bigotti A, De Martino C, Natali P, et al: Malignant lentigo-melanoma: A comparative histological and electron microscopy study. *J Surg Oncol* 1981; 18:31-37.
128. Bleehen SS, Hartley L, Senior J: Proceedings: Lentigo-maligna melanoma: An ultrastructural study. *Br J Dermatol* 1976; (Suppl 14) 95:14-15.
129. Lupulescu A, Pinkus H, Birmingham DJ, et al: Lentigo maligna of the fingertip: Clinical, histologic and ultrastructural studies of two cases. *Arch Dermatol* 1973; 107:717.
130. Jimbow K, Szabo G, Fitzpatrick TB: Ultrastructure of giant pigment granules (macromelanosomes) in the cutaneous pigmented macules of neurofibromatosis. *J Invest Dermatol* 1973; 61:330-339.
131. Frenk E: Étude ultrastructurale des taches pigmentaires due syndrome d'Albright. *Dermatologica* 1971; 143:12-20.
132. Konrad K, Honigsmann H, Wolff K: Naevus spilus: Ein Pigmentnaevus mit Riesenmelanosomen. *Hautarzt* 1974; 25:585-593.
133. Konrad K, Wolff K, Hongismann H: The giant melanosome: A model of deranged melanosome-morphogenesis. *J Ultrastruc Res* 1974; 48:102-123.
134. Konrad K, Honigsmann H: Riesenmelanosomen in Naevus-Zellnaevi und in normaler menschlicher Epidermis. *Wien Klin Wochenschr* 1975; 87:173-177.
135. Weiss LW, Zelickson AS: Giant melanosomes in multiple lentigens syndrome *Arch Dermatol* 1977; 113:491-494.
136. Ortonne JP, Perrot H: Giant melanin granules in vitiliginous achromia with malignant melanoma. *Acta Derm Venereol* 1978; 58:475-480.
137. Burgess JH, Warner TF, Mohs FE, et al: Giant melanosomes in the B-K mole syndrome. *J Cutan Pathol* 1982; 9:241-248.
138. Pritchett RM, Pritchett PS: Zosteriform speckled lentiginous nevus with giant melanosomes. *Cutis* 1982; 30:329-334.
139. Mishima YH, Matsunaka M: Pagetoid premalignant melanosis and melanoma: Differentiation from Hutchinson's melanotic freckle. *J Invest Dermatol* 1975; 65:434-440.
140. Juarez EP, Tso MOM: An ultrastructural study of melanocytomas (magnocellular nevi) of the optic disk and uvea. *Am J Ophthalmol* 1980; 98:48-62.
141. O'Donnell FE Jr, Hambrick GW, Green WR, et al: X-linked ocular albinism: An oculocutaneous macromelanosomal disorder. *Arch Dermatol* 1976; 94:1883-1892.
141a. Rennie IG, Bleehen SS: Melanosis oculi: An ultrastructural study of an affected iris. *Arch Ophthalmol* 1983; 101:1912-1916.
142. Schreiner E, Wolff K: Die Ultrastruktur des benignen juvenilen Melanoms. *Arch Klin Exp Dermatol* 1970; 237:749-768.
143. Curran RC, McCann BG: The ultrastructure of benign pigmented naevi and melanocarcinoma. *J Pathol* 1976; 119:119-135..
144. Niizuma K: Juvenile melanoma: A case report and histogenetic investigation. *Arch Dermatol Res* 1977; 259:83-89.
145. Eady R, Gilkes JJH, Wilson-Jones E: Eruptive naevi: Report of two cases. *Br J Dermatol* 1977; 97:267-278.
146. Tsuji T, Saito T: Multiple naevocellular naevi in brothers with albinism. *Br J Dermatol* 1978; 98:685-692.

147. Wiborn WH, Montes LF: A tridimensional view of intradermal nevi as revealed by scanning electron microscopy. *J Cutan Pathol* 1979; 6:170-179.
148. Abrahams C, Skudowitz RB: An ultrastructural study of a juvenile melanoma. *S Afr J Med* 1979; 55:47-49.
149. Wassilew S, Janner M, Schaeg G: Familial multiple naevogene melanomas: Electron microscopical findings in pigmented moles of the B-K mole syndrome. *Z Hautkr* 1981; 56:931-941.
150. Nagao S, Sonoda K, Ikjima S: An electron microscopic study of juvenile melanoma: With special reference to the ultrastructure of epithelioid cell nevus and its resemblance to hypertrophic astrocytes. *Nippon Hifuka Gakkai Zasshi* 1980; 90:17-28.
151. Fresko A, Sidhu GS: Clefts in intradermal nevi: An ultrastructural study. *Ultrastruct Pathol* 1980; 1:361-366.
152. Erlandson RA, Rosen PP: Elecron microscopy of a nevus cell aggregate associated with an axillary lymph node. *Cancer* 1982; 49:269-272.
153. Hashimoto K, Bale GF: An electron microscopic study of balloon cell nevus. *Cancer* 1972; 30:530-540.
154. Okun MR, Donnellan B, Edelstein L: An ultrastructural study of balloon cell nevus. *Cancer* 1974; 34:613-625.
155. Sondergaard K, Henschel A, Hou J, et al: Metastatic melanoma with balloon cell changes: An electron microscopic study. *Ultrastruct Pathol* 1980; 1:357-360.
156. Rodrigues MM, Shields JA: Malignant melanoma of the choroid with balloon cells: A clinicopathologic study of three cases. *Can J Ophthalmol* 1976; 11:208-216.
157. Jakobiec FA, Shields J, Desjardins L, et al: Balloon cell melanomas of the ciliary body. *Arch Ophthalmol* 1979; 97:1687-1692.
158. Khalil MK: Balloon cell malignant melanoma of the choroid: Ultrastructural studies. *Br J Ophthalmol* 1983; 67:579-584.
159. Klug H, Gunther W: Ultrastructural differences in human malignant melanoma: An electron microscopic study. *Br J Dermatol* 1972; 86:395.
160. Clark WH Jr, Heggeler B, Bretton R: Electron microscopic observations of human cutaneous melanomas correlated with their biologic behaviour, in McCarthy WH (ed): *Melanoma and Skin Cancer*. Sydney, Australia, Blight, 1972, p 121.
161. Hunter JAA, Zaynoon S, Paterson WD, et al: Cellular fine structure in the invasive nodules of different histogenetic types of malignant melanoma. *Br J Dermatol* 1978; 98:255-272.
162. Hunter JAA, Paterson WD, Fairley DJ: Human malignant melanoma: Melanosomal polymorphism and the ultrastructural dopa reaction. *Br J Dermatol* 1978; 98:381-389.
163. Mintzis M, Silvers D: Ultrastructural study of superficial spreading melanoma and benign simulants. *Cancer* 1978; 45:502-511.
164. Allen AC: A reorientation on the histogenesis and clinical significance of cutaneous nevi and melanomas. *Cancer* 1949; 2:28-56.
165. Allen AC, Spitz S: Malignant melanoma: A clinicopathological analysis of the criteria for diagnosis and prognosis. *Cancer* 1952; 6:1-45.
166. Drzewiecki KT: Cutaneous malignant melanoma: Studies on the fine structure of cells and their surface morphology in relation to tumour types and clinical course. *Scand J Plast Reconstr Surg* 1979; 13:321-332.
167. Tschang TP, Kasin JV, Parnell D, et al: Annulate lamellae in human malignant tumors: Report of three cases. *Arch Pathol Lab Med* 1978; 102:426-430.
168. Sun CN, White HJ: Annulate lamellae in human tumor cells. *Tissue Cell* 1979; 11:139-146.
169. Mackay B, Ayala AG: Intracisternal tubules in human melanoma cells. *Ultrastruct Pathol* 1980; 1:1-6.
170. Tillman LJ: Metastatic malignant melanoma: A case study with unusual ultrastructure. *Dermatologica* 1981; 163:343-347.
171. Mazur MT, Katzenstein AL: Metastatic melanoma: The spectrum of ultrastructural morphology. *Ultrastruct Pathol* 1980; 1:337-356.

172. Riehl R, Wilsen W: Morphological aspects of human malignant skin tumours: A freeze-fracture study. *J Cutan Pathol* 1982; 9:225-240.
173. Fidler IJ, Gerstein DM, Hart IR: The biology of cancer invasion and metastasis. *Adv Cancer Res* 1978; 28:149-250.
174. Merkow LP, Burt RC, Hayeslip DW, et al: A cellular and malignant blue nevus. *Cancer* 1969; 24:886-896.
175. Hernandez E: Malignant blue nevus. *Arch Dermatol* 1973; 107:741-744.
176. Mishima Y: Cellular blue nevus: Melanogenic activity and malignant transformation. *Arch Dermatol* 1970; 101:104-110.
177. Boudreaux D, Waisman J: Clear cell sarcoma with melanogenesis. *Cancer* 1978; 41:1381-1394.
178. Tsuneyoshi M, Enjoji M, Kubo T: Clear cell sarcoma of tendons and aponeuroses: A comparative study of 13 cases with a provisional subgrouping into the melanotic and synovial types. *Cancer* 1978; 42:243-252.
179. Diaz de Molnar AM, Guralnick M, Ferenczy A: Blue nevus of the endocervix: Report of two cases and ultrastructure. *Gynecol Oncol* 1978; 6:373-382.
180. Uff JS, Hall M: Blue naevus of the cervix: Report of two cases and review of the literature. *Histopathology* 1978; 2:291-299.
181. Okawa Y, Yokota R, Yamauchi A: On the extracellular sheath of dermal melanocytes in nevus fusco-ceruleus acromiodeltoideus (Ito) and Mongolian spot: An ultrastructural study. *J Invest Dermatol* 1979; 73:224-230.
182. Bhawan J, Chang WH, Edelstein LM: Cellular blue nevus: An ultrastructural study. *J Cutan Pathol* 1980; 7:109-122.
183. Hori Y, Ohara K, Nirmura M, et al: Electron microscopy: Ultrastructural observations of the extracellular sheath of dermal melanocytes in the nevus of Ota. *Am J Dermatopathol* 1982; 4:245-251.
184. Lovas GL, Wysocki GP, Daley TD: The oral blue nevus: Histogenetic implications of its ultrastructural features. *Oral Surg* 1983; 55:145-150.
185. Kudo M, Nagayama T, Miura M, et al: Blue nevus of the uterine cervix: An ultrastructural study of two cases. *Arch Pathol Lab Med* 1983; 107:87-90.
186. Fu YS, Kaye GI, Lattes R: Primary malignant melanocytic tumors of the sympathetic ganglia, with an ultrastructural study of one. *Cancer* 1975; 36:2029-2041.
187. Mennemeyer RP, Hallman KO, Hammar SP, et al: Melanotic schwannoma: Clinical and ultrastructural studies of three cases with evidence of intracellular melanin synthesis. *Am J Surg Pathol* 1979; 3:3-10.
188. Elder DE, Ainsworth AM, Goldman LI, et al: Malignant-melanocytic schwannoma: Report of a case with some histologic features of desmoplastic melanoma, in Ackerman AB (ed): *Pathology of Malignant Melanoma*. New York, Masson Publishing, 1981, pp 251-262.
189. Janzer RC, Makek M: Intraoral malignant melanotic schwannoma: Ultrastructural evidence for melanogenesis by Schwann's cells. *Arch Pathol Lab Med* 1983; 107:298-301.
190. Valensi OJ: Desmoplastic malignant melanoma: A light and electron microscopic study of two cases. *Cancer* 1979; 43:1148-1155.
191. Bryant E, Ronan SG, Felix EL, et al: Desmoplastic malignant melanoma: A study by conventional and electron microscopy. *am J Dermatopathol* 1982; 4:467-474.
192. Warner TF, Gafez GR, Finch RE, et al: Schwann cell features in neurotropic melanoma. *J Cutan Pathol* 1981; 3:177-187.
193. Reed R, Leonard D: Neurotropic melanoma: A variant of desmoplastic melanoma. *Am J Surg Pathol* 1979; 3:301-311.
194. Cruickshank CND, Harcourt SA: Pigment donation *in vitro*. *J Invest Dermatol* 1964; 42:183-184.
195. Mottaz JH, Zeilickson AS: Melanin transfer: A possible phagocytic process. *J Invest Dermatol* 1967; 49:605-610.

196. Austin P, Jakobiec FA, Iwamoto T: Elastodysphasia and elastodystrophy as the pathologic bases of ocular pterygia and pinguecula. *Ophthalmology* 1983; 90:96-109.
197. Noor Sunba MS, Rhai AH, Garner A, et al: Tumours of the anterior uvea: III. Oxtalan fibres in the differential diagnosis of leiomyoma and malignant melanoma of the iris. *Br J Ophthalmol* 1980; 64:867-874.
198. Folberg R, McLean I, Zimmerman L: Conjunctival melanosis and melanoma. *Ophthalmology* 1984; 91:673-678.
199. Charles NG, Stenson S, Taterka HB: Epibulbar malignant melanoma in a black patient. *Arch Ophthalmol* 1979; 97:316-318.
200. Kielar RA: Limbal melanoma in a negro. *Arch Ophthalmol* 1972; 87:392-395.
201. Leonard D, Lund H: Pathology of malignant melanoma, in Andrade R, Gumport S, Popkin G, et al (eds): *Cancer of the Skin*. Philadelphia, WB Saunders Co, 1976, vol 2, pp 1019-1044.
202. McGovern VJ, Shaw HM, Milton GW, et al: Cell type and pigment content as prognostic indicators in cutaneous malignant melanoma, in Ackerman AB (ed): *Pathology of Malignant Melanoma*. New York, Masson Publishing, 1981, pp 327-332.
203. Silvers D, Becker L, Hiles E: Epidermal melanocytes in eruptive xanthomas. *Arch Dermatol* 1973; 107:847-853.
204. Paul E, Illig L: Melanin-producing dendritic cells and histogenesis of malignant melanoma. *Arch Dermatol Res* 1976; 257:163-177.
205. Lewis PN, Zimmerman LE: Delayed recurrence of malignant melanoma of the bulbar conjunctiva. *Am J Ophthalmol* 1958; 55:536-543.
206. Grove AS Jr: Melanomas of the conjunctiva. *Int Ophthalmol Clin* 1980; 20:161-175.