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MECHANISM OF DEPIGMENTATION BY HYDROQUINONE*

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ABSTRACT

Histochemical (dopa reaction) and electron microscopic studies were carried out to elucidate the nature of the chemical depigmentation produced by hydroquinone (HQ). Depigmentation was induced by topical application or subcutaneous injection of HQ in black guinea pigs.

The present study showed that HQ preferentially affected the nonfollicular and follicular melanocyte system. It caused decreased formation of melanosomes, a marked alteration in the internal structure of melanosomes, an increased degradation of melanosomes, and, finally, a destruction of the membranous organelles in the melanocytes.

These findings indicate that HQ affects not only the formation, melanization, and degradation of melanosomes, but that it affects also the membranous structures of melanocytes and eventually causes necrosis of whole melanocytes.

Application of electron microscopy to the study of biology of melanin pigmentation has led to significant advances during the last decade. As a result, the morphogenesis of melanosomes and the four processes—formation, melanization, transfer, and degradation of melanosomes—that occur during melanin pigmentation in man are now reasonably well understood. These advances provide a sound basis for investigation of the influence of chemical agents that selectively influence, at different stages, the four processes of melanin pigmentation. The biology of melanin pigmentation can be examined by either stimulation of melanocytes and of melanogenesis, or by inhibition of the biogenesis of melanin pigmentation.

Inhibition of the melanin pigmentation can be achieved by various chemical agents, some of which are monobenzyl ether of hydroquinone [1]; monomethyl ether of hydroquinone [2]; hydroquinone [3]; N-(2-mercaptoethyl)-dimethylamine hydrochloride (MEDA); 2-mercaptoethylamine hydrochloride (MEA) [2, 3]; monoethyl ether of hydroquinone (MEH) [3, 4]; 4-isopropyl catechol (4-IPC) [5]; and several phenolic compounds (*p*-tertiary butyl- and amyl-phenol ditertiary butylcatechol, and butylated hydroxy toluene) [6–8].

Topical application or local injection of hydroquinone (HQ) produces a reversible depigmentation of skin and hair in humans, mice, and guinea pigs [9–12], and a reversible regression of melanotic tumors in fish and mice [13, 14]. Presently HQ is the only safe depigmenting agent that can be recommended for the treatment of melasma and other conditions of hyperpigmentation. The pathogenesis of the depigmentation produced by HQ is not known; depigmentation can result from a variety of actions of HQ on the melanocyte.

This study was designed to elucidate the precise nature of the depigmentation produced by HQ. The cytologic events that proceed *pari passu* with the depigmentation in skin and hair of black guinea pigs after topical application or subcutaneous injection of HQ are described.

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MATERIALS AND METHODS

Skin. To determine the effects of topically applied HQ on the skin, epilated backs of adult black guinea pigs (600–1,000 gm) of both sexes received daily application for 6 days of each week, of creams containing, respectively, 2% and 5% HQ in an oil-in-water emulsion base. Detailed procedures of the bioassay for evaluation of the depigmenting potency were reported previously from this laboratory [11]. Biopsy specimens for electron microscopy and histochemistry were obtained at intervals of 1, 2, and 3 weeks from five guinea pigs treated topically with 2% and 5% HQ. The following biopsy specimens were obtained from epilated skin of each of five animals: (a) untreated (control); (b) treated with an emulsion-base cream that contained all the ingredients except HQ; (c) treated with a cream containing 2% HQ, and (d) treated with a cream containing 5% HQ. The remaining 19 guinea pigs were used only for evaluation of depigmentation.

Hair. The depigmentary effects on the melanocytes of the hair matrix were evaluated after daily subcutaneous injection of HQ solution. Two ml of 1% HQ in normal saline were injected subcutaneously every day for 8 days around both nonepilated and epilated hair matrices. Biopsy specimens were obtained on days 4, 8, and 15 after the first injection. Specimens designated as "controls" were obtained from the nonepilated and epilated hair follicles with and without the injection of 2 ml saline solution. Solutions of HQ were prepared daily and sterilized through a Millipore filter. Each hair follicle was dissected under a stereoscopic microscope.

Each biopsy specimen was divided into two parts for histochemical studies of the dopa (3,4-dihydroxyphenylalanine) reaction of skin, and electron microscopic

studies of skin and hair follicles. The dopa reaction was carried out according to the procedure of Staricco et al [15]. For electron microscopic studies, specimens of skin and hair follicles were prefixed in a solution containing a mixture of formaldehyde and glutaraldehyde in 0.1 M cacodylate buffer adjusted to pH 7.4 [16], and were subsequently postfixed in 2% osmium tetroxide made in 0.1 M cacodylate buffer at pH 7.4. Some of the tissues were fixed in 2% osmium tetroxide made in 0.1 M collidine buffer at pH 7.2. Fixed tissues were dehydrated by ethanol solution and were embedded in epoxy resin. Tissue sectioning was carried out on an LKB or a Porter-Blum MT-2 microtome with a glass or a diamond knife attached. Sections were stained with uranyl acetate and lead citrate and observed in a Siemens Elmiskop 1-A.

RESULTS

Effect of Topical Application of HQ on Skin

Topical application of 2% and 5% HQ to skin induced not only depigmentation but also inflammatory changes and thickening of the epidermis. Depigmentation was visible within 8–10 days and was maximum between 14–20 days. Rarely, total depigmentation of the skin could be observed. Figure 1 shows depigmented skin 3 weeks after topical application of 2% and 5% HQ. Preparations containing 2% and 5% HQ were less effective in inducing depigmentation than those containing 3% 4-isopropyl-catechol, a potent depigmenting agent reported previously from this laboratory [5]. Five percent HQ caused more marked depigmentation and scaling than did 2% HQ.

Examination of the epidermal sheets after incubation in dopa solution showed a decrease in the number of dopa-positive melanocytes proportional to the duration of application (Figs. 2, 3). Both 2% and 5% HQ induced a marked diminution of these dendritic cells (Table).

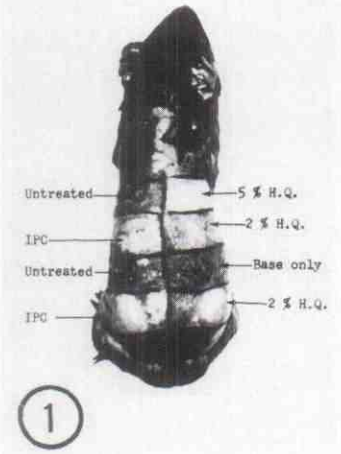
Effect of HQ on melanocytes. The untreated skin or skin treated with cream without HQ revealed many melanocytes and fully melanized melanosomes (Figs. 4, 5). Occasionally, melanosomes in the developmental stages were also seen. These melanocytes revealed well-developed membranous organelles such as Golgi apparatus and endoplasmic reticulum.

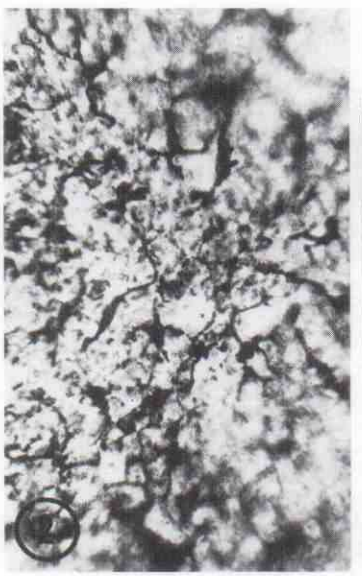
Topical application of HQ for 3 weeks resulted in a marked diminution in the number of melanized melanosomes and in the number of actively functioning melanocytes (Fig. 6). Most of the dendritic cells were devoid of any melanized melanosomes.

TABLE

Effect of hydroquinone on melanocytes of black guinea pig skin (3 weeks' application)

Guinea pig number	Number of melanocytes/mm² ± S.E. mean			
	Control	2% hydroquinone unstabilized	2% hydroquinone stabilized with Na_2S	5% hydroquinone unstabilized
1	962 ± 45	917 ± 52	471 ± 42	0
2	1062 ± 49	882 ± 36	630 ± 40	0
3	913 ± 51	484 ± 59	107 ± 16	29 ± 11
4	1374 ± 65	212 ± 27		
5	471 ± 27	7 ± 7	7 ± 5	





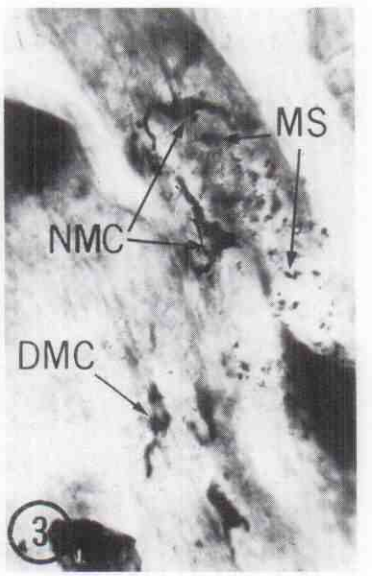
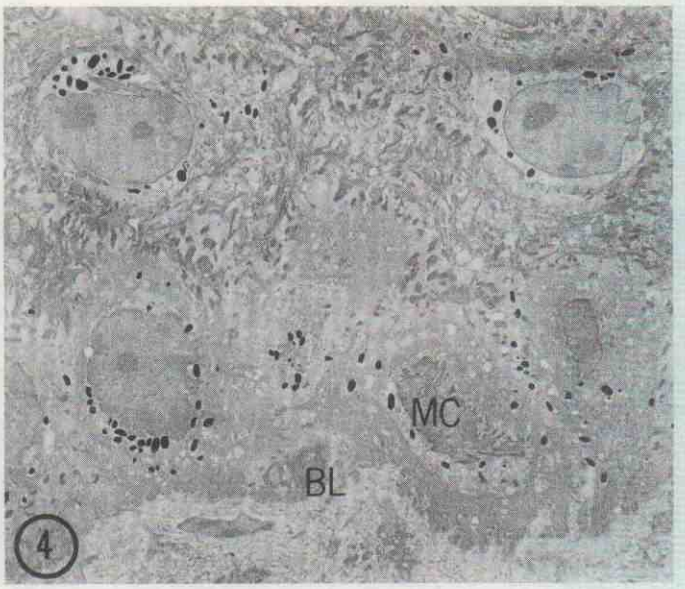
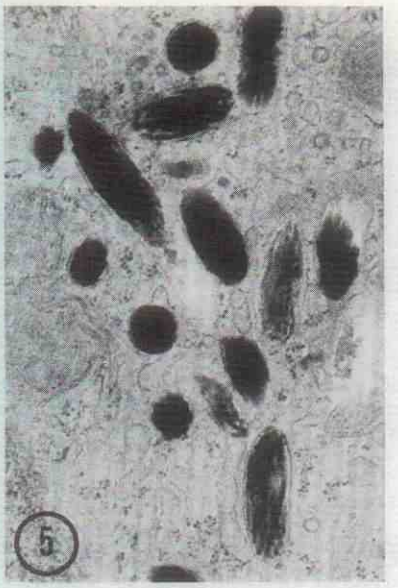


FIG. 1: Depigmentation of guinea pig skin 3 weeks after topical application of hydroquinone (HQ) and 3% 4-isopropyl-catechol (IPC) in an oil-in-water emulsion base. Base only: control area treated with emulsion base.

FIG. 2: NaBr-split, dopa-incubated specimen of the untreated normal skin of black guinea pig. (× 300)

FIG. 3: NaBr-split, dopa-incubated specimen obtained from depigmented site treated with 5% HQ for 2 weeks. Besides normal-appearing melanocytes (NMC), clumped melanosomes (MS) and degenerating melanocytes (DMC) are also visible. (× 300)





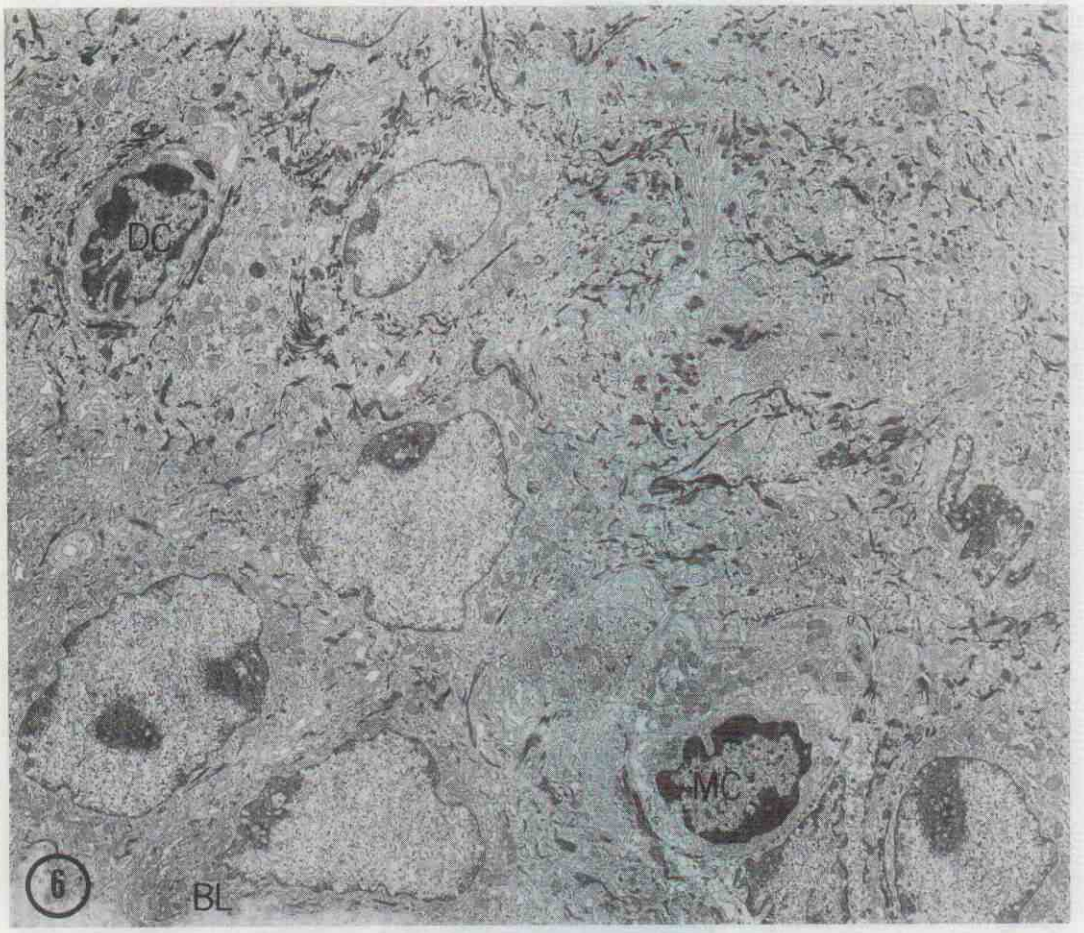


FIG. 4: Normal, untreated skin of black guinea pig. Note that ellipsoidal, highly electron-dense melanosomes are present in the entire epidermis. The melanocyte (MC) contains many mature melanosomes. BL = basal lamina. (× 5,100)

FIG. 5: Normal black guinea-pig skin. The melanosomes are ellipsoidal, highly melanized, and electron-dense, with an internal core of melanized lamellae. (× 18,750)

FIG. 6: Depigmented skin 3 weeks after topical application of 5% HQ. The mature, melanized melanosomes are greatly decreased in number and are hard to discern. The melanocyte (MC) is devoid of any melanized melanosomes and is poorly developed. In the malpighian layer there is a dendritic cell (DC) of unknown nature. BL = basal lamina. (× 4,000)

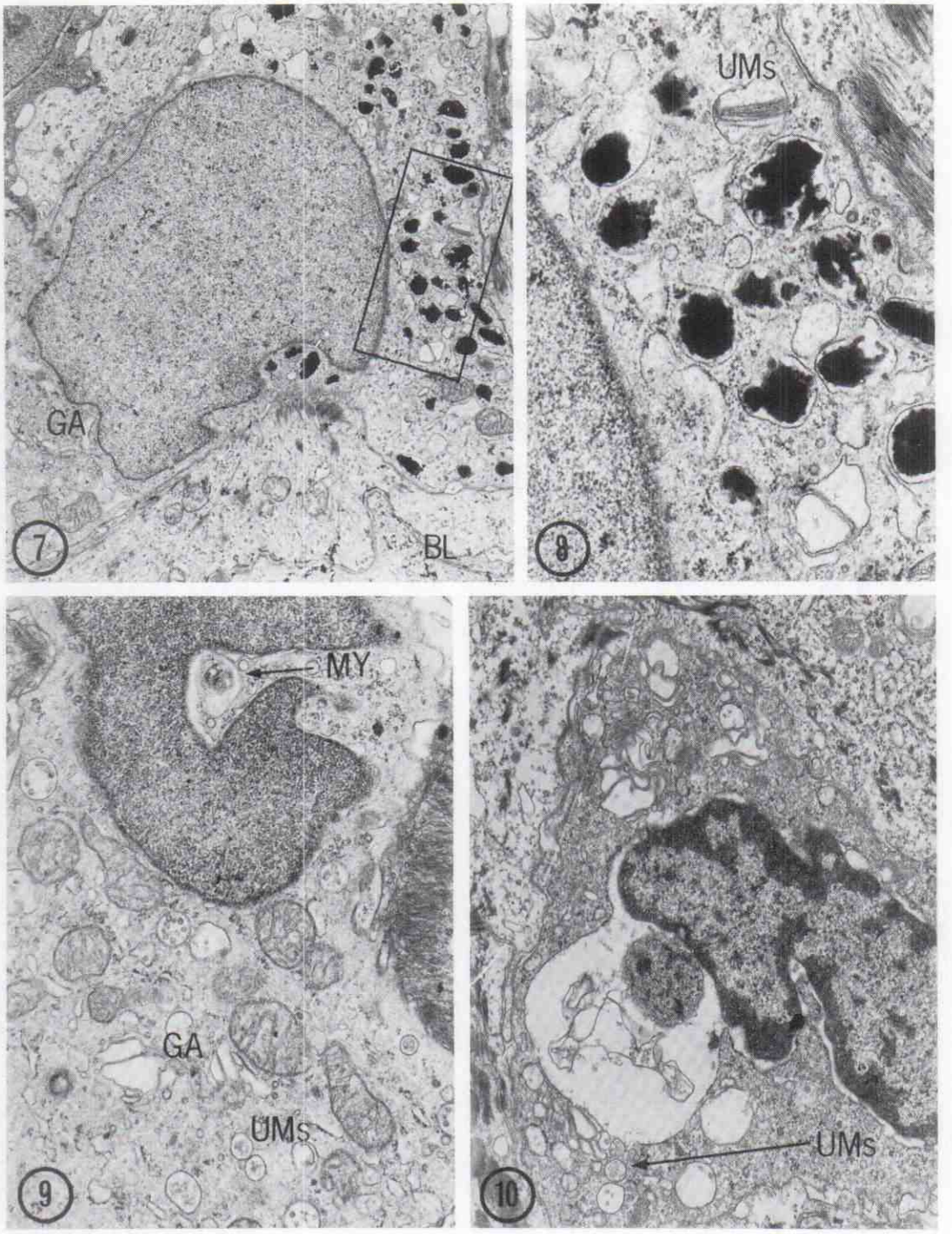


FIG. 7: "Type-1 melanocyte" in the depigmented skin after topical application of 5% HQ for 3 weeks. It contains melanosomes in abundance and well-developed Golgi apparatus (GA). The melanosomes are irregular in shape and show a peculiar configuration when compared with those in Fig. 5. BL = basal lamina. (× 11,250)

FIG. 8: A high-power view of the portion enclosed within the rectangle in Fig. 7. The unmelanized melanosome (UMs) shows a normal lamellar structure with regular striations. The melanized melanosomes contain highly irregular or disorganized lamellae. The outer membranes of these melanosomes are swollen and show an irregular shape. (× 22,500)

FIG. 9: "Type-2 melanocyte" remaining in the depigmented skin after topical application of 5% HQ for 3 weeks. The melanosomes are unmelanized (UMs) and reveal poorly developed lamellae. There are also vacuolated Golgi cisternae (GA). MY = myelin granule. (× 16,000)

FIG. 10: "Type-3 melanocyte" present in the depigmented skin after topical application of 5% HQ for 3 weeks. The nuclear envelope and the other membranous organelles are remarkably vacuolated. Only unmelanized melanosomes (UMs) can be seen. (× 11,000)

Some of them showed poorly developed intracytoplasmic organelles. Melanocytes of the depigmented sites could be differentiated into three types on the basis of their ultrastructure.

The first type of melanocyte contained much cytoplasm with well-developed intracytoplasmic organelles including fairly numerous melanosomes (Fig. 7). The melanosomes in these melanocytes were found at the unmelanized stage or at the early stage of melanization and exhibited a lamellar pattern of regular striations; they were very similar to those of normal melanosomes from the control sites. The outer membrane surrounding the lamellae, however, often was irregular. The melanosomes during the late stage of melanization showed significant changes in their shape. Instead of having the internal core of several melanizing lamellae with regular striations, they contained disorganized or highly irregular lamellae. This suggests that some sort of a degradation process was occurring or, alternatively, that melanin deposition was proceeding irregularly at certain foci only. The outer membranes of the melanosomes were also swollen and detached from their internal core (Fig. 8).

The second type of melanocyte remaining in the depigmented skin revealed a significant reduction in the formation of melanosomes and manifested poorly developed, frequently vacuolated, intracytoplasmic organelles. The melanocytes often showed aggregation of myelin-like granules in the cytoplasm. The melanosomes in these melanocytes revealed poorly developed lamellae (Fig. 9). Fully melanized melanosomes were very rarely seen.

The third type of melanocyte showed changes indicative of cellular destruction. The degenerative changes were characterized by vacuolation of the cytoplasm, swelling and disruption of membranous organelles, such as the endoplasmic reticulum and the nuclear envelope. Myelin-like granules were often seen around the degenerating melanocytes, and most had poorly developed, unmelanized lamellae (Fig. 10).

In contrast to the first type of melanocytes described above, which showed well-developed cytoplasmic organelles and appeared to be actively engaged in melanogenesis, those of the second and third revealed ultrastructural changes indicative of some sort of cellular degeneration. The formation of melanosomes appeared to be inhibited.

Electron microscopic observations of the depigmented skin specimens obtained at 1–2 weeks after HQ application apparently explain why the number of dopa-positive melanocytes had decreased when the specimens were examined by light microscopy. In the light microscopic studies of the specimens obtained within the first two weeks of daily application of HQ, we sometimes could see melanocytes that appeared to be degenerating (Fig. 3). The membranous organelles of these degenerating melanocytes, when examined under the electron microscope, appeared to be markedly swollen and disrupted. Myelin-like granules could be found around these membranes. Keratinocytes adjacent to the degenerating melanocytes contained aggregates of cell débris (Fig. 11) and sometimes a large aggregate of melanosomes in various developmental stages (Fig. 12).

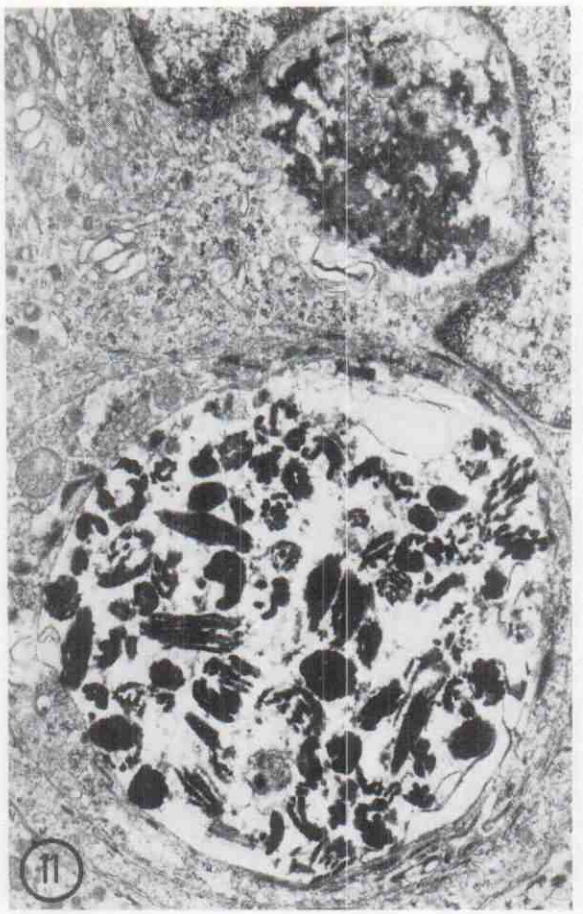
These findings suggest that some of the melanocytes were directly affected by HQ and were eventually destroyed and discharged from the skin with the exfoliating scales (Fig. 13).

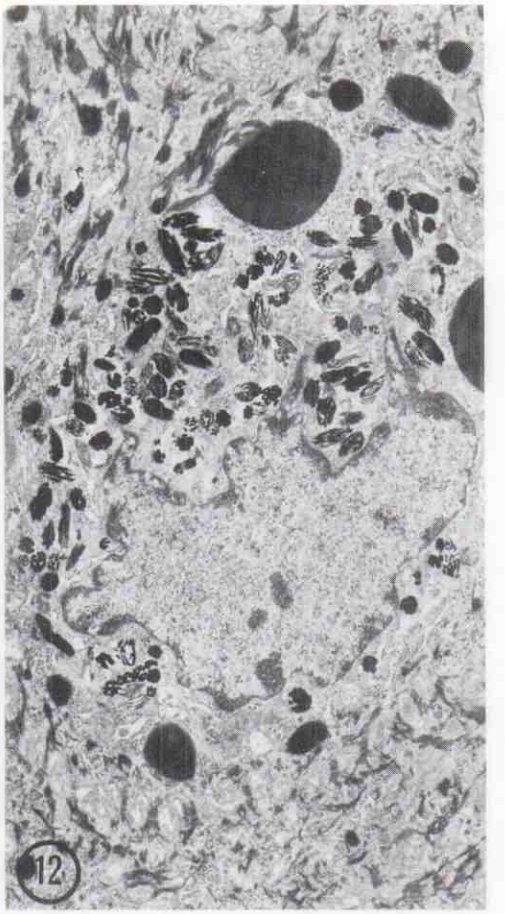
Effect of HQ on the degradation of melanosomes in keratinocytes. In the control specimens of skin, many of the melanosomes transferred from melanocytes into keratinocytes were frequently distributed singly and rarely showed any degradative changes (Figs. 4, 14); they were seen most frequently in the cells of the basal and the suprabasal malpighian layers, although sometimes they could be seen also in the cells of the granular and horny layers. The melanosomes in the upper epidermis showed no evidence of any degradative changes and appeared as electron-dense, amorphous bodies surrounded by a unit membrane. In contrast to this, in the depigmented area treated with HQ for 3 weeks, there was a significant reduction in the number of transferred melanosomes in the keratinocytes (Fig. 6). The melanosomes in the depigmented skin 3 weeks after treatment revealed definite evidence of degradation, and were often seen as broken-down, incomplete lamellae or bizarre electron-dense bodies with small, round, electron-lucent structures. Instead of revealing a normal distribution pattern of single melanosomes within keratinocytes, most of these melanosomes of the depigmented skin were aggregated (Fig. 15).

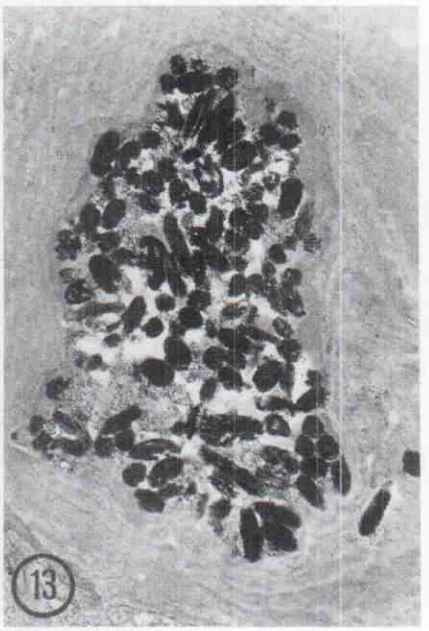
Effect of HQ on Langerhans cells. The Langerhans cells of the control, or untreated, sites could be detected in the basal and malpighian layers by the presence of dendritic processes and characteristic Langerhans granules [17]. The Langerhans granules were, however, relatively few and not so prominent as they are in the Langerhans cells of normal human skin. In the HQ-treated depigmented skin, Langerhans cells could be found easily in the depigmented area and showed well-developed dendritic processes more clearly than those seen in the control, pigmented skin. The cells contained a large number of lysosomal granules of

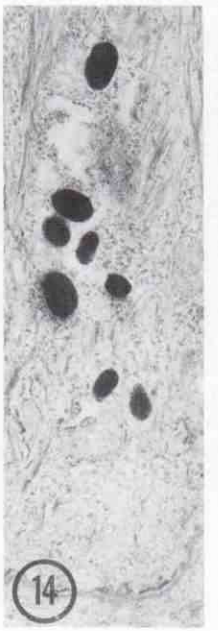
FIG. 11: A degenerating melanocyte and the aggregates of cell debris present in the malpighian layer after topical application of 5% HQ for 2 weeks. The cytoplasm and membranous organelles of the melanocyte are completely disrupted and vacuolated. (× 15,000)

FIG. 12: Depigmented skin after topical application of 5% HQ for 2 weeks showing aggregates of melanosomes present in the keratinocytes of the granular layer. (× 15,750)









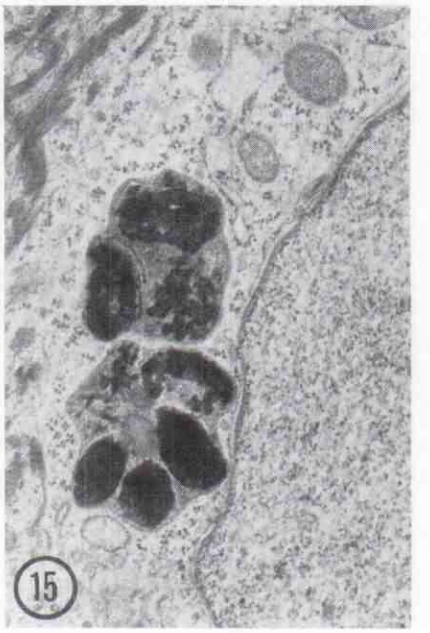


FIG. 13: Depigmented skin after topical application of 5% HQ for 3 weeks. Melanosomes in various developmental stages are aggregated in the horny layer of the epidermis. (× 29,000)

FIG. 14: Melanosomes in the keratinocyte of the basal layer of the epidermis of a normal black guinea pig. They are singly distributed, highly electron-dense, and show little degradative change. (× 13,000)

FIG. 15: Depigmented skin after topical application of 2% HQ for 3 weeks. Instead of being singly distributed, the melanosomes are present in the aggregated form in the keratinocyte. They are highly irregular and show marked degradative change. (× 14,500)

various sizes (Figs. 16, 17) and pinocytotic vesicles very similar to those in the Langerhans cells of human lymph nodes in patients with lipomelanotic reticulosis [18]. They also contained several multivesicular bodies. The increased number of these organelles and the highly developed dendritic processes may be related to the increased phagocytic activity of Langerhans cells. This increased activity could be the result of the direct effect of HQ on Langerhans cells or could result from an indirect effect of HQ due to an inflammatory reaction of the skin. The degraded melanosomes were rarely found in the lysosomal granules of Langerhans cells.

Effect of HQ on epidermal inflammatory cells. Topical application of 2% and 5% HQ induced a mild inflammation characterized by the migration of dermal cells into the epidermis. The migrating cells were mainly histiocytic cells; some lymphocytic cells and polymorphous nuclear leukocytes were also seen. The histiocytic cells sometimes contained aggregates of melanosomes (Fig. 18). These cells were seen in the basal, suprabasal, and upper malpighian layers of the epidermis. After application of HQ for 3 weeks, a certain number of these cells could be found in the totally depigmented skin. In control tissues that were treated with base alone, such a migration of dermal cells was minimal, suggesting that the migration of the inflammatory cells was induced by the topical application of HQ.

Among the migrating dermal cells, we could see some peculiar dendritic cells that were difficult to classify; they were found to be located in the basal and lower malpighian layers and were devoid of any special organelles such as desmosomes, Langerhans granules, melanosomes, and lysosomes. Like Langerhans cells and melanocytes, these peculiar dendritic cells had indented nuclei with a dense aggregate of chromatin along the nuclear envelope. They usually did not show any well-developed membranous organelles, although intracytoplasmic free ribosomes were fairly abundant (Fig. 18). The role of these cells in the inflammatory and depigmenting processes remains unknown.

Effect of HQ on keratinocytes. In a previous report it was noted that epilation caused a thickening of the epidermis [5]. Skin treated with HQ also showed marked epidermal thickening, particularly of the granular layer. Clinically, desquamation was prominent and was often seen within 1 week after HQ application.

In comparison to the marked ultrastructural changes of the melanocytic system, the epidermal keratinocytes did not show any apparent cellular degeneration as a result of HQ application. Only rarely, as shown in Figure 19, could increased density of nucleoplasm and cytoplasm be seen in the keratinocytes. There was no abnormal aggregation of the tonofilament bundles, nor was there any individual cell dyskeratosis. These few cellular degenerative changes of the keratinocytes suggest that HQ has selective action on the melanocytes.

Effect of Local Injection of HQ to Hair

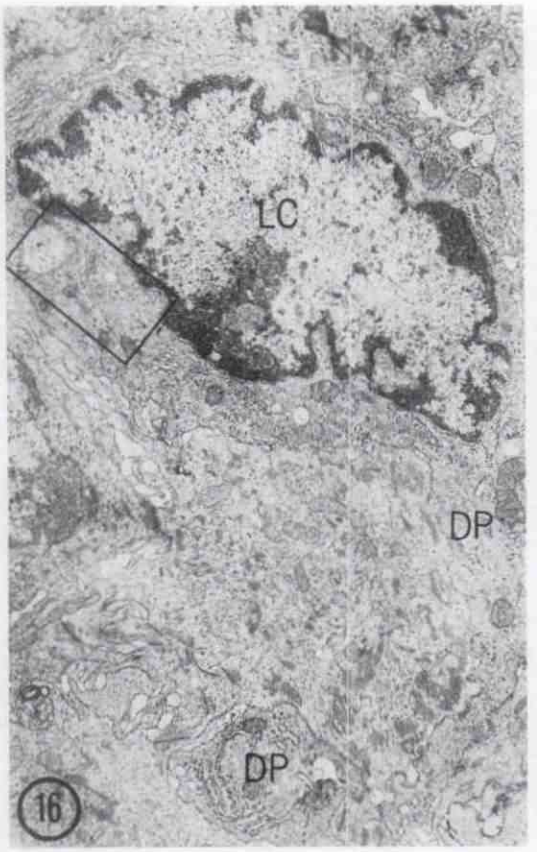
In the epilated or nonepilated skin, daily subcutaneous injection of 2 ml of 1% HQ for 8 consecutive days resulted in depigmentation of the hair. The emerging hair shaft by days 10–12 after injection appeared distinctly depigmented when compared to the surrounding normal hair (Fig. 20). The new-growing hair follicles rarely showed total loss of color.

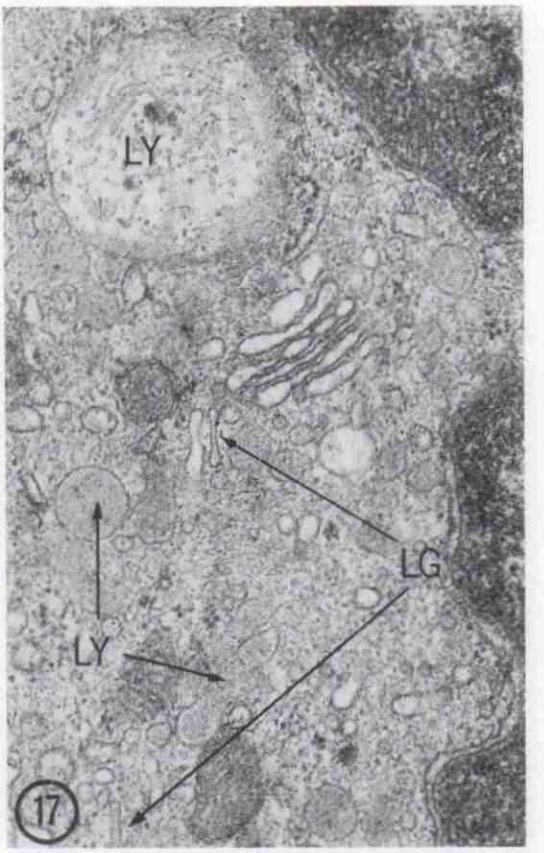
Depigmentation of hair follicles by local injection of HQ appears to result from a selective destruction of melanocytes, decreased synthesis of melanosomes, and synthesis of abnormal-appearing melanosomes. To differentiate the degenerating changes of melanocytes resulting from HQ treatment, it is, however, essential to distinguish the ultrastructural changes that ensue as a result of saline injection or epilation. These changes are, therefore, discussed sequentially.

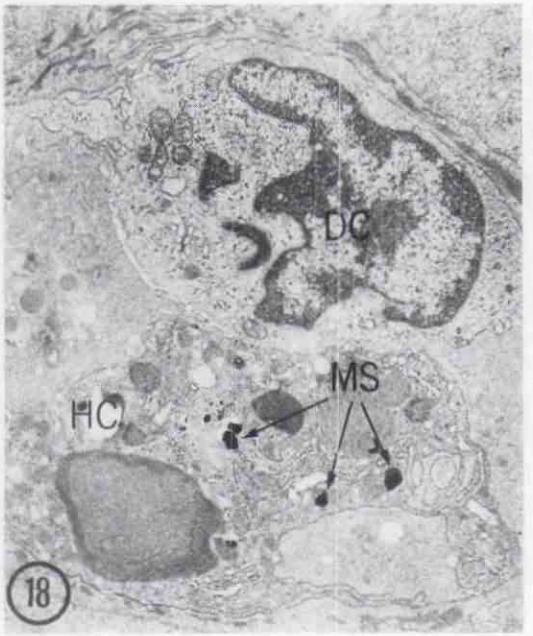
Effect of saline injection on melanocytes and keratinocytes in nonepilated hair. The melanocytes of hair matrices are located above the basal lamina of the dermal papilla, and, in the growing hair, they appear to be actively engaged in melanogenesis. They exhibit well-developed long dendrites and contain melanosomes in various developmental stages. Highly melanized melanosomes are particularly prominent (Fig. 21). The melanosomes are usually singly dispersed in keratinocytes and are found in the cortical as well as the medullary cells. Within cortical and medullary keratinocytes, the melanosomes were markedly electron-dense and ellipsoidal in shape. There was no evidence of any degradation of melanosomes.

The daily injection of 2.0 ml of saline for 8 days induced widening of the intercellular spaces between differentiated keratinocytes in the outer root sheath and of the immature, undifferentiated keratinocytes above the basal lamina in the hair matrix. The melanocytes and keratinocytes inside the hair matrix did not, however, show any changes. Neither the fine structure nor the developmental sequence of melanization was affected, although some melanosomes occasionally showed a widening and swelling of the outer membranes.

Effect of epilation on melanocytes and keratinocytes. Silver et al [19] observed marked mitotic activity in the hair matrix 18 hr after plucking of hair follicles. It is known that regrowing epilated hair follicles are darker than nonepilated follicles [11]. On day 8 after epilation, the melanocytes in the hair matrix appeared to be actively engaged in the formation of new melanosomes. Although the melanosomes were increased in number and highly electron-dense (indicating increased melanization), in shape and size they







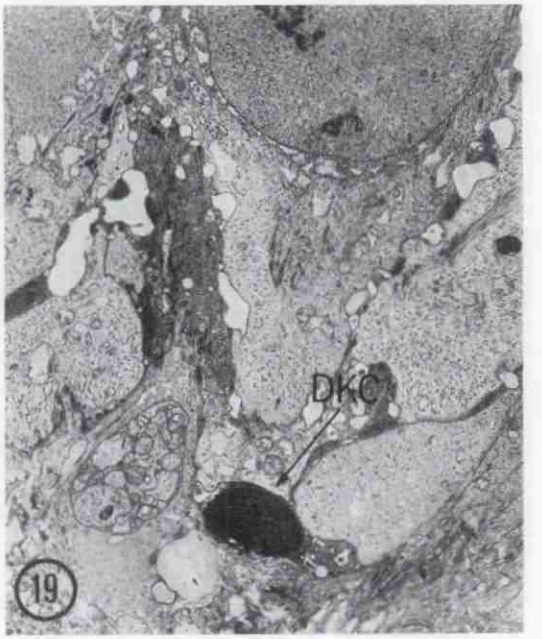


FIG. 16: A Langerhans cell in the depigmented skin after topical application of 2% HQ for 3 weeks. Note that the dendritic processes (DP) of the Langerhans cell (LC) are well developed. (× 10,250)

FIG. 17: A high-power view of the Langerhans cell shown in Fig. 16. Lysosomal granules (LY) of various sizes are abundant; Langerhans granules (LG) are few. (× 24,000)

FIG. 18: Depigmented skin after topical application of 5% HQ for 3 weeks. There are two dendritic cells, a histiocytic cell (HC), and a dendritic cell of unknown nature (DC). The histiocytic cell contains the aggregates of melanosomes (MS). (× 9,500)

FIG. 19: Depigmented skin after topical application of 5% HQ for 3 weeks. There are no easily recognizable melanosomes at the epidermodermal junction. A degenerating keratinocyte (DKC) is seen. (× 6,800)

were similar to those of the melanosomes of the nonepilated hair.

The prominent difference observed between the epilated and nonepilated hairs without HQ injection was that keratinocytes in the upper hair matrix of the epilated hair contained dense aggregates of melanosomes in various developmental stages. There were also some large vacuolated bodies, similar in structure to those observed in necrotic cell debris. Large aggregates of melanosomes were often seen in the papillary cells and fibroblastic cells around the hair follicles. Although small aggregates of the degraded melanosomes can sometimes be seen in normal nonepilated hair follicles, these large aggregates of melanosomes and dense bodies observed in the epilated hair were unusual and may be due to cellular destruction resulting from the mechanical process of epilation.

Effect of HQ on melanocytes and melanosomes in the nonepilated hair. After 8 days of daily injection of HQ, the melanocytes in the nonepilated hair matrix showed marked ultrastructural changes in comparison with the surrounding keratinocytes. The undifferentiated or the differentiated keratinocytes revealed no remarkable intracellular changes. The ultrastructure of their intracytoplasmic organelles appeared normal.

Hydroquinone appeared to affect the follicular melanocytes directly either through destruction and decreased formation of melanosomes or through the formation of abnormal melanosomes. We could see a number of melanocytes that exhibited apparent destruction of membranous organelles and aggregation of nuclear chromatin (Fig. 22). The degeneration of the cytoplasmic membrane resulted in the dissociation of melanocytes from the surrounding normal-appearing melanocytes and keratinocytes. Among these dissociated intercellular spaces, myelin-like granules were seen. Degenerating melanocytes were sometimes observed as early as day 4 after HQ injection.

The remaining functioning melanocytes, which were usually more numerous than the degenerating ones when observed at low magnification, appeared to synthesize normal-looking melanosomes and contained normal-appearing membranous organelles as well as mitochondria. At high magnification, however, these melanosomes showed great abnormality in their ultrastructure, very similar to that observed in epidermal melanocytes (Fig. 23).

The melanosomes in the early stages of development had unmelanized lamellae with normal, regular striations, but in the later stages of development, when melanization occurred, their lamellae became irregular and disorganized. The outer membrane of unmelanized and melanized melanosomes showed degenerative changes and were swollen. Between the degenerating membranes and lamellae, myelin-like granules were aggregated.

Biopsy specimens taken at one week after the cessation of HQ injections showed clearly the reversible nature of HQ depigmentation. The hair matrix above the basal lamina was replaced by actively functioning melanocytes and normal-appearing melanosomes. The degenerating melanocytes were pushed aside and appeared at the upper part of the hair matrix surrounded by cortical keratinocytes (Fig. 24).

Effect of HQ on melanocytes and keratinocytes after epilation. The two stimuli, mechanical (epilation) and chemical (HQ injection), caused marked destruction of melanocytes and keratinocytes (Fig. 25). Huge aggregates of melanosomes, both immature and mature, and cell debris were found in the keratinocytes and intercellular spaces among the keratinocytes. The keratinocytes containing these aggregates of melanosomes also showed degenerative changes such as intracytoplasmic vacuolation, aggregation of myelin-like granules, and sometimes disruption of cellular membranes. The large, round dense bodies containing aggregated tonofilaments, degraded melanosomes, and cell debris were also often found in the intercellular spaces (Fig. 26). The melanocytes not completely destroyed by HQ injection showed less-developed cytoplasmic organelles and very few melanosomes, suggesting decreased activity of melanosome formation. In the papillary and perifollicular areas, aggregates of melanosomes and cell debris were found.

Effect of topical application of HQ to hair. After a long period of topical application of HQ to the epilated skin, depigmented (brownish) hair occurred rarely in the depigmented skin. In these hair follicles, melanocytes could be found above the dermal papillae (Fig. 27). These melanocytes contained abnormal-appearing oval melanosomes and the melanosomes transferred into the keratinocytes of cortex and medulla were remarkably degraded (Fig. 28).

It has been reported that the topical application of depigmenting agents did not affect the melanocytes of the hair follicles [5]. Our present observations, however, revealed that the follicular melanocytes occasionally do show definite ultrastructural changes after a long period of topical application of HQ (more than 4 weeks).

DISCUSSION

Previous studies have suggested that the primary action of HQ is directed at the tyrosinase in the melanocytes [9]. This selective inhibition of the enzyme by HQ presumably affects melanogenesis and the melanocytes, resulting in the subsequent cessation of melanin formation and depigmentation (enzyme-mediated depigmentation) [9]. Additional studies [13, 14] revealed that the primary action of HQ is on some essential subcellular components or vital metabolic proc-

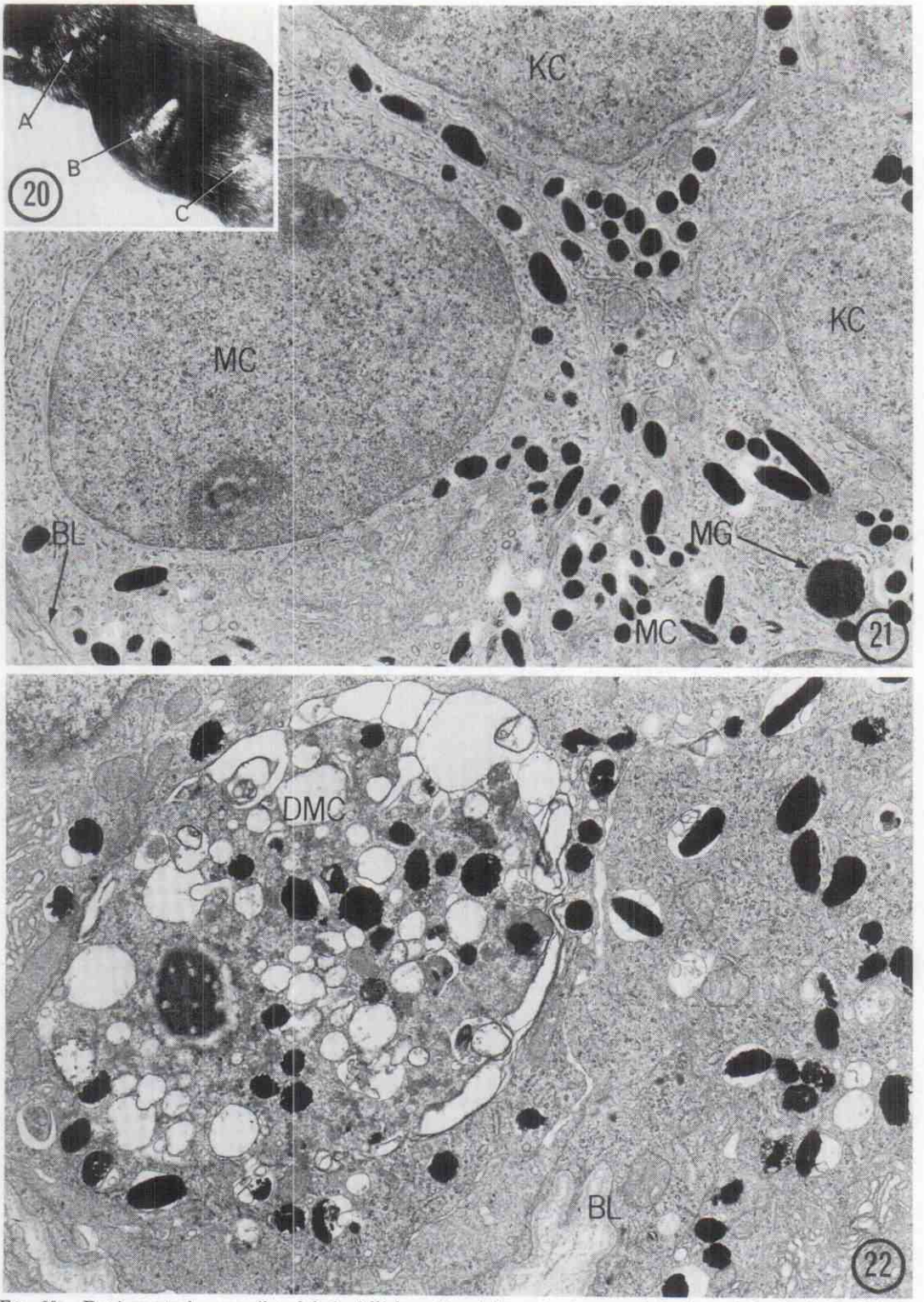
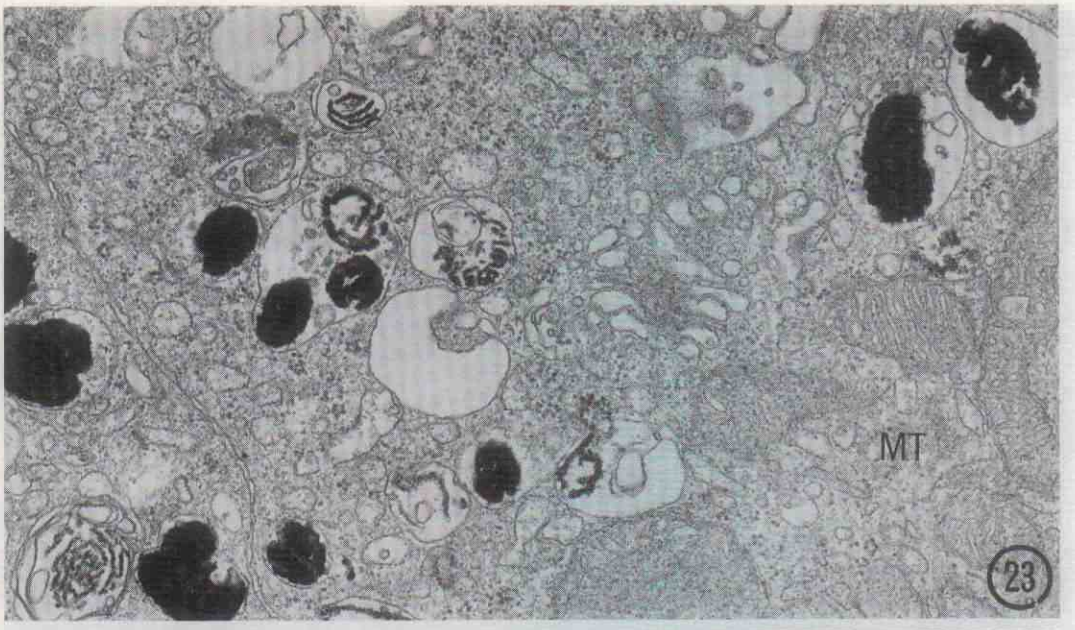


FIG. 20: Depigmented, nonepilated hair follicles after daily subcutaneous injection of 2 ml of 1% HQ for 8 consecutive days. *A:* 12 days after cessation of HQ injection; *B:* 7 days after cessation of HQ injection; *C:* on day of cessation of HQ injection.

FIG. 21: Normal nonepilated hair bulb of a black guinea pig. The melanosomes in the melanocytes (MC) are mostly fully melanized and ellipsoidal. They are highly electron-dense. The melanocytes have well-developed membranous organelles. Melanosomes in the keratinocytes (KC) show no degradation and are singly distributed. BL = basal lamina, MG = medullary granule in the medullary keratinocyte. (× 7,100)

FIG. 22: Depigmented, nonepilated hair bulb after 1% HQ injection for 8 days. There is a degenerating melanocyte (DMC) above the basal lamina (BL). (× 12,800)



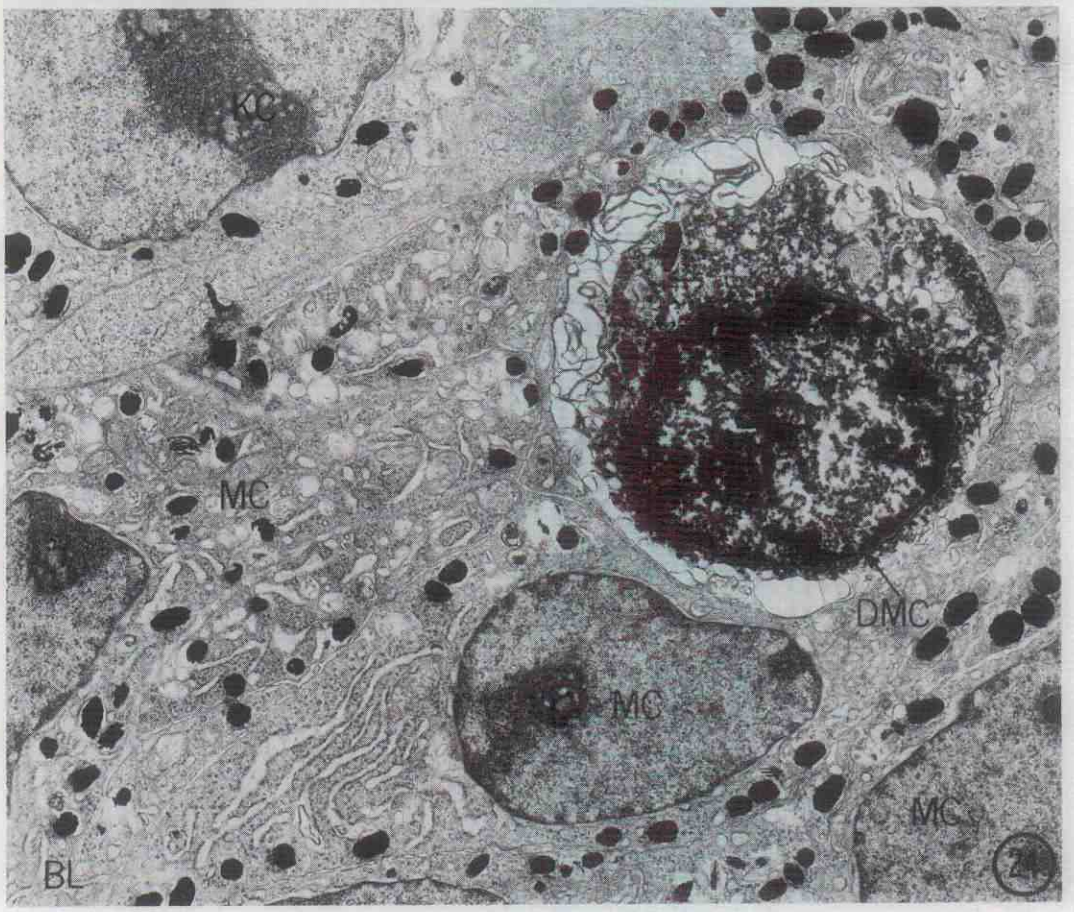


FIG. 23: A high-power view of the surviving, functional melanocyte found in the hair matrix immediately after the cessation of HQ injection for 8 days. Although the melanocyte has normal-looking membranous organelles and mitochondria (MT), it has melanosomes containing irregular and disorganized lamellae. Their outer membrane is swollen, and myelin-like granules are aggregated between the swollen membrane and disorganized lamellae. (× 47,500)

FIG. 24: Growing hair follicle biopsied at day 7 after cessation of HQ injections. The melanocytes (MC) above the basal lamina (BL) show active melanogenesis. The degenerating melanocyte (DMC) is now pushed to the upper part of the hair matrix near the cortical keratinocytes (KC). (× 9,300)

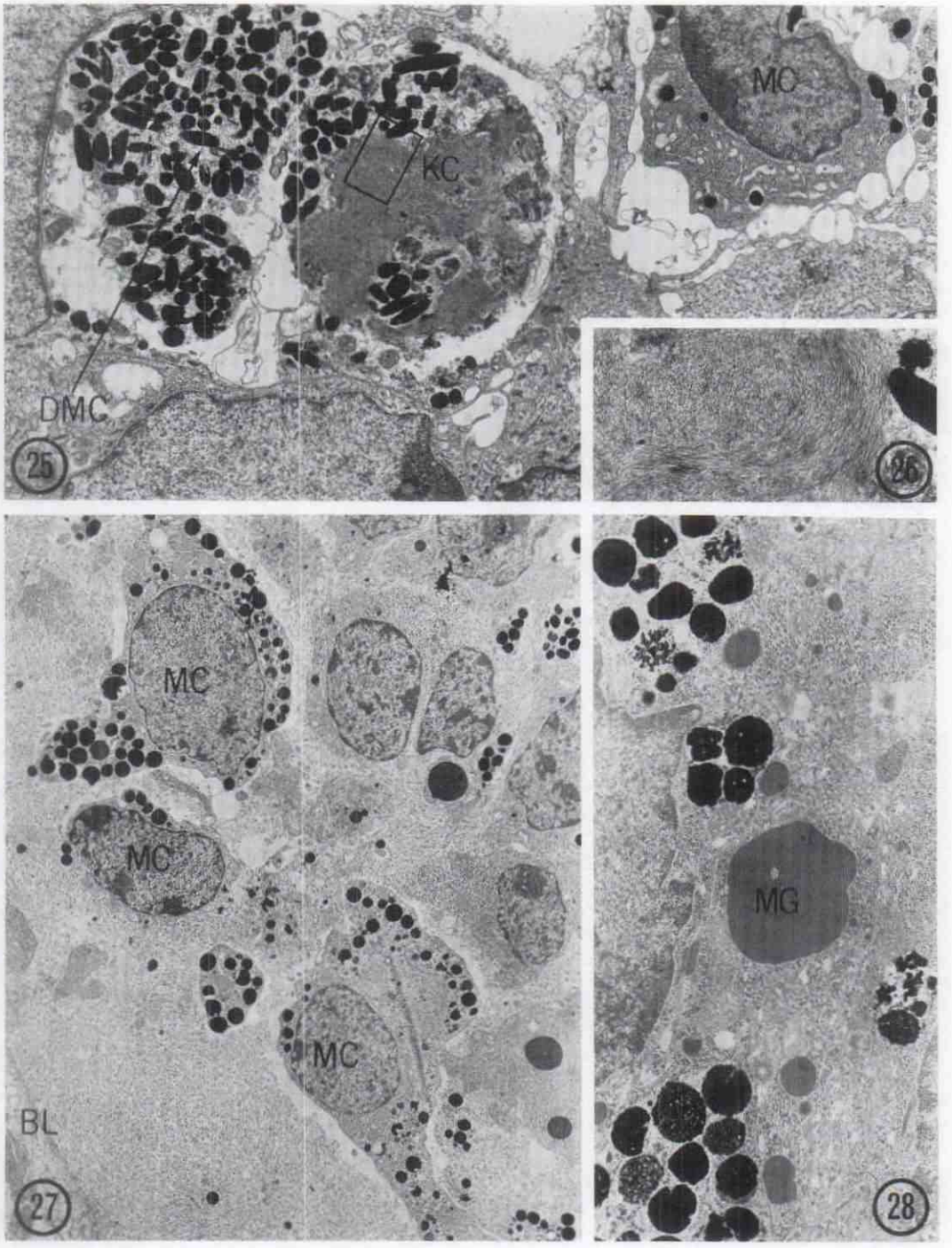


FIG. 25: Depigmented hair matrix occurring after frequent epilation and HQ injection for 8 days. There are aggregated masses with a degenerating melanocyte (DMC) and a degenerating keratinocyte (KC). The surviving, functional melanocyte (MC) appears to be less active in melanogenesis when compared with those melanocytes shown in Figs. 23 and 24. (× 9,000)

FIG. 26: A high-power view of the dense aggregates of tonofilaments encircled in Fig. 25. (× 48,000)

FIG. 27: Hair matrix of depigmented (brownish) hair follicles which occurred in the depigmented skin after topical application of 5% HQ for 4 weeks. Above the basal lamina (BL), there are a few functioning melanocytes (MC) containing abnormal oval melanosomes. (× 3,500)

FIG. 28: The melanosomes in the medullary keratinocytes of the depigmented (brownish) hair in Fig. 27. The melanosomes are aggregated within the membrane-limited vacuoles and show degradation of various degrees. MG = medullary granule. (× 36,750)

esses of melanocytes, with resultant cytolysis (non-enzyme-mediated depigmentation).

The ultrastructural findings reported here establish that the primary action of HQ is directed to the nonfollicular and follicular melanocytes. The migration of the dermal inflammatory cells and an increased function of Langerhans cells can be attributed to a nonspecific inflammation induced by HQ, inasmuch as topical application of HQ and its derivatives is known to cause irritation of the skin [11, 12, 19].

Inhibition of melanogenesis by HQ was documented by demonstration of a decrease in the number of melanized melanosomes and by the formation of abnormally melanized melanosomes. These melanosomes had a markedly altered internal core and appeared to be easily degraded after their transfer to the keratinocytes. The direct intracellular damage to the melanocytes by HQ was demonstrated by the disruption and lytic changes of the membranous organelles of these secretory cells. These events eventually result in the necrosis of the melanocytes.

It has been reported that HQ affects tyrosinase activity and inhibits the enzymatic oxidation of tyrosine to dopa and its subsequent conversion to melanin. Studies in vitro using tyrosine from mouse melanoma revealed that HQ inhibited only the first step in the melanogenesis (conversion to tyrosine to dopa) [9]. The observations of Iijima et al [20] indicated that the second step (oxidation of dopa to melanin by tyrosinase) was also affected by HQ. We believe that the decreased melanization of melanosomes can be attributed to this inhibitory effect of HQ on tyrosinase. It is possible that the inhibition of tyrosinase by HQ subsequently causes cessation of melanin formation, and results in decreased synthesis of melanized melanosomes.

Abnormal melanization of melanosomes could result from many other causes. For example: (1) HQ could, like tyrosine, behave like a substrate and act as a competitive inhibitor of tyrosinase; (2) HQ could combine chemically with the intermediates of melanin and inhibit further melanization; and (3) HQ might cause a focal degradation of melanosomes by generation of free radicals in melanin or its intermediates. All of these possibilities could cause the formation of abnormal melanosomes found in the present study.

Lerner et al [21] reported that HQ as a substrate was relatively less effective than tyrosine or dopa for mammalian tyrosinase, but it could participate as a substrate in partial melanization in the presence of tyrosine. It has been reported that during oxidation of HQ, reactive free radicals (semiquinone-like), capable of disrupting membranes through the enzyme-catalyzed oxidation, can be generated [22]. Lerner et al [21] reported that HQ was readily oxidized in the presence of an active tyrosine–tyrosinase system. In mammalian melanocytes, three types of tyrosinase have been reported: soluble tyrosinase (T1 and T2) and insoluble tyrosinase (T3). T1 and T2 are the enzymes in combination with a structural protein of the melanocytes; T3 is the enzyme associated with the melanosomes [23]. It is conceivable that HQ, when applied topically or injected locally, can produce reactive free radicals in the tyrosine–tyrosinase system of melanosomes and melanocytes; these free radicals could interfere with the oxidation of tyrosine and melanization of melanosomes, and cause a focal degradation of melanosomal matrix and distortion in the configuration of the outer membrane of melanosomes. Indeed, Riley [24] demonstrated that phenol derivatives can give rise, through the enzymatic oxidation by tyrosinase, to free radicals that cause lipid peroxidation and consequent cellular damage. In the present ultrastructural study, nuclear and cytoplasmic membranes of melanocytes were found to be preferentially affected by HQ. Melanocyte necrosis was found to be initiated by the disruption and degradation of the membranous organelles. Further studies are needed, however, to elucidate the biochemical basis of this selective action of HQ on the nonfollicular and follicular melanocytes of guinea pigs.

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