0022-202X/82/7802-0108$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 78:108–115, 1982

Vol. 78, No. 2
Printed in U.S.A.

New Melanogenesis and Photobiological Processes in Activation and Proliferation of Precursor Melanocytes after UV-Exposure: Ultrastructural Differentiation of Precursor Melanocytes from Langerhans Cells

KOWICHI JIMBOW, M.D., PH.D., AND TAKASHI UESUGI, M.D., PH.D.

Department of Dermatology, Sapporo Medical College, Sapporo, Japan

Photobiological processes involving new melanogenesis after exposure to ultraviolet (UV) light were experimentally studied in C57 black adult mice by histochemistry, cytochemistry, and autoradiography. The trunk and the plantar region of the foot, where no functioning melanocytes were present before exposure, were exposed to UV-A for 14 consecutive days. Both regions revealed a basically similar pattern for new melanogenesis which involved an activation of precursor melanocytes. Essentially all of "indeterminate" cells appeared to be precursor melanocytes, the fine structure of which could be differentiated even from poorly developed Langerhans cells. New melanogenesis was manifested by 4 stages of cellular and subcellular reactions of these cells as indicated by histochemistry of dihydroxyphenylalanine (dopa) and autoradiography of thymidine incorporation: (*a*) an initial lag in the activation of precursor melanocytes with development of Golgi cisternae and rough endoplasmic reticulum followed by formation of unmelanized melanosomes (day 0 to 2); (*b*) synthesis of active tyrosinase accumulated in Golgi cisternae and vesicles with subsequent formation of melanized melanosomes in these cells (day 3 to 5); (*c*) mitotic proliferation of many of these activated cells, followed by an exponential increase of new melanocytes (day 6 to 7); and (*d*) melanosome transfer with differentiation of 10 nm filaments and arborization of dendrites, but without any significant change in the melanocyte population (day 8 to 14). The melanosome transfer was, however, not obvious until after 7 days of exposure. The size of newly synthesized melanosomes was similar to that of tail skin where native melanocytes were present before exposure.

New melanogenesis caused by repeated exposure to ultraviolet (UV) light is characterized by a numerical increase in functioning melanocytes in the epidermis as measured by histochemistry of the L-3,4-dihydroxyphenylalanine (dopa) reaction [1]. The trunk and the plantar skin of the adult black mouse may provide an ideal model for elucidating the photobiological processes involving this numerical increase [2]. As early as 1954, Reynolds reported that functioning melanocytes are present in the trunk skin of the neonatal mouse and that these melanocytes disappear at about day 12 postpartum, but that they persist at fixed sites in the epidermis in an "amelanotic state" in the adult [3]. Later, fragments of melanocytes were found in heterophagic vacuoles of keratinocytes after day 4 postpartum [4], indicating that at least some functioning melanocytes are programmed for destruction. However, light microscopic study of repeated exposure of the trunk and plantar skin of the same adult mouse to UV-light (280-315 nm) revealed a numerical increase in functioning melanocytes and suggested that this increase derives from both activation and proliferation of melanocytes [5]. Electron microscopic studies of epidermal dendritic cells indicated that the trunk skin in the hairless mouse possesses "indeterminate" dendritic cells which may give rise to either Langerhans cells or melanocytes occurring after topical application of chemical agents (dinitrochlorbenzene: DNCB) and/or exposure to UV-light (257.7 nm) [6, 7]. In a recent autoradiographic study with cumulative labeling of thymidine it was proposed that essentially all of the increase in epidermal melanocytes in the ear skin of the black mouse after UV-exposure (290-350 nm) resulted from the proliferation of functioning melanocytes [8].

These previous studies, however, have left several questions unanswered. These are (a) what are the ultrastructural characteristics indicative of the precursor melanocytes in terms of differentiation from Langerhans cells, (b) what are the sequential subcellular processes during the activation of the precursor melanocytes from dopa-negative to dopa-positive states and (c) when and how does mitotic replication of melanocytes occur during the course of UV-exposure? To elucidate answers to these questions, we have carried out experiments in which UV-A was exposed to 2 sites on the adult black mouse: (a) the dorsal skin of the trunk, where hair follicles are abundant, and (b) the plantar skin of the hind foot which is the site essentially free from hair follicles and sweat glands.

MATERIALS AND METHODS

Animals and UV-Light

The dorsal surface of the trunk skin and the plantar surface of the hind foot of inbred C57 black male mice (C57BL/6, 8- to 10-weeks old) were daily exposed to UV-light (300–400 nm) by a Dermaray M-DMR-1 (Toshiba Inc., Japan). Approximately 98% of the emission energy of the lamp was above 320 nm, with its peak emission being 352 nm. The mice were irradiated for 30 min on each of 14 consecutive days at a distance of 15 cm from light source to object. Each exposure delivered 6.12 J/cm^2 of energy. During exposure, each mouse was fixed on a board. At 3 weeks before the experiments, the hair of the exposed areas was plucked by a paraffin-Rosin mixture and the hair cycle was synchronized. At 2 days before exposure, the hair was again plucked. During the course of the experiments, cages were shielded to avoid exposure to any external light except during the time of the experiments.

Tissue Preparation

Under ether anesthesia, nonexposed and UV-exposed skin was biopsied 12 hr after 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, and 14 daily exposures to UV-light. The biopsied specimens were cut into small pieces and processed for (a) light microscopy of hematoxylin-eosin and Fontana-Masson's silver staining, dopa histochemistry and autoradiography of thymidine incorporation combined with dopa histochemistry, and (b) routine electron microscopy and dopa-cytochemistry.

Histochemistry of dopa: Dopa histochemistry was carried out on vertical and horizontal preparations of the biopsied tissues. Vertical

Manuscript received March 16, 1981; accepted for publication June 30, 1981.

This work was supported by Grants-in-Aid for Cancer Research (#548215) from the Ministry of Education, Science and Culture, and (#55-22) from the Ministry of Health and Welfare, Japan.

Reprint requests to: Kowichi Jimbow, M.D., Ph.D., Department of Dermatology, Sapporo Medical College, Minami 1, Nishi 16, Sapporo 060, Japan.

sections were made by sectioning frozen tissues of 7 μm in thickness after fixing with 5% formalin in 0.1 M phosphate buffer, pH 7.4 for 1 hr, with the sections then being incubated with 0.1% L-3,4-dopa in 0.1 M phosphate buffer, pH 7.4, for 4 hr, at 37°C. A horizontal view of the whole epidermal sheet was prepared by means of a split-dopa technique [9] in which the epidermal sheet of the unfixed tissues was split with 2 N NaBr solution at 37°C and was incubated with 0.1% L-3,4-dopa in 0.1 M phosphate buffer, pH 7.4, for 4 hr. The dopa preparation was then fixed with 5% neutral formalin solution, dehydrated by alcohol solutions and counterstained. Control specimens for dopa histochemistry included (a) heat-inactivated tissues (60°C, for 2 min) and (b) tissues in which substrate was omitted.

Electron microscopy: For routine electron microscopy, small pieces of tissue were fixed with 2% osmium tetroxide in s-collidine buffer, pH

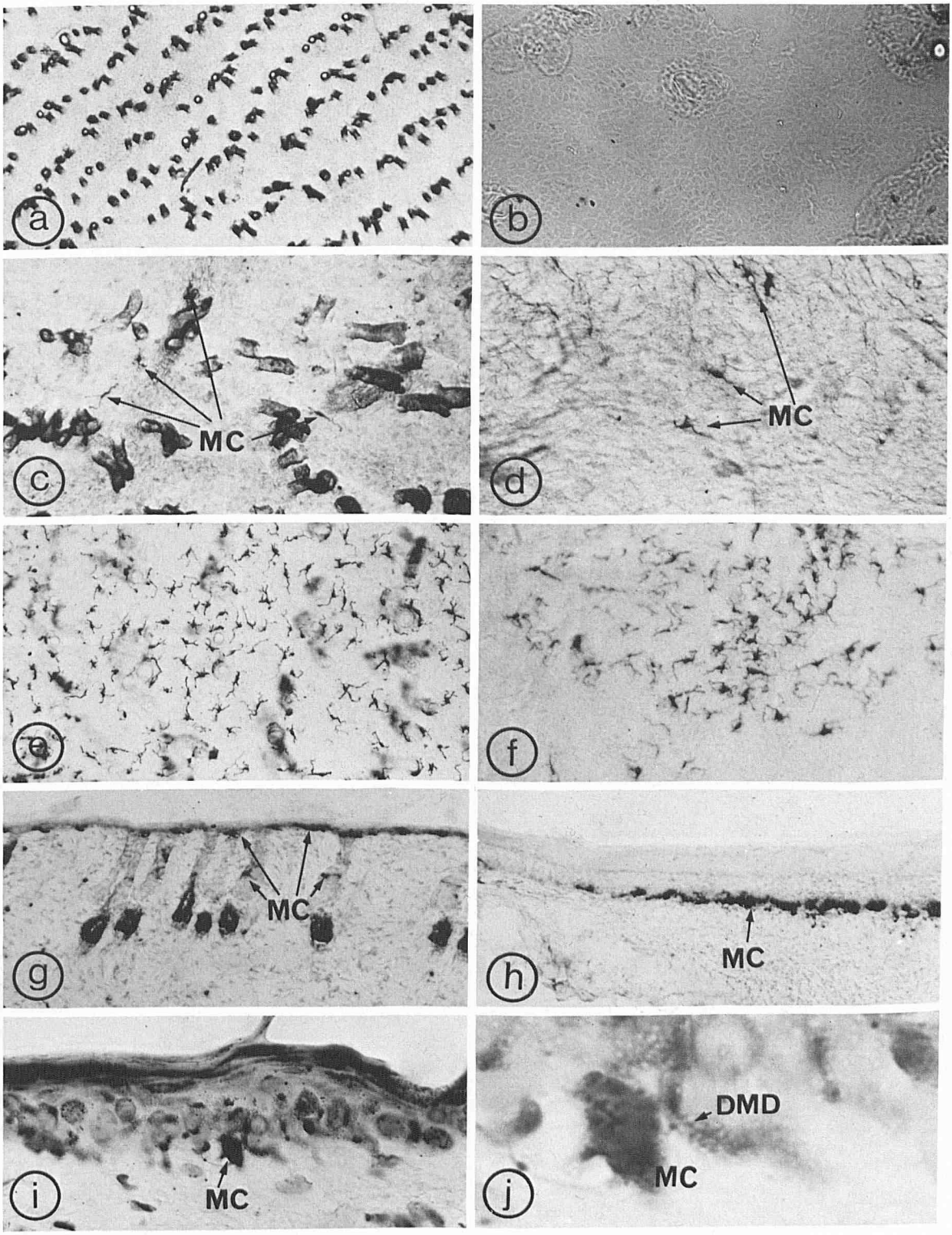

FIG 1. Dopa histochemistry of melanocytes in dorsal and plantar areas of skin before and after UV-exposure. *a–f*, Split dopa-preparation, *g–j*, vertical dopa-preparation. *a*, Dorsal skin before exposure (× 320); *b*, Plantar skin before exposure (× 320); *c*, dorsal skin at day 3 (× 320); *d*, Plantar skin at day 3 (× 320); *e*, dorsal skin at day 14 (× 280); *f*, Plantar skin at day 10 (× 320); *g*, dorsal skin at day 14 (× 320); *h*, Plantar skin at day 10 (× 280); *i*, dorsal skin at day 7 with thymidine labeling, focussed in perikaryon (× 450); *j*, the same specimen of Fig 1-*i*, but focussed in silver grain (× 960). *MC*, dopa-positive melanocytes and *DMD*, dopa-positive melanotic dendrite.

7.2, for 2 hr in ice. For cytochemistry of the dopa reaction, tissues were first fixed with 0.2% glutaraldehyde-0.2% formaldehyde in 0.1 M cacodylate buffer, pH 7.4, for 2 hr in the ice, washed extensively with 0.1 M phosphate buffer, pH 7.4 and incubated with 0.1% L-3,4-dopa in 0.1 M phosphate buffer, pH 7.4 for 4 hr at 37°C. They were then postfixed with 1% osmium tetroxide in 0.1 M cacodylate buffer, pH 7.2, for 2 hr in ice. The fixed tissues were stained en bloc with 1.5% uranyl acetate in 0.1 M veronal buffer, pH 7.2, for 30 min, dehydrated by alcohol solutions, mounted in epoxy resin, ultrasectioned with a Porter Blum microtome, counterstained with lead citrate and observed with a Hitachi electron microscope.

Autoradiography of thymidine incorporation combined with dopa histochemistry: Thymidine (2 μCi/g weight of [methyl-^{3}H] thymidine, specific activity: 20 Ci/m mole, New England Nuclear Inc., Boston) was prepared in normal saline and was injected subcutaneously into the dorsal skin just before 3, 6, and 10 exposures to UV. At 6 hr after irradiation, the exposed skin was biopsied and fixed with 0.2% glutaraldehyde-0.2% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.2, for 2 hr. The fixed tissues were sliced, washed with 0.1 M phosphate buffer, pH 7.4, incubated with 0.1% dopa in 0.1 M phosphate buffer, pH 7.4, for 4 hr at 37°C, dehydrated with alcohol solutions, mounted in paraffin, sectioned and processed to the autoradiography of our previously described method [9]. Briefly, the dopa-incubated sections were exposed to NTB-2 emulsion (Eastman Kodak Co., Rochester, NY) for 14 days at 4°C, developed with D19 solution (Eastman Kodak Co.) and counterstained with hematoxyline solution. The control specimens for dopa histochemistry were the same as those of the split-dopa preparations. Only nuclei with 10 or more grains were counted as labeled.

RESULTS

Light and electron microscopic findings were essentially the same in both dorsal and plantar skin and will be described together.

Before Exposure to UV-A

Light microscopy: The dorsal and plantar skin of black mice was pale and amelanotic. Split and vertical preparations with dopa histochemistry did not reveal any dopa-positive melanocytes in either the epidermis or dermis, including the outer root sheath of hair follicles of the dorsal skin (Fig 1-*a, b*). In contrast to a previous report by Gerson and Szabo [10], the plantar skin of our experimental mice did not contain any melanocytes in the epidermis as well as dermis. Similarly, no argyrophylic granules of melanin were shown in any of the tissues examined by Fontana's silver staining. Preliminary experiments indicated that repeated epilation does not cause any visible melanin pigmentation nor does it produce any functioning melanocytes with melanin synthesis during the entire course of experiments.

Electron microscopy: No melanosomes were identified in the entire skin before irradiation. In the basal layer of the dorsal and plantar skin, however, there were a certain number of "indeterminate" dendritic cells (Fig. 2-*a, b*). In general, these cells were found among about 8 to 10 keratinocytes along the

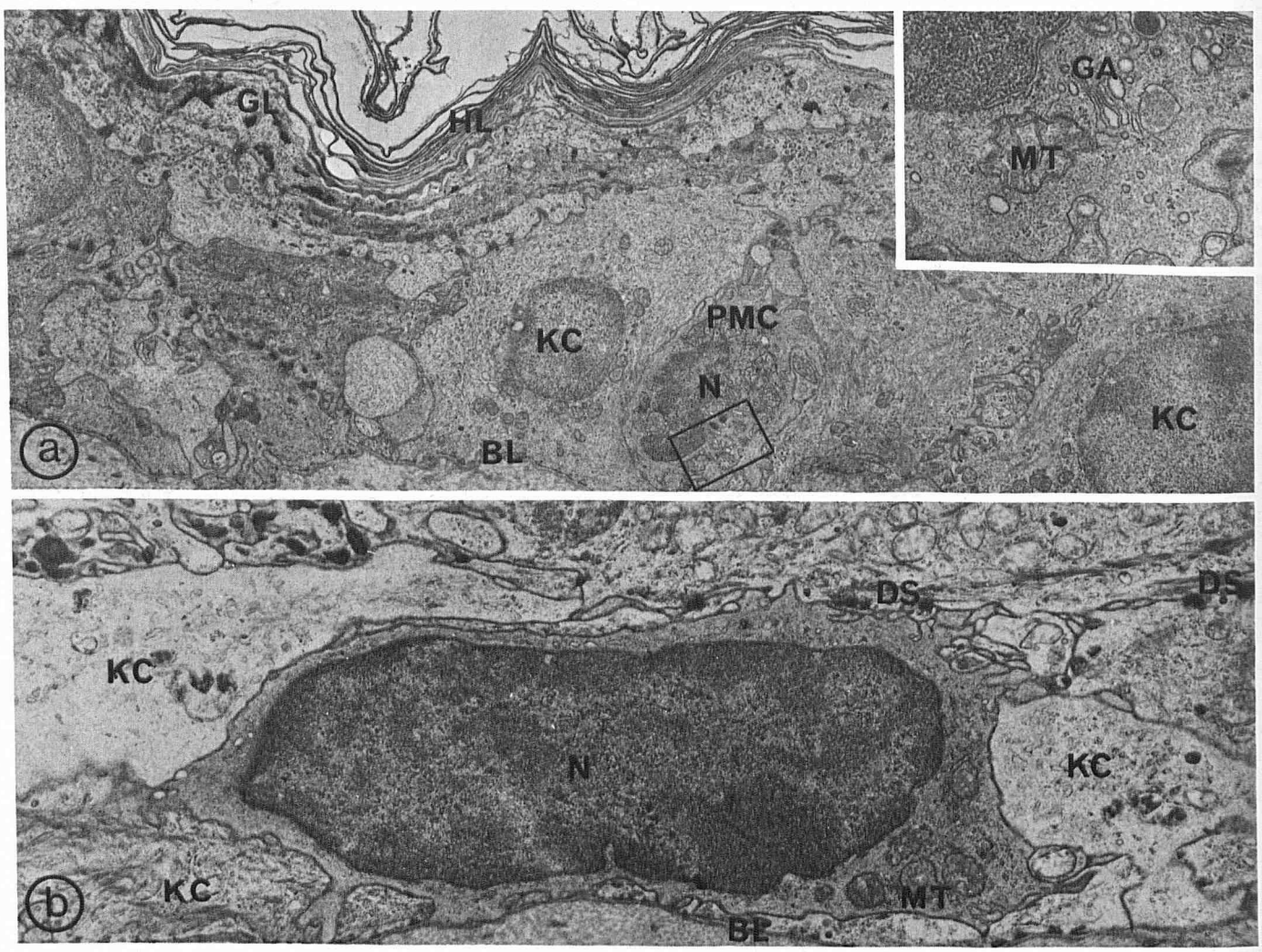

FIG 2. Fine structure of precursor melanocytes ("indeterminate" cells) before UV-exposure. *a*, A low power view of the entire epidermis demonstrating the location of the precursor melanocyte (*PMC*). An *insert* (× 29,000) indicates a high-power view of the *encircled area of Fig 2-a* showing a poorly developed Golgi apparatus (*GA*) (× 3,400). *b*, A precursor melanocyte above the basal lamina (*BL*) showing poorly developed organelles and dendrites, which, however, appear rather as pseudopods (× 16,000). *HL*, horny layer; *GL*, granular layer; *N*, nucleus; *KC*, keratinocyte; *MT*, mitochondria; and *DS*, desmosome.

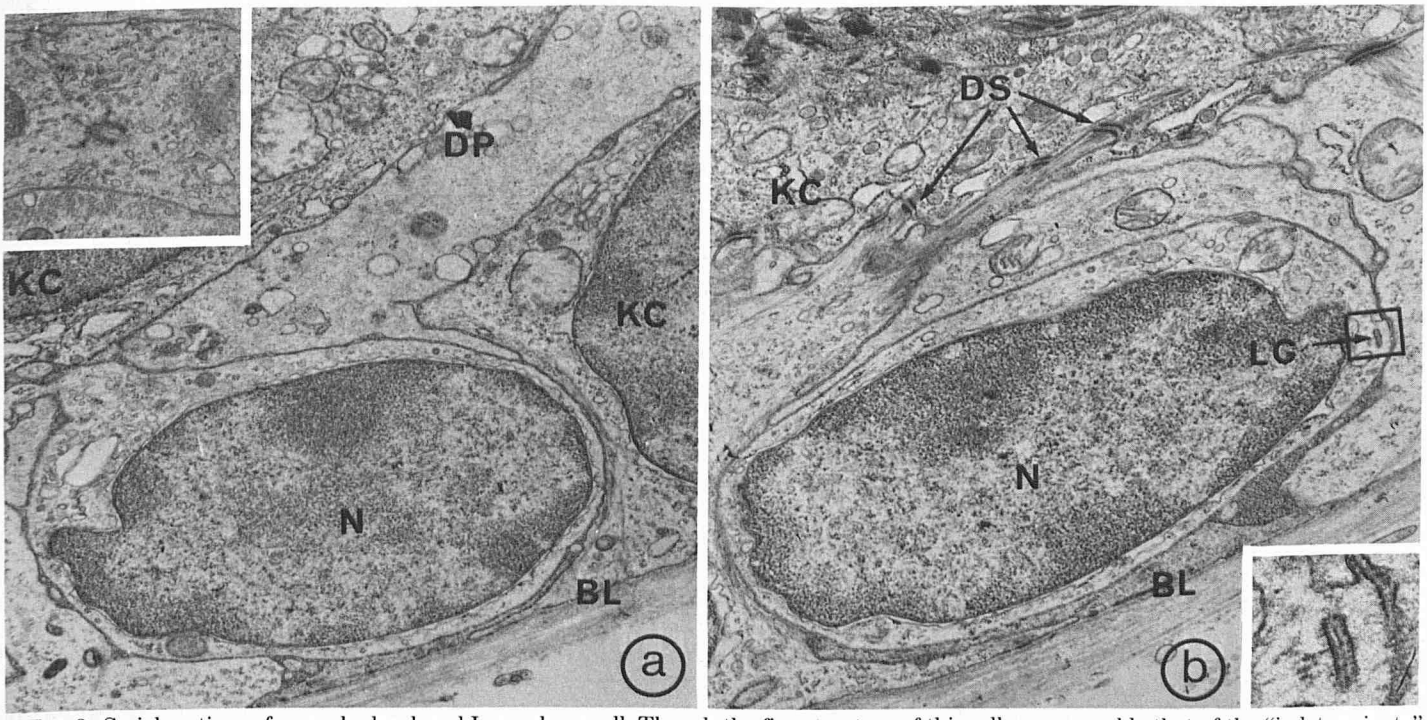

FIG 3. Serial sections of a poorly developed Langerhans cell. Though the fine structure of this cell may resemble that of the "indeterminate" cell, the cell was identified as a Langerhans cell by serial sectioning. The biopsy was obtained before exposure. *a*, The cell with a well-developed, large dendritic process (*DP*), but without Langerhans-cell granules (× 11,000). The *insert* shows serial section of the cytoplasm of the same cell,which reveals a relatively well-developed organelles (× 12,500). *b*, The cell with one Langerhans-cell granule (*LG: insert*, × 77,000) and a poorly developed dendrite (× 12,000). *KC*, keratinocyte; *BL*, basal lamina; and *N*, nucleus.

basal lamina and were devoid of any specific organelles such as melanosomes, Langerhans-cell granules or desmosome complexes (Fig 2-*a*). Cytoplasmic organelles such as endoplasmic reticula, ribosomes and Golgi complexes were poorly developed (Fig 2-*b*). Usually Golgi complexes were not visible, and if seen, formed 2 to 3 layers of poorly differentiated cisternae (Fig 2-*a*). Rarely the dendritic cells were found among 4 to 5 basal keratinocytes. However, one of these cells would often reveal better developed cytoplasmic organelles and, by serial sectioning, a few specific granules such as seen in Langerhans cells (Fig. 3-*a, b*).

The nucleus of the "indeterminate" cells was large, occupying almost the whole cytoplasm (Fig 2-*b*), and was usually oval and smooth in contour. Heterochromatin was coarsely scattered, and the nucleolus was poorly developed and hardly visible. The dendritic processes were poorly developed, occasionally appearing as pseudopods or villous processes because of poor development. Microtubules were rarely seen. Several cells revealed a small number of 10 nm filaments which tended to aggregate around the nucleus. None of these cells demonstrated any tyrosinase activity by dopa cytochemistry. Absence of Langerhans-granules was always confirmed by serial sectioning.

The "indeterminate" cells were easily differentiated from Langerhans cells which were usually seen in the suprabasal layer. The Langerhans cells revealed a nuclear contour and chromatin pattern similar to those of the indeterminate cells (Fig 3-*a, b*), but always possessed better developed dendrites and organelles such as rod and/or racket shaped granules, dense tubules, and 10 nm filaments. In several cases, the organelles of Langerhans cells were poorly developed and no Langerhans-cell granules were seen. These cells, however, always revealed better developed dendrites and a few Langerhans-cell granules by serial sectioning (Fig 3-*a, b*).

After UV-A Exposure

Light microscopy: The dorsal and plantar skin became brownish pigmented after 7–8 consecutive exposures to UV-A. Dopa-preparations and Fontana's silver staining indicated that

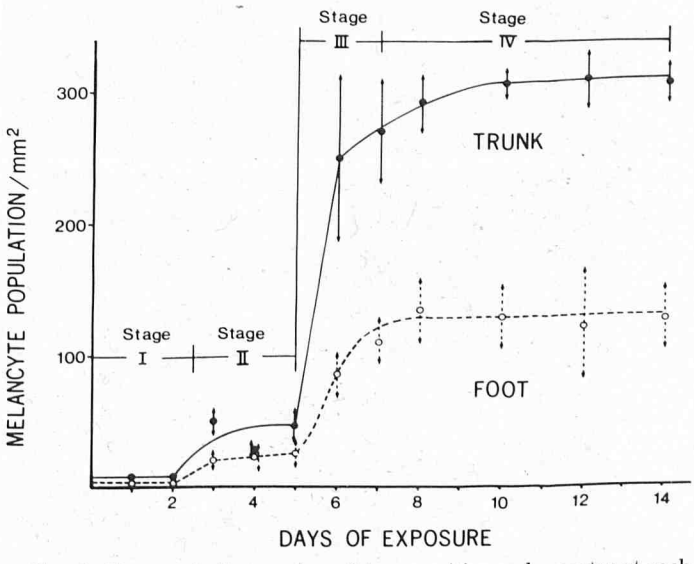

FIG 4. Changes in the number of dopa-positive melanocytes at each of 14 consecutive exposures. Stage I: day 0 to 2; Stage II: day 3 to 5; Stage III: day 6 to 7; Stage IV: day 8 to 10.

the brownish pigmentation of the skin after UV-exposure was related to the presence of melanin pigments produced by functioning melanocytes in the basal layer of the skin (Fig 1-*c, d, e, f, g, h*). No dermal melanocytes were found in either the dorsal or plantar skin during the entire course of UV-exposure. In the dorsal skin, however, dopa-positive melanocytes were also seen in the hair follicles (Fig 1-*g, h*). Split-dopa preparations of both dorsal and plantar skin revealed the same pattern of numerical increase in functioning melanocytes (Fig 1-*c, d, e, f*). Their numerical increase per mm^2 of skin was arbitrarily divided into 4 stages (Fig 4); In stage I, i.e., from day 0 to 2 of exposure, no functioning melanocytes were yet visible; In stage II, from day 3 to 5, a small number of melanocytes became visible; In stage

III, from day 6 to 7, the number of melanocytes increased exponentially, reaching almost 5- to 7-fold as compared with that at stage II; later on, however, at stage IV, i.e., from day 8 to 14, the numerical increase of melanocytes became stationary. Melanocytes in the dorsal skin were more numerously seen than in the plantar skin, due to the fact that both epidermal and follicular melanocytes of the dorsal skin were often counted in the same specimen (Fig 1-*e*, *g*, and 4). These findings indicated that new melanogenesis after UV-exposure on the dorsal and plantar skin is due to numerical increases in functioning melanocytes in the epidermis and in the melanin pigments elaborated by them.

To examine whether a rapid numerical increase in functioning melanocytes at stage III of UV-exposure derives from the

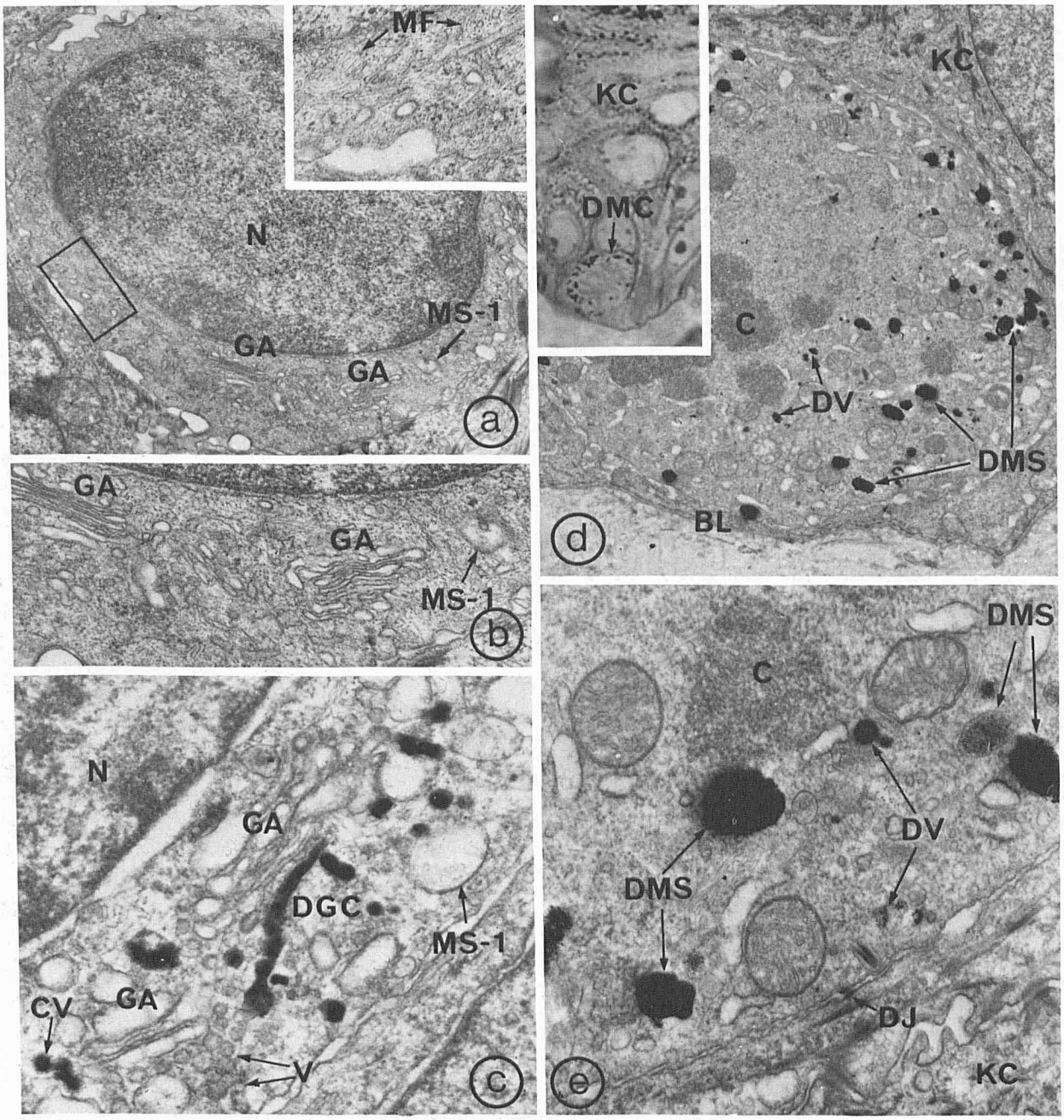

FIG 5. Dopa cytochemistry demonstrating the subcellular processes in activation and proliferation of precursor melanocytes at stages II and III of UV-exposure. *a*, A slightly activated precursor melanocyte at day 3 of exposure. A control specimen of dopa cytochemistry in which the substrate was omitted. No melanized melanosomes were yet synthesized, though Golgi apparatus (*GA*) became multilayered, rough endoplasmic reticulum became better developed, and a large vacuole accumulated with tiny vesicles and proteinaceous material such as seen in the earliest stage of melanosomes development (MS-1) was present. An *insert* (× 95,000) shows an aggregation of 10 nm microfilaments (*MF*) around the nucleus (*N*) (× 14,000). *b*, A high-power view of Golgi apparatus (*GA*) and a vacuole resembling the earliest stage of melanosome (*MS-1*) seen in Fig 5-*a* (× 96,000). *c*, An activated, dopa-positive melanocyte at day 5 of exposure. The outermost cisterna of Golgi apparatus (*GA*) is dopa-positive (dopa-positive Golgi cisterna: *DGC*). Numerous vesicles (*V*), one of which is coated and dopa-positive (coated vesicle: *CV*), are scattered around the Golgi apparatus. Around the organelles, the vacuole resembling the earliest stage of melanosome (*MS-1*) is present (× 23,000). *d*, A mitotic melanocyte at day 7 of exposure. It locates above the basal lamina (*BL*) and carries dopa-positive vesicles (*DVC*) and melanosomes (*DMS*). An *insert* (× 650) shows the location of a dividing melanocyte (*DMC*) seen in the electron micrograph (× 8,600). *e*, A high-power view of a dividing melanocyte. Around the nuclear chromatin (*C*), dopa-positive vesicles (*DV*) and melanosomes (*DMS*) in various stages of development are seen. The cell also possesses a desmosome-like junction (*DJ*) with surrounding keratinocyte (*KC*) (× 38,000).

activation of pre-existing precursor melanocytes or from a combination of activation and mitotic proliferation of these melanocytes, the melanocytes in the S-phase of mitosis were labeled with thymidine and identified by a dopa histochemistry. The mitotic index of dopa-positive melanocytes by a single labeling of the thymidine was 2.15% ± 1.45% at day 6 and 1.65 ± 1.20% at day 10. None of the dopa-positive melanocytes showed significant thymidine labeling at day 3 of exposure.

Electron microscopy: Sequential events that might be indicative of activation of the "indeterminate" cells to functioning melanocytes were observed by electron microscopy. At stage I of UV-exposure, none of the "indeterminate" cells revealed any obvious ultrastructural changes indicative of melanogenesis, though rarely they did possess a large vacuole with tiny vesicles such as those reported in the earliest developmental stage of melanosomes [11]. At stage II, however, these cells became more activated and produced many organelles (Fig 5-*a, b, c*). The differentiation of melanogenic organelles appeared to initiate from the better development of Golgi cisternae, which became composed of 4 to 5 layers of cisternae, and of rough endoplasmic reticulum. The outermost of the cisternae was dopa-positive. Around the cisternae, there were numerous vesicles, some of which were either coated, dopa-positive or both (Fig 5-*c*). These cells, however, had not yet produced any melanized melanosomes with characteristic lamellar patterns. In addition, these cells revealed a number of large dopa-negative vacuoles which were aggregated with proteinaceous materials and tiny vesicles such as seen in the earliest form of melanosomes (Fig 5-*b, c*). Beside better developed Golgi complexes, these cells showed 10 nm filaments more numerously (Fig 5-*a*). At stage III, the dopa-positive cells became more activated, having several dopa-positive Golgi cisternae and numerous dopa-positive vesicles as well as melanosomes in various stages of development. However, the melanosome transfer from melanocytes to keratinocytes was not obvious until day 7 of exposure. On several occasions, melanocytes under replication were seen at stage III (Fig 5-*d, e*). Dividing melanocytes possessed dopa-positive melanosomes, Golgi cisternae and vesicles. These cells occasionally revealed desmosome-like junctions with neighboring keratinocytes (Fig 5-*e*).

At stage IV, the melanocytes were full of dopa-positive melanosomes, Golgi cisternae and vesicles (Fig 6-*c, d*). Often the entire cytoplasm was diffusely occupied with dopa-reaction products. The melanosomes transferred into keratinocytes became numerous, forming either the aggregated (melanosome complex) or non-aggregated form. The size of the melanosomes, which was calculated by our previously reported method [12], was similar to that seen in control, nonexposed tail skin; It was estimated as 453 ± 86 nm (standard deviation) in the long axis with 228 ± 45 nm in the short axis at day 3, 486 ± 96 nm in the long axis with 301 ± 57 nm in the short axis at day 7 and 489 ± 118 nm in the long axis with 276 ± 51 nm in the short axis at day 14. Control specimens of tail skin before exposure revealed melanosomes of similar sizes ranging from 429 ± 99 nm in the long axis and 219 ± 32 nm in the short axis.

DISCUSSION

This study confirmed that previously held concept that the epidermis of the adult black mouse contains a fixed population of precursor melanocytes and indicated that new melanogenesis after repeated UV-exposure involves both activation and proliferation of these cells. Our previous study [13] has already ruled out the possibility that new melanocytes originate from either dermis, sweat glands or from both, though the precursor melanocytes from hair follicles may account for some source of new melanocytes in the hairy trunk skin. This study, by electron microscopy and cytochemistry, differentiated the fine structure of precursor melanocytes from that of Langerhans cells and characterized the subcellular processes during activation and proliferation of precursor melanocytes after UV-exposure.

Wolff and Winkelmann [14], demonstrated by histochemistry that repeated exposure of guinea pig skin to UV does not involve any significant changes in population, distribution, cellular appearance or enzymic activities of the Langerhans cells whereas it does cause a marked alteration of these cellular characteristics in melanocytes. If the recently held concept that the Langerhans cells and melanocytes represent two distinct and independent cell lines fitted our experimental system, it might not be necessary to speculate upon the presence of the precursor Langerhans cells and it would be possible to expect to see some ultrastructural differences between the precursor ("indeterminate") cells of melanocytes and the Langerhans cells. In fact, this study indicated that essentially all of the "indeterminate" cells may be delineated to melanocytes. The fine structure of the poorly developed Langerhans cells, though they may resemble "indeterminate" cells and may be located in the basal layer, was differentiated from that of precursor melanocytes by large, much better developed dendrites, numerous vesicular and tubular structures, dense tubules and a few Langerhans-cell granules. Even in situations in which a whole view of the dendrites and Langerhans-cell granules was not observed in one section, the serial sectioning of the same specimen confirmed the presence of one or more of these ultrastructural characteristics, including Langerhans-cell granules (see Fig 3). However, this view may still be speculative and needs to be confirmed by such studies as (a) statistical calculation of "indeterminate" cells and activated melanocytes before and after UV-exposure, and (b) labeling of membrane and/or cytoplasm of these cells by enzyme markers. Nonetheless, this might be further supported by several previous studies in different animals and humans with the following results: (a) epidermal dendritic cells reactive with α-naphthyl esterase, i.e., one of histochemical markers for Langerhans cells [6], did not change their number, but rather declined, after repeated exposure of the hairless mice to UV [7], and (b) the decrease in the number of "indeterminate" cells as well as Langerhans cells was confirmed by electron microscopic linear scanning of the epidermis after repeated daily exposure of human skin to UV [15].

In this study, the subcellular processes responsible for new melanogenesis after UV-exposure were basically similar to those reported in human skin [16]. Recently it was proposed that the site of synthesis for structural matrix proteins and tyrosinase is different; structural proteins are accumulated in a "condensing vacuole" deriving from rough endoplasmic reticulum and tyrosinase is transported from Golgi apparatus to this vacuole [17]. This study, however, indicated that the activation of the synthesis in structural matrix proteins and tyrosinase appears to occur quite rapidly inasmuch as various combinations in synthesis of these 2 units were seen from day 3 to 5 of exposure. The melanosomes transferred into keratinocytes became obvious later at day 7 of exposure. Our previous study [18] suggested that 10 nm filaments are responsible for both dendrite arborization and melanosome transfer. Another important finding in this study was that the dividing melanocytes occasionally reveal desmosome-like junctions with neighboring keratinocytes. The same structure was reported in human melanocytes under replication [9]. This study, however, failed to demonstrate half-desmosome-like structures with basal lamina as reported in depigmented human skin [19].

Previously, we have shown that the melanocytes in the tail skin of the adult black mouse possess a low thymidine index (0.75%) under conditions (epilation) known to produce mild stimulation of the keratinocyte mitotic index (15%) [9]. We tentatively hypothesized that UV-light or other external stimuli known to increase the number of melanocytes in skin may do so by directly stimulating DNA synthesis and mitosis in differentiated melanocytes, as these stimuli do in keratinocytes. Later, Rosdahl and Szabo [8], exposing ear skin of the same species of black mouse, at which a small native population of melanocytes is present, to UV-light (290–350 nm) found that all new melanocytes had taken up thymidine by cumulative

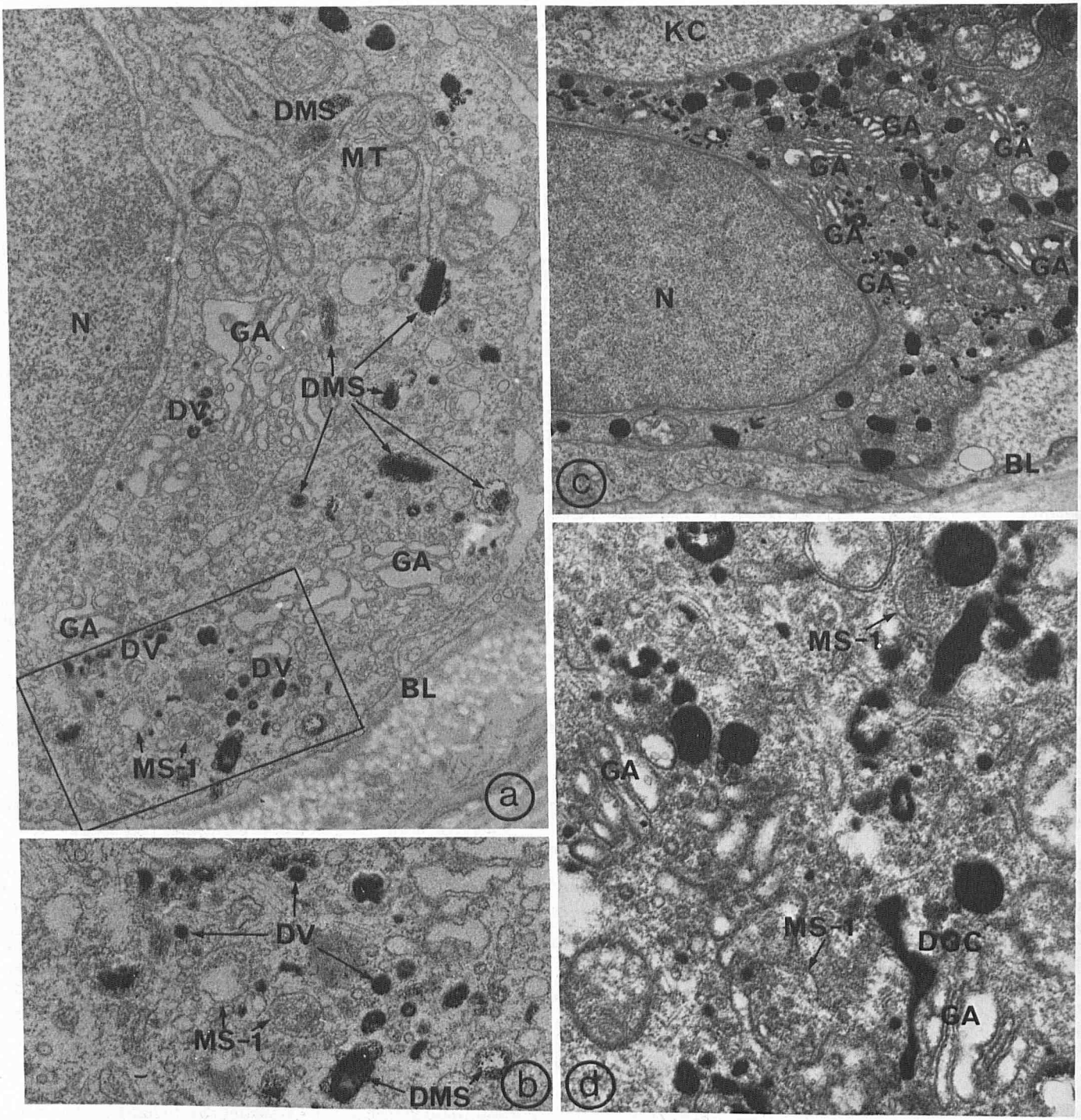

FIG 6. Dopa cytochemistry showing various aspects of new melanogenesis at stages III and IV of exposure. *a*, A melanocyte at day 7 of exposure. The cytoplasm becomes enlarged and aggregated with a large number of Golgi apparatus (*GA*), dopa-positive melanosomes (*DMS*) in various stages of development, dopa-positive vesicles (*DV*), and mitochondria (*MT*) around the nucleus (*N*) (× 39,000). *b*, A high-power view of the *encircled area* in Fig 6-*a*. The melanosomes in the earliest stage of development (*MS-1*) such as seen in Fig 5-*a, b, c* are seen around the Golgi apparatus (*GA*) and dopa-positive melanosomes (*DMS*). The deposition of dopa melanin is not obvious in these melanosomes (× 71,000). *c*, A melanocyte at day 14 of exposure. The cytoplasm is aggregated with a large number of melanosomes and Golgi apparatus (*GA*) as compared with that shown in Fig 6-*a, b*. (× 10,700). *d*, A high-power view of a portion of the melanocyte shown in Fig 6-*c*. Around the Golgi apparatus (*GA*), numerous vesicles, some of which are coated and dopa-positive, and melanosomes in the earliest stage of development (*MS-1*) which have again little reacted with dopa cytochemistry; compare the degree of the dopa reaction product with that of the dopa-positive Golgi cisterna (*DGC*) (× 42,800). *KC*, keratinocyte and *BL*, basal lamina.

labeling. In this study of the dorsal and plantar skin, where no native functioning melanocytes were present, a numerical increase in melanocytes was accompanied by (a) a lag phase (from stage I to II; day 0 to 5) for activation of precursor melanocytes (thymidine index: 0%) and (b) an exponential phase (stage III; day 6 to 7) for proliferation of many activated melanocytes (thymidine index: 2.1%). A similar process may be operative in the case of the numerical increase of melanocytes after UV-exposure in the depigmented skin of vitiligo vulgaris, in which, similar to our experimental conditions, no functioning melanocytes are present.

This study, however, left one paradox unsolved, i.e., there

was no significant increase in the population of functioning melanocytes at stage IV, from day 8 to 14 of exposure. Recently, studies with the rhesus monkey [20,21] revealed a basically similar pattern for a numerical increase in melanocytes in the epidermis after UV-exposure; Peak melanogenic activity was attained after 23 exposures and, after 46 continued exposures, the population of melanocytes declined to about one half as many as that found at 23 exposures. Our previous study [22] showed that repeated exposure of human skin to UV-A, UV-B or UV-A plus psoralen causes a numerical increase in melanocytes, which, however, never becomes more than a 2–3 fold increase as compared with that before exposure. Whether a "fixed" or "declined" population of melanocytes after repeated UV-exposure occurs as a result of either direct cellular damage or some alterations in the physico-chemical properties of DNA, RNA and/or chromatin complexes is unknown, though none of the cellular and nuclear damage reported previously in UV-exposed skin was seen [23]. In this connection, it should also be mentioned that there was a striking increase in the thickness of the entire epidermis and in the number of melanosomes transferred to keratinocytes at stage IV. Further study is necessary to solve this paradox.

The authors express many thanks to Drs. Y. Takeda, M. Katoh, and M. Jimbow in the Department of Dermatology, Sapporo Medical College, for their help in carrying out this study, to Mr. S. Mori for preparation of the micrographs, and to Miss T. Niikawa for her typing of the manuscript and preparing figures.

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