Genetic Factors Controlling the Proliferative Activity of Mouse Epidermal Melanocytes During the Healing of Skin Wounds

Tomohisa Hirobe

Division of Biology, National Institute of Radiological Sciences, Anagawa, Chiba, 260 Japan

Manuscript received December 26, 1987
Accepted June 27, 1988

ABSTRACT

A cut was made on the middorsal skin of newborn mice of strains C57BL/10J, C57BL/10J-*A/A*, and C3H/He using fine iridectomy scissors. In the epidermis within 1 mm of the wound edge in C57BL/10J and C57BL/10J-*A/A*, the melanocyte population positive to the dopa reaction as well as the melanoblast-melanocyte population positive to the combined dopa-premelanin reaction increased dramatically until the 3rd day, then gradually decreased. In contrast, the melanocyte population of C3H/He did not increase after wounding, despite that the melanoblast-melanocyte population increased. Pigment-producing melanocytes in mitosis were frequently found in C57BL/10J and C57BL/10J-*A/A*, but not in C3H/He. The F_1, F_2, and backcross matings were performed to get some information about the genetic basis of the difference between C57BL/10J and C3H/He. In the F_1 generation the offspring from reciprocal crosses exhibited intermediate values in both populations on the 3rd day after wounding. The F_2 generation included the C3H/He type, F_1 type, and C57BL/10J type in a ratio of 1:2:1 in both populations. Moreover, both reciprocal backcrosses gave 1:1 ratios of parent type to F_1 type in both populations. These results indicate that the proliferative activity of mouse epidermal melanocytes during the healing of skin wounds are controlled by semidominant genes.

IN mice, melanoblasts, precursor of melanocytes, originate from the neural crest and migrate into the epidermis of all body regions in early embryonic life (RAWLES 1947). By 13 or 14 days of gestation melanoblasts sufficiently colonize the epidermis (MAYER 1973). Mouse epidermal melanoblasts begin the production of unmelanized melanosomes on 14 days and begin to differentiate into melanocytes by the appearance of tyrosinase activity on 16 days of gestation (HIROBE 1984). Melanocytes increase in number until 4 days after birth and then decrease (HIROBE 1984). However, little is known as to how the proliferation of epidermal melanoblasts and melanocytes is regulated in the process of differentiation.

Recent studies using newborn mice of strain C57BL/10J showed that the epidermal melanocytes were capable of proliferating in wounded skin (HIROBE 1983). However, it is unclear whether the proliferative response of epidermal melanocytes to skin wounding differ in other strains of mice. Previous study showed that the number of epidermal melanocytes of newborn mice of strain C3H/He was much fewer than that of C57BL/10J (HIROBE 1982a). The object of this study is to investigate in detail whatever difference exists in the proliferative response of epidermal melanocytes of C3H/He to skin wounding as compared with C57BL/10J. In order to get insights into the genetic control of the melanocyte proliferation the F_1, F_2, and backcross matings were performed. In this way we expect to better understand the genetic control of the proliferation of mouse epidermal melanocytes in the process of differentiation.

MATERIALS AND METHODS

The animals used in this study were the house mouse, *Mus musculus*, of strain C57BL/10J (substrain C57BL/10JHir), C3H/He (substrain C3H/HeJmsHir), and C57BL/10J-*A/A* (substrain C57BL/10JHir-*A/A*). A congenic C57BL/10J-*A/A* (N12F4-N12F9) was made from the cross between C3H/He and C57BL/10J. Agouti mice in the backcross generation between F_1 and C57BL/10J were crossed with C57BL/10J, and repeatedly backcrossed. Then *A/A* homozygous mice were sibmated. Thus, the *A* allele at the agouti locus in C3H/He was subsequently transferred to C57BL/10J genetic background by repeated backcross mating. The three strains of mice were given water, fed *ad libitum* on a commercial diet (Clea Japan), and maintained at 24 ± 1° with 40–60% relative humidity; 12 hr of fluorescent light was provided daily.

The method of skin wounding was reported previously (HIROBE 1983). A full-thickness cut 7 mm long was made anteroposteriorly on the middorsal skin of 1.5-day-old mice using fine iridectomy scissors. The incision extended from the epidermis to the deepest layer of macrophages under the panniculus carnosus muscle. Immediately after the incision was made, the margins retracted and wound cavity widened to about 2 mm. The wounds were not sutured and not dressed. The animals were killed at various times thereafter. The entire wound area, including the bed, was then removed from the animals. Biopsy specimens from the wounded skins and from corresponding field of skin from intact control animals were fixed with 16% formalin in

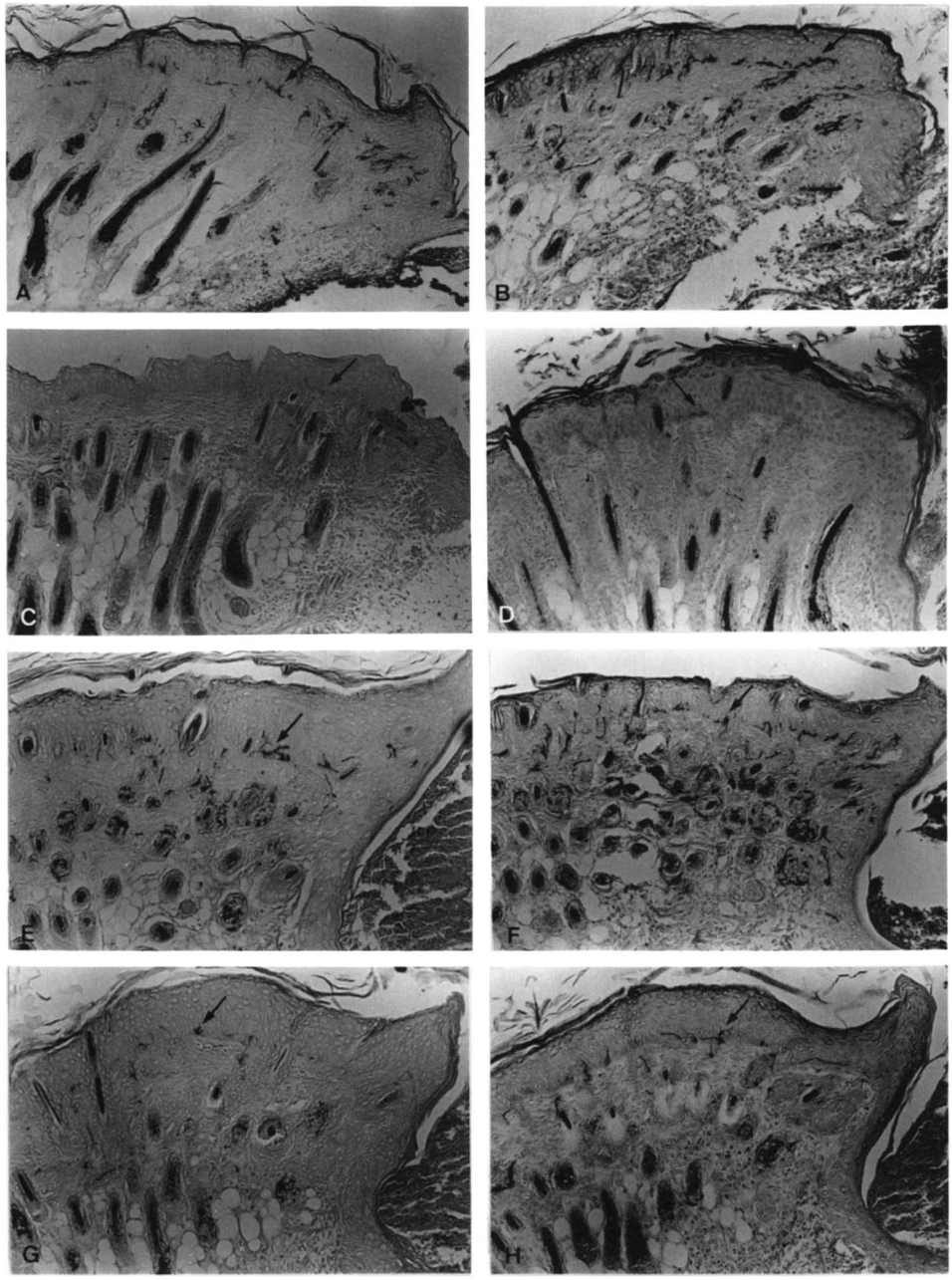

FIGURE 1.—Vertical sections of the dorsal skins of C57BL/10J (A, B), C3H/He (C, D), C57BL/10J-*A*/*A* (E, F), and F_1 (C57BL/10J × C3H/He; G, H) mice during the wound healing. A cut was made on the middorsal skins of 1.5-day-old mice. Cells positive to the dopa reaction (A, C, E, and G) as well as to the combined dopa-premelanin reaction (B, D, F, and H) are shown on the 3rd day after wounding. Epidermal melanocytes or melanoblasts are seen in the vicinity of a wound (arrows). The number of epidermal melanocytes or melanoblasts in F_1 is fewer than that in C57BL/10J and C57BL/10J-*A*/*A*. The number in C3H/He is much fewer than that in F_1. The right sides of all figures indicate the wound edge and regenerating wound epidermis (×80).

phosphate buffer (pH 7.0) for 20–24 hr at 2°. Each age group was represented by three mice and one sample was obtained from the wounded skin of each animal. One wounded skin consisted of two regenerating epidermes, namely right and left sides of the wound bed. The experiments were repeated three times. The specimens were washed with distilled water and incubated with 0.1% L-dopa (3,4-dihydroxyphenylalanine, Wako) solution in phosphate buffer (pH 7.4) for 20–24 hr at 37°. This staining reveals tyrosinase containing differentiated melanocytes (HIROBE 1982b). The specimens were oriented transversely to the wound edge, and 10 μm serial sections were deparaffinized and counterstained with eosin. For combined dopa-premelanin reaction (combined dopa-ammoniacal silver nitrate staining), deparaffinized sections after the dopa treatment were incubated with 10% ammoniacal silver nitrate (Wako) solution for 10 min at 58° (MISHIMA 1960; HIROBE 1982a). This preferential staining reveals undifferentiated melanoblasts that contain unmelanized stage I and II melanosomes in addition to tyrosinase containing differentiated melanocytes (MISHIMA 1964; HIROBE 1982b). The specimens were also counterstained with eosin.

The number of melanocytes (cells positive to the dopa reaction) and the number of stage I and II melanosome-containing melanoblasts plus melanocytes (cells positive to the combined dopa-premelanin reaction) were estimated per 0.1 mm^2 of the epidermis of each section of skin, and the calculations based on 10 consecutive sections with the width of 1 mm covering the area 0.1 mm^2 of the skin.

In some cases, the specimens from wounded and control animals were fixed with Bouin's fixative, and sectioned transversely to the wound edge. Numerous serial sections, 8 μm in thickness, were stained with hematoxylin and eosin. Pigment-producing melanocytes in resting phase and mitosis were examined with the light microscope.

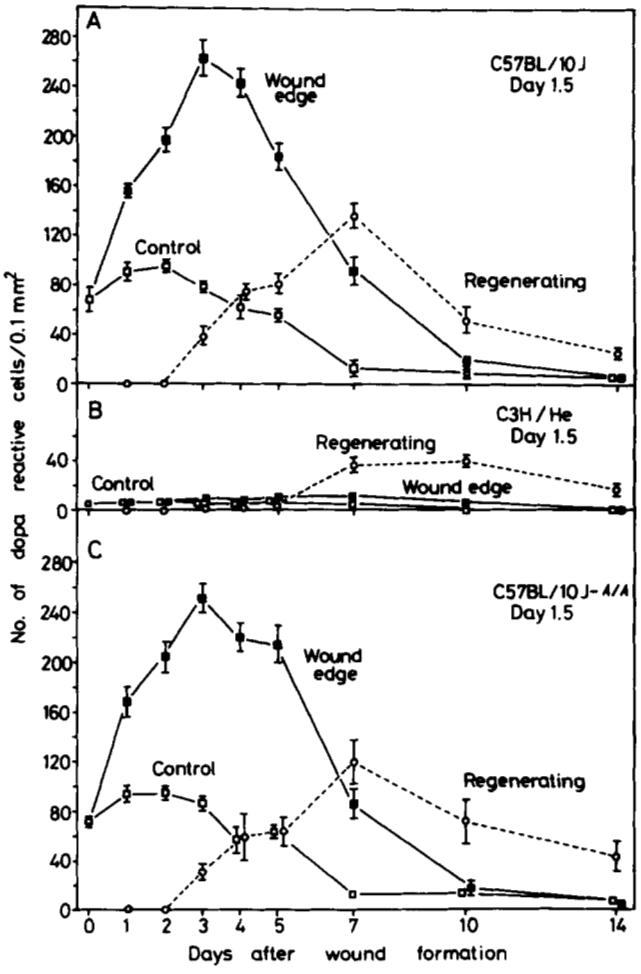

FIGURE 2.—Changes in the number of melanocytes (cells positive to the dopa reaction) of the dorsal skin of 1.5-day-old mice of strains C57BL/10J (A), C3H/He (B), and C57BL/10J-*A/A* (C) after wounding per 0.1 mm² of the epidermis within 1 mm of the wound edge (■), the control epidermis (□), and the regenerating wound epidermis (○). Bars indicate SEM.

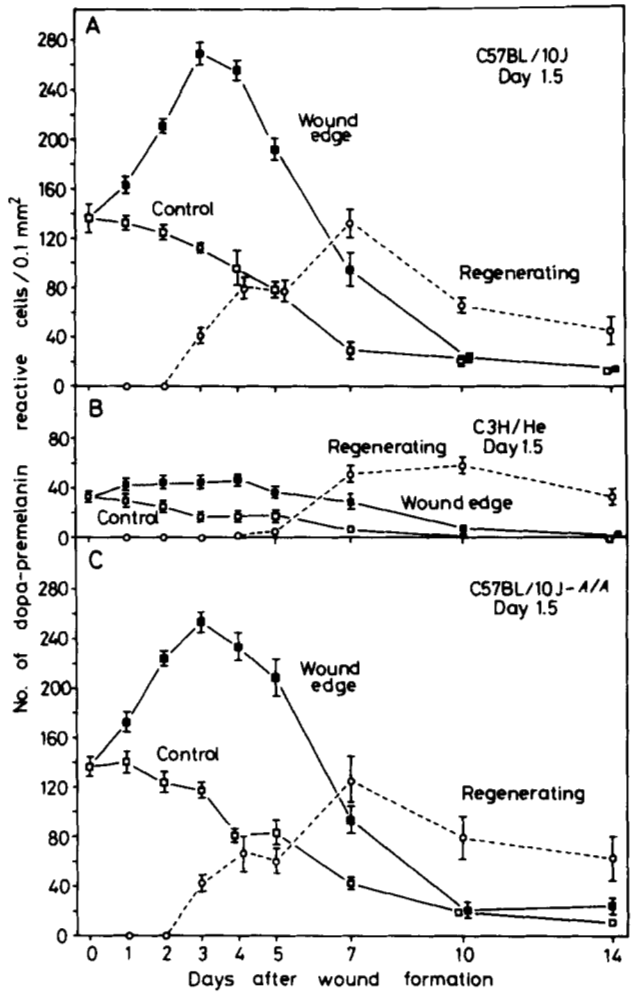

FIGURE 3.—Changes in the combined number of melanoblasts and melanocytes (cells positive to the combined dopa-premelanin reaction) of the dorsal skin of 1.5-day-old mice of strains C57BL/10J (A), C3H/He (B), and C57BL/10J-*A/A* (C) after wounding per 0.1 mm² of the epidermis within 1 mm of the wound edge (■), the control epidermis (□), and the regenerating wound epidermis (○). Bars indicate SEM.

RESULTS

Changes in the melanocyte and melanoblast-melanocyte populations in the epidermis of newborn mice after wounding: When the dorsal skins of 1.5-day-old mice of strain C57BL/10J were wounded, the melanocyte population (Figure 1A) positive to the dopa reaction as well as the melanoblast-melanocyte population (Figure 1B) positive to the combined dopa-premelanin reaction increased dramatically in the epidermis adjacent to a skin wound. On the 1st day after wounding the melanocyte and melanoblast-melanocyte populations in the epidermis within 1 mm of the wound edge significantly ($P < 0.05$) exceeded the controls on the zero and the 1st day (Figures 2A and 3A). Both populations showed maximal number on the 3rd day, then gradually decreased (Figures 2A and 3A). From the 3rd day, both populations were observed in the roots of hair follicles in addition to the basal layer of epidermis. This suggests that the epidermal melanoblasts or melanocytes migrate into hair follicles. In all stages of wound healing, the size of the melanocyte population did not differ significantly from that of the melanoblast-melanocyte population, suggesting that all melanoblasts differentiate into melanocytes in the epidermis adjacent to a skin wound.

The melanocyte and melanoblast-melanocyte populations appeared in the regenerating wound epidermis on the 3rd day after wounding, and increased in number. Both populations were observed in the roots of the advancing epidermal sheets from the 3rd day, and lagged behind their forward edges. This suggests that epidermal melanoblasts or melanocytes increase in number adjacent to a skin wound and, thereafter, migate into the regenerating wound epidermis. Both populations showed a maximal number on the 7th day and decreased thereafter. From the 7th day, both populations were observed in the roots of hair follicles

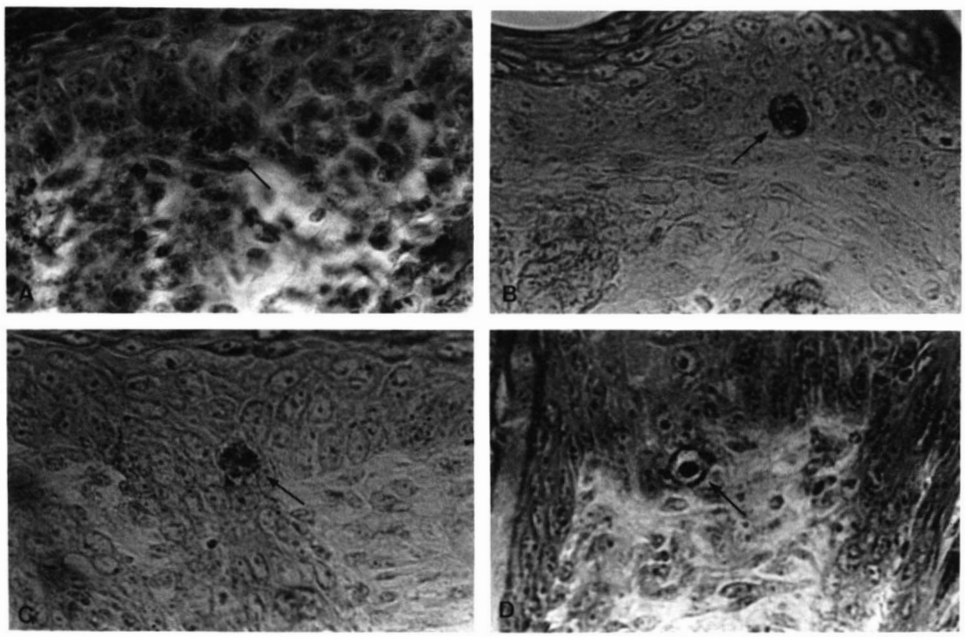

FIGURE 4.—Vertical sections of the dorsal skins of 1.5-day-old mice of strains C57BL/10J (A), C57BL/10J-*A*/*A* (B, C), and F_1 (C57BL/10J × C3H/He, D) on the 3rd day after wounding. Melanocytes in metaphase (B and D), telophase (C), and anaphase (A) are observed in the epidermis (arrows) adjacent to a skin wound. All specimens were fixed with Bouin's fixative and stained with hematoxylin and eosin. No dopa treatment or silver staining (×360).

TABLE 1

Mitotic indices of pigment-producing epidermal melanocytes on the 3rd day after wounding

Strain	Mitotic indices[a] % (melanocyte mitosis/total)	
	Control epidermis	Epidermis within 1 mm of the wound edge
C57BL/10J	0% (0/2388)	0.57% (14/2452)
C3H/He	0% (0/1031)	0% (0/1123)
C57BL/10J-*A*/*A*	0% (0/2053)	0.60% (26/4318)
F_1 (C57BL/10J × C3H/He)	0% (0/2162)	0.55% (11/2008)

[a] A cut was made on the middorsal skin of 1.5-day-old mice of various strains. Specimens were fixed on the 3rd day after wounding with Bouin's fixative. No dopa treatment or silver staining. Mitotic indices of pigment-producing melanocytes in the control epidermis as well as the epidermis within 1 mm of the wound edge are shown.

in addition to the basal layer of the regenerating wound epidermis, suggesting that the epidermal melanoblasts or melanocytes migrate into hair follicles. In all stages of wound healing, the melanocyte and melanoblast-melanocyte populations did not differ significantly in size, suggesting that all melanoblasts differentiate into melanocyte in the regenerating wound epidermis.

When the dorsal skin of congenic C57BL/10J-*A*/*A* mice were wounded, similar changes were observed in the melanocyte (Figures 1E and 2C) and melanoblast-melanocyte (Figures 1F and 3C) populations. In all stages of wound healing, the size of the melanocyte and melanoblast-melanocyte populations of C57BL/10J-*A*/*A* did not differ significantly from that of C57BL/10J in the epidermis within 1 mm of the wound edge, the control epidermis, and the regenerating wound epidermis (Figures 2 and 3).

When the dorsal skins of 1.5-day-old mice of strain C3H/He were wounded, the melanocyte population in the epidermis within 1 mm of the wound edge did not increase significantly (Figures 1C and 2B). In contrast, the melanoblast-melanocyte population in the epidermis within 1 mm of the wound edge increased significantly as compared with the control epidermis (Figures 1D and 3B). The population from the 2nd to the 10th day was significantly ($P < 0.05$) greater than that of the control. The population on the 4th day significantly ($P < 0.05$) exceeded the initial density, but the population on the other day did not exceed the initial density (Figure 3B). In all stages of wound healing, the size of the melanocyte population was significantly ($P < 0.05$) smaller than that of the melanoblast-melanocyte population, except the 10th day.

In the regenerating wound epidermis, the melanocyte and melanoblast-melanocyte populations appeared on the 3rd day after wounding, and increased in number. They showed a maximal number on the 10th day and decreased thereafter. In all stages of wound healing, the melanocyte and melanoblast-melanocyte population did not differ significantly in size, suggesting that all melanoblasts differentiate into melanocytes in the regenerating wound epidermis. These results indicate that the melanoblasts of C3H/He increased in the vicinity of a wound and thereafter migrate into the regenerating wound epidermis and differentiate into melanocytes there.

Changes in the mitotic indices of epidermal melanocytes of newborn mice after wounding: When the dorsal skin of 1.5-day-old mice of strains C57BL/10J (Figure 4A), C57BL/10J-*A*/*A* (Figure 4, B and C) and F_1 (C57BL/10J × C3H/He, Figure 4D) were wounded, melanocytes in mitotic division were fre-

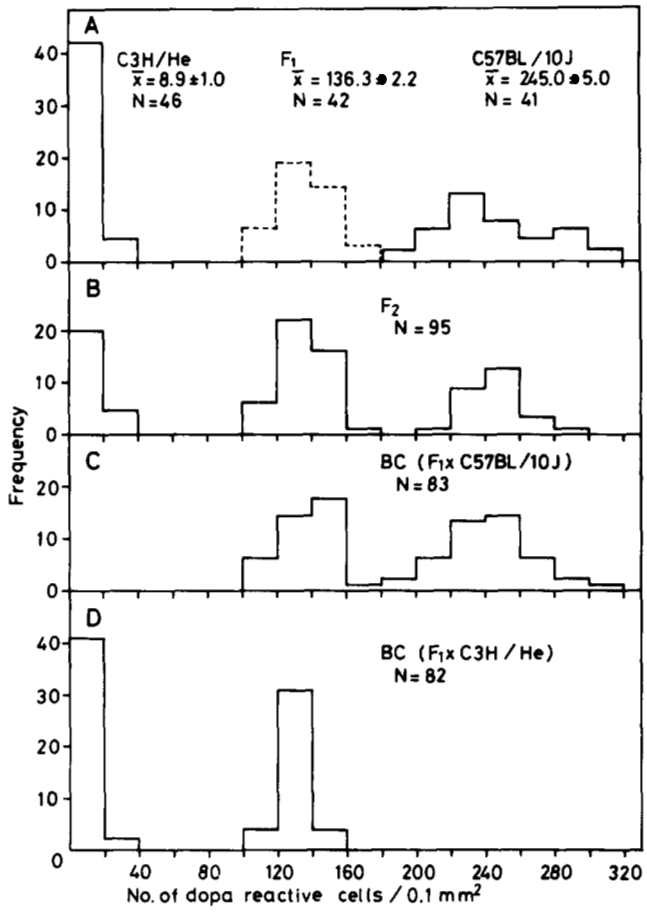

FIGURE 5.—Frequency histograms showing the number of melanocytes (cells positive to the dopa reaction) per 0.1 mm² of the epidermis within 1 mm of the wound edge of C57BL/10J, C3H/He, and F_1 (dotted line) (A) as well as F_2 (B) and backcross [F_1 × C57BL/10J, (C); F_1 × C3H/He, (D)]. Dorsal skins of 1.5-day-old mice were wounded and fixed with buffered formalin on the 3rd day after wounding and subjected to the dopa reaction. Each value is the mean of the number of melanocytes of right and left sides of the wound bed.

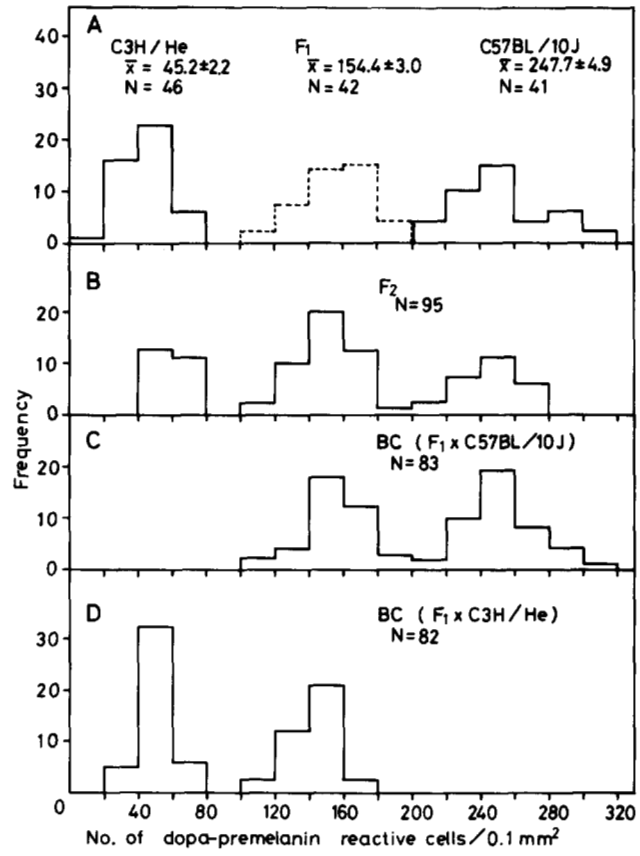

FIGURE 6.—Frequency histograms showing the combined number of melanoblasts and melanocytes (cells positive to the combined dopa-premelanin reaction) per 0.1 mm² of the epidermis within 1 mm of the wound edge of C57BL/10J, C3H/He, and F_1 (dotted line) (A) as well as F_2 (B) and backcross [F_1 × C57BL/10J, (C); F_1 × C3H/He, (D)]. Dorsal skins of 1.5-day-old mice were wounded and fixed with buffered formalin on the 3rd day after wounding and subjected to the combined dopa-premelanin reaction. Each value is the mean of the combined number of melanoblasts and melanocytes of right and left sides of the wound bed.

quently observed in the epidermis within 1 mm of the wound edge on the 3rd day. Similar mitotic index was observed among these strains (0.55–0.60, Table 1). However, mitotic melanocytes were never found in the epidermis within 1 mm of the wound edge of C3H/He mice (Table 1). Mitotic melanocytes were never found in the control epidermis of four strains (Table 1).

Segregation of the melanocyte and melanoblast-melanocyte populations in the epidermis within 1 mm of the wound edge of offspring in the F_1, F_2, and backcross generations: In order to get some information about the genetic basis of the difference between C57BL/10J and C3H/He in the proliferative response of epidermal melanocytes to skin wounding, the F_1, F_2, and backcross matings were performed. The dorsal skins of C57BL/10J and C3H/He as well as offspring in the F_1, F_2, and backcross generations were excised on the 3rd day after wounding and subjected to the dopa reaction and to the combined dopa-premelanin reaction. The observed number of melanocytes (Figure 1G) and melanoblasts plus melanocytes (Figure 1H) from the reciprocal crosses was intermediate between the numbers of the parent strains. Histograms of the distribution of the melanocyte (Figure 5A) and melanoblast-melanocyte populations (Figure 6A) show that both strains exhibit nonoverlapping distributions, while the F_1 values locate between the two strains in both cases.

The distributions of the number of melanocytes (Figure 5B) and melanoblasts plus melanocytes (Figure 6B) in the F_2 showed multimodal distributions. The distributions of the melanocyte (Figure 5C) and melanoblast-melanocyte (Figure 6C) populations in offspring of the backcross between F_1 and C57BL/10J showed bimodal distributions. Similarly, the distributions of the melanocyte (Figure 5D) and melanoblast-melanocyte (Figure 6D) populations in offspring of the backcross between F_1 and C3H/He were bimodal.

TABLE 2

Segregation of the number of melanocytes in the epidermis within 1 mm from the wound edge of the dorsal skin of offspring in the F_2 and backcross generations on the 3rd day after wounding

Generation	Low (0–40)		Intermediate (100–180)		High(181–320)		χ^2	P
	Observed	Expected	Observed	Expected	Observed	Expected		
F_2	24[a]	23.75	45	47.5	26	23.75	0.347	$0.80 < P < 0.90$
	12.5 ± 1.3[b]		134.9 ± 2.8		242.9 ± 2.5			
Backcross 1 (F_1 × C57BL/10J)			39	41.5	44	41.5	0.301	$0.50 < P < 0.70$
			135.0 ± 2.5		242.7 ± 3.3			
Backcross 2 (F_1 × C3H/He)	43	41	39	41			0.195	$0.50 < P < 0.70$
	8.6 ± 0.8		130.3 ± 1.6					

[a] Number of mice with specified melanocyte density.

[b] Number of melanocytes (cells positive to the dopa reaction) per 0.1 mm^2 of the epidermis within 1 mm from the wound edge of the dorsal skin on the 3rd day after wounding. Each value is the mean ± SE. Each value is the mean of two different values (right and left sides of the wound bed) per each animal.

TABLE 3

Segregation of the combined number of melanoblasts and melanocytes in the epidermis within 1 mm from the wound edge of the dorsal skin of offspring in the F_2 and backcross generations on the 3rd day after wounding

Generation	Low (0–80)		Intermediate (100–200)		High(201–320)		χ^2	P
	Observed	Expected	Observed	Expected	Observed	Expected		
F_2	24[a]	23.75	45	47.5	26	23.75	0.347	$0.80 < P < 0.90$
	57.6 ± 1.8[b]		148.8 ± 2.3		242.9 ± 4.5			
Backcross 1 (F_1 × C57BL/10J)			39	41.5	44	41.5	0.301	$0.50 < P < 0.70$
			154.1 ± 3.0		251.7 ± 3.3			
Backcross 2 (F_1 × C3H/He)	43	41	39	41			0.195	$0.50 < P < 0.70$
	49.2 ± 1.5		141.4 ± 2.1					

[a] Number of mice with specified melanoblast-melanocyte density.

[b] Combined number of melanoblasts and melanocytes (cells positive to the combined dopa-premelanin reaction) per 0.1 mm^2 of the epidermis within 1 mm from the wound edge of the dorsal skin on the 3rd day after wounding. Each value is the mean ± SE. Each value is the mean of two different values (right and left sides of the wound bed) per each animal.

χ^2 Analysis of the ratio of segregation of the melanocyte and melanoblast-melanocyte populations in the epidermis within 1 mm of the wound edge of offspring in the F_2 and backcross generations: In order to obtain the ratio of segregation in F_2 and backcross, distributions of the melanocyte and melanoblast-melanocyte populations in the epidermis within 1 mm of the wound edge were divided into three (F_2) or two (backcross) classes based on the observed ranges in the parental strains and F_1. When the melanocyte populations of the offspring in the F_2 generation were classified into the three groups of the histogram of Figure 5B, namely 0–40 (C3H/He type, low), 100–180 (F_1 type, intermediate), and 181–320 (C57BL/10J type, high), the ratio of segregation was 1:2:1 ($\chi^2 = 0.347$, $0.80 < P < 0.90$, Table 2). Similarly, a 1:2:1 ratio of segregation of the melanoblast-melanocyte population was confirmed (Table 3) when these populations were classified into the three groups of the histogram of Figure 6B, namely, 0–80 (C3H/He type, low) 100–200 (F_1 type, intermediate), and 201–320 (C57BL/10J type, high). Similarly, two types (intermediate and high) of individuals were present in the backcross generation between F_1 and C57BL/10J in a ratio of 1:1 (Table 2 for the melanocyte population and Table 3 for the melanoblast-melanocyte population). The backcross generation between F_1 and C3H/He included low and intermediate types in a ratio of 1:1 (Tables 2 and 3). These results indicate that the melanocyte and melanoblast-melanocyte populations in the epidermis within 1 mm of the wound edge are controlled by semidominant genes.

DISCUSSION

The results of the present study suggest that the melanocyte and melanoblast-melanocyte populations in the epidermis adjacent to a skin wound are controlled by semidominant genes, since the genetic

analysis showed satisfactory agreements between the observed data and the expected ratios, and the allelic difference at the agouti locus between C57BL/10J and congenic C57BL/10J-*A*/*A* showed no difference in the two populations.

The present study also demonstrated that pigment-producing melanocytes were stimulated to undergo mitosis adjacent to a skin wound in mice of C57BL/10J, C57BL/10J-*A*/*A*, and F_1 (C57BL/10J × C3H/He), whereas pigment-producing melanocytes of C3H/He mouse were not stimulated to undergo mitosis. This suggests that differentiated melanocytes in C57BL/10J mouse are capable of proliferating after wounding, but not in C3H/He mouse. The increase in the size of the melanoblast-melanocyte population in the epidermis adjacent to a skin wound of C3H/He seems to be the result of the division of melanoblasts, since the number of melanocytes did not increase and mitosis of pigment-producing melanocytes were not observed. The melanocytes of C57BL/10J are thought to possess the proliferative activity even at the late stage of differentiation, while the activity is restricted to the early stage of differentiation in the melanocytes of C3H/He. Therefore, it seems reasonable that the genes controlling the proliferative activity of epidermal melanocytes during the healing of skin wounds determine how long the epidermal melanocyte retains its proliferative capacity during the process of differentiation. However, precise mechanism of the action of the genes remains to be investigated in a future study.

The numbers of melanocytes and melanoblast-melanocyte in the dorsal epidermis of 3-day-old C3H/He mouse were reported to be fewer than those of C57BL/10J mouse (HIROBE 1982a). The F_1 from reciprocal crosses between C3H/He and C57BL/10J exhibited an intermediate value in both populations. The F_2 gave a 1:2:1 ratio of C3H/He type, to F_1 type, to C57BL/10J type in both populations. Both reciprocal backcrosses gave ratios of parent type to F_1 type that did not differ significantly from 1:1 in either population. Moreover, the allelic difference at the agouti locus between the two strains showed no difference in both population. These results suggested that semidominant genes were involved in regulating the melanocyte and melanoblast-melanocyte populations in the epidermis of the newborn mouse skin (HIROBE 1982a). It seems likely that the genes controlling the melanocyte and melanoblast-melanocyte populations in the epidermis of newborn mouse skin are identical to the genes controlling the proliferative activity of epidermal melanocytes during the healing of skin wounds, since the similar histogram pattern of melanocyte and melanoblast-melanocyte populations between the previous study (HIROBE 1982a) and the present study was observed in the offspring of F_1, F_2, and backcross generations. The genes are thought to determine the proliferative activity of epidermal melanocytes. However, this hypothesis remains to be investigated in a future study.

It has been reported that the epidermal melanocytes of the adult mouse ear skin were normally in a continuous process of renewal (ROSDAHL and LINDSTRÖM 1980; ROSDAHL and BAGGE 1981), and that they were stimulated to proliferate by ultraviolet irradiation (ROSDAHL and SZABO 1976, 1978; ROSDAHL 1978). The epidermal melanocytes of the dorsal skin of adult guinea pig (GIACOMETTI and ALLEGRA 1967) and of the scalp skin of the young rhesus monkey (GIACOMETTI *et al.* 1972) were reported to be induced to proliferate by skin wounding. Also, JIMBOW *et al.* (1975) reported that the epidermal melanocytes of adult human skin underwent mitotic division normally. These studies indicate that the differentiated melanocytes in the epidermis of mammalian skin are capable of proliferating in both normal and experimental conditions. However, it is unknown at present whether the proliferative activity of the differentiated melanocytes differs in other strains of animals or in other races of men. The solution of these problems is expected to clarify the mechanism of the genetic control of the proliferation of mammalian epidermal melanocytes.

This work was supported in part by grant 58740290 from the Ministry of Education, Japan.

LITERATURE CITED

GIACOMETTI, L., and F. ALLEGRA, 1967 The effect of wounding upon the uptake of ^{3}H-thymidine by guinea-pig epidermal melanocytes. Adv. Biol. Skin **8:** 89–95.

GIACOMETTI, L., W. MONTAGNA, M. BELL and F. HU, 1972 Epidermal melanocyte proliferation in skin wound healing and after ultraviolet irradiation in rhesus monkeys. pp. 451–459. In: *Pigmentation: Its Genesis and Biologic Control*, Edited by V. RILEY. Appleton-Century-Crofts, New York.

HIROBE, T., 1982a Genes involved in regulating the melanocyte and melanoblast-melanocyte populations in the epidermis of newborn mouse skin. J Exp. Zool. **223:** 257–264.

HIROBE, T., 1982b Origin of melanosome structures and cytochemical localizations of tyrosinase activity in differentiating epidermal melanocytes of newborn mouse skin. J. Exp. Zool. **224:** 355–363.

HIROBE, T., 1983 Proliferation of epidermal melanocytes during the healing of skin wounds in newborn mice. J. Exp. Zool. **227:** 423–431.

HIROBE, T., 1984 Histochemical survey of the distribution of the epidermal melanoblasts and melanocytes in the mouse during fetal and postnatal periods. Anat. Rec. **208:** 589–594.

JIMBOW, K., S. I. ROTH, T. B. FITZPATRICK and G. SZABO, 1975 Mitotic activity in non-neoplastic melanocytes in vivo as determined by histochemical, autoradiographic, and electron microscope studies. J. Cell Biol. **66:** 663–670.

MAYER, T. C., 1973 The migratory pathway of neural crest cells into the skin of mouse embryos. Dev. Biol. **34:** 39–46.

MISHIMA, Y., 1960 New technic for comprehensive demonstration of melanin, premelanin, and tyrosinase sites—combined dopa-premelanin reaction. J. Invest. Dermatol. **34:** 355–360.

MISHIMA, Y., 1964 Electron microscopic cytochemistry of melanosomes and mitochondria. J. Histochem. Cytochem. **12:** 784–790.

RAWLES, M. E., 1947 Origin of pigment cells from the neural crest in the mouse embryo. Physiol. Zool. **20:** 248–266.

ROSDAHL, I. K., 1978 Melanocyte mitosis in UVB-irradiated mouse skin. Acta Derm. Venereol. **58:** 217–221.

ROSDAHL, I., and U. BAGGE, 1981 Vital microscopy of epidermal melanocytes. Acta Derm. Venereol. **61:** 55–58.

ROSDAHL, I. K., and S. LINDSTRÖM, 1980 Morphology of epidermal melanocytes in different stages of mitosis. Acta Derm. Venereol. **60:** 209–215.

ROSDAHL, I. K., and G. SZABO, 1976 Thymidine labelling of epidermal melanocytes in UV-irradiated skin. Acta Derm. Venereol. **56:** 159–162.

ROSDAHL, I. K., and G. SZABÓ, 1978 Mitotic activity of epidermal melanocytes in UV-irradiated mouse skin. J. Invest. Dermatol. **70:** 143–148.

Communicating editor: R. E. GANSCHOW