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DISTRIBUTION OF MELANOCYTES IN THE DORSAL COAT OF MOUSE CHIMERAS

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The study of interaction between cells and its role in the formation of the ultimate phenotype of an organ is a difficult task in developmental biology. The value of chimeras for the study of these problems is that the experimenter can combine for the convenience of his own examination several genetically different cell populations in the same animal chimera. However, such an analysis largely depends on the presence of suitable markers. One such marker is pigment, genetically controlled variants of which are carried by melanocytes. The first genetic marker which was used when aggregation chimeras were obtained was melanocyte pigment [3, 11].

Mintz [6, 7] suggested that melanocytes of the skin arise from 17 paired clones distributed randomly along the length of the neural crest. The number of melanoblast clones arising on each side of the neural crest should correspond to the number of ancestral cells in the neural crest at the beginning of melanoblast migration, and not to their number during determination. Mouse chimeras in certain combinations of melanocyte genotypes give identical patterns of coat pigmentation; pigmented and white regions, moreover, are distributed more or less uniformly over their whole body. In chimeras, however, the distribution of pigmented skin is not uniform [12]. The ability of chimeras to form definite transverse stripes, and not a speckled pattern, of coat color varies in different combinations of lines. A striped pattern was evident for all combinations studied by Mintz [3, 4, 6, 7] and also for certain combinations described by McLaren and Bowman [2]. Meanwhile the striping in CBA—CBA T6T6 chimeras was less marked [9]. It is possible that genetic differences between components of chimeras prevent the haphazard migration of cells. This shows that the ultimate phenotypic effect of pigmentation in chimeras depends on the genotypes of the interacting cells. Variants of pigment distribution in the coat of mouse chimeras shed light on the way in which different clones of melanocytes are distributed in particular regions of skin.

The aim of the present investigation was to analyze the distributions of pigmentation in the dorsal coat of C57BL/Mib↔AKR and C57BL/Mib↔c/c mouse chimeras.

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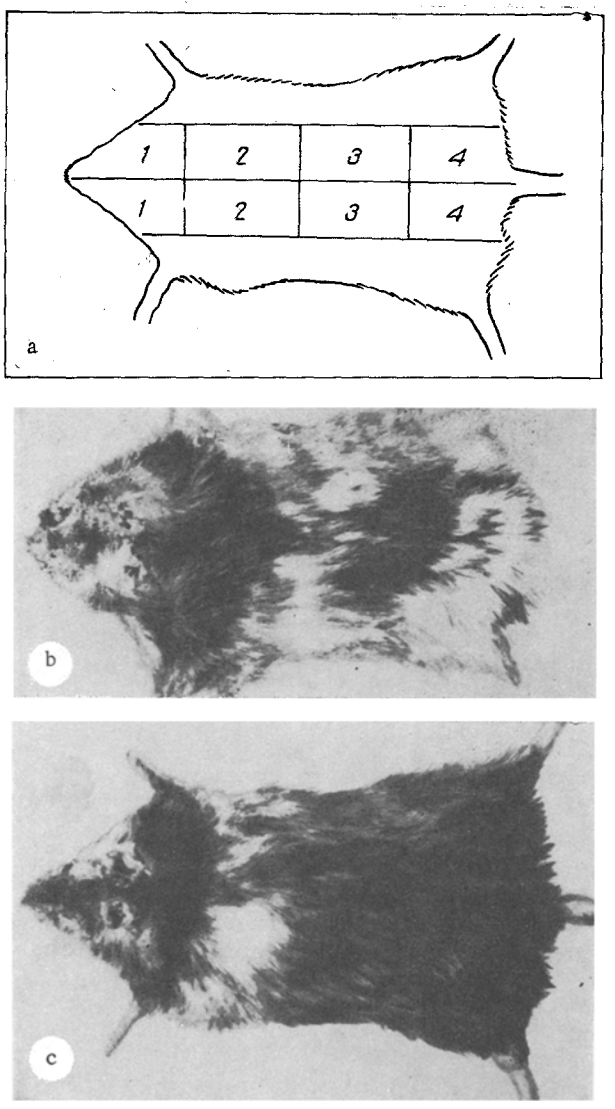

Fig. 1. Pigmentation of coat of chimeras. a) Dorsal part of coat; b) C57BL/Mib↔AKR; c) C57BL/Mib↔c/c.

EXPERIMENTAL METHOD

Mouse chimeras were obtained by the method in [11], modified by Mintz [3-5], by aggregation of two embryos of different genotypes. Two groups of chimeras were obtained by aggregation of 8-cell morulas from pigmented and nonpigmented mice. The pigmented mice were of the inbred line C57BL/Mib, the nonpigmented were mice of inbred line AKR or outbred albinos (c/c). Spontaneously ovulating females were crossed with males of the same genotype. On the 3rd day after discovery of a vaginal plug the c/c, C57BL/Mib, and AKR donor females were killed (the day of discovery of the vaginal plug was considered as the first day of pregnancy), and the oviducts and both uterine cornua were removed. The embryos were flushed out of the oviducts with warm physiological saline on to a watch glass. Under a binocular stereoscopic microscope 8-blastomere embryos were selected. The zona pellucida was removed with pronase. Embryos of the two genotypes were brought into contact in Biggers' medium [1] under mineral oil and placed in a container at 37°C, through which a gas mixture (5% CO_2 in air) was passed to maintain the pH of the medium. Aggregated embryos, developing as far as the blastocyst stage, were transferred surgically into the cornu of a pseudopregnant female of the inbred A/He line of mice. Pseudopregnant females were obtained by mating with A/He males, whose sterility had been verified. Sterile males were obtained after division and ligation of the spermatic cords. Altogether 11 C57BL/Mib—AKR and eight C57BL/Mib—c/c mouse chimeras were obtained.

TABLE 1. Dorsal Pigmentation in 10-Month Chimeras (in %)

Chimeras	Whole dorsal region	Side of dorsal region	
		right	left
C57Bl/Mib ↔ AKR (11)	42,6*	42,6*	42,7*
C57Bl/Mib ↔ c/c (8)	62,5	62,0	63,0

Legend. Number of animals in parentheses.
*$P < 0.01$.

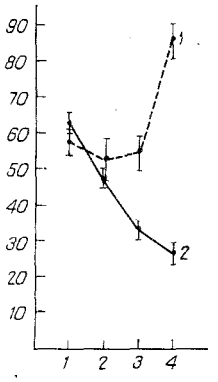

Fig. 2. Distribution of dorsal skin pigmentation among zones in chimeras. 1) C57BL/Mib↔c/c; 2) C57BL/Mib↔AKR. Ordinate, pigmentation (in %); abscissa, zones of dorsal region.

The chimeras were killed at the age of 10 months with petroleum ether, an incision was made through the skin in the midline of the abdomen, and the coat was removed, sprayed with boric acid, and dried pinned to a foam rubber slab for 8-10 days. Each coat was photographed; pigmentation of the coat was evaluated in photographs taken by the method suggested by West and McLaren [12]. The intensity of pigmentation of the spots was classified on a 5-point scale: a) absolutely white areas, b) white areas with a few pigmented hairs, c) areas with equal numbers of pigmented and white hairs, d) pigmented areas with a few white hairs, e) completely pigmented areas.

An axial line was drawn on the photographs from the tip of the nose to the tail and divided into four segments of equal length. At distances equal to one-quarter of the width of the coat two other lines were drawn parallel to the axial line and on its right and left sides in the central part. In this way the dorsal part of the coat was divided into eight regions (Fig. 1).

The area of each spot was determined gravimetrically and the percentage of pigmentation calculated by the formula:

$$\frac{(1/4\,b + 1/2\,c + 3/4\,d + e)}{a + b + c + d + e} \cdot 100.$$

The calculation was done for each of the eight regions, after which the percentage of pigmentation of the dorsal part of the coat as a whole, for the right and left sides of the coat, and for each separate zone was obtained by summation of the parameters for individual regions or, in the latter case, by summation of parameters of the corresponding region on the right and left sides.

Altogether 11 C57BL/Mib↔AKR chimeras and eight C57BL/Mib↔c/c chimeras were investigated. Statistical differences were evaluated by the Kolmogorov–Smirnov test.

EXPERIMENTAL RESULTS

The dorsal coat of C57BL/Mib↔AKR chimeras was much less densely pigmented than in C57BL/Mib↔c/c chimeras (Table 1). Comparison of the left and right sides of the dorsal region of these chimeras showed no statistically significant differences. However, differences between pigmentation of the dorsal coat were still found between C57BL/Mib↔AKR and C57BL/Mib↔c/c chimeras. The pigment in the coat of these chimeras did not form distinct transverse stripes, but instead it formed discrete spots with different types of pigmentation. The striped character of chimeras in different combinations of mouse lines is known to vary [2, 6, 9]. This

evidently depends on genetic differences between the components of the chimera. In the chimeras now studied melanocytes of the AKR genotype were much more numerous than C57BL/Mib melanocytes, by contrast with melanocytes of the outbred albino (c/c).

When the distribution of pigments was studied among zones 1-4 from head to tail in the C57BL/Mib↔AKR chimeras a considerable decrease in pigmentation was found from the head toward the sacral region of the spine (Fig. 2). Differences were observed between zones 1 and 2 ($P < 0.01$) and were possible between zones 2 and 3 ($P = 0.05$), whereas between zones 3 and 4 differences were not statistically significant ($P > 0.05$) and their pigmentation was similar. The distribution of dorsal pigmentation among these same four zones of the C57BL/Mib↔c/c chimeras also was irregular and the considerable variability of pigmentation was due to genetic heterogeneity of the outbred albinos. In the first three dorsal zones the percentage of pigmentation was practically identical ($P > 0.05$), and in the 4th zone the percentage of pigmentation was increased ($P < 0.01$). Pigmentation of the first two zones in C57BL/Mib↔AKR and C57BL/Mib↔c/c chimeras was practically identical ($P > 0.05$), whereas differences in pigmentation were observed in the 3rd and 4th zones. Melanocytes of outbred albino c/c mice in the C57BL/Mib↔c/c chimeras were represented chiefly in the anterior half of the body. Melanocytes of AKR mice were present in the anterior part of the body of C57BL/Mib↔AKR chimeras, just as in C57BL/Mib↔c/c chimeras, and they predominated in the posterior half of the body.

Rawles [10] showed that migration of melanoblasts from the neural crest begins in its anterior part and spreads toward the caudal part. Differences observed in the distribution of melanocytes may mean that c/c and AKR melanoblasts differ in the times and pathways of their migration from C57BL/Mib melanoblasts.

West and McLaren [12] observed a "recessive" effect (the term used by West and McLaren) in (C57BL × C3H)F_1 chimeras, similar to the distribution of pigment in the dorsal skin of C57BL/Mib↔c/c chimeras. They explained the "unbalanced" distribution of pigment on the grounds that recessive melanoblasts begin to migrate from the neural crest sooner than pigmented (C57Bl × C3H)F_1 melanoblasts, and for that reason these clones can occupy a large area of skin and hairs in the anterior part of the body. If recessive melanoblasts migrate in the posterior direction more slowly than pigmented melanoblasts, they will not succeed in occupying the posterior regions of the dorsal coat.

A similar version, namely a shift of the selective predominance of one or other component in time, was postulated by Moore and Mintz [8] to explain the anteroposterior gradient observed in C57BL↔C3H chimeras. A definite rule was found with regard to the ratio between the two components and their distribution in these chimeras. The cranial bones were more frequently of the C57BL type, whereas the vertebrae, especially in the lumbosacral region, were more often of the C3H type. These workers interpreted this distribution as evidence of the "autonomous, line-specific selective predominance of C57BL sclerotomal cells in the early-formed anterior somites and of C3H cells in the posterior somites, which are formed later." They emphasize that their observation is in good agreement with the clonal model of development of the spine and cranial bones.

In the C57BL/Mib↔AKR chimeras studied in the present experiments pigmentation became less dense in the posterior parts of the body, although in AKR mice the melanocytes, like those of outbred mice, are recessive for pigmentation. This may perhaps be explained by the fact that melanoblasts migrate posteriorly faster in AKR mice than in C57BL/Mib mice and succeed in occupying a large area of skin in chimeras. Evidently genetic differences exist between AKR melanoblasts and melanoblasts of c/c outbred mice, and these influence their migration.

The presence of clones of c/c melanocytes in the posterior half of the body of C57BL/Mib↔c/c chimeras, and of clones of C57BL/Mib melanocytes in C57BL/Mib↔AKR chimeras in the form of four or five small spots suggests that primary melanoblasts are distributed more or less uniformly in the neural crest and that differences in the pigmentation of these regions are due to different velocities of melanoblast migration.

Consequently, the distribution of pigment in the chimeras studied in this investigation depends on the migration rate of the melanoblasts, which is under genetic control.

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