# COAT COLOR AND ACTION OF THE OCULAR RETARDATION GENE IN THE EYES OF CHIMERIC or/or ↔ AKR MICE

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Chimeric animals, including those obtained experimentally, are of organisms with two or more genetically different cell clones. Chimeras are convenient objects in which the origin and fate of cells forming tissues and organs and also interaction between genetically different cells in these processes can be studied during individual development by making use of the different cell populations. Genetic mouse chimeras were first obtained by Tarkowski [14] by fusion of two early embryos of different genotype. This method was lately modified by Mintz [6-8]. By now chimeric mice have been obtained in several laboratories in the West.

The creation of chimeras with genetically different cells makes it possible to discover whether the phenotype of an individual cell is determined by its own genotype or by the cell environment. It is thus possible to discover how cells collaborate during the construction of tissues and organs and to investigate the phenotype of the whole animal.

The object of this investigation was to obtain chimeric mice and to study interaction between cells participating in the formation of the eye and skin.

## EXPERIMENTAL METHOD

Chimeric mice were obtained by Tarkowski's method [14] and modified by Mintz [6-8], by aggregating two embryos of different genotypes. Spontaneously ovulating females, homozygous for the mutant ocular retardation gene (symbol or) and females of the inbred albino AKR line were mated with males of the same genotype. The or/or mice passed through 12 back-crosses with C57BL/Mib mice in the writers' laboratory.

The or gene inhibits growth of the retinal anlage in homozygous mice [1, 2].

On the 3rd day after discovery of a vaginal plug the or/or and AKR donor mice were killed (the day of discovery of the vaginal plug was taken to be the first day of pregnancy) and the oviducts and both uterine cornuae were removed. The embryos were flushed out of the oviducts with warm physiological saline onto 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 [4] 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, having developed to the blastocyst stage, were transferred surgically into the uterine cornua of a pseudopregnant mouse of the inbred A/He line. Pseudopregnant mice were obtained by crossing with C57BL/Mib males, previously verified for sterility. Sterile males were obtained after division and ligation of the spermatic cords.

Altogether 25 newborn chimeric mice were obtained and reared by C57BL/Mib nurse mice with their own newborn progeny.

The weight of the newborn chimeric mice was determined and compared with that of normal individuals of the same age. Material was fixed in alcohol–formol and the eye structure was studied in histological sections. Mice aged 6-8 months were killed for histological study of

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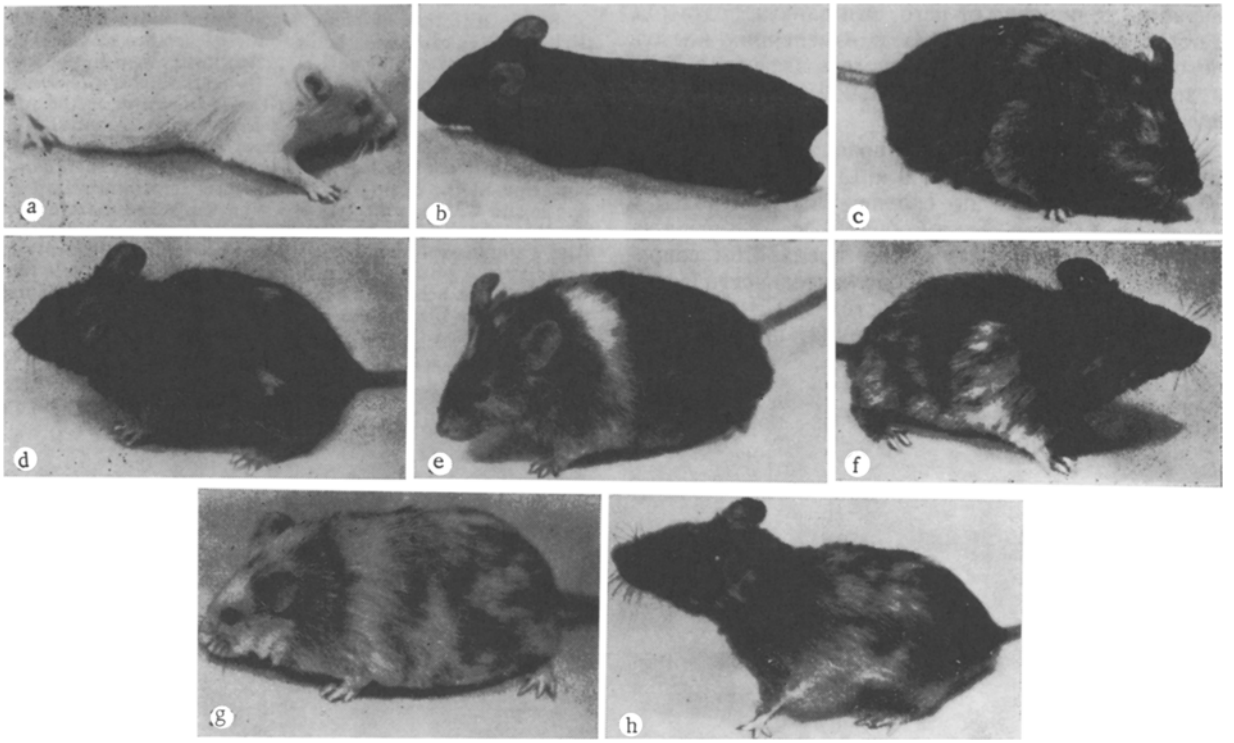

Fig. 1. Mice of different genotypes: a) AKR, b) or/or, c-h) chimeric or/ or ↔ AKR mice.

the eye and skin. The eyes were weighed, measured along and transversely to the optical axis, and the percentage of cells containing melanin in the pigmented epithelium was determined.

## EXPERIMENTAL RESULTS

Hair color of the adult or/or ↔ AKR Chimeras varied from one or several small white spots against a black background to unusually speckled. Black strips and zones in the coat of the chimera were formed from clones of or/or cells with normally colored melanocytes, and the white stripes and zones consisted of clones of albino AKR cells. The symmetry of arrangement of the white spots or regular alternation of black and white zones was thus observed in the hair color of these mice. However, the combination and arrangement of the white spots on the right side of the chimeric mice very often differed significantly from that on the left side (Fig. 1). This indicates inconstancy of distribution of the clones in the skin of the chimeric mice. Absence of symmetry and of regular alternation of zones in the coat color of chimeric mice has also been observed by other workers [5, 9, 10, 11, 16]. In all cases the skin on the digits was not pigmented in the chimeras. There could be two main reasons for this: either the skin in this area is formed entirely on account of the albino clone or migrating normal melanoblasts appear in this zone later, when formation of the hair bulbs is already complete and they remain outside the hair follicle, not differentiating into melanocytes.

The newborn chimeric mice weighed 1.58 ± 0.08 g compared with 1.62 ± 0.06 g for normal mice. The weight of the chimeric mice did not differ statistically significantly from that of the normal mice. A decrease in weight of chimeric mice at birth and at later stages of development was found by McLaren and Bowman [5] and also by ourselves [3]. However absence of statistically significant differences in the weight of newborn chimeric and normal mice also have been reported [9, 13, 14]. Whatever the case, the viability of the chimeras was not reduced.

The weight and dimensions of the right and left eyes were indistinguishable from normal in the chimeras. The pigmented epithelium of these eyes contained from 32 to 40% of cells with melanin. A decrease in the weight and size of the eyes was observed in seven chimeric animals. In three of these cases the eye lesion was asymmetrical. In the pigmented epithelium of these eyes from 63 to 84% of cells contained melanin (Table 1). Depending on the number of or/or cells in the clones forming the eyes variability of eye size was noted. Microph-

TABLE 1. Effect of Ocular Retardation Gene in Eyes of 6-8-Month or/or ⟷ AKR Chimeras ($x \pm S_{\bar{x}}$)

| Genotype | Right eye | | | | | Left eye | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | weight, mg | dimensions of eye, mm: along optical axis | dimensions of eye, mm: across optical axis | % of cells with melanin in pigmented epithelium | *n* | weight, mg | dimensions of eye, mm: along optical axis | dimensions of eye, mm: across optical axis | % of cells with melanin in pigmented epithelium |
| or/or | 15 | 3,8±0,71 | 1,6±0,08 | 1,6±0,07 | 100 | 15 | 3,7±0,84 | 1,6±0,08 | 1,6±0,07 | 100 |
| AKR | 11 | 26,8±1,82 | 3,2±0,09 | 3,0±0,07 | 0 | 11 | 26,1±2,24 | 3,2±0,09 | 3,0±0,09 | 0 |
| or/or⟷AKR | 18 | 27,3±1,32 | 3,2±0,12 | 3,0±0,14 | 32±5,4 | 18 | 26,7±1,48 | 3,2±0,14 | 3,0±0,20 | 40±5,8 |
| or/or⟷AKR | 4 | 20,1±1,52 | 2,8±0,30 | 2,8±0,31 | 63±4,6 | 6 | 19,2±1,48 | 2,8±0,30 | 2,8±0,26 | 64±7,8 |
| or/or⟷AAR | 3 | 14,8±1,20 | 2,6±0,18 | 2,6±0,20 | 84±5,3 | 1 | 13,5 | 2,6 | 2,6 | 82 |

thalmia and asymmetry were due to the action of the or gene in retinal cells of the chimeras.

Microphthalmia and asymmetry of the eye in $Mi^{wh}/Mi^{wh}\leftrightarrow+\leftrightarrow/+$ chimeras also were observed in our previous investigation [3]. Variability of distribution of clones forming the eyes in chimeras been demonstrated by several workers [12, 15]. However, asymmetry of the eye lesion was not noted in chimeras by these workers. The reason is that they did not study the effects of mutant genes controlling the early stages of eye formation in the chimeras.

The effect of the mutant or gene in our or/or↔AKR chimeras was thus manifested only when the mutant clone predominated in the neural structures of the eye, but even then its action was much weaker than in or/or homozygotes.

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