myotic drive, owing its origin to certain regions of heterochromatin in the genome, was described long ago in *Drosophila* and *Zea mays*. It has also been shown that deficiency of sex chromosome heterochromatin in *Drosophila* may affect the nonrandom character of segregation not only of the sex chromosomes, but also of autosomes [6, 8]. The possibility that an analogous phenomenon may be found in other organisms cannot be ruled out.

The results thus show that reciprocal variation of the content of Q heterochromatin is possible in human chromosomes. One result of this process is evidently maintenance of the stability of the genome with regard to the heterochromatin content and the balance between its components.

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EXPRESSION OF eyeless MUTANT GENES IN PRIMORDIAL MOUSE RETINA

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Autorecessive genes eyeless-1 (ey-1) and eyeless-2 (ey-2), in the homozygous state, determine a severe disturbance of morphogenesis of the eye in mice, leading to anophthalmia or microphthalmia [2, 3]. In mice of the LRDCT-AN line, with ey-1/ey-1 ey-2/ey-2 genotype, bilateral anophthalmia is observed in about 70% of cases and microphthalmia in the rest [4, 6]. Disturbance of eye development in LRDCT-AN mice takes place as early as at the optic vesicle stage. In 10-day-old mutant embryos the anlagen of the lenses are smaller than normally and, as a rule, they are located outside the cavity of the optic cup, which is abnormal in shape and orientation. In the majority of ZRDCT-AN mouse embryos the anlaga of the lens and the optic cup undergo resorption. Several hypotheses have been put forward regarding the primary mechanism of disturbance of eye development in ZRDCT-AN mice, and in particular: depression of growth of the optic vesicle, blocking out the process of lens induction by mesenchymal cells, or inability of the lenticular ectoderm to respond to inductive influences from the optic vesicle [1, 3, 4, 6, 7]. Salaün [5] reported that her data indicate expression of eyeless genes in cellular systems not involved in eye formation.

The aim of this investigation was to determine the site of action of eyeless mutant genes in mice.

METHODS

Mouse embryos, aged 10 days, of the mutant genotype ey-1/ey-1 and ey-2/ey-2 (line LRDCT-AN) were used for the experiments, and embryos of the same age, of normal genotype (line CC57BR)

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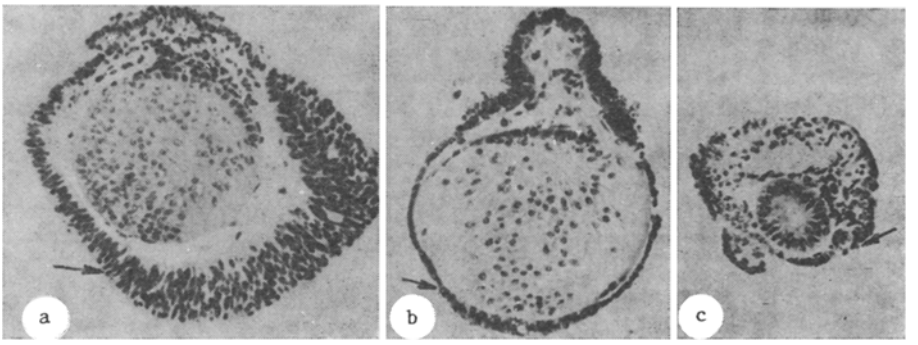

Fig. 1. Sections through anlagen of eyes isolated from 10-day mouse embryos of ZRDCT-AN line and cultured *in vitro* for 6 days. a) Retina and lens indistinguishable from control; b) retina approximately one-sixth as thick as in the control, but lens is differentiated into fibrous and epithelial parts; c) retina and lens damaged, development of lens is blocked at the vesicle stage. Arrow indicates retina. Hematoxylin, 190×

as the control. Mice of the LRDCT-AN line were generously provided by Professor H. Chase (Brown University, Providence, USA). The day of discovery of a vaginal plug was taken as Day 0 of pregnancy. The anlagen of the eyes were freed from surrounding mesenchyme and pigmented epithelium and cultured in airtight glass flasks 2 cm in diameter at 37°C in 1 ml of nutrient medium of the following composition: 0.9 ml of Eagle's medium with glutamine and 0.1 ml of uninactivated embryonic calf serum. The nutrient medium was aerated beforehand with a mixture of air and 5% CO_2. After culture for 3 or 6 days (in the latter case the nutrient medium was changed after 72 h) the anlagen of the eyes were fixed in Carnoy's fluid and embedded in paraffin wax; serial sections cut to a thickness of 5 μ were stained with Carazzi's hematoxylin. Differentiation of the lens and retina was studied in the cultured anlagen of the eyes.

RESULTS

After culture for 3 days, all 22 primordial eyes of mouse embryos of the CC57BR line (control) had a well marked retina and lens, similar in structure to those of 13-day mouse embryos of the same genotype. The lens was differentiated into fibrous and epithelial parts, and mitotically dividing cells could be seen in the retina. In the course of 6 days 14 anlagen of the eyes of CC57BR mice were cultured. These anlagen were enlarged, and the retina in many cases showed evidence of differentiation of the ganglionic layer. However, under these circumstances pycnotic nuclei were observed in the retinal cells with lysis of the lens fibers. Despite destructive changes in some cells after 6 days of culture, development of the lens and retina in anlagen of the eyes of normal genotype in vitro was similar to that *in situ*. These findings indicate that the conditions of culture were optimal for development of eye anlagen.

The results of culture of primordial eyes of mouse embryos of the ZRDCT-AN line are given in Table 1: 18 eye anlagen were cultured for 3 days and 59 for 6 days. After both 3 and 6 days of culture, a high percentage of cases of abnormal development of this retina and lens were observed. After culture for 6 days the lesion of the retina was much more evident and was present in a higher percentage of cases than disturbance of lens development. Development of the retina and lens in 18 of the cultured anlagen of the eyes was similar to the control (Fig. 1a). In nine other cases development of the retina was severely disturbed, for it consisted of only one layer of cells and was 4-6 times thinner than in the control. In these eye anlagen, however, the lens had differentiated into epithelial and fibrous parts (Fig. 1b). The lens in 13 eye anlagen consisted of a vesicle, the cavity of the optic cup was filled with mesenchymal cells, and the thin retina was undifferentiated and infiltrated by pigment (Fig. 1c). In 37 eye anlagen no signs of formation of the lens or retina could be discovered after 3 or 6 days of culture, but instead there were collections of cells, some of which were pigmented.

Thus of 77 high anlagen cultured only 18 (23.3%) had relatively normal differentiation of the retina and lens. Severe disturbance of retinal development was observed in all the other 59 eye anlagen. Disturbance of development of the lens was not observed in every case.

TABLE 1. Culture of Primordial Eyes of 10-Day Mouse Embryos of LRDCT-AN line *in vitro*

Duration of culture, days	Number of eye anlagen	Retina		Lens	
		C	A	C	A
3	18	6	12	6	12
6	59	12	47	21	38

Legend. C) Development similar to control; A) abnormal development, structure of retina or lens disturbed and differs sharply from control.

For instance, in nine eye anlagen the retina was much thinner than in the control, although the lenses had differentiated into fibrous and epithelial parts. Consequently, in these cases induction of the lens and differentiation of its cells were undisturbed, and the effect of mutant eyeless genes was manifested only as inhibition of growth of the retina. In those eye anlagen in which disturbance of development of the lens and retina was observed, the lesion of the lens could be due to a disturbance of its induction as a result of expression of the mutant eyeless genes in cells of the optic vesicle. The results show that development of eye anlagen of 10-day mouse embryos of the ZRDCT-AN line *in vitro* is disturbed in about the same percentage of cases as *in situ*. Salaün [5] observed disturbance of retinal development in eye anlagen from 10-day mouse embryos of the ZRDCT-AN line in culture only in 10% of cases. A smaller percentage of disturbed development of eye anlagen in culture in Salaün's study compared with the results of the present investigation can evidently be attributed to the fact that Salaün cultured eye anlagen together with their surrounding mesenchyme and pigmented epithelium, as the result of which the eyeless mutant genes were not expressed in all the eye anlagen.

The results of the present investigation thus show that mutant eyeless genes act in cells of the primordial retina. The time of initiation of expression of mutant eyeless genes evidently varies, and it is this which determines the development of different anomalies of the eye in LRDCT-AN mice. If the mutant eyeless genes are expressed in cells of the optic vesicle this leads to anophthalmia, but if these genes begin to be expressed later — at the stage of formation of the optic cup — microphthalmia arises.

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