Differentiation of the optic cups 
from an anophthalmic murine strain, in culture 
and in intrafoetal grafts

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SUMMARY

The optic anlage of the anophthalmic murine strain ZRDCT/an has been studied. In this strain, 70% of the adults are anophthalmic, 30% are one-eyed. The eye primordium appears and starts to differentiate at day 9 of gestation, then it degenerates. At 13 days of gestation, it has practically disappeared.

When isolated and cultured in vitro, the 10-day anophthalmic anlage develops in the same way as a control optic-cup culture from a Swiss or C57B16 mouse.

When grafted in the back of a 15–17 day Swiss foetus, this 10-day anophthalmic anlage also develops: retina and lens differentiate, the structures which grow are very similar to those obtained when C57B16 optic cups are grafted in the same conditions.

It appears from these results that the eye regression of the mutant in situ is not a process intrinsic to the anlage. If withdrawn from its local environmental influences, the eye primordium is able to express its aptitude for differentiation.

INTRODUCTION

In the anophthalmic murine strain, first described by Chase & Chase (1941) and by Chase (1942, 1944, 1945), 90% of the adults are anophthalmic whilst the remaining 10% are either one-eyed or microphthalmic. The eye primordia appear and start differentiating on day 9, but later on they degenerate and in most cases are regressed completely at day 13. Various hypotheses, based mainly on morphological observations, have been put forward to explain the defect. For Silver & Hughes (1974), the malformation results from the non-degeneration, between the optic cup and the presumptive lens ectoderm, of mesenchymal cells, which normally disappear. According to Harch, Chase & Gonsalves (1978), the location of the lens in the optic cup is essential for the eye's development. If ectopic, the lens disappears and subsequently the optic rudiment regresses. Whatever the process of involution (leading to the expression of the mutation) may be, the problem which remains to be solved is whether the eye is doomed to regress, or if it can be rescued when withdrawn from the environment of the mutant embryo. We tried to answer this question through

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two experimental approaches: in vitro organ culture, to reveal the possible self-differentiating capacities of mutant eye primordia; and intrafoetal grafting of mutant eye anlagen in normal hosts, to determine if a normal foetal environment can modify the expression of the mutation.

METHODS

(I) Animals

The strain used in this study was the eyeless strain ZRDCT/an, derived from the initial strain used by Chase; this strain was kindly supplied by Dr Kaiserman-Abramof, and is now currently bred in our laboratory. This line is genotypically non-agouti, brown, pink-eyed and dilute (aabbddpp) (Beck, 1963). It is characterized by the fact that 70% of the adults are completely anophthalmic, the remaining 30% being unilateral or bilateral microphthalmics. However, already from 10 days of gestation onwards, the difference between the primordia doomed to regress and the ones able to differentiate is conspicuous; only the first category of rudiments was chosen for experimental purpose.

Two different strains were used as controls: the pigmented C57BL6 strain and the albinos Swiss strain. Mice were placed with males at 6 p.m. and were checked for vaginal plugs the next morning, which was designated as day 0 of gestation. The pregnant mice were killed by cervical dislocation at chosen times. The foetuses were removed and collected in phosphate-buffered saline. The eye anlagen were dissected; some of them were fixed immediately in Bouin’s mixture for routine microscopy. The others were used either for organ culture or for intrafoetal grafting.

(II) Organ culture

Dulbecco-modified Eagle’s medium, plus 15% non-inactivated foetal calf serum, was used. Eye primordia were taken from 10-day-old foetuses. They were explanted with the surrounding mesenchyme on to Millipore filters (pore size: 0.22 μm) and incubated at 38 °C in humidified air +10% CO₂, for 6 to 8 days.

(III) Intra-foetal grafting

Eye primordia for grafting were taken from 10-day-old foetuses. Hosts were 14- to 17-day-old foetuses. The latter remained inside the uterus during the whole procedure, which was performed according to the technique of Jost (1946), as follows: the pregnant mouse was anaesthetized with Imalgène 200 Mérieux (0.5 to 0.7 ml i.p.). The uterus was then exteriorized and one foetus

Fig. 1. Optic cup of 10-day Eyeless embryo. Bar = 100 μm.
Fig. 2. Optic cup of 10-day SWISS embryo. Bar = 100 μm.
Fig. 3. Residual eye rudiment from a 13-day Eyeless embryo. Bar = 100 μm.
Fig. 4. Differentiating eye from a 13-day SWISS embryo. Bar = 100 μm.
Anophthalmic optic cup in vitro and in intrafoetal grafts
Anophthalmic optic cup in vitro and in intrafoetal grafts

Table 1

<table>
<thead>
<tr>
<th>Number of cases</th>
<th>Pigment</th>
<th>Retina</th>
<th>Lens</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZRDCT/an</td>
<td>43</td>
<td>43 (100%)</td>
<td>39 (90%)</td>
</tr>
<tr>
<td>SWISS</td>
<td>38</td>
<td>0</td>
<td>38 (100%)</td>
</tr>
<tr>
<td>C57BL6</td>
<td>23</td>
<td>23 (100%)</td>
<td>23 (100%)</td>
</tr>
</tbody>
</table>

Differentiation of the eye cup of ZRDCT/an, SWISS, C57BL6, in vitro. The eye was explanted from 10-day embryos for 6 to 8 days.

chosen as a host. A small area facing this foetus was defined on the uterus by means of a very thin surgical thread (Ethibond-Ethnor Paris), and the rudiment to be grafted – marked with charcoal powder – was inserted in this area, through the uterus, under the skin of the foetus as near as possible to the head. The abdominal wall of the mouse was then stitched; in most cases the pregnancy went on normally. It is frequent for newborn operated animals to be eaten by the mother. Therefore, they were mostly recovered by cesarean section and transferred to an adoptive mother from a tolerant strain (for example strain 129). They were sacrificed a few days later. Charcoal-marked grafted primordia were easily recognizable. They were dissected away and processed for routine microscopy.

RESULTS

(I) Development in situ of normal and mutant optic cup

We first compared the successive stages of eye differentiation of the mutant strain with those of the two control strains: C57BL6 and Swiss, differing by the presence or the absence of pigment granules in the pigmented layer of the retina. As shown in Fig. 1, on day 10 of gestation the optic cup of the mutant strain was present, but was already smaller than in controls (Fig. 2). As development proceeded, differences between the mutant and the normal strains became more and more noticeable. On day 11 of gestation, whereas pigment granules started to appear in the C57BL6 eye, none were found in the mutant; moreover, in the latter, the optic cup was reduced and irregular in shape. Finally on day 13, whereas in the controls typical lenses and retina have partially differentiated,

Fig. 5. SWISS optic cup explanted at 10 days and cultivated for 6 days. Bar = 100 μm.
Fig. 6. C57BL6 optic cup explanted at 10 days and cultivated for 6 days. Notice the pigment granules. Bar = 100 μm.
Figs. 7, 8. Eyeless optic cup explanted at 10 days and cultivated for 6 days. Notice the dark pigmented layer (Fig. 7), and the scattered pigment granules (Fig. 8). Bar = 100 μm.
in the mutant only a very rudimentary eye with a degenerated lens was left (Figs. 3 and 4).

(II) In vitro differentiation of normal and mutant optic cup

When control eye rudiments were explanted in vitro, they differentiated almost as in vivo: in explants from C57BL6 mice, the pigment became very abundant (of course none differentiated in SWISS eye rudiments), neural retina became thicker, and in most cases ganglion cells appeared at the inner limiting layer. On the other hand, although lenses sometimes degenerated (9/38 in the SWISS strain, 5/23 in the C57 mice), in most cases, typical fibres differentiated (Figs. 5 and 6).

When the eye rudiments from the ZRDCT/an strain were explanted in vitro, the differentiation process was closer to normal than in situ; the three main structures of the eye, lens, neural retina, and pigmented layer, developed in most cases. Dark-brown pigment formed, generally visible macroscopically, it was either homogeneously spread over the cultured eye, or scattered into patches. Lens differentiation was less constant. However in a number of explants, normal fibres appeared; in that case, the cultured mutant eye rudiments looked very similar to the controls (Figs. 7 and 8). These results are summarized in Table 1.

(III) Differentiation in intrafoetal grafts of normal and mutant optic cups.

The eye anlagen from 10-day C57BL6 and ZRDCT/an embryos were grafted under the skin of the back of 16- or 17-day Swiss foetuses (Fig. 9). Mortality was high in experimental foetuses, and newborn animals bearing a graft were very difficult to raise. (a) 39 grafts of C57BL6 were performed. Only 10 transplants were recovered (25 %), in six of which neural retina was differentiated (60 %) (Fig. 10); (b) from 62 grafts of ZRDCT/an to Swiss foetuses, 14 transplants were recovered (22 %); in 9 of these grafts, there was a well-differentiated neural retina, (64 %) (Figs, 11 and 12).

In both kinds of transplants, lens only appeared in one third of cases. Often, the topography of the different eye tissues was disturbed, probably because of the ectopic site and the local growth of the host tissues.

As far as pigmentation is concerned, a marked difference was observed between the two kinds of grafts, especially by comparison with the in vitro results; whereas pigment granules differentiated in all C57BL6 optic cups in graft as well as in culture, the appearance of dense pigment in Eyeless anlage grafts never occurred, although it was always present in the cultures.

Fig. 9. C57BL6 10-day optic cup, grafted to the back of a 16 day SWISS foetus, and photographed 2 days after birth. Bar = 500 μm.

Fig. 10. C57BL6 10-day optic cup grafted to the back of a 16-day SWISS foetus, and studied histologically the day after birth. Bar = 100 μm.

Figs. 11, 12. Eyeless 10-day optic cup, transplanted to the back of a 16-day SWISS foetus and studied histologically the day after birth. Bar = 100 μm.
These results show that (1) the anophthalmic anlage, when withdrawn from its specific environment, is able to differentiate much better than in situ; (2) the anophthalmic anlage and the normal anlage, in two different experimental situations, grow and differentiate exactly in the same way. This is true in the two kinds of transplantations performed in our experiments, i.e. in vivo and in vitro. In both conditions, a fairly typical differentiation of the retina was obtained. Lens developed also in some cases but it seemed to be the most labile structure, in the anophthalmic anlage as well as in the normal one. Nevertheless, there are differences between the in vivo and in vitro results. In the grafts, the whole topography of the organ is more deeply disturbed, even if the different tissues are well differentiated. Growth and morphogenetic processes of the host may lead to partial disorganization of the graft. This may account for the disappearance of the lens in some cases. As has been well known for a long time and has recently been confirmed in the mouse, the induction of lens and its maintenance are dependant on the neural retina (Muthukkaruppan, 1965). An abnormality either in the relative spacial organization or in the time sequence in inductions (Konyukhov & Vakhrusheva, 1969) leads to the non-development of the lens. In the case of the in vitro cultures, the whole explant flattens; this may explain why also in this case, lenses occur less frequently than retinas. Pigmentation is always obtained with the C57BL6 cup, in vitro as well as in intrafoetal grafts. Conversely, in the case of the ZRDCT/an optic cup, the dark pigmentation appears only in vitro. In this connexion, Sidman & Pearlstein (1965) noticed an increased pigmentation in normal eye cultures of adult mice homozygous for the p gene. In situ, these eyes appear grossly pink, with unpigmented or partially pigmented granules. The ZRDCT/an strain is also homozygous for the p gene. Therefore, we can conclude that in our culture conditions the mutant anlage fully expresses its capacities and especially the p gene properties.

As clearly demonstrated by our results, the potentiality for differentiation exists even after inhibition in situ has taken place; (see for comparison 10-day mutant and control anlage, Figs/ 1 and 2). This situation differs from that described by Sidman (1961) in another inherited dystrophy of the eye, where evolution of the organ is identical in vitro and in vivo.

This author concluded that the disease must be intrinsic to the eye. In the ZRDCT/an mutation, if the rudiment isolated from the rest of the mutant organism, it is able to express its potentialities, which are strongly restricted in situ. Hence it seems likely that regression of the eye is not the expression of an intrinsic deficiency in the organ. Rather it results from deleterious influences of the environment. Whether these influences are of local (head of the mutant) or general origin in the ZRDCT/an strain organism is not known. Grafting of Eyeless optic cups in the dorsal skin of Eyeless mutants are presently in progress to solve this problem.
RESUME
Différenciation en culture et en greffe intrafoetale de cupules optiques issues d'une souche de souris anophtalmique

La différenciation de l'oeil de la souche de souris anophtalmique ZRDCT/an a été étudiée par les techniques de culture in vitro et de greffe intrafoetale. Dans cette souche, 70% des adultes naissent anophtalmes, les 30% restants étant borgnes. Chez l'embryon, la cupule optique apparait à 9 jours puis elle subit une involution, et à 13 jours de gestation, elle a pratiquement disparu.

Isolée en culture in vitro, l'ébauche prélevée sur un embryon ZRDCT/an de 10 jours, se développe de façon analogue à celle de cupules optiques de souris SWISS et C57BL6 du même âge, placées dans les mêmes conditions. Cette même ébauche greffée dans le dos de foetus SWISS de 15 à 17 jours de gestation, se développe également : la rétine et le cristallin se différencient, et les structures formées sont tout à fait comparables à celles obtenues avec les greffons de cupules optiques témoins.

Il semble donc que la régression de l'œil in situ n'est pas un phénomène intrinsèque à l'organe. Soustraite à l'influence de l'environnement local, la cupule optique ZRDCT/an est capable d'exprimer ses capacités de différenciation.

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REFERENCES


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