**In vitro Culture of the Eye and the Retina of the Mouse and Rat**

by D. R. LUCAS and O. A. TROWELL

From the Medical Research Council Radiobiological Unit, Harwell, and Group for Research in Ophthalmological Genetics, Royal College of Surgeons, London

WITH TWO PLATES

**INTRODUCTION**

Strange & Fell (1926) and Dorris (1938) showed that embryonic chick eyes could be cultivated successfully *in vitro* on plasma clots, and Harrison (1951) and Reinbold (1954) obtained satisfactory results with an agar-tyrode medium containing embryo extract. The retina in these eyes was undifferentiated and, though little or no increase in the size of the eyes occurred, the authors agreed that cytological differentiation proceeded at a more or less normal rate.

Tansley (1933) reported the successful cultivation on plasma clots of whole eyes from rat embryos of 14–17 days and of isolated retina from rats up to 5 days old. Cytological differentiation proceeded at a normal rate, at least for the first few days in culture, but excessive growth of mesodermal elements tended to occur after 12 to 14 days. Rosette formation was a prominent feature in these cultures.

The present work differs from previous studies in that most of the material cultured was more mature and a fluid medium free from embryo extract has been used. The maturity of the material was governed chiefly by the fact that it is at this stage of retinal differentiation that retinal dystrophies, which will be the subject of a separate study, occur (Sorsby et al., 1954; Lucas et al., 1955).

**MATERIAL AND METHODS**

*Material*

CBA mice aged 8–14 days. Albino rats aged 1–2 and 9–14 days. Both lines are maintained on a cubed diet at the M.R.C. Radiobiological Unit, Harwell.

The animals were decapitated and the eyes removed aseptically. In the case of retinal cultures, the retina was dissected out in culture fluid. In mice the retina was divided into two pieces, and in rats it was divided into four.

---

1 Authors' address: M.R.C. Radiobiological Unit, A.E.R.E., Harwell, Berkshire, U.K.

Culture technique

The culture chamber was made of Perspex (Lucite) and contained a shallow dish in which was a sheet of lens paper supported on a tantalum wire grid exactly as described by Trowell (1954). The culture medium was the synthetic medium (TACPI) described by Trowell (1955) with the addition of 15 per cent. rat serum. The volume of culture medium was 6 ml. and the medium was changed after 4 days. The chambers were either circulated very slowly with a mixture of 97 per cent. oxygen and 3 per cent. CO₂ or left containing air. When the chambers contained air the side tubes were sealed off, and the pH of the culture medium was controlled by periodically opening one of the side tubes to allow escape of CO₂.

Histological technique

The tissues were fixed in acid Zenker’s fluid, dehydrated in cellosolve, and embedded in ester wax (Chesterman & Leach, 1956). Sections were cut at 5 μ and stained with Mayer’s haemalum and alcoholic eosin.

RESULTS

Whole eyes

Some excellent results were obtained with the eyes of mice 8–11 days old, cultured in 97 per cent. oxygen with the cornea uppermost. In some of these all layers of the retina survived in good condition for up to 8 days (Plate 1, figs. A, B). Limited areas of necrosis were, however, sometimes present at the posterior pole. There was some folding and tunnel formation in the outer nuclear layer, but, if the eye was not punctured when planted, no herniation of retina through the sclera occurred as reported by Strangeways & Fell (1926) and Tansley (1933). The deformation of the outer nuclear layer made it difficult to ascertain whether any cells had been lost, but any such loss was certainly less than in the isolated retina (see below).

In the eyes of rats, aged 1–2 days, cultured in air or oxygen for 4–8 days, most of the retina became necrotic whichever way up the eye was placed. Such areas of retina as survived usually showed some structural disorganization.

The other ocular tissues, including lacrimal gland, survived well for the period of cultivation whether under 97 per cent. oxygen or air. The centre of the lens underwent necrosis, but the epithelium and adjacent lens fibres remained intact.

Isolated retina

The retina became doubled over during dissection and division into two or four pieces and, during culture, the lower layer usually degenerated, but the upper layer survived remarkably well. Retinae of both rats and mice of age 9–14 days cultured in air for up to 10 days—the longest period tried—showed good histological preservation (Plate 1, fig. D).
The number of cells in both inner and outer nuclear layers was reduced in the cultured retinae by up to one-third as compared with in vivo controls. The thickness of the inner fibre layers, which normally is increasing at this age, was also less than in controls (Plate 1, fig. C). In some mouse retinae the rod nuclei were apparently being replaced by nuclei with a much more open chromatin pattern resembling cone nuclei, but more likely to be glial elements. Mitotic figures were very rarely seen.

Higher concentrations of oxygen were found to be toxic. Retinae cultured in 97 per cent. or even 60 per cent. oxygen were totally necrotic after a few days, though the ciliary epithelium (pars ciliaris retinae) survived satisfactorily (Plate 2, fig. H). The ciliary epithelium arises from the anterior part of the retina during post-natal development and it is interesting that this epithelium should be resistant to high concentrations of oxygen, whereas all the elements of the retina proper were completely destroyed.

In an entirely synthetic medium (TACPI) rat retinae survived quite well for 7 days (Plate 2, fig. I), but mouse retinae did rather poorly (Plate 2, fig. J).

Differentiation of visual cells

In rats and mice aged 9–11 days, the nuclei of the visual cells are ovoid (Plate 2, fig. E), and the outer segments only just evident. During the ensuing few days rapid elongation of the rod elements normally occurs (Detwiler, 1932) and slower differentiation of the nuclei (Sorsby et al., 1954; Lucas et al., 1955). The nuclear change consists of coalescence of the chromatin, first to two or more large masses (Plate 2, fig. F), and eventually, after several weeks, to a single spherical mass surrounded by a delicate crenated membrane. In the cultures this nuclear differentiation appeared to proceed exactly as in vivo (Plate 2, fig. G), although no further growth of the rod elements occurred.

Discussion

The advantages of this culture method have already been discussed (Trowell, 1954) and the present results show that by this technique the retina of the rat and mouse can be maintained in good histological condition for about 10 days. Under these conditions, however, the fibre layers and sensory organelles do not grow further. The loss of nuclei from the nuclear layers may well be due to loss of cells from trauma during dissection. Retinae examined shortly after dissection showed a certain number of pycnotic nuclei.

The adverse effect of 97 per cent. oxygen on the isolated retina is one of the most interesting features of the present work. In the case of the whole-eye cultures, the outer layers would consume oxygen and also hinder its diffusion to the retina, so the better survival of the retina when 97 per cent. oxygen was used was not unexpected. The somewhat variable results obtained when the eye was placed cornea uppermost may well have been due to the difficulty of achieving
a satisfactory balance between oxygen-deprivation and oxygen-poisoning over the whole retinal field. The poor results obtained when the eye was placed cornea downwards are likely to have been due to oxygen-poisoning, since the anterior part of the retina was often spared.

Other workers have shown that the retina, and also the cerebral cortex, are peculiarly susceptible to oxygen poisoning. Thus, Gyllensten & Hellström (1955) kept 10-day-old mice in an atmosphere of oxygen and found that this caused atrophy of the inner retinal layers. Noell (1955) kept adult rabbits in an atmosphere of oxygen and found widespread necrosis of the visual cells within a few days. Dickens (1946) incubated brain slices in Warburg manometers, using a gas phase of pure oxygen, and found that respiration was slowly and irreversibly poisoned after a few hours.

One of us (Trowell, to be published) has found that the following organs of the young rat can be successfully cultured for at least a week, using the technique described here and a gas phase of 97 per cent. oxygen and 3 per cent. CO₂: lymph node, thyroid, parathyroid, pituitary, pineal, ovary, uterus, fallopian tube, vas deferens, prostate, ureter, trachea, lung, adipose tissue, submandibular gland, and sympathetic ganglion. For all these organs culture in oxygen gave more satisfactory results than culture in air, and there was no sign of oxygen-poisoning. The retina is the only exception which we have so far encountered.

Strain C3H mice exhibit a genetically determined retinal dystrophy which begins to appear at age 11 days. The results of culturing potentially dystrophic C3H retinae will be reported in a subsequent paper.

SUMMARY

1. By means of an organ culture technique previously described, whole eyes of 8–11-day-old mice have been successfully maintained in vitro for 8 days. Isolated retinae from both rats and mice aged 9–14 days have been similarly maintained for 10 days.

2. Isolated retinae survived best in a gas phase of air; 60 per cent. oxygen was toxic. In the whole eye, on the other hand, the retina survived better when pure oxygen was used, doubtless because the outer coats of the eye both consume oxygen and hinder its diffusion to the retina.

3. Isolated rat retinae survived well in an entirely synthetic medium, but mouse retinae did not.

4. Differentiation of the visual cell nuclei proceeded normally in vitro.

ACKNOWLEDGEMENT

The histological sections were prepared by Mr. W. R. Lush and Miss E. Peakman, whose skill we gratefully acknowledge.
REFERENCES


EXPLANATION OF PLATES

**PLATE 1**

Fig. A. Eye of mouse aged 11 days after 7 days' culture in 97 per cent. oxygen. × 15.

Fig. B. Retina of the eye shown in fig. A. × 335.

Fig. C. Retina of mouse aged 21 days. For comparison with B and D. × 335.

Fig. D. Retina of mouse aged 11 days after 10 days' culture in air. A double layer was cultured, the upper layer has survived well, the under layer is atrophied and entangled with lens paper. × 335.

**PLATE 2**

Fig. E. Visual cell nuclei of mouse aged 11 days. × 1,840.

Fig. F. Visual cell nuclei of mouse aged 21 days. × 1,840.

Fig. G. Visual cell nuclei of retina of mouse aged 11 days after 10 days' culture in air. Compare with E and F. × 1,840.

Fig. H. Retina of rat aged 13 days after 7 days' culture in 60 per cent. oxygen. All elements of the neuroretina are dead but the ciliary epithelium is healthy. × 335.

Fig. I. Retina of rat aged 13 days after 7 days' culture in synthetic medium in air. × 335.

Fig. J. Retina of mouse aged 10 days after 7 days' culture in synthetic medium in air. × 335.

(Manuscript received 30: vii: 57)