INFLUENCE OF EMBRYONIC IMPLANTS UPON LENS REGENERATION IN RABBITS*†

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DURING the early embryological development of the crystalline lens, the lens invaginates to form the lens vesicle. As a rule, a few cells of the surface ectoderm become trapped in the lens vesicle and eventually disappear by cytolysis. Some authors (Sikharulidze, 1956; Chanturishvili, 1958; Stewart, 1960), claim that these perishing cells release substances essential for the proper continued development of the lens. Their experiments seem to indicate that implantation of cytolyzing ectodermal cells of the embryo into the anterior segment leads to larger regenerates more frequently.

Experiments published during the 19th century and our own experiences, which demonstrate the possibility of adequate lens regeneration without cytolyzing embryonic material, seem to be in disagreement with these statements.

To clarify this question, experiments on lens regeneration with embryonic implants were performed on rabbits.

Methods and Materials

Adult rabbits were used. Bicillin injections were given on the day before surgery. Anaesthesia was obtained by intravenous Pentothal injection. Great care was taken to perform surgery under strictly sterile conditions.

After preparing a fornix-based conjunctival flap, the anterior chamber was opened by a corneo-scleral section reaching from 9 over 12 to 3 o'clock. A key-hole iridectomy was performed in the 12 o'clock position. To preserve the suspensory ligament, the lens capsule was opened by a semicircular incision parallel to the lens equator and just central (anterior) to the insertion of the zonule fibres (Fig. 1).

FIG. 1.—Incision of anterior lens capsule central (anterior) to insertion of zonule fibres.

* Received for publication July 17, 1961.

† This study was supported by research grant B-2678 of the National Institutes of Health, Bethesda, Maryland, U.S.A.

The lens was extracted through this opening, and a small piece of embryonic lid ectoderm measuring 1–2 mm. was implanted into the capsule sac (Fig. 2).

FIG. 2.—Cytolysing ectodermal embryonic material in evacuated lens capsule (arrow) 24 hrs after extracapsular lens extraction.

The corneo-scleral wound was closed with gut sutures and the iris carefully replaced. The conjunctival flap was pulled over the wound and anchored with wing sutures at 5 and 7 o'clock at the limbus. Bacitracin ointment was applied daily for the first 10 post-operative days.

The implants were taken from the eye region of rabbit embryos of 16 days ovulation age and stored at refrigerator temperatures under sterile conditions for 5 days.

Of the 42 rabbit eyes thus treated, six had to be discarded because of severe post-operative reaction. Eyes which did not show signs of lens regeneration for 3 months were enucleated. Also enucleated were eyes which showed some early regeneration but which failed to continue growing for as long as 3 months. The longest observation time was 12 months.

All 36 eyes included in this study were frequently examined with ophthalmoscope and slit lamp. All were processed histologically to determine the final size of the regenerates.

Results

As compared with a previously reported control group (Binder, Binder, Wells, and Katz, 1961), in which the aqueous of the anterior chamber cleared on an average within 10 days post-operatively, the eyes used in these experiments took one week longer for the aqueous flare to clear.

Although it was the aim to place the embryonic implants inside the evacuated lens capsule, in about two-thirds of the eyes the implants were found outside the lens capsule one week after surgery.

The implants of embryonic material disappeared by absorption within 1 to 4 weeks in 23 eyes. In six eyes the implants remained in their original position, became apparently attached to their environment (cornea, iris, lens capsule), and gradually shrank to small densely-white nodules. In the remaining seven eyes, however, the implants gradually enlarged; they reached

considerable proportions within several weeks and caused mechanical difficulties. Three of these monstrosities resembled an eye (Fig. 3), while others were amorphous, showing hair growth, areas of pigmentation, and chalky-white spots.

FIG. 3.—Enlarging conglomeration of ocular embryonic tissue (I) in anterior chamber 6 weeks after surgery.

Nine of the regenerates were “large”, the newly-formed tissue filling the capsule rather completely in a frontal plane, while the antero-posterior diameter did not reach the thickness of a normal lens (Fig. 4, and Figs 5 and 6, opposite).

FIG. 4.—Two “large” lens regenerates. The eyeballs were halved at the equator after fixation and the lenses were photographed from behind. Arrows point to 12 o'clock position of lens equator.

They could be readily recognized on routine examination. With the exception of one, these regenerates showed a varying degree of distortion of the surface in the scar area. Slit-lamp examination showed linear or spotty opacifications where the capsules had been opened. Vacuoles were found frequently (Fig. 7, opposite). Fundus detail could be seen readily through the regenerates, but in an optically distorted way.

FIG. 5.—“Large” lens regenerate (L) after 27 wks.
A.C. anterior chamber.

FIG. 6.—“Large” lens regenerate 21 wks after surgery (same eye as in Fig. 3).

L lens regenerate not clinically visible

C cornea.

I conglomeration of embryonic ocular tissues.

FIG. 7.—“Large” lens regenerate seen in transillumination of freshly enucleated eye. The light source was held against the posterior pole.
V lenticular vacuoles.

Twelve eyes produced “sizeable” regenerates which filled at least half of the frontal plane of the lens capsule without reaching full thickness in the antero-posterior diameter (Fig. 8, overleaf). These regenerates were clear, but vacuolar and of irregular optical density. Their optical value was further limited by the fact that they filled only part of the pupillary area.

Smaller regenerates resembling a Soemmerring’s ring were found in seven cases and five eyes failed to show any definite signs of lens regrowth.

FIG. 8. "Sizeable" lens regenerate (L).
A.C. anterior chamber.
Arrow points to inferior equator.

Discussion

While some regenerates developed rapidly within the first few post-operative months, others enlarged more hesitantly, showing periods of rapid enlargement alternating with periods of apparent cessation of growth. Some of the regenerates had already reached their final size within 18 weeks, while others took a full year for the same amount of growth to occur.

It was difficult to decide whether the fate of the implant or its post-operative location inside or outside the lens capsule had influenced the results, since there was no regular association of those circumstances with the size or quality of the regenerates.

The longer duration of post-operative irritation as evidenced by a cloudy aqueous humour on slit-lamp examination seemed to be rather regularly associated with "large" and "sizeable" regenerates. The average time which elapsed before the complete clearing of the aqueous was 20 days (longest 29, shortest 14).

In the eyes which contained small regenerates or which failed to grow a new lens, the aqueous had already cleared by an average of 12.6 days (longest 26, shortest 9).

It had been previously observed (Randolph, 1900) that the hyperaemia of chronic post-operative irritation is helpful for lenticular regrowth. This is understandable if one considers that the growing embryonic lens is also surrounded by a vascular tunic during part of the period of gestation.

The refractive qualities of the lenses did not lend themselves to satisfactory retinoscopy because of gross optical irregularity, although as a rule the fundi could be well observed.

Summary

In the series under discussion, 25 per cent. of the regenerates were "large" or "sizeable". In a control series (Binder, 1961) without embryonic implants, "large" and "sizeable" regenerates were obtained in 28 per cent. of the eyes after extracapsular lens extraction.

Prolonged post-operative irritation, most likely caused by the presence of cytolyzing embryonic tissue, seemed to promote lenticular regrowth.

We conclude from this study, first that in our hands the implantation of cytolyzing ectodermal embryonic material does not offer any advantage over simple lens regeneration, and secondly that the implantation of embryonic material may sometimes lead to the development of disturbing intra-ocular monstrosities.

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