INTRALAMELLAR HETEROGRAFTS OF FROG CORNEA INTO RABBIT CORNEA*

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THE idea of heterograft keratoplasty was first conceived by Himly (1813). Early experimental work (Reisinger, 1824; Bigger, 1837; Dieffenbach, 1831; Lesser, 1908) and clinical application (Bigger, 1837; Power, 1873; Sellerbeck, 1878; Fuchs 1894), however, both proved unsatisfactory and in some cases disastrous. Because of these failures and the success of homotransplants, heterogeneous corneal grafting was almost abandoned, until the work of Babel and Bourquin (1952) and Choyce (1952) revived interest in experimental heterografts. Basu and Ormsby (1957) found that the antibody-antigen reactions were less frequent with avian than with mammalian corneae and surmised that the farther apart in the scale the donor and recipient tissues were the less likelihood there was of antigen-antibody reaction. Tsutsui and Watanabe (1959) studied intralamellar fish heterografts in the rabbit with encouraging results, and this prompted our experiments with amphibian corneae.

Methods and Material

Adult albino rabbits weighing from 2 to 4 kg. were used as recipients. Graft tissue was taken from pithed Indian frog (*Rana tigrina*) before the animal was dead. A 6 mm. circular disc of the entire thickness of the cornea was taken out with Castroviejo's trephine and kept in penicillin-saline solution (50,000 units/ml.) for up to 45 minutes before use.

The recipient rabbit was anaesthetized by intraparenteral Pentothal. The two lids were separated by lid sutures. A curved incision about 7 mm. in length was made from 10 to 2 o'clock at the limbus or 0.5 to 1 mm. inside it through three-quarters of the thickness of the cornea. The cornea was then split from the incision making a sufficiently large intralamellar pocket extending well below the lower margin of the pupil into which a graft could be placed in the centre of the cornea. Every effort was made to keep the line of separation between the same two lamellae during this procedure. The graft was then spread evenly in position with the help of a spatula, the corneal incision was closed with two or three sutures, and the two lids were sutured together by a single stitch. Soon after the operation, 150,000 units of penicillin were given intra-muscularly and another 25,000 units subconjunctively. No local or systemic antibiotics or steroids were used post-operatively.

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Frog heterografts were inserted into twelve rabbit eyes, and eight chicken and two dog heterografts were inserted by the same technique as controls.

For the first 3 days the cornea of the eye behind the stitched lid was examined daily at the side of the stitch. On the fourth day the suture was removed and the eye was examined directly with the unaided eye and with the slit lamp. The eyes were observed on alternate days for the first 3 weeks and then weekly.

Observations

Period of Observation.—This varied, as some eyes were enucleated for histological purposes, but it extended in some instances up to 20 weeks.

Clinical Results.—These are given in the Table. All the operated eyes showed mild to moderate conjunctival reaction in the early post-operative period, but this gradually subsided.

TABLE
RESULTS IN 12 FROG AND 10 CONTROL EXPERIMENTS

Donor	Experiment No.	Period of Observation (wks)	Results				Remarks
			Opacity	Vascularization	Ectasia	Infection	
Frog	1	14	+	±	—	—	Enucleated
	2	8	—	—	—	—	Enucleated
	3	8	—	—	—	—	Enucleated
	4	8	—	—	—	—	Enucleated
	5	8	—	—	—	—	Enucleated
	6	8	—	—	—	—	Enucleated
	7	4	—	—	—	—	Enucleated
	8	14	—	—	—	—	Under observation
	9	4	—	—	—	—	Enucleated
	10	12	—	—	—	—	Under observation
	11	12	—	—	—	—	Under observation
	12	12	—	—	—	—	Under observation
Chicken	1	19	—	—	—	—	Under observation
	2	10	+	—	—	—	Under observation
	3	19	++	+	—	—	Under observation
	4	19	+	+	—	—	Under observation
	5	10	++	++	—	—	Enucleated
	6	19	++	+	—	—	Under observation
	7	8	+	+	—	—	Enucleated
	8	17	+	+	—	—	Under observation
Dog	1	5	+++	+++	+	—	Under observation
	2	5	+++	+++	+	—	Under observation

Frog (Amphibian) Series.—In eleven of the twelve eyes the donor disc became transparent and in most of them it was difficult to differentiate macroscopically between the grafted and normal eye (Fig. 1). The slit lamp showed that the corneal thickness was increased opposite the disc and the margins of the disc could just be made out in some places. Optically the grafts were perfectly clear, and the fundus details could be seen distinctly.

In only one eye was the graft slightly hazy and the margins obvious, but in this eye also the iris, pupil, and fundus details could still be seen with ease (Fig. 2).

FIG. 1.—Intralamellar graft of frog cornea into rabbit eye. The graft is clear and no reaction is seen.

FIG. 2.—Frog heterograft into rabbit eye, showing slight opacification.

Control (Avian and Mammal) Series.—Only one of the eight chicken grafts remained completely transparent (Fig. 3). The opacity was slight in four and moderately dense in three; vascularization was associated with these opacities (Fig. 4).

FIG. 3.—Chicken heterograft into rabbit eye, showing clear and transparent cornea.

FIG. 4.—Chicken heterograft into rabbit eye, showing opacification and vascularization.

Both the dog grafts became densely opaque with extensive vascularization (Fig. 5).

FIG. 5.—Dog heterograft into rabbit eye, showing complete opacification and extensive vascularization.

Histological Studies

Frog Heterografts

HOST.—The epithelium, stroma, and endothelium appeared normal. The stromal fibres were regular and showed no increase in the cellular contents.

DONOR.—The donor disc took a higher stain and appeared more hyaline in the cellular elements. The disc seemed to have become devoid of epithelium and endothelium and its margins at places became merged with the host stroma, so that the line of union could be seen only with difficulty. There was no cellular reaction and no blood vessel was seen growing into the cornea (Figs 6 and 7).

FIG. 6.—Intralamellar frog heterograft into rabbit eye, showing clear and transparent host cornea and frog cornea. Haematoxylin and eosin. Magnification $\times 106$.

FIG. 7.—Intralamellar frog heterograft into rabbit eye, showing clear and transparent host cornea and frog cornea, with no inflammatory reaction. Clefts in superficial parts of host cornea are artefacts. Haematoxylin and eosin. Magnification $\times 266$.

Chicken Heterografts.—The one clear result is shown in Fig. 8 (opposite).

HOST.—Epithelium and endothelium showed no abnormality. The stromal fibres were slightly wavy and the cellular contents were increased only in a few places.

DONOR.—The disc was quite distinct. It took a lighter stain and showed moderate increase in cellular contents. At a few points there was also an accumulation of a few inflammatory cells. No blood vessels were seen growing into the disc.

Chicken heterografts with opaque results are shown in Figs 9 to 11 (opposite).

HOST.—The corneal epithelium and endothelium were irregular and thick in places, the stromal fibres were wavy and showed a marked increase in cellular contents. The cells were more numerous in the immediate vicinity of the disc and especially in the stroma lying in front of the disc.

DONOR.—The disc seemed to be disintegrated and partly absorbed. There was a large collection of basophils in the pocket made for the graft. Some cells were distended with the nucleus pushed to one side, which is evidence of phagocytic activity. Some of the cells showed fibroblastic activity. Several blood vessels were invading the disintegrated donor disc.

Discussion

When amphibian heterografts were used in the rabbit's eye the percentage of success was very high. Eleven out of twelve remained absolutely clear, and even in the one slightly hazy graft the central portion remained clear and fundus details could be easily seen. Our results compare very favourably with those of other workers. Babel and Bourquin (1952) obtained 50 per cent. success in grafting from one mammalian species to another (*i.e.* human and cat corneae were transplanted into rabbits' eyes). Choyce (1952) reported similar results. Basu and Ormsby (1957) obtained 66·6 per cent. success with avian grafts and 51·8 per cent. with mammalian grafts into rabbit's eyes. Tsutsui and Watanabe (1959) obtained 88 per cent. success with fish grafts into rabbits' eyes. It appears that the tissue and serum of cold-blooded animals usually produce an insignificant antigenic response. Our results indicate, however, that the amphibian cornea transplants even better than the fish cornea. The difference in the antigenicity of the cornea in various groups of animals suggests that the corneal proteins are not organ-specific. Kamata (1957) suggested pre-operative serum treatment for heterografts, but the results in the series here reported do not indicate any necessity for this. The corneae in this series were not stored but were transplanted directly from the frog to the rabbit, and the donor-recipient reaction was almost nil. Tsutsui and Watanabe (1959) suggested that the thickness of the graft should be less than 0·6 mm., saying that if the thickness exceeded 0·7 mm. there was a greater risk of opacity and vascularization, but we used the entire thickness of the frog cornea with excellent results.

FIG. 8.—Chicken heterograft into rabbit eye, showing clear and transparent host cornea and chicken cornea. Haematoxylin and eosin. Magnification $\times 106$.

FIG. 9.—Chicken heterograft into rabbit eye, showing opaque cornea, partly broken down stroma of the graft, and inflammatory cells. Haematoxylin and eosin. Magnification $\times 106$.

FIG. 10.—Chicken heterograft into rabbit eye, showing vascularization and inflammatory cells. Haematoxylin and eosin. Magnification $\times 106$.

FIG. 11.—Chicken heterograft into rabbit eye, showing inflammatory and distended (bladder) cells. Magnification $\times 266$.

It is possible that certain animals show different reactions to corneal tissue from different species, and that this might be established by a simple intra-dermal test.

We are now investigating the concentration of corneal antigen and the antigenic response in man, and we are also examining the antigens in pooled corneae from various species by agar gel electrophoresis.

We are sure that the opacification and vascularization of heterografts depend upon an antigen-antibody reaction, and feel that they are probably unrelated to the species from which the grafts are taken.

Summary

- (1) Data on the intralamellar grafting of corneal tissue from an amphibian (*Rana tigrina*) into the rabbit eye are presented.
- (2) Most of the amphibian grafts remained clear.
- (3) Opacification and vascularization of the graft is attributed to antigen-antibody reaction.
- (4) It is suggested that this reaction is unrelated to the difference of species.
- (5) The possibility of an intradermal test of antigenicity is suggested.

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