FATE OF ENDOTHELIUM IN CORNEAL HOMOGRAFTS AN EXPERIMENTAL STUDY

BY

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STUDIES of the fate of donor tissues in transparent and opaque corneal grafts and especially of the endothelium in penetrating grafts have been published by Basu and Ormsby (1960), Espirtu, Kara, and Tabowitz (1961), Polack and Smelser (1962), Polack, Smelser, and Rose (1964), and Chi, Teng, and Katzin (1965). In the course of our work, a modified technique for sex chromatin staining has also been evolved, which gives better and more consistent results than those of techniques previously described (Culling, 1966).

Material and Methods

Two groups of adult albino rabbits weighing 1–2 kg. were used for the following procedures:

- (1) Standardization of sex chromatin counts in normal corneal endothelium in five male and five female rabbits.
- (2) Sex chromatin counts in animals in which penetrating corneal grafts had been transferred from one sex to the other: cornea from a male rabbit was transplanted into a female rabbit and *vice versa*.

Technique of Keratoplasty

The rabbits were anaesthetized with sodium pentothal supplemented when necessary by a retrobulbar injection of 0.5 ml. 2 per cent. Xylocaine. A 5-mm. full-thickness graft was removed from a male rabbit and transplanted into a female rabbit and *vice versa*. Direct suturing was performed as a routine, but if good apposition was not obtained with direct suturing, indirect sutures were applied and a piece of egg-membrane was slipped between the graft and the indirect sutures to increase the support to the grafted cornea.

No corticosteroids were used post-operatively. The following observations were recorded:

- (1) Transparency of the graft;
- (2) Iridocyclitis;
- (3) Anterior synechiae formation;
- (4) Corneal vascularization.

The animals were killed at intervals ranging from 10 days to a maximum of 5 months for the endothelium studies.

Fixation of Corneal Tissue

The central disc and the peripheral parts of the cornea were removed separately from the anaesthetized animals and fixed immediately in modified Davidson's solution (95 per cent. alcohol 30 ml., 40 per cent. formalin 20 ml., glacial acetic acid 10 ml., distilled water 30 ml.). Fixation for 3 hours gave the best results in our series.

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Scraping of Endothelium

Two drops of modified Davidson's fixation solution were placed on an albumen-coated slide. The cornea was placed on the slide with the epithelial surface downwards. The cornea was fixed at one end with a hypodermic needle, and the complete endothelial layer was scraped off with a single gentle sweep of a curved iris repositor. The scraped endothelium floated in the fixative fluid on the slide. Excess fluid was removed by means of a filter paper. The whole procedure was carried out under a dissecting microscope. The slides were kept in a clean container overnight and stained with Feulgen stain as follows:

Staining Technique.—The Feulgen reaction is based upon the cleavage of the purine-deoxyribose bond by mild acid hydrolysis to expose a reactive aldehyde group, which may then be detected by the use of a Schiff reagent. This leucofuchsin gives a reddish-purple colour in the presence of chromatin owing to the formation of quinoid compound. The reaction is specific for chromatin and sex chromatin.

Special Reagents

(i) *I/N HCl*: Add 82.5 ml. HCl of specific gravity 1.19 to 1 litre distilled water.

(ii) *Fuchsin Sodium Bisulphite*: Dissolve 1 g. powdered fuchsin in 200 ml. distilled water brought to boiling point. Shake the solution for 5 minutes and cool to 50°C. Filter the solution. Add 20 ml. I/N HCl. Cool the solution to 25°C. and add 1 g. sodium bisulphite. Add 1 g. animal charcoal. Filter. Apply stopper tightly. Decolourization will be complete in a few hours, but the bottle must be kept in a dark place for at least 24 hours. Filter and use.

(iii) *Sulphate Rinse Composition*:

Distilled water—200 ml.

10 per cent. anhydrous sodium bisulphite—10 ml.

I/N HCl solution—10 ml.

Mix the three solutions and shake well.

Procedure

- (1) Keep the smears in running water for 2 hours.
- (2) Transfer them to I/N HCl for 12 minutes at 60°C. This time interval was found to be the ideal.
- (3) Rinse in distilled water for 5 minutes.
- (4) Transfer to Schiff reagent for 60 minutes.
- (5) Transfer slides to sulphate rinse for 1 minute.
- (6) Transfer slides to second sulphate rinse for 2 minutes.
- (7) Transfer slides to third sulphate rinse for 3 minutes.
- (8) Rinse in distilled water for 5 minutes.
- (9) Dehydrate through 50, 70, and 90 per cent., and absolute alcohol.
- (10) Give two washes of xylene for 4 minutes each.
- (11) Mount the slides.

All solutions have to be freshly prepared every time. The slides are studied under oil immersion, and the power of the microscope and the percentage counts of sex chromatin are calculated. At least 500 cells were studied in each slide.

Observations

(1) **Sex Chromatin Counts in Normal Female and Male and Rabbit Corneal Endothelium (20 eyes)**

In the normal female rabbit corneal endothelium, 40 to 60 per cent. of the endothelial

cells showed the presence of sex chromatin (Table I). In most of the nuclei the position of the sex chromatin was eccentric, *e.g.* in contact with the nuclear membrane, only in 0.1 per cent. of cells was its location near the centre (Figs 1 and 2, overleaf).

In the normal male rabbit, sex chromatin was seen only in 0 to 2 per cent. of corneal endothelial cells. Therefore, by a comparison of the sex chromatin, it was possible to see whether the donor graft persisted intact or was invaded by cells from the host.

TABLE I
PERCENTAGE SEX CHROMATIN IN 500 CORNEAL ENDOTHELIUM CELLS IN EACH
OF 10 RABBITS (20 EYES)

Sex	Animal No.	Eye	Percentage Sex Chromatin	Sex Chromatin Position	
				Peripheral	Central
Female	1	Right	50	245	5
		Left	52	258	2
	2	Right	45	220	5
		Left	47	230	5
	3	Right	40	200	0
		Left	40	200	0
Male	4	Right	60	300	0
		Left	60	298	2
	5	Right	54	264	6
		Left	53.2	260	6
	6	Right	1.0	5	0
		Left	0.8	4	0
	7	Right	0.0	0	0
		Left	0.0	0	0
	8	Right	0.0	0	0
		Left	0.0	0	0
	9	Right	1.6	8	0
		Left	2.0	10	0
	10	Right	1.2	6	0
		Left	1.6	8	0

(2) Sex Chromatin Counts in Donor and Host Tissue in Transparent Grafts (4 eyes)

Table II (overleaf) shows the sex chromatin counts in the donor disc and the host cornea. It is evident that in the clear grafts the donor endothelial cells retained their identity for at least 5 months. There was no attempt at replacement on the part of the host endothelium even at the periphery.

(3) Sex Chromatin Counts in Donor and Host Tissues in Opaque Corneal Grafts (7 eyes)

Table II shows that, in opaque grafts, the endothelium was completely replaced by the host endothelium in 10 days.

FIG. 1.—Monolayer of corneal endothelium.
× 100.

FIG. 2.—Sex chromatin, indicated by arrows, in corneal endothelial cells. × 1,000.

TABLE II

FATE OF ENDOTHELIUM SHOWN BY PERCENTAGE SEX CHROMATIN IN 500 CELLS IN ELEVEN CORNEAL GRAFTS

Graft	Animal No.	Age of Graft	Sex of Tissue		Percentage of Sex Chromatin	
			Graft	Host	Graft	Host
Clear	11	4 wks	M	F	0	50
	12	6 wks	F	M	54	2
	20	8 wks	F	M	50	0
	21	5 mths	M	F	0	55
Opaque	13	10 days	M	F	50	50
	14	2 wks	M	F	60	61
	15	4 wks	F	M	2	1.6
	16	5 wks	F	M	1	1
	17	8 wks	M	F	54	56
	18	3 mths	M	F	54	53
	19	5 mths	M	F	48	50

Discussion

The sex chromatin count in normal female corneal endothelium found in our series ranged from 40 to 60 per cent. Lee, Mueller, and Trevor-Roper (1967) obtained the same results, but Espirtu and others (1961) and Chi and others (1965) have reported sex chromatin counts as high as 80 to 95 per cent. These variations do not impair the useful-

ness of this method of identifying the donor and recipient tissue, since in the male rabbit corneal endothelium the sex chromatin was identified in only 2 per cent. of cells.

In the transparent corneal grafts we found that the donor endothelial cells retained their identity for as long as 5 months. These observations are in accordance with those of Basu and Ormsby (1960), Polack and Smelser (1962), Polack and others (1964), and Chi and others (1965), but Espirtu and others (1961) observed that the donor endothelial cells began to be replaced by the end of the 4th month and that the replacement was complete by the 7th month.

In opaque corneal grafts sex chromatin studies revealed that the donor endothelium was completely replaced by that of the recipient, often after only 10 days.

This early replacement of donor endothelium in cases in which the grafts become opaque must be due to faulty surgical technique, causing unnecessary trauma and such post-operative complications as iridocyclitis, anterior synechia formation, or immunological reactions.

Summary

(1) The fate of donor endothelium cells in transparent and opaque corneal grafts has been studied by the identification of sex chromatin in the host and donor cells.

(2) A modified Feulgen-staining technique for sex chromatin gave reliable results in rabbit endothelial studies.

Our technique differed from previous methods in the following ways:

(a) Instead of acetyl alcohol, we used a modified Davidson's solution as a fixative. This assists the separation of corneal endothelium and the flat preparation so obtained is smoother.

(b) Smears were washed for 2 hours in running water.

(c) Smears were kept in I/N HCl at 60°C. for 12 minutes.

(d) Smears were immersed in Schiff reagent for 1 hour.

(3) It was observed that in clear grafts the donor endothelial cells retained their identity for at least 5 months, but that in opaque grafts replacement occurred within 10 days.

(4) Damage to the donor endothelium may be the reason why certain grafts become opaque.

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