COMMUNICATIONS

EXPERIMENTAL RESEARCH WITH CORNEAL HETEROGRAFTS*

BY

J. BABEL AND J. B. BOURQUIN

*Laboratory of the Ophthalmological Clinic, University of Geneva
(Director: Prof. A. Franceschetti)*

KERATOPLASTY still poses problems, and the more we extend our experiments to try to elucidate the biology of the tissue fragments incorporated into the cornea, the more the biology appears complex. Until now, it has been almost dogma, because of the failure of most previous attempts, to state that corneal heterografts are not possible; that first they become opaque, and then they are replaced by reactive tissue originating in the receiver. A few workers have succeeded in maintaining fragments of clear transplanted cornea in the cornea of another species, but these experiments have not been very extensive (Bonnefon, 1914; Galante, 1938), and similar work done since has given negative results (Thomas, 1936; Castroviejo, 1937). Though this question seemed to be finally closed, we began further experiments after receiving a personal communication from Dr. Bock, now a collaborator of Professor Maumenee in San Francisco. Bock, while trying to explain the mechanism of opacification of corneal grafts, proposed a technique of implantation which differs from that of perforation and lamellar grafts. The former is followed all too often in the rabbit by infection or inclosure of the iris; with the latter it is difficult to achieve fixation.

Technique

The new method[†] consists in using a spatula with thin edges to cut the corneal stroma of the receiver starting from a limbal incision, and to slide into the slit so made a rounded edge of the cornea to be grafted. Thus the graft is included between lamellae of the corneal stroma of the recipient, which obviously implies totally different biological conditions from those obtaining with the usual types of graft; *i.e.*, those which are in contact with the surface, and also, in the case of perforating grafts, with the aqueous humour.

This technique, which is simple to perform, has given us in the past 6 months some unexpected results which pose a whole series of new biological problems.

Experiments

We have transplanted into rabbit corneae (by means of Franceschetti's trephine) rounded slivers of cornea (5 mm. in diameter) taken from various species: ox, sheep, horse, pig, guinea-pig, rabbit, and man. The corneae from the first four species, since they were too thick to be introduced as such, were sometimes split, so that some animals received a part of the parenchyma with epithelium, others the posterior part of the stroma

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[†] Choyce (1949) used a somewhat analogous technique for homografts, and has also used it since then for heterografts. The results of his most recent experiments are described in the adjoining article in this issue of the B.J.O.

with Descemet's membrane and endothelium, and others pieces of full-thickness cornea. The donor-globes were removed at the slaughter-house immediately after the animals' death, put into Ringer's solution (holofusine), and refrigerated for 24 hours. The grafts taken at the moment of transplantation were sometimes slightly opalescent, at others quite transparent. A local antibiotic treatment (aureomycin ointment 3 per cent.) generally served to prevent infection. We watched the evolution of the grafts by repeated slit-lamp control, at first daily for several weeks, and then bi-weekly.

These repeated biomicroscopical controls were supplemented in some cases, by a histological study of the transplants after a period ranging from 8 days to 3½ months. The other animals are still alive.

Results

We operated on 22 rabbits, some bilaterally, and performed a total of 33 grafts. Three grafts were expelled, either exteriorly or into the anterior chamber, but the remaining thirty may be classified into three groups:

(1) Transparent Heterografts.—Some heterogeneous grafts remained clear throughout the period of observation, which ranged from 6 to 3½ months for the most recent (Figs 1 and 2). Their presence in the rabbit cornea produced no inflammatory, oedematous, or vascular reaction. This favourable evolution was observed in twelve eyes which received the following heterografts:

four ox (anterior portion, 3; posterior, 1), two horse (anterior, 1 (Fig. 1); posterior, 1), two pig (total cornea, 1; posterior, 1), one guinea-pig (total cornea, 1), three sheep (total, 1; anterior, 1; posterior, 1). Two rabbit homografts were also successful.

FIG. 1.—Rabbit 121, right eye. Horse interlamellar graft (anterior part of cornea): still clear 6 months after implantation.

FIG. 2.—Rabbit 124, right eye. Human interlamellar graft, 8 days old, no opacification; (reaction from receiver and opacification of graft began in this case after 2 weeks).

This tolerance of a heterogenous tissue does not appear to be a phenomenon peculiar to a given species, nor is it related to the presence of a given portion of the cornea (stroma, anhistic membranes, epithelium), since grafts which come from very different species far removed in the animal series are sometimes tolerated perfectly.

The histological examination of the grafts which remained transparent in the few animals which died between the first week and 3½ months, showed the corneal stroma of the rabbit to be unaffected by the introduction of a foreign body (Figs 3 and 4). Neither inflammatory cellular infiltration, nor vascular neoformation was noted. The fragment of tissue is enclosed between lamellae of normal aspect, though its structure is not altogether normal; *i.e.*, some lamellae are irregular and in places dissociated. This phenomenon is no doubt largely due to the method of implantation and also to the lack of mechanical tension on the tissue. The cells appear rarefied, but, on sections perpendicular to the corneal surface at least, show hardly any pyknosis or other nuclear modifications. The aspect of these corneal fragments can be compared to what is observed when the corneae are kept without fixation for 1 or 2 days at a temperature of 18–20°C. When Descemet's membrane is included in the graft, it frequently folds upon itself, and at times is displaced and partially surrounds the graft. The endothelium is often conserved, at least in part, and does not present any manifest signs of activity. The epithelium, on the other hand, undergoes modifications: in most cases it proliferates and produces important cellular collections in the recipient

FIG. 3.—Rabbit 140, right eye. Sheep interlamellar graft (full thickness), 12 days old. No reaction in receiver's eye, rabbit's and sheep's epithelia mixed.

FIG. 4.—Rabbit 133, right eye. Ox interlamellar graft (anterior part) 5 weeks old. Enormous epithelial proliferation of graft, no reaction in receiver's eye.

cornea. These are already visible macroscopically and form dull well-defined white spots, which grow slowly and ordinarily provoke no inflammatory or vascular reaction in the host.

Microscopically, these proliferating, heterogeneous, epithelial cells constitute a sort of *in vivo* tissue culture (Fig. 4). When we introduce the heterogenic graft the vitality of its epithelium is still great, a proliferation of the latter in the interlamellar space made by the cleavage is the result. Histological sections of grafts taken between the 18th and 36th day show these epithelial cells to present degenerative changes, atypia, nuclear irregularities, and multinucleated giant cells.

On the other hand, if the cleavage has been done very superficially, small erosions or bullae which burst, and then fill up again, may appear on the surface. In such cases, histological sections show that the graft is well-covered with epithelium, and that it is so embodied within the recipient epithelium that it is impossible to tell which tissue comes from where (Fig. 3).

The vitality of grafted epithelium is a phenomenon already known in the homografts of human cornea we have already described as failures in keratoplasty due to a disordered proliferation of the epithelium of the graft and its host, preventing the normal union of the parenchyma (Babel, 1950; Hervouët, 1952).

(2) Opacified Heterografts.—A certain number of grafts became opaque more or less immediately after their introduction (Fig. 5), and all these produced oedema of the stroma, and pannus-type vascularization in the recipient. These vessels have in common that as a general rule they grow relatively quickly and develop most in that portion of the corneal parenchyma which is anterior to the implant. We have not noted rapid

FIG. 5.—Rabbit 139, right eye. Pig interlamellar graft (full thickness), 6 months old, opaque. Note epithelial bubble in inferior region of graft.

FIG. 6.—Rabbit 133, left eye. Pig interlamellar graft (full thickness). Graft into left eye made 2 weeks after graft into right eye (Fig. 4). Important reaction in rabbit cornea from 6th day, no reaction in graft.

vascular invasion into the graft in most of our cases, except in one animal in which there was an infection and resulting necrosis of the graft. Under prolonged observation, the graft is finally seen to be invaded by neoformed vessels.

This reaction on the recipient sometimes starts very early (Rabbit 47: 2 days), but in most cases it begins between the 5th and 12th day. It manifests itself as a small pannus *en epaulette*, but can also be widespread, and progress very quickly. Since this vascularization is accompanied by turbidity of the corneal parenchyma in the recipient, which is partly due to the oedema, and partly to a cellular infiltration (Fig. 6), we treated a series of animals with injections of trypan blue in order to follow the path of the wandering cells. A number of cells in the recipient part of the cornea are coloured blue, but we have not seen this coloration take in the implanted tissue, except in one human corneal graft. In two animals (one with a human corneal graft after 3 days' refrigeration in physiological saline, and the other with a graft of pig cornea after 24 hours) the injection of trypan blue checked the oedematous reaction in the recipient, and brought about an immediate and lasting clearing of the parenchyma, without, however, supressing the infiltrative cellular reaction. The neoformed vessels gradually emptied of their blood, so that after several months they were scarcely visible. Also, even among those animals which did not react with trypan blue to give an immediate clarification, a tardy clearing was seen and the implanted tissue remained transparent for several weeks.

In one case, the reaction was so intense that we thought an abscess had formed, and the animal was killed after 8 days. Histological examination showed that there was no infection but a very violent anaphylactic reaction with a massive eosinophilic infiltration into the tissue of the host and the beginning of an infiltration into the graft (Fig. 7). The sixteen cases of this group may be divided as follows:

- one horse cornea (anterior),
- four ox cornea (posterior, 2;
anterior, 2),
- two sheep's cornea (full-
thickness),
- five pig's cornea (anterior, 1;
posterior, 1; full-thick-
ness, 3),
- one human cornea,
- three grafts of fragments of
cornea preserved in form-
alin (rabbit and pig) or
plunged into alcohol for
a few minutes (pig).

These last experiments, which are to be repeated, showed that in two cases the graft was completely invaded and destroyed by elements from the recipient;

and that in the third (rabbit cornea preserved for several weeks in formalin) the cornea, opaque at the moment of the graft, provoked an inflammatory reaction in

FIG. 7.—Rabbit 47, right eye. Sheep interlamellar graft (full thickness), 8 days old. Massive stroma infiltration of rabbit's cornea by eosinophilic cells, oedema of graft, and epithelial proliferation.

the recipient which after several weeks began to clear up steadily, without being invaded by neovascular formations (time of observation: 4 months).

A few workers have already had a partial success with formalinized perforating or lamellar grafts, which can be explained only by the replacement of tissue from the host (Salzer, 1937; Babel, 1950). A few workers have risked implantation of grafts treated with formalin even in man (Scherschewskaya, 1940).

(3) Bilateral Heterografts.—The animals which received grafts to both eyes present interesting data. We treated nine animals in this manner; six had their graft to the second eye 2, 3, or 4 weeks after the first, and all but one received corneae from different species:

ox-pig,	ox-pig,
horse-sheep,	ox-ox,
guinea-pig-sheep,	pig-ox.

With the exception of this last case where the two grafts are still transparent 5 and 4½ months after operation, all the grafts introduced into the second eye became opaque, the reaction beginning between the 5th and 10th day. These cases were similar in evolution and aspect as the single grafts which were badly tolerated. On the other hand, none of the grafts which were clear at the time of the second operation became turbid later (the length of the longest observation was 6 months). One graft was expelled after 14 days.

Histological examination of a few cases shows changes similar to those of grafts which were badly tolerated as a whole: namely, that the reaction is marked in the region of the recipient (infiltration and vascularization) but that the graft takes little part in the process. In a case examined 3½ months after the second intervention, the clear graft was quite free from reactional changes, while in the second eye there was partial invasion by neoformed vessels and cellular elements, lymphocytes, and fibroblasts.

Three other animals received simultaneous bilateral grafts:

horse-horse,
ox-ox,
rabbit (refrigerated 72 hrs)- rabbit (formalin).

Only two grafts remained clear, one in the first and one in the third animal. All the others provoked a strong reaction and became turbid.

Discussion

It may be concluded that interlamellar heterografts into the rabbit's cornea are either well tolerated and remain clear, or induce a defence reaction in the recipient eye. In a total of thirty heterografts, 14 were successful. In twelve animals in which one eye only had been operated upon, five heterografts remained clear while seven were not well tolerated. In eighteen bilateral cases, nine corneae remained clear. As a whole, we may say that about half of our heterografts have been successful. The lack of defence reaction is observed as well in the living animal with the aid of the slit lamp as in histopathological studies. The question whether these transparent heterografts are still living remains to be answered. Histological studies are not much help alone unless special methods are used.*

* The phosphatase reaction, which as a rule can be used as a proof of vitality, is of no value in the corneal tissue, which, except in the epithelium, does not normally contain this enzyme (Süllmann and Payot, 1949; François and Rabaey, 1951).

We have not yet been able to explain the ultimate evolution of these corneal opacifications in the recipient which sometimes clear up and thus differ from the behaviour of the implanted tissues. Indeed, the latter maintains a relative transparency even when its bed becomes opaque.

Evidence is lacking to permit definite conclusions regarding this phenomenon. Corneal opacification is nearly always produced in the second eye, both when we graft pieces of cornea from the same species to both eyes in succession (bilateral graft of ox cornea for example), and when we employ the cornea of different species (ox on one side, for example, and pig on the other). It appears as if in the second eye the heterografts provoked an anaphylactic type of reaction, due not so much to the animal species involved, as to the corneal tissue itself. This point warrants further research. The hypothesis that the existence of tissue groups equivalent to blood groups might explain certain intolerances has already been put forward (Babel, 1950). This should be brought into line with the observations of Maumenee and his school (Müller and Maumenee, 1951a, b) to explain the mechanism of secondary opacification of corneal grafts in the rabbit (homografts) through the influence of an allergic reaction provoked by a subcutaneous implantation of skin from the same donor (experiments which we have been able to confirm personally).

Conclusions

This work, which has only just been begun, and must be followed up by further research before its clinical applications can be considered, has produced the following facts:

- (1) a corneal heterograft is feasible, provided a proper technique is utilized, in which the implanted tissues submit to unusual biological conditions (interlamellar graft),
- (2) it is possible in this manner to maintain in a transparent state for several months, often without provoking a reaction in the recipient, fragments of corneae coming from different species.

The question of knowing if transplants are alive and remain so, has not been resolved by these initial experiments. The reaction phenomena which they provoke in a certain number of cases appear to be of an allergic type.

Specifically, when bilateral heterografts are performed, the later graft provokes, within the space of a few days, a reaction on the part of the recipient cornea, which at times can be violent and which cannot be explained by an infection but rather by an anaphylactic reaction.

There is a great difference in behaviour between grafted connective tissue stroma which apparently remains passive, and the epithelium which on the contrary proliferates, as it would perhaps in a tissue culture.

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