Fungal contamination of donor eyes

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In March, 1965, the first case of fungal infection occurred in a lamellar graft performed by the Corneo-Plastic Unit, Queen Victoria Hospital, East Grinstead (Case 1). Subsequently two other cases of infection in lamellar grafts were attributed to fungi (Cases 2 and 3).

In Case 1 the infection could be seen to arise from the lamellar bed and extend forward into the graft (Figs 1 and 2). It was believed, therefore, that the introduction of the fungus took place at the time of operation, either from the atmosphere or from the donor material used. Instrument sterilisation was satisfactory and preoperative culture of the recipient's eye was negative. It was decided to investigate the frequency of fungal contamination of donor material and the efficacy of the preoperative sterilizing solution in current use at East Grinstead.

Case 1

A man aged 28 years had a lamellar graft for corneal dystrophy. One week after operation deep localized white opacity was noted in the graft and this opacity continued to spread laterally and anteriorly. Mycelial type opacities of a branching thread-like character were observed in the stroma of the graft and a provisional diagnosis of fungal infection was made. Cultures from corneal scrapings on Sabouraud's media were consistently negative for fungi. Despite treatment with Nystatin drops the opacity continued to increase in size and 3 weeks postoperatively the lamellar graft was removed. Part of the graft was cultured giving a growth of *Aspergillus nidulans* and part was sectioned displaying fungal elements (Figs 1 and 2). The lamellar bed was thermally cauterized and treatment continued with local Amphotericin B and Nystatin drops. The infection resolved satisfactorily leaving a dense central opacity. Subsequently, successful full-thickness keratoplasty was performed.

Cases 2 and 3

These were patients with infections in lamellar grafts which yielded *Pullaris pullarium* and *Trichophyton rubrum*, respectively, on culture of corneal scrapings. Both cleared up satisfactorily on treatment with Amphotericin B, Nystatin, and Soframycin drops. No lamellar material was available for histological examination.

Material and Methods

The donor eyes examined were those collected by the Eye Bank at East Grinstead and not used for keratoplasty. The sites of enucleation varied from private homes to hospital wards and hospital and public mortuaries. The majority of the eyes were collected by the staff of the Eye Bank using aseptic technique, but with no cleansing or sterilizing solution at the time of collection. The remainder of the eyes were supplied by peripheral hospitals in the area. The eyes were placed

FIG. 1 *Aspergillus nidulans* in lamellar graft. $\times 75$

FIG. 2 *Aspergillus nidulans* in lamellar graft. $\times 375$

direct into empty sterile screw-topped glass jars and were stored at 0–4°C. The jars remained unopened until the time of examination.

After an interval which varied from 1 to 4 weeks, each whole eye was immersed in a jar containing standard nutrient broth for a few minutes, and then removed and placed in an empty sterile jar. The eye was covered with a sterilizing solution for a given time, following which the solution was poured off and the eye rinsed with sterile normal saline. The eye was then placed in another jar containing nutrient broth. Subsequently both jars of nutrient broth were cultured and examined for organisms. Aseptic technique was observed through the procedure.

Results

These are shown in the Table (overleaf), where they are grouped according to the sterilizing solution used and the time of immersion therein.

Types of organisms identified were *Staph. pyogenes*, *Strept. viridans*, *Strept. faecalis*, *E. coli*, *Proteus vulgaris*, *B. subtilis*, *N. crassus*, micrococci, yeasts, and fungi. It was noted that most *Staph. pyogenes* were penicillin resistant but sensitive to Gentamicin.

Results in Group A indicated that a high degree of bacterial contamination existed in the donor eyes and that it was satisfactorily controlled by the penicillin and Gentamicin

solution. Fungal contamination remained unaffected. When Amphotericin B 0.01 per cent. was added to the solution in an attempt to deal with the problem, it proved ineffective.

Increasing the strength of Amphotericin B in Group C and its length of use in Group D effected only a slight decrease in fungal contamination.

In Group E a change of media to one specifically for fungi gave substantially increased positive results for fungi.

Table *Results of sterilization experiments*

Group	No. of eyes examined	Sterilizing solution	Immersion time (min.)	No. infected			
				With bacteria		With fungi	
				Pre-immersion	Post-immersion	Pre-immersion	Post-immersion
A	20	Penicillin (1,000 u./ml.) Gentamicin (0.15 per cent.)	15	12	0	4	4
B	28	Penicillin (1,000 u./ml.) Gentamicin (0.15 per cent.) Amphotericin B (0.01 per cent.)	15	20	0	9	9
C	28	Penicillin (1,000 u./ml.) Gentamicin (0.15 per cent.) Amphotericin B (0.1 per cent.)	15	23	0	5	4
D	12	Penicillin (1,000 u./ml.) Gentamicin (0.15 per cent.) Amphotericin B (6.1 per cent.)	30	10	0	2	1
E*	16	Penicillin (1,000 u./ml.) Gentamicin (0.15 per cent.) Amphotericin B (0.1 per cent.)	30	13	0	9	7

* Culture media was changed from standard nutrient broth to malt peptone broth for this group only

Discussion

The occurrence of one proven and two suspected cases of fungal infection in grafts in East Grinstead led to the investigation outlined above. The results obtained indicate that fungal contamination of donor material may be a more serious hazard than commonly accepted at present and may be an unsuspected cause of some postoperative failures in keratoplasty. The inadequacy of the current antibiotics in use to deal with fungi pre-operatively is demonstrated. Investigation is proceeding to find a more effective pre-operative sterilizing solution.

Previously attention has been concentrated on bacterial contamination and its control (Doctor and Hughes, 1958; Boberg-Ans, Badsberg, and Rasmussen, 1962; Rollins and Stocker, 1965; Rycroft, 1965; Polack, Locatcher-Khorazo, and Gutierrez, 1967).

In such a small series the high proportion of fungal contamination found in Group E is not statistically significant, especially as many of the eyes examined were pairs and hence subject to simultaneous infection or contamination. Nevertheless it might be expected that fungal contamination should rival that from bacteria for several reasons.

One of these is the high frequency and ubiquity of fungi in our environment. The sites of eye collection such as dark and damp mortuaries are especially likely to contain large numbers of fungi. Moreover, storage of donor material at 0-4°C. is more suitable for fungal than for bacterial growth. Also bacterial inhibition of fungal growth may be reduced at this temperature. Even under normal conditions, up to 27.4 per cent. of eyes

have been found to harbour fungi (Fazakas, 1953; Mitsui and Hanabusa, 1955; Hammeke and Ellis, 1960; Azevedo, 1962; Ainley and Smith, 1965). The incidence of fungal infection has been found to be greater in the elderly than in the young (Hammeke and Ellis, 1960). A high proportion of donors come from hospitals where routine treatment has commonly consisted of antibiotics and perhaps also steroids, both of which have been stated by numerous authors to increase the incidence of fungal infection (Williamson, Gordon, Wood, Dyer, and Yahya, 1968).

Summary

A high incidence of fungal contamination was found in donor material for keratoplasty. The inadequacy of antibiotic solutions to deal with fungi has been demonstrated. A case of fungal infection after keratoplasty is described.

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Sodium benzyl penicillin was supplied by Glaxo (Crystapen)

Gentamicin was supplied by Aspro-Nicholas (Genticin)

Amphotericin B was supplied by Squibb (Fungizone)

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