

USE OF DIMETHYL SULPHOXIDE FOR PRESERVING CORNEAL TISSUE*†

BY

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SINCE it was discovered by Polge, Smith, and Parkes (1949) that living cells could be protected during freezing and thawing by adding glycerol to the storage medium, various substances and methods have been used in an attempt to achieve better results (Fleming,‡ 1952; Eastcott, Cross, Leigh, and North, 1954; Lovelock, 1954).

We have been concerned with retaining the viability of corneal tissue after freezing and in the past (Reed, Stanfield, and Nagel-Leindl, 1960) have corroborated the findings of Eastcott and others (1954) and of Cockeram, Basu, and Ormsby (1957), who have shown that a 15 per cent./V solution of glycerol is the optimum concentration for the preservation of cornea at dry ice temperatures. The value of this freezing method has been demonstrated clinically (Eastcott and others, 1954) as well as experimentally, using the growth of the corneal tissue in tissue culture as the criterion for viability (Reed and others, 1960; Ormsby and others, 1957).

Lovelock (1954) experimented with various neutral solutes and has demonstrated that living cells are afforded protection only when the solute is permeable to the cell. In more recent experiments, Lovelock and Bishop (1959) used dimethyl sulphoxide, a neutral solute with a low molecular weight which is more readily permeable to living cells than glycerol. However, it was found to be less effective than glycerol in the protection of bull spermatozoa during freezing.

Our need to maintain a supply of stored corneae for keratoplasty prompted us to set up an experiment to evaluate the usefulness of dimethyl sulphoxide in preserving the viability of cornea during freezing.

Experiment.—Our purpose was to compare the preserving qualities of dimethyl sulphoxide with those of glycerol during the storage of corneal tissue at -64° C. Concentrations of 15 per cent./V glycerol and 15 per cent./V dimethyl sulphoxide, both in Hank's balanced salt solution, were used.

Corneal material from rabbits, cats, and dogs was obtained from eyes removed as soon as possible after death, usually within 30 minutes. Pig and ox corneae were obtained from eyes removed approximately 4 hours after death.

Method.—The eyes were soaked for 10 minutes in a balanced salt solution containing 200 units penicillin and 75 mg. streptomycin per ml. Then the corneae were removed and washed again in balanced salt solution and antibiotics. Each

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‡ Chapters by P. B. Medawar and R. E. Billingham; R. D. Passey and L. Dmochowski; B. J. Luyet; A. S. Parkes.

cornea was divided into three or four small pieces and each piece was placed separately in a small screw-capped bijou bottle containing the appropriate storage medium. The corneae were allowed to soak in the storage medium for approximately one hour at room temperature to allow penetration of the preservative. The bottles were then placed directly into a dry-ice chest in which the temperature fluctuated between -64° and -67° C.

The viability of the corneal fragments was determined by tissue culture. At weekly intervals specimens were removed from the storage chest and thawed in a 37° C. water bath for 5 to 10 minutes. Each piece of corneal tissue was removed from the preserving fluid and was cut into three or four smaller fragments which were dipped into chick embryo extract and separately planted in pre-cooled (4° C.) tubes each containing a drop of chick plasma. The drop was then allowed to set, forming a clot in which the small corneal fragment was embedded. To each tube 2.5 to 3.0 ml. of the following propagating medium was added:

- 20 per cent. rabbit serum
- 20 per cent. tryptose phosphate broth
- 60 per cent. Hank's balanced salt solution.

The cultures were incubated at 37° C. for 48 hours, after which they were examined daily with the microscope. The cultures were discarded as soon as a moderate outgrowth from the explant appeared or at the end of 14 days if there was no outgrowth.

Results

Rabbit Corneae (Fig. 1).—Ten to fifteen pieces of corneal tissue were tested at the end of each storage period. The glycerol solution was found to be much more effective as a preserving medium than dimethyl sulphoxide solution.

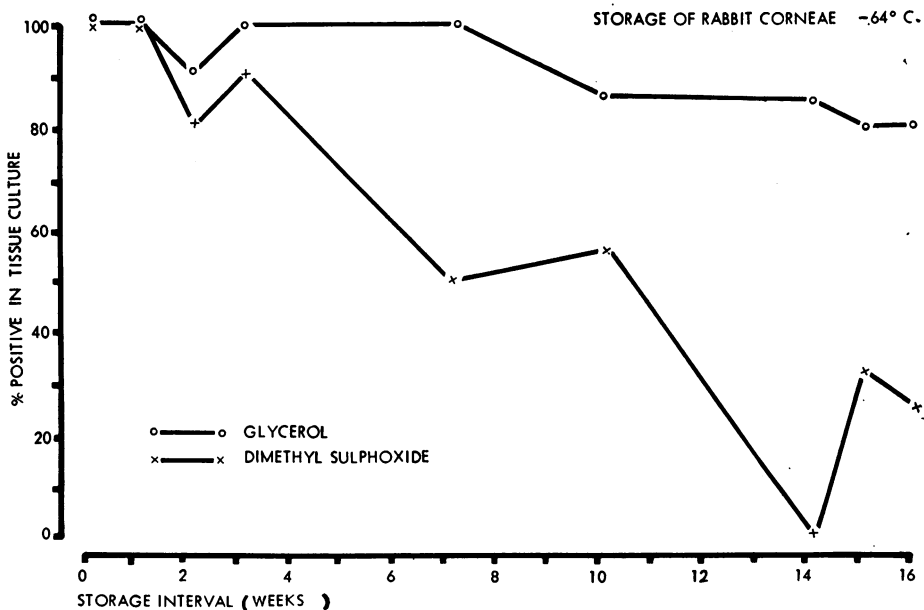


FIG. 1.—Rabbit corneal tissue.

Dog and Cat Corneae (Figs 2 and 3).—Six to ten pieces of corneal tissue were tested at the end of each storage period. The percentages of viable cat and dog corneae dropped considerably in both storage media and neither was effective over a long storage period. However, results obtained with the glycerol solution were still significantly better than those obtained with the dimethyl sulphoxide solution.

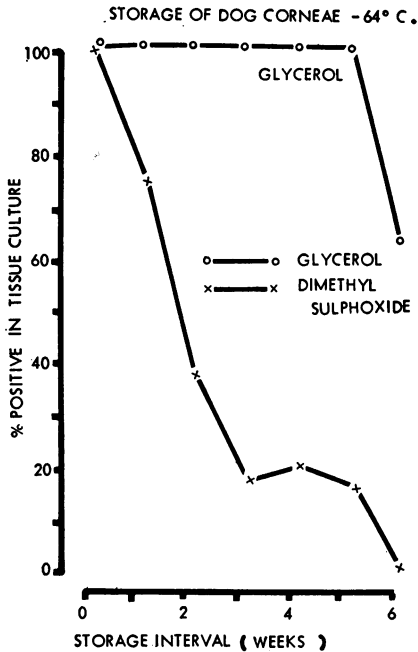


FIG. 2.—Dog corneal tissue.

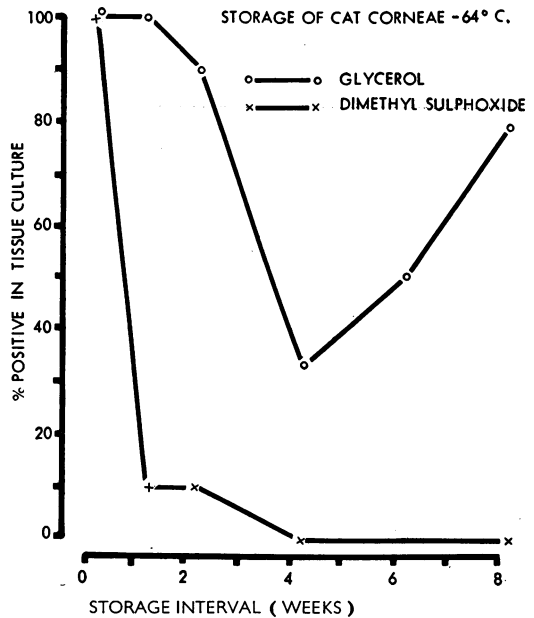


FIG. 3.—Cat corneal tissue.

Pig Corneae (Fig. 4, opposite).—Eight to ten pieces of corneal tissue were tested at the end of each storage period. No specimen frozen in 14 per cent./V dimethyl sulphoxide was viable in tissue culture at the end of one week of storage and of those frozen in 15 per cent./V glycerol none was viable after 3 weeks.

Ox Corneae (Fig. 5, opposite).—Six pieces of corneal tissue were tested at the end of each storage period. No specimens were viable after 3 weeks of storage in either medium.

The poor results obtained with the ox and pig corneae were probably due to the length of time which had elapsed between death and the removal of the eyes.

Discussion

It was observed that the outgrowth of cells from explants stored in 15 per cent./V dimethyl sulphoxide was delayed in comparison with that of cells from explants stored in 15 per cent./V glycerol. The lag was also more

pronounced in all specimens as the length of the storage period increased. In many cases microscopic examination of the cultures revealed that the cells of the explant appeared to be alive but, as no outgrowth was observed, these specimens were recorded as being non-viable. Initially, the propagating medium used for the tissue culture of each type of corneal material (excluding the pig) contained the corresponding homologous

serum. No difference in the number of viable specimens or in the rate and amount of outgrowth was observed with heterologous rabbit serum, however,

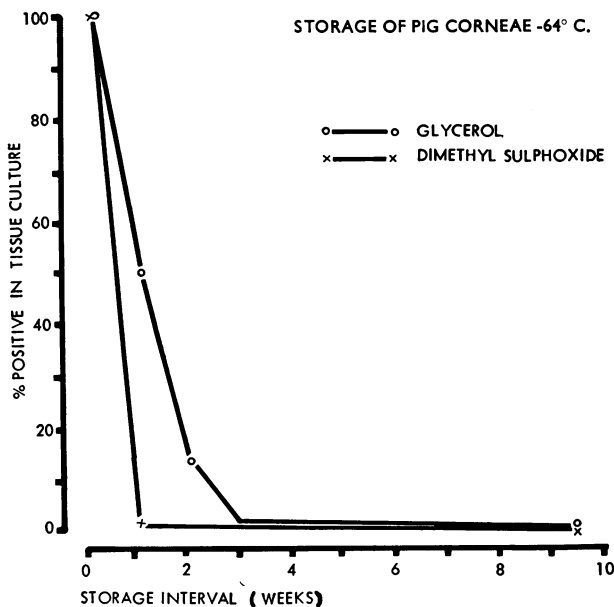


FIG. 4.—Pig corneal tissue.

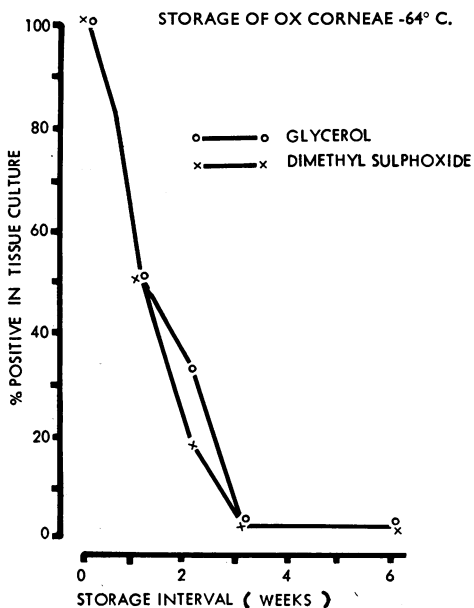


FIG. 5.—Ox corneal tissue.

and the tissue culture was continued using only one type of propagating medium. This agrees with the opinion of Stocker, Eiring, Georgiade, and Georgiade (1959a), who observed that fresh cultures of rabbit corneae grew equally well using autologous, homologous, and heterologous plasma and sera.

Previous work in this laboratory (Reed and others, 1960) indicated that a slow freezing procedure was needed in order to preserve the viability of corneal tissue; in this series, however, the storage containers were not wrapped in any insulating material before being placed in the freezing chest.

Perhaps -45°C. should have

been tested as a storage temperature in this experiment, since work by Stocker and others (1959b) showed it to be the most favourable temperature for storage over a wider range of glycerol concentrations and more effective over longer intervals of storage. It is also possible that a different concentration might prove to be more effective than the 15 per cent./V used in these experiments.

Recent work suggests that, for lamellar grafts in particular, living corneal tissue is not essential. It is possible, therefore, that corneal material stored in dimethyl sulphoxide might give equal or better results than that stored in glycerol despite its lessened viability.

Conclusions

15 per cent./V glycerol in a balanced salt solution appears to be a better medium than a 15 per cent./V solution of dimethyl sulphoxide for the storage of corneae at -64°C . when viability is determined by tissue culture.

Summary

The effectiveness of a 15 per cent./V solution of glycerol in Hank's balanced salt solution was compared with a 15 per cent./V solution of dimethyl sulphoxide as a suspension medium for corneae during freezing.

The specimens were stored in a commercial CO_2 chest at -67°C . Moderately rapid freezing and rapid thawing procedures were employed and the success of these procedures was evaluated by determining the viability of the corneal material in tissue culture after storage.

Under the conditions employed, glycerol was more effective as a preservative than dimethyl sulphoxide.

REFERENCES

- COCKERAM, A. M., BASU, P. K., and ORMSBY, H. L. (1957). *Amer. J. Ophthalm.*, **43**, 380.
 EASTCOTT, H. H. G., CROSS, A. G., LEIGH, A. G., and NORTH, D. P. (1954). *Lancet*, **1**, 237.
 FLEMING, A. (1952). Session II, "The Preservation of Mammalian Cells at Low Temperatures", in "Freezing and Drying—a Symposium, 1951", ed. R. J. C. Harris, pp. 55–106. Institute of Biology, London.
 LOVELOCK, J. E. (1954). *Biochem. J.*, **56**, 265.
 ——— and BISHOP, M. W. H. (1959). *Nature (Lond.)*, **183**, 1394.
 POLGE, C., SMITH, A. U., and PARKES, A. S. (1949). *Ibid.*, **164**, 666.
 REED, H., STANFIELD, F. J., and NAGEL-LEINDL, L. (1960). *Manitoba med. Rev.*
 STOCKER, F. W., EIRING, A., GEORGIADIS, R., and GEORGIADIS, N. (1959a). *Amer. J. Ophthalm.*, **46**, No. 1, Pt 2, p. 294.
 ———, ———, ———, ———, (1959b). *Ibid.*, **47**, 772.